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Thermogenetic tools for remote control of cellular functions

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Abstract

Precise spatial and temporal control of cellular functions is essential for investigating complex biological processes in living cells and organisms. Thermogenetics combines temperature-responsive molecular tools with externally delivered heat to remotely control cellular activities. Recent advances have expanded thermogenetic toolbox from thermosensitive ion channels and heat-responsive transcription to direct regulation of protein function. In parallel, emerging heating technologies enable precise thermal control across different tissue depths. Here, we review the molecular principles, engineering strategies, and heating modalities underlying thermogenetic control, and discuss key challenges in tool development and future applications. Integrating targeted delivery, engineered thermoswitches, and precise thermal control could establish thermogenetics for determining which cells respond, and where and when their functions are activated.

1. Introduction

Thermogenetics enables remote control of cellular functions by coupling temperature-responsive molecular tools with externally delivered heat. Temperature is a fundamental parameter that influences numerous biological processes, including protein conformation, molecular interactions, and biochemical reactions. By harnessing these temperature-dependent properties, thermogenetics converts thermal stimuli into defined biological responses. Although temperature changes can broadly affect cellular physiology, engineered thermogenetic systems aim to selectively regulate biological activities within a defined temperature for activation, providing opportunities to investigate cellular functions with spatial and temporal precision.

The molecular toolbox of thermogenetics has expanded considerably over the past decade. Early approaches exploited thermosensitive ion channels, such as transient receptor potential vanilloid 1 (TRPV1) channel, to regulate cation influx and neuronal excitability, or endogenous heat-shock pathways to control gene expression.[1] Advances in synthetic biology subsequently introduced engineered temperature-controlled gene switches that function independently of endogenous heat-shock transcriptional pathways.[2] More recently, protein engineering has enabled direct thermal control of protein function through changes in molecular assembly,[3] conformation,[4] and phase separation.[5] Together, these developments have broadened the scope of thermogenetics beyond ion-channel activation and gene expression to include cellular signaling, genome regulation, and programmed cell death.

A feature of thermogenetics is that the molecular thermoswitch can be activated using different heating modalities. Localized temperature changes can be generated through photothermal approaches, focused ultrasound, or magnetic field,[6] allowing the heating strategy to be tailored to tissue depth and experimental requirements. This modularity provides a framework in which heating determines where and when activation occurs, whereas engineered thermoswitch determines how cells respond.

In this review, we discuss the molecular principles and engineering strategies underlying thermogenetic tools, examine heating technologies for localized thermal stimulation, and highlight representative applications in cellular and animal models. We further discuss key engineering challenges and future directions for advancing thermogenetics as a versatile platform for remote control of cellular functions. For broader discussions of thermogenetic principles and applications, we refer readers to previously published comprehensive reviews.[2,7,8]

2. Molecular tools for thermogenetic control

2.1. Thermosensitive ion channels

Thermosensitive ion channels were among the first genetically encoded actuators for converting heat into cellular activity. The best-established example is the TRPV1 channel, a non-selective cation channel activated by heat and other stimuli[1] (Fig. 1A). Local heating opens TRPV1 and induces cation influx, including calcium ions (Ca^{2+}), thereby coupling temperature changes to membrane depolarization and downstream Ca^{2+}-dependent signaling. This principle has enabled remote activation of endogenous TRPV1-expressing cells using infrared light,[9,10] radiofrequency,[11] or alternating magnetic fields[12,13] in combination with magnetic nanoparticles for neuromodulation in freely behaving animals. Beyond neuronal activation, TRPV1-mediated Ca^{2+} influx has been employed to synthetic Ca^{2+}-responsive transcriptional circuit to remotely regulate insulin production and glucose homeostasis in mice.[11]

Thermosensitive channels provide rapid responses and substantial signal amplification through ion flux. However, their outputs are inherently linked to membrane excitability and intracellular Ca^{2+} signaling, restricting the range of cellular functions that can be directly regulated. In addition, the relatively high thermal activation threshold of human TRPV1 (approximately 43°C)[14] requires heating to temperatures that may induce cellular stress, thereby narrowing the window between effective channel activation and heat-induced damage. These limitations have motivated the development of thermogenetic systems that regulate gene expression or protein function without relying on ion-channel activation.

2.2. Endogenous heat-responsive transcriptional systems

The cellular heat-shock response provides a broadly applicable mechanism for converting temperature changes into gene expression (Fig. 1B). Upon heating, heat-shock factor 1 (HSF1) is activated and binds to heat-shock elements (HSEs), inducing transcription from heat-responsive promoters. Gene of interests (GOIs) placed under the control of these promoters can be regulated by heating. As an example, engineered promoters derived from the human heat shock protein family A member 6 (*HSPA6)* locus exhibited low basal activity at 37°C and strong transcriptional induction at 40–42°C.[15] Notably, the output genes can be exchanged without redesigning the temperature-sensing mechanism, providing a modular platform for controlling diverse cellular functions. In addition, heat-responsive transcriptional systems have been implemented in primary human T cells,[16] used to regulate CRISPR–dCas9-mediated

transcriptional activation and repression,[17] and combined with photothermal heating[18] or focused ultrasound[19] to achieve spatially controlled activation of engineered immune cells *in vivo*.

A major advantage of transcription-based thermogenetics is biological amplification: a transient thermal input can induce the production of many copies of a target protein. Different target genes can be regulated by exchanging the GOI without redesigning the temperature-sensing mechanism. However, because the systems require de novo synthesis of the effector protein through transcription and translation, a delay occurs between thermal stimulation and the functional response. Deactivation is also constrained by the lifetime of the induced mRNA and protein. Furthermore, heating also activates endogenous heat-shock pathways in addition to the engineered circuit.

2.3. Synthetic temperature-controlled gene switches

Engineered thermoresponsive proteins provide an alternative strategy for synthetic temperature-controlled gene expression without relying on endogenous heat-shock pathways (Fig. 1C). In these systems, temperature-induced conformational changes in engineered proteins are coupled to synthetic transcriptional machinery, enabling thermal stimuli to be translated into regulated gene expression. This approach allows the temperature-sensing component to be engineered independently of endogenous heat-shock transcriptional systems and provides opportunities to tune the activation temperature.

An example is the temperature-inducible gene regulation (TIGR) system, in which the bacterial thermosensor RheA was converted into a synthetic transcription factor to control gene expression in vertebrate cells in response to temperature changes.[20] Recently, the human enhanced gene activation thermometer (HEAT) was developed for heat-inducible gene expression within the physiologically relevant temperature range.[21] HEAT used an engineered variant of the bacterial temperature-sensitive protein TlpA coupled to a synthetic transcriptional activator. Temperature-dependent conformational changes in TlpA regulated transcriptional activity, enabling gene expression within a narrow temperature range of 37–40°C.[21] The system was demonstrated across several mammalian cell lines and was further used to control insulin production from implanted designer cells in diabetic mice.[21] TlpA has also been incorporated into split T7 RNA polymerases to generate Thermal-T7RNAPs,[22] in which temperature-dependent protein interactions directly regulate polymerase activity. Engineered variants enabled reversible and tunable control of gene expression between 30 and 42°C.[22]

Unlike endogenous heat-shock promoter-based systems, synthetic temperature-controlled gene switches can regulate gene expression without relying on endogenous heat-shock transcriptional pathways. Their thermal response can also be tuned through protein engineering, providing flexibility in defining the activation temperature and reducing dependence on cell-specific heat-shock responses. However, the functional output still depends on de novo synthesis of the effector protein, and the delay between thermal stimulation and the resulting response remains.

2.4. Direct thermogenetic control of protein function

Beyond transcriptional regulation, temperature can directly control protein function (Fig. 1D). In these systems, temperature-induced changes in protein assembly, localization, conformation, or phase behavior directly alter the activity of an effector protein. Recent studies have demonstrated distinct strategies for direct protein-level control. The membrane localization using temperature (Melt) platform exploited the intrinsic thermosensitivity of *Botrytis cinerea* LOV4 (BcLOV4), which oligomerized and translocated to the plasma membrane in response to decreasing temperature.[3] Engineered Melt variants with switching temperatures between 30 and 40°C enabled reversible regulation of diverse cellular functions, including signaling, protein localization, proteolysis, and cell death.[3] Similarly, engineered *Avena sativa* LOV2 (AsLOV2) domains have been inserted directly into target proteins to generate thermoresponsive allosteric switches.[4] Temperature-induced conformational changes in AsLOV2 directly modulated effector activity within a narrow range of 37–41°C for thermal control of diverse proteins, including CRISPR–Cas genome editors in mammalian cells.[4] Elastin-like polypeptides (ELPs) provide a complementary strategy based on heat-induced phase separation. ELPs consist of repetitive pentapeptide sequences $(VPGXG)_n$, where X represents any amino acid except proline, and n denotes the number of repeats. ELPs undergo reversible phase separation above their transition temperature (T_t), which can be tuned by modifying X or n,[23] enabling thermal regulation across a broad temperature range. For example, fusion of the ELP[V60] ($[VPGVG]_{60}$) to the catalytic domain of caspase-8 promoted clustering above T_t and activated programmed cell death in mammalian cells.[5]

Unlike transcription-based systems, direct protein-level thermogenetics can provide rapid and reversible regulation of protein activity because the effector proteins are already expressed in cells. However, the performance of these systems depends on the molecular properties of individual thermoswitches, while efficient delivery and potential immunogenicity remain challenges for *in vivo* applications.

3. Delivering heat to thermogenetic systems

3.1. Photothermal heating

Photothermal agents convert absorbed light into heat, providing localized thermal stimulation with high spatial and temporal precision (Fig. 2A). Near-infrared (NIR) wavelengths are commonly used because they penetrate biological tissues more effectively than visible light. Photothermal materials, including chemical dyes,[24,25] gold nanostructures,[26,27] silver-iron oxide[28,29] and other nanoparticles,[30,31] can further enhance local light-to-heat conversion, allowing localized temperature increases while minimizing heating of surrounding tissues. For deeper tissue applications, interstitial optical fibers or fiber-optic diffusers delivered through catheters or endoscopes can provide localized NIR illumination.[32,33] Similar light-delivery strategies have been explored in clinical studies of NIR photoimmunotherapy (NIR-PIT),[34,35] demonstrating the feasibility of delivering NIR light to inaccessible lesions. Photothermal heating has been combined with heat-responsive gene circuits to achieve spatially confined gene expression and to control engineered immune cells *in vivo*.[15,18] For example, gold nanorods have been used to convert NIR

light into local hyperthermia and activate heat-inducible gene circuits in engineered T cells for spatial control of immunomodulatory proteins within tumors.[18]

3.2. Focused ultrasound

Focused ultrasound (FUS) provides a non-invasive approach for thermal control in deep tissues (Fig. 2B). Acoustic energy can be focused within a defined tissue volume and converted into heat through tissue absorption, enabling localized heating without requiring exogenous photothermal agents. FUS is already used clinically for thermal ablation,[36,37] providing an established technological foundation for thermogenetic applications. Unlike thermal ablation, which typically uses temperatures above ~ 50–60°C to induce irreversible tissue damage, thermogenetic stimulation requires only a modest and reversible temperature increase, typically to ~ 39–42°C. FUS has been used to induce localized chimeric antigen receptor (CAR) expression in engineered T cells for spatial control of CAR-T cell activity and enhanced tumor suppression in mice.[19] More recently, an integrated ultrasound-guided FUS system combined localized heating with real-time anatomical imaging and ultrasound-based temperature monitoring to activate thermal gene switches in engineered T cells *in vitro* and in tumor-bearing mice, while maintaining mild hyperthermia at 39–43°C.[38] When combined with magnetic resonance imaging (MRI), MRI-guided FUS can provide real-time temperature mapping and feedback control,[39,40] allowing precise regulation of thermal exposure to achieve effective thermogenetic activation while minimizing the risk of heat-induced tissue damage.

3.3. Magnetic heating

Magnetic heating, also known as magnetic hyperthermia, uses magnetic nanoparticles to convert energy from an alternating magnetic field (AMF) into heat (Fig. 2C). Because magnetic fields penetrate biological tissues with minimal attenuation, this approach allows thermal stimulation at depths that are difficult to access optically. Magnetic hyperthermia has been extensively investigated for cancer treatment (see review[41,42]) and has also been adapted for thermogenetic control. For example, AMF-induced heating of magnetic nanoparticles has been used to activate TRPV1-expressing neurons and achieved wireless deep-brain stimulation in freely moving animals.[12,13,43]

3.4. Radiofrequency heating

Radiofrequency (RF) electromagnetic fields provide another approach for remote thermogenetic control (Fig. 2D). The ability of RF fields to penetrate deep tissues enables remote heat generation through their interaction with RF-absorbing materials (e.g., iron oxide nanoparticles[11]) or direct energy deposition in tissues. For thermogenetic demonstration, RF exposure at 465 kHz, a relatively low frequency that minimizes direct tissue heating, was used to stimulate iron oxide nanoparticles targeted to TRPV1, inducing Ca^{2+} influx and activating a Ca^{2+}-responsive insulin expression circuit that reduced blood glucose in mice.[11] In another study, interventional RF hyperthermia was applied through needle electrodes to directly heat tumors carrying an HSP-promoter-controlled HSV-TK therapeutic gene.

Localized heating enhanced heat-induced gene expression and antitumor effects both *in vitro* and *in vivo*.[44]

No single heating modality is optimal for all thermogenetic applications. Photothermal approaches provide high spatial and temporal precision but are constrained by tissue penetration. FUS enables localized heating in deep tissues but can be affected by acoustic attenuation and tissue heterogeneity, complicating precise temperature control. Magnetic hyperthermia provides excellent tissue penetration but typically require exogenous magnetic nanoparticles, with spatial control determined largely by their distribution. RF-based approaches can also reach deep tissues but may require implanted electrodes or RF-responsive materials for localized heating. The modular design of thermogenetic systems allows molecular actuator and heating modalities to be selected according to specific experimental requirements.

4. Applications of thermogenetic control

4.1. Spatial control of gene expression

Temperature-controlled gene expression is among the most extensively developed applications of thermogenetics. By placing target genes under the control of temperature-responsive transcriptional circuits, gene expression can be induced selectively in heated cells or tissues. Coupling these circuits with localized thermal stimulation provides a strategy for spatially restricted protein production, allowing gene expression to be regulated at selected locations and times.

A demonstration example coupled nanoparticle-mediated heating to TRPV1 activation and a Ca^{2+}-responsive synthetic transcriptional circuit. RF heating of iron oxide nanoparticles induced TRPV1-mediated Ca^{2+} influx, which subsequently activated Ca^{2+}-dependent insulin expression system to regulate blood glucose in mice.[11] Thermal gene switches based on the endogenous heat-shock response were developed to provide more direct control of gene expression. An HSPA6-derived switch exhibited low basal activity and strong heat-induced transcription, and photothermal heating of implanted engineered cells enabled spatially localized gene expression *in vivo*.[15] Delivery of heat as repeated pulses improved cellular tolerance compared with continuous heating, highlighting the importance of thermal dose in designing thermogenetic stimulation protocols.[15] Engineered thermoresponsive proteins provide another route to temperature-controlled gene expression without relying directly on endogenous heat-shock transcription. The HEAT platform, which uses an engineered TlpA-derived thermosensor coupled to a synthetic transcription factor, enabled gene expression between 37 and 40°C.[21] Furthermore, microencapsulated designer cells carrying a HEAT-controlled insulin cassette were implanted subcutaneously in mice with experimental type-1 diabetes. Topical heating using an electrothermal patch induced insulin production, reduced blood glucose and restored normoglycemia (Fig. 3A).[21]

Although transcription-based thermogenetic systems can amplify a transient thermal stimulus by inducing the production of multiple copies of a target protein, this amplification comes at the expense of temporal precision because activation requires transcription and translation, whereas deactivation depends on the persistence of expressed proteins. Temperature-controlled transcription may therefore be suited to applications requiring sustained protein production, whereas direct protein-level thermogenetics offers an alternative for applications requiring faster responses.

4.2. Remote control of engineered immune cells

Engineered immune cells provide a compelling application for thermogenetics because their activity can be difficult to restrict spatially. CARs are engineered receptors that enable T cells to recognize specific antigens and initiate immune responses against target cells. Conventional CAR-T cells recognize their target antigen wherever they encounter it, potentially causing on-target, off-tumor toxicity when the antigen is also expressed by healthy tissues. Thermogenetic circuits provide an additional layer of spatial regulation by allowing immune-cell functions to be activated selectively at heated sites.

One strategy places the CAR expression under thermal control. Wu *et al.* combined a heat-responsive CAR expression cassette with magnetic resonance (MR)-guided FUS. Short FUS pulses generated localized mild heating at 43°C within tumors and induced CAR expression in engineered T cells. In mice bearing subcutaneous tumors, FUS activation enhanced tumor suppression while reducing on-target, off-tumor activity compared with constitutive CAR expression.[19] Importantly, CAR expression decreased after thermal stimulation was withdrawn and could be re-induced by repeated heating, demonstrating reversible control of CAR expression.[19]

A complementary strategy maintains constitutive CAR-mediated tumor recognition while placing additional immunomodulatory functions under thermal control. Miller *et al.* engineered primary human T cells with thermal gene switches activated by mild hyperthermia at 40–42°C.[18] Gold nanorods were used to convert NIR light into localized heat within tumors, inducing gene expression preferentially at the heated site. Thermal control of an interleukin-15 (IL-15) superagonist enhanced T-cell proliferation and antitumor activity, whereas thermal expression of a bispecific T-cell engager targeting NKG2D ligands expanded tumor-cell recognition and reduced antigen escape.[18] This study illustrates two distinct architectures: temperature can control whether engineered immune cells recognize their target, through regulation of CAR expression, or what additional functions they deploy, through regulation of cytokines or bispecific engagers. In principle, similar circuits could be employed to regulate proliferation, migration, or safety switches.

4.3. Thermally controlled genome regulation and editing

Genome regulation and editing represent another area in which external thermal control could improve spatial and temporal specificity. Because CRISPR-based systems can produce persistent or irreversible biological effects, restricting their activity to the intended tissue could provide an additional layer of safety beyond molecular targeting and delivery.

An initial strategy used temperature to control expression of the CRISPR machinery. Gamboa *et al.* combined an HSPA6-derived thermal switch with dCas9-based transcriptional activators (dCas9-VP64) and repressors (KRAB-dCas9).[17] A brief temperature increase of approximately 3–5°C above baseline temperature of 37°C induced expression of dCas9 complexes. The magnitude of transcriptional regulation could be adjusted through repeated thermal pulses, providing a means of controlling the output through thermal dose. Furthermore, localized NIR photothermal heating using gold nanorods enabled KRAB-dCas9-mediated repression of d2GFP expression in subcutaneously implanted engineered cells in mice (Fig. 3B).[17] However, temperature controlled the expression of the dCas9 machinery rather than directly regulating Cas protein activity. More recently, direct thermal regulation of CRISPR-associated proteins has been demonstrated. Kroell *et al.* engineered thermoresponsive allosteric proteins by inserting optimized AsLOV2 domains into *Spy*Cas9 or the anti-CRISPR protein AcrIIA5.[4] Temperature-dependent conformational changes of AsLOV2 allowed Cas9 activity to be regulated between 37 and 40°C. Thermally controlled Cas9 systems produced temperature-dependent genome editing at endogenous *EMX1*, *GRIN2B*, *CFTR* and *CCR5* loci in HEK293T cells.[4] Unlike transcription-based approaches, this strategy directly couples temperature to the activity of the genome-editing machinery.

Direct thermal control could be particularly valuable for genome editing. Combining cell- or tissue-selective delivery with localized heating could create an AND-gate-like architecture in which editing requires both the presence of the CRISPR machinery and exposure to the activating temperature. This strategy could reduce editing outside the thermally targeted region. However, direct thermogenetic genome editing remains at an early stage. Current evidence establishes molecular feasibility in mammalian cells, but whether thermal gating can improve editing specificity, efficacy, and safety in living organisms remains to be demonstrated.

4.4. Thermally controlled programmed cell death

Temperature can also be used to directly control programmed cell-death pathways. Unlike transcription-based thermogenetic systems, direct thermal regulation of pre-existing cell-death proteins bypasses de novo protein synthesis, potentially accelerating the cellular response. Thermogenetic control of programmed cell death could offer a strategy for remotely eliminating pathological cells and may eventually serve as a safety mechanism for engineered cell therapies.

The Melt platform demonstrates how temperature-dependent protein assembly and localization can regulate cell fate.[3] Melt was engineered from the thermosensitive BcLOV4 protein that undergoes oligomerization and plasma-membrane recruitment when temperature is decreased. Fusion of Melt to cell-death effectors of Caspase-1 (MeltCasp1-37) enabled cooling-induced activation of programmed cell death. In a mouse xenograft model using human lung cancer cells expressing MeltCasp1–37, localized cooling of one tumor resulted in a marked reduction in viable tumor cells relative to the untreated tumor.[3] Melt demonstrates that thermogenetic control is not restricted to heat activation: either heating or cooling can serve as the input when coupled to an appropriately engineered thermoswitch.

Heat-induced phase separation provides a complementary strategy. ELPs undergo a lower-critical-solution-temperature phase transition from a soluble state to coacervates upon heating. Vu and Arai fused the thermoresponsive ELP[V60] to the catalytic domain of human caspase-8 (CASP8), an initiator caspase whose activation depends on molecular proximity and dimerization.[5] Heating induced ELP coacervation, increasing the local concentration of CASP8 and promoting its activation and subsequent apoptosis. Cell death depended on both temperature and heating duration, demonstrating that the biological output can be regulated by thermal dose.[5] Localized irradiation with a 1470-nm laser, combined with fluorescence lifetime imaging microscopy (FLIM)-based thermometry, further enabled CASP8 activation and apoptosis in individual target cells while neighboring cells exposed to lower temperatures showed delayed or no activation (Fig. 3C).[5]

Thermogenetic cell killing is conceptually distinct from conventional thermal ablation. Thermal ablation relies on sufficiently high temperatures to directly produce irreversible tissue damage, whereas thermogenetics uses temperature as an information-carrying signal that activates a genetically encoded death program. Molecular amplification within apoptotic pathways could allow cellular elimination at substantially lower temperatures than those required for direct thermal destruction.

5. Challenges and engineering strategies for thermogenetic systems

5.1. Engineering an appropriate thermal activation window

The activation temperature is a fundamental parameter of a thermogenetic system. A switch that activates too close to physiological temperature risks unintended activation during fever, inflammation, or exercise. In contrast, a switch requiring excessive heating reduces the distinction between thermogenetics and conventional hyperthermia, and increases the risk of thermal damage. For cellular applications, mild hyperthermia around 39–42°C provides a practical operating range. Heat-responsive transcriptional systems and several recently engineered protein thermoswitches can function within this range.[3,4,16] However, the activation threshold alone is insufficient to describe performance. An ideal switch should exhibit a steep temperature-response curve, allowing a small temperature increase to produce a large change in activity (Fig. 4A). The difference in activity between the physiological and stimulated conditions is as important as the absolute temperature at which switching occurs. Low basal activity is particularly important when the thermogenetic output is highly potent. Leakage of a cytokine, genome editor, or pro-apoptotic protein could produce biological effects even without deliberate heating. Switch design should therefore prioritize both high induced activity and a low OFF-state signal.

Thermogenetic switches should respond selectively to temperature changes while minimizing sensitivity to other factors, including protein concentration, light exposure, and cellular conditions such as pH, ionic strength, and molecular crowding. Phase-separating systems, such as those based on ELPs, are particularly sensitive to concentration,[5,45] whereas endogenous heat-shock pathways can vary between cell types and physiological states.[46] Thermogenetic switches should therefore be characterized across

relevant expression levels, environmental conditions, cell types, and heating durations rather than at a single temperature under optimized culture conditions.

5.2. Reversibility and repeated activation

The need of reversibility depends on the intended application. Regulation of signaling, migration, or protein localization may benefit from rapid and reversible switching, whereas apoptosis or permanent genome editing requires only transient activation, because the downstream consequence is irreversible. Protein-level thermoswitches are candidates for reversible control because temperature can directly alter localization, assembly, or conformation.

Repeated activation is another consideration, particularly for applications requiring multiple heating cycles over extended periods. Heat-shock-based transcriptional circuits could be influenced by cellular adaptation to repeated thermal stress, whereas repeated phase transitions or conformational switching could affect protein stability or aggregation. Long-term studies examining switch performance, cellular fitness, and toxicity across repeated activation cycles will be necessary for assessing the reliability of thermogenetic systems (Fig. 4B).

5.3. Thermal dose, heat diffusion, and thermometry

Thermogenetic activation is determined by both temperature and exposure time. A brief exposure to 42°C is biologically different from maintaining the same tissue at that temperature for an extended period. Consequently, reporting only the maximum temperature can be misleading. Temperature history, heating and cooling rates, exposure duration, and repeated stimulation should be considered when defining the thermal dose.

Spatial control of heat creates an additional challenge. External energy can be focused, but the generated heat subsequently diffuses into surrounding tissues. Thus, energy localization does not necessarily equal temperature localization. Blood perfusion further alters heat distribution and can produce substantial differences between cultured cells, small-animal tumors, and human tissues. A thermogenetic system that performs well under uniform heating *in vitro* may behave differently when activated within a heterogeneous temperature field *in vivo*.

Real-time thermometry will be important for precise thermal control. FLIM-based thermometry offers a promising approach for quantitative temperature mapping in living cells with high spatial resolution. Because fluorescence lifetime is largely independent on probe concentration and excitation intensity,[47] FLIM-based thermometers are suited for quantitative measurements in heterogeneous biological environments. When combined with multiphoton excitation, FLIM-based thermometry enables temperature measurements to optically accessible depths within living tissues and facilitates the characterization of local temperature gradients around thermogenetically activated cells. At larger tissues scales, MRI-based thermometry can be combined with MRI-guided FUS to monitor temperature distributions in deep tissues.[40,48] Integrating real-time thermometry with closed-loop systems, in which

measured tissue temperature is used to adjust energy delivery, could improve the reproducibility and safety of thermogenetic activation (Fig. 4C).

5.4. Delivery and immunogenicity

Thermogenetics requires the temperature-responsive machinery to be present in target cells. Effective and selective delivery of thermogenetic tools in animals or tissues represents a major challenge (Fig. 4D). Viral vectors can provide efficient and sustained expression but have limitations, including restricted cargo capacity, immunogenicity, and prolonged transgene expression.[49] Non-viral DNA and mRNA delivery provide alternative strategies. mRNA is well suited to applications requiring only transient expression of thermogenetic tools because it does not involve genomic integration and has a limited intracellular lifetime.[50] Cell therapies offer another route because the thermogenetic circuit can be introduced *ex vivo* before administration. CAR-T cells are a prominent example: cells can be genetically engineered, characterized, and expanded before infusion, after which localized heating provides an additional layer of spatial control.[18]

The origin and size of thermosensitive domains should also be considered. Current thermogenetic switches are derived from non-human proteins, including bacterial TlpA,[22] fungal photoreceptor BcLOV4[3] and AsLOV2.[4] These foreign proteins could elicit immune responses during prolonged expression, although this risk has not yet been systematically evaluated for thermogenetic applications. Engineering compact thermosensitive domains, minimizing non-human sequences, and exploring human-derived thermoswitches could improve the biocompatibility and versatility of thermogenetic systems.

5.5. Safety should emerge from multiple layers of control

Thermogenetic control should not rely on temperature alone for specificity. A more robust strategy is to combine multiple independent layers of control. Tissue- or cell-selective delivery can determine which cells contain the thermogenetic machinery. Molecular recognition, such as antigen recognition by engineered immune cells, can provide an additional layer of selectivity. Localized heating further controls where and when the machinery is activated (Fig. 4E). These architectures establish biological “AND” gate systems in which cellular responses require multiple conditions to be functioned.

6. Perspectives and conclusion

Thermogenetics provides an approach for remote control of cellular functions. Compared with optogenetics, a well-established technology that uses light-sensitive proteins to regulate cellular functions, thermogenetics remains at an early stage of development. The significance of optogenetics was recently recognized by the 2026 Nobel Prize in Physiology or Medicine, and its applications have advanced to clinical trials for vision restoration.[51] Although optogenetics offers high spatial and temporal precision, light absorption and scattering limit its application in deep tissues without invasive optical delivery. In contrast, thermogenetics can employ FUS or magnetic fields to generate localized heating in deeper tissues, potentially overcoming limitations of light-based control. Recent advances in

molecular engineering and heating technologies have further expanded the capabilities of thermogenetics for regulating biological processes in living cells and organisms. Furthermore, integrating thermogenetics with optogenetics, chemogenetics, and magnetogenetics could enable multiplexed control of cellular functions. Combining molecular tools responsive to distinct physical or chemical stimuli could allow different signaling pathways or protein activities to be regulated independently or sequentially within the same biological system.

A future direction is the development of thermogenetic systems that directly regulate protein function. Although transcriptional switches provide signal amplification and are well suited to sustained protein production, direct control of protein localization, assembly, or activity bypasses transcription and translation, potentially allowing faster and more reversible responses. Further engineering of thermoswitches with tunable activation temperatures, low basal activity, and improved reversibility could expand the range of proteins and cellular processes accessible to thermal regulation. Rather than replacing transcription-based systems, protein-level thermogenetics offers complementary mechanisms suited to different biological functions and timescales.

Thermogenetics holds promise for therapeutic applications requiring spatially and temporally controlled cellular activity. Potential applications include regulating therapeutic gene expression, controlling engineered immune cells, and activating proteins involved in programmed cell death. Engineered cell therapies offer a promising starting point because thermogenetic circuits can be introduced and characterized *ex vivo* before administration. Advances in targeted viral vectors, lipid nanoparticles, and other delivery technologies further enable selective expression of thermogenetic components in specific cell populations *in vivo*. However, clinical translation will require coordinated optimization of molecular thermoswitches, delivery strategies, heating technologies, and quantitative thermometry. Further studies addressing long-term safety, immunogenicity, performance under repeated activation, and reproducibility in relevant preclinical models will be essential.

In conclusion, thermogenetics provides a modular framework in which temperature-responsive molecular tools determine the biological response and externally delivered thermal energy controls their location and timing. Continued advances in molecular engineering, targeted delivery, and precision heating could establish thermogenetics as a versatile platform for investigating and remotely regulating cellular functions in living systems.

Declarations

Author contributions

C.Q.V. conceptualized the review and wrote the original manuscript draft with input from H.T.T. and S.A. All authors reviewed, edited, and approved the final version of the manuscript.

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Conflict of Interest

The authors declare no conflicts of interest.

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Figures

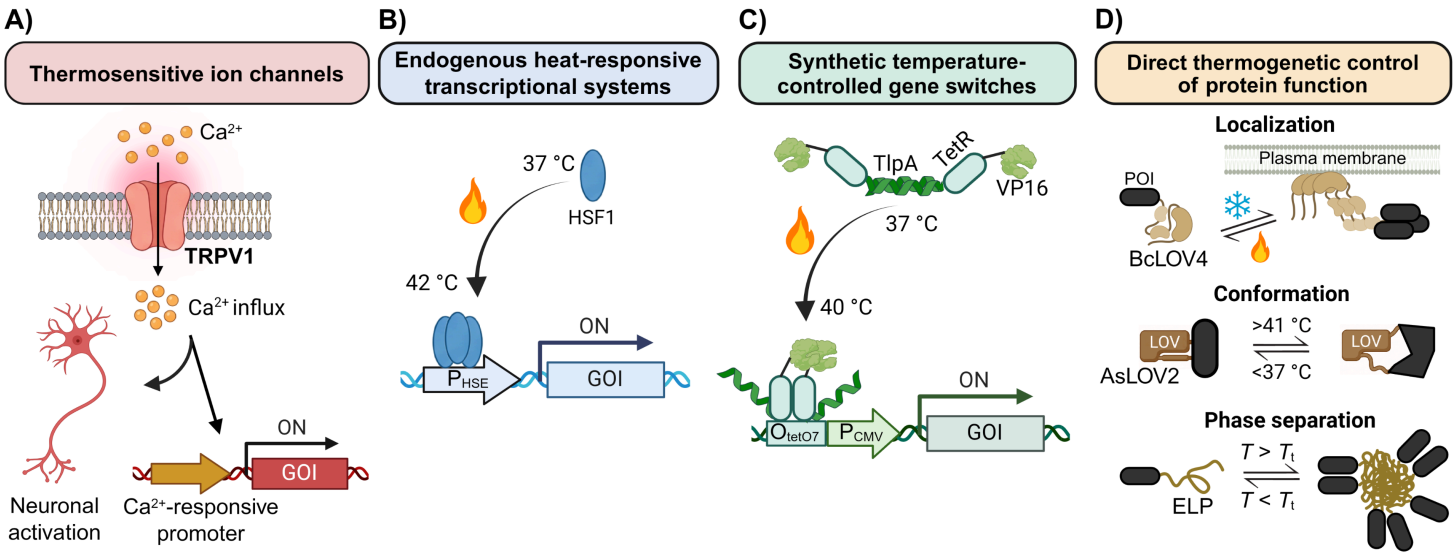


Figure 1

Molecular tools for thermogenetic control. A) Thermosensitive ion channels. Heating activates temperature-sensitive channels such as TRPV1, inducing cation influx, including Ca^{2+}, and triggering membrane depolarization and downstream signaling. **B) Endogenous heat-responsive transcriptional systems.** Heating activates endogenous heat-shock factor 1 (HSF1), which binds heat-shock elements (HSEs) and drives expression of a gene of interest (GOI) from a heat-responsive promoter. **C) Synthetic temperature-controlled gene switches.** Engineered thermoresponsive proteins, exemplified by TlpA-derived systems, undergo temperature-dependent conformational changes that regulate synthetic transcriptional machinery and gene expression. **D) Direct thermogenetic control of protein function.** Temperature-dependent changes in protein assembly and localization, conformation, or phase separation directly regulate protein activity without requiring de novo protein synthesis. Representative mechanisms include temperature-dependent membrane recruitment, thermoresponsive LOV-domain-based allosteric regulation, and elastin-like polypeptide (ELP)-mediated phase separation. Illustrations were created with BioRender.com.

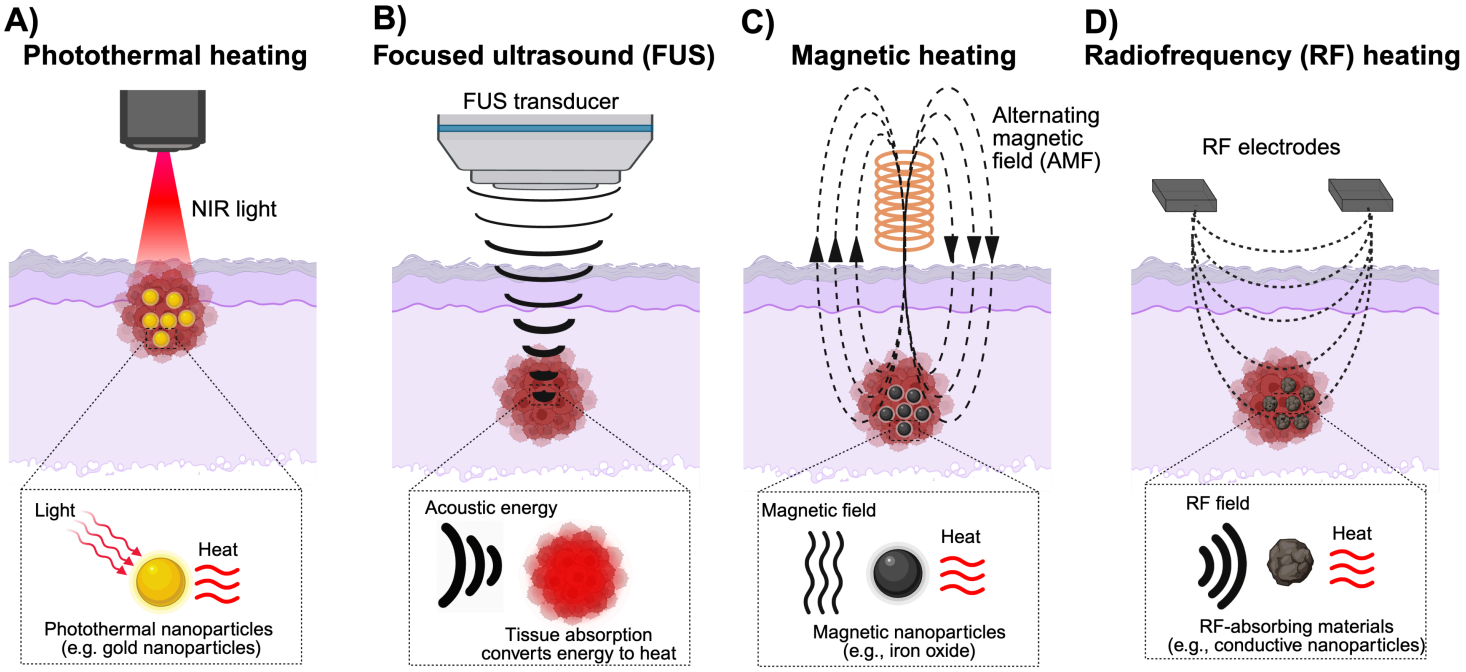


Figure 2

External heating modalities for thermogenetic control. A) Photothermal heating. Near infrared (NIR) light is absorbed by photothermal agents, such as gold nanoparticles, and converted into heat, enabling localized thermal stimulation with high spatial and temporal precision. B) Focused ultrasound (FUS). Acoustic energy is focused within a defined tissue volume and converted into heat through tissue absorption, allowing localized heating in deep tissues without requiring exogenous heating agents. C) Magnetic heating.Magnetic nanoparticles exposed to an alternating magnetic field (AMF) dissipate energy as heat. D) Radiofrequency (RF) heating. RF electromagnetic energy penetrates biological tissues and generates heat through interactions with RF-absorbing materials. Illustrations were created with BioRender.com.

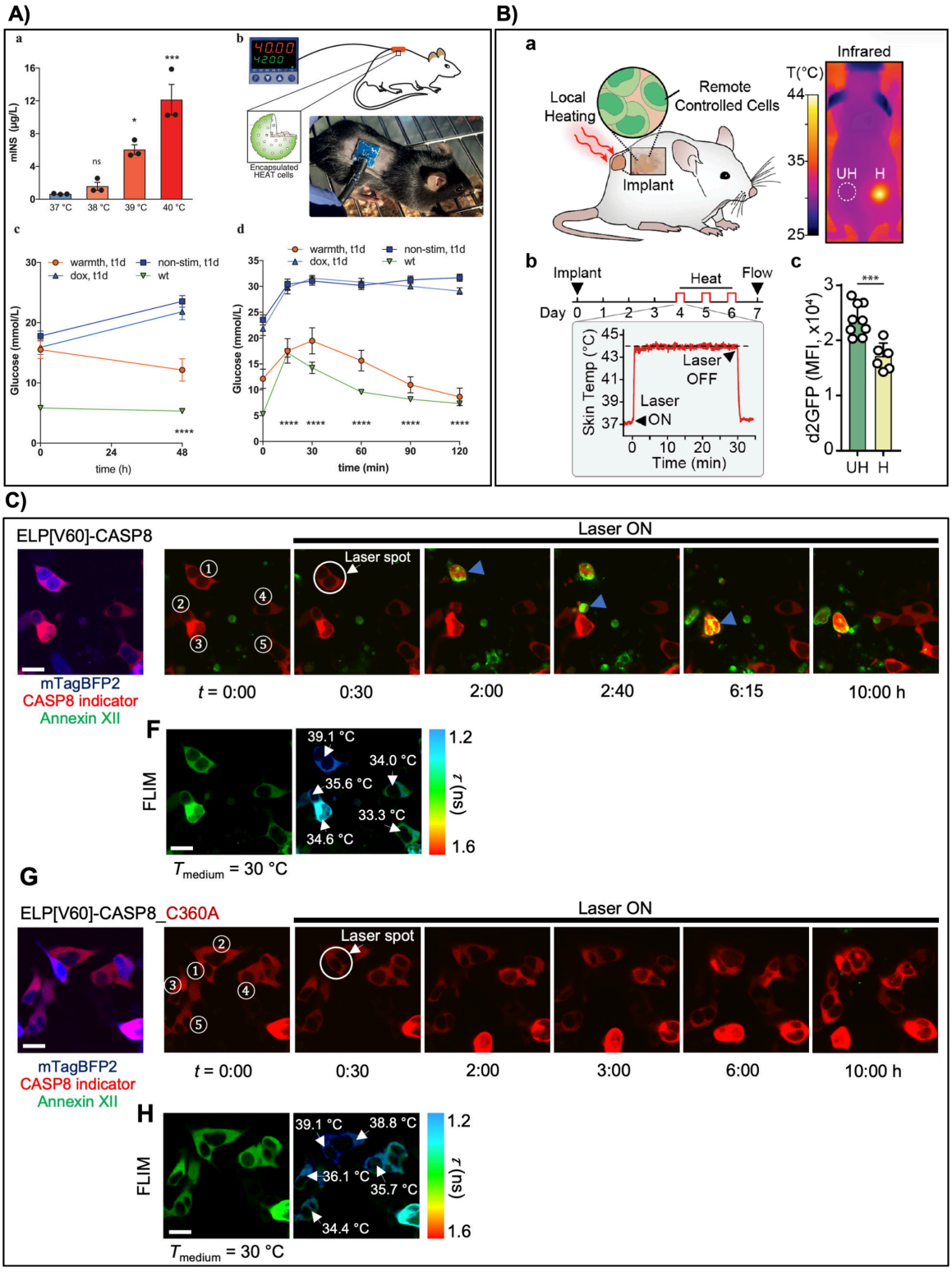


Figure 3

Representative applications of thermogenetic control. A) Heat-controlled insulin production. The human enhanced gene activation thermometer (HEAT), using an engineered TlpA-based thermoresponsive transcriptional system, enabled temperature-dependent insulin expression within 37–40 °C. Microencapsulated designer cells carrying a HEAT-controlled insulin expression cassette were implanted subcutaneously in mice with experimental type 1 diabetes. Local heating with an

electrothermal skin patch induced insulin production and release, reduced blood glucose and restored normoglycemia (reproduced with permission from Ref.[21]). **B) Photothermal control of CRISPR-mediated gene repression. Engineered cells containing a heat-inducible KRAB-dCas9 system and gold nanorods were implanted subcutaneously in mice. Localized NIR laser heating activated KRAB-dCas9 expression, resulting in repression of d2GFP fluorescence in the implanted cells (reproduced with permission from Ref.[17]). C) Heat-triggered activation of caspase-8 (CASP8).** Local laser heating induced coacervations of an ELP-CASP8 fusion protein, increasing the local concentration of **CASP8** and triggering apoptosis. FLIM-based thermometry enabled quantitative measurement of intracellular temperature during spatially controlled activation (reproduced with permission from Ref.[5]).

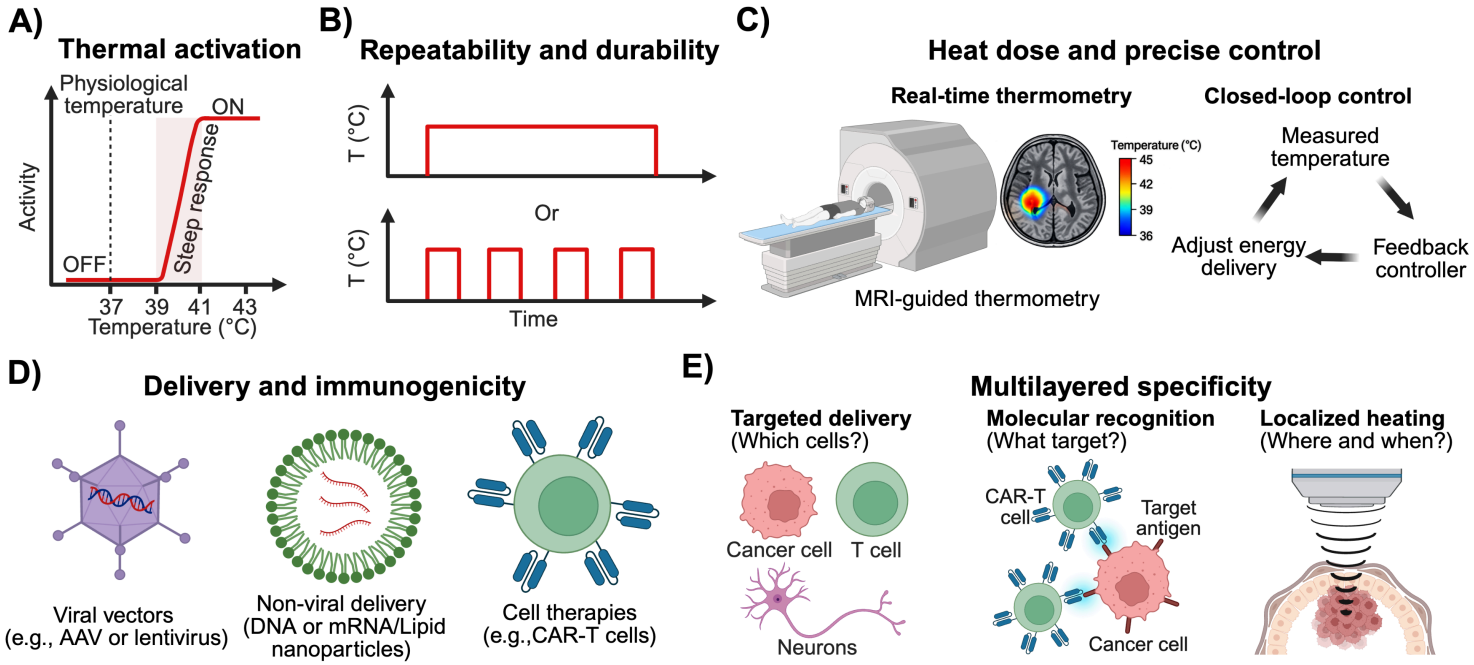


Figure 4

Engineering strategies for robust thermogenetic control. A) Thermal activation window. Thermogenetic switches should exhibit low basal activity at physiological temperature and a steep activation response within a physiologically tolerable temperature range, ideally around 39–41 °C. B) Thermal stimulation protocols. Continuous or pulsed heating can be applied to regulate the duration and magnitude of thermogenetic activation while minimizing thermal exposure. C) Heat dose and quantitative thermometry. MRI-based thermometry enables spatial mapping of temperature during localized heating, supporting precise thermal control and feedback-guided stimulation. D) Delivery strategies. Viral vectors, lipid nanoparticles, and engineered cells provide complementary approaches for introducing thermogenetic machinery into target cells or tissues. E) Multilayered specificity. Combining targeted delivery, molecular recognition, and localized heating creates biological AND gates that restrict thermogenetic activation to specific cells, locations, and conditions. Illustrations were created with BioRender.com.