

Bioinspired nanotransducers for neuromodulation

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ABSTRACT

The field of neuromodulation has experienced significant advancements in the past decade, owing to breakthroughs in disciplines such as materials science, genetics, bioengineering, photonics, and beyond. The convergence of these fields has resulted in the development of nanotransducers, devices that harness the synergies of these diverse disciplines. These nanotransducers, essential for neuromodulation, often draw inspiration from energy conversion processes found in nature for their unique modalities. In this review, we will delve into the latest advancements in wireless neuromodulation facilitated by optical, magnetic, and mechanical nanotransducers. We will examine their working principles, properties, advantages, and limitations in comparison to current methods for deep brain neuromodulation, highlighting the impact of natural systems on their design and functionality. Additionally, we will underscore potential future directions, emphasizing how continued progress in materials science, neuroscience, and bioengineering might expand the horizons of what is achievable with nanotransducer-enabled neuromodulation.

KEYWORDS

bioinspired synthesis, neuromodulation, mechanoluminescence, focused ultrasound, nanotransducers, sono-optogenetics

1 Introduction

Neuromodulation techniques allow for the modification and regulation of neural activity using external stimuli or interventions. These methods often aim to alter the functionality of neural circuits and nervous systems for both neuroscience research and therapeutic applications [1]. Clinical neuromodulation, often used to alleviate symptoms or improve function in neurological and psychiatric disorders, is typically achieved through electrical stimulation with an implanted electrode. However, this modality of stimulation lacks precision, as it cannot be localized to a single neuron. Moreover, it does not provide cell-type specificity in neuromodulation [2]. Alternatively, optogenetics employs light to control and modify the activity of specific neuron types. This technique facilitates a detailed investigation of complex neural circuits, offering exceptional spatial and temporal precision [3]. Since brain tissue significantly scatters light in the visible range, traditional optogenetics usually requires an optical fiber to deliver light effectively deep into the brain.

While implantable electrical or optical devices offer a robust means for precise and controlled stimulation of targeted brain tissue, several challenges remain. Both methods, whether involving the invasive implantation of an electrode or an optical fiber, carry the risk of causing irreversible acute damage and eliciting chronic immune responses in the neural tissue. Specifically, the mechanical mismatch between the rigid implantable device and the soft brain tissue can trigger chronic immune responses, resulting in the formation of glial scars that encapsulate the device.

This process not only displaces target neurons but also causes structural changes and functional disturbances in the neural network around the device, thus compromising its overall performance. Consequently, these challenges render implantable devices suboptimal for both neuromodulation research and clinical applications [4]. Thus, there is a pressing need to develop neuromodulation techniques that provide high spatiotemporal resolution and deep brain penetration with minimal invasiveness, since a wired and tethered interface can restrict and alter the animal's natural behavior.

To mitigate the challenges of current neuromodulation techniques, recent advancements in materials science have inspired the use of bioinspired nanotransducers (NTs) as novel interfaces for brain neuromodulation [5]. Recent studies have demonstrated that implantable devices with feature sizes on par with or smaller than individual neurons and neurites (1–10 μm) can overcome the challenges of conventional implantable device [6]. Following this developmental trajectory, NTs with even smaller sizes (< 100 nm) emerge as promising candidates to reduce both acute damage and chronic immune responses upon *in vivo* delivery. Inspired by natural biological processes, these NTs can wirelessly convert external stimulation energy—be it light, magnetic fields, or ultrasound—into localized forms of energy such as light, heat, electricity, and mechanical force (Fig. 1(a)). This converted energy then interacts with native or bioengineered ion channels, including light-sensitive, thermo-sensitive, voltage-gated, and mechanosensitive ion channels. This process regulates neuronal activity, facilitating the implementation of optogenetic, thermal, electrical, and mechanical neuromodulation methods,

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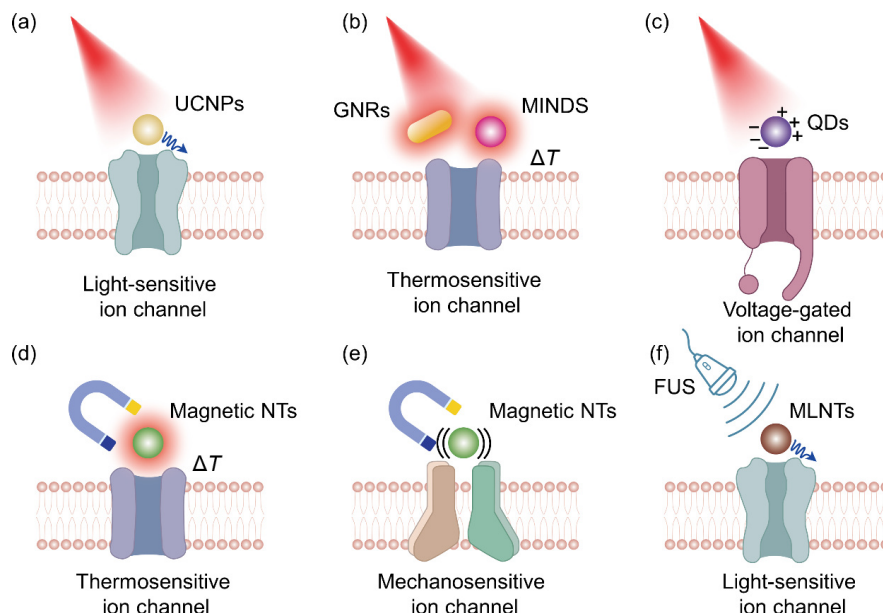


Figure 1 Schematic diagrams of NT-enabled neuromodulation. (a) UCNPs-mediated optogenetics. (b) Photothermal NTs-mediated thermogenetics. (c) QDs-mediated electrical neuromodulation. (d) Magnetic NTs-mediated thermogenetics. (e) Magnetic NTs-mediated mechanogenetics. (f) MLNTs-mediated sono-optogenetics.

respectively. By harnessing forms of external stimulation energy with deep tissue penetration capabilities, these NTs meet the demand for non-invasive neuromodulation, eliminating the need for physical tethering or invasive procedures.

Based on their response to different external stimuli, NTs are primarily categorized into three types: optical, magnetic, and mechanical NTs (Table 1). For instance, optical NTs, including upconversion nanoparticles (UCNPs), gold nanorods (GNRs), macromolecular infrared nanotransducers for deep-brain stimulation (MINDS), and quantum dots (QDs), have the capability to transform light into photons of a different wavelengths, heat, and electricity, respectively (Figs. 1(a)–1(c)). Furthermore, magnetic NTs have the ability to transform a local magnetic field into heat and mechanical force (Figs. 1(d) and 1(e)). Lastly, mechanoluminescent NTs (MLNTs) can transduce focused ultrasound (FUS) into light emission of desired wavelengths (Fig. 1(f)). The efficacy of neuromodulation enabled by NTs largely depends on the properties of the NTs, their mechanisms of energy transformation, and the foundational principles governing their response to external stimuli.

In this review, we will first discuss both the established optical and magnetic NTs employed in recent wireless neuromodulation techniques, encompassing optogenetics, thermogenetics, electrical neuromodulation, and mechanogenetics. We will delve into their operating principles, often inspired by biology, as well as their characteristics, primary advantages, and limitations. Additionally, MLNTs-enabled optogenetic neuromodulation, termed sono-optogenetics, has recently emerged as a promising strategy to address the challenges of light delivery in traditional fiber-based optogenetics. We will highlight the subject of sono-optogenetic neuromodulation, with a particular focus on bio-inspired synthetic methods for MLNTs. Lastly, we will discuss the challenges faced, suggest potential strategies for enhancement, and consider future prospects to boost the efficacy of NT-enabled neuromodulation.

2 UCNPs for neuromodulation

2.1 Optogenetics

Optogenetics, a technique that combines genetic engineering and

optical methods, was introduced by Deisseroth et al. in 2005 and has since revolutionized neuroscience research [7]. Specifically, optogenetics uses light to precisely control the activity of genetically modified neurons that express light-sensitive ion channels (e.g., cation and anion channels) and ion pumps (e.g., H^+ , Cl^- , and Na^+ pumps) [8]. Utilizing genetic tools for targeted cell transfection, optogenetics facilitates precise and cell-specific modulation of transgenic neurons through light stimulation.

For successful optogenetic neuromodulation, effective light delivery to the targeted neurons is required. The one-photon excitation wavelengths of the current opsin toolbox are confined to visible light, which cannot penetrate deep brain regions due to the scattering and absorption properties of brain tissue [9]. To deliver light to the deep brain, traditional optogenetic neuromodulation relies on a fiber optics interface. This approach presents a notable challenge in experimental design since it can induce acute damage and chronic immune responses at the fiber-tissue interface [10]. While radio frequency-powered micro-light emitting diodes (μ -LEDs) have been developed for wireless optogenetics, the implantation of these optical devices into the brain remains necessary, inevitably leading to brain damage [11–13]. To this end, various approaches have been developed to address the challenges of light delivery in the mouse brain for optogenetics. For example, the development of red-shifted opsins (e.g., ChRmine) has enabled transcranial deep-brain optogenetic neuromodulation without the need for intracranial surgery. This unique advantage arises from the reduced scattering of the 635 nm red light (cf. 470 nm blue light) and the enhanced light sensitivity of ChRmine [14, 15]. Additionally, ultrasensitive opsins can be engineered to improve the efficiency of optogenetic neuromodulation under low-intensity light excitation, particularly in deep brain regions where light is attenuated by overlying tissues [16, 17].

2.2 UCNPs

Besides these genetic engineering techniques, UCNPs-enabled photon conversion offers an alternative method to address the challenges of light delivery in optogenetic neuromodulation. Upconversion is an anti-Stokes shift process in which two or more low-energy photons are sequentially absorbed, resulting in the emission of a single high-energy photon. UCNPs typically

Table 1 Summary of nanotransducers for neuromodulation^a

External physical stimuli	Nano-transducers	Size	Penetration depths	Operation distance	Energy conversion efficiency	References
Light	UCNPs	1–100 nm	From < 1 to ~ 6 mm for wavelengths from blue to NIR-II	2 mm above the skull/through the eye	QY: < 5%	[29, 30, 33]
	GNRs	10–80 nm		6 mm from the cornea	PCE: 95%	[41, 42]
	MINDS	~ 40 nm		~ 1 m above the head	PCE: 71%	[40]
	QDs	2–10 nm		<i>In vitro</i> demonstration only	QY: 29.1% ± 2.8%	[45, 46]
Magnetic field	Iron oxide nanoparticles	6–30 nm	At least 5.5 mm	< 16 cm	N/A	[50–53]
Ultrasound	MLNTs	10–100 nm	> 1 cm, frequency dependent	On the scalp	N/A	[4, 59, 60, 63, 71]

^a N/A: not applicable and PCE: photothermal conversion efficiency.

comprise a host lattice with low phonon energy, designated sensitizers for near-infrared (NIR) light absorption, and a low concentration of activators that collect excitation energy from the sensitizers and then emit visible light. Commonly used activators include lanthanide ions such as Er³⁺, Tm³⁺, and Ho³⁺, whereas Yb³⁺ ions are typically employed as sensitizers because their absorption band aligns with the excitation wavelength of the 980-nm laser. The NIR excitation light at 980 nm experiences reduced scattering, thus overcoming the constraint of shallow tissue penetration associated with visible light. Meanwhile, the careful pairing of lanthanide ions can adjust the emission color to encompass the activation spectra of current opsins, underscoring the potential of UCNPs as a versatile toolkit for optogenetic neuromodulation. For example, Tm³⁺ ions have characteristic upconversion emission at ca. 470 nm (¹G₄-³H₆) and 450 nm (¹D₂-³F₄), ideal for activating channelrhodopsin-2 (ChR2). Additionally, Er³⁺ ions emit at 650 nm (⁴F_{9/2}-⁴I_{15/2}) and 540 nm (⁴S_{3/2}-⁴I_{15/2}), suitable for activating *Natronomonas pharaonis* halorhodopsin (NpHR) and chimeras of *Chlamydomonas reinhardtii* ChR1 and *Volvox carteri* ChR1 (C1V1), respectively.

2.3 UCNPs-mediated optogenetics

UCNPs-mediated optogenetics was first proposed by Deisseroth et al. in a patent application in 2011 [18]. Several years later, the first experimental demonstration of upconversion optogenetics was reported by Yawo et al. in 2015 [19]. Upon the excitation by a 976 nm laser, NaYF₄:Sc/Yb/Er particles emit green luminescence at 543 nm, which in turn activates C1V1 and *Volvox*-channelrhodopsin-1 (VChR1)-expressing neurons. Subsequently, a series of experiments were conducted involving optogenetics mediated by UCNPs. These experiments were performed both *in vitro* at the cellular level and *in vivo*, using simple organisms such as *Caenorhabditis elegans* or zebrafish [20–25]. Following these demonstrations, Shi et al. utilized an implanted microdevice based on UCNPs to demonstrate tetherless multiplexed control of neural activity via NIR light stimulation in free-moving mice [26–28]. However, tetherless optogenetics enabled by UCNP microdevices was restricted to shallow brain regions due to the relatively low upconversion quantum yield (QY). To address this challenge, Liu and McHugh et al. created blue-emitting NaYF₄:Yb,Tm/NaYF₄ UCNPs using a core/shell strategy, achieving an upconversion QY as high as ca. 2.5% (Figs. 2(a)–2(d)) [29]. Under excitation of an 980-nm laser (2 W), 470-nm emission with a power density of 0.063 mW·mm⁻² was detected at a depth of 4.2 mm in the mouse brain. The authors demonstrated that transcranial UCNP-mediated optogenetics can effectively achieve both neuron activation and inhibition (inhibition (Figs. 2(c) and 2(d)). In addition to modulating transgenic opsin-expressing neurons, the sub-retinal injection of photoreceptor-binding UCNPs (pbUCNPs) enabled NIR light sensation and pattern vision of native retinal opsins in mice (Figs. 2(e)–2(h)) [30].

Besides UCNPs-based optogenetics, Sheng et al. reported an optoelectronic upconversion ultraminiaturized device for *in vitro* and *in vivo* optogenetic neuromodulation [31]. To overcome the limitations of conventional nonlinear upconversion in UCNPs, this upconversion microdevice integrated semiconductor heterostructures to convert NIR (810 nm) illumination to visible (630 or 590 nm) emission with a linear response. This device exhibited a QY of ~ 1.5% under incoherent low power excitation at 10 mW·cm⁻². This approach offers an alternative method, alongside UCNPs, to achieve high-efficiency upconversion-based optogenetics with low-power excitation.

Previously reported UCNP-mediated optogenetics can only be activated or inhibited with UCNPs that emit a single peak wavelength for a fixed composition. In contrast to this single peak emission, different opsins exhibit distinct activation spectra. To address this discrepancy, Zhang et al. recently prepared orthogonally emitting NaYbF₄:Er³⁺/Tm³⁺@NaYF₄:Yb³⁺ UCNPs, which exhibited tunable red or green emission by simply adjusting laser excitation at 980 or 808 nm [32]. These UCNPs can thus activate two synergistic light-gated ion channels (i.e., VChR1 and Jaws) in a programmable manner in the same cell. Later, Zhang et al. synthesized trichromatic UCNPs with excitation-specific luminescence via a core/multi-shell structure design (Fig. 2(i)) [33]. By switching the excitation wavelength, the corresponding blue, green, and red emission enabled selective activation of ChR2, C1V1, and ChrimsonR, respectively.

Despite recent advances in UCNPs-mediated optogenetics, the upconversion QY of UCNPs remains low, necessitating the use of high-power 980 nm lasers for deep-brain optogenetics. This requirement is problematic as the 980 nm laser coincides with a significant water absorption peak, resulting in a heating effect in the brain tissue. This excessive heat can alter neural activity and potentially lead to chronic tissue damage, thus confounding the interpretation of experimental results. Therefore, control experiments must be carefully designed and performed to rule out these competing mechanisms.

3 Photothermal nanotransducers for neuromodulation

Temperature, like light, is a vital stimulus that influences neural activity. The influence of temperature on biology is primarily due to its capacity to activate thermosensitive ion channels and modify membrane capacitance. Within the transient receptor potential (TRP) ion channel family, several members exhibit temperature-dependent behavior across a wide temperature spectrum. These members include the transient receptor potential cation channel subfamily V member 1 (TRPV1), transient receptor potential ankyrin 1 (TRPA1), and the temperature-gated chloride channel, anoctamin 1 (TMEM16A). Each of these channels has been validated for their specific temperature responsiveness.

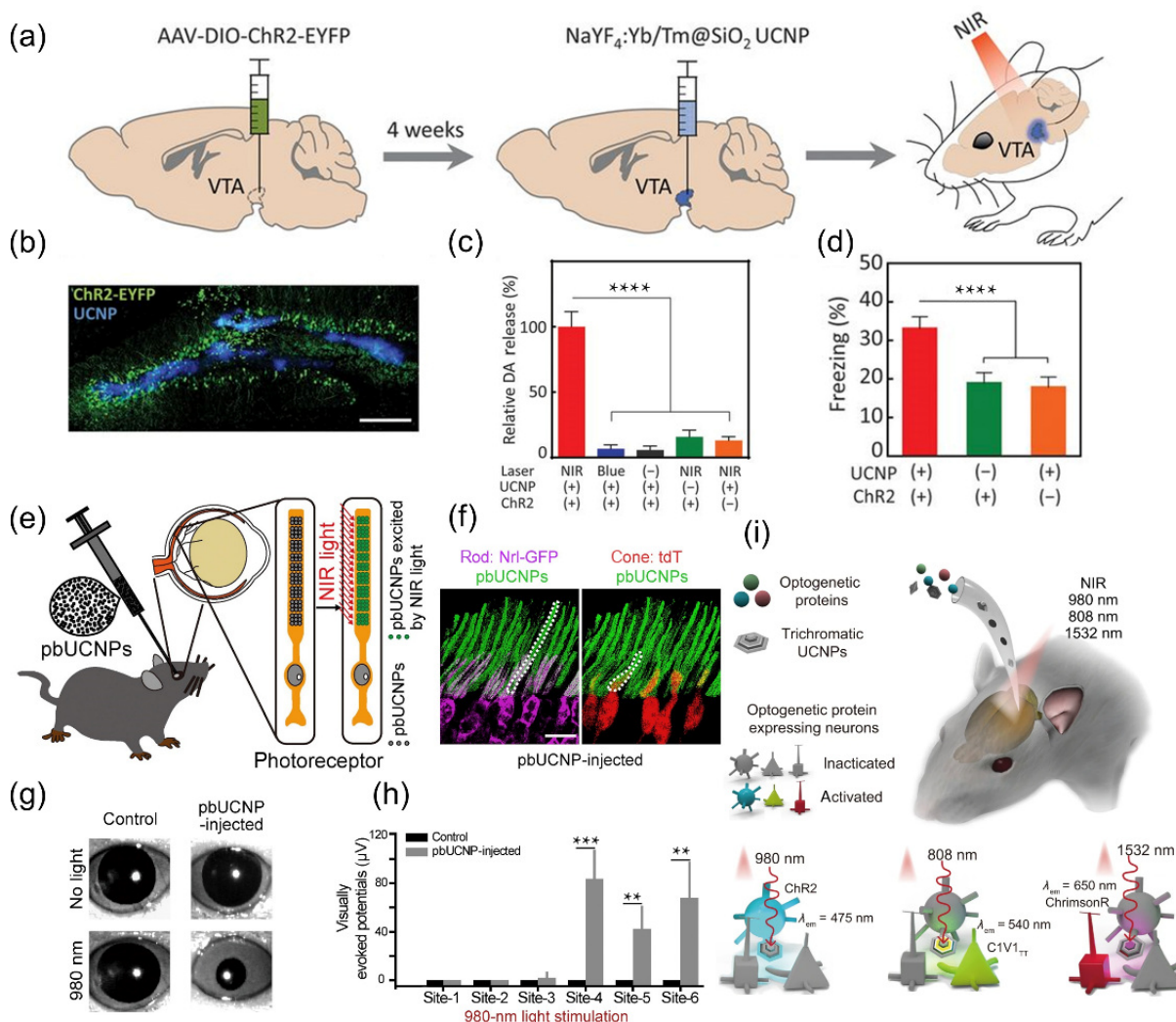


Figure 2 UCNPs-mediated optogenetics. (a) Schematic for optogenetic neuromodulation of the VTA in the mouse brain via NIR-excited up conversion. (b) Confocal image showing the overlay of UCNPs and ChR2 expression in the dentate gyrus (DG) of a mouse brain. The scale bar represents 200 μm . (c) Cumulative dopamine (DA) release in the mouse brain after optogenetic neuromodulation with five different conditions. (d) Freezing levels of mice after optogenetic neuromodulation with three different conditions. Reproduced with permission from Ref. [29], © Chen, S. et al. 2018. (e) Schematic of sub-retinal injection of pbUCNPs in mice. The inset shows the binding of pbUCNPs to the outer segments of photoreceptors, producing green emission of pbUCNPs under NIR excitation. (f) Overlaid green (pbUCNPs)/violet (rods) and green (pbUCNPs)/red (cones) fluorescence images of the retina from pbUCNP-injected mice. The dashed lines show the segments of a rod and a cone, respectively. The scale bar represents 10 μm . (g) Images showing pupil constriction from mice injected with pbUCNPs upon 980 nm illumination (40 s) in comparison to no light control. The intensity of 980 nm light was 1.21×10^8 photons $\mu\text{m}^{-2}\text{s}^{-1}$. (h) Visually evoked potentials recorded at different sites in the visual cortex of mice stimulated by 980 nm light. Reproduced with permission from Ref. [30], © Elsevier Inc. 2019. (i) Schematic of multicolor optogenetics mediated by trichromatic UCNPs. Channelrhodopsins and trichromatic UCNPs were administered into specific brain regions. NIR light at 980, 808, and 1532 nm can effectively penetrate brain tissue and selectively excite the trichromatic UCNPs. As a result, these UCNPs emit colors at 475, 540, and 650 nm, respectively, thereby activating the corresponding neuronal populations. Reproduced with permission from Ref. [33], © Liu, X. et al. 2021.

3.1 Photothermal nanotransducers

Photothermal NTs can convert incident light into thermal energy for localized heating. A variety of these NTs have been developed recently, including plasmonic nanoparticles, carbon-based nanomaterials, semiconducting polymer nanoconjugates, and silicon nanomaterials. Their high photothermal conversion efficiency makes them particularly beneficial for thermal-related biological applications [34–36]. One prominent example is GNRs, which rely on surface plasmon resonance (SPR) to transform photon energy into heat. The SPR effect in GNRs is influenced by their size, shape, and aspect ratio, allowing for the adjustment of the SPR absorption peak from visible to NIR spectra. The NIR spectrum is especially favored for deeper tissue penetration. A notable limitation of GNRs, however, is their tendency to transform into nanodots after multiple heating and cooling cycles, leading to compromised thermal stability and changed absorption characteristics.

Semiconducting polymers represent a promising class of emerging photothermal NTs. They stand out due to their enhanced biocompatibility, photothermal stability, and size-independent optical properties. The idea to employ these photothermal NTs in neuromodulation is drawn from the infrared-sensing abilities of rattlesnakes in biology. Specifically, rattlesnakes possess a distinctive pit organ that converts incoming infrared light into a transient and localized temperature increase. Such a temperature rise then triggers heat-sensitive ion channels, notably the TRPA1 channel [37]. This biological mechanism has inspired the design and application of photothermal NTs in neuromodulation.

The underlying photothermal action of semiconducting polymers arises from nonradiative electron relaxation. When electrons transition from an excited state back to the ground state, heat is produced [38]. The efficiency of this photothermal conversion in semiconducting polymers hinges on their electron donor–acceptor π -conjugated backbone structures. Fortunately,

activating the thermosensitive cation channel, TRPV1, using the photothermal effect (Fig. 3(b)) [41]. In their experiments, the GNRs produced a localized heat upon exposure to 780-nm light. This heat prompted a Ca^{2+} influx through TRPV1 activation, a phenomenon captured in fluorescence images of TRPV1-expressing HEK293T cells (Fig. 3(c)).

Building on this foundation, Roska et al. innovatively employed GNRs in conjunction with a modified TRPV1.465-6x-His variant, which they termed rTRPV1, aiming for *in vivo* photothermal neuromodulation to rehabilitate visual function (Figs. 3(d)–3(f)) [42]. Remarkably, NIR-I light at 915 nm was found capable of stimulating various layers of a human retina transfected with rTRPV1. Drawing inspiration from rattlesnakes' ability to perceive infrared light, this approach effectively endows mammalian eyes with NIR vision capabilities [42]. Such research charts a promising course, leveraging bioinspired methods to potentially reinstate vision in individuals suffering from retinal degeneration diseases.

Beyond plasmonic GNRs, organic semiconducting polymer NTs are gaining attention in thermogenetics because of their notable biocompatibility. For instance, Pu et al. introduced semiconducting polymer nanobioconjugates that absorb at 808 nm. Impressively, these conjugates outperformed GNRs in terms of photothermal conversion efficiency [35]. Their swift heating properties enabled the rapid, reversible, and safe activation of intracellular Ca^{2+} influx in neuronal cells.

In our own research, we exploited the reduced tissue attenuation at 1064 nm in the NIR-II window. Specifically, we synthesized MINDS for deep-brain photothermal neuromodulation in freely moving mice using low-power-density NIR-II illumination (Figs. 3(g)–3(i)) [40]. We selectively transduced TRPV1 into dopaminergic neurons in the ventral tegmental area (VTA), a crucial zone in the mouse brain's reward circuit (Fig. 3(h)). Afterward, we injected the VTA with the MINDS solution and assessed its neuromodulatory effect using a Y-maze conditioned place preference test. By positioning an NIR-II light source one meter above a terminal of the Y-maze, we detected a marked place preference in mice after three days of conditioning. In contrast, the control group showed no such preference (Fig. 3(i)). This experiment underscores the potential of photothermal NTs in deep-brain modulation of dopaminergic neurons through brain-penetrant NIR-II illumination.

4 Quantum dots for neuromodulation

Beyond the photothermal effect, optical stimulation can also generate electricity by harnessing the photovoltaic effect in semiconductor NTs, notably QDs. This mechanism mirrors that of solar cells, where absorbed photons generate electron/hole pairs. These electrons and holes can then shift, thus affecting the spatial charge distribution. During this process, the resultant electric voltage or current has the potential to modulate voltage-gated ion channels (Fig. 1(c)).

4.1 QDs

Colloidal QDs are semiconductor nanoparticles characterized by a unique size-adjustable bandgap, a result of the quantum confinement effect. They also boast a high absorption coefficient, making them adept at light absorption [43]. Furthermore, QDs are optically stable, with little susceptibility to photobleaching or chemical breakdown [44]. Such advantages have piqued significant interest in their potential for electrical neuromodulation.

4.2 QD-mediated electrical neuromodulation

Nizamoglu et al. were the first to integrate type-II InP/ZnO

core/shell QDs into a photoelectrode structure with the aim of neural activation [45]. When exposed to blue LED light, this photoelectrode produces a bioelectrical current capable of stimulating a single neuron to fire. Impressively, the power density of the blue light source, at $4 \mu\text{W}\cdot\text{mm}^{-2}$, is 26 times lower than the ocular safety limit for continuous exposure to visible light. This discovery suggests the promising role of QDs in photoelectrode systems for potential retinal implants. Subsequently, the team transitioned from the visible-absorbing InP/ZnO core/shell QDs to NIR-absorbing PbS QDs, broadening the photoelectrode's response wavelength to 780 nm in the tissue-penetrating NIR-I window [46]. This advanced structure consistently triggered action potentials in primary hippocampal neurons.

However, it is crucial to note that QD-mediated electrical neuromodulation has only been reported *in vitro*. A possible reason is that electrical neuromodulation may result in undesired modulation of neighboring cells, leading to off-target effect especially in three-dimensional neural tissue *in vivo*. Moreover, for QDs to function properly, they need to be part of a multilayered photovoltaic device for charge injection to neurons. The relatively large size of these devices could limit their practical application in living subjects.

5 Magnetic nanotransducers for neuromodulation

Optogenetic, thermogenetic, and electrical neuromodulation techniques, as discussed above, heavily rely on light's ability to penetrate tissues, yet this penetration is usually confined to a few millimeters. In contrast, magnetic fields, which interact only weakly with biological tissues, can penetrate deeply without significant energy loss. Moreover, their non-contact nature makes magnetic fields a safe and noninvasive alternative for neuromodulation.

5.1 Magnetic nanotransducers

Magnetic NTs, such as iron oxide nanoparticles, can produce heat when subjected to an alternating magnetic field [47]. This phenomenon, termed magnetic hyperthermia, arises from the energy dissipated during repeated cycles of magnetizing magnetic materials, essentially due to hysteretic power loss. Magnetic hyperthermia has been clinically applied for cancer therapy by MagForce AG [48]. Besides the thermal effects, magnetic fields can also exert mechanical forces to align the magnetic nanoparticles to the magnetic field. The generation and application of these mechanical forces in biological systems have spurred interest in the study of sensory mechanisms, encompassing touch, balance, proprioception, and hearing.

5.2 Magnetic NT-mediated thermogenetics and mechanogenetics

Magnetic NTs, when subjected to a magnetic field, can induce both thermal and mechanical effects. Consequently, they can facilitate neuromodulation by triggering either thermosensitive or mechanosensitive ion channels. As mentioned in Section 3, thermosensitive ion channels respond to changes in temperature. On the other hand, mechanosensitive ion channels activate in response to mechanical deformations of cell membranes, playing a pivotal role in certain sensory systems. To date, several mechanosensitive channels have been identified, including the piezo-type mechanosensitive channels (PIEZO) and the transient receptor potential cation channel subfamily V member 4 (TRPV4). The operational mechanisms of these channels can be understood through two primary models: the membrane tension

model and the tether model [49]. The former suggests that force applied directly to the membrane leads to channel opening, while the latter posits that the force is transmitted to the ion channel via connections to either the extracellular matrix or the cortical cytoskeleton.

In 2010, Pralle et al. first reported the precise control of neurons expressing TRPV1 through heating of ferrite nanoparticles in an alternating current (AC) magnetic field (8.4×10^{-4} T) [50]. Subsequently, Anikeeva et al. demonstrated a wireless magnetothermal deep brain stimulation technique (Fig. 4(a)), in which iron oxide nanoparticles were injected into the mouse brain

for activating TRPV1 under an AC magnetic field (1.8×10^{-2} T) [51]. The expression of *c-fos* in the VTA provided evidence of the efficacy of the wireless magnetic NTs-driven thermogenetics (Figs. 4(b) and 4(c)). Nonetheless, the mice in these experiments had to remain stationary, with their heads affixed within the toroidal field coil for magnetic stimulation. Addressing this limitation, Pralle et al. later introduced a larger magnetic coil, facilitating wireless deep brain thermogenetic neuromodulation in awake mice that could move freely [52]. When exposed to an AC magnetic field of 9.4×10^{-3} T, these mice displayed rotational movements around their

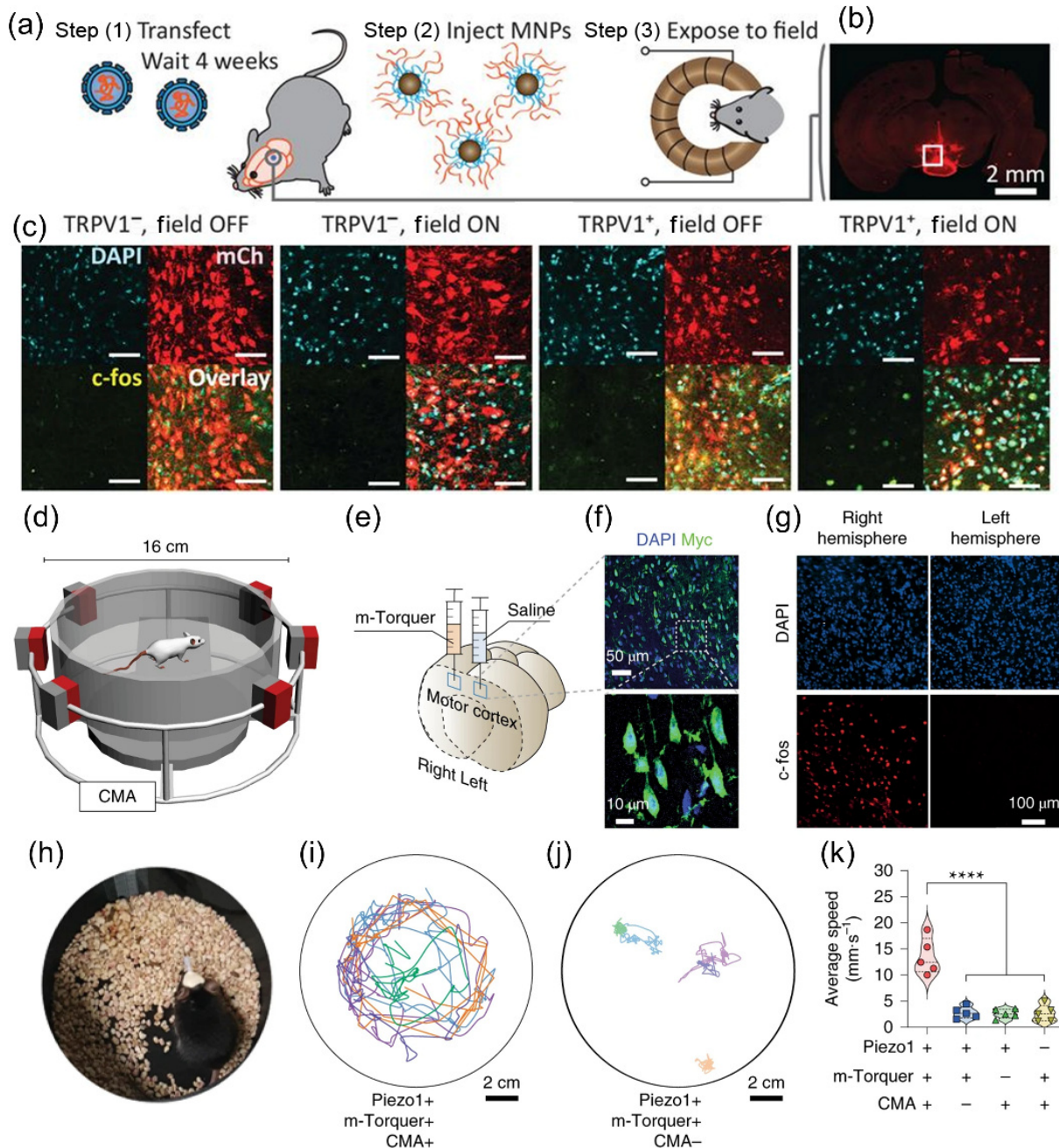


Figure 4 Magnetic NTs for neuromodulation. (a) Schematic for *in vivo* magnetothermal neuromodulation. (b) Confocal image showing representative TRPV1-p2A-mCherry expression in the VTA of a mouse brain transfected with TRPV1. (c) Confocal images of brain regions for quantification of neuromodulation with staining for 4',6-diamidino-2-phenylindole (DAPI) (blue), mCherry (red), *c-fos* (green), and overlay. All mice were injected with magnetic nanoparticles. Scale bars: 25 μm. Reproduced with permission from Ref. [51], © American Association for the Advancement of Science 2015. (d) Schematic of the CMA setup for *in vivo* neuromodulation with m-Torquer. (e) Administration of m-Torquer and saline to both sides of the mouse's motor cortex. (f) Fluorescence images showing the expression of Myc-Piezo1 in mouse cortical neurons. (g) Fluorescence images showing DAPI staining (blue) and *c-fos* expression (red) within the motor cortex after m-Torquer stimulation. (h) Photograph of the m-Torquer setup for mouse neuromodulation. (i) The trajectories of five mice administered with m-Torquer in the premotor cortex of the right hemisphere, along with the application of a rotating magnetic field (on/off cycle of 2 s, repeated 15 cycles). (j) The trajectories of control mice without CMA application. (k) Statistical evaluation of average speed of mouse movement ($n = 5$, **** $P_{\text{adj}} < 0.0001$). Reproduced with permission from Ref. [53], © Lee, J. U. et al. 2021.

body axis. Remarkably, the duration of this behavior displayed a strong correlation with the intensity of the applied magnetic field.

In addition to activating thermosensitive ion channels, magnetic NTs can be harnessed to remotely control mechanosensitive ion channels by transforming a magnetic field into a targeted mechanical force. A notable recent advancement was made by Cheon et al., who introduced the m-Torquer system designed for manipulating the mechano-sensitive ion channel Piezo1 in freely moving mice (Figs. 4(d)–4(k)) [53]. The m-Torquer system is composed of a magnetic torquer—a structured assembly of iron oxide nanoparticles with a total diameter of ~ 500 nm—and a rotating circular magnet array (CMA) (Fig. 4(d)). This setup produces a consistent and uniform rotating magnetic field in the range of $2\text{--}5 \times 10^{-2}$ T, capable of exerting piconewton-scale forces at an impressive distance of up to 70 cm. With its long working distance, m-Torquer has demonstrated its efficacy in mechanogenetics through both c-fos staining results (Figs. 4(e)–4(g)) and behavioral studies (Figs. 4(h)–4(k)).

It is worth noting that the current energy conversion efficiency of thermogenetics or mechanogenetics mediated by magnetic NTs is limited, necessitating the use of strong magnetic fields provided by sophisticated and costly equipment [54]. Moreover, the intense magnetic fields required for efficient neuromodulation can potentially cause artifacts, unintended side effects, or even harm to living tissues.

6 Mechanoluminescent nanotransducers for neuromodulation

6.1 MLNTs

Besides light and magnetic fields, FUS has emerged as a promising addition to the repertoire of physical stimuli for neuromodulation. Piezoelectric transducers are adept at converting electrical energy into mechanical waves, specifically ultrasonic waves. Given that these mechanical waves can penetrate deeply into tissues,

ultrasound has become a staple in various clinical healthcare settings [55–57]. Leveraging this deep-penetrating capability, our lab utilized FUS to stimulate MLNTs to produce localized light emissions [4, 58]. Typically, MLNTs consist of inorganic host materials, doped transition metal or lanthanide ions as luminescence centers, and substitutional atoms or vacancies acting as trap states (Fig. 5(a)). This trap-controlled mechanoluminescence is commonly achieved by first capturing charge carriers in the trap states, and then releasing them upon FUS stimulation, resulting in light emission.

A potential disadvantage of trap-controlled mechanoluminescence arises from the nature of these MLNTs, which are usually constructed from non-degradable inorganic materials, thus imposing toxicity concerns for *in vivo* biomedical applications. In an attempt to make biocompatible and degradable MLNTs, we collaborated with Wang et al. to develop liposome-based organic MLNTs, named Lipo@IR780/L012, designed specifically for light delivery in the brain using FUS (Fig. 5(b)) [59]. These organic MLNTs are formulated by encapsulating a chemiluminescent compound (L012) and a sonosensitizer (IR780) within a liposome. Notably, the light-emission mechanism of these organic MLNTs differs from the traditional trap-controlled mechanoluminescence observed in inorganic MLNTs. When exposed to FUS, the sonosensitizer IR780 produces reactive oxygen species (ROS), which, in turn, activates the chemiluminescent compound L012, resulting in light emission. An added advantage of these organic MLNTs is the elimination of the typical ultraviolet (UV) charging process required for their inorganic counterparts, simplifying their application, especially in living organisms.

6.2 Bioinspired synthesis of MLNTs

Many of the optical and magnetic NTs discussed earlier were produced using conventional synthetic methods. In this section, we will highlight our recent advancements in MLNT synthesis, utilizing a bioinspired demineralization approach.

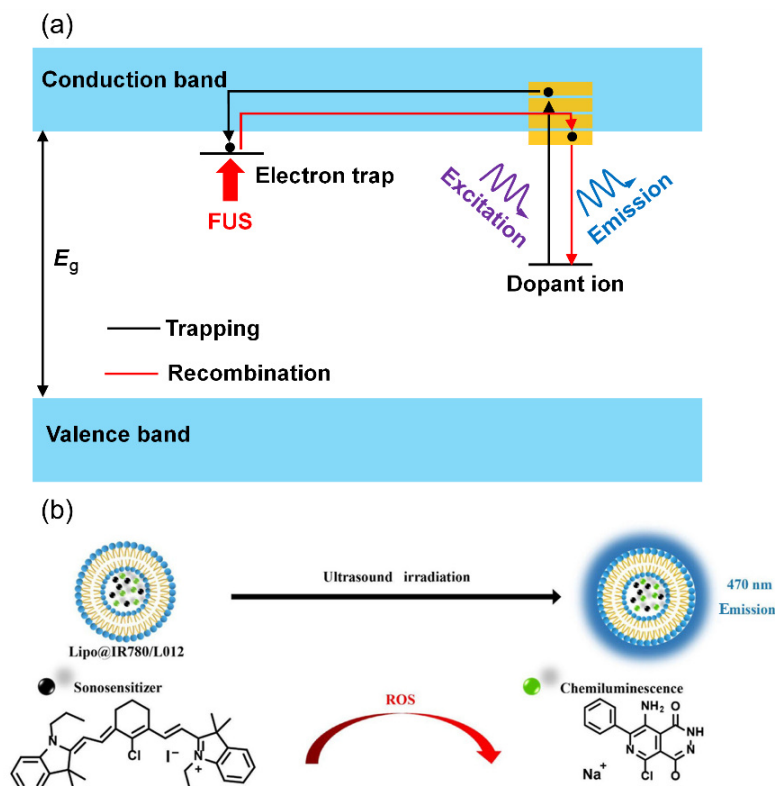


Figure 5 Schematic of mechanoluminescence mechanisms of (a) inorganic MLNTs and (b) organic liposome-based MLNTs under FUS stimulation. Reproduced with permission from Ref. [59], © American Chemical Society 2023.

Traditionally, mechanoluminescent materials have been difficult to synthesize at low temperatures using wet chemical techniques. Desired point defects and polymorphs for mechanoluminescence are typically achieved through solid-state reactions or post-annealing processes in sol-gel methods and ultrasonic spray pyrolysis techniques, requiring temperatures above 1000 °C. As a result, these high-temperature processes often yield mechanoluminescent materials with large grain sizes ($> 10 \mu\text{m}$), making them unsuitable for forming stable water solutions. While high-energy ball milling can produce nanoparticles from solid-state powders, the process can introduce stresses and dislocations that suppress mechanoluminescence emission, rendering it unsuitable for MLNT production. Other methods like pulsed laser deposition also fall short in synthesizing water-dispersible MLNTs. Consequently, a method to produce colloidal nanoparticles while retaining their mechanoluminescent properties has remained elusive.

In our quest to produce colloidal MLNTs, we took inspiration from the natural world. We observed a fascinating phenomenon in the demineralization process of biominerals like bone and tooth enamel (Fig. 6(a)) [60, 61]. In certain undersaturated aqueous conditions, these biominerals can dissolve into nanoparticles while exhibiting a remarkable self-preservation behavior. Surprisingly, these released nanoparticles resist further dissolution, even in environments where continued dissolution should be thermodynamically favorable (Gibbs free energy change (ΔG) < 0). Drawing inspiration from this unique self-preservation phenomenon observed in biominerals, we set out to produce stable MLNTs using a similar suppressed dissolution process in undersaturated aqueous solutions. Additionally, our calculations suggest that many mechanoluminescent ceramics possess a critical size between 10 to 100 nm. This finding indicates the potential to generalize the dissolution method for creating colloidal MLNTs of tens of nanometers in size.

We then implemented the suppressed dissolution method to produce colloidal MLNTs from an array of bulk mechanoluminescent materials, including silicate, sulfide, and titanate. In this process, bulk micrometer-sized mechanoluminescent materials, as illustrated in Fig. 6(b) and synthesized through solid-state reactions, were mixed with an undersaturated citrate solution until a metastable equilibrium was attained. This simple and generalizable approach yielded MLNTs ranging in size from 10 to 110 nm, as shown in Figs. 6(c) and 6(d). This size range well aligns with our predictions and is also within a safe range that ensures prolonged circulation time for *in vivo* biomedical applications [62]. The negative charged citrate anions presented in the undersaturated solution adsorb on MLNTs during the suppressed dissolution process. As a result, MLNTs exhibit exceptional colloidal stability, which is further evidenced by the negligible precipitations both in water and cell culture medium (Figs. 6(e) and 6(f)).

Importantly, our approach maintains the strong mechanoluminescence of the original bulk material. This retention is attributed to the mild top-down process that preserves the crystallinity and optical properties of the bulk materials in the resulting colloidal MLNTs. This method stands in stark contrast to the harsh high-energy ball milling technique, which often leads to dislocation-induced emission quenching. Therefore, our bioinspired demineralization strategy represents a unique and distinctive method for producing stable and intensely luminescent colloidal MLNTs. These colloidal MLNTs are particularly suitable for a variety of biomedical applications, including sono-optogenetics, which we will discuss in greater detail in the subsequent section.

6.3 Sono-optogenetics

As discussed above, effective light delivery in living organisms, especially in sizeable mammals, remains a longstanding challenge, primarily due to the severe scattering and absorption of visible light within biological tissues. In order to mitigate this challenge, many conventional optical neuromodulation techniques resort to invasive measures, such as surgically removing scalp tissue or implanting optical fibers directly into the brain. Alternatively, red-shifted opsins (e.g., ChRmine) and NIR-absorbing NTs (e.g., UCNPs and MINDS) represent promising strategies that enable light to penetrate deeper into the brain for neuromodulation with less invasiveness. However, these emerging strategies still require invasive procedures, such as injecting transgenes and NTs directly into the brain.

Sono-optogenetics mediated by FUS offers a viable solution to these light delivery challenges. MLNTs, used in this approach, can convert deeply penetrating ultrasound energy (up to several centimeters) into localized light emissions. Moreover, MLNTs can be introduced into the body through intravenous administration, a significantly less invasive method compared to direct brain injections. Additionally, the light emission spot in the brain can be easily shifted to target different regions by simply adjusting the focus of the ultrasound. This level of flexibility is hard to achieve with permanently implanted optical fibers or light-emitting devices.

In a pivotal study, our team introduced the idea of “sono-optogenetics” by implementing a non-invasive ultrasound-mediated *in vivo* light delivery method in live mice [63]. Specifically, we synthesized ZnS:Ag,Co/ZnS core/shell MLNTs that emit light at 470 nm when stimulated by FUS. These MLNTs were chosen because their emission closely matches the absorption spectrum of ChR2. We introduced these MLNTs into the mice via intravenous injection, allowing them to be recharged within superficial blood vessels (Fig. 7(a)). When we applied FUS to the mouse brain through the scalp (Fig. 7(b)), these MLNTs were discharged to release the stored energy as visible light, which then activated ChR2-expressing neurons. By utilizing this circulating light source enabled by MLNTs, we successfully produced contralateral hindlimb motion synchronized with FUS pulses in the motor cortex, only in mice expressing ChR2 and injected with MLNTs (Fig. 7(c)). Importantly, this limb motion was not observed in the absence of ChR2 or MLNTs (Fig. 7(d)), ruling out the possibility of nonspecific neural activation with FUS alone. Our findings mark the first instance where sono-optogenetic neuromodulation was achieved non-invasively using FUS and circulating MLNTs in live mice without necessitating brain implants or scalp surgeries.

In addition to our demonstration, Chang et al. showcased the use of mechanoluminescence for optogenetic neuromodulation through a magneto-luminescence microdevice [64]. This innovative device combines bulk CaZnOS:Tb^{3+} mechanoluminescent phosphors with a free-moving magnet bar inside a container. When subjected to an external magnetic field, the magnet bar rotates, stressing the ML phosphors and inducing a light emission at 544 nm. This green mechanoluminescent light is compatible with the activation spectra of two proteins: C1V1 and KillerRed. Upon implanting the magneto-luminescence microdevice into mice that express the red-shifted channelrhodopsin C1V1, effective optogenetic neuron activity was observed under mechanoluminescence illumination. Moreover, the mechanoluminescence emission could activate the photosensitive protein KillerRed, leading to the production of ROS that can be utilized for subcutaneous antitumor treatments. This research exemplifies the cutting-edge and wireless

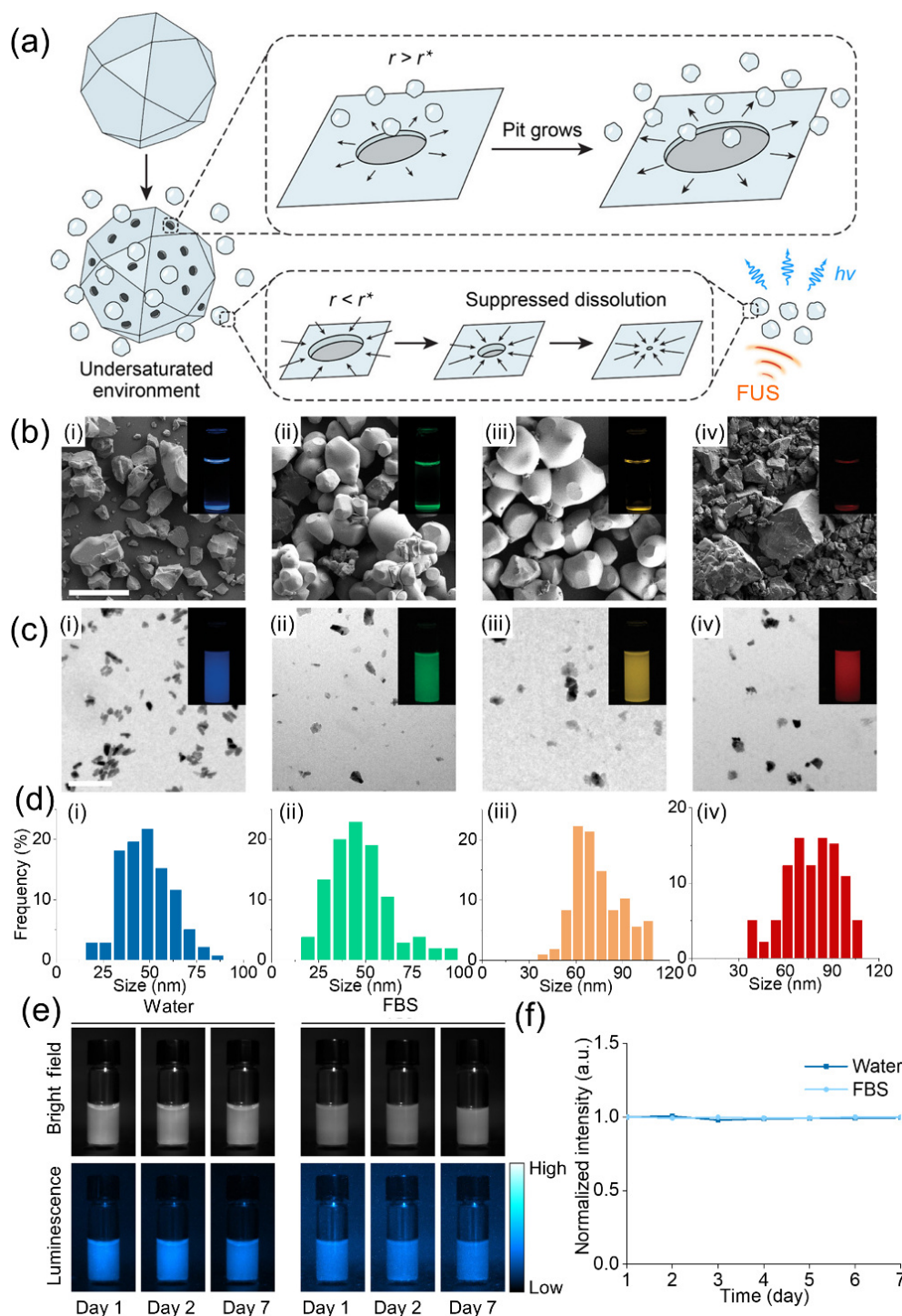


Figure 6 Bio-inspired synthesis of MLNTs. (a) The biomineral-inspired method leverages a kinetically preserved dissolution mechanism, originally found in natural biominerals, to produce colloidal MLNTs as a rechargeable light source upon FUS stimulation. (b) Scanning electron microscopy (SEM) images of (i) bulk $\text{Sr}_2\text{MgSi}_2\text{O}_7:\text{Eu,Dy}$, (ii) $\text{ZnS}:\text{Cu,Al}$, (iii) $\text{ZnS}:\text{Mn}$, and (iv) $\text{CaTiO}_3:\text{Pr}$ materials prepared by solid-state reactions. Insets: fluorescence images of the corresponding bulk mechanoluminescent material in an aqueous suspension. The scale bar represents 50 μm . (c) Transmission electron microscopy (TEM) images of (i) $\text{Sr}_2\text{MgSi}_2\text{O}_7:\text{Eu,Dy}$, (ii) $\text{ZnS}:\text{Cu,Al}$, (iii) $\text{ZnS}:\text{Mn}$, and (iv) $\text{CaTiO}_3:\text{Pr}$ MLNTs produced by the biomineral-inspired suppressed dissolution approach. Insets: fluorescence images of the corresponding MLNTs in an aqueous solution. The scale bar represents 200 nm. (d) Histograms of the size distributions of (i) $\text{Sr}_2\text{MgSi}_2\text{O}_7:\text{Eu,Dy}$, (ii) $\text{ZnS}:\text{Cu,Al}$, (iii) $\text{ZnS}:\text{Mn}$, and (iv) $\text{CaTiO}_3:\text{Pr}$ MLNTs. (e) Brightfield (top row) and luminescence (bottom row) images of $\text{Sr}_2\text{MgSi}_2\text{O}_7:\text{Eu,Dy}$ MLNTs in water (left) and fetal bovine serum (FBS) (right). (f) Luminescence intensity of $\text{Sr}_2\text{MgSi}_2\text{O}_7:\text{Eu,Dy}$ MLNTs over one week duration in water and FBS. Reproduced with permission from Ref. [60], © American Chemical Society 2022.

neuromodulation possibilities that mechanoluminescent materials can offer.

As previously mentioned, our team utilized a bioinspired approach to produce colloidal MLNTs, deriving them from various bulk materials. These MLNTs, when stimulated by FUS, display multi-color emissions. Specifically, they emit at 470 nm ($\text{Sr}_2\text{MgSi}_2\text{O}_7:\text{Eu,Dy}$), 534 nm ($\text{ZnS}:\text{Cu,Al}$), 578 nm ($\text{ZnS}:\text{Mn}$), and

610 nm ($\text{CaTiO}_3:\text{Pr}$), spanning the complete visible spectrum as depicted (Fig. 8(a)). Our approach preserves the crystallinity of the original materials, which ensures the resultant MLNTs to have strong mechanoluminescence. Notably, the brightness of $\text{Sr}_2\text{MgSi}_2\text{O}_7:\text{Eu,Dy}$ significantly surpasses that of $\text{ZnS}:\text{Ag,Co}@/\text{ZnS}$ MLNTs, which were synthesized using combined hydrothermal and post-annealing techniques. When $\text{Sr}_2\text{MgSi}_2\text{O}_7:\text{Eu,Dy}$ MLNTs

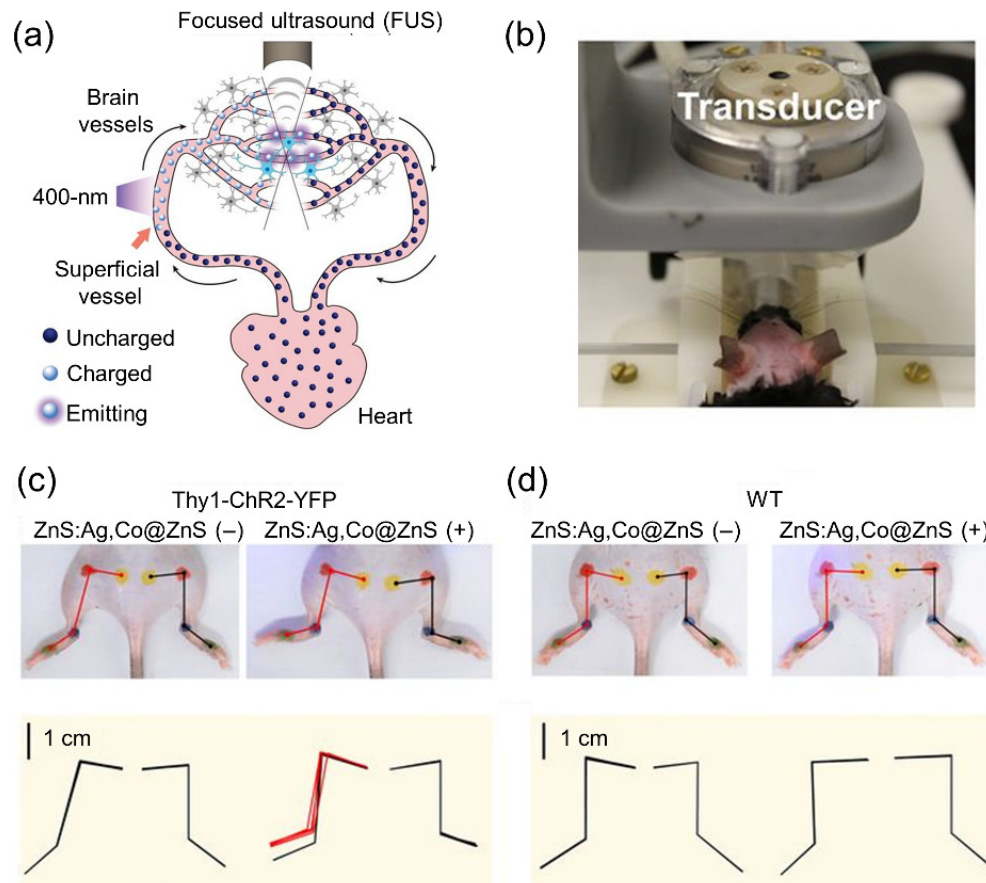


Figure 7 Sono-optogenetics enabled by ZnS:Ag,Co@ZnS MLNTs. (a) Schematic showing blood circulation of ZnS:Ag,Co@ZnS MLNTs, in which 400-nm photoexcitation energy received by superficial vessels is transported into deep-brain regions to generate 470-nm emission for optogenetic neuromodulation. (b) Photograph showing the *in vivo* sono-optogenetic neuromodulation setup, where the FUS transducer is placed on top of a shaved mouse head with the intact scalp. Photographs and hindlimb kinematics of (c) a Thy1-ChR2-YFP mouse and (d) a wild-type (WT) mouse during sono-optogenetic neuromodulation through the intact scalp before (left) and after (right) injection of ZnS:Ag,Co@ZnS MLNTs. In the photographs, the red and black lines denote the kinematics of left and right hindlimbs, respectively. In the bottom kinematic diagrams, the red lines highlight the activation of contralateral limb by sono-optogenetic stimulation. Reproduced with permission from Ref. [63], © Wu, X. et al. 2019.

are systemically introduced, discernible blue emissions are seen in various mice organs, including the liver, kidneys, and brain, when stimulated with tissue-penetrating FUS (Fig. 8(b)). This internally generated light within the mouse organs exhibits a high spatial resolution of $< 500 \mu\text{m}$, comparable to that of standard optical fibers. Importantly, successful neural activation in the brain of Thy1-ChR2-YFP mice administered with $\text{Sr}_2\text{MgSi}_2\text{O}_7\text{:Eu,Dy}$ MLNTs and stimulated by FUS was confirmed by immunohistochemical staining of c-fos expression (Figs. 8(c)–8(e)). These findings complement our earlier experiments with ZnS:Ag,Co@ZnS MLNTs, bolstering the evidence for the effectiveness of FUS-mediated optogenetic neuromodulation using systemically administered MLNTs.

Due to the limited degradability of inorganic MLNTs after administration, we collaborated with Wang et al. to develop organic MLNTs encapsulated in liposomes, termed Lipo@IR780/L012, as detailed in Section 6.1 [59]. These Lipo@IR780/L012 MLNTs have an average size of approximately 120 nm and carry a negative charge on their surface. When subjected to FUS, they emit luminescence around 470 nm, which closely aligns with the absorption spectrum of ChR2. Following the intravenous administration of these MLNTs, neurons in the motor cortex of Thy1-ChR2-YFP transgenic mice respond to repeated FUS exposures. This suggests that the Lipo@IR780/L012 MLNTs can enable non-invasive sono-optogenetic neuromodulation.

7 Silicon-based nanotransducers for neuromodulation

Neuromodulation techniques utilizing NTs discussed in previous sections primarily depend on genetic engineering to impart specific neurons with responsiveness to light, heat, and mechanical force. Delivery of transgenes could introduce unpredictable or even permanent side effects when applied to non-human primates and humans. In contrast, non-genetic photostimulation methods leverage the temporary physicochemical changes of NTs to modulate the activity of native neurons, thereby avoiding the introduction of foreign genes.

Silicon-based NTs are emerging as a promising solution for this non-genetic optical neuromodulation due to their attributes: excellent electrical conductivity, ease of fabrication, efficient absorption in the visible spectrum, and high biocompatibility [65]. The photovoltaic, photoelectrochemical, and thermal traits of silicon have been widely researched in material and energy sciences. As an example, Tian et al. introduced a biocompatible and degradable mesoporous silicon structure suitable as a photothermal agent, enabling optical modulation of individual dorsal root ganglia neurons without genetic modification [66]. Besides the use of silicon-based NTs in the extracellular space, the same team pioneered silicon nanowires as agents for non-genetic and light-controlled modulation within cells, specifically targeting organelles [67]. Shifting from photothermal effects, Sheng et al. developed thin-film silicon-based pn diodes that generate electrical stimuli upon light exposure [68]. This silicon-based optoelectronic

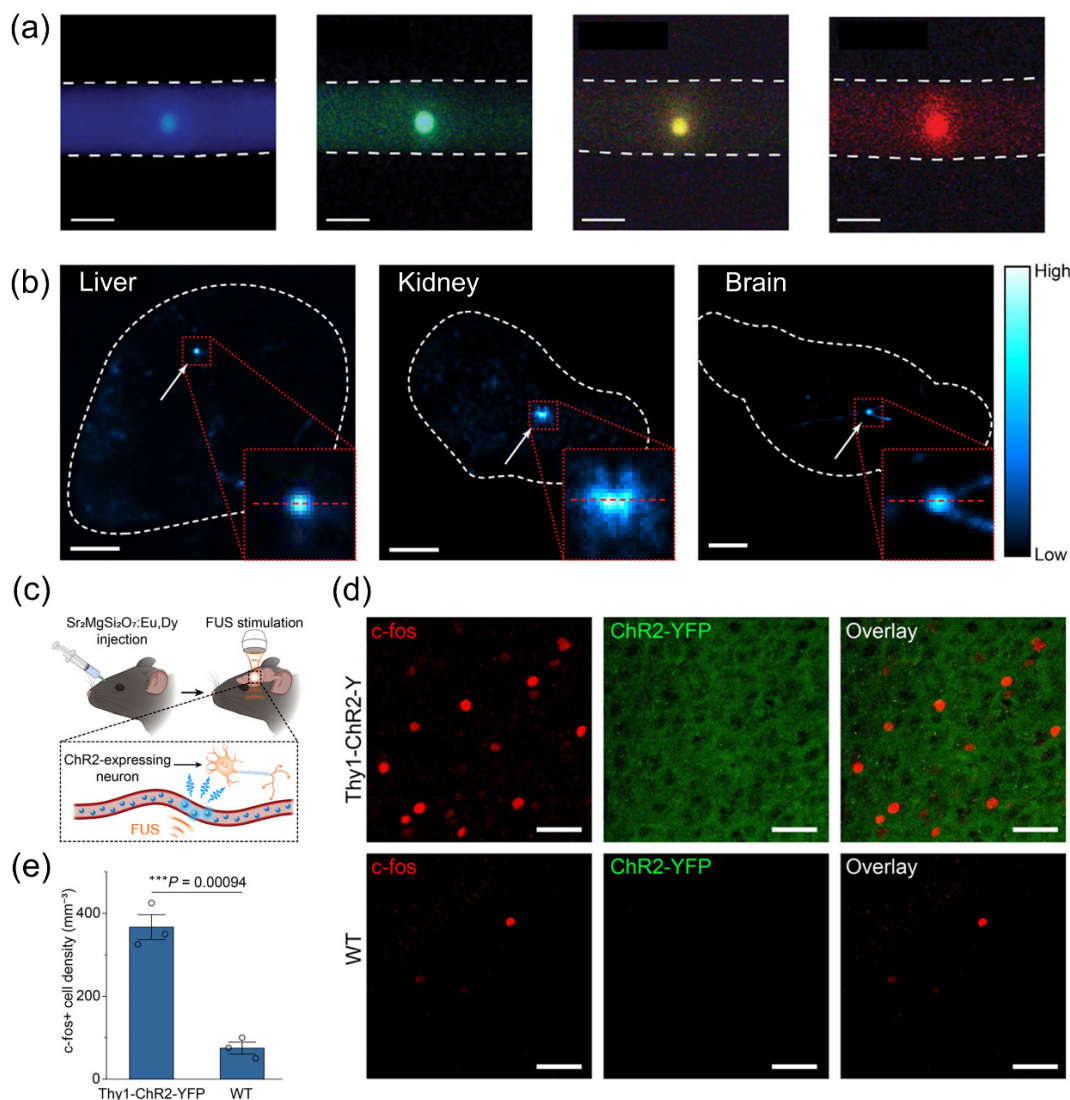


Figure 8 Sono-optogenetics enabled by MLNTs synthesized by bioinspired approach. (a) True-color photographs of a tubing filled with MLNTs composed of $\text{Sr}_2\text{MgSi}_2\text{O}_7:\text{Eu,Dy}$, $\text{ZnS}:\text{Cu,Al}$, $\text{ZnS}:\text{Mn}$, and $\text{CaTiO}_3:\text{Pr}$ and exposed to FUS. All scale bars represent 1 mm. (b) Luminescence images of the liver, kidney, and brain of a mouse, with the blood vessels of these organs filled with $\text{Sr}_2\text{MgSi}_2\text{O}_7:\text{Eu,Dy}$ MLNTs and exposed to FUS. All scale bars represent 2 mm. (c) Schematic for the experimental procedure of FUS-mediated optogenetic neuromodulation with $\text{Sr}_2\text{MgSi}_2\text{O}_7:\text{Eu,Dy}$ MLNTs. (d) Representative confocal images of c-fos (left), ChR2-YFP (middle), and their overlay (right) of the same motor cortex area in Thy1-ChR2-YFP (top) and WT (bottom) mice systemically administered with $\text{Sr}_2\text{MgSi}_2\text{O}_7:\text{Eu,Dy}$ MLNTs and stimulated by FUS. All scale bars represent 40 μm . (e) Statistical analysis of c-fos+ cell density for the Thy1-ChR2-YFP and WT mice in (d). All data are presented as mean $\pm$ standard deviation (SD). $n = 3$ per group. One-way analysis of variance (ANOVA), $***P < 0.001$. Reproduced with permission from Ref. [60], © American Chemical Society 2022.

device can be affixed to specific regions such as the mouse's sciatic nerve or cerebral cortex, facilitating precise activation or suppression of neural activity in both peripheral and central nervous systems.

8 Conclusions and perspective

Traditional electrical and optogenetic neuromodulation often involve invasive brain implants or head tethering in animals, limiting their utility in both neuroscience research and clinical scenarios. The pursuit of wireless neuromodulation using NTs represents a promising emerging direction due to its potential to address these limitations. NT-facilitated neuromodulation offers non-invasiveness, cell-type specificity, and deep-brain penetration. These strengths are achieved by leveraging the NTs' ability to transform remote energy into local stimuli that modulate the activity of the neural system. While this approach bypasses many hurdles of prior methods, it is not without challenges as it advances. In this section, we will outline these challenges, suggest potential improvements, and highlight promising avenues from the intersections of material science, neuroscience, and

bioengineering for future research in NT-facilitated neuromodulation.

8.1 NTs

Biocompatibility is a paramount concern when considering the long-term use of NTs, yet many studies have overlooked this important factor. Several elements influence biocompatibility: physicochemical properties (e.g., size, shape, surface charge, and chemical composition), surface modifications, and interactions with biological systems [69]. Specifically, when discussing NTs in comparison to implantable devices, a distinct aspect to consider is the retention and clearance of NTs in the brain, which is crucial for effective and safe design of NTs for neuromodulation. Rigorous analysis and thorough characterization of NT diffusion and metabolism are essential to guarantee their biocompatibility. The use of organic NTs demonstrates improved biocompatibility compared to their inorganic counterparts (Fig. 9(a)). Additionally, shifting towards genetically encoded protein-based NTs rather than chemically synthesized ones could further mitigate toxicity risks. Another challenge arises from the energy conversion

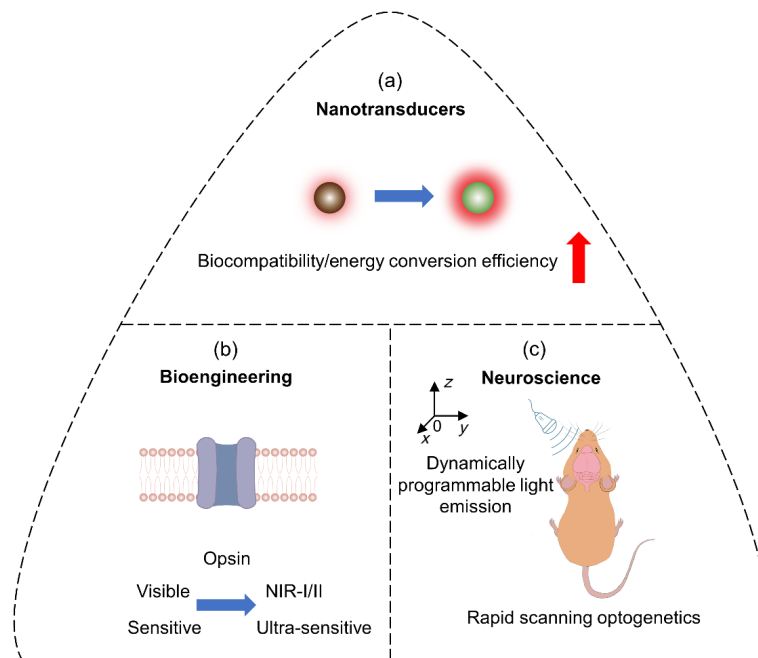


Figure 9 Future directions for NTs-enabled neuromodulation. (a) Synthesis of biocompatible nanotransducers with high energy conversion efficiency. (b) Engineering tissue penetrant NIR-I/II responsive and ultra-sensitive opsin variants. (c) Designing dynamically programmable light emission patterns throughout the entire brain of live mice to achieve rapid scanning optogenetics.

efficiency in certain NTs. For instance, UCNPs smaller than 20 nm often have a QY below 1% [70]. The mechanoluminescence of many MLNTs is also suboptimal, not meeting the intensity demands for optogenetics. Strategies such as the core/shell approach and surface plasmon sensitization may boost upconversion efficiency. Similarly, a piezoelectric polymer coating on MLNTs might enhance ML emission intensity.

8.2 Bioengineering

While opsin variants have evolved to be sensitive to illumination up to 635 nm-red light, there are currently no reported variants responsive to longer wavelengths in the NIR-I and NIR-II regions. However, drawing inspiration from the genetically encoded, thermosensitive TRP channels' infrared sensitivity, we foresee the creation of new endogenous and genetically encoded methods for making neurons responsive to light in the NIR-I and NIR-II regions (Fig. 9(b)). At the same time, advancing ultra-sensitive opsins offers a promising avenue for achieving neuromodulation through light-sensitive ion channels using low-power illumination. This can complement efforts to enhance the energy conversion efficiency of NTs.

8.3 Neuroscience

Delivering light into deep tissues poses a significant challenge for optical neuromodulation. Conventional implants, such as electrodes, optical fibers, and LEDs, once positioned in the brain, cannot be relocated in the brain. This limitation means that these implanted devices are unable to dissect the synergistic contributions of different brain regions to a certain behavior or disease with high throughput. In contrast, MLNTs, as systemically delivered light source circulating in the bloodstream, can emit light at any desired location or depth in the mouse brain gated by tissue penetrant FUS for sono-optogenetic neuromodulation (Fig. 9(c)). This leads to the exciting potential to establish protocols that allow on-the-fly and dynamic programming of light patterns across the entire brain. Such a system could swiftly activate or deactivate various brain regions in sequence with high efficiency. Furthermore, there is a growing interest in developing wearable FUS devices for freely behaving animals, especially since current

sono-optogenetic techniques are used on stationary and head-fixed mice.

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