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Nanoplasmonic gene regulation

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This review focuses on the recent developments in nanoplasmonic gene regulations. Types of nanoplasmonic carriers and DNA/RNA cargo are described. Strategies to liberate cargo from their carriers using NIR and enable on-demand silencing of endogenous intracellular genes are reviewed. In addition to inhibitory effects, exogenous foreign genes are also introduced and expressed on-demand using nanoplasmonic optical switches. The magnitude and timing of genetic activities can therefore be systematically controlled on-demand remotely. Equipped with new nanoplasmonic optics to directly probe the intracellular space, quantitative approaches should capture many dynamic activities within living systems that were otherwise previously impossible to control using conventional methods.

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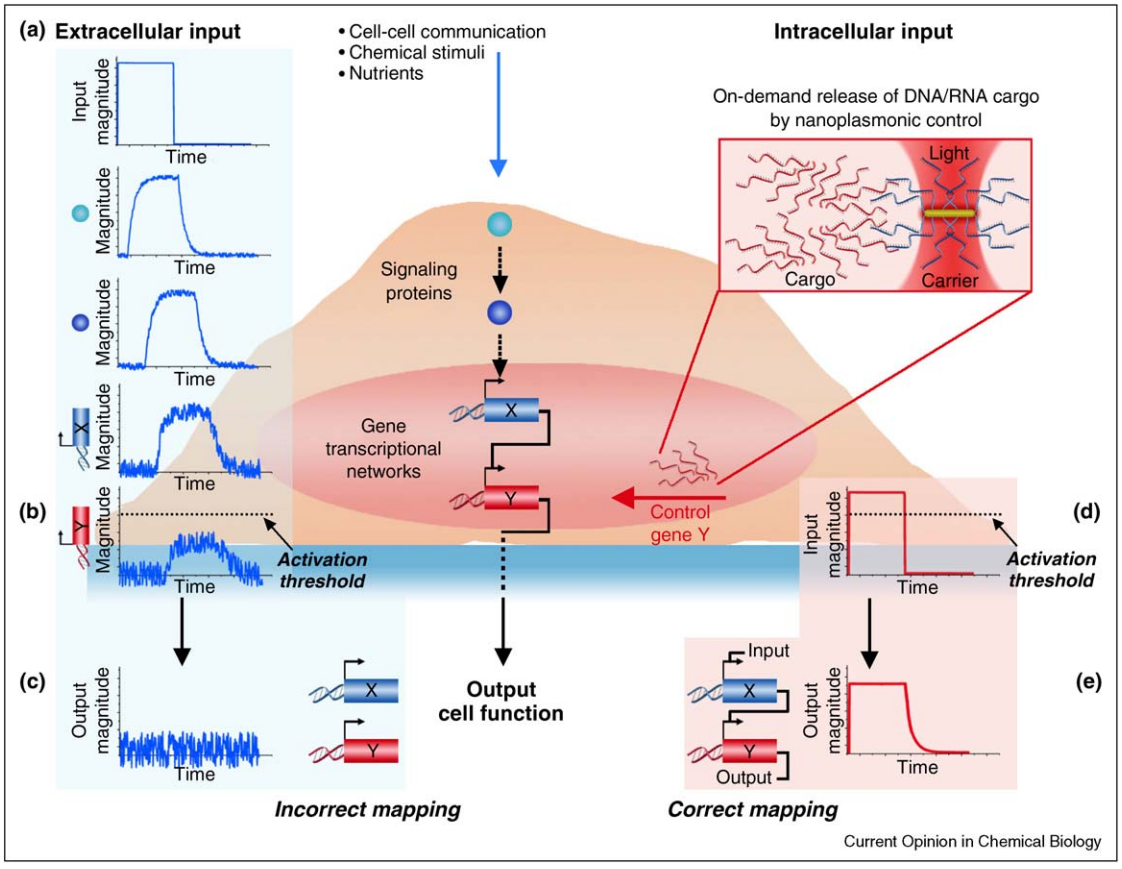
Introduction

A single living cell is a dynamic system that is capable of constantly sensing and responding to its perpetually changing environment. As such, it is an integrated system consisting of extracellular input signals streaming in from the local external environment, interlinked signaling cascades of internal connections, and gene transcriptional networks that respond by producing the appropriate proteins to give rise to cell function (Figure 1). Quantitatively understanding the inner workings of these systems is the quest set forth by quantitative cell biology and systems biology. By systematically perturbing with extracellular stimuli, specific internal connections can be mapped and the resulting cell function can be observed in response to the single environmental change. Spatial, temporal, and systematic variation of extracellular stimuli has been shown to be absolutely critical for developmental processes [1], growth [2], differentiation [3], apoptosis, and stem cell fate decisions [4].

In addition to extracellular control of environmental stimuli, intracellular control of the actual internal connections themselves can provide unparalleled insight into the inner workings of these systems. When a cell is treated as input–output 'black box,' ideally, the output response is directly correlated to the input signal. However, signal distortions – time delays, noise, and signal magnitude reductions – can confound this input–output relationship. Time delays, due to inherent time-dependent interactions, transcriptional rates, and translational rates, are associated with each interconnected step inside the 'black box.' Noise, from stochastic fluctuations in proteins, is introduced at each stage inside the 'black box.' Signal magnitude reductions, due to degradation, dilution and diffusion effects, are also inevitable. These signal distortions can have significant implications on the output response. Consider a simple transcriptional network, where gene X regulates gene Y, and gene Y in turn regulates the output. In the presence of its input signal, X becomes active. Threshold effects are known to govern many gene regulatory processes [5]. Thus, when the concentration of X is greater than the threshold concentration required for activating Y, Y is produced. Subsequently, when the concentration of Y is greater than the threshold concentration required for activating the output, then the output is produced. Now, suppose that the input signal is externally introduced (Figure 1a). As this extracellular input signal traverses through each step of the extracellular-to-intracellular cascade, signal distortions are introduced. If the signal is significantly confounded by noise or if the signal magnitude is significantly reduced such that the activity of Y no longer satisfies the threshold condition (Figure 1b), the output is not produced (Figure 1c). This result can give the misleading impression that no relationship exists between the input signal and Y, when in fact they are related. Internal connections can be incorrectly mapped. Suppose now that Y is directly controlled at the intracellular level (Figure 1d). Steps in the extracellular-to-intracellular cascade are essentially bypassed, thereby minimizing signal distortions. As a result, the output is reliably produced (Figure 1e). Internal connections can therefore be correctly mapped and modeled.

By delving directly into the intracellular 'black box,' the magnitude and timing of intracellular processes can be precisely controlled. Interfering oligonucleotides, such as antisense DNA, single-stranded RNA, short hairpin RNA (shRNA), and small interfering RNA (siRNA), enable

Figure 1



Concept of nanoplasmonics-enabled on-demand and systematic intracellular gene regulation for quantitative biology. **(a)** Extracellular control. Extracellular input signals, such as chemical stimuli or nutrients, are externally introduced. As this extracellular input signal traverses through each step of the extracellular-to-intracellular cascade, signal distortions are introduced. **(b)** The activity of Y does not satisfy the threshold condition due to signal distortions. **(c)** The output is not produced. This result gives the misleading impression that no relationship exists between the input signal and Y, when in fact they are related. Internal connections are incorrectly mapped. **(d)** Intracellular control. Nanoplasmonic technologies are used as carriers of oligonucleotide cargo. Using light illumination as a remote trigger to release free oligonucleotides and 'activate' their functionality, Y is directly controlled at the intracellular level. Signal distortions are minimized since steps in the extracellular-to-intracellular cascade are essentially bypassed. As a result, the output is reliably produced and internal connections are correctly mapped.

direct, sequence-specific control of intracellular genes, but alone, lack the temporal control necessary for precise manipulation. Recent advancements in chemical biology, nanotechnology, and plasmonics now enable new light-sensitive tools of sub-nanometer and nanometer size scales to directly interface with intracellular processes. For example, nanoplasmonic technologies can be used as carriers of oligonucleotide cargo (Figure 1d). Initially, oligonucleotide functionality is inactivated. Using light illumination as a remote trigger to release free oligonucleotides and 'activate' their functionality, endogenous intracellular genes can be silenced on-demand. In addition to the inhibitory effects of interfering oligonucleotides, exogenous foreign genes can be also introduced and expressed on-demand. In this way, the magnitude and timing of genetic activities can be systematically varied on-demand. Signal distortions can be minimized since light-sensitive tools are activated from within the intracellular space, and therefore, steps in the extracellular-to-intracellular cascade are bypassed at the time of activation. Equipped with new tools to directly probe the intracellular space, quantitative and systematic approaches should capture many dynamic activities within the living cell that were otherwise previously impossible to detect using conventional methods.

Caging technologies

Caging is also an effective means to temporarily inactivate oligonucleotide functionality by incorporating photolabile protective groups, otherwise known as caging groups, into the bases or the phosphate backbone [6•]. Ultra-violet (UV) irradiation removes the caging groups and restores oligonucleotide functionality. UV-activated gene silencing using caged antisense DNA has been demonstrated in mouse NIH 3T3 fibroblast cells [7]. UV-activated gene silencing using caged siRNA has also

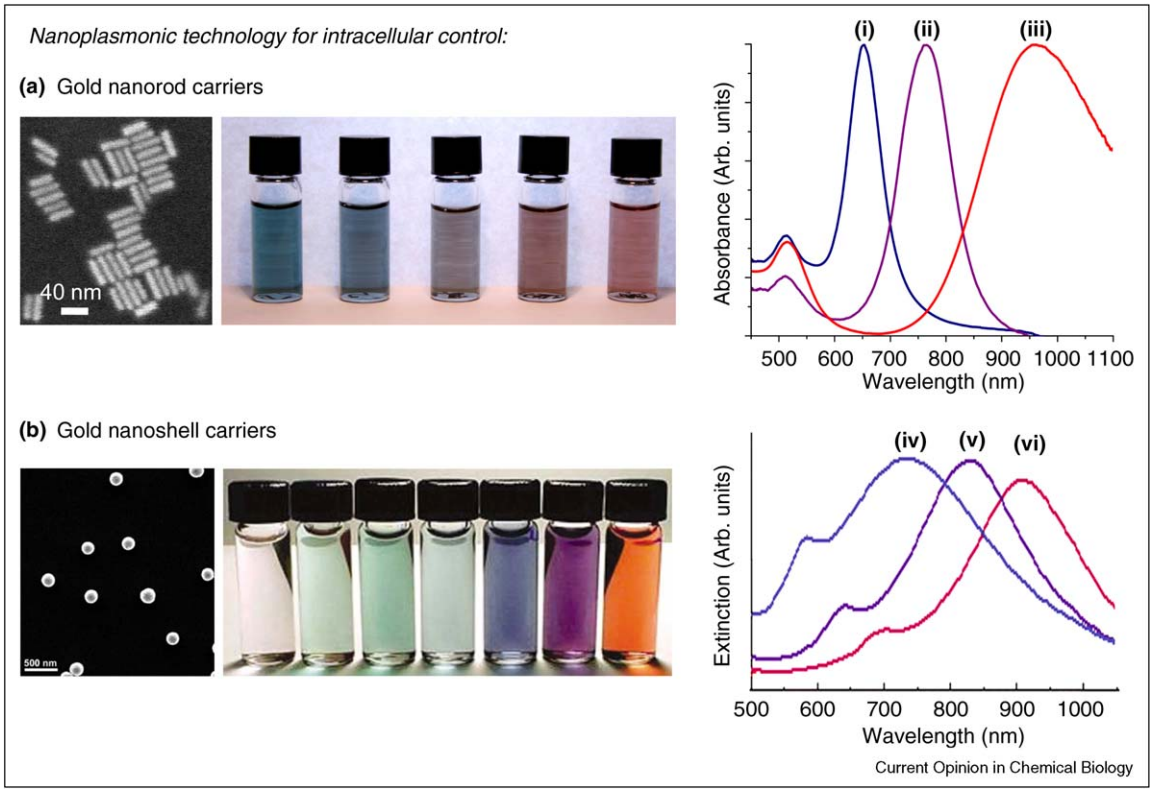
been demonstrated in human HeLa cervical carcinoma cells [8]. While caging enables excellent spatiotemporal control of intracellular genes, the particular use of UV wavelengths is of some concern since intracellular proteins and nucleic acids are widely known to absorb, crosslink, and mutagenize in the presence of UV irradiation. Therefore, activation using less biologically harmful wavelengths of light is highly desirable.

NIR nanoplasmonic technologies

Gold nanoplasmonic particles (GNPs), in the near-infrared (NIR) spectral region, are attractive candidates for intracellular control. The NIR wavelength regime is well suited for biological and biomedical applications since tissues and cells are essentially transparent between 700 and 1300 nm [9]. Due to their strong and sharp resonance peak in their optical properties, GNPs efficiently convert light energy into surface-localized heat, otherwise known as photothermal conversion [10–12], when the incident light is matched to their plasmon resonance wavelength. In the presence of this incident light, the conduction band electrons of the GNPs collectively oscillate in phase on resonance and subsequently make collisions with the metal lattice, thereby dissipating heat [12]. Heat transfer from the surface of GNPs to the surrounding cellular environment is highly localized, decaying exponentially within a few nanometers [10,13••,14] and therefore is thought to have minimal adverse effects on cells. Among the GNPs, gold nanorods [13••,15,16] and gold nanoshells [17••,18], are of widespread interest and pervasive use due to their large absorption cross-section, facile tunability of their plasmon resonance wavelength on the basis of geometry, and large-scale synthesis with uniform distribution (Figure 2).

Because of their large surface-to-volume ratio, GNPs are ideal carriers of cargo, such as interfering oligonucleotides. While attached to their carriers, cargo is rendered inactive due to steric hindrances between the tightly packed cargos. For 150 nm diameter gold nanoshells, for example, the surface coverage of dsDNA cargo was determined to be 6400 dsDNA molecules/nanoshell [17••]. In the presence of light that is matched to their plasmon resonance wavelength, GNPs photothermally

Figure 2



Nanoplasmonic technologies for intracellular control. **(a)** Scanning electron microscopy image showing large-scale synthesis with uniform distribution, visual image showing facile tunability of the plasmon resonance wavelength, and UV–vis absorbance spectra showing large absorption cross-sections of gold nanorod carriers. In the UV–vis absorbance spectra, the plasmon resonance wavelengths correspond to aspect ratios (AR): (i) 2.3 AR, (ii) 3.5 AR, (iii) 5.0 AR [13••] **(b)** Scanning electron microscopy image showing large-scale synthesis with uniform distribution, visual image showing facile tunability of the plasmon resonance wavelength, and UV–vis absorbance spectra showing large absorption cross-sections of gold nanoshell carriers. In the UV–vis absorbance spectra, the plasmon resonance wavelengths correspond to nanoshell thicknesses (60 nm core diameter): (iv) 20 nm, (v) 10 nm, (vi) 5 nm [17••,18].

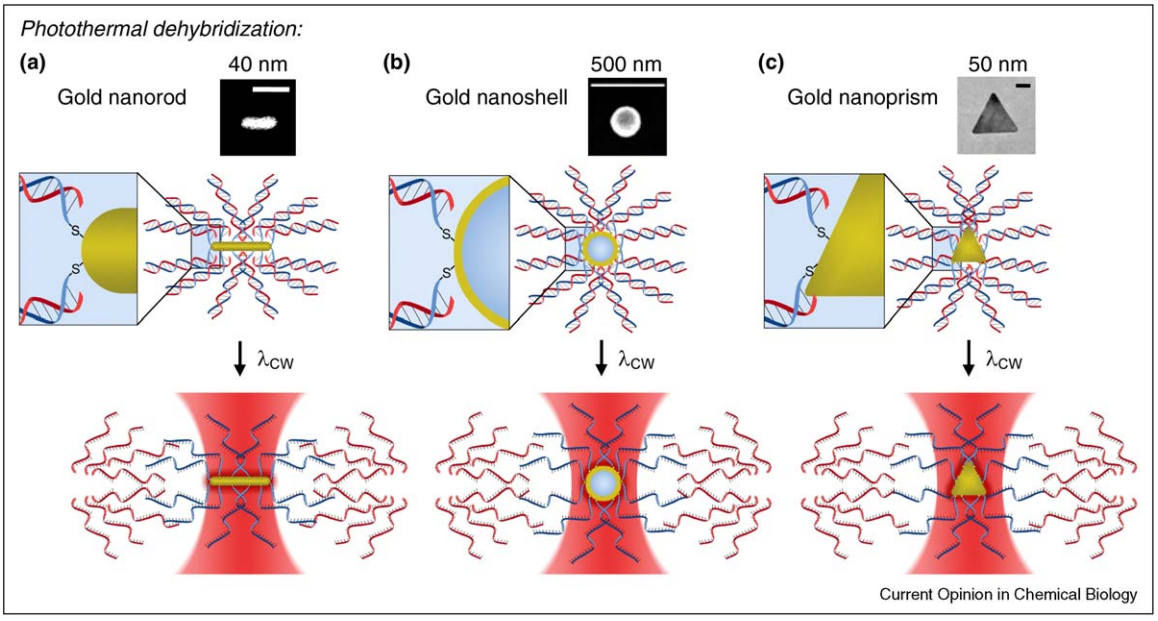
Table 1

Carrier and cargo strategies for nanoplasmonic technologies

Genetic cargo	Target	Cargo length	Cell type	Carrier type	Carrier size	Carrier plasmon resonance	Surface conjugation	NIR light wavelength	Light type	Mechanism	Ref.
dsDNA	ERBB2	15 bp	Human BT474	Gold nanorod	*l* = 50 nm *d* = 15 nm	780 nm	Thiol-gold bond	785 nm	CW	Antisense DNA dehybridization; gene silencing of ERBB2 in BT474 cells	[13••]
dsDNA	Unspecified	30–70 bp	n/a	Gold nanoshell	*d* = 120 nm	800 nm	Thiol-gold bond	800 nm	CW	Antisense DNA dehybridization	[17••]
dsDNA	Unspecified	15 bp	n/a	Gold nanoprism	*l* = 120 nm *t* = 7.5 nm	1200 nm	Thiol-gold bond	1064 nm	CW	Antisense DNA dehybridization	[19•]
ssDNA	Unspecified	40 bases	n/a	Gold nanorod	*l* = 44 nm *d* = 11 nm	800 nm	Thiol-gold bond	800 nm	Pulse	ssDNA release	[23••]
ssDNA	Unspecified	40 bases	n/a	Gold nanorod	l = 89 nm *d* = 17 nm	1100 nm	Thiol-gold bond	1100 nm	Pulse	ssDNA release	[23••]
Linearized plasmid DNA	EGFP-N1	4700 bp	Human HeLa	Gold nanorod	*l* = 42 nm *d* = 11 nm	782 nm	Thiol-gold bond	800 nm	Pulse	Linearized plasmid release; Induction of EGFP-N1 expression in HeLa cells	[24]
Circular plasmid DNA	EGFP-C1	7000 bp	n/a	Gold nanorod	*l* = 65 nm *d* = 11 nm	900 nm	Electrostatic interaction	1064 nm	Pulse	Circular plasmid release	[28]
Circular plasmid DNA	Luciferase	Unspecified	n/a	Gold nanorod	*l* = 65 nm *d* = 11 nm	900 nm	Electrostatic interaction	1064 nm	Pulse	Circular plasmid release; *in vitro* luciferase expression assay	[27]
siRNA	EGFP-N1	25 bp	Mouse C166-EGFP	Gold hollow nanoshell	*d* = 40 nm	800 nm	Thiol-gold bond	800 nm	Pulse	siRNA release; Gene silencing of EGFP-N1 inC166-EGFP cells	[25••]
siRNA	NF-κB p65	21 bp	Human HeLa	Gold hollow nanoshell	*d* = 40 nm	800 nm	Thiol-gold bond	800 nm	Pulse	siRNA release; Gene silencing of NF-κB p65 in HeLa xenografts of mice	[26••]

Abbreviations: continuous-wave (CW), length (*l*), diameter (*d*), thickness (*t*), base pairs (bp).

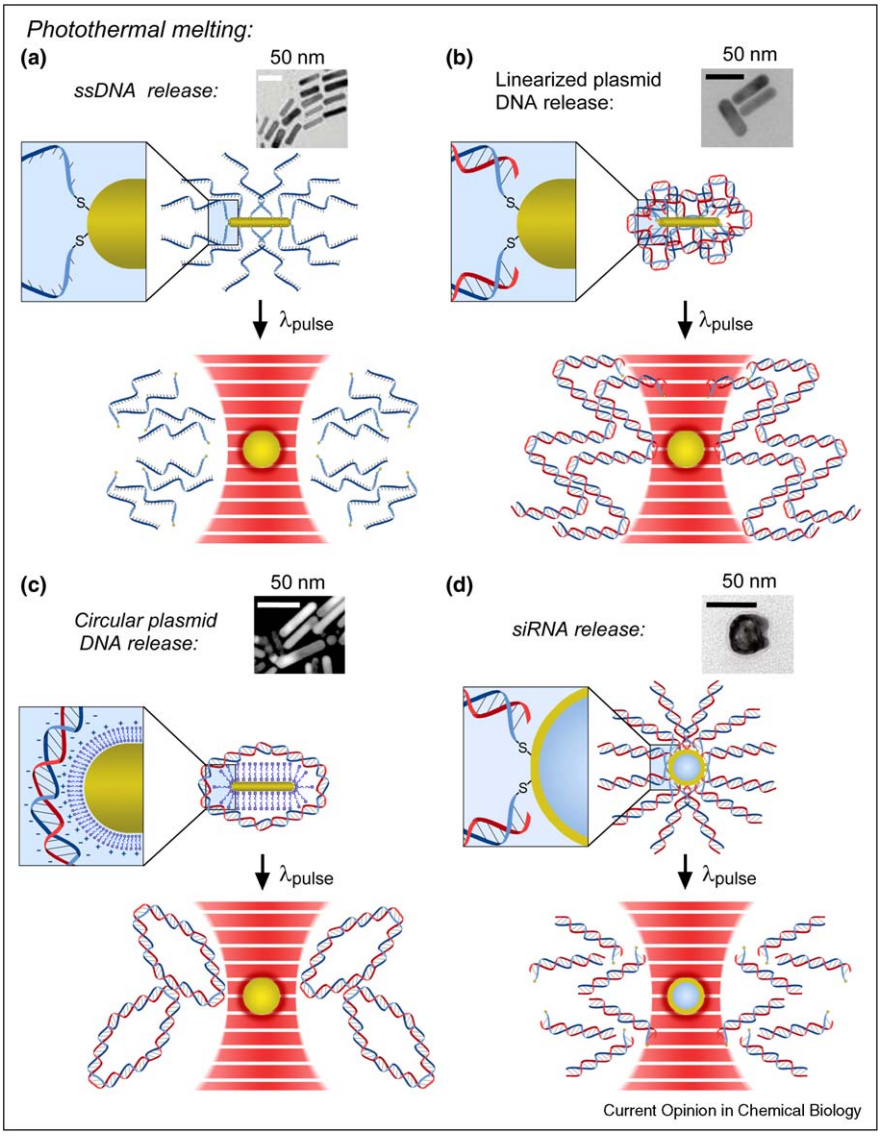
Figure 3



Photothermal dehybridization using continuous-wave illumination. **(a)** Scanning electron microscopy image of a gold nanorod carrier [13••]. The duplex of thiol-modified sense and antisense DNA is covalently bound to the gold nanorod carrier. In the presence of continuous-wave illumination, the antisense DNA strands dehybridize while the thiol-modified sense strands remain attached to the carrier. **(b)** Scanning electron microscopy image of a gold nanoshell carrier [17••]. The duplex of thiol-modified sense and antisense DNA is covalently bound to the gold nanoshell carrier. In the presence of continuous-wave illumination, the antisense DNA strands dehybridize while the thiol-modified sense strands remain attached to the carrier. **(c)** Scanning electron microscopy image of a gold nanoprism carrier [19•]. The duplex of thiol-modified sense and antisense DNA is covalently bound to the gold nanoprism carrier. In the presence of continuous-wave illumination, the antisense DNA strands dehybridize while the thiol-modified sense strands remain attached to the carrier.

release their cargo to freely interact with the local environment. Several strategies, employing different carrier and cargo types, have been demonstrated (Table 1). For example, it has been shown that short single-stranded DNA, otherwise known as antisense DNA, can be hybridized to a thiolated complementary sense strand, bound to a gold nanorod's surface through the gold-thiol covalent bond, and photothermally dehybridized using continuous-wave incident light that is matched to the plasmon resonance wavelength of the gold nanorods [13••] (Figure 3a). Antisense DNA has also been photothermally dehybridized from gold nanoshells [17••] (Figure 3b) and gold nanoprisms [19•] (Figure 3c) using continuous-wave illumination. This strategy of photothermal dehybridization using continuous-wave illumination offers several notable advantages. Firstly, no chemical modifications are made to the antisense DNA strand itself since a thiolated complementary strand is used to directly conjugate to the GNP's surface. Because chemical modifications can interfere with nucleic acid functionality and gene silencing efficacy, unmodified antisense DNA is highly desirable. Secondly, gold-thiol covalent bonds are stable after illumination, such that the GNP's surface remains covered with the thiolated complementary sense strands. With respect to cytotoxicity, this surface coating of complementary strands after illumination is critical. While the gold core is widely accepted as being biocompatible, bare GNPs have been shown to interact with proteins and induce mis-folding at physiological conditions [20•]. Maintaining surface coverage with complementary strands after illumination also prevents reattachment of antisense DNA strands back onto the GNP carrier since rehybridization events are thermodynamically unfavorable due to steric hindrances and electrostatic repulsive forces at the GNP's surface [21]. Finally, the structural integrity of GNPs is uncompromised after illumination. Maintaining structure after illumination is crucial for *in vivo* applications, where the size, geometry, coating material, and core material of nanoparticles are precisely designed and carefully characterized for proper biodistribution [22]. Maintaining structure after illumination also allows unique nano-scale optical properties to be retained, thereby enabling the same incident light wavelength to be used. Repetitive or finely graded release of cargo is conceivable for future applications requiring precise temporal patterns of cargo release. For example, the delivery of a drug at its most effective concentration profile is an on-going challenge. When a drug is introduced, its concentration steadily decreases, producing a large concentration swing between the intervals at which the drug is administered. Photothermal cargo release from GNPs can be used to

Figure 4

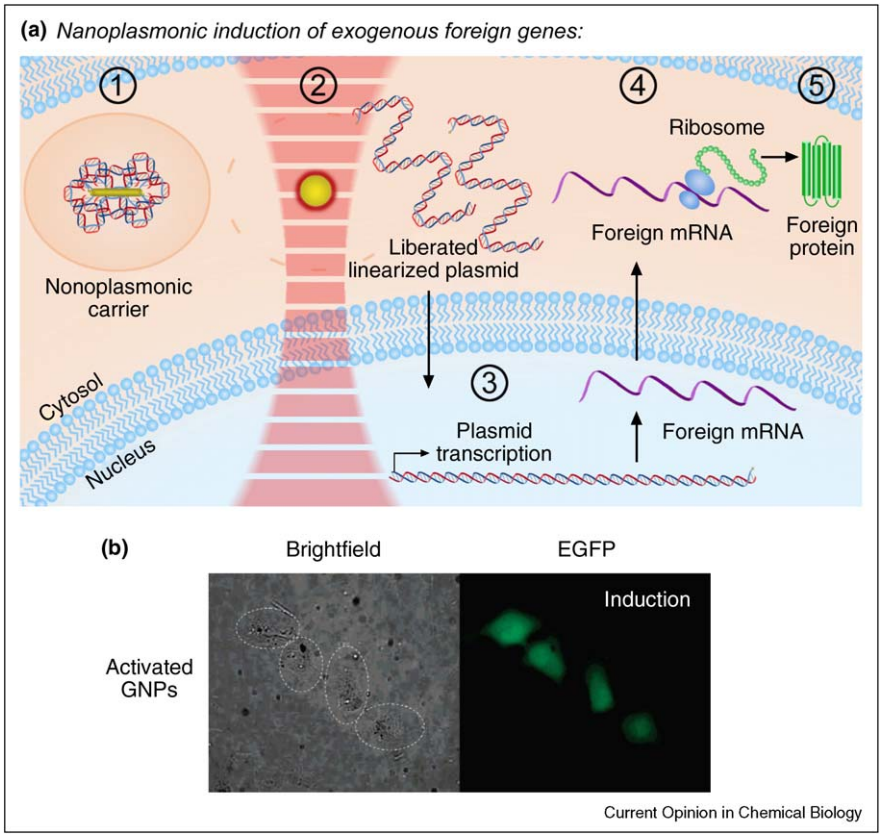


Photothermal melting using pulsed illumination. **(a)** Scanning electron microscopy image of gold nanorod carriers [23••]. Thiol-modified antisense DNA is covalently bound to the gold nanorod carrier. Pulsed illumination is used to photothermally melt gold nanorods into spheres, thereby destabilizing the thiol-gold bond and releasing the antisense DNA. **(b)** Scanning electron microscopy image of gold nanorod carriers [24]. Thiol-modified linearized plasmid DNA is covalently bound to the gold nanorod carrier. Pulsed illumination is used to photothermally melt gold nanorods into spheres, thereby destabilizing the thiol-gold bond and releasing the plasmid. **(c)** Scanning electron microscopy image of gold nanorod carriers [27,28]. Circular plasmid DNA is electrostatically attached to the gold nanorod carrier. Pulsed illumination is used to photothermally melt gold nanorods into spheres, thereby destabilizing the electrostatic interaction and releasing the plasmid. **(d)** Scanning electron microscopy image of gold hollow nanoshell carriers [25••]. Thiol-modified siRNA is covalently bound to the gold hollow nanoshell carrier. Pulsed illumination is used to photothermally melt gold hollow nanoshells, thereby destabilizing the thiol-gold bond and releasing the siRNA.

temporally control drug release for delivering programmable drug concentration profiles that are tailored to specific patients and applications. In addition to temporal control, spatial control of photothermal cargo release from GNPs can also be utilized for localized gene therapy at the tumor location. Genetic abnormalities at the tumor location can be corrected without adversely affecting neighboring, functional organs.

Alternatively, thiol-modified antisense DNA can be covalently bound to gold nanorods directly. It has been demonstrated that pulsed incident light can photothermally melt gold nanorods into spheres, thereby destabilizing the gold-thiol covalent bond and releasing thiol-modified antisense DNA [23••] (Figure 4a). Photothermal melting has also been effective at releasing thiol-modified linearized plasmid DNA from gold nanorods [24]

Figure 5



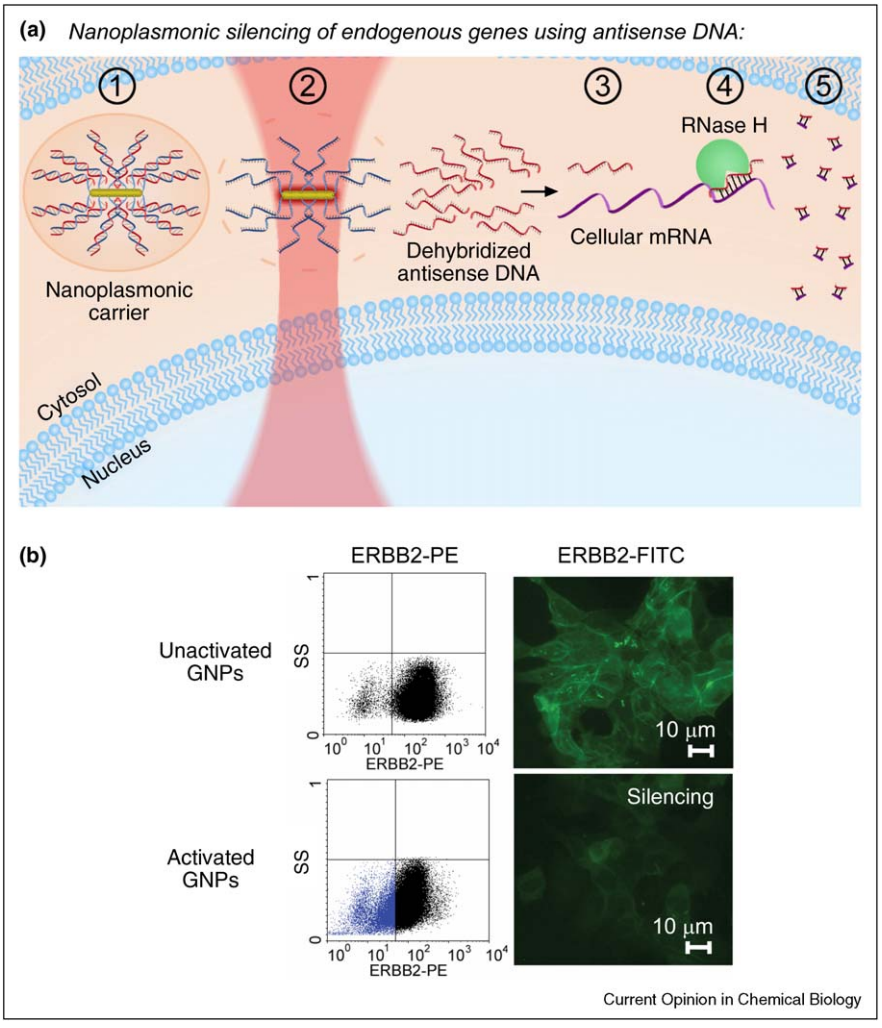
Nanoplasmonic induction of exogenous foreign genes. **(a)** Concept of nanoplasmonic induction of exogenous EGFP in HeLa cells. In step 1, thiol-modified, linearized EGFP-N1 plasmid DNA is covalently bound to gold nanorods and internalized in HeLa cells. In step 2, pulsed illumination is used to photothermally melt gold nanorods into spheres, thereby destabilizing the thiol-gold bond and releasing the plasmid into the cytosol. In step 3, the plasmid transports to the nucleus and foreign mRNA is then transcribed from the plasmid. In step 4, the foreign mRNA is exported out of the nucleus and translated into the corresponding amino acids. In step 5, this primary sequence of amino acids is then folded into the final EGFP protein structure. **(b)** Brightfield and fluorescent image after nanoplasmonic induction of EGFP in HeLa cells [24].

(Figure 4b) and thiol-modified siRNA from gold hollow nanoshells [25••,26••] (Figure 4d). Additionally, photothermal melting has been shown to destabilize electrostatically attached circular plasmid DNA from gold nanorods [27,28] (Figure 4c). This strategy of photothermal melting using pulsed illumination presents several unique functionalities. Firstly, photothermal melting ensures complete release of the entire cargo. Secondly, the resulting shape change from rods to spheres enables a distinct and defined shift in the plasmon resonance wavelength. A second light source can be easily employed to re-match to the plasmon resonance wavelength of these shape-transformed GNPs. These functionalities may be useful for future studies that involve multifunctional release and detection schemes. Conceivably, cargo can be completely released from gold nanorods using pulsed illumination, and after a shape transformation, binding activity can then be detected using the now bare gold spherical surface. In the case of gold nanoshells, their silica cores deform after pulsed illumination due to high temperature heating (lattice temperatures reaching ~1064 °C, the melting point of bulk gold) [29]. As a result, their plasmon resonance wavelength can also shift after illumination, but in a less distinct and controlled manner compared with photothermally melted gold nanorods. Re-matching the incident light wavelength can therefore be considerably more difficult if the plasmon resonance wavelength cannot be easily controlled after shape transformation.

Nanoplasmonic optical control

GNPs enable 'nanoplasmonic optical control' of genetic activities with sequence-specificity and spatiotemporal resolution. GNPs carrying genetic cargo are internalized in living cells by endocytosis. While attached to their carriers, the cargo is temporarily 'inactive'. The cargo is also protected from degradation by nucleases due to steric hindrances between the tightly packed cargo at the GNPs' surface [30]. Illumination matched to the plasmon resonance wavelength of GNPs is used to 'activate' cargo by photothermally disrupting encapsulating endosomes [13••,31–34] and photothermally releasing free cargo into

Figure 6



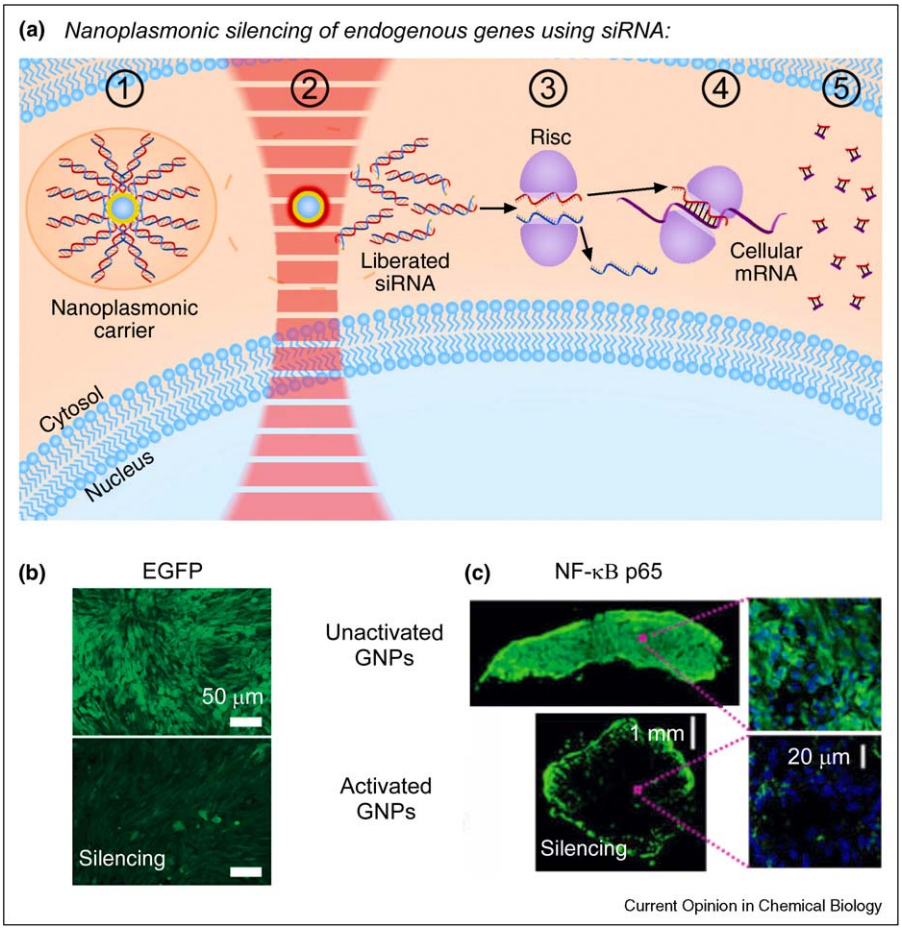
Nanoplasmonic silencing of endogenous genes using antisense DNA. **(a)** Concept of nanoplasmonic silencing of endogenous ERBB2 in BT474 cells using antisense DNA. In step 1, the duplex of thiol-modified sense and unmodified antisense DNA is covalently bound to gold nanorods (GNPs) and internalized in BT474 cells. In step 2, in the presence of continuous-wave illumination, antisense DNA strands dehybridize and release into the cytosol, while the thiol-modified sense strands remain attached to the gold nanorods. In step 3, the unbound antisense DNA then binds to a portion of the mature mRNA corresponding to ERBB2. In step 4, once the mRNA/antisense DNA heteroduplex is formed, it is recognized by RNase H enzymes. In step 5 RNase H enzymes degrade the heteroduplex, thereby silencing ERBB2. **(b)** Flow cytometric data and immunofluorescent images showing nanoplasmonic silencing of ERBB2 using activated GNPs in BT474 cells. Flow cytometric data and immunofluorescent images of the control showing no silencing using unactivated GNPs [13••].

the cytosol. In this way, exogenous foreign genes can be introduced and expressed on-demand. Endogenous intracellular genes can also be silenced on-demand.

Nanoplasmonic induction of exogenous EGFP expression has been demonstrated in human HeLa cervical carcinoma cells [24]. This mechanism is illustrated in Figure 5a. Thiol-modified, linearized EGFP-N1 plasmid DNA is covalently bound to gold nanorods and internalized in HeLa cells. Pulsed illumination is used to photothermally melt gold nanorods into spheres, thereby destabilizing the thiol-gold bond and releasing the plasmid into the cytosol. The plasmid transports to the nucleus. Exogenous mRNA is then transcribed from the plasmid, exported out the nucleus, and translated into the corresponding EGFP protein. The nanoplasmonic gene induction of EGFP expression is shown in the fluorescent image in Figure 5b [24]. Thus, expression of exogenous foreign genes can be induced on demand using nanoplasmonic optical control.

In addition to inducing exogenous expression, endogenous intracellular genes can also be silenced on demand. Nanoplasmonic silencing of endogenous ERBB2 expression using antisense DNA has been demonstrated in human BT474 breast carcinoma cells [13••]. As illustrated

Figure 7



Nanoplasmonic silencing of endogenous genes using siRNA. **(a)** Concept of nanoplasmonic silencing of EGFP using siRNA in C166 cells stably expressing EGFP. In step 1, thiol-modified siRNA targeting EGFP is covalently bound to gold hollow nanoshells (GNPs) and internalized in endothelial cells stably expressing EGFP. In step 2, pulsed illumination is used to photothermally melt gold hollow nanoshells, thereby destabilizing the thiol-gold bond and releasing siRNA into the cytosol. In step 3, the unbound siRNA sequentially triggers cytosolic RISC to unwind the duplex. In step 4, cytosolic RISC binds single-stranded RNA to its complementary mRNA. In step 5, mRNA is degraded and EGFP expression is silenced. **(b)** Fluorescent image showing nanoplasmonic silencing of EGFP using activated GNPs in C166 cells stably expressing EGFP. Fluorescent image of the control showing no silencing using unactivated GNPs [25••]. **(c)** Fluorescent image showing nanoplasmonic silencing of NF-κB p65 using siRNA in HeLa xenografts in mice [26••].

in Figure 6a, the duplex of thiol-modified sense and unmodified antisense DNA is covalently bound to gold nanorods and internalized in BT474 cells. In the presence of continuous-wave illumination, antisense DNA strands dehybridize and release into the cytosol, while the thiol-modified sense strands remain attached to the gold nanorods. The unbound antisense DNA then binds to a portion of the mature mRNA corresponding to ERBB2. Once the mRNA/antisense DNA heteroduplex is formed, it is recognized and degraded by RNase H enzymes in the cytosol, thereby silencing ERBB2. As seen in Figure 6b, nanoplasmonic silencing of ERBB2 expression is qualitatively demonstrated using immunofluorescence imaging and quantitatively shown using flow cytometry [13••]. To ensure light illumination itself and photothermally generated heat had no adverse effects on cells, viability studies were conducted using a fluorescence assay [13••]. Since RNase H is ubiquitously present in both the cytosol and the nucleus [35,36], an alternative model [35] suggests that unbound antisense DNA transports to the nucleus. In the nucleus, the antisense DNA binds to the premature mRNA. Once this heteroduplex is formed, it is recognized and degraded by RNase H enzymes in the nucleus.

Intracellular genes can also be silenced on-demand using siRNA. siRNA also signals the degradation of specifically targeted mRNA; however, degradation is through a different mechanism compared to antisense DNA. Nanoplasmonic silencing of EGFP expression using siRNA has been shown in mouse endothelial C166 cells stably expressing EGFP [25••]. In Figure 7a, thiol-modified

siRNA targeting EGFP is covalently bound to gold hollow nanoshells and internalized in endothelial cells stably expressing EGFP. Pulsed illumination is used to photothermally melt gold hollow nanoshells, thereby destabilizing the thiol-gold bond and releasing siRNA into the cytosol. The unbound siRNA sequentially triggers cytosolic RNA-inducing silencing complex (RISC) to unwind the duplex, binds to complementary mature mRNA, and silences EGFP expression. Nanoplasmonic silencing of EGFP in cells stably expressing EGFP is shown in the fluorescent images in Figure 7b [25••]. Nanoplasmonic silencing of endogenous genes using siRNA has also been demonstrated *in vivo* [26••]. Thiol-modified siRNA targeting NF-κB p65 is covalently bound to hollow gold nanoshells, internalized in Hela cells, and transplanted in mice as xenografts. Pulsed illumination is used to photothermally melt gold hollow nanoshells, thereby destabilizing the thiol-gold bond and releasing the siRNA. Nanoplasmonic silencing of NF-κB p65 is shown in the fluorescent images in Figure 7c [26••]. Since key components of RISC have been discovered in both the nucleus and cytosol [37], another model [38] suggests that unbound siRNA transports to the nucleus. In the nucleus, siRNA triggers RISC to unwind the duplex, binds to premature mRNA, and silences gene expression. Other models [38] also propose that RISC actively transports siRNA from the cytosol to the nucleus for degradation.

Future directions and conclusions

In order to create widespread acceptance of nanoplasmonic technologies by the biology community, optimal plasmonic properties, colloidal stability, and internalization efficiencies of nanoplasmonic technologies must be demonstrated under physiological conditions. Multilayering cargo-carriers with cationic lipids have been shown to improve colloidal stability and internalization under physiological conditions [25••]. Thus, in addition to the importance of cargo conjugation to carriers, additional protective surface coating materials also deserve critical attention [39,40]. It must also be demonstrated to the biology community that nanoplasmonic technologies are indeed biocompatible and nontoxic. Toxicology, biodistribution, and environmental studies on the long-term effects of nanoplasmonic materials are necessary to promote these technologies and move the fields of nanoplasmonics-enabled quantitative biology and on-demand gene therapeutics forward.

Finally, both extracellular and intracellular controls are necessary to systematically perturb living systems. This integration of extracellular and intracellular controls can provide unprecedented insight into the dynamic nature of living systems for gene regulation and cell reprogramming. For example, microfluidic technologies can provide precise spatial and temporal control of the local external environment of a cell while nanoplasmonic technologies systematically perturb the intracellular space. Dynamic activities can be controlled and biological questions can be answered that were otherwise previously impossible using conventional methods.

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- • of special interest
- •• of outstanding interest

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