

Photothermal Genetic Engineering

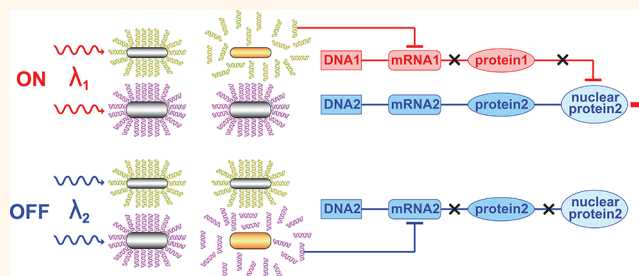
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Over the past decade, a wealth of optical tools have been introduced to address a wide spectrum of biological questions. A few examples include optical tweezers for dynamic characterization of molecular motors¹ and on-chip surgery,² use of two-photon microscopy for intact tissue imaging,^{3,4} optical pacemakers,⁵ and microbial opsins for optogenetic deconstruction of neural circuits.⁶ The latter method employs light-sensitive proteins of archaeal and bacterial origin to create sensitivity of metazoan neurons to visible light.⁷ Optogenetics with these light-activated ion channels and pumps takes advantage of cell-specific genetic targeting and the millisecond precision of optical hardware, allowing for precise delineation of the causal role of neural electrical activity. However, genetic pathways provide another avenue wherein optical methods could accelerate discovery.

Until recently, protein engineering remained a dominant method for design of photonically controlled genetic interventions. The majority of available optical tools for gene activation and silencing consist of a light-sensitive antenna domain and an expression regulator domain (e.g., DNA binding protein/transcription factor).⁸ This structure is conceptually similar to that of optoXRs, chimeric proteins composed of light-sensitive cores of opsins and intracellular loops of G-protein coupled receptors enabling optical control of cellular signaling.^{9,10} A number of studies have employed optically sensitive light-oxygen-voltage (LOV) domains,¹¹ blue-light-utilizing flavin adenine dinucleotide (BLUF FAD),¹² photoactive yellow protein (PYP),¹³ or light-sensor modules from phytochromes (Phy)^{14,15} bound to transcription factors or DNA-binding proteins to activate or to silence biochemical signaling or gene expression. Consequently, currently available methods for gene or neural pathway engineering tend to rely on the genetic modification of tissue as well as delivery of visible light. However, many biological tissues

ABSTRACT



Optical methods for manipulation of cellular function have enabled deconstruction of genetic and neural circuits *in vitro* and *in vivo*. Plasmonic gold nanomaterials provide an alternative platform for external optical manipulation of genetic circuits. The tunable absorption of gold nanoparticles in the infrared spectral region and straightforward surface functionalization has led to applications in intracellular delivery and photorelease of short RNAs, recently enabling bidirectional photothermal modulation of specific genes *via* RNA interference (RNAi). We discuss recent advances in optical gene circuit engineering and plasmonic nanomaterials, as well as future research opportunities and challenges in photothermal gene manipulation.

are highly scattering and absorptive in the visible part of the spectrum, which necessitates invasive approaches for delivery of visible light into deep tissue. Near infrared (NIR) light ($\lambda = 650\text{--}1064\text{ nm}$) penetrates almost 2 orders of magnitude deeper into tissue due to the NIR windows in absorption spectra of water and hemoglobin. Consequently, developing a method for NIR control of gene activation and silencing could enable minimally invasive strategies for cell manipulation.

Nanomaterials composed of noble metals can efficiently absorb visible or NIR photons into surface plasmon modes and dissipate the absorbed energy as heat.¹⁶ Because of efficient absorption that is tunable across the NIR spectrum, gold-based plasmonic nanomaterials (AuPNMs: nanoparticles, nanorods, nanospheres, and nanoshells) have been widely adopted as enablers of localized targeted photoheating.^{16,17} Gold-based plasmonic nanomaterials are ubiquitous in cancer research and are undergoing translation to

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clinical applications.¹⁸ These nanomaterials have been shown to accumulate preferentially within tumors and to enable selective photothermal tumor ablation by deeply penetrating NIR light.^{19,20} Another advantage of AuPNMs is the ability to target specific cells or tissues *via* straightforward surface functionalization using thiol conjugation chemistry.^{21,22}

Over the past 10 years, AuPNMs also have been employed as drug-delivery vehicles. Directly linking a single-stranded nucleic acid (NA) to the AuPNM surface and coupling to a therapeutic agent *via* NA hybridization enables optically driven agent release through photothermal melting of the NA interstrand bonds.²³ It has also been shown that deep blue light ($\lambda = 400 \text{ nm}$)²⁴ or pulsed NIR light ($\lambda = 800 \text{ nm}$)²⁵ can be used to dissociate gold–thiol bonds, releasing a therapeutic payload from the AuPNM carrier. The latter feature of thiol-functionalized AuPNMs has recently led to applications in gene silencing *via* photothermal activation of RNA interference (RNAi).^{25,26}

RNAi is a general method for gene silencing, which operates by hybridization of short interfering RNA (siRNA) or microRNA strands to mRNA (mRNA), preventing protein synthesis and activating mRNA cleavage. While RNAi is a widely accepted tool for deconstruction of genetic circuits, the methods for siRNA delivery and spatiotemporal control of activity remain a challenge. Gold-based plasmonic nanomaterials now provide a pathway toward spatially and temporally precise NIR control of RNAi. Recent studies by Braun *et al.*²⁵ and Lu *et al.*²⁶ employed gold nanoshells to deliver short surface-coupled siRNA into the cells and then used photothermal excitation to release the siRNA and reduce transcription of a specific gene *in vitro* and *in vivo*. While these pioneering studies demonstrated AuPNM gene silencing, the photothermal genetic engineering toolbox remained incomplete without a complementary gene activation mechanism.

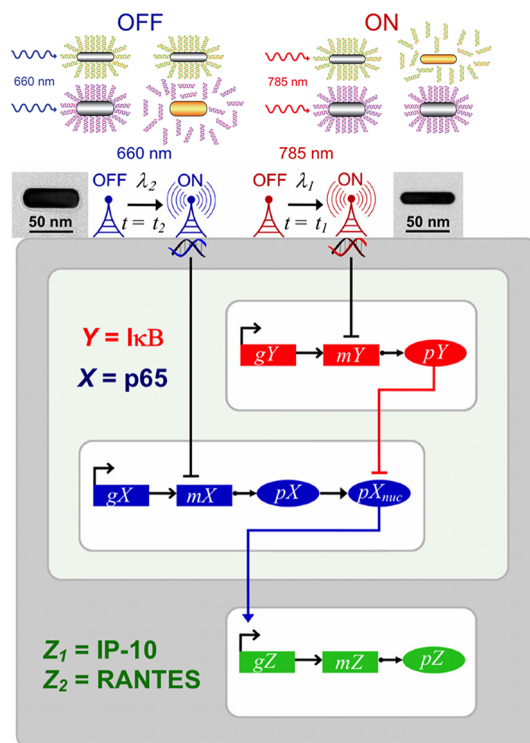


Figure 1. Schematic illustration of the photothermal gene circuits designed by Lee *et al.* Figure reprinted from ref 27. Copyright American Chemical Society.

In this issue of *ACS Nano*, Lee *et al.* extend the photothermal approach for RNAi-mediated gene silencing to multistep bidirectional control of specific gene expression.

In this issue of *ACS Nano*, Lee *et al.*²⁷ extend the photothermal approach for RNAi-mediated gene silencing to multistep bidirectional control of specific gene expression (Figure 1). In this elegant study, the authors take advantage of the tunability of the longitudinal plasmon resonance wavelength in gold nanorods (GNRs), which is enabled simply by adjusting the aspect ratio. The advantage of GNRs is demonstrated through the construction of three photonic gene

circuits of increasing complexity. As a first step, the authors create a one-part photonic “OFF” switch. In this experiment, siRNA to the activated isoform of NF κ B-p65 (p65) was coupled to GNRs and delivered into HeLa cells. The expression of p65 was then compared for no illumination and illumination with resonant wavelengths. Consistent with the earlier study by Lu *et al.*,²⁶ activation of the photonic “OFF” switch resulted in a decreased expression of p65. However, the authors then took advantage of the modular structure of genetic circuits and designed a two-part photonic “ON” switch. In the absence of external stimuli, p65 is normally sequestered in the cytosol by the inhibitor I κ B before translocation into the nucleus. The authors constructed a photothermal “OFF” switch for I κ B by coupling I κ B siRNA to GNRs. By inhibiting the inhibitor, this photonic circuit acted as an “ON” switch for p65 in the nucleus. Finally, taking advantage of the plasmon resonance differences between GNRs with different aspect ratios,

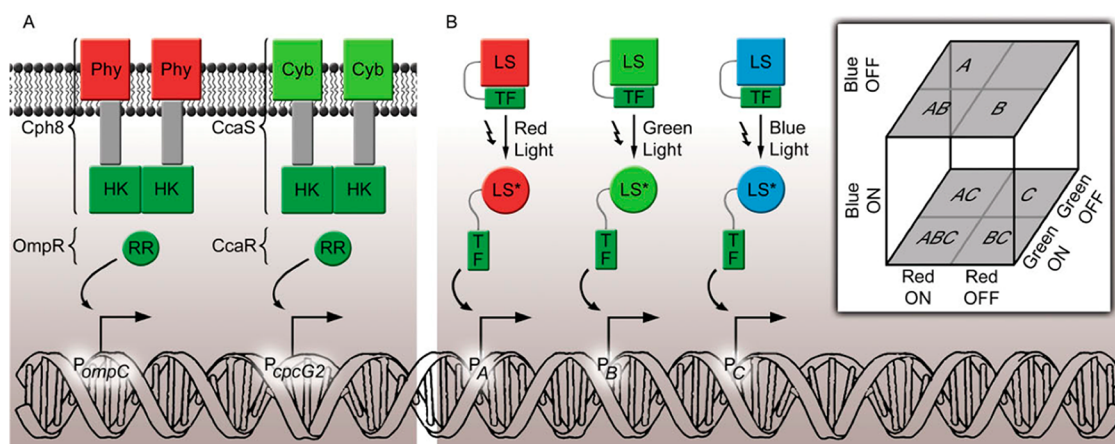


Figure 2. Multiplexed photonic gene circuits. Reproduced with permission from ref 8. Copyright 2011 Wiley-VCH Verlag GmbH & Co.

the authors constructed a three-part gene circuit for bimodal photothermal control of expression. In this experiment, the GNRs with plasmon resonance at wavelength $\lambda = 785$ nm were coupled to *l*kB siRNA, enabling an optical boost of p65 levels in the nucleus and the GNRs with plasmon resonance at a wavelength $\lambda = 660$ nm were coupled to p65 siRNA, enabling optical inhibition of p65 production. The efficacy of the bimodal photothermal control was demonstrated through measurements of the levels of expression of IP-10 and RANTES—early and late response genes activated by nuclear p65.

This two-wavelength approach to bimodal control of cellular activity is in some ways analogous to that of the step function opsins (SFOs), genetically engineered light-sensitive proteins that take advantage of separate wavelengths to assume “ON” and “OFF” states.²⁸ In optogenetic experiments, neurons that are genetically modified to express SFOs can be activated by a short pulse of blue light ($\lambda = 473$ nm) and returned back to the inactive state by a short green, yellow, or amber ($\lambda = 532$ – 594 nm) light pulse. A number of studies have recently demonstrated the utility of SFOs in deconstruction of neural circuits;²⁹ a bidirectional photothermal approach enabled by siRNA coupled GNRs could similarly illuminate the structure and logic of biochemical and genetic circuits.

The work by Lee *et al.*²⁷ may be also viewed as an alternative to the recently developed two-color phytochrome-based transcription regulation scheme by Tabor *et al.*³⁰ In the latter study, the authors took advantage of the cyanobacterial two-component system consisting of a photosensitive cyanobacteriochrome antennae CcaS and its response regulator CcaR. The CcaS–CcaR system activates transcription from the promoter *cpcG2* when exposed to green light, and this function can be terminated by red light. Combination of this green light-sensitive transcription scheme with the red-sensitive phytochrome system designed earlier within the same laboratory allowed multiplexed optical control of transcription. Taken together, it is now possible to envision combinatorial gene regulation approaches involving blue-light-sensitive LOV or BLUF domains, green-sensitive cyanobacterial chromophores, and red-sensitive phytochromophores (Figure 2).⁸

Outlook and Future Challenges. In optogenetic deconstruction of neural circuits, opsins enable reversible neural excitation and inhibition with millisecond precision during neural activity and behavior. As nature's optoelectronic biological nanomaterials, microbial opsins are optimized to sense light with visible wavelengths near the peak of the solar spectrum. To enable minimally invasive NIR control of cellular functions, artificial nanostructures that approach opsin performance

in optical sensitivity and temporal precision are needed. While single-component optoXR-like structures that can be directly activated by NIR light are ultimately desirable, photoactivated siRNA provides an important initial step. Recent advances demonstrate the potential of photothermal RNAi-mediated gene silencing in minimally invasive manipulation of gene circuits *in vivo*. The advantages of plasmonic gold nanomaterials as siRNA delivery vehicles and photothermal RNAi switches include the ability to function in genetically unmodified tissues (*i.e.*, they do not require foreign proteins), generalizable applicability to selective silencing of essentially arbitrary genes, and compatibility with multiplexed gene regulation schemes due to tunable plasmon resonances. However, a number of challenges need to be overcome in order to enable efficient photothermal deconstruction of gene circuits in real time.

Recent advances demonstrate the potential of photothermal RNAi-mediated gene silencing in minimally invasive manipulation of gene circuits *in vivo*.

First, gene circuits involved in normal cellular function or pathological conditions are often changing and, consequently, our ability to investigate and to control these circuits relies on the temporal precision and reversibility of interrogation. In the present state, the photothermal mechanism does not allow for reversible gene silencing/activation as the siRNA is permanently released from the plasmonic carrier upon illumination with resonant frequency light. Special attention should be dedicated to the development of photoswitchable siRNA–nanomaterial complexes that can release multiple siRNA payloads in a stepwise manner. The addition of a scavenging complex may enable rapid removal of siRNA and enable reversible gene silencing. Alternatively, a photothermal method for temporary siRNA activation could be implemented. Such a method may involve the combination of multiple nanomaterial–siRNA complexes, which can reversibly interact with each other upon illumination, or a more sophisticated siRNA design, allowing for reversible hybridization.

Reversible photothermal activation of RNAi would also require careful characterization of siRNA release dynamics. Multiple release mechanisms (e.g., thiol bond cleavage, hydrogen bond dissociation) need to be considered to isolate the critical elements (such as Au–thiol bonds, hybridization, and siRNA length), which may provide additional optical handles to the siRNA–nanomaterial complexes. Chemistries beyond thiols may need to be considered for siRNA attachment to enable intensity-dependent photorelease of the payload from the gold surface. While Jain *et al.*²⁴ make a step toward understanding the optoelectronic origins underlying RNA release via blue-light-induced dissociation of the gold–thiol bonds, additional detailed spectroscopic studies, akin to those by Alper *et al.*,³¹ are needed to investigate the surface changes evoked by NIR light.

While Lee *et al.*²⁷ provide a first demonstration of multiplexed photothermal interrogation, additional experiments will be required to eliminate the existing cross-talk between the plasmonic structures. Physical modeling of the dependence of the absorption spectra and the shape of the surface plasmon mode on the nanostructure geometry may enable computer-guided design of plasmonic nanomaterials with narrow resonances. Such materials may allow simultaneous interrogation of multiple gene circuit elements as well as provide a route toward reversibility. Special attention should be dedicated to the extension of the photothermal genetic engineering approach to living animal models. The work by Lu *et al.*²⁶ demonstrating photothermal activation of p65 RNAi in HeLa xenografts in nude mice provides a path toward applying the method to tumors; however, the general adoption of photothermal gene regulation would require demonstrations in healthy tissues as well as in diseases such as disseminated cancers.

The development of a robust photothermal gene regulation approach will ultimately rely on multidisciplinary efforts with materials scientists, biologists, chemists, and bioengineers working side-by-side to tailor the properties of plasmonic nanomaterials to designer transcription factors and siRNA. Overcoming the current challenges associated with long-term reversible implementation of photothermal genetic intervention, *in vivo* could not only enable a new generation of treatments but also could deepen the understanding of genetic and biochemical function and dysfunction within behaving mammals.

Conflict of Interest: The authors declare no competing financial interest.

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