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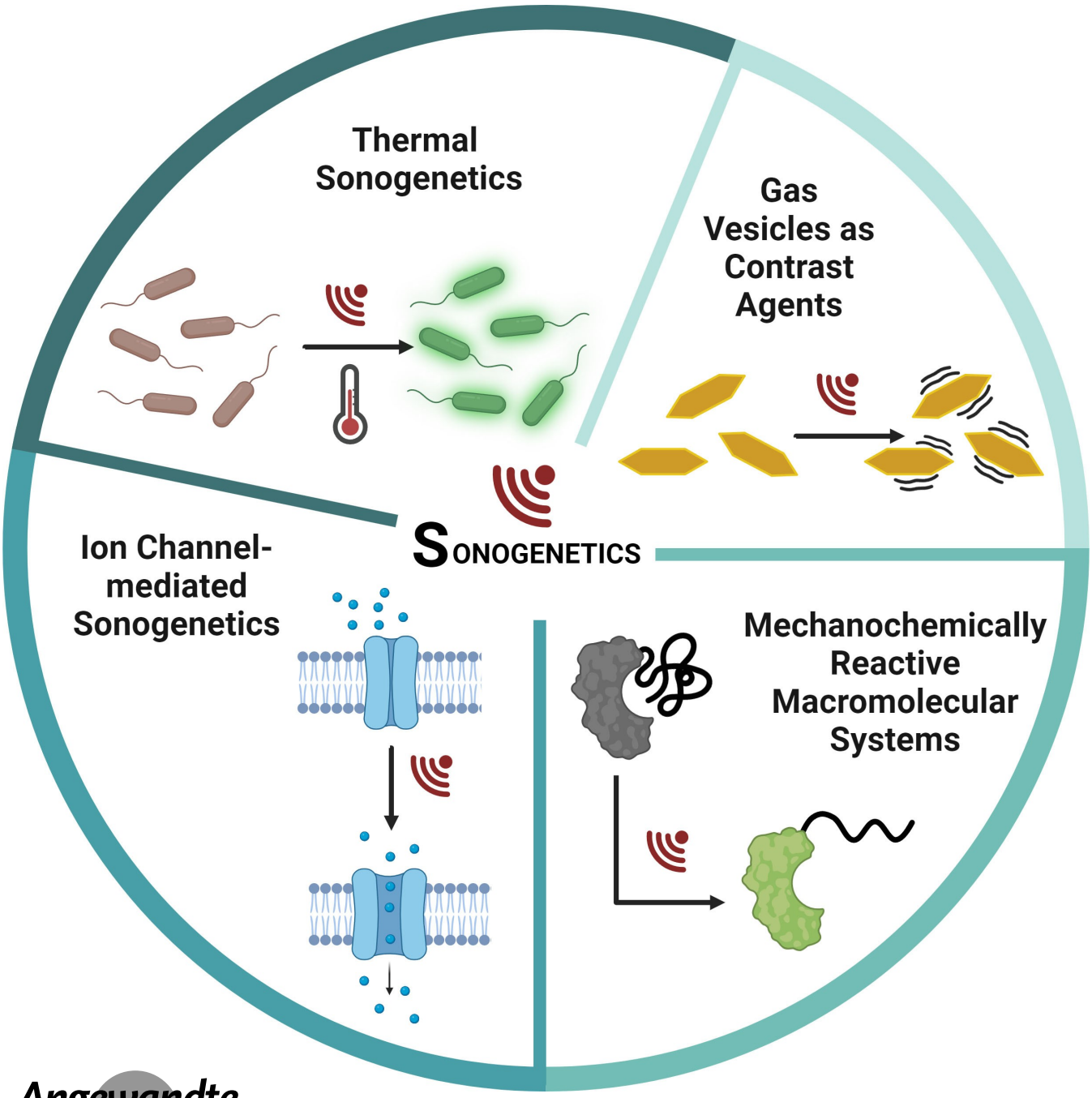
Sonogenetics

Sonogenetics for Monitoring and Modulating Biomolecular Function by Ultrasound

Johannes Hahmann[+], *Aman Ishaqat*[+], *Twan Lammers, and Andreas Herrmann**



Abstract: Ultrasound technology, synergistically harnessed with genetic engineering and chemistry concepts, has started to open the gateway to the remarkable realm of sonogenetics—a pioneering paradigm for remotely orchestrating cellular functions at the molecular level. This fusion not only enables precisely targeted imaging and therapeutic interventions, but also advances our comprehension of mechanobiology to unparalleled depths. Sonogenetic tools harness mechanical force within small tissue volumes while preserving the integrity of the surrounding physiological environment, reaching depths of up to tens of centimeters with high spatiotemporal precision. These capabilities circumvent the inherent physical limitations of alternative *in vivo* control methods such as optogenetics and magnetogenetics. In this review, we first discuss mechanosensitive ion channels, the most commonly utilized sonogenetic mediators, in both mammalian and non-mammalian systems. Subsequently, we provide a comprehensive overview of state-of-the-art sonogenetic approaches that leverage thermal or mechanical features of ultrasonic waves. Additionally, we explore strategies centered around the design of mechanochemically reactive macromolecular systems. Furthermore, we delve into the realm of ultrasound imaging of biomolecular function, encompassing the utilization of gas vesicles and acoustic reporter genes. Finally, we shed light on limitations and challenges of sonogenetics and present a perspective on the future of this promising technology.

1. Introduction

Understanding and controlling the molecular and cellular processes that occur within living organisms is essential for investigating biology and disease. Observing and manipulating these events can be facilitated through external triggers, enabling the remote control of cellular function with high spatiotemporal precision. This advancement offers selective investigational, diagnostic and therapeutic capabilities. Such technologies have expanded in recent years to include optogenetics, magnetogenetics and sonogenetics, providing new methods to modulate biological functions using external stimuli. Notably, these techniques are particularly beneficial when dealing with challenging-to-reach organs, such as the brain,[1] thereby opening new avenues for scientific exploration and medical intervention.

Over half a century ago, microbiology underwent a groundbreaking discovery. Scientists revealed the operative mechanism of bacteriorhodopsin in halophilic bacteria, working as an ion pump that can be swiftly activated by visible light.[2] This delineation was a major milestone that laid the foundation for optogenetics—a field that uses genetically engineered light-responsive cells controlled by opsin-based photoreceptors.[1a,3] Although important biological questions could be answered by applying optogenetics,[4] serious limitations result from the low penetration depth of light and its scattering within opaque tissue.[5] Thus, optic fibers implanted into the brain through invasive surgical procedures were needed.

Alternatively, magnetogenetics leverages thermosensitive transient receptor potential (TRP) channels, which can be stimulated through the application of radio-frequency alternating magnetic field (AMF) to heat the TRP-attached magnetic nanoparticles.[6] Nevertheless, the precise control of cellular function using magnetogenetics presents considerable challenges, owing to its inherent nonspecific induction, limited precision and dependence on intricate infrastructure that generates powerful magnetic fields, often resulting in unwanted artifacts.[7] In fact, the reproducibility of fundamental studies in the field of magnetogenetics relying on genetically encoded ferritin nanoparticles[8] has been a subject of debate,[9] with some even questioning their compliance with the laws of physics.[10] This however does not apply for the highly reproducible key study by Huang *et al.* which involved synthetic nanoparticles.[6]

In 2014, Heureaux *et al.* reported the initial observation of sonogenetics,[11] but it was not until 2015 that Ibsen *et al.* coined the term, thereby introducing this innovative technique to the scientific community.[12] Sonogenetics utilizes ultrasound (US) to manipulate, image and control cellular activities. It employs genetically-encoded US-sensitive elements,[13] as well as proteins that indirectly influence genetic readouts, thereby broadening its scope of influence and potential applications. Sonogenetics offers a seamless avenue for the translation of remote-control technologies into clinical applications by presenting several advantages when compared to optogenetics[14] and magnetogenetics. A primary distinction lies in sonogenetics' superior tissue penetration depth, extending up to tens of centimeters, while concurrently maintaining precise control. Moreover, US is already widely used in healthcare and chemistry, making it adaptable for novel applications from both fields.

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It operates with high spatiotemporal precision, demonstrating cell-specific and non-invasive properties, while exhibiting minimal signal attenuation in complex tissues.[1b,13,15] Multiple studies have showcased the efficacy and safety of US in various systems, including neurons,[16] brain tissue,[17] nematodes,[18] rodents,[17b,19] primates,[20] and humans.[21]

The depth and resolution of US can be efficiently tuned to match the system's needs across different length and time scales through varying its frequency. In principle, US and acoustic waves are produced by the propagation of vibration induced by a transducer, which converts an electrical signal to mechanical vibrations within the medium. US frequency typically falls within the range of 20 kHz to several GHz. However, sonogenetics predominantly employs frequencies ranging from 1 to 10 MHz due to their safety profile.

In water, these frequencies correspond to comparably small wavelengths—approximately 15 mm at 100 kHz, 1.5 mm at 1 MHz and 0.15 mm at 10 MHz.[1b] This characteristic renders them particularly effective for interacting with small-scale systems. Additionally, the associated time scales are exceedingly brief ($\tau \approx 1/f$), making US an effective tool for energy exchange with fast phenomena.[1b] For a comprehensive understanding of how US interacts with molecular and cellular structures, along with insights into the underlying physical principles, we recommend referring to an in-depth review[22] covering these aspects.

Two main effects arise from the propagation of acoustic waves through tissues: thermal and mechanical ones. Thermally, US induces mild hyperthermia (37 °C to 43 °C) or thermal ablation (>43 °C). Mild hyperthermia increases blood flow and vascular permeability,[23] while thermal ablation causes cell death and found applications in the treatment of cancer and neurodegenerative diseases.[24]

On the other hand, mechanical effects of US include streaming, cavitation and acoustic radiation forces (ARF), which all play a significant role in controlling biological functions.[1b,13] Cavitation (both stable and inertial) instigates different biophysical effects such as sonoporation and disruption of tight junctions,[25] resulting in transient permeabilization of membranes[26] even for difficult barriers such as the blood brain barrier.[15] Cavitation utilizes either endogenous gas nuclei present in inter- and intracellular spaces, or exogenous nano- and microbubbles co-administered with the system[27] to enhance its response to US.[28] In inertial cavitation, the oscillation of these bubbles leads to their collapse, thereby transferring a significant amount of energy into the system, resulting in shearing forces that can be used productively. Polymer mechanochemistry is a research field that exploits the conversion of such forces to stretch polymers and ultimately result in either bond cleavage or isomerization at the site of mechanically sensitive molecules within polymers, called mechanophores, usually located at the polymer's center.[29]

US has been used for applications in sonopharmacology,[30] drug activation[31] and US-facilitated payload delivery.[32] Excellent reviews offering deep insights into these topics have been published recently.[1b,33] Therefore, we exclude them from our discussion.

This article provides a comprehensive review of recent advancements in sonogenetics, highlighting its unique fea-

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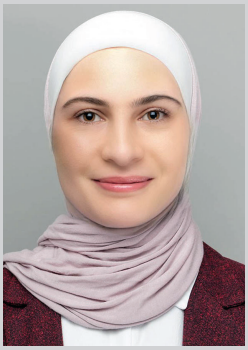

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Twan Lammers obtained a D.Sc. in Radiation Oncology from Heidelberg University in 2008 and a Ph.D. in Pharmaceutical Technology from Utrecht University in 2009. In 2014, he was appointed as full professor of nanomedicine and theranostics at RWTH Aachen University Clinic. His group aims to individualize and improve disease treatment by combining drug targeting with imaging. To this end, image-guided drug delivery systems are being developed, as well as materials and methods to monitor tumor growth, angiogenesis, inflammation, fibrosis and metastasis.

Andreas Herrmann studied chemistry at the University of Mainz, pursued his Ph.D. studies at the Max Planck Institute for Polymer Research in Mainz and a postdoctoral stay at the Swiss Federal Institute of Technology in Zurich. Since 2017, he holds the chair for Macromolecular Materials and Systems at RWTH Aachen University and is presently director of the DWI – Leibniz-Institute for Interactive Materials. His group develops novel molecular technologies using nucleic acids, engineered proteins and synthetic polymers for applications such as sonopharmacology and sonogenetics.

tures and advantages that significantly expedite the impact of US on healthcare technology and remote-control systems. We discuss the current state-of-the-art sonogenetic molecular toolkits, their capabilities and the diverse range of applications they encompass. These toolkits (Figure 1) are categorized based on the underlying mechanism; either thermal or mechanical sonogenetics.

We distinguish between systems that depend on mechanosensitive (MS) ion channels and mechanoresponsive macromolecular systems. We extend our scope beyond merely defining sonogenetics and its applications to explore the emerging frontiers, pressing challenges and prevailing unresolved questions central to this rapidly evolving field.

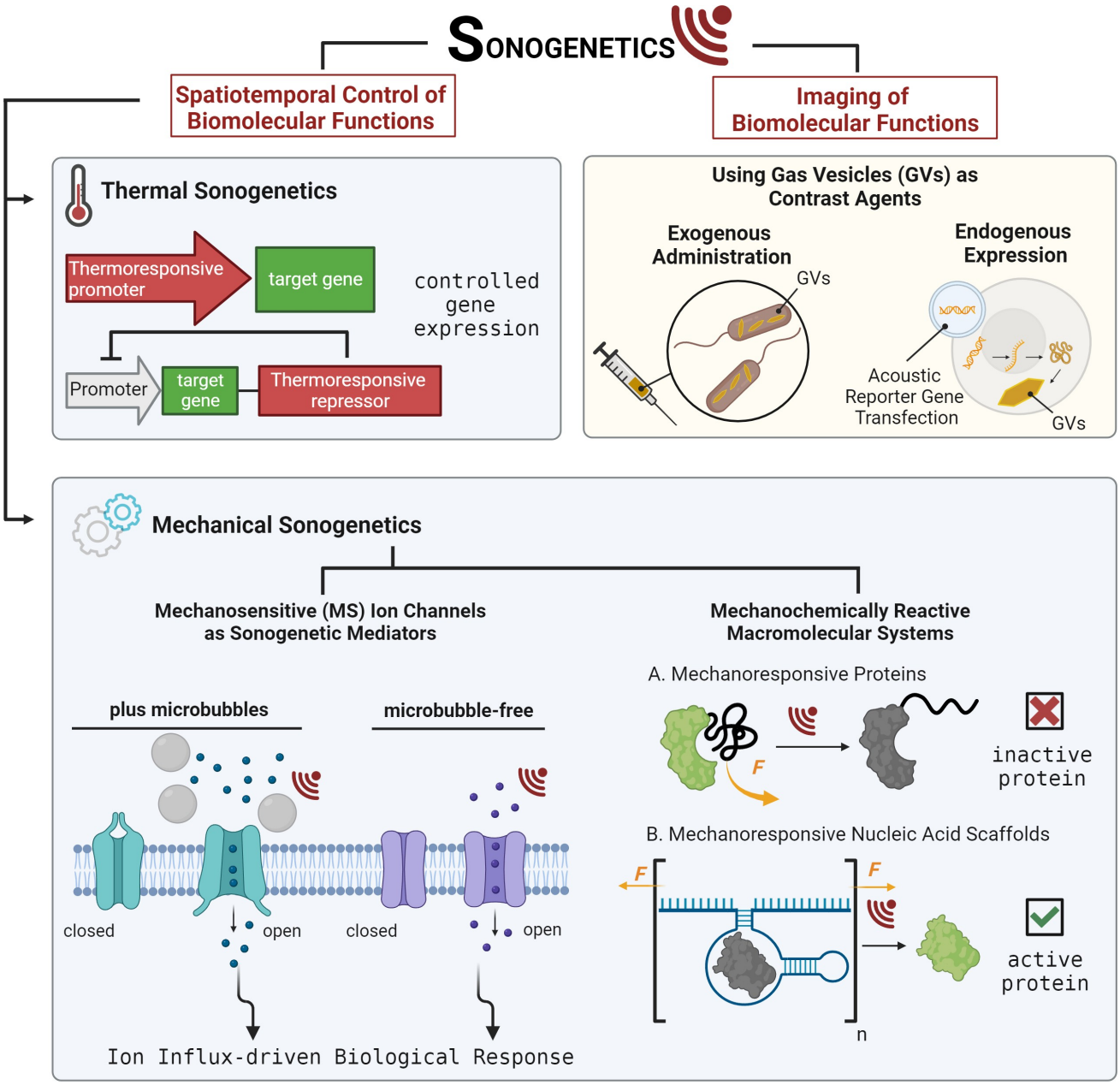

Figure 1. A general scheme summarizing the scope of sonogenetics including sonogenetic tools for either controlling or imaging biomolecular functions. Manipulating cellular functions can be achieved using thermal or mechanical sonogenetics. Heat-responsive promoters and repressors are utilized in the former, while mechanosensitive (MS) ion channels and mechanochemically reactive macromolecular systems are employed in the latter. These strategies ultimately lead to controlled protein activity, signalling pathways or gene expression. On the other hand, gas vesicles (GVs) have been devised as contrast agents to image biomolecular functions at the cellular level, by means of either exogenous administration of gas vesicle-producing microorganisms or genetically engineering target cells to express GVs endogenously.

2. Sonogenetics Mediators

A set of biomolecular tools that connects US to cellular and molecular function is needed to make sonogenetics feasible. These sonogenetic mediators are usually found in the form of MS ion channels,[28b,34] which were discovered in a wide spectrum of living organisms, ranging from bacteria to humans. Various MS ion channels developed unique structures and mechanotransduction mechanisms tailored to suit their specific biological functions.[35]

The activation of MS ion channels is essential for various physiological processes, including sensory perception, cellular signaling and adaptation to mechanical stress. They help cells sense and respond to their mechanical environment, allowing them to maintain proper function and adapt to different conditions.[36] Upon US exposure, both excitatory and inhibitory effects on MS ion channels in different experimental and disease conditions have been documented.[37]

Regarding their mechanism of action, it is widely acknowledged that the application of mechanical stimuli to a cell's membrane results in the distribution of stress across various cellular components, collectively influencing MS ion channels and causing a transition from their closed to open states. Two potential mechanisms have been proposed: the bilayer model and the tether model.[38] In the bilayer model, an increase in the plasma membrane tension is believed to lead to its thinning. This thinning, in turn, exposes hydrophobic residues of the channels that were previously embedded within the lipid bilayer. Consequently, this mechanical alteration induces a tilting of the channel subunits, thereby prompting their opening.

Conversely, in the tether model, the stress experienced by the membrane is transduced to the ion channel through auxiliary proteins, such as those associated with the cytoskeleton or the extracellular matrix. These proteins are believed to directly engage with the MS ion channel subunits, hence facilitating the mechanosensitive response.[38a]

Despite their broad occurrence, few MS ion channels are valuable tools in sonogenetics, such as Piezo1,[28b,39] large conductance mechanosensitive ion channel (MscL),[11,34b,40] mechanosensory ion channel (MEC)-4,[18c] *hs*TRPA1,[34e] TRP-4,[12] $Na_v1.5$,[41] voltage-gated calcium channels (VGCC)[42] and the two-pore potassium channel (K2P) family.[34a,f]

The first demonstration of US-mediated opening of MS ion channels was reported by Heureaux *et al.* for the bacterial MscL expressed in mammalian cells. A G22S mutant of MscL that can be activated at a lower US threshold was used in combination with integrin-bound microbubbles in retinal pigment epithelial cells.[11] One year later, the term sonogenetics was introduced by Ibsen *et al.*, in a study revealing that neural circuits in *Caenorhabditis elegans* (*C. elegans*) exhibit responsiveness towards low-pressure US stimulation when coupled with microbubbles.[12] The crucial role of TRP-4 MS ion channel was underscored as its absence resulted in reduced sensitivity. Overexpressing TRP-4 in specific neurons modulated their response to US and novel roles for ASH, AWC and PVD sensory neurons and AIY interneurons were discovered.[12]

Furthermore, MEC-4 and MEC-6 MS ion channels have been activated with high frequency US, resulting in reversal behavior and neural activity in *C. elegans*.[43] Towards this end, surface acoustic waves (SAWs) were generated by an interdigital transducer (IDT). While it has been demonstrated that SAWs can directly modulate neural activity,[44] the specific SAW mode responsible for ion channel activation remained elusive. Subsequently, further investigations have revealed that TRP-4 and MEC-4, are necessary for eliciting responses to US in *C. elegans*, with evidence suggesting their cooperative involvement in this process.[45]

Most microorganisms have members of the MscL family, which act as osmotic release valves in response to osmotic shock.[46] The best studied example is the MscL from *Escherichia coli* (*E. coli*) and efforts to express it in mammalian cells opened new opportunities for introducing new mechanotransduction pathways and employing sonogenetics in mammalian systems.

Multiple types of MS ion channels have been identified in mammals. Notably, the K2P family consists of six subfamilies including TWIK (tandem of pore domains in a weak inward-rectifying K^+ channel) and TREK (TWIK-related K^+ channel) subfamilies. In particular, three key members: TREK-1, TREK-2 and TRAAK (TWIK-related arachidonic acid activated K^+ channel) are primarily expressed in sensory neurons and found to be responsive to a wide array of mechanical stimuli.[38b]

The Piezo family represents another crucial MS ion channel family, demonstrating diverse roles in mechanosensory, developmental and regulatory processes.[38b] It is worth noting that a mild mutation in Piezo1 confers resistance to malaria in approximately one-third of individuals of African descent.[47] TMEM63A channels, present in the human nervous system, have been associated with myelination defects in infants carrying mutated proteins.[48]

TRP channels have also been postulated to be directly activated by mechanical stimuli. Furthermore, acid sensing ion channel 1a (ASIC1a) stands as the sole member within its family known to be activated by US,[49] and there is some emerging evidence suggesting that ASIC3 may also be US-responsive.[50] The utilization of these MS ion channels with the framework of sonogenetics for various biomedical applications will be elaborated upon in section 4.

3. Thermal Sonogenetics

US can be localized to deliver precise and concentrated energy to a specific target area within the body. This focused US (FUS) and its resulting thermal effects have been medically utilized for different applications such as cancer cell killing,[51] uterine fibroid removal,[52] neurosurgery[53] and kidney stone fragmentation,[54] where it offers several advantages when compared to traditional surgical procedures such as reduced infection risk, faster recovery and less damage to healthy tissue.

Apart from thermally destroying tissue, FUS can be used to exhibit control over gene expression in combination with temperature-sensitive repressors and promoters.[55] A pioneering example is the mammalian heat shock protein 70 (HSP70), which is severely repressed in its off-state but readily thermally-induced using mild heating protocols for few minutes at 43 °C without causing tissue damage.[55a,56] In 2017, a new class of tunable, orthogonal temperature sensitive repressors (TSRs) was reported, which demonstrated hundred-fold change in gene expression in response to temperature change.[57] These bioswitches were also integrated into logic circuits and spatially activated by FUS in mammalian hosts (Figure 2A) with high precision.[57] Additionally, they were used in the field of probiotic immunotherapy by combining the tumor-infiltrating capabilities of therapeutic bacteria with the spatiotemporal control of FUS (Figure 2B).[58]

Recently, significant advances in using FUS to activate HSPs in combination with chimeric antigen receptor (CAR) T cells *in vivo* have been reported.[59] Short pulses of FUS

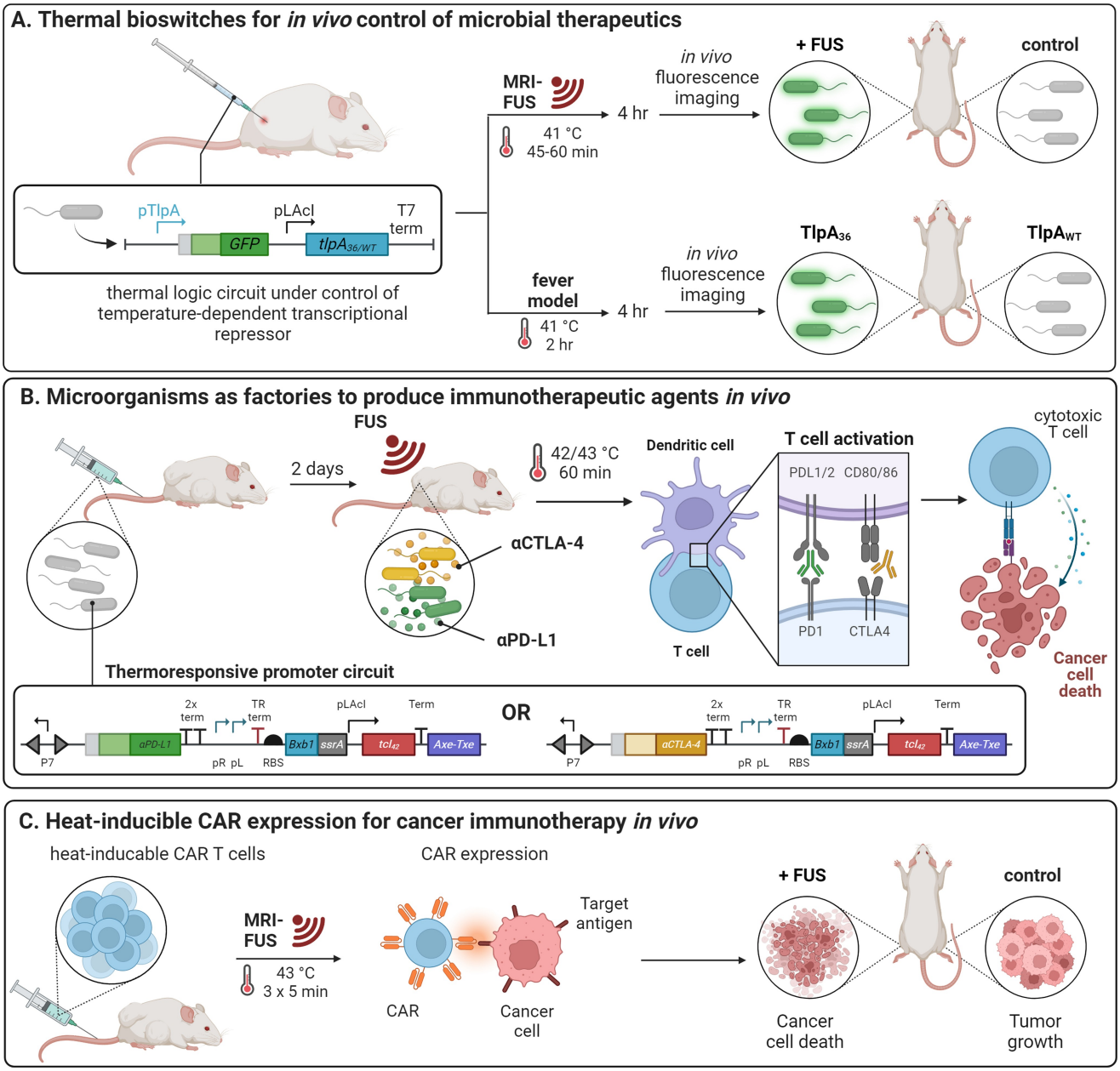

Figure 2. Thermal sonogenetics. (A) Microbial therapeutic approach in which temperature-dependent transcriptional repressors control bacterial gene expression integrated into thermal logic circuits and utilized *in vivo* to induce spatially precise gene activation using magnetic resonance imaging (MRI)-guided focused ultrasound (FUS) and modulation of activity in response to host fever. (B) Similar approach used to trigger expression of αCTLA-4 and αPDL-1 as immunotherapeutic agents that activate T cells against tumors. (C) Heat-inducible chimeric antigen receptor (CAR) expression in tumor models *in vivo*, leading to suppressed cancer development. Images recreated with BioRender.com from Refs. [57,58a,59] respectively.

can reversibly induce CAR expression to suppress tumor growth in mice with a high spatiotemporal control guided by magnetic resonance imaging (MRI) (Figure 2C). The biocompatibility of the HSP, combined with the non-invasive induction of transient gene expression utilizing FUS, can be considered as a safe alternative to conventional gene therapy. In this case, the immune response is localized, thereby minimizing overstimulation of the immune system and unwanted side effects.

In a combined sonothermogenetic approach, FUS was employed to selectively activate genetically modified neurons expressing TRPV1 ion channels in the brains of mice *in vivo*, evoking locomotor behaviour. The non-invasive nature of this approach was confirmed by evaluating neuronal integrity and assessing markers of inflammation and apoptosis.[60]

4. Mechanical Sonogenetics

4.1. Ion Channel-mediated Sonogenetics

4.1.1. Microbubbles as Sono-adjuvants

To activate mammalian cells overexpressing MS ion channels via low-frequency US, microbubbles can be used as acoustic actuators that sensitize the cells towards low-energy US. Such systems have been widely utilized for various ranges of applications in different biological systems.

In the context of cancer immunotherapy, T cells that endogenously express the Piezo1 MS ion channel were coated with streptavidin to allow their subsequent binding to biotinylated microbubbles.[28b] Activation of Piezo1 ion channel results in calcium influx, which then activates the calcium-dependent phosphatase calcineurin that phosphorylates NFAT transcription factors. NFATs translocate to the nucleus and activate an NFAT-dependent promoter that triggers the expression of anti-CD19 CAR gene in T cells (Figure 3A). This enabled these T cells to engage with target tumor cells that express CD19 on their surface, leading to subsequent activation of T cells to secrete cytokines that ultimately results in cancer cells' apoptosis.[28b]

In the realm of neuromodulation, Shen *et al.* employed microbubbles designed for precise targeting of the extracellular domains of Piezo1 on the cellular membrane of sensitized N2A cells and primary cultured neurons. To achieve this, they initially conjugated biotinylated microbubbles with avidin. Subsequently, they functionalized the surface by binding to biotinylated anti-Piezo1 antibodies. The utilization of these specialized targeting microbubbles facilitated the stimulation of Piezo1 ion channels at significantly reduced US intensities.[61]

4.1.2. Microbubble-free Ion Channel Stimulation

Mechanical activation of ion channels could also be achieved without the administration of microbubbles. However, either a higher frequency US or a more sensitive MS ion channel mutant is needed. Due to its extensive investigation, MscL has been the channel of choice for expression in mammalian cells in several studies. Ye *et al.* used the gain-of-function mutation I92L to sensitize MscL towards low-pressure US pulses, expressed it in a primary culture of rat hippocampal neurons and achieved programmable excitation of neurons.[34b] The same MscL mutant was used for US-controlled tumor apoptosis in HeLa, B16 and 4T1 cells. Moreover, tumor growth was suppressed in a B16 melanoma mouse model. Continuous sonication resulted in excessive calcium influx, which then activates cells' apoptosis.[40b]

Similarly, to trigger cancer immunotherapy via US, iron-alginate nanogels containing poly(ethyleneimine) were used to deliver the MscL gene to cancer cells *in vivo* to render them US-responsive. Excessive calcium influx led to cell apoptosis, which in turn liberated tumor associated antigens that can promote dendritic cell maturation and T cell activation leading to tumor treatment and potential prophylactic effect in mice.[62]

Furthermore, Qiu *et al.* successfully stimulated primary neurons, which express the MscL G22S mutant that has lower voltage gating threshold (Figure 3B). They demonstrated targeted stimulation of MscL G22S expressing excitatory neurons in mice cortices, which led to a stronger muscular response in their limbs.[40a] The G22S-MscL was the first MscL demonstrating neuromodulation in the right cortex and right dorsomedial striatum of mice brain *in vivo* using US without any effects on other regions. Cadoni *et al.* advanced the technology by fine-tuning the spatiotemporal resolution to a precision of 400 μm at a frequency of 15 MHz.[63] This significant breakthrough, in line with the stringent requirements of brain-machine interfaces, was the activation of neurons in the visual cortex of mice, generating a behavior indicative of light perception.

A recent study introduced an innovative method in sonogenetic applications by leveraging music characterized by low-frequency auditory stimuli as low as 50 Hz to modulate insulin levels in type 1 diabetic mice implanted with MscL-transgenic cells. This pioneering approach resulted in near wild-type blood insulin concentrations,[64] showcasing its potential in therapeutic contexts. These findings necessitate a reassessment of the conventional narrow frequency spectrum utilized in sonogenetic studies.

In neuroscience, the establishment of a non-invasive technique capable of perturbing neuronal activity with high spatial resolution has been a long-standing challenge. US stands out as a superior alternative to conventional neuromodulation methods as it effortlessly traverses an intact skull. For instance, the auditory sensing protein Prestin was engineered to respond to US in transfected cells and induce calcium influx both *in vitro* and in mouse brain *in vivo*.[34c] In the same context, Wu *et al.* harnessed low-frequency US for a dual purpose; gene delivery and neuromodulation. They genetically engineered an ultra-sensitive mutant of the Prestin protein and coupled its plasmid with microbubbles to achieve transcranial US targeting of neurons buried in deep brain.[34d]

In the quest of non-invasive Parkinson's disease treatment, a sonogenetic approach employed an engineered

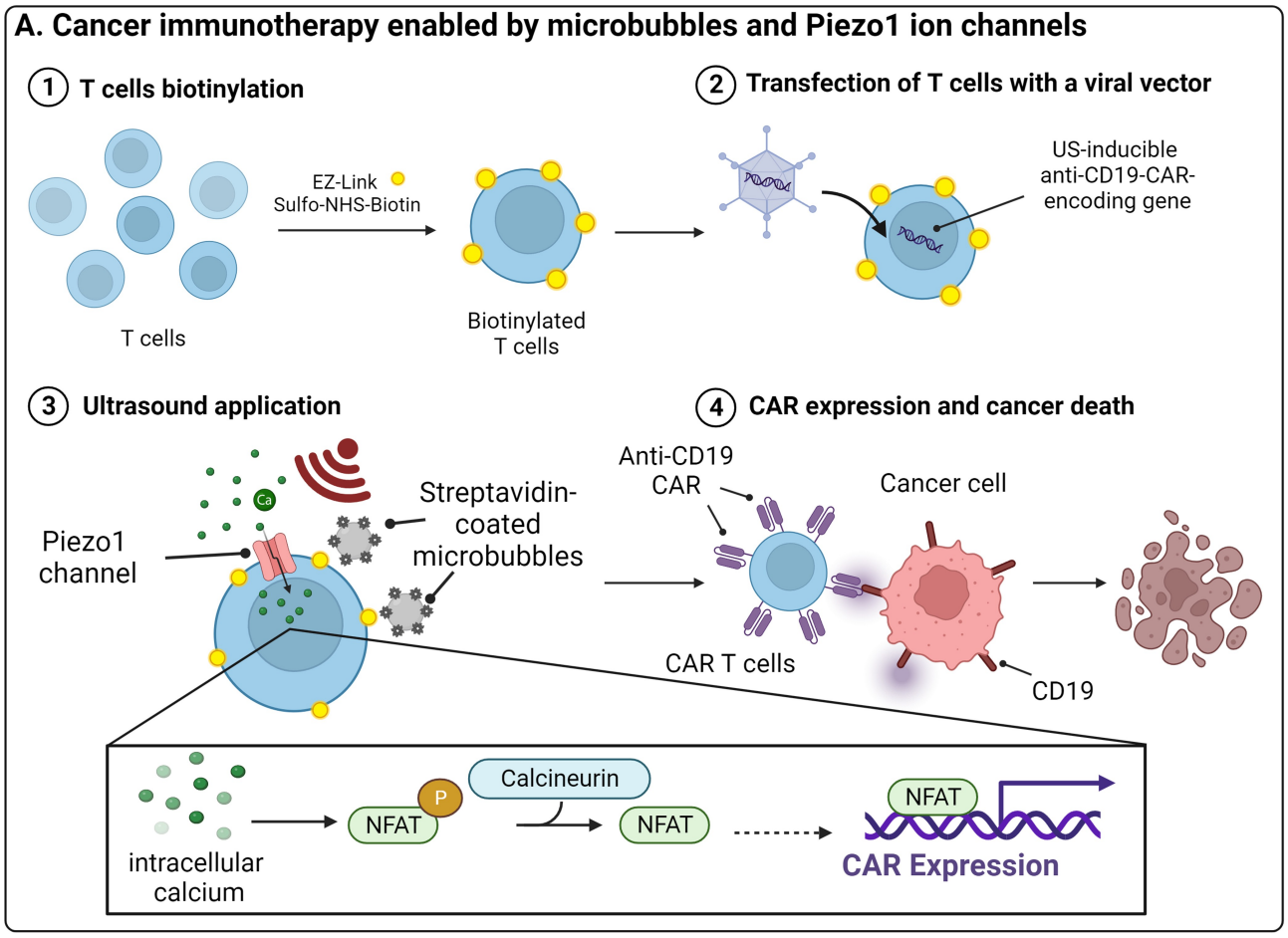

Figure 3. Ion channel-mediated sonogenetics. (A) Microbubbles attached to cell surfaces act as ultrasound (US) amplifiers to mechanically activate Piezo1 ion channels in chimeric antigen receptor (CAR) T cells. Calcium influx leads to NFAT dephosphorylation by calcineurin and the stimulation of anti-CD19 CAR expression enabling cancer immunotherapy. (B) Expression of the mechanosensitive (MS) ion channel MscL-G22S *in vivo* and sonication with low-intensity 0.3 MPa US. Calcium influx is triggered and neurons are activated, which evokes muscular response and c-Fos expression. (C) Engineered auditory-sensing protein mPrestin is expressed in dopaminergic neurons of Parkinson's disease mice. US (0.5 MHz) is exerted repeatedly for localized brain stimulation to ameliorate the dopaminergic neurodegeneration and mitigate Parkinson's symptoms. Images recreated with BioRender.com from Refs. [28b,40a,65] respectively.

Prestin in mice (Figure 3C) to ameliorate the dopaminergic neurodegeneration by 10-folds upon US stimulation.[65] A significant 4-fold alleviation of symptoms was observed, suggesting a clinical potential towards the treatment of neurodegenerative diseases.

As one of the principal mechanoreceptor channels in mammalian cells, Piezo ion channels were expressed for US targeted modulation of cellular activity in different studies. Prieto *et al.* could stimulate Piezo1 channels expressed in mammalian cells selectively using a focused 43 MHz transducer.[39a] A study by Qui *et al.* revealed that US alone could stimulate both heterologous and endogenous Piezo1, leading to calcium influx and increased nuclear c-Fos expression in primary neuron cell culture. This was accompanied by an additional increase in the expression of crucial proteins like phospho-CaMKII, phosphor-CREB and c-Fos in the neuronal cell line.[39b] Liao *et al.* have developed a vertically deployed SAW platform to trigger Piezo1 channels expressed in HEK293T cells. Their study concluded that the ion channel response depends on the pulse repetition frequency (PRF) or burst duration of US.[66]

Furthermore, Piezo1 was expressed in osteoblast precursor cells and shown to be selectively stimulated by US to induce the corresponding downstream signaling pathways leading to a significant increase in cell migration and proliferation. Intracellular calcium acts as a second messenger and the increase of its levels leads to the activation of ERK1/2 phosphorylation, which in turn regulates cell proliferation. Structural remodeling was also observed after US stimulation of Piezo1-transfected cells, demonstrated by the accumulation of actin filaments in the perinuclear region.[67]

Recent investigation of the underlying biological mechanisms of ultrasonic neuromodulation's demonstrated the functional expression of Piezo1 channel in different regions of the brain, including both the right motor cortex and central amygdala with the latter exhibiting greater sensitivity to US.[68]

In the same context of ultrasonic neuromodulation, Yoo *et al.* investigated the biomolecular and cellular mechanisms by which FUS excites primary cortical neurons. Their results established that US excites neurons via direct mechanical force leading to extracellular calcium influx. The roles of TRPC1 and TRPP2 as MS ion channels and TRPM4 as calcium-dependent amplifier were confirmed through specific pharmacological and genetic inhibition of different types of MS ion channels. Overexpression of the involved channels was shown to sensitize the target cells to lower US intensities, thereby expanding the sonogenetics toolbox to cells that don't inherently respond to US.[34f]

Another member of the TRP family, TRPA1, is endogenously expressed in the central nervous system, particularly in astrocytes found in regions such as the hippocampus, cortex and trigeminal caudal nucleus. Oh *et al.* identified a novel mechanism of US-induced neuromodulation in which sonication triggered the activation of TRPA1 channels. This in turn leads to intracellular calcium increase followed by Best1-mediated release of gliotransmitters, including glutamate. The latter stimulated NMDA receptors within the nearby synapses, thereby initiating action potentials. It was further proposed that the TRPA1-Best1-NMDAR pathway might be involved in pathological processes such as brain concussion, characterized by direct mechanical effects on cells.[69]

Within the same TRP family, Burks *et al.* mechanically activated a Na^+-containing TRPC1 current using FUS, thereby stimulating cyclooxygenase-2 (COX2)-dependent pathways and inducing mesenchymal stem cell (MSC) tropism to kidneys and muscles.[42]

Ultrasonic neuromodulation of TRAAK ion channels, a member of the K2P family, was observed in genetically engineered neurons.[34a] This could open the door for further studies in which TRAAK ion channels might be used as tools in sonogenetics in the future.

Finally, Lim *et al.* determined that ASIC1a and cytoskeletal proteins played crucial roles in mechanotransduction and activation of cultured neurons upon stimulation by low-intensity US. The role of ASIC1a was more profound than that of Piezo or TRP proteins. The proposed mechanism differed between *in vitro* and *in vivo* scenarios. *In vitro*, activation of the sodium channel ASIC1a likely led to the stimulation of voltage-gated calcium channels, resulting in increased intracellular calcium levels. *In vivo*, ASIC1a activation was attributed to a cytoskeletal-dependent mechanism arising from the mechanical effects of acoustic pressure exerted on extracellular matrix proteins.[49]

4.2. Mechanochemically Reactive Macromolecular Systems

A novel and distinctive approach towards sonogenetics involves the design and development of macromolecular systems that inherently respond to US, without using MS ion channels or co-administering nano- and microbubbles as sono-adjuvants. This methodology expands the scope of sonogenetics and broadens its definition by incorporating mechanochemical strategies that utilize US as a trigger for controlling cellular functions at various levels.

These approaches were inspired by the realm of polymer mechanochemistry, where US-mediated mechanical forces induce chemical reactions and transformations in polymers,[70] aiming to create new materials,[71] modify existing ones,[72] or facilitate reactions otherwise unattainable under standard conditions.[73] Force-responsive moieties named mechanophores have been designed to selectively react to applied US.[74] Additionally, supramolecular mechanophores have emerged, in which assemblies formed through weak, dynamic, non-covalent interactions dissociate upon exposure to mechanical forces.[75]

Transferring these concepts from polymer mechanochemistry to sonogenetics requires meticulous consideration of the physicochemical properties of the involved biological macromolecules. These approaches mark significant advancements in the field of sonogenetics, offering tailored solutions for manipulating cellular functions and paving the way for their application in medical research and therapy.

Within this burgeoning landscape, we describe two recent strategies employed to modulate cellular activity at

the protein level by US, either by *de novo* designing the protein to become mechanoresponsive, or by activating the protein from a mechanoresponsive nucleic acid scaffold on-demand.

4.2.1. Proteins Engineered with Supercharged Unfolded Polypeptide Chains

It is well-established in polymer mechanochemistry that the application of US as a source of mechanical shear stress can trigger transformations at the molecular level via the collapse of US-generated cavitation bubbles.[76] The kinetics of bond scission induced by US depend on the degree of polymerization of the polymer.[77] Therefore, for proteins to become mechanosensitive, a long flexible domain is needed. This can be achieved with so-called supercharged elastin-like polypeptide chains (SUPs),[78] which can be fused to proteins[79] to increase their sensitivity towards US.

An example for this is an engineered green fluorescent protein (GFP) variant modified with an internal long unfolded pentapeptide repeat unit $(VPGEG)_n$ linked to the 11th beta-strand that is part of the beta-barrel (Figure 4A).[80] Mechanical force due to aforementioned shear stress leads to changes in the protein's tertiary structure, displacing the beta-strand from the barrel and extinguishing fluorescence, while maintaining the secondary structure of GFP (Figure 4C). In the same work, Zhou *et al.* activated trypsin by the release of a genetically modified inhibitor fused with a cationic SUP domain that acts as a biophysical transducer for the shear forces originating from ultrasonication (Figure 4B). As a result, the protein-protein interaction between trypsin and its inhibitor is cleaved, therefore, restoring trypsin activity (Figure 4D).

In these two examples, the unprecedented activation and deactivation of the optical and catalytic activities of genetically engineered proteins was revealed. The strategy of equipping proteins with SUPs enabled the tuning of their mechanosensitivity to render them controllable by US-induced shear forces.

4.2.2. Proteins Activated from Nucleic Acid Scaffolds

Apart from outfitting proteins with mechanosensitive domains via protein engineering, nucleic acid scaffolds made of polynucleic acid aptamers (poly aptamers) can be utilized to inhibit and restore enzymatic activity in combination with

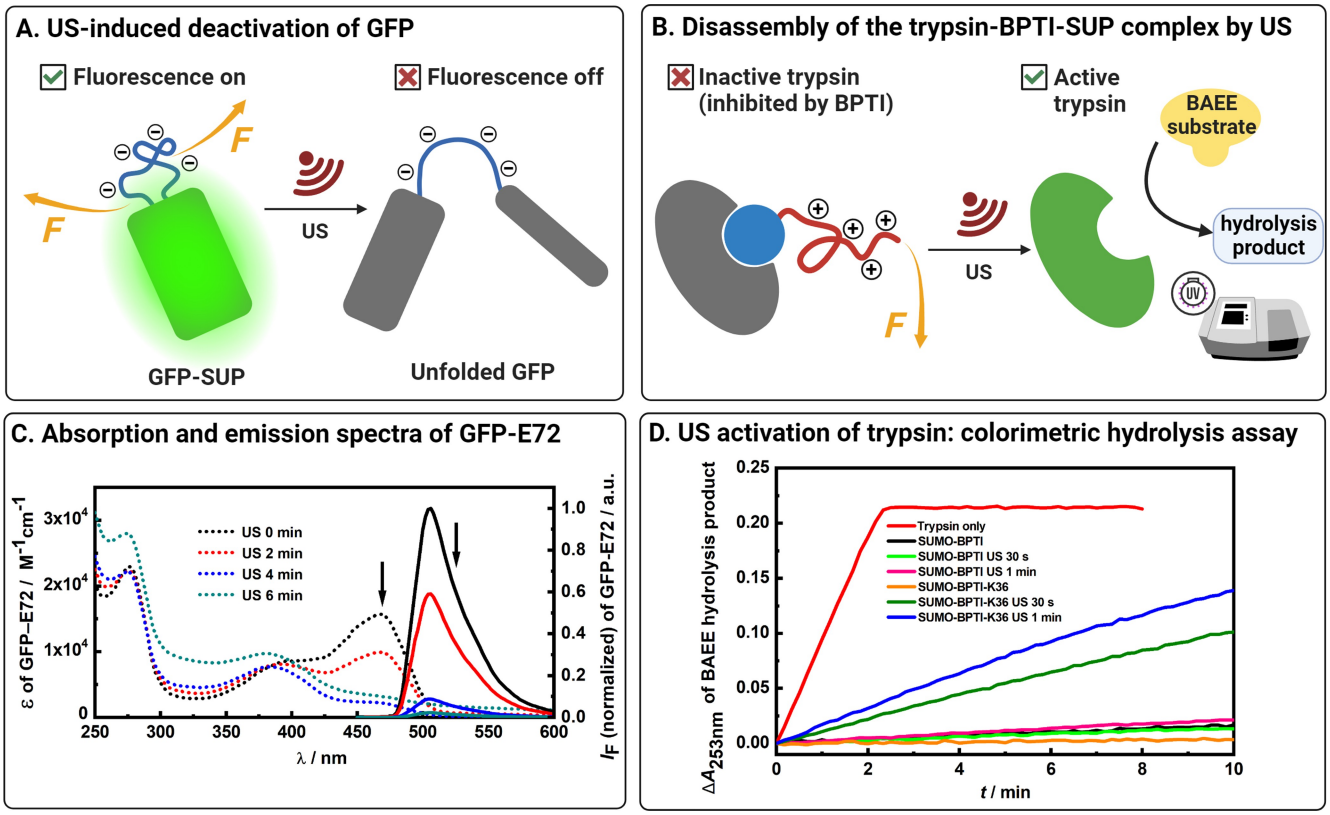

Figure 4. *De novo* design of ultrasound (US)-sensitive proteins to control optical and catalytic activity. (A) Illustration of US-induced unfolding of green fluorescent protein (GFP)-supercharged elastin-like polypeptide chains (SUP) fusion and switching off fluorescence properties. (B) Concept of US-mediated disassembly of trypsin inhibited by bovine pancreatic trypsin inhibitor (BPTI)-SUP complex, which can be followed by the hydrolysis of the substrate N_α-benzoyl-L-arginine ethyl ester (BAEE) yielding a product absorbing at 253 nm. (C) Absorption (dashed) and normalized fluorescence emission (solid) spectra of anionic GFP-E72 at different sonication times using 20 kHz US. (D) The enzymatic activity of trypsin, trypsin-SUMO-BPTI and trypsin-SUMO-BPTI-K36 complexes for different times of sonication. Image recreated with BioRender.com from Ref. [80].

low frequency US and high frequency medical low-intensity FUS (LIFU).[81] This approach precludes the need for genetically engineering cells and can be potentially used to control the activity of a wide range of proteins.

High molar mass poly aptamer chains, prepared through rolling circle amplification (RCA), bind to thrombin and inhibit its catalytic activity (Figure 5A). FUS is used similarly as explained earlier—to induce shear stress by collapsing cavitation bubbles.

This stretches the long poly aptamer chains resulting in scission of non-covalent thrombin-aptamer interactions such as hydrogen bonds and hydrophobic interactions (Figure 5B). This was demonstrated in both irreversible and reversible manner, with the latter involving a gold nanoparticles system with split aptamers protruding from their surface (Figure 5C). This was the first time to report protein activation from their aptamer-bound form using US as a source for mechanical force.

The utilization of non-invasive US as an external cue to regulate protein activity has been infrequently explored in the past. Currently, there is no widely agreed-upon strategy for its application in this field, despite its advantages such as biocompatibility, exceptional tissue penetration depth and high spatiotemporal precision. Although this field is still in its early stages, it holds significant potential for future approaches concerning the remote control of protein activity both within cells and in living organisms.

5. US Imaging of Biomolecular Functions

Aspiring towards imaging of specific cells' function deep inside intact organisms is essential for addressing both basic research questions and therapeutic approaches. Optical imaging techniques that utilize fluorescent and luminescent protein reporters face challenges due to light scattering and absorption.

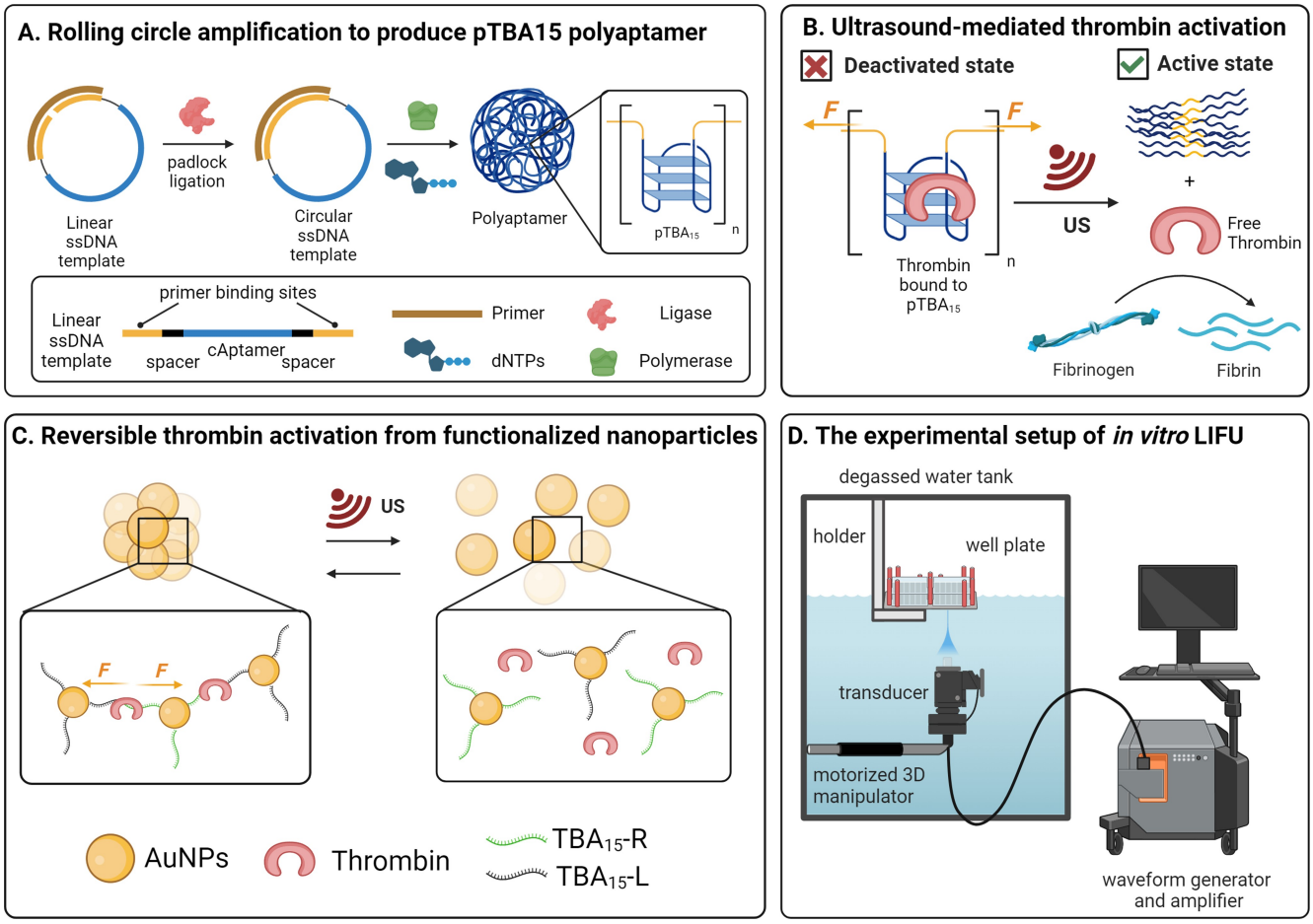

Figure 5. Mechanochemically responsive macromolecular systems. (A) Synthesis of pTBA15 polyaptamer by rolling circle amplification (RCA). (B) Thrombin binding and inactivation by pTBA15 and subsequent release and activation with ultrasound (US) to catalyze the formation of fibrin from fibrinogen. (C) Aggregation of gold nanoparticles (AuNPs) functionalized with either the split aptamers TBA15-L or TBA15-R in presence of thrombin. Sonication leads to reversible disaggregation to achieve thrombin release and activation. (D) Low-intensity focused ultrasound (LIFU) setup using 2.5 MHz transducer (60 mm diameter, 50 mm focal length and 0.9 mm −6 dB focal diameter) which is positioned through a custom-made motorized 3D-manipulator inside a 150 L degassed water tank with a pre-determined distance from the bottom of a sonographic well plate that contains the samples. The well plate is fixed in its place using a holder. Images recreated with BioRender.com from Ref. [81].

On the other hand, US imaging stands as the most commonly employed modality in the realm of diagnostics in medical practice, with a range of imaging modes applicable in the biomolecular context.[82] Compared to other modalities, US possesses remarkable temporal resolution while also providing adaptable and frequency-dependent spatial resolution. To obtain high-quality images with enhanced lateral and axial resolution, it is essential to employ high center frequency and wide bandwidth. US medical imaging does not necessitate high intensity but instead relies on frequent samplings with a high PRF.[83]

In soft tissues, the frequencies conventionally used in US imaging (ranging from 3 to 25 MHz) correspond to wavelengths spanning from 60 to 500 μm and penetration depths of 10 to 1 cm, respectively. Given that both spatial resolution and penetration depth are contingent upon frequency,[84] attaining a high submicron resolution—akin to optical imaging—requires employing US frequencies in the GHz range. However, this escalation in frequency significantly limits tissue penetration to around 1 mm. This presents a pivotal trade-off in US imaging that demands thorough scrutiny in the subsequent section.

5.1. Gas Vesicles as US Contrast Agents

Conventional US contrast agents in the form of synthetic microbubbles have been used for several decades.[85] Nevertheless, challenges associated with their size and stability limited their application when it comes to molecular imaging and observation of dynamic cellular processes.[86] In 2014, Shapiro *et al.* proposed a novel class of air-filled protein nanostructures, named gas vesicles (GVs) as biomolecular contrast agents for US imaging.[87] GVs are cylindrical or spindle-shaped gas-filled protein nanostructures, with lengths ranging from 100 nm to 2 μm and widths of 45 to 200 nm.[88] They are constituted out of a 2 nm-thick amphiphilic protein shell, selectively permeable to gas that can freely enter and leave the hollow structure while water is excluded (Figure 6B).[89]

Due to the larger acoustic impedance difference of aqueous media and the GVs' gaseous interior, US contrast can be achieved both *in vitro* and *in vivo*. GVs are physically remarkably stable and pressure resistant, withstanding up to 1.3 MPa before disrupting.[90] Initially, GVs were used as US contrast agents after purification and subsequent incubation with cells or injection *in vivo*.[87] Since then, substantial advances have been reported aimed to understand and engineer the acoustic characteristics of GVs at the molecular level, as well as to improve their targeting properties and their detection by US.[91]

Furthermore, GVs were used in imaging in oncology[92] and neuroscience[93] and their stability was further enhanced by PEG-modification of their surface.[94] GVs were also modified to operate as acoustic biosensors that show dynamic variations in US contrast in response to enzymatic activity.[95] One of the surface proteins of the GV shell was engineered to be cleaved by different proteases which reduces the collapse pressure and enhances nonlinear US contrast.

5.2. Acoustic Reporter Genes

The ability of GVs to be genetically modified led to the development of acoustic reporter genes (ARGs), as acoustic analogues to optical reporter genes, such as GFP, in which the contrast detected signal can be correlated to their expression.[96] By combining structural GV proteins from *Anabaena flos-aquae* with assembly factors from *Bacillus megaterium*, a hybrid operon was successfully expressed recombinantly in *E. coli* resulting in strong US contrast in cells. Engineering various genetic variants of GVs enabled multiplex imaging by modulating and tuning their response to US.

A major challenge was the translation of ARGs from prokaryotes to eukaryotes. In 2019, ARGs were expressed in mammalian cells for the first time, allowing high-resolution imaging of gene expression inside living organisms.[97] Due to the limited sensitivity with which ARG-expressing cells can be visualized, an imaging paradigm was developed to improve the cellular detection limit.[98] This new tool, termed burst US reconstructed with signal templates (BURST), increased the detection limit of US to image ARG-expressing cells by more than 1000-fold in comparison with conventional methods. Furthermore, it enabled the detection of gene expression in single cells in dilute cell suspensions.[98]

Recently, a second-generation of ARGs was developed for both bacterial and mammalian cells (Figure 6A) through a genomic mining approach.[99] These are ARGs with enhanced properties compared to their first-generation counterparts, as they offer stronger US contrast (9-fold and 38-fold, respectively), generate distinguishable non-linear signals in the presence of background tissue and maintain stable long-term expression. Employing these novel ARGs, Hurt *et al.* conducted non-invasive imaging of in situ tumor colonization and monitored gene expression within tumor-targeting therapeutic bacteria. Additionally, they tracked gene expression and tumor growth within a breast cancer mouse model.[99] It is speculated that the improved properties and performance of second-generation ARGs could facilitate a wider range of *in vivo* applications in the future. While many capabilities of ARGs are similar to optical reporter genes, as for example the imaging of cellular dynamics by promoter-driven expression, ARGs can be resolved non-invasively without any surgical procedures.

The realm of optical microscopy applications expanded beyond the initial scope envisioned when GFP was utilized in biotechnology. In a manner akin to the enhancements and modifications made to fluorescent proteins, the methodical advancement of both ARGs and accompanying US-based imaging technologies holds the potential to unlock the possibilities of highly sensitive, high-resolution and non-invasive imaging of cellular function within mammals.

Another study showcased GVs to serve as biomolecular agents for inertial cavitation, enabling remote activation to

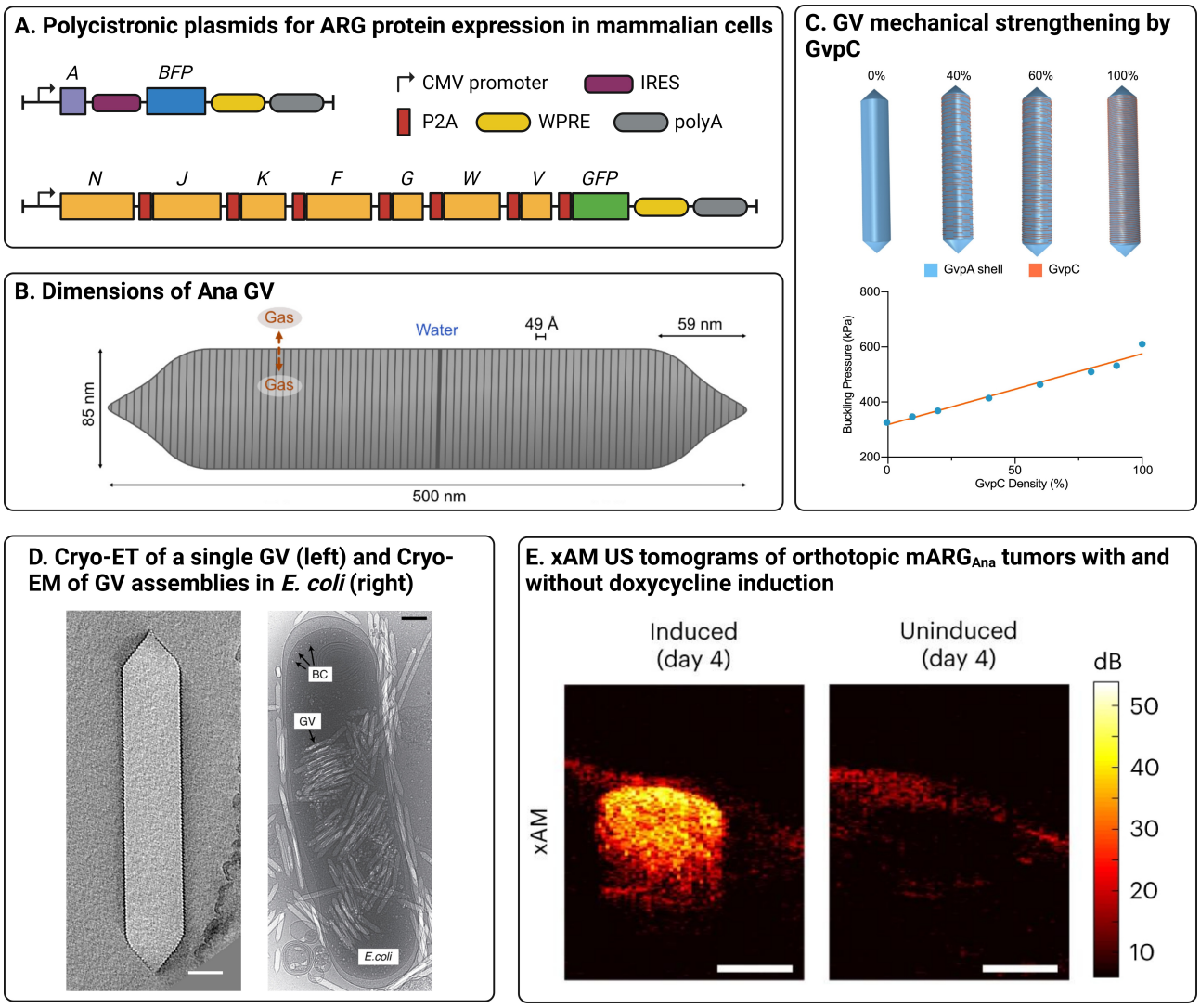

Figure 6. Genetically encoded acoustic protein nanostructures. (A) Schematic polycistronic plasmids encoding the mammalian acoustic reporter gene (ARG) adapted from *Anabaena flos-aquae* ($mARG_{Ana}$). *A*, *N*, *J*, *K*, *F*, *G*, *W*, *V* encode for gas vesicle (GV) proteins (gvpVs). *gvpA* is supplied on a separate plasmid to enable stoichiometric tuning. Blue fluorescence protein (BFP) and green fluorescence protein (GFP) are used for fluorescent analysis and sorting (recreated with BioRender.com from Ref. [99]). (B) Illustration of an Ana GV highlighting its dimensions (adapted from Ref. [89a], CC BY 4.0). (C) Solid mechanics simulations on GV shell models reveal a linear relationship between buckling and GvpC density, consistent with experimental data (adapted from Ref. [89a], CC BY 4.0). (D) Cryo-electron tomography (cryo-ET) of an individual Ana GV (left, adapted from Ref. [89a], CC BY 4.0) and cryo-electron microscopy (cryo-EM) of *Escherichia coli* (*E. coli*) engaged in the heterologous production of *Bacillus megaterium* GVs (right, adapted from Ref. [89b], CC BY 4.0). Scale bar 50 nm (left) and 100 nm (right). (E) In situ $mARG_{Ana}$ expression enables non-destructive ultrasound (US) imaging of orthotopic tumors. Representative middle sections of cross-amplitude modulation (xAM) US tomograms of MDA-MB-231 $mARG_{Ana}$ tumors induced with doxycycline (left) and uninduced control (right) imaged on day 4. Scale bars 2 mm. (adapted from Ref. [99], CC BY 4.0.)

destroy cells and tissue.[100] This facilitates genetically engineered cells to lyse, release molecular payloads and cause localized mechanical damage upon command. In this case, GVs act as precision mechanical warheads, activated by FUS with millimeter accuracy. In the future, this approach could improve drug delivery to targeted tissues and enhance effectiveness of immunotherapy treatments.

The latest findings have illuminated the potential of GVs to serve as actuators of ARF,[101] a phenomenon witnessed for the first time through genetic manipulation. Such a facet, not only facilitates the direct and selective manipulation of bacterial cells utilizing ARF, enhancing scope for acoustic trapping, translation and holographic patterning, but also extends to the selective acoustic maneuvering of mammalian cells predicated on their genotype. These discoveries herald applications in biomaterials and biotechnology, including dynamic 3D cellular patterning for tissue engineering and living materials, leveraging GVs' acoustic properties for enhanced and reconfigurable fabrication techniques and offering new avenues in acoustofluidic devices for cellular separation based on gene expression.

6. Challenges and Future Outlook

Sonogenetics is an emerging field, having developed within the last decade, that explores the use of US to manipulate and control biological function with high spatiotemporal precision. Sonogenetics not only allows the steering of cellular processes but also enables their real-time imaging. Achieving these functionalities involves mechanosensitive biomolecules and macromolecular architectures that respond to sonication and elicit a down-stream biological activity through various mechanisms, encompassing thermal, mechanical, or acoustic effects.

Recent research highlights the versatility and potential of sonogenetics, with notable achievements including the neuromodulation in mice brain,[37] the MRI-guided reversible CAR T cell expression in mice to suppress tumor growth,[59] and the expression of ARGs in mammalian cells enabling imaging of gene expression *in vivo*.[99] While these recent accomplishments showcase the remarkable potential of sonogenetics, ongoing research and technological developments hold the key to unlocking even more exciting possibilities and addressing current challenges in this evolving field.

One of the primary challenges in sonogenetics is the identification of appropriate US mediators, aimed at expanding the repertoire of available tools. A frequently employed strategy revolves around the utilization of MS ion channels, necessitating the discovery and subsequent genetic expression of these channels within target cells.[102] Two primary methods are employed in this endeavor: top-down screening and bottom-up channel engineering. The latter approach, though highly effective, demands considerable labor input,[103] while the former relies predominantly on calcium imaging as its readout, which is characterized by limited sensitivity. To advance our understanding and further identify novel MS ion channels, there is a pressing need to develop techniques such as voltage-based imaging[34f] and low-frequency US compatible patch clamping.

Furthermore, the exploration of inhibitory MS ion channels is paramount for achieving bidirectional control over cellular activity through US.[104] In this regard, the investigation of US-responsive potassium or chloride channels assumes significance. Novel MS ion channel mutants characterized by heightened sensitivity and enhanced single-channel conductance would offer the advantage of direct utilization in engineered cells, without the need for the administration of microbubbles as sonoadjuvants.

The systematic and hypothesis-driven advancement of sonogenetic mediators would not only enable the engineering of novel variants endowed with specific mechanosensitive properties and ion selectivity, but also provide a valuable avenue for gaining profound insights into the mechanisms governing their activation and regulation, as the precise mechanism underlying this stress-induced transition has been a subject of ongoing debate.[38b]

Significant technical challenges are associated with the utilization of MS ion channel-dependent approaches in sonogenetics, including safety profiles and substantial costs. Specifically, when genetically engineering neurons and other cell types using viral vectors, a range of safety concerns emerges, notably the risk of triggering an immune response. Furthermore, unintended side effects can occur due to inadvertent stimulation of non-target endogenous MS ion channels, which can compromise efficiency. For example, US was reported to activate the ascending auditory system via a cochlear pathway[105] and was shown to exhibit secondary mechanical effects similar to that evoked by audible sound[19d] in animal models. Moreover, transcranial US stimulation was found to provoke auditory signals in human subjects.[106] These findings revealed the need to take this auditory confound into consideration while developing ultrasonic neuromodulation as a tool in neuroscience research in the future.

Additionally, the application of high-intensity FUS (HIFU) carries the potential to cause irreversible tissue damage. These safety-related issues could significantly limit the translational potential of such sonogenetic tools for clinical applications. Moreover, as with any gene-based therapy, critical aspects such as targeted delivery, biodistribution and liver clearance must be thoroughly investigated *in vivo*.[107]

Another challenge lies in the fabrication of designer proteins that are inherently sensitive to low-intensity US. The need for such proteins stems from the desire for enhanced safety and minimal tissue disruption during therapeutic interventions. This sensitivity would be particularly advantageous in minimizing potential adverse effects and broadening the applications of US-responsive proteins. Given the myriad of potential protein candidates, it is crucial to identify which proteins exhibit both structural and functional compatibility with US responsiveness. Efficient and advanced high-throughput screening methods would allow for the systematic assessment and optimization of these proteins, followed by a rigorous investigation of their function within cellular milieus and *in vivo*. Such insights would offer a clearer understanding of their physiological implications, potential side-effects and overall therapeutic efficacy.

Moreover, a generalized approach to protein activation and deactivation is sought after. The goal is to formulate strategies that enable the modulation of any given protein without the arduous task of individually engineering each one. Such universal strategies would provide a more streamlined and adaptable approach to therapeutic applications.

In the context of developing sonoresponsive nucleic acid carriers, their successful deployment mandates stabilization. Drawing from techniques established in the context of therapeutic nucleic acids, these carriers must remain robust and functional across diverse cellular contexts and *in vivo*. Furthermore, the demonstrated sonoactivation of proteins accentuates the need to extend this capability to other molecules, including small molecule bioactives and therapeutic nucleic acids. Broadening this spectrum of activatable molecules will undoubtedly increase the versatility of US-responsive therapeutic modalities. Also, a lacuna in understanding persists regarding the exact mechanisms driving the sonoactivation of these carriers. When utilizing LIFU, the established role of cavitation in mediating cellular effects

requires reconsideration. Concurrently, identifying the intrinsic physical properties of these soft colloids remains a subject of intensive research.

Gas-filled microbubbles have been employed to augment the mechanical effects induced by US.[12,108] However, microbubbles exhibit a limited lifetime *in vivo* and a restricted capacity to cross biological barriers.[109] In contrast, a growing trend involves the utilization of microbubble-free macromolecular systems. These systems are mechanoresponsive by-design and obviate the necessity for co-administering microbubbles.

On the other hand, ARGs present a very promising modality to be used especially in US imaging. However, to achieve the impact that GFP and similar fluorescent reporter genes have in the field of biological imaging, GVs require extensive modifications to broaden their applicability across a diverse range of cell types. Moreover, their acoustic properties must be optimized to enhance their detection in living organisms with high sensitivity and selectivity.[99]

In the context of thermoresponsive promoters, it is imperative to consider unwanted stimulation of endogenous thermoresponsive proteins, such as ion channels. Prior to triggering the promoter of interest, it is crucial to identify the genes that express these temperature-sensitive proteins, which may necessitate knockout procedures.

A final set of challenges pertains to the US devices and procedures currently employed in sonogenetics. These challenges encompass the pressing need for standardization and achieving high-resolution US focusing coupled with precise parameter control. These factors are important for realizing uniform mechanical and thermal effects within biological tissues, a prerequisite for successful and safe sonogenetic applications. To exert precise control over cellular activity, it is essential to focus US waves with high spatial resolution, which requires advancements in transducer technology and imaging techniques to concentrate acoustic energy precisely on the target cells or tissues.

Achieving precise focusing is essential in neuromodulation, especially when targeting the complex structures of the brain or accessing its deeper regions. Two main strategies exist: first, one can express the sono-sensitive component specifically in deep brain areas. Alternatively, one can direct the ultrasonic waves to target deep brain regions while allowing the protein to be expressed more broadly. This approach emphasizes the critical balance between precision and breadth required for successful neuromodulation.

Furthermore, sonogenetics demands meticulous control over various US parameters, including frequency, intensity, duration and waveform. Achieving the desired mechanical and/or thermal effects while avoiding unintended tissue damage or adverse effects necessitates fine-tuning of these parameters. Developing user-friendly interfaces and algorithms for parameter adjustment and monitoring is important for researchers and clinicians. Consequently, precise parameter control ensures the safety of sonogenetic interventions. Researchers need to carefully evaluate the potential biological consequences of US exposure, including tissue heating, cavitation and acoustic streaming. Understanding the thermal and mechanical effects on different tissues and cell types is essential for minimizing risks and optimizing the therapeutic or experimental outcome.

In conclusion, sonogenetics exhibits significant potential and exciting opportunities for non-invasive modulation of cellular activity. While it does confront several challenges and constraints, such as specificity, sensitivity, safety, mechanistic comprehension and delivery methods, these obstacles are seen as avenues for growth rather than unsurmountable barriers. The prospect of elucidating fundamental biological processes and creating novel therapies, previously unachievable with current technologies, serves as a powerful incentive for these ongoing efforts to fully harness the capabilities of sonogenetics.

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Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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[1] a) T. Matsubara, T. Yanagida, N. Kawaguchi, T. Nakano, J. Yoshimoto, M. Sezaki, H. Takizawa, S. P. Tsunoda, S.-i. Horigane, S. Ueda, S. Takemoto-Kimura, H. Kandori, A. Yamanaka, T. Yamashita, *Nat. Commun.* **2021**, *12*, 4478; b) A. G. Athanassiadis, Z. Ma, N. Moreno-Gomez, K. Melde, E. Choi, R. Goyal, P. Fischer, *Chem. Rev.* **2022**, *122*, 5165–5208.

[2] D. Oesterhelt, W. Stoeckenius, *Nature New Biol.* **1971**, *233*, 149–152.

[3] M. Mansouri, T. Strittmatter, M. Fussenegger, *Adv. Sci.* **2019**, *6*, 1800952.

[4] a) K. Deisseroth, *Nat. Methods* **2011**, *8*, 26–29; b) J.-A. Sahel, E. Boulanger-Scemama, C. Pagot, A. Arleo, F. Galluppi, J. N.

Martel, S. D. Esposti, A. Delaux, J.-B. de Saint Aubert, C. de Montleau, *Nat. Med.* **2021**, *27*, 1223–1229; c) A. V. Kravitz, B. S. Freeze, P. R. Parker, K. Kay, M. T. Thwin, K. Deisseroth, A. C. Kreitzer, *Nature* **2010**, *466*, 622–626; d) E. S. Boyden, F. Zhang, E. Bamberg, G. Nagel, K. Deisseroth, *Nat. Neurosci.* **2005**, *8*, 1263–1268.

[5] A. N. Bashkatov, E. A. Genina, V. I. Kochubey, V. V. Tuchin, *J. Phys. D* **2005**, *38*, 2543.

[6] H. Huang, S. Delikanli, H. Zeng, D. M. Ferkey, A. Pralle, *Nat. Nanotechnol.* **2010**, *5*, 602–606.

[7] S. Nimpf, D. A. Keays, *EMBO J.* **2017**, *36*, 1643–1646.

[8] a) M. A. Wheeler, C. J. Smith, M. Ottolini, B. S. Barker, A. M. Purohit, R. M. Grippo, R. P. Gaykema, A. J. Spano, M. P. Beenhakker, S. Kucenas, M. K. Patel, C. D. Deppmann, A. D. Güler, *Nat. Neurosci.* **2016**, *19*, 756–761; b) S. A. Stanley, J. Sauer, R. S. Kane, J. S. Dordick, J. M. Friedman, *Nat. Med.* **2015**, *21*, 92–98.

[9] a) K. Kole, Y. Zhang, E. J. R. Jansen, T. Brouns, A. Bijlsma, N. Calcini, X. Yan, A. d S Lantyer, T. Celikel, *Nat. Neurosci.* **2020**, *23*, 1044–1046; b) G. Wang, P. Zhang, S. K. Mendu, Y. Wang, Y. Zhang, X. Kang, B. N. Desai, J. J. Zhu, *Nat. Neurosci.* **2020**, *23*, 1047–1050; c) F.-X. Xu, L. Zhou, X.-T. Wang, F. Jia, K.-Y. Ma, N. Wang, L. Lin, F.-Q. Xu, Y. Shen, *Nat. Neurosci.* **2020**, *23*, 1041–1043.

[10] M. Meister, *eLife* **2016**, *5*, e17210.

[11] J. Heureaux, D. Chen, V. L. Murray, C. X. Deng, A. P. Liu, *Cell. Mol. Bioeng.* **2014**, *7*, 307–319.

[12] S. Ibsen, A. Tong, C. Schutt, S. Esener, S. H. Chalasani, *Nat. Commun.* **2015**, *6*, 8264.

[13] Y. Pan, S. Yoon, L. Zhu, Y. Wang, *Curr. Opin. Biomed. Eng.* **2018**, *7*, 64–70.

[14] M. Provansal, K. Marazova, J. A. Sahel, S. Picaud, *Transl. Vis. Sci. Technol.* **2022**, *11*, 18–18.

[15] C. Rabut, S. Yoo, R. C. Hurt, Z. Jin, H. Li, H. Guo, B. Ling, M. G. Shapiro, *Neuron* **2020**, *108*, 93–110.

[16] Y. Hu, W. Zhong, J. M. Wan, C. Alfred, *Ultrasound Med. Biol.* **2013**, *39*, 915–925.

[17] a) Z. Lin, L. Meng, J. Zou, W. Zhou, X. Huang, S. Xue, T. Bian, T. Yuan, L. Niu, Y. Guo, *Theranostics* **2020**, *10*, 5514–5526; b) Z. Lin, T. Bian, W. Zhou, Y. Wang, X. Huang, J. Zou, H. Zhou, L. Niu, J. Tang, L. Meng, *IEEE. Trans. Ultrason. Ferroelectr. Freq. Control.* **2021**, *68*, 1752–1761.

[18] a) Q. Xian, Z. Qiu, S. Kala, K. F. Wong, J. Guo, L. Sun, *IEEE. Trans. Ultrason. Ferroelectr. Freq. Control.* **2021**, *68*, 2150–2154; b) W. Zhou, J. Wang, K. Wang, B. Huang, L. Niu, F. Li, F. Cai, Y. Chen, X. Liu, X. Zhang, *Lab Chip* **2017**, *17*, 1725–1731; c) J. Kubanek, P. Shukla, A. Das, S. A. Baccus, M. B. Goodman, *J. Neurosci.* **2018**, *38*, 3081–3091.

[19] a) Y. Tufail, A. Matyushov, N. Baldwin, M. L. Tauchmann, J. Georges, A. Yoshihiro, S. I. H. Tillery, W. J. Tyler, *Neuron* **2010**, *66*, 681–694; b) H. Zhou, L. Meng, X. Xia, Z. Lin, W. Zhou, N. Pang, T. Bian, T. Yuan, L. Niu, H. Zheng, *IEEE Trans. Biomed. Eng.* **2021**, *68*, 3375–3387; c) H. Guo, H. Salahshoor, D. Wu, S. Yoo, T. Sato, D. Y. Tsao, M. G. Shapiro, *iScience* **2023**, *26*, 108372; d) T. Sato, M. G. Shapiro, D. Y. Tsao, *Neuron* **2018**, *98*, 1031–1041.

[20] J. Kubanek, J. Brown, P. Ye, K. B. Pauly, T. Moore, W. Newsome, *Sci. Adv.* **2020**, *6*, eaaz4193.

[21] W. Legon, T. F. Sato, A. Opitz, J. Mueller, A. Barbour, A. Williams, W. J. Tyler, *Nat. Neurosci.* **2014**, *17*, 322–329.

[22] D. Maresca, A. Lakshmanan, M. Abedi, A. Bar-Zion, A. Farhadi, G. J. Lu, J. O. Szablowski, D. Wu, S. Yoo, M. G. Shapiro, *Annu. Rev. Chem. Biomol. Eng.* **2018**, *9*, 229–252.

[23] C. Song, H. Park, C. K. Lee, R. Griffin, *Int. J. Hyperthermia* **2005**, *21*, 761–767.

[24] Y. Meng, K. Hynynen, N. Lipsman, *Nat. Rev. Neurol.* **2021**, *17*, 7–22.

[25] S. Mura, J. Nicolas, P. Couvreur, *Nat. Mater.* **2013**, *12*, 991–1003.

[26] a) J. Deprez, G. Lajoinie, Y. Engelen, S. De Smedt, I. Lentacker, *Adv. Drug Delivery Rev.* **2021**, *172*, 9–36; b) S. S. Low, C. N. Lim, M. Yew, W. S. Chai, L. E. Low, S. Manickam, B. T. Tey, P. L. Show, *Ultrason. Sonochem.* **2021**, *80*, 105805.

[27] S. Roovers, T. Segers, G. Lajoinie, J. Deprez, M. Versluis, S. C. De Smedt, I. Lentacker, *Langmuir* **2019**, *35*, 10173–10191.

[28] a) S. Hernot, A. L. Klibanov, *Adv. Drug Delivery Rev.* **2008**, *60*, 1153–1166; b) Y. Pan, S. Yoon, J. Sun, Z. Huang, C. Lee, M. Allen, Y. Wu, Y.-J. Chang, M. Sadelain, K. K. Shung, *Proc. Natl. Acad. Sci. USA* **2018**, *115*, 992–997.

[29] J. Li, C. Nagamani, J. S. Moore, *Acc. Chem. Res.* **2015**, *48*, 2181–2190.

[30] M. Zou, P. Zhao, J. Fan, R. Göstl, A. Herrmann, *J. Polym. Sci.* **2022**, *60*, 1864–1870.

[31] a) S. Huo, P. Zhao, Z. Shi, M. Zou, X. Yang, E. Warszawik, M. Loznik, R. Göstl, A. Herrmann, *Nat. Chem.* **2021**, *13*, 131–139; b) S. Huo, Y. Zhou, Z. Liao, P. Zhao, M. Zou, R. Göstl, A. Herrmann, *Chem. Commun.* **2021**, *57*, 7438–7440.

[32] a) M. Zou, P. Zhao, S. Huo, R. Göstl, A. Herrmann, *ACS Macro Lett.* **2022**, *11*, 15–19; b) Z. Shi, Q. Song, R. Göstl, A. Herrmann, *Chem. Sci.* **2021**, *12*, 1668–1674; c) Y. Yao, M. E. McFadden, S. M. Luo, R. W. Barber, E. Kang, A. Bar-Zion, C. A. Smith, Z. Jin, M. Legendre, B. Ling, *Proc. Natl. Acad. Sci. USA* **2023**, *120*, e2309822120.

[33] a) E. Stride, C. Coussios, *Nat. Rev. Phys.* **2019**, *1*, 495–509; b) D. Yildiz, R. Göstl, A. Herrmann, *Chem. Sci.* **2022**, *13*, 13708–13719.

[34] a) B. Sorum, R. A. Rietmeijer, K. Gopakumar, H. Adesnik, S. G. Brohawn, *Proc. Natl. Acad. Sci. USA* **2021**, *118*, e2006980118; b) J. Ye, S. Tang, L. Meng, X. Li, X. Wen, S. Chen, L. Niu, X. Li, W. Qiu, H. Hu, *Nano Lett.* **2018**, *18*, 4148–4155; c) Y.-S. Huang, C.-H. Fan, N. Hsu, N.-H. Chiu, C.-Y. Wu, C.-Y. Chang, B.-H. Wu, S.-R. Hong, Y.-C. Chang, A. Yan-Tang Wu, *Nano Lett.* **2020**, *20*, 1089–1100; d) C.-Y. Wu, C.-H. Fan, N.-H. Chiu, Y.-J. Ho, Y.-C. Lin, C.-K. Yeh, *Theranostics* **2020**, *10*, 3546–3561; e) M. Duque, C. A. Lee-Kubli, Y. Tufail, U. Magaram, J. Patel, A. Chakraborty, J. Mendoza Lopez, E. Edsinger, A. Vasan, R. Shiao, C. Weiss, J. Friend, S. H. Chalasani, *Nat. Commun.* **2022**, *13*, 600; f) S. Yoo, D. R. Mittelstein, R. C. Hurt, J. Lacroix, M. G. Shapiro, *Nat. Commun.* **2022**, *13*, 493.

[35] B. Martinac, *J. Cell Sci.* **2004**, *117*, 2449–2460.

[36] P. Jin, L. Y. Jan, Y.-N. Jan, *Annu. Rev. Neurosci.* **2020**, *43*, 207–229.

[37] T. Zhang, N. Pan, Y. Wang, C. Liu, S. Hu, *Front. Hum. Neurosci.* **2021**, *15*, 749162.

[38] a) M. Chalfie, *Nat. Rev. Mol. Cell Biol.* **2009**, *10*, 44–52; b) J. Kefauver, A. Ward, A. Patapoutian, *Nature* **2020**, *587*, 567–576.

[39] a) M. L. Prieto, K. Firouzi, B. T. Khuri-Yakub, M. Maduke, *Ultrasound Med. Biol.* **2018**, *44*, 1217–1232; b) Z. Qiu, J. Guo, S. Kala, J. Zhu, Q. Xian, W. Qiu, G. Li, T. Zhu, L. Meng, R. Zhang, H. C. Chan, H. Zheng, L. Sun, *iScience* **2019**, *21*, 448–457.

[40] a) Z. Qiu, S. Kala, J. Guo, Q. Xian, J. Zhu, T. Zhu, X. Hou, K. F. Wong, M. Yang, H. Wang, L. Sun, *Cell Rep.* **2020**, *32*, 108033; b) T. Wang, H. Wang, G. Pang, T. He, P. Yu, G. Cheng, Y. Zhang, J. Chang, *ACS Appl. Mater. Interfaces* **2020**, *12*, 56692–56700.

[41] J. Kubanek, J. Shi, J. Marsh, D. Chen, C. Deng, J. Cui, *Sci. Rep.* **2016**, *6*, 24170.

[42] S. R. Burks, R. M. Lorsung, M. E. Nagle, T. W. Tu, J. A. Frank, *Theranostics* **2019**, *9*, 5517–5531.

[43] W. Zhou, X. Wang, K. Wang, U. Farooq, L. Kang, L. Niu, L. Meng, *IEEE. Trans. Ultrason. Ferroelectr. Freq. Control.* **2021**, *69*, 473–479.
[44] a) Z. Lin, W. Zhou, X. Huang, K. Wang, J. Tang, L. Niu, L. Meng, H. Zheng, *Adv. Biosyst.* **2018**, *2*, 1800041; b) Z. Lin, X. Huang, W. Zhou, W. Zhang, Y. Liu, T. Bian, L. Niu, L. Meng, Y. Guo, *Front. Pharmacol.* **2019**, *10*, 544.
[45] U. Magaram, C. Weiss, A. Vasan, K. C. Reddy, J. Friend, S. H. Chalasani, *PLoS One* **2022**, *17*, e0267698.
[46] a) S. L. Grage, A. M. Keleshian, T. Turdzeladze, A. R. Battle, W. C. Tay, R. P. May, S. A. Holt, S. A. Contera, M. Haertlein, M. Moulin, *Biophys. J.* **2011**, *100*, 1252–1260; b) A. Anishkin, S. Sukharev, J. M. Vanegas, *Biophys. J.* **2021**, *120*, 232–242.
[47] a) S. Ma, S. Cahalan, G. LaMonte, N. D. Grubaugh, W. Zeng, S. E. Murthy, E. Paytas, R. Gamini, V. Lukacs, T. Whitwam, *Cell* **2018**, *173*, 443–455; b) C. N. Nguetse, N. Purington, E. R. Ebel, B. Shakya, M. Tetard, P. G. Kremsner, T. P. Velavan, E. S. Egan, *Proc. Natl. Acad. Sci. USA* **2020**, *117*, 9074–9081.
[48] H. Yan, G. Helman, S. E. Murthy, H. Ji, J. Crawford, T. Kubisiak, S. J. Bent, J. Xiao, R. J. Taft, A. Coombs, *Am. J. Hum. Genet.* **2019**, *105*, 996–1004.
[49] J. Lim, H.-H. Tai, W.-H. Liao, Y.-C. Chu, C.-M. Hao, Y.-C. Huang, C.-H. Lee, S.-S. Lin, S. Hsu, Y.-C. Chien, *eLife* **2021**, *10*, e61660.
[50] Y.-C. Chu, J. Lim, C.-H. Lai, M.-C. Tseng, Y.-S. Chu, J.-L. Wang, *Ultrasound Med. Biol.* **2021**, *47*, 1775–1784.
[51] a) E. M. Knavel, C. L. Brace, *Tech. Vasc. Interv. Radiol.* **2013**, *16*, 192–200; b) Y.-F. Zhou, *World J. Clin. Oncol.* **2011**, *2*, 8–27.
[52] K. Funaki, H. Fukunishi, T. Funaki, K. Sawada, Y. Kaji, T. Maruo, *Am. J. Obstet. Gynecol.* **2007**, *196*, 184, e1.
[53] a) E. Martin, D. Jeanmonod, A. Morel, E. Zadicario, B. Werner, *Ann. Neurol.* **2009**, *66*, 858–861; b) S. A. Quadri, M. Waqas, I. Khan, M. A. Khan, S. S. Suriya, M. Farooqui, B. Fiani, *Neurosurg. Focus* **2018**, *44*, E16.
[54] S. Yoshizawa, T. Ikeda, A. Ito, R. Ota, S. Takagi, Y. Matsumoto, *Med. Biol. Eng. Comput.* **2009**, *47*, 851–860.
[55] a) R. Deckers, B. Quesson, J. Arsaut, S. Eimer, F. Couillaud, C. T. Moonen, *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 1175–1180; b) E. Guilhon, B. Quesson, F. Moraud-Gaudry, H. de Verneuil, P. Canioni, R. Salomir, P. Voisin, C. T. Moonen, *Mol. Imaging* **2003**, *2*, 11–17; c) D. P. Madio, P. van Gelderen, D. DesPres, A. W. Olson, J. A. de Zwart, T. W. Fawcett, N. J. Holbrook, M. Mandel, C. T. Moonen, *J. Magn. Reson. Imaging* **1998**, *8*, 101–104; d) R. C. Smith, M. Machluf, P. Bromley, A. Atala, K. Walsh, *Hum. Gene Ther.* **2002**, *13*, 697–706; e) S. Wang, V. Zderic, V. Frenkel, *Future Oncol.* **2010**, *6*, 1497–1511.
[56] a) E. Guilhon, P. Voisin, J. De Zwart, B. Quesson, R. Salomir, C. Maurange, V. Bouchaud, P. Smirnov, H. De Verneuil, A. Vekris, *J. Gene Med.* **2003**, *5*, 333–342; b) D. E. Kruse, M. A. Mackanos, C. E. O'Connell-Rodwell, C. H. Contag, K. W. Ferrara, *Phys. Med. Biol.* **2008**, *53*, 3641–3660.
[57] D. I. Piraner, M. H. Abedi, B. A. Moser, A. Lee-Gosselin, M. G. Shapiro, *Nat. Chem. Biol.* **2017**, *13*, 75–80.
[58] a) M. H. Abedi, M. S. Yao, D. R. Mittelstein, A. Bar-Zion, M. B. Swift, A. Lee-Gosselin, P. Barturen-Larrea, M. T. Buss, M. G. Shapiro, *Nat. Commun.* **2022**, *13*, 1585; b) Y. Chen, M. Du, Z. Yuan, Z. Chen, F. Yan, *Nat. Commun.* **2022**, *13*, 4468.
[59] Y. Wu, Y. Liu, Z. Huang, X. Wang, Z. Jin, J. Li, P. Limsakul, L. Zhu, M. Allen, Y. Pan, R. Bussell, A. Jacobson, T. Liu, S. Chien, Y. Wang, *Nat. Biomed. Eng.* **2021**, *5*, 1336–1347.
[60] Y. Yang, C. P. Pacia, D. Ye, L. Zhu, H. Baek, Y. Yue, J. Yuan, M. J. Miller, J. Cui, J. P. Culver, *Brain Stimul.* **2021**, *14*, 790–800.
[61] X. Shen, Z. Song, E. Xu, J. Zhou, F. Yan, *Ultrason. Sonochem.* **2021**, *73*, 105494.
[62] T. He, H. Wang, T. Wang, G. Pang, Y. Zhang, C. Zhang, P. Yu, J. Chang, *J. Chem. Eng.* **2021**, *407*, 127173.
[63] S. Cadoni, C. Demené, I. Alcala, M. Provansal, D. Nguyen, D. Nelidova, G. Labernède, J. Lubetzki, R. Goulet, E. Burban, J. Dégardin, M. Simonutti, G. Gauvain, F. Arcizet, O. Marre, D. Dalkara, B. Roska, J. A. Sahel, M. Tanter, S. Picaud, *Nat. Nanotechnol.* **2023**, *18*, 667–676.
[64] H. Zhao, S. Xue, M.-D. Hussherr, P. Buchmann, A. P. Teixeira, M. Fussenegger, *Lancet Diabetes Endocrinol.* **2023**, *11*, 637–640.
[65] C. H. Fan, K. C. Wei, N. H. Chiu, E. C. Liao, H. C. Wang, R. Y. Wu, Y. J. Ho, H. L. Chan, T. A. Wang, Y. Z. Huang, T. H. Hsieh, C. H. Lin, Y. C. Lin, C. K. Yeh, *Nano Lett.* **2021**, *21*, 5967–5976.
[66] D. Liao, F. Li, D. Lu, P. Zhong, *Biochem. Biophys. Res. Commun.* **2019**, *518*, 541–547.
[67] G. Zhang, X. Li, L. Wu, Y.-X. Qin, *Bone Res.* **2021**, *9*, 16.
[68] J. Zhu, Q. Xian, X. Hou, K. F. Wong, T. Zhu, Z. Chen, D. He, S. Kala, S. Murugappan, J. Jing, *Proc. Natl. Acad. Sci. USA* **2023**, *120*, e2300291120.
[69] S.-J. Oh, J. M. Lee, H.-B. Kim, J. Lee, S. Han, J. Y. Bae, G.-S. Hong, W. Koh, J. Kwon, E.-S. Hwang, *Curr. Biol.* **2019**, *29*, 3386–3401.
[70] R. T. O'Neill, R. Boulatov, *Nat. Chem. Rev.* **2021**, *5*, 148–167.
[71] A. L. Black, J. M. Lenhardt, S. L. Craig, *J. Mater. Chem.* **2011**, *21*, 1655–1663.
[72] a) E. Ducrot, Y. Chen, M. Bulters, R. P. Sijbesma, C. Creton, *Science* **2014**, *344*, 186–189; b) A. L. B. Ramirez, Z. S. Kean, J. A. Orlicki, M. Champhekar, S. M. Elsakr, W. E. Krause, S. L. Craig, *Nat. Chem.* **2013**, *5*, 757–761.
[73] a) Y. Liu, S. Holm, J. Meisner, Y. Jia, Q. Wu, T. J. Woods, T. J. Martinez, J. S. Moore, *Science* **2021**, *373*, 208–212; b) J. Wang, T. B. Kouznetsova, Z. Niu, M. T. Ong, H. M. Klukovich, A. L. Rheingold, T. J. Martinez, S. L. Craig, *Nat. Chem.* **2015**, *7*, 323–327.
[74] a) Y. Chen, G. Mellot, D. van Luijk, C. Creton, R. P. Sijbesma, *Chem. Soc. Rev.* **2021**, *50*, 4100–4140; b) G. De Bo, *Macromolecules* **2020**, *53*, 7615–7617.
[75] A. P. Haehnel, Y. Sagara, Y. C. Simon, C. Weder, *Polym. Mech.* **2015**, *369*, 345–375.
[76] G. Cravotto, E. C. Gaudino, P. Cintas, *Chem. Soc. Rev.* **2013**, *42*, 7521–7534.
[77] P. A. May, N. F. Munaretto, M. B. Hamoy, M. J. Robb, J. S. Moore, *ACS Macro Lett.* **2016**, *5*, 177–180.
[78] A. Kolbe, L. L. del Mercato, A. Z. Abbasi, P. Rivera Gil, S. J. Gorzini, W. H. Huibers, B. Poolman, W. J. Parak, A. Herrmann, *Macromol. Rapid Commun.* **2011**, *32*, 186–190.
[79] D. Pesce, Y. Wu, A. Kolbe, T. Weil, A. Herrmann, *Biomaterials* **2013**, *34*, 4360–4367.
[80] Y. Zhou, S. Huo, M. Loznik, R. Göstl, A. J. Boersma, A. Herrmann, *Angew. Chem. Int. Ed.* **2021**, *60*, 1493–1497.
[81] P. Zhao, S. Huo, J. Fan, J. Chen, F. Kiessling, A. J. Boersma, R. Göstl, A. Herrmann, *Angew. Chem. Int. Ed.* **2021**, *60*, 14707–14714.
[82] H.-D. Liang, J. A. Noble, P. N. Wells, *Interface Focus* **2011**, *1*, 475–476.
[83] V. Chan, A. Perlas, *Atlas of Ultrasound-Guided Procedures in Interventional Pain Management* **2011**, Springer, Cham.
[84] F. S. Foster, C. J. Pavlin, K. A. Harasiewicz, D. A. Christopher, D. H. Turnbull, *Ultrasound Med. Biol.* **2000**, *26*, 1–27.
[85] V. Paefgen, D. Doleschel, F. Kiessling, *Front. Pharmacol.* **2015**, *6*, 197.
[86] H. Yusefi, B. Helfield, *Front. Phys.* **2022**, *10*, 791145.
[87] M. G. Shapiro, P. W. Goodwill, A. Neogy, M. Yin, F. S. Foster, D. V. Schaffer, S. M. Conolly, *Nat. Nanotechnol.* **2014**, *9*, 311–316.

[88] P. Dutka, D. Malounda, L. A. Metskas, S. Chen, R. C. Hurt, G. J. Lu, G. J. Jensen, M. G. Shapiro, *Protein Sci.* **2021**, *30*, 1081–1086.
[89] a) P. Dutka, L. A. Metskas, R. C. Hurt, H. Salahshoor, T.-Y. Wang, D. Malounda, G. J. Lu, T.-F. Chou, M. G. Shapiro, G. J. Jensen, *Structure* **2023**, *31*, 518–528; b) S. T. Huber, D. Terwiel, W. H. Evers, D. Maresca, A. J. Jakobi, *Cell* **2023**, *186*, 975–986.
[90] A. E. Walsby, *Microbiol. Rev.* **1994**, *58*, 94–144.
[91] a) A. Lakshmanan, A. Farhadi, S. P. Nety, A. Lee-Gosselin, R. W. Bourdeau, D. Maresca, M. G. Shapiro, *ACS Nano* **2016**, *10*, 7314–7322; b) A. Lakshmanan, G. J. Lu, A. Farhadi, S. P. Nety, M. Kunth, A. Lee-Gosselin, D. Maresca, R. W. Bourdeau, M. Yin, J. Yan, C. Witte, D. Malounda, F. S. Foster, L. Schroder, M. G. Shapiro, *Nat. Protoc.* **2017**, *12*, 2050–2080; c) E. Cherin, J. M. Melis, R. W. Bourdeau, M. Yin, D. M. Kochmann, F. S. Foster, M. G. Shapiro, *Ultrasound Med. Biol.* **2017**, *43*, 1016–1030; d) D. Maresca, A. Lakshmanan, A. Lee-Gosselin, J. M. Melis, Y.-L. Ni, R. W. Bourdeau, D. M. Kochmann, M. G. Shapiro, *Appl. Phys. Lett.* **2017**, *110*, 073704.
[92] J. Yan, M. Yin, F. S. Foster, C. E. Démoré, *Ultrasound Med. Biol.* **2020**, *46*, 359–368.
[93] D. Maresca, T. Payen, A. Lee-Gosselin, B. Ling, D. Malounda, C. Demené, M. Tanter, M. G. Shapiro, *NeuroImage* **2020**, *209*, 116467.
[94] a) G. Wang, L. Song, X. Hou, S. Kala, K. F. Wong, L. Tang, Y. Dai, L. Sun, *Biomaterials* **2020**, *236*, 119803; b) B. Ling, J. H. Ko, B. Stordy, Y. Zhang, T. F. Didden, D. Malounda, M. B. Swift, W. C. Chan, M. G. Shapiro, *Nano Lett.* **2023**, *23*, 10748–10757.
[95] A. Lakshmanan, Z. Jin, S. P. Nety, D. P. Sawyer, A. Lee-Gosselin, D. Malounda, M. B. Swift, D. Maresca, M. G. Shapiro, *Nat. Chem. Biol.* **2020**, *16*, 988–996.
[96] R. W. Bourdeau, A. Lee-Gosselin, A. Lakshmanan, A. Farhadi, S. R. Kumar, S. P. Nety, M. G. Shapiro, *Nature* **2018**, *553*, 86–90.
[97] A. Farhadi, G. H. Ho, D. P. Sawyer, R. W. Bourdeau, M. G. Shapiro, *Science* **2019**, *365*, 1469–1475.
[98] D. P. Sawyer, A. Bar-Zion, A. Farhadi, S. Shivaei, B. Ling, A. Lee-Gosselin, M. G. Shapiro, *Nat. Methods* **2021**, *18*, 945–952.
[99] R. C. Hurt, M. T. Buss, M. Duan, K. Wong, M. Y. You, D. P. Sawyer, M. B. Swift, P. Dutka, P. Barturen-Larrea, D. R. Mittelstein, Z. Jin, M. H. Abedi, A. Farhadi, R. Deshpande, M. G. Shapiro, *Nat. Biotechnol.* **2023**, *41*, 919–931.
[100] A. Bar-Zion, A. Nourmahnad, D. R. Mittelstein, S. Shivaei, S. Yoo, M. T. Buss, R. C. Hurt, D. Malounda, M. H. Abedi, A. Lee-Gosselin, *Nat. Nanotechnol.* **2021**, *16*, 1403–1412.
[101] D. Wu, D. Baresch, C. Cook, Z. Ma, M. Duan, D. Malounda, D. Maresca, M. P. Abundo, J. Lee, S. Shivaei, D. R. Mittelstein, T. Qiu, P. Fischer, M. G. Shapiro, *Sci. Adv.* **2023**, *9*, eadd9186.
[102] Y.-C. Chu, J. Lim, A. Chien, C.-C. Chen, J.-L. Wang, *Ultrasound Med. Biol.* **2022**, *48*, 1981–1994.
[103] J. Geng, W. Liu, H. Zhou, T. Zhang, L. Wang, M. Zhang, Y. Li, B. Shen, X. Li, B. Xiao, *Neuron* **2020**, *106*, 438–451.
[104] D. P. Darrow, P. O'Brien, T. J. Richner, T. I. Netoff, E. S. Ebbini, *Brain Stimul.* **2019**, *12*, 1439–1447.
[105] H. Guo, M. Hamilton, S. J. Offutt, C. D. Gloeckner, T. Li, Y. Kim, W. Legon, J. K. Alford, H. H. Lim, *Neuron* **2018**, *98*, 1020–1030.
[106] V. Braun, J. Blackmore, R. O. Cleveland, C. R. Butler, *Brain Stimul.* **2020**, *13*, 1527–1534.
[107] a) J. T. Bulcha, Y. Wang, H. Ma, P. W. Tai, G. Gao, *Signal Transduction Targeted Ther.* **2021**, *6*, 53; b) J. R. Mendell, S. A. Al-Zaidy, L. R. Rodino-Klapac, K. Goodspeed, S. J. Gray, C. N. Kay, S. L. Boye, S. E. Boye, L. A. George, S. Salabarria, *Mol. Ther.* **2021**, *29*, 464–488.
[108] M. Xuan, J. Fan, V. N. Khiêm, M. Zou, K. O. Brenske, A. Mourran, R. Vinokur, L. Zheng, M. Itskov, R. Göstl, A. Herrmann, *Adv. Mater.* **2023**, *14*, 11941–11954.
[109] J. K. Willmann, Z. Cheng, C. Davis, A. M. Lutz, M. L. Schipper, C. H. Nielsen, S. S. Gambhir, *Radiology* **2008**, *249*, 212–219.

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