

Near-Infrared Manipulation of Membrane Ion Channels via Upconversion Optogenetics

Zhimin Wang, Ming Hu, Xiangzhao Ai, Zhijun Zhang, and Bengang Xing*

Membrane ion channels are ultimately responsible for the propagation and integration of electrical signals in the nervous, muscular, and other systems. Their activation or malfunctioning plays a significant role in physiological and pathophysiological processes. Using optogenetics to dynamically and spatiotemporally control ion channels has recently attracted considerable attention. However, most of the established optogenetic tools (e.g., channelrhodopsins, ChRs) for optical manipulations, are mainly stimulated by UV or visible light, which raises the concerns of potential photodamage, limited tissue penetration, and high-invasive implantation of optical fiber devices. Near-infrared (NIR) upconversion nanoparticle (UCNP)-mediated optogenetic systems provide great opportunities for overcoming the problems encountered in the manipulation of ion channels in deep tissues. Hence, this review focuses on the recent advances in NIR regulation of membrane ion channels via upconversion optogenetics in biomedical research. The engineering and applications of upconversion optogenetic systems by the incorporation multiple emissive UCNPs into various light-gated ChRs/ligands are first elaborated, followed by a detailed discussion of the technical improvements for more precise and efficient control of membrane channels. Finally, the future perspectives for refining and advancing NIR-mediated upconversion optogenetics into *in vivo* even in clinical applications are proposed.

1. Introduction


As essential cell surface components, membrane ion channels are primarily responsible for the propagation and integration of electrical signals in the nervous, muscular, and other systems, their activation or malfunctioning plays significant roles in physiological and pathophysiological processes, such as brain thinking, muscle contraction, and channelopathies.^[1] Precisely regulating various membrane channels and spatiotemporally balancing the relevant dynamic processes are crucial to understand the biological implications of cellular ion channels, as well as provide insight into the

future therapy of neurological and other life-threatening diseases.^[2] Conventional strategies through chemical, genetic or electrical approaches have been well developed for modulation of ion channel activity and conductance in biochemical research.^[3] Despite the initial success in practice, these commonly used methods are faced with challenges of dissecting the role of dynamic nature. For instance, nonspecific accumulation and action of chemical drugs with the blood circulation would be inevitable, which may compromise the spatial accuracy of the control.^[4] In addition, the irreversibility of chemical or genetic perturbations would be another inherent concern that impedes the patterned stimulation with a high temporal resolution.^[3b] Furthermore, although the electrical patterns show great promise for spatiotemporal mapping and modulation of voltage-gated ion channels, even for deep brain's stimulation (DBS), the highly invasive implantation of electrodes or chips, and associated adverse events are considerable concerns for the clinical application.^[5] Therefore, the development of unique and reliable

techniques, which exhibit high spatiotemporal precision, minimal invasion as well as broad applicability for membrane ion channels modulation and future translational studies are highly desirable.

In recent years, using light to control biomolecules and biological processes has gained much attention based on its unsurpassable flexibility and spatiotemporal precision.^[6] One typical technology, termed as “optogenetics,” has been extensively utilized for optical manipulation neural activity in neuroscience with high specificity and temporal resolution (millisecond-timescale).^[7] Most importantly, the rapid evolution of light-sensitive microbial opsins also opens up opportunities for driving optogenetic ion channels manipulation into more complex systems, ranging from single cell level *in vitro* to brain circuitries-mediated behavioral analyses in freely moving animals.^[8] However, despite the remarkable achievements, currently established opsins or other optogenetic tools for membrane channels modulation are mainly sensitized in visible window (Scheme 1a), which significantly limits their uses for *in vivo* applications due to the unsatisfactory light penetration and less controlling efficacy caused by biological tissues absorption and scattering (Scheme 1b).^[7c,9] Although

Z. Wang, M. Hu, Dr. X. Ai, Dr. Z. Zhang, Prof. B. Xing
Division of Chemistry and Biological Chemistry
School of Physical & Mathematical Sciences
Nanyang Technological University
Singapore 637371, Singapore
E-mail: Bengang@ntu.edu.sg

 The ORCID identification number(s) for the author(s) of this article can be found under <https://doi.org/10.1002/adbi.201800233>.

DOI: 10.1002/adbi.201800233

the approach by optical fiber or LED implantation could partly achieve deep tissue stimulations, the highly invasive procedure raises tremendous safety concerns.^[10] Hence, the engineering of novel optogenetic technique that allows minimized invasion in deep tissues for controlling of membrane channels is of great importance.

Notably, extensive efforts have been devoted to achieve the goal for in vivo optogenetic stimulation, in which shifting the excitation wavelength to the near infrared (NIR) window is considered to be favorable for deeper tissue penetration.^[9b,13] Although significant progress has been acquired in genetically engineering of red-shifted ChRs,^[14] current optogenetics is still constrained within the visible wavelength window (e.g., <650 nm). Alternatively, by employing a combination of NIR light responsive nanomaterials and optogenetic tools encourages the possibility of enhanced penetration, where the nanomaterials mainly serve as photon transformer in optogenetic stimulation.^[15] To this end, lanthanide-doped upconversion nanoparticles (UCNPs) as emerging photonic nanomaterials with their fascinating capability to convert NIR light (e.g., at 980 or 808 nm, **Scheme 2a,b**) into multiple emissions ranging from UV–vis to NIR region, have attracted numerous interests in bioimaging and nanomedicine due to the less scattering and deeper penetration depth into tissues.^[16] It is noted that the development of NIR light-triggered optogenetics through incorporating multiple emissive UCNPs into various light-sensitive opsins, terms as upconversion optogenetics (Scheme 2c), have been witnessed in the past few years, relevant progress and results in membrane ion channels control have already been widely reported.^[15a,17]

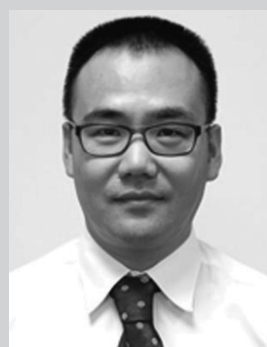
Therefore, in this review, we focus on the recent advances of upconversion optogenetics for NIR manipulation of membrane ion channels in biomedical research. The engineering of upconversion optogenetic systems by incorporation specific UCNPs into desired ChRs or other light-sensitive proteins will be first elaborated, followed by detail discussion of their broad applications as well as technique improvements, such as enhancing photon-converting efficacy of UCNPs, minimizing heating effect and localized regulation for more precise and efficient optogenetic manipulation of membrane channels. Finally, the conclusion and future perspectives for refining and advancing NIR-mediated upconversion optogenetic manipulations of ion channels into in vivo applications, even in clinical studies are proposed.

2. Advances of Upconversion Optogenetics in NIR Membrane Ion Channel Manipulations

The combination of upconversion nanotechnology and optogenetics provides great opportunities for NIR manipulation of membrane ion channels in either neuronal or nonneuronal research. In particular, significant efforts in neuroscience have been made by utilization of upconversion optogenetics, which lifts such flexible optical interrogation tools to a level of broader applicability.^[15c,21] Hence, the recent advances of diverse applications are discussed from the following perspectives: upconversion optogenetic systems, stimulation platforms, and applied models.



Zhimin Wang received his bachelor's degree and master's degree from China Pharmaceutical University in 2013 and 2016. He is currently pursuing his Ph.D. under the supervision of Prof. Bengang Xing in the Division of Chemistry and Biological Chemistry, School of Physical and Mathematical Sciences, Nanyang Technological University. His research focuses on the development of near infrared photon-converting nano-platforms for bioimaging and biofunctional regulation.

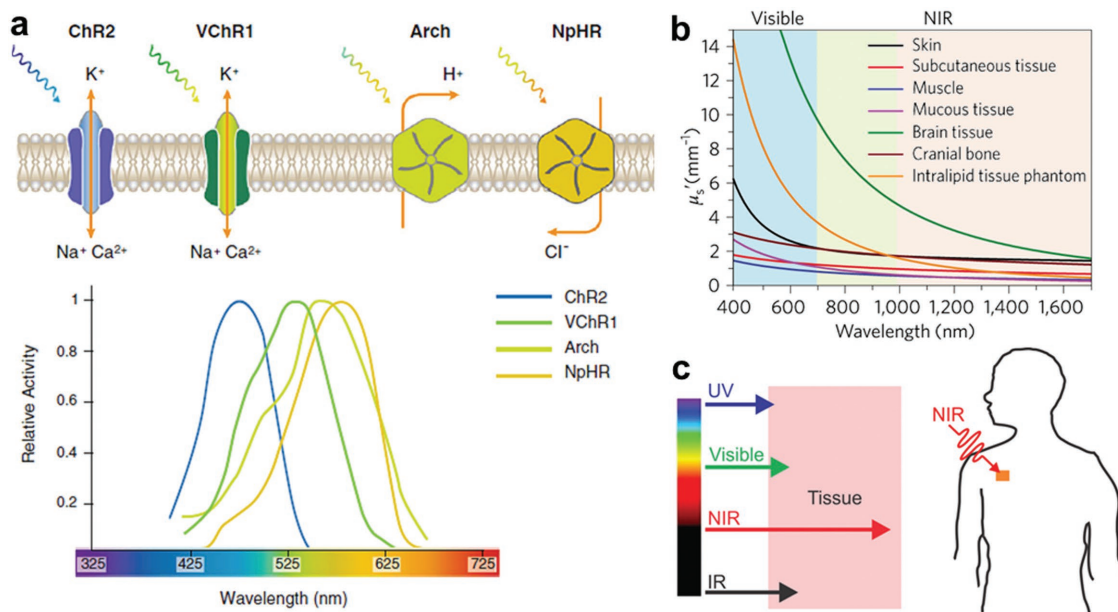


Bengang Xing received his Ph.D. degree from Nanjing University in 2000, after which he did his postdoctoral studies in HKUST, UCLA, and Stanford University. He joined Nanyang Technological University in 2006. Currently he is an associate professor in the School of Physical & Mathematical Sciences. His research interest is at the interface of bioimaging, nanomedicines, and chemical biology, focusing on the development of “smart” small molecules, peptides, and nanomaterials for biological process monitoring and diagnosis.

2.1. Upconversion Optogenetics

As summarized in **Table 1**, by incorporation appropriate UCNPs into light-sensitive opsins, flexible and multiplexed activation/inhibition of specific ion channels can be achieved in response to NIR stimulation. The idea proposed first by Deisseroth and Anikeeva in a patent application in 2011,^[22] where compositions and methods for noninvasively delivering light to light-responsive opsins-expressed neurons on the plasma membrane via the use of various UCNPs are provided. In 2013, Han et al. also proposed in a grant to develop the wireless upconversion optogenetics for in vivo neuronal manipulations.^[23]

Hereafter, successful implementation of upconversion optogenetics was reported in 2015 by different research groups independently.^[17,25] For example, Yawo and co-workers used rare-earth elements doped UCNPs as luminous donors to activate ChRs, in which different types of upconversion optogenetic systems (termed “LNP-ChRs,” **Figure 1**) were established for membrane ion channels and neural activities manipulation.^[17] Upon NIR (976 nm) irradiation, the LNP(NaYF₄: Sc/Yb/Er) emitted green light (550 nm) that in turn activated C1V1 or mVChR1 to generate a photocurrent in



Scheme 1. Optogenetic tools—channelrhodopsins for membrane ion channels manipulation and spectral working properties. a) channelrhodopsin-2 from *Chlamydomonas reinhardtii* (ChR2) and channelrhodopsin-1 *Volvox carteri* (VChR1) from nonselective cation channels leading to depolarization of target cells; Archaelhodopsin-3 (Arch) from *Halorubrum sodomense* works as a proton pump and leads to hyperpolarization of the target cell such as the chloride pump NpHR (NpHR) from *Natronomonas pharaonis*. Reproduced with permission.^[8a] Copyright 2012, Springer-Verlag. b) Reduced scattering coefficients of various tissues plotted as a function of wavelength covering 400–1700 nm. Reproduced with permission.^[11] Copyright 2017, Nature Publishing Group. c) Tissue penetration depth of light with different wavelengths. Reproduced with permission.^[12] Copyright 2017, Royal Society of Chemistry.

the expressing cells (Figure 1b). Similarly, the blue emissive (peaks at 450 and 480 nm) LNP(NaYF₄:Sc/Yb/Tm@NaYF₄) could effectively activate PsChR ion channel with significant photocurrent generation (Figure 1c). Moreover, the results of NIR stimulations also demonstrated time-dependent and power-dependent manners. However, there remains room for refinements of this study, such as the light-upconverting efficiency of UCNP-ChR systems, the biosafety of UCNPs and NIR optics, to further boost upconversion optogenetics into in vivo applications.

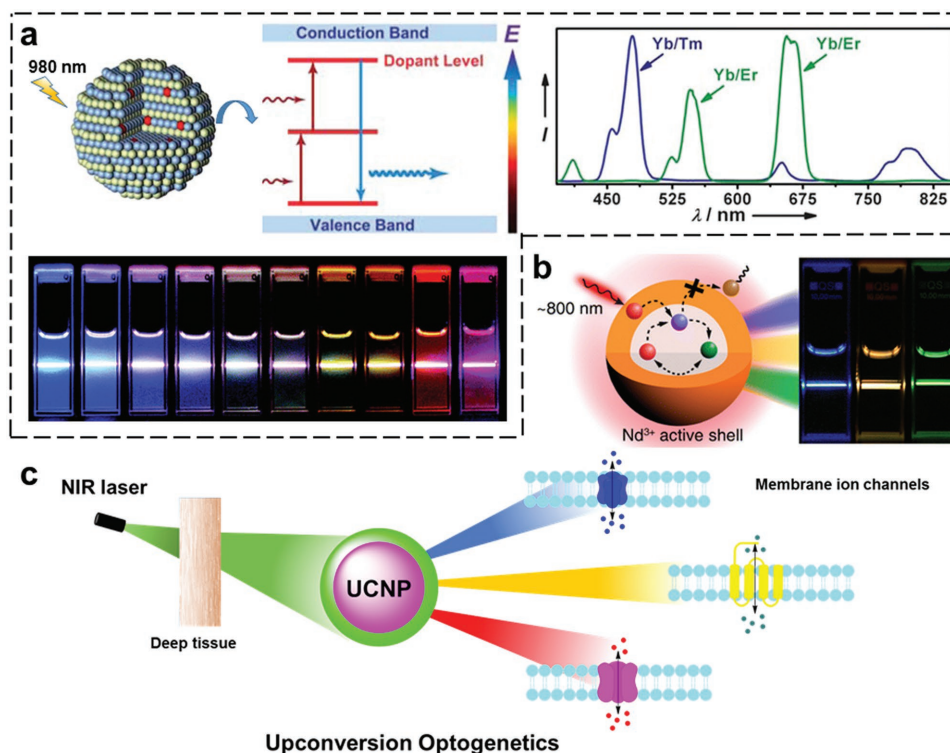
In addition to the superior UCNP-ChR systems for NIR manipulation ion channels, Zhou and co-workers demonstrated another type of UCNP-based optogenetic platform, termed “Opto-CRAC” (Figure 2a).^[25b] Such unique optogenetic system was capable of remote and selective control Ca²⁺ influx, subsequent Ca²⁺-dependent gene expression and further photomodulate of immune-inflammatory responses both in vitro and in vivo. In which, blue emissive NaYF₄:Yb/Tm@NaYF₄ UCNPs upon 980 nm excitation was applied to manipulate Ca²⁺ gate-controlling protein that was genetically engineered to enable blue light-activation (Figure 2b–d). Moreover, the optogenetic module-LOVSoC^[27] can reversibly generate both sustained and oscillatory Ca²⁺ signals through the light input adjustment (e.g., laser pulse and intensity). Most importantly, Opto-CRAC mediated light-generated Ca²⁺ signaling pathway can lead to specific physiological responses in cells of the immune system and further enable its potential application for (patho)physiological studies in animal models.

The lanthanide-doped UCNPs with tunable emission can be applied to activate/inhibit a wide range of genetically

photosensitive ion channel proteins (e.g., ChRs and CRAC). In combination with the deep light delivery in vivo, such promising and multiplexed upconversion optogenetics will provide great opportunities to specifically and precisely control ion-dependent signaling pathways, biological activities and even the ultimate goal of disease therapy.

2.2. Stimulation Platforms

Despite the flexible adaptability of upconversion optogenetic systems, great efforts have addressed that the development of preferable stimulation platforms could intensively facilitate efficient NIR stimulations of light-sensitive ion channels. As shown in Figure 3a, Lee and co-workers reported hybrid upconversion nanomaterials, UCNPs (NaYF₄:Yb/Tm@NaYF₄) embedded poly(lactic-co-glycolic acid) (PLGA) 0.5 mm films, which were used as underlying scaffolds for neurons culture and optogenetic stimulation.^[25a] Such hybrid polymeric scaffolds converted NIR light to blue luminescence as the internal excitation light source, and subsequently activated blue light-sensitive channelrhodopsin-2 (ChR2)-expressed neurons. The neurons generated time-locked, sustained naturalistic impulses with millisecond resolution in response to 980 nm laser pulses at 1, 5, and 10 Hz. Most critically, this upconversion platform could be further optimized for NIR-mediated optogenetic control by balancing multiple physicochemical features of the nanomaterial (e.g., morphology, size, emission spectra, surface modification, concentration), thus enabling an early demonstration of rationally



Scheme 2. Optical properties of UCNPs and upconversion optogenetics designing. a) UCNPs composed of a crystalline host and lanthanide dopant ions embedded in the host lattice. Typical energy level diagram and emission spectra under 980 nm laser excitation. UC multicolor fine-tuning through the use of lanthanide-doped NaYF_4 nanoparticles with varied dopant ratios (Yb/Tm/Er). Schematic diagrams of the nanoparticle and the energy transfer are reproduced with permission.^[18] Copyright 2010, Royal Society of Chemistry. Emission spectra and luminescent photos are reprinted with permission.^[19] Copyright 2008, American Chemical Society. b) Nd^{3+} -sensitized UCNPs with multicolor UC luminescence upon 800 nm excitation. Reproduced with permission.^[20] Copyright 2013, American Chemical Society. c) Upconversion optogenetics for NIR manipulation of membrane ion channels.

designing nanomaterial-based strategies for advanced neural applications.

Additionally, relevant studies regarding the effective optogenetic light delivery by different stimulation platforms and methods were also demonstrated in Yawo and co-workers.^[17] The UCNPs were set close to the recording cell by either Method 1 or Method 2 (Figure 3b). Both two methods showed inward photocurrent response in a manner dependent on the laser power, which indicated that the upconverting luminescence (green emission) emitted from UCNPs effectively activated the ChR-based membrane channel (C1V1). However, Method 1 showed higher efficiency than Method 2 upon the same power of NIR excitation, it is suggested that UCNPs should be positioned as close as possible to the ChR protein since the power density is proportional to the reciprocal square of the distance. This result provides valuable information to clarify optimized conditions for further applications of upconversion optogenetic systems.

Other than the progress of developing upconversion platforms for in vitro optogenetic stimulation, Shi et al. first packaged UCNPs into glass micro-optrodes to form implantable transducers that can convert NIR energy to visible light and subsequently stimulate neurons with different ChRs expression (Figure 3c).^[28] These microdevices showed superb

long-term biocompatibility and allowed remotely NIR modulation of brain function, even to control complex animal behaviors. Most significant, such fully implantable microdevices not only represented the feasibility of NIR optogenetic practical applications in living animals but also opens up new possibilities for remote and multiplexed control of neural activities in the brains.

Very recently, Sheng et al. reported another type of infrared-to-visible upconversion concept-based microscale optoelectronic devices for highly efficient optogenetic neuromodulation (Figure 3d).^[29] The thin-film, ultraminiaturized devices realize NIR to visible red or yellow upconverting emission that was linearly dependent on incoherent, low-power excitation, with a quantum yield of about 1.5%. Moreover, these microscale devices showed promising biocompatibilities in the heterogeneous biological environment. Most notably, in vitro and in vivo studies demonstrated the possible utility of such injectable microdevices for optogenetic neuromodulation upon implanted in behaving animals. Compare to UCNP-based systems, this type of optoelectronic platform provides another versatile route to achieve photon-upconversion throughout the entire visible spectral range with higher efficiency. It is also feasible for optogenetic stimulation various ChRs in living animals via upconversion spectral tuning.

Table 1. Summary of commonly used opsins and upconversion nanoparticles for engineering upconversion optogenetic systems.

Opsin	$\lambda_{\max}^a)$	Threshold limit value ^{b)}	Functions	UCNP	X ^{c)}	Ex.	Em. ^{d)}
ChR2	480 nm	P: $\approx 1.1 \text{ mW mm}^{-2}$ S: $\approx 1.05 \text{ mW mm}^{-2}$	Excitatory cation channel; native from <i>Chlamydomonas reinhardtii</i>	NaYF ₄ : Yb/X@NaYF ₄	Tm	980 nm	450, 475, 800 nm
VChR1	545 nm	Not tested	Red-shifted, excitatory cation channel; native from <i>Volvox carteri</i>		Er		545, 665 nm
ChIEF	450 nm	P: $\approx 1.65 \text{ mW mm}^{-2}$ S: $\approx 1.38 \text{ mW mm}^{-2}$	Fast kinetics, excitatory cation channel; chimera of ChR1 and VChR1		Pr		480 606 nm
ChETA	470 nm	P: $\approx 5.02 \text{ mW mm}^{-2}$ S: $\approx 0.62 \text{ mW mm}^{-2}$	Fast kinetics, excitatory cation channel; substitutions in ChR2		Tb		480–650 nm
Step function opsins	470 nm on 590 nm off	Not tested	Excitatory cation channel; single mutations in ChR2		Eu		570–720 nm
Chronos	500 nm	$\approx 0.3 \text{ mW mm}^{-2}$	Fast kinetics, excitatory cation channel; native from <i>Stigeoclonium helveticum</i>		Sm		601 nm
Chrimson	590 nm	$\approx 4.0 \text{ mW mm}^{-2}$	Red-shifted, excitatory cation channel; Native from <i>Chlamydomonas noctigama</i>		Ho		540 nm
C1V1	540 nm	$\approx 7.2 \text{ mW mm}^{-2}$	Red-shifted, excitatory cation channel; chimera of ChR1 and VChR1		Dy		486, 575 nm
NpHR	590 nm	$\approx 21.7 \text{ mW mm}^{-2}$	Inhibitory chloride pump; native from <i>Natronomonas pharaonis</i>	NaGdF ₄ : Yb/X@NaGdF ₄	As above		As above
ChloC	480 nm	Not tested	Inhibitory anion channel; mutagenesis of ChR2				
GtACR2	520 nm	Not tested	Inhibitory anion pump; native from <i>Guillardia theta</i>	NaYF ₄ : Nd/Yb/X@NaYF ₄ : Nd	As above	980 nm or 808 nm	As above
ArCh	570 nm	Not tested	Inhibitory proton pump; native from <i>Halobacterium sodomense</i>				

^{a)}Peak λ indicates the wavelength of light that maximally activates the opsin; ^{b)}P and S indicate peak response and steady-state response, respectively; ^{c)}X indicates the doped lanthanide ions within UCNPs; ^{d)}Upconverting emissions with corresponding lanthanide ion-doped UCNPs. The table of both the optogenetic tools and UCNPs is reproduced with permission.^[9c,24] Copyright 2010, The Authors, Journal compilation copyright, 2011, The Physiological Society. Copyright 2016, John Wiley & Sons. Copyright 2013, Royal Society of Chemistry.

2.3. Applied Models

Upconversion optogenetic techniques have been successfully applied into broad biomedical research in a highly controlled manner and spatiotemporal precision. Such NIR manipulating system via incorporating UCNPs with genetically expressed photosensitive ion channels that in principle overcome the limitations, including low penetration of excitation light and the invasiveness of light source implantation, encountered in commonly used optogenetics in vivo.^[15a] Considering the inherent complexity of living conditions, so far, it has remained extremely difficult to experimentally clarify the fundamental physiological/pathophysiological implications based on current NIR optogenetics and other technical approaches. Evolutionary studies on upconversion optogenetics for NIR manipulation membrane ion channels and relevant biological activities in different animal models and varying purposes are discussed further below.

Other than the successful upconversion optogenetic manipulation of membrane channels in vitro, Zhang and co-workers utilized quasi-continuous wave NIR excitation approach to improve the efficacy and applicability of optogenetic neuromodulation in *Caenorhabditis elegans* model expressing ChR2 (Figure 4).^[30] This work offers possibility to enhance

multiphoton emissions from UCNPs, furthermore, it achieves NIR light-triggered upconversion optogenetic activation of the membrane channel in vitro and manipulation of touch-akin reversal behavior of *C. elegans* in vivo. Moreover, such noninvasive NIR optogenetic control strategy also exhibits flexibility for advancing optogenetic operations in other animal models and living system studies.

Zebrafish have proved to be a suitable in vivo model for biomedical studies in bioimaging and theranostics due to their physiological and genetic homology with mammals, high fecundity and easy operability.^[31] Therefore, the utilization of zebrafish in optogenetic manipulation would be highly convenient for spatiotemporally regulating and monitoring biological events. Xing and co-workers creatively applied upconversion optogenetics in living zebrafish for remotely activating the ion-channel (ChR2) and Ca²⁺ mediated biological functions under NIR (808 nm) light stimulation (Figure 5).^[32] Furthermore, site-specific covalent localization of UCNPs on the cell membrane by means of glycan metabolic labeling enabled more precise and efficient optogenetic regulation. It was revealed that the feasibility of NIR-light-mediated activation of ion channels to evoke apoptosis in vitro and in vivo. Very recently, another optogenetic study in living zebrafish from Xing et al. was reported to precisely manipulate membrane ion channel and

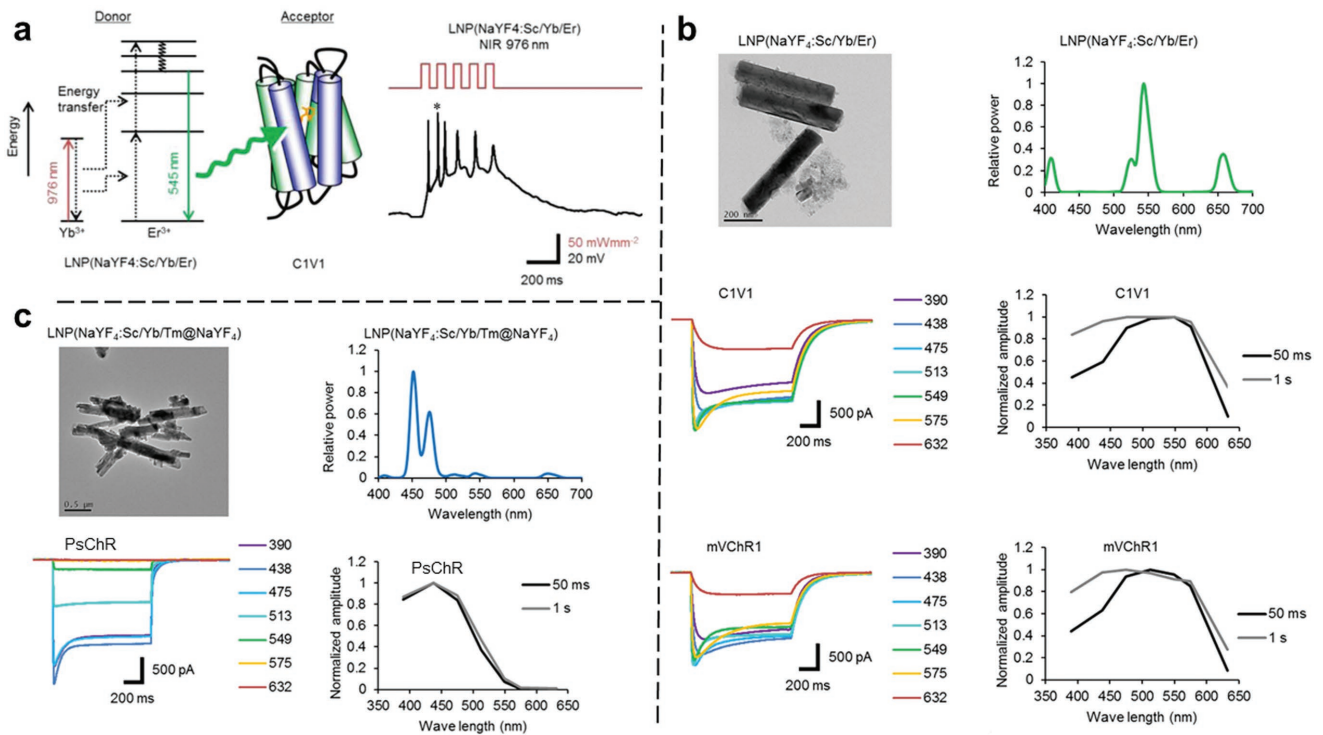


Figure 1. Upconversion optogenetic systems for opsin-based membrane ion channels manipulation. a) The visible light upconverted from donor UCNP through NIR laser excitation that is absorbed by the acceptor (C1V1) to activate the cation channel. Reproduced with permission.^[26] Copyright 2015, SPIE. b) Donor-acceptor matched upconversion optogenetics for yellow light-gated ion channels (C1V1 and mVChR1) manipulation. c) NIR upconversion activation of blue light-sensitive ion channel (PsChR). Reproduced under the terms and conditions of the Creative Commons Attribution 4.0 International License.^[17] Copyright 2015, Macmillan Publishers Limited.

Ca²⁺ influx triggered signaling pathway.^[33] As mentioned above, the practicality and reliability of zebrafish model in upconversion optogenetic studies would greatly promote further applications on the manipulation of biological events.

Over the past few decades, the mouse has emerged as a prevalent model system in biomedical research considering its high genomic and physiological homology with humans as well as enormous technical advances in genetic engineering

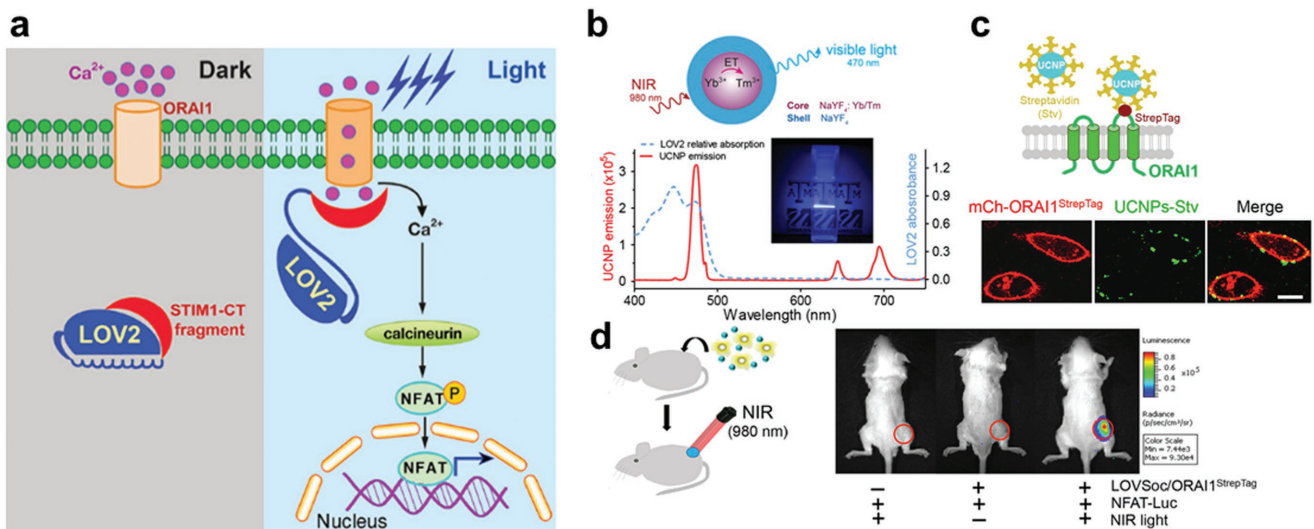


Figure 2. Opto-CRAC system for optogenetic manipulation of membrane Ca²⁺ channel. a) LOV2-mediated photoactivatable Ca²⁺ entry and NFAT nuclear translocation. b) Physicochemical properties of UCNP (NaYF₄: Yb/Tm@NaYF₄). c) Schematic showing the interaction between streptavidin-coated UCNP and the engineered ORAI1 Ca²⁺ channel on the plasma membrane. d) NFAT-dependent luciferase expression in vivo triggered by NIR light mediated Ca²⁺ influx. Reproduced under the terms and conditions of the Creative Commons Attribution License.^[25b] Copyright 2015, The Authors, eLife Sciences Publications.

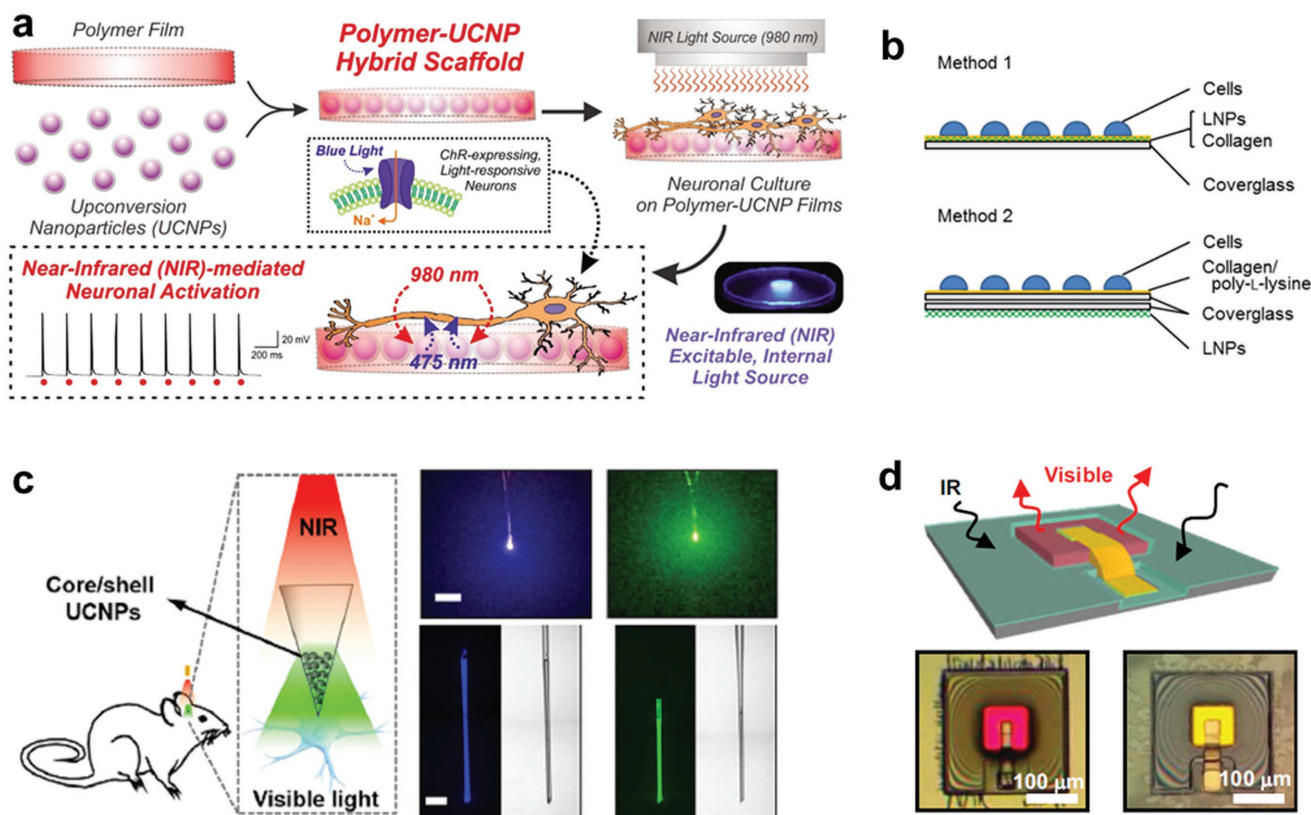


Figure 3. UCNP platforms for NIR optogenetic stimulation. a) The generation and application of polymer–UCNP hybrid scaffolds for optogenetic neuronal activation. Reproduced with permission.^[25a] Copyright 2015, Royal Society of Chemistry. b) Schematic illustration of two UCNP platforms applied in ChRs activation with different methods. Reproduced under the terms and conditions of the Creative Commons Attribution 4.0 International License.^[17] Copyright 2015, Macmillan Publishers Limited. c) Upconversion-based microdevices for tetherless neural optogenetic stimulation. Reproduced with permission.^[28] Copyright 2017, Elsevier Ltd. d) The microscale optoelectronic upconversion devices as injectable light sources for optogenetic neuromodulation. Reproduced with permission.^[29] Copyright 2018, National Academy of Sciences.

and characterization techniques.^[34] Although the application of mice in deep-tissue optical stimulation is substantially dependent on special techniques and complex manipulations, recent progress in optogenetics and upconversion nanotechnology have addressed the possibility of NIR optogenetic control mouse (or rat) brain activity.

Shi et al. reported an all-optical method by utilizing UCNP based microdevices to remote control neurons expressing different opsin proteins (ChR2 or C1V1) in rat brain (Figure 6). Upon NIR illumination with the robotic laser projection system, this neural stimulation technique was able to reliably trigger spiking activity and spatiotemporally modulate brain activity in various regions, including the striatum, ventral tegmental area, and visual cortex. Moreover, this study provided an innovative tetherless upconversion optogenetic strategy for brain stimulation in behaving rats. Notably, another work by Shi et al. applied the similar upconversion based strategy for multiplexed optogenetic neural stimulation both in vitro and in vivo by fabricating spectrum-selective UCNP.^[35] Such unique technique would significantly benefit both fundamental physiological regulation and translational neuroscience research.

Furthermore, several other upconversion optogenetic studies are also significantly contributing to this field. For instance,

McHugh and Liu recently explored feasibility of using UCNP as optogenetic actuators to miniinvasively stimulate deep neurons in the mouse brain (Figure 7).^[36] Moreover, the NIR-mediated upconversion optogenetics could evoke dopamine release from genetically tagged neurons in the ventral tegmental area (VTA), and induce brain oscillations through activation of inhibitory neurons in the medial septum (MS). Most importantly, such upconversion optogenetics can also silence seizure by inhibition of hippocampal excitatory cells, and thus trigger memory recall. Such a promising technology will enable NIR manipulation of neuronal activity with the great potential for remote therapy of the neurological disease.

3. Strategies to Improve Upconversion Optogenetics

Despite the great promise, upconversion optogenetics is still facing challenges of low photon-conversion efficacy and heating effect caused by NIR illumination.^[15a,37] To address these issues, multitudinous strategies have been established to increase the upconversion luminescence, including surface passivation, material design, engineering of the excitation source, or a

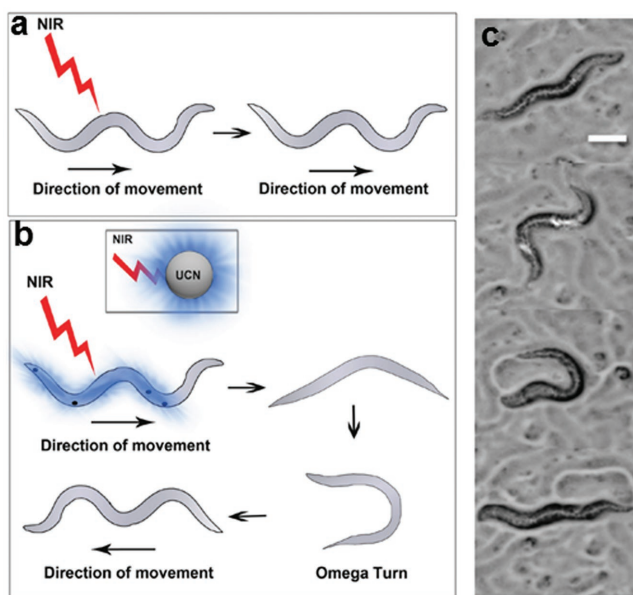


Figure 4. Upconversion optogenetic manipulation of *Caenorhabditis elegans* expressing ChR2 by quasi-continuous wave NIR excitation. a) Different movement behavior in worms expressing ChR2 in their mechanosensory neurons with NIR light illumination alone (no UCNP) and b) NIR light illumination in the presence of UCNP. c) Representative images showing the reversal process under NIR optogenetic stimulation. Reproduced with permission.^[30] Copyright 2016, Wiley-VCH.

combination of multiple parameters.^[38] Moreover, site-specific optogenetic manipulation by internalizing or localizing UCNP inside cell or on cell surface has proved to be an alternative method for overcoming above limitations.^[32] These improvement approaches provide exciting opportunities to promote NIR-mediated upconversion optogenetics for more efficient and precise biomedical modulations, and enabling future translational and clinical research.

3.1. Enhancing Photon-Converting Efficacy of UCNP

As summarized in **Table 2**, the recent advances to enhance photon-converting efficacy of UCNP provide flexible solutions for further improving upconversion optogenetics. Among numerous strategies, surface passivation by preparing core-shell nanostructured UCNP was an effective and commonly used approach in majority of NIR optogenetic studies. Besides, very recent efforts from Wang and co-workers have been made to enhance the luminescence of UCNP through fabricating core-shell-shell structure and optimizing the doping contents of ytterbium ions (Yb^{3+}) (**Figure 8a**),^[39] which achieved nearly threefold luminescent enhancement over traditional core-shell UCNP and were further utilized as optical transducers to develop a fully implantable upconversion-based device for in vivo optogenetic inhibition in eNpHR-expressed behaving mice. Meanwhile, another previous work by Prasad and co-workers applied a new type of core-shell UCNP (NaYbF_4 : Tm@NaYF_4)

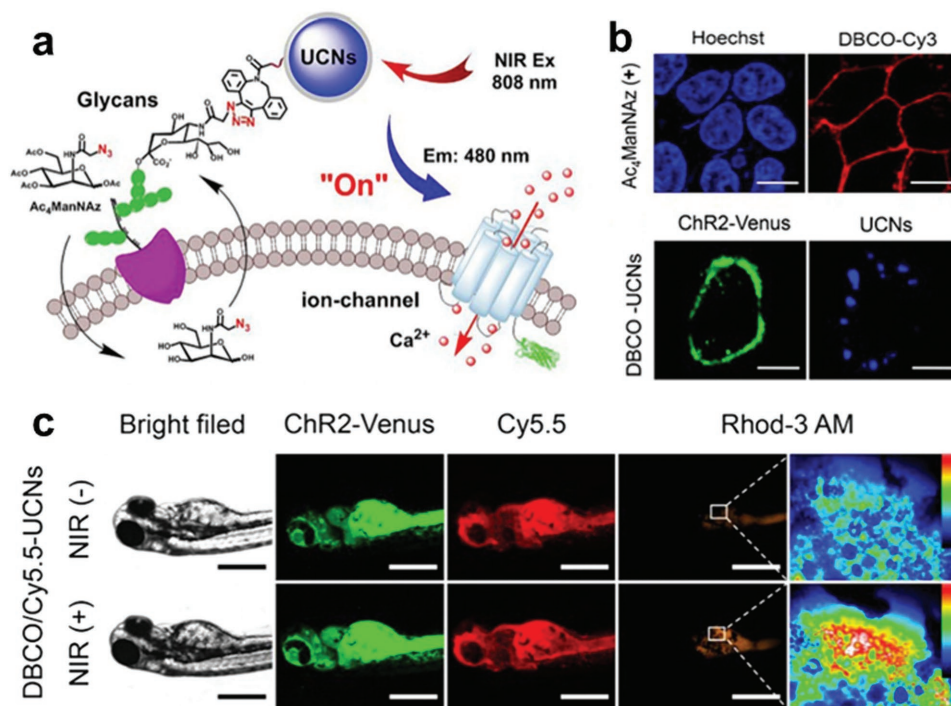


Figure 5. Membrane localization of upconversion optogenetics for precise ion channel stimulation in zebrafish. a) Metabolic labeling strategy for site-specific covalent localization of UCNP on the cell membrane to enable the precise optogenetic regulation. b) Imaging of membrane localization of UCNP through glycan metabolic strategy. c) In vivo optogenetic activation of ChR2 in zebrafish. Reproduced with permission.^[32] Copyright 2017, Wiley-VCH.

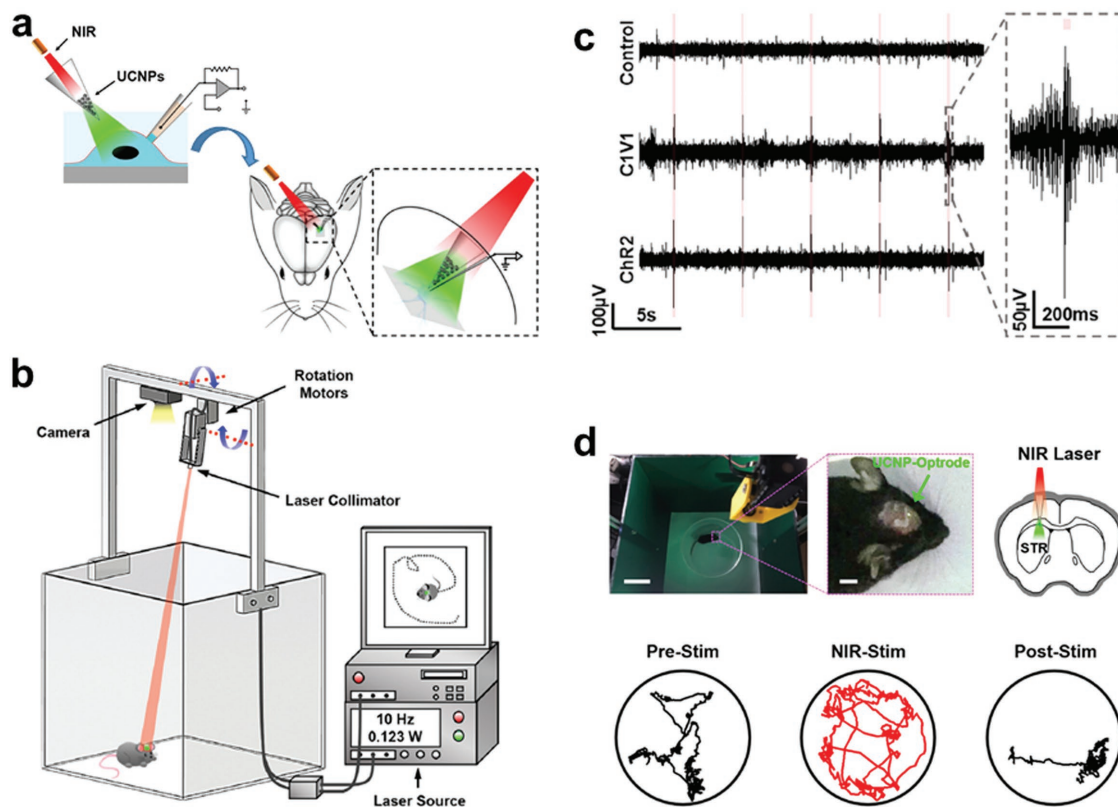


Figure 6. NIR optogenetic control of rat brain activity based on upconversion neural stimulation. a) NIR stimulation of ChR-expressing neurons using UCNP in vitro and in vivo. Reproduced with permission.^[35] Copyright 2017, Wiley-VCH. b) A robotic laser projection system for automatic and consistent NIR irradiation of the heads of behaving animals. c) Representative recordings of NIR-driven spiking traces in rats brain expressing different ChR variants (ChR2 and C1V1). d) Tetherless all-optical brain stimulation in behaving animals. Reproduced with permission.^[28] Copyright 2017, Elsevier Ltd.

in NIR optogenetics, such photon-transformers presented exceptionally high, about 6-times higher luminescent intensity than conventional $\text{NaYF}_4:\text{Yb/Tm}@ \text{NaYF}_4$ UCNP.^[40] Other than the aspect of UCNP optimization, Zhang and co-workers enhanced the blue emission intensity of UCNP

by employing quasi-continuous wave as excitation source to achieve efficient optogenetic neuroregulations.^[30] These progresses regarding upconversion luminescence enhancement provide great opportunities for the feasibility of using UCNP in NIR optogenetics.

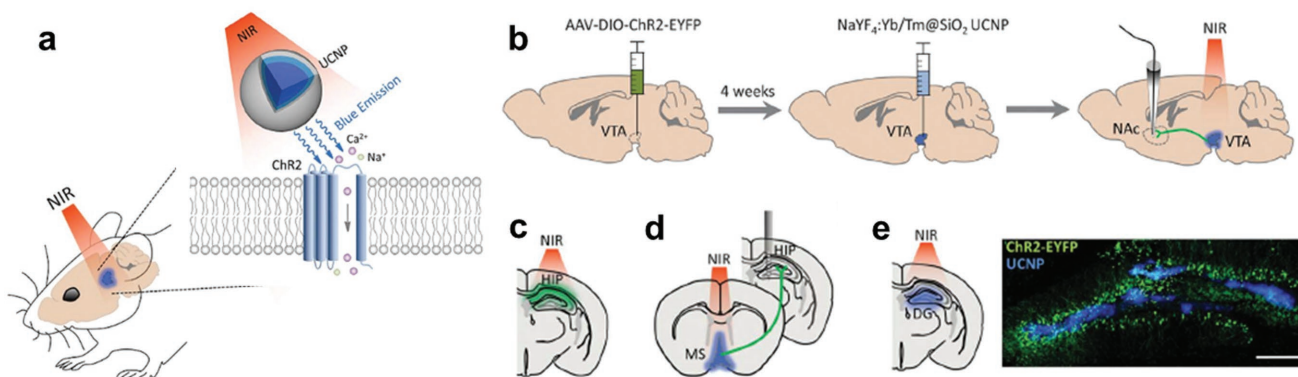


Figure 7. UCNP-mediated NIR upconversion optogenetics for deep brain stimulation. a) Principle of blue emissive UCNP-mediated NIR upconversion optogenetics for ChR2-expressed neurons excitation in TH-Cre transgenic mice. b) Experimental scheme of AAV-DIO-ChR2-EYFP and UCNP injection and NIR optogenetic stimulation in deep brain tissue. c) Illustration of transcranial NIR inhibition of hippocampal (HIP) activity during chemically induced seizure, e) Medial septum (MS) for generation of theta oscillations and d) hippocampal engram for memory recall. Reproduced with permission.^[36] Copyright 2018, The Authors, some rights reserved, exclusive licensee American Association for the Advancement of Science.

Table 2. The typical strategies for enhancing UCNPs' photon-converting efficacy.

Upconversion enhancement

Strategies	Representative example	Enhancement factor ^{a)}	Refs.
A	Lithium ion or other transition-metal ions doping to improve the coordination environment and crystal field splitting of lanthanides	$\text{Na}_x\text{Li}_y\text{YF}_4: \text{Yb}^{3+}, \text{Er}^{3+}$	≈ 30 (540 nm) [41]
		$\text{Y}_2\text{O}_3: \text{Er}^{3+}, \text{Li}^+$	≈ 60 (410 nm), ≈ 25 (540 nm) [42]
		$\text{NaGdF}_4: \text{Yb}^{3+}, \text{Er}^{3+}, \text{Li}^+$	≈ 47 (540 nm), ≈ 23 (660 nm) [43]
B	Incorporating transition metals to control the energy transfer processes	Er-Mo: $\text{Yb}_2\text{Ti}_2\text{O}_7$	≈ 250 (540 nm) [44]
		Er-Mn: $\text{Yb}_3\text{Al}_5\text{O}_{12}$	≈ 260 (540 nm), ≈ 20 (660 nm) [45]
		Optimizing Tm doping concentration	$\text{NaYF}_4: \text{Yb}^{3+}, \text{Tm}^{3+}$
	Increasing Yb doping concentration	$\text{NaTm}_{0.02}\text{Lu}_{0.98-x}\text{Yb}_x\text{F}_4$	13.6 (340 nm), 23.3 (450 nm) [47]
C	Encapsulating Au or Ag for plasmonic enhancement of upconversion luminescence	$\text{NaYF}_4: \text{Yb}^{3+}, \text{Tm}^{3+}@ \text{Au}$	≈ 50 (450 nm), ≈ 30 (470 nm) [48]
		$\text{NaYF}_4: \text{Yb}/\text{Er}/\text{Tm}@ \text{Ag}$	≈ 21 (450 nm), ≈ 22 (540 nm) [49]
D	Amorphous shell coating	$\text{Y}_2\text{O}_3: \text{Tm}^{3+}, \text{Yb}^{3+}@ \text{SiO}_2$	≈ 200 (480 nm) [50]
	Crystalline-shell coating	$\text{KYF}_4: \text{Yb}^{3+}, \text{Er}^{3+}@ \text{KYF}_4$	> 20 (540, 660 nm) [51]
E	Incorporation of broadband sensitizers	$\text{NaGdF}_4: \text{Yb}^{3+}, \text{Tm}^{3+}@ \text{NaGdF}_4$	≈ 450 (480 nm) [52]
		$\text{NaYF}_4: \text{Yb}/\text{Tm}@ \text{NaYF}_4: \text{Nd}$	≈ 7 (450/480, 540, 650 nm) [20]
		$\text{NaYF}_4: \text{Yb}/\text{Er}@ \text{NaYF}_4: \text{Nd}$	
	$\text{NaYF}_4: \text{Yb}/\text{Ho}@ \text{NaYF}_4: \text{Nd}$		
	$\text{NaYF}_4: \text{Yb}/\text{Er}@ \text{IR806}$	3300 (550, 650 nm) [53]	
F	Adding UCNPs to photonic crystals	PMMA OPCs/ $\text{NaYF}_4: \text{Yb}^{3+}, \text{Tm}^{3+}$	≈ 32 (450, 480 nm) [54]

^{a)}Fold enhancement of the upconversion luminescence at specific wavelength. The schematic diagram was reproduced with permission.^[38b] Copyright 2014, Wiley-VCH.

3.2. Controlling Heating Effects

The utilization of UCNPs can effectively activate light-sensitive membrane ion channels in deep tissues. However, it should be noted that conventional UCNPs were excited by 980 nm laser, such NIR illumination possibly leads to potential thermal damage to the local tissue due to the strong water absorption.^[38a] To this end, shifting the excitation wavelength to 800 nm (where water absorption was largely reduced) could be useful for minimizing laser-induced local overheating effect.^[55] Accordingly, Han et al. used 800 nm NIR light to activate ion channel protein (ReaChR) in hippocampal neurons, which were cultured on thin films of poly(methylmethacrylate) embedded with dye-sensitized core/shell UCNPs (Figure 8b).^[56] This strategy not only amplifies the upconversion luminescence efficiency but also significantly

reduces the temperature increasing during NIR light stimulation in a mouse model. Another method by exploring Nd^{3+} sensitized UCNPs with 808 nm laser excitation could also overcome the heating effect.^[55] Such type of UCNPs was successfully applied in NIR optogenetic regulation of membrane cation channel (ChR2) in cells and in zebrafish by Xing and co-workers.^[32] On the other hand, high power density or long-time laser irradiation can apparently enhance upconversion optogenetic efficiency, but it will result in potential heat damage. As shown in Table 3, a moderate power density ($< 50 \text{ mW mm}^{-2}$) of NIR optogenetic stimulation was applied for most of optogenetic studies in cells or in the zebrafish model. In addition, engineering UCNPs as implantable microdevices also demonstrated efficient transcranial neural stimulation at various depths in mouse or rat brains with relative low NIR laser power density ($< 10 \text{ mW mm}^{-2}$). It should be noted that

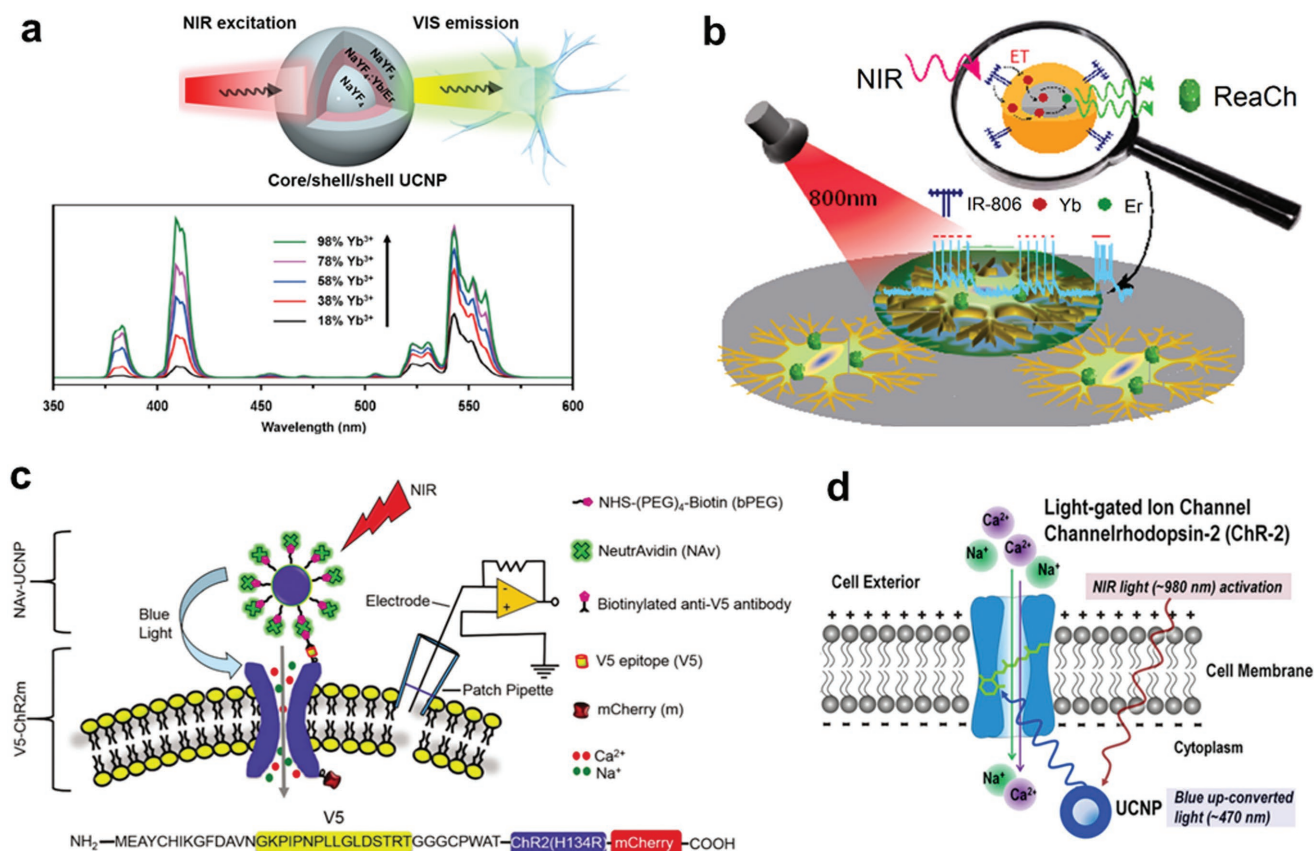


Figure 8. Strategies to improve the efficacy and applicability of upconversion optogenetics. a) Core-shell-shell UCNPs with enhanced emission for wireless NIR optogenetic inhibition. Reproduced with permission.^[39] Copyright 2018, American Chemical Society. b) Dye-sensitized core/active shell UCNPs for NIR (800 nm) optogenetics. Reproduced with permission.^[56] Copyright 2016, American Chemical Society. c) Targeted and efficient activation of ChRs expressed in living cells via specifically bound UCNPs. Reproduced with permission.^[57] Copyright 2017, Royal Society of Chemistry. d) Subcellular Optogenetics enacted by targeted nanotransformers of NIR light. Reproduced with permission.^[40] Copyright 2017, American Chemical Society.

a very recent study for deep brain stimulation via upconversion optogenetic in mouse model was well performed with a power density of 1.4 W mm^{-2} .^[36] As above, although the noninvasive

deep-tissue optogenetic regulations without opening skin or skull show great promising in the future translational study, the potential temperature increase needs to be tightly controlled due to the relative high power density of 980 nm laser irradiation.^[37] Overall, the advances in rational refinements of UCNPs and stimulation platforms will greatly promote upconversion optogenetics in practical biomedical research and even clinical translations.

Table 3. The NIR power density of current upconversion optogenetics in vitro and in vivo.

Upconversion optogenetics	Power density of NIR stimulation ^{a)}		Ex./Em. wavelength	Refs.
	In vitro	In vivo		
UCNP-ChR2	8.22 W mm^{-2} slice	1.4 W mm^{-2} mouse	980 nm/470 nm	[36]
	22.6 mW mm^{-2} cell	–		[57]
	8 mW mm^{-2} cell	8 mW mm^{-2} zebrafish	808 nm/470 nm	[32]
	7 mW mm^{-2} cell	7 mW mm^{-2} rat	980 nm/470 nm	[35]
UCNP-C1V1	5.3 mW mm^{-2} cell	4.4 mW mm^{-2} rat	980 nm/540 nm	
	$50\text{--}100 \text{ W mm}^{-2}$ cell	–	975 nm/550 nm	[17]
UCNP-mVChR1			975 nm/450 nm	
UCNP-PsChR			800 nm/540 nm	[56]
UCNP-ReaChR	20 mW mm^{-2} cell	–	800 nm/540 nm	[56]
UCNP-eNpHR3.0	–	6 mW mm^{-2} rat	980 nm/540 nm	[39]
“Opto-CRAC”	30 mW mm^{-2} cell	50 mW mm^{-2} mouse	980 nm/470 nm	[25b]

^{a)}The stimulation platform and the distance between the laser source and the subject vary from different studies.

3.3. Localized Optogenetic Manipulation

Other than the improvements of UCP-based photon-transducers, localized stimulations could be additional approaches that contribute to more efficient and remote optogenetic manipulations. Recent study from Chen and co-workers demonstrated that reducing the distance between UCNPs and light-sensitive ion channel proteins (ChRs) to the molecular level could minimize the NIR energy and achieve highly specific optogenetic stimulation (Figure 8c).^[57] The demonstrated study

provides a generic approach on the basis of specific binding between NAV-UCNPs and V5-ChR2m on the cell membrane, which can be further duplicated for various optogenetic systems. Apart from this, other different strategies have been reported to realize the cell surface localized optogenetic regulations. Of which Zhou and co-workers performed the membrane-localized optogenetic manipulation by specifically anchoring streptavidin-conjugated UCNPs to engineered ORAI1 channels (Figure 2b).^[25b] Xing et al. innovatively applied glycan metabolic strategy and click chemistry for covalently localizing UCNPs on the cell surface (Figure 5a).^[32] Moreover, Prasad et al. demonstrated that subcellular optogenetics also exhibits high precision of ion channel activation, owing to upconverted light generated in situ by intracellular UCNPs in close vicinity to the optogenetic proteins (Figure 8d).^[40]

These efforts from different aspects definitely promote the development of NIR optogenetics. However, current studies in this field are still at their infant stage. Therefore, more extensive research needs to be fully engaged to identify the significances or potential disadvantages, and to further clarify the feasibilities of these evolutions within upconversion optogenetics. For example, the horizontal comparison and analysis of various labeling strategies as well as different localized stimulations in the same conditions would be of great interest and importance.

4. Summary and Perspectives

Currently, the rapid development of upconversion optogenetics for NIR manipulation of membrane ion channels has been witnessed over the past few years. Such unique hybrid techniques have advanced a broad range of applications in neuroscience research and beyond, including neural/brain activity control, intracellular signaling regulation, pathophysiological profiling and therapeutic purposes.^[15a,c] However, despite the significant achievements, there are still some challenges remain in current biomedical study and future clinical translation.

First of all, the efficiency of upconversion optogenetic manipulations should be further improved due to the low quantum yields and poor targeting capability of UCNPs. So far, the highest NIR-to-visible upconverting efficiencies in UCNPs are approximately 5% under the excitation source power density below 100 W cm^{-2} , while the quantum yields of UCNPs are typically <1% in practical bio-applications considering the maximum permissible exposure of skin (e.g., 980 nm, MPE < 1 W cm^{-2}).^[38a,58] Recent research efforts demonstrated the possibility for enhancing the photon-upconverting efficacy by manipulating the local environment or improving the energy transfer in UCNPs, including surface passivation by synthesizing core-shell structures, surface modifications by sensitizers/activators and the excitation source engineering.^[38a,b,59] Although these findings have disclosed that the low upconverting luminescence efficiency of UCNPs is attributed to multiple factors, the detail investigation and innovative strategy are highly desired to fundamentally resolve this issue and achieve efficacious optogenetic manipulations.

Secondly, the targeting capability of UCNPs is an additional limitation for the remote regulation of cell-type/tissue-specific ion channels.^[60] For example, optogenetic manipulations in deep brain where the blood-brain barrier (BBB) is a huge

obstacle for foreign substance delivery. Therefore, UCNPs should be ultimately designed and modified to cross the BBB. Recent studies revealed that several major parameters show clear correlations among nanoparticles (NPs) and BBB penetrability: 1) size (less than 100 nm); 2) shape (usually spherical); 3) zeta potential (preferably moderate to high negative potential); 4) ligand modification (ligands that mediate NPs' pharmacokinetics, biodistribution and clearance pathway).^[61] Besides, the remote and sustained delivery of NPs could also be beneficial for effective and prolonged CNS targeting.^[62] Despite the prominent progress in nanomaterial field, so far, very few well-conjugated UCNPs have been applied in optogenetics for the noninvasive and selective photo-transformer delivery and optogenetic modulation of ion channel-mediated neural activity, demonstrating that the research in upconversion optogenetics is still at its early stage. Further research is necessary to develop brighter and targeted UCNPs for more precise and effective optogenetic manipulation of tissue-specific ion channels, which would significantly advance upconversion optogenetics into a broader range of applications and potential clinical translation.

The third key issue critical for facilitating upconversion optogenetics in biomedicine and even clinical applications is the potential toxicity or biocompatibility issues of the metallic nanomaterials' utilization in vivo.^[63] Extensive studies investigated the nanotoxicity of UCNPs with varying morphology, chemical composition, size distribution and surface modifications in vitro and in vivo.^[64] In spite of the feasibility for predicting and assessing nanotoxicity, these data are difficult to evaluate the long-term toxicological aspects of UCNPs, such as NPs induced immune response and mutagenic effects.^[65] In fact, there is no report to date addresses the long term (i.e., a few animal generations) toxicity of the UCNPs itself or relevant reagents and ligands used in the surface functionalization. Nevertheless, based on current research results, the possible strategies to circumvent the toxicity concern of UCNPs are 1) synthesis of ultrasmall size (<10 nm) NPs for enhancing the biological clearance;^[66] 2) surface modification with suitable ligands or detoxifying agents, such as polyethylene glycol (PEG), polyethylene imine (PEI), SiO₂ and other biofunctional materials for improving the biocompatibility;^[64] 3) development of high luminescence efficiency UCNPs to lower the dosage in practice. Furthermore, the high power density of lasers causes heating effects and leads to possible tissue damage,^[67] which may represent another potential threat for ion channels' manipulation by upconversion optogenetics. Notably, the shift of the UCNPs excitation wavelength to 808 nm could be a key step to reduce overheating effects caused by the relatively strong absorption of water at 980 nm.^[55] Besides, necessary efforts in revolutionizing and standardizing the stimulation methods including the excitation source, power density, irradiation time, and frequency are also highly required. Notwithstanding the fact that several studies claimed the negligible or low toxicity of UCNPs by carefully selecting the chemical composition (of NPs alone), optimizing surface state and utilizing in appropriate dosage.^[64a] It is expected that future advances in UCNPs nanotechnology will encourage the clinical research of upconversion optogenetics in the area of biomedicine and pharmacy.

In the fourth place, despite the initial success in neuronal or nonneuronal modulation via upconversion optogenetics,

ion-selective (e.g., Ca²⁺, Na⁺ or K⁺) and cell-specific manipulations are considerable challenges in biomedical applications. There have been few reports that underpin the possible strategies to address the issues: one way is to genetically engineer novel ChRs or other optogenetic toolboxes for selective ion channel modulation, in which Ca²⁺ release-activated Ca²⁺ (CRAC) channel-based optogenetic platform (Opto-CRAC) represented promising capability to achieve selective Ca²⁺ regulation in vitro and in vivo;^[25b,68] another approach is the employment of targeted optogene delivery system,^[69] recent progress in cell/tissue-specific lentiviral infection technique has greatly boosted the optogenetic manipulation in clinical research.^[70] In terms of the evolution of ChR-based optogenetic tools is still on the journey, which would significantly benefit remote ion channels manipulation with higher precision and specificity.

Finally, other than perspectives of improving UCNPs and optogenetic tools for NIR ion channels manipulation, the reliable instrumentation is of great importance in practical upconversion optogenetic applications. To date, most of the instruments used in optical characterizations of UCNPs are custom built. In addition, specialized instruments toward NIR laser-mediated optogenetic stimulation/monitor in animal models are also very limited.^[71] Considering the unsatisfied reproducibility and robustness of upconversion optogenetic manipulations vary from different research groups, the development of commercial instrumentation, such as spectrometers, microscopes and stimulators, is a critical step for standardizing the protocols and enabling comparative results. Overall, as upconversion optogenetic research is a highly multidisciplinary field, the cross-continental constructive cooperation and integration from academia and industry would also be great helpful to translate this technology into real-world applications.

In summary, the emerging upconversion optogenetics by incorporating multiple emissive UCNPs into various light-sensitive optogenetic toolboxes allow more precise NIR manipulation of membrane ion channels with deeper penetration, higher spatiotemporal resolution, and less invasiveness, which provides great opportunities for overcoming the problems encountered in the classical optogenetic approaches. Along with remarkable efforts in improving the efficacy, minimizing the potential toxicity and developing the dependable instrumentation of upconversion optogenetics, we envision that such powerful techniques will deepen our understanding toward physiological and pathophysiological implications of ion channels, as well as provide insight into clinical translations for the future therapy of neurological and other life-threatening disorders.

Acknowledgements

This work was partially supported by NTU-AIT-MUV NAM/16001, RG110/16 (S), NTU-JSPS JRP grant (M4082175.110) and Merlion 2017 program (M408110000) awarded in Nanyang Technological University (NTU) and National Natural Science Foundation of China (NSFC) (No. 51628201).

Conflict of Interest

The authors declare no conflict of interest.

Keywords

channelrhodopsins, membrane ion channels, near-infrared manipulation, optogenetics, upconversion nanoparticles

Received: August 4, 2018

Revised: September 27, 2018

Published online:

- [1] a) W. R. Loewenstein, *Physiol. Rev.* **1981**, *61*, 829; b) O. P. Hamill, D. McBride, *Pharmacol. Rev.* **1996**, *48*, 231; c) S. P. Alexander, A. Mathie, J. A. Peters, *Br. J. Pharmacol.* **2011**, *164*, S137.
- [2] a) M. J. Ackerman, D. E. Clapham, *N. Engl. J. Med.* **1997**, *336*, 1575; b) J. Dunlop, M. Bowlby, R. Peri, D. Vasilyev, R. Arias, *Nat. Rev. Drug Discovery* **2008**, *7*, 358.
- [3] a) H. Reuter, *Nature* **1983**, *301*, 569; b) C. Miller, *Neuron* **1989**, *2*, 1195; c) C. M. Armstrong, B. Hille, *Neuron* **1998**, *20*, 371; d) M. A. Nicolelis, *Nat. Rev. Neurosci.* **2003**, *4*, 417.
- [4] G. W. Zamponi, *Nat. Rev. Drug Discovery* **2016**, *15*, 19.
- [5] a) B. Erwin, M. M. Jr., K. K. Baker, *Neurol. Res.* **2000**, *22*, 259; b) M. L. Kringelbach, N. Jenkinson, S. L. Owen, T. Z. Aziz, *Nat. Rev. Neurosci.* **2007**, *8*, 623.
- [6] a) A. Gautier, C. Gauron, M. Volovitch, D. Bensimon, L. Jullien, S. Vriz, *Nat. Chem. Biol.* **2014**, *10*, 533; b) N. Ankenbruck, T. Courtney, Y. Naro, A. Deiters, *Angew. Chem., Int. Ed.* **2018**, *57*, 2768; c) D. G. Grier, *Nature* **2003**, *424*, 810.
- [7] a) L. Fenno, O. Yizhar, K. Deisseroth, *Annu. Rev. Neurosci.* **2011**, *34*, 389; b) O. Yizhar, L. E. Fenno, T. J. Davidson, M. Mogri, K. Deisseroth, *Neuron* **2011**, *71*, 9; c) N. A. Repina, A. Rosenbloom, A. Mukherjee, D. V. Schaffer, R. S. Kane, *Annu. Rev. Chem. Biomol. Eng.* **2017**, *8*, 13.
- [8] a) M. L. Rein, J. M. Deussing, *Mol. Genet. Genomics* **2012**, *287*, 95; b) F. Zhang, J. Vierock, O. Yizhar, L. E. Fenno, S. Tsunoda, A. Kianianmomeni, M. Prigge, A. Berndt, J. Cushman, J. Polle, *Cell* **2011**, *147*, 1446; c) K. Deisseroth, *Nat. Neurosci.* **2015**, *18*, 1213; d) X. Han, *ACS Chem. Neurosci.* **2012**, *3*, 577.
- [9] a) M. Häusser, *Nat. Methods* **2014**, *11*, 1012; b) T. Knöpfel, M. Z. Lin, A. Levskaya, L. Tian, J. Y. Lin, E. S. Boyden, *J. Neurosci.* **2010**, *30*, 14998; c) J. Y. Lin, *Exp. Physiol.* **2011**, *96*, 19.
- [10] a) R. P. Kale, A. Z. Kouzani, K. Walder, M. Berk, S. J. Tye, *Neurophotonics* **2015**, *2*, 031206; b) M. A. Rossi, V. Go, T. Murphy, Q. Fu, J. Morizio, H. H. Yin, *Front. Integr. Neurosci.* **2015**, *9*, 8.
- [11] G. Hong, A. L. Antaris, H. Dai, *Nat. Biomed. Eng.* **2017**, *1*, 0010.
- [12] S. Wu, H.-J. Butt, *Phys. Chem. Chem. Phys.* **2017**, *19*, 23585.
- [13] a) K. D. Piatkevich, F. V. Subach, V. V. Verkhusha, *Chem. Soc. Rev.* **2013**, *42*, 3441; b) T. A. Redchuk, E. S. Omelina, K. G. Chernov, V. V. Verkhusha, *Nat. Chem. Biol.* **2017**, *13*, 633.
- [14] a) D. Urmann, C. Lorenz, S. M. Linker, M. Braun, J. Wachtveitl, C. Bamann, *Photochem. Photobiol.* **2017**, *93*, 782; b) F. Zhang, M. Prigge, F. Beyrière, S. P. Tsunoda, J. Mattis, O. Yizhar, P. Hegemann, K. Deisseroth, *Nat. Neurosci.* **2008**, *11*, 631.
- [15] a) K. Huang, Q. Dou, X. J. Loh, *RSC Adv.* **2016**, *6*, 60896; b) S. Shah, A. Solanki, K.-B. Lee, *Acc. Chem. Res.* **2016**, *49*, 17; c) D. Scaini, L. Ballerini, *Curr. Opin. Neurobiol.* **2018**, *50*, 50.
- [16] a) F. Wang, X. Liu, *Chem. Soc. Rev.* **2009**, *38*, 976; b) G. Chen, H. Qiu, P. N. Prasad, X. Chen, *Chem. Rev.* **2014**, *114*, 5161; c) N. M. Idris, M. K. G. Jayakumar, A. Bansal, Y. Zhang, *Chem. Soc. Rev.* **2015**, *44*, 1449; d) S. Wen, J. Zhou, K. Zheng, A. Bednarkiewicz, X. Liu, D. Jin, *Nat. Commun.* **2018**, *9*, 2415; e) Y. Yang, Q. Shao, R. Deng, C. Wang, X. Teng, K. Cheng, Z. Cheng, L. Huang, Z. Liu, X. Liu, *Angew. Chem.* **2012**, *124*, 3179.
- [17] S. Hososhima, H. Yuasa, T. Ishizuka, M. R. Hoque, T. Yamashita, A. Yamanaka, E. Sugano, H. Tomita, H. Yawo, *Sci. Rep.* **2015**, *5*, 16533.

- [18] F. Wang, D. Banerjee, Y. Liu, X. Chen, X. Liu, *Analyst* **2010**, *135*, 1839.
- [19] F. Wang, X. Liu, *J. Am. Chem. Soc.* **2008**, *130*, 5642.
- [20] X. Xie, N. Gao, R. Deng, Q. Sun, Q.-H. Xu, X. Liu, *J. Am. Chem. Soc.* **2013**, *135*, 12608.
- [21] a) Y. Zhang, L. Huang, Z. Li, G. Ma, Y. Zhou, G. Han, *ACS Nano* **2016**, *10*, 3881; b) P. Tan, L. He, G. Han, Y. Zhou, *Trends Biotechnol.* **2017**, *35*, 215.
- [22] K. Deisseroth, P. Anikeeva, Leland Stanford Junior University, *US9522288B2*, **2016**.
- [23] G. Han, *Wireless Optogenetics by relay nano-illuminators*, **2013**, <http://grantome.com/grant/NIH/R01-MH103133-01>, (accessed: September 2013).
- [24] a) C. Towne, K. R. Thompson, *Curr. Protoc. Pharmacol.* **2016**, *75*, 11.19. 1; b) X. Huang, S. Han, W. Huang, X. Liu, *Chem. Soc. Rev.* **2013**, *42*, 173.
- [25] a) S. Shah, J.-J. Liu, N. Pasquale, J. Lai, H. McGowan, Z. P. Pang, K.-B. Lee, *Nanoscale* **2015**, *7*, 16571; b) L. He, Y. Zhang, G. Ma, P. Tan, Z. Li, S. Zang, X. Wu, J. Jing, S. Fang, L. Zhou, *eLife* **2015**, *4*, e10024.
- [26] S. Hososhima, H. Yuasa, T. Ishizuka, H. Yawo, *Proc. SPIE* **2015**, *9305*, <https://doi.org/10.1117/12.2078875>.
- [27] a) D. Strickland, X. Yao, G. Gawlak, M. K. Rosen, K. H. Gardner, T. R. Sosnick, *Nat. Methods* **2010**, *7*, 623; b) S. M. Harper, L. C. Neil, K. H. Gardner, *Science* **2003**, *301*, 1541.
- [28] Y. Wang, X. Lin, X. Chen, X. Chen, Z. Xu, W. Zhang, Q. Liao, X. Duan, X. Wang, M. Liu, F. Wang, J. He, P. Shi, *Biomaterials* **2017**, *142*, 136.
- [29] H. Ding, L. Lu, Z. Shi, D. Wang, L. Li, X. Li, Y. Ren, C. Liu, D. Cheng, H. Kim, N. C. Giebink, X. Wang, L. Yin, L. Zhao, M. Luo, X. Sheng, *Proc. Natl. Acad. Sci. USA* **2018**, *115*, <https://doi.org/10.1073/pnas.1802064115>.
- [30] A. Bansal, H. Liu, M. K. G. Jayakumar, S. Andersson-Engels, Y. Zhang, *Small* **2016**, *12*, 1732.
- [31] a) X. Li, M. Li, *Anal. Methods* **2018**, *10*, 818; b) R. Sood, M. A. English, M. Jones, J. Mullikin, D.-M. Wang, M. Anderson, D. Wu, S. C. Chandrasekharappa, J. Yu, J. Zhang, *Methods* **2006**, *39*, 220; c) S. E. Lepage, A. E. Bruce, *Int. J. Dev. Biol.* **2010**, *54*, 1213.
- [32] X. Ai, L. Lyu, Y. Zhang, Y. Tang, J. Mu, F. Liu, Y. Zhou, Z. Zuo, G. Liu, B. Xing, *Angew. Chem., Int. Ed.* **2017**, *56*, 3031.
- [33] B. Xing, L. LYU, M. Hu, A. Fu, *Bioconjugate Chem.* **2018**, *29*, 2715.
- [34] a) P. McGonigle, B. Ruggeri, *Biochem. Pharmacol.* **2014**, *87*, 162; b) J. G. Fox, S. Barthold, M. Davison, C. E. Newcomer, F. W. Quimby, A. Smith, *The Mouse in Biomedical Research: Normative Biology, Husbandry, and Models*, Vol. 3, Elsevier, USA **2006**.
- [35] X. Lin, Y. Wang, X. Chen, R. Yang, Z. Wang, J. Feng, H. Wang, K. W. Lai, J. He, F. Wang, P. Shi, *Adv. Healthcare Mater.* **2017**, *6*, 1700446.
- [36] S. Chen, A. Z. Weitemier, X. Zeng, L. He, X. Wang, Y. Tao, A. J. Huang, Y. Hashimoto-dani, M. Kano, H. Iwasaki, L. Parajuli, S. Okabe, D. Teh, A. All, I. Kimura, K. Tanaka, X. Liu, T. McHugh, *Science* **2018**, *359*, 679.
- [37] N. Vogt, *Nat. Methods* **2018**, *15*, 242.
- [38] a) S. Wilhelm, *ACS Nano* **2017**, *11*, 10644; b) S. Han, R. Deng, X. Xie, X. Liu, *Angew. Chem., Int. Ed.* **2014**, *53*, 11702; c) Y. Li, X. Liu, X. Yang, H. Lei, Y. Zhang, B. Li, *ACS Nano* **2017**, *11*, 10672.
- [39] X. Lin, X. Chen, W. Zhang, T. Sun, P. Fang, Q. Liao, X. Chen, J. He, M. Liu, F. Wang, *Nano Lett.* **2018**, *18*, 948.
- [40] A. Pliss, T. Y. Ohulchanskyy, G. Chen, J. Damasco, C. E. Bass, P. N. Prasad, *ACS Photonics* **2017**, *4*, 806.
- [41] H.-Q. Wang, T. Nann, *ACS Nano* **2009**, *3*, 3804.
- [42] a) G. Chen, H. Liu, G. Somesfalean, Y. Sheng, H. Liang, Z. Zhang, Q. Sun, F. Wang, *Appl. Phys. Lett.* **2008**, *92*, 113114; b) G. Chen, H. Liu, H. Liang, G. Somesfalean, Z. Zhang, *Solid State Commun.* **2008**, *148*, 96.
- [43] Q. Cheng, J. Sui, W. Cai, *Nanoscale* **2012**, *4*, 779.
- [44] B. Cao, Y. He, Z. Feng, Y. Li, B. Dong, *Sens. Actuators, B.* **2011**, *159*, 8.
- [45] Z. Li, B. Dong, Y. He, B. Cao, Z. Feng, *J. Lumin.* **2012**, *132*, 1646.
- [46] J. Zhao, D. Jin, E. P. Scharfner, Y. Lu, Y. Liu, A. V. Zvyagin, L. Zhang, J. M. Dawes, P. Xi, J. A. Piper, *Nat. Nanotechnol.* **2013**, *8*, 729.
- [47] X. Zhai, S. Liu, Y. Zhang, G. Qin, W. Qin, *J. Mater. Chem. C* **2014**, *2*, 2037.
- [48] N. Liu, W. Qin, G. Qin, T. Jiang, D. Zhao, *Chem. Commun.* **2011**, *47*, 7671.
- [49] P. Yuan, Y. H. Lee, M. K. Gnanasammandhan, Z. Guan, Y. Zhang, Q.-H. Xu, *Nanoscale* **2012**, *4*, 5132.
- [50] Q. Lü, F. Guo, L. Sun, A. Li, L. Zhao, *J. Appl. Phys.* **2008**, *103*, 123533.
- [51] H. Schäfer, P. Ptacek, O. Zerzouf, M. Haase, *Adv. Funct. Mater.* **2008**, *18*, 2913.
- [52] F. Wang, J. Wang, X. Liu, *Angew. Chem.* **2010**, *122*, 7618.
- [53] W. Zou, C. Visser, J. A. Maduro, M. S. Pshenichnikov, J. C. Hummelen, *Nat. Photonics* **2012**, *6*, 560.
- [54] Z. Yin, Y. Zhu, W. Xu, J. Wang, S. Xu, B. Dong, L. Xu, S. Zhang, H. Song, *Chem. Commun.* **2013**, *49*, 3781.
- [55] Y.-F. Wang, C.-Y. Liu, L.-D. Sun, J.-W. Xiao, J.-C. Zhou, C.-H. Yan, *ACS Nano* **2013**, *7*, 7200.
- [56] X. Wu, Y. Zhang, K. Takle, O. Bilsel, Z. Li, H. Lee, Z. Zhang, D. Li, W. Fan, C. Duan, E. M. Chan, C. Lois, Y. Xiang, G. Han, *ACS Nano* **2016**, *10*, 1060.
- [57] K. Yadav, A.-C. Chou, R. K. Ulaganathan, H.-D. Gao, H.-M. Lee, C.-Y. Pan, Y.-T. Chen, *Nanoscale* **2017**, *9*, 9457.
- [58] a) P. J. Parker, S. P. Parker, in *Lasers in Dentistry—Current Concepts*, Springer International Publishing AG, **2017**, p. 87; b) D. H. Sliney, J. Mellerio, *Safety with Lasers and Other Optical Sources: A Comprehensive Handbook*, Springer Science & Business Media, Springer-Verlag, USA **2013**.
- [59] M. Saboktakin, X. Ye, U. K. Chettiar, N. Engheta, C. B. Murray, C. R. Kagan, *ACS Nano* **2013**, *7*, 7186.
- [60] a) J. G. Huang, T. Leshuk, F. X. Gu, *Nano Today* **2011**, *6*, 478; b) J. G. Bernstein, P. A. Garrity, E. S. Boyden, *Curr. Opin. Neurobiol.* **2012**, *22*, 61.
- [61] a) S. Wang, Y. Meng, C. Li, M. Qian, R. Huang, *Nanomaterials* **2015**, *6*, 3; b) J. W. Seo, J. Ang, L. M. Mahakian, S. Tam, B. Fite, E. S. Ingham, J. Beyer, J. Forsayeth, K. S. Bankiewicz, T. Xu, *J. Controlled Release* **2015**, *220*, 51; c) D. Wang, L.-P. Wu, *Mater. Sci. Eng., C* **2017**, *70*, 1039.
- [62] H. L. Wong, X. Y. Wu, R. Bendayan, *Adv. Drug Delivery Rev.* **2012**, *64*, 686.
- [63] C. Buzzea, I. I. Pacheco, K. Robbie, *Biointerphases* **2007**, *2*, MR17.
- [64] a) Y. Sun, W. Feng, P. Yang, C. Huang, F. Li, *Chem. Soc. Rev.* **2015**, *44*, 1509; b) A. Gnach, T. Lipinski, A. Bednarkiewicz, J. Rybka, J. A. Capobianco, *Chem. Soc. Rev.* **2015**, *44*, 1561.
- [65] a) G. Vecchio, A. Galeone, V. Brunetti, G. Maiorano, L. Rizzello, S. Sabella, R. Cingolani, P. P. Pompa, *Nanomed.: Nanotechnol. Biol. Med.* **2012**, *8*, 1; b) P. H. Hoet, I. Brüske-Hohlfeld, O. V. Salata, *J. Nanobiotechnol.* **2004**, *2*, 12.
- [66] K. Zarschler, L. Rocks, N. Licciardello, L. Boselli, E. Polo, K. P. Garcia, L. De Cola, H. Stephan, K. A. Dawson, *Nanomed.: Nanotechnol. Biol. Med.* **2016**, *12*, 1663.
- [67] a) Q. Zhan, J. Qian, H. Liang, G. Somesfalean, D. Wang, S. He, Z. Zhang, S. Andersson-Engels, *ACS Nano* **2011**, *5*, 3744; b) K. M. McNally, B. S. Sorg, A. J. Welch, J. M. Dawes, E. R. Owen, *Phys. Med. Biol.* **1999**, *44*, 983.
- [68] G. Ma, J. Liu, Y. Ke, X. Liu, M. Li, F. Wang, G. Han, Y. Huang, Y. Wang, Y. Zhou, *Angew. Chem., Int. Ed.* **2018**, *57*, 7019.
- [69] C. E. Thomas, A. Ehrhardt, M. A. Kay, *Nat. Rev. Genet.* **2003**, *4*, 346.
- [70] a) J. A. Cardin, M. Carlén, K. Meletis, U. Knoblich, F. Zhang, K. Deisseroth, L.-H. Tsai, C. I. Moore, *Nat. Protoc.* **2010**, *5*, 247; b) S. Zhao, J. T. Ting, H. E. Atallah, L. Qiu, J. Tan, B. Gloss, G. J. Augustine, K. Deisseroth, M. Luo, A. M. Graybiel, *Nat. Methods* **2011**, *8*, 745.
- [71] a) J. Y. Lin, *Progress in Brain Research*, Vol. 196, Elsevier B. V. **2012**, 29; b) A. Klimas, E. G. Entcheva, *J. Biomed. Opt.* **2014**, *19*, 080701; c) E. Papagiakoumou, *Biol. Cell* **2013**, *105*, 443.