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# Biomolecular plasmonics for quantitative biology and nanomedicine

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Free electrons in a noble metal nanoparticle can be resonantly excited, leading to their collective oscillation termed as a surface plasmon. These surface plasmons enable nanoparticles to absorb light, generate heat, transfer energy, and re-radiate incident photons. Creative designs of nanoplasmonic optical antennae (i.e. plasmon resonant nanoparticles) have become a new foundation of quantitative biology and nanomedicine. This review focuses on the recent developments in dual-functional nanoplasmonic optical antennae for label-free biosensors and nanoplasmonic gene switches. Nanoplasmonic optical antennae, functioning as biosensors to significantly enhance biochemical-specific spectral information via plasmon resonance energy transfer (PRET) and surface-enhanced Raman spectroscopy (SERS), are discussed. Nanoplasmonic optical antennae, functioning as nanoplasmonic gene switches to enable spatiotemporal regulation of genetic activity, are also reviewed. Nanoplasmonic molecular rulers and integrated photoacoustic–photothermal contrast agents are also described.

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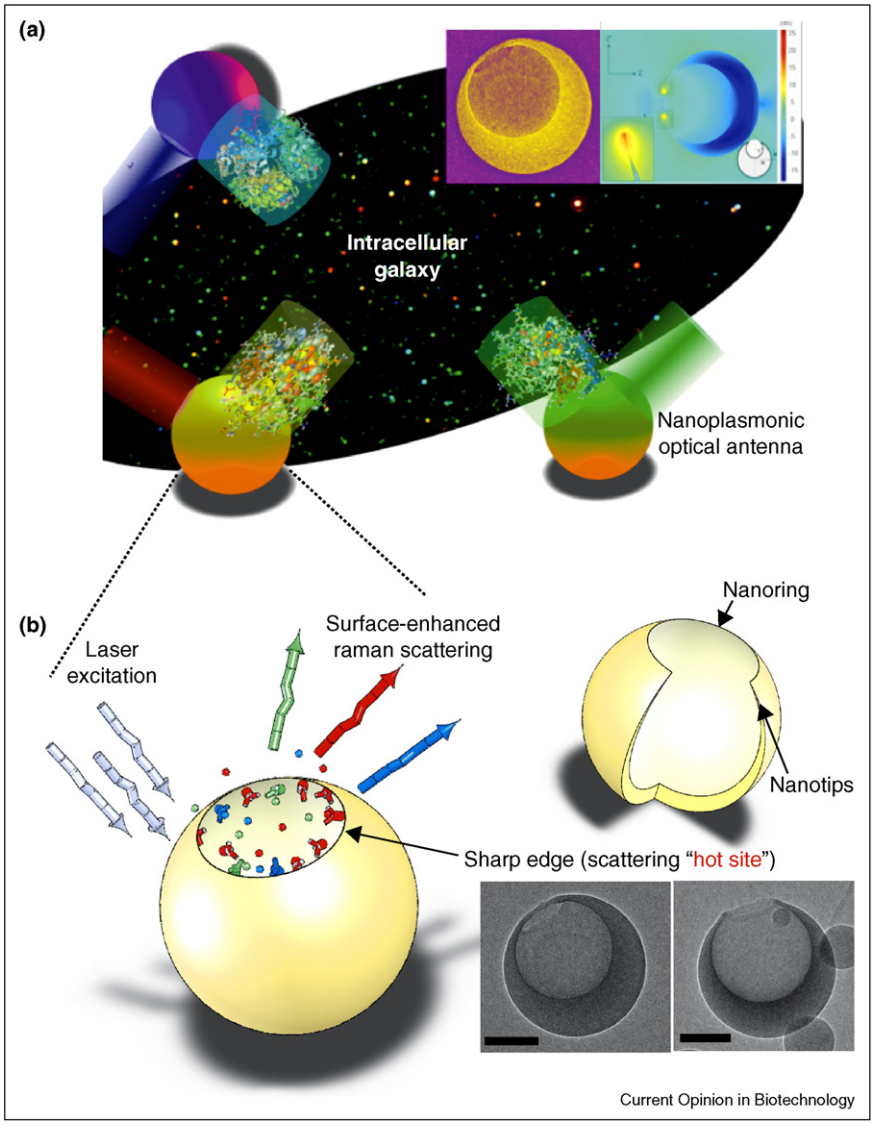
## Introduction

Our understanding of biological systems is increasingly dependent on our ability to visualize and precisely measure the dynamics of molecular, biological, biophysical events with high spatial and temporal resolution, within the context of a living cell. The living cell dynamically responds to its perpetually changing environment, such that signaling proteins, transcription factors, and enzymes are constantly synthesized, transported from one organelle to another, and finally shuttled to their appropriate locations to give rise to cell function. The intracellular distribution of these molecular complexes is spatially non-uniform and dynamically changing over time in response to environmental cues [1]. Quantitative knowledge of the intracellular biochemical distribution is crucial for understanding intracellular organization and function in developmental processes, growth, differentiation, apoptosis, and disease. In this regard, the development of nanoplasmonic optical antennae for cellular and molecular imaging techniques, as well as nanoplasmonic gene switches, are of considerable interest in many areas of research, from molecular and cellular biology to molecular diagnostics to nanomedicine. Label-free nanoplasmonic optical antennae, also referred to as nanomechanical probes, offer multiple advantages over traditional molecular imaging techniques: stability, biocompatibility, selectivity, and spectroscopic imaging capability. By visualization and wireless communication via nanoplasmonic optical antennae within a living cell, we can obtain quantitative spectral snapshots of what we refer to as the *intracellular galaxy* (Figure 1a).

By focusing on a specific antenna within this intracellular galaxy, we can probe localized biochemical data to explore the living intracellular environment (Figure 1b). Intracellular manipulation in conjunction with real-time imaging can provide unparalleled insight into the dynamic biochemical distribution as a result of local environmental changes. Recent advancements in nanotechnology and nanoplasmonics now enable subnanometer and nanometer tools to directly interface with intracellular processes. By focusing electromagnetic fields down to dimensions smaller than the diffraction limit, nanoplasmonic optical antennae – functioning as nanoplasmonic gene switches – enable spatiotemporally precise regulation of genetic activity to give rise to location-specific function [2••,3••,4••]. Nanoplasmonic optical antennae – functioning as biosensors – also focus electromagnetic fields to significantly enhance spectral information for plasmon resonance energy transfer (PRET) [5••,6••,7••], surface-enhanced Raman spectroscopy (SERS) [8–16], nanoplasmonic molecular rulers [17••], and integrated photoacoustic–photothermal contrast agents [18••]. In this way, quantitative spectral snapshots of the intracellular biochemical distribution can be obtained over time as function of changes in the local environment. In this review, the dual functions of nanoplasmonic optical antennae, as nanoplasmonic gene switches and biosensors, for quantitative biology and nanomedicine, are discussed (Figure 2).

**Figure 1**

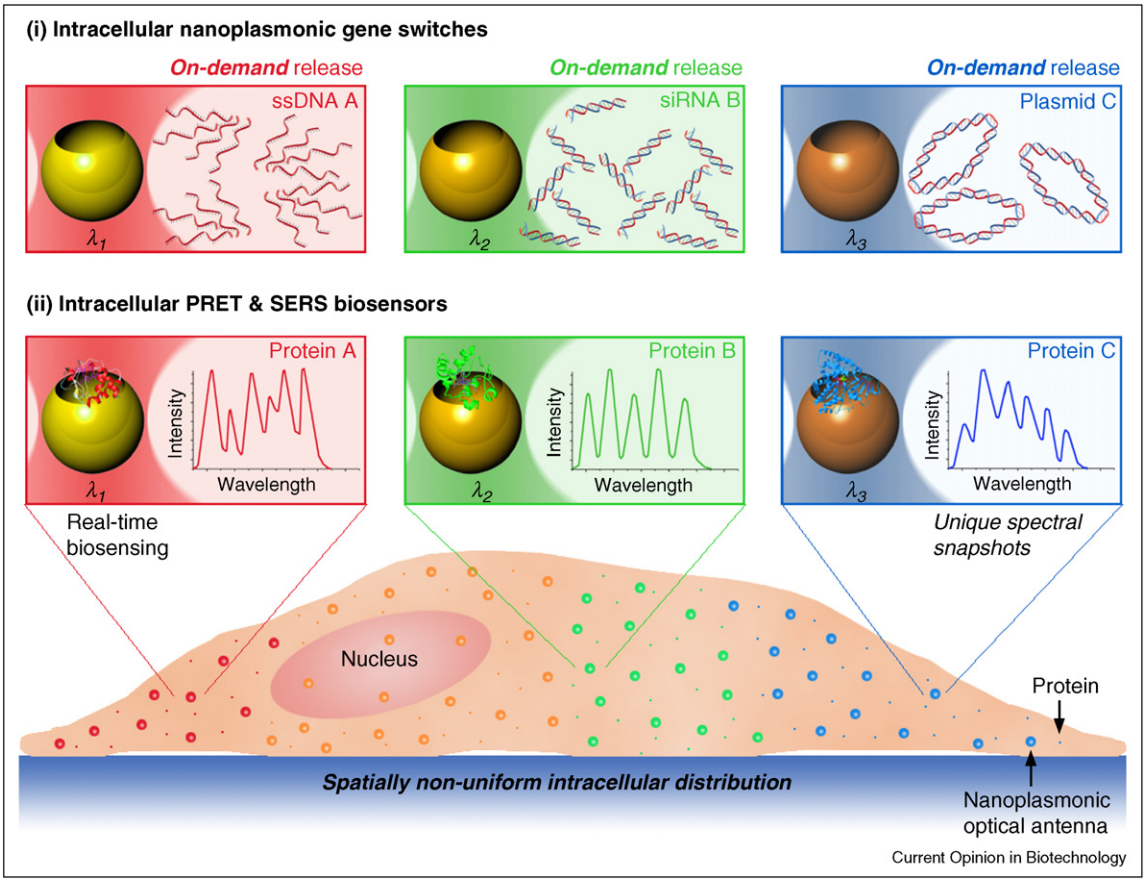



Wireless communication via nanoplasmonic optical antennae. **(a)** Concept of visualization and wireless communication between the real world and the intracellular galaxy using nanoplasmonic optical antennae. Such antennae enable label-free spectroscopic imaging by methods such as plasmon resonance energy transfer (PRET) and surface-enhanced Raman scattering (SERS). **(b)** A gold nanocrescent antenna within the intracellular galaxy. The gold surface is functionalized with target ligands to recognize specific molecular complexes. High local field enhancement is achieved owing to the lightning rod effect at the sub-10 nm sharp edges and plasmon coupling between the closely spaced crescent tips. The nanocrescent antenna enhances the Raman scattering intensity of molecular complexes in proximity of the antenna. Transmission electron microscope images of gold nanocrescent antenna. The scale bar is 100 nm.

## Dual functions of nanoplasmonic optical antennae

Dual-functional nanoplasmonic optical antennae are powerful biological tools for on-demand gene regulation and label-free biosensing. A nanoplasmonic optical antenna receives, focuses, and transmits incoming optical and near-infrared (NIR) electromagnetic radiation as an analogous, classical antenna receives, focuses, and transmits radio-frequency electromagnetic radiation. A nanoplasmonic optical antenna focuses incoming electromagnetic radiation down to dimensions smaller than the diffraction limit by coupling the incoming electromagnetic radiation to the localized excitation of conduction electrons at the conductor–dielectric interface. This antenna effect is prominent when the incoming electromagnetic radiation is matched to the plasmon resonance of the nanoplasmonic optical

**Figure 2**




Concept of nanoplasmonic optical antennae for on-demand gene regulation and real-time imaging. The intracellular distribution is spatially non-uniform and dynamically changing over time in response to environmental cues. Nanoplasmonic optical antennae – functioning as nanoplasmonic gene switches – enable on-demand and spatially precise gene regulation to give rise to location-specific function. Switches with distinct plasmon resonance wavelengths can selectively release cargo (ssDNA, siRNA, plasmid DNA) using incident light wavelengths $\lambda_1$, $\lambda_2$, and $\lambda_3$. Nanoplasmonic optical antennae – functioning as biosensors – enhance spectral information for PRET and SERS. 'Spectral snapshots' of the dynamically changing intracellular biochemical distribution can be obtained over time using multiple nanoplasmonic optical antennae with distinct plasmon resonance wavelengths matched to incident light wavelengths $\lambda_1$, $\lambda_2$, and $\lambda_3$.

antenna, and as a result, the conduction electrons at the conductor–dielectric interface of the nanoplasmonic optical antenna collectively oscillate in phase on resonance.

Nanoplasmonic optical antennae, functioning as nanoplasmonic gene switches, utilize the antenna effect to convert absorbed light energy into surface-localized heat, otherwise known as photothermal conversion [19–21]. For efficient photothermal conversion, nanoplasmonic gene switches are geometrically designed such that their absorption cross-sections dominate over their scattering cross-sections [22]. Therefore, when the incoming electromagnetic radiation is coupled to the localized excitations of conduction electrons at the conductor–dielectric interface of the nanoplasmonic gene switch, these conduction electrons are excited from the ground (unexcited) state. Energy is then transferred from the excited conduction electrons to the lattice through electron–phonon collisions. As the system relaxes back to the ground state, the absorbed energy is finally dissipated as heat through phonon–phonon interactions. This photothermally generated heat transfer from the surface of nanoplasmonic gene switches' to the surrounding cellular environment is highly localized, decaying exponentially within a few nanometers [3••,19,23] and therefore is thought to have minimal adverse effects on cells. Additionally, the plasmon resonance of the nanoplasmonic gene switches is also tuned to the NIR, since tissues and cells are essentially transparent in the NIR wavelength regime [24]. Nanoplasmonic gene switches utilize photothermally generated heat to liberate surface-bound cargo, such as single-stranded DNA, short interfering RNA (siRNA), or plasmid DNA, *in a highly localized manner*.

Nanoplasmonic optical antennae can also be employed as label-free biosensors. To increase biosensor sensitivity, the geometry and structure of biosensors are specifically designed to substantially enhance the antenna effect by utilizing (1) the plasmon coupling between closely positioned geometrical features of the biosensor and (2) the lightning rod effect [25] at sharp geometrical features of the biosensor. In contrast to nanoplasmonic gene switches, biosensors are designed such that their scattering cross-sections dominate over their absorption cross-sections in order to substantially enhance scattering spectra of molecular complexes in proximity of the biosensors. Therefore, when the incoming electromagnetic radiation is coupled to the localized excitations of conduction electrons at the conductor–dielectric interface of the biosensor, intense scattered radiation is generated. Molecules in proximity undergo a momentary transition from the ground state to a virtual state. Transitions are related to the biochemical composition. Enhanced Raman scattering, utilized in SERS, results when the transition is immediately to a vibrational level of the ground state. Enhanced Rayleigh scattering, utilized in PRET, results when the transition is immediately back to the ground state. In this way, biosensors enable a highly sensitive and label-free spectral readout of the biochemical composition of the local environment.

## Nanocrescent antennae for SERS

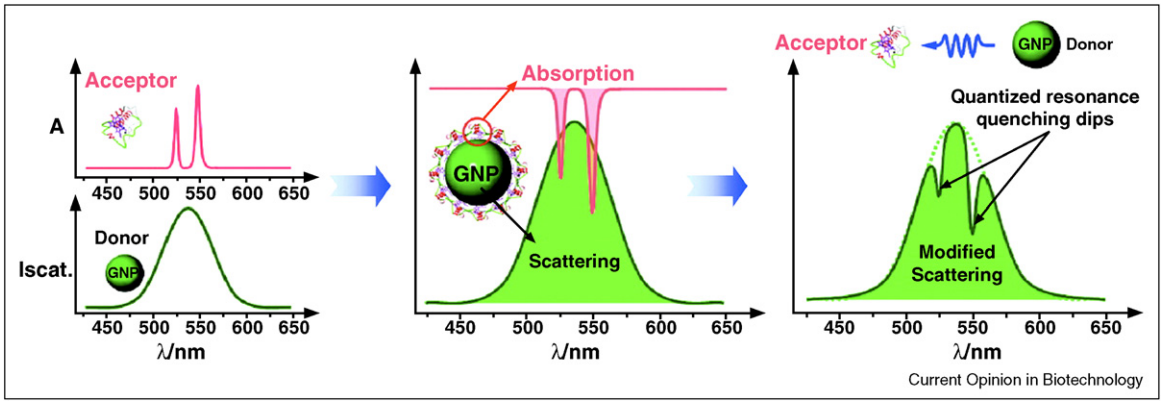
For biological and biomedical applications, the ideal biologically functional nanoplasmonic optical antenna must exhibit non-toxicity, plasmon resonance in the NIR regime, high local field enhancement, and mobility under physiological conditions. Therefore, the material, size, and structure of the nanoplasmonic optical antenna are designed to simultaneously achieve the aforementioned features. Gold is selected since it is widely accepted as a biocompatible material. The nano-scale size and dimensions of the nanoplasmonic optical antenna are selected to achieve plasmon resonance tunability in the NIR regime between 700 and 1300 nm, where tissues and cells are essentially transparent [24]. Finally, a nanocrescent structure is specifically designed to substantially enhance the antenna effect by utilizing the plasmon coupling between closely spaced crescent tips and the lightning rod effect [25] at the sharp geometrical features of the nanocrescent. A systematic numerical analysis has been used to optimize the geometry of the nanocrescent to show high local field enhancement and plasmon resonance tunability in the NIR regime. A finite element model has been utilized to solve the time-harmonic Maxwell equations over the domain-of-interest. Significant plasmon band tuning can be seen by varying the overall nanocrescent size or by varying the cavity offset, while keeping the other parameters constant [26]. Gold nanocrescent antennae have been shown to significantly enhance the Raman scattering of Rhodamine 6G by a Raman enhancement factor of larger than $10^{10}$ [12]. Since the local field enhancement is dependent on the orientation of asymmetrical antennae with respect to the incoming electromagnetic radiation, magnetic-gold nanocrescent antennae have also been created and externally controlled using magnetic fields. High local field enhancement has been demonstrated when the nanocrescent antenna's structural symmetry line is parallel with the propagation direction of the incoming NIR electromagnetic radiation [11].

## Intracellular nanoplasmonic gene switches

Nanoplasmonic gene switches enable temporal and spatial regulation of intracellular genetic activity. Using remote-controlled NIR light as a trigger to release free oligonucleotides and 'activate' their functionality, endogenous intracellular genes can be silenced on demand. In addition to the inhibitory effects, exogenous foreign genes can also be introduced and expressed on demand.

Because of their large surface-to-volume ratio, nanoplasmonic gene switches are ideal carriers of oligonucleotides, such as *single-stranded DNA* (*ssDNA*), *siRNA*, *and plasmid DNA*. For example, short ssDNA, otherwise known as antisense DNA, can be hybridized to thiolated complementary sense DNA and bound to the switch's surface through the gold–thiol covalent bond [3••]. While attached to their carriers, oligonucleotides are rendered inactive owing to steric hindrances between the tightly packed DNA. In the presence of continuous-wave incident light that is matched to their plasmon resonance wavelength, antisense DNA is photothermally dehybridized from its carrier to freely interact with the local environment. Antisense DNA has also been photothermally dehybridized from other antenna structures, such as gold nanoshells [27•] and gold nanoprisms [28•]. Release of circular plasmid DNA [29,30], linearized plasmid DNA [4••], siRNA [2••], and directly conjugated single-stranded DNA [31•] has also been demonstrated by photothermally melting the carrier. This strategy of photothermal dehybridization 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 switch'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 switch's surface remains covered with the thiolated complementary sense strands. With respect to cytotoxicity, this surface coating of complementary strands after illumination is crucial. While the gold core is widely accepted as being biocompatible, bare nanoparticles have been shown to interact with proteins and induce mis-folding at physiological conditions [32]. Maintaining surface coverage with complementary strands after illumination also prevents reattachment of

**Figure 3**




Mechanism of PRET. Plasmon resonance energy is transferred from nanoplasmonic optical antennae to cytochrome *c* biomolecules in proximity. Typical Rayleigh scattering spectrum of bare gold nanoparticles. Typical absorption spectra of cytochrome *c* biomolecules in bulk solution. When the plasmon resonance spectrum of the biosensors is intentionally matched to the absorption spectrum of biomolecules, plasmon resonance energy transfer (PRET) results in wavelength-specific quenching in the Rayleigh scattering spectra of the biosensors that is specific to the biomolecules (Ref. [7••]).

antisense DNA strands back onto the switch since rehybridization events are thermodynamically unfavorable owing to steric hindrances and electrostatic repulsive forces at the switch's surface [33]. Finally, the structural integrity of the switches is uncompromised after illumination. Maintaining structure after illumination 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. Maintaining structure after illumination is also 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 [34] and eventual environmental distribution [35].

Using antisense DNA-conjugated nanoplasmonic gene switches, endogenous intracellular genes can be silenced on demand. On-demand silencing of ERBB2 expression has been qualitatively demonstrated using immunofluorescence imaging and quantitatively shown using flow cytometry [3••]. Intracellular genes can also be silenced on demand using siRNA-conjugated nanoplasmonic gene switches [2••]. In addition to the inhibitory effects of interfering oligonucleotides, exogenous foreign genes can also be expressed on demand using plasmid-conjugated nanoplasmonic gene switches [4••]. In this way, nanoplasmonic gene switches can enable spatially precise regulation of intracellular activity to give rise to location-specific function.

## Intracellular PRET biosensors

In addition to on-demand gene regulation, nanoplasmonic optical antennae can also serve as label-free biosensors to significantly enhance spectral information for PRET. Plasmon resonance energy can be transferred from nanoplasmonic optical antennae to biomolecules in proximity. When the plasmon resonance spectrum of an antenna is intentionally matched to the absorption spectrum of the biomolecules, energy transfer by PRET [5••,6••,7••] results in wavelength-specific quenching in the Rayleigh scattering spectrum (Figure 3). For instance, when the plasmon resonance energy of the biosensors is transferred to adsorbed cytochrome *c*, wavelength-specific quenching is observed in the Rayleigh scattering spectrum of the biosensor [5••,7••]. The quenching positions exactly correspond to the absorbance peaks of cytochrome *c*.

Real-time production of cytochrome *c* in living HepG2 cells has been dynamically imaged using PRET spectroscopy [7••]. It is well known that cytochrome *c* is released from the mitochondria to the cytoplasm in response to pro-apoptotic stimuli, such as ethanol, owing to increased permeability of the outer membrane of the mitochondria [36]. Therefore, when biosensors are internalized into HepG2 cells and cells are then exposed to ethanol, the production of intracellular cytochrome *c* results in distinguishable wavelength-specific quenching in the scattering spectra over time. Highly sensitive and selective metal ion sensing has also been enabled by PRET spectroscopy [6••]. In addition to offering high spatial resolution owing to the small nanometer-scale size of the biosensor, this method is 100–1000 times more sensitive than organic reporter-based methods.

## Nanoplasmonic molecular rulers

Biosensors functioning as nanoplasmonic molecular rulers enable label-free measurement of DNA length, real-time kinetic studies of nuclease activity, and real-time detection

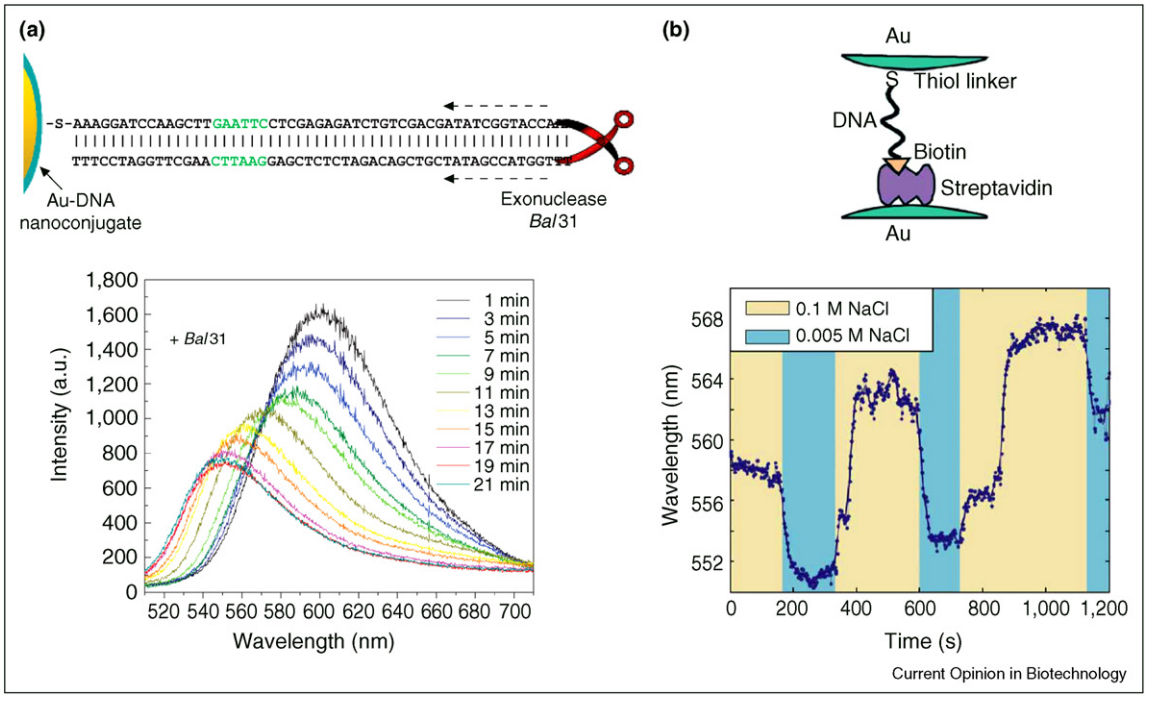
of specific binding activities between proteins and DNA. A nanoplasmonic molecular ruler utilizes a single gold nanoparticle with tethered double-stranded DNA containing cleavage sites for nucleases. DNA digestion by nucleases resulted in changes in the dielectric constant of the medium locally surrounding the gold nanoparticle. Therefore, changes in DNA length, due to nuclease activity, correlated to wavelength shifts in the plasmon resonance of the nanoparticle over time. An average plasmon wavelength shift of approximately 1.24 nm was observed per DNA base pair [17••]. Using this nanoplasmonic molecular ruler, nuclease enzymatic kinetics were studied in real-time. Nanoplasmonic molecular rulers are ideal for long-term kinetic studies because they do not suffer from photobleaching or blinking. Furthermore, the ability to resolve a single nanoparticle without the need for radioactive or fluorescent labeling also makes the integration of biosensors into microfluidic devices for high-throughput screening possible [16,37,38] (Figure 4).

A pair of gold nanoparticles can also be utilized as a nanoplasmonic molecular ruler to measure biomolecular distances between the nanoparticles on the basis of plasmon coupling. The biomolecular distance was set by a single-strand of DNA tethered between the pair of gold nanoparticles. The biomolecular distance was then be modulated by changing the ionic strength of the solution. Low salt concentrations resulted in the increase of electrostatic repulsion between the charged gold nanoparticles and therefore a blue shift in the plasmon resonance of the nanoparticle pairs [39••]. Plasmon coupling-based measurement of biomolecular distances is not limited to spherical nanoparticle pairs, but can also be potentially achieved using other geometries, such as gold nanorod pairs [40]. Nanoplasmonic molecular rulers based on plasmon coupling are advantageous because long distances (up to 70 nm) can be measured between nanoparticle pairs. Additionally, no photobleaching occurs and therefore, measurements can be made continuously over long periods of time.

## Integrated photoacoustic–photothermal contrast agents

Photoacoustic imaging is a non-invasive technique to image the distribution of optical absorption in tissues. As one of the promising methods for *in vivo* medical imaging, it is based on the optical absorption of photons. The release of localized heat and the local thermal expansion produces pressure transients. A photoacoustic pulse provides the information of location, absorption, and dimension of the source area. The integration of photoacoustic and photothermal imaging provides optical, acoustic, and thermal

**Figure 4**




Nanoplasmonic molecular rulers. **(a)** A single gold nanoparticle, with tethered double-stranded DNA containing cleavage sites for nucleases, is utilized as a nanoplasmonic molecular ruler. Changes in DNA length, due to nuclease activity, can be correlated to shifts in the plasmon resonance of the nanoparticle over time (Ref. [17••]). **(b)** A pair of gold nanoparticles can also be utilized as a nanoplasmonic molecular ruler to measure biomolecular distances based on plasmon coupling. Low salt concentrations resulted in the increase of electrostatic repulsion between the charged gold nanoparticles and therefore a blue shift in the plasmon resonance of the nanoparticle pairs (Ref. [39••]).

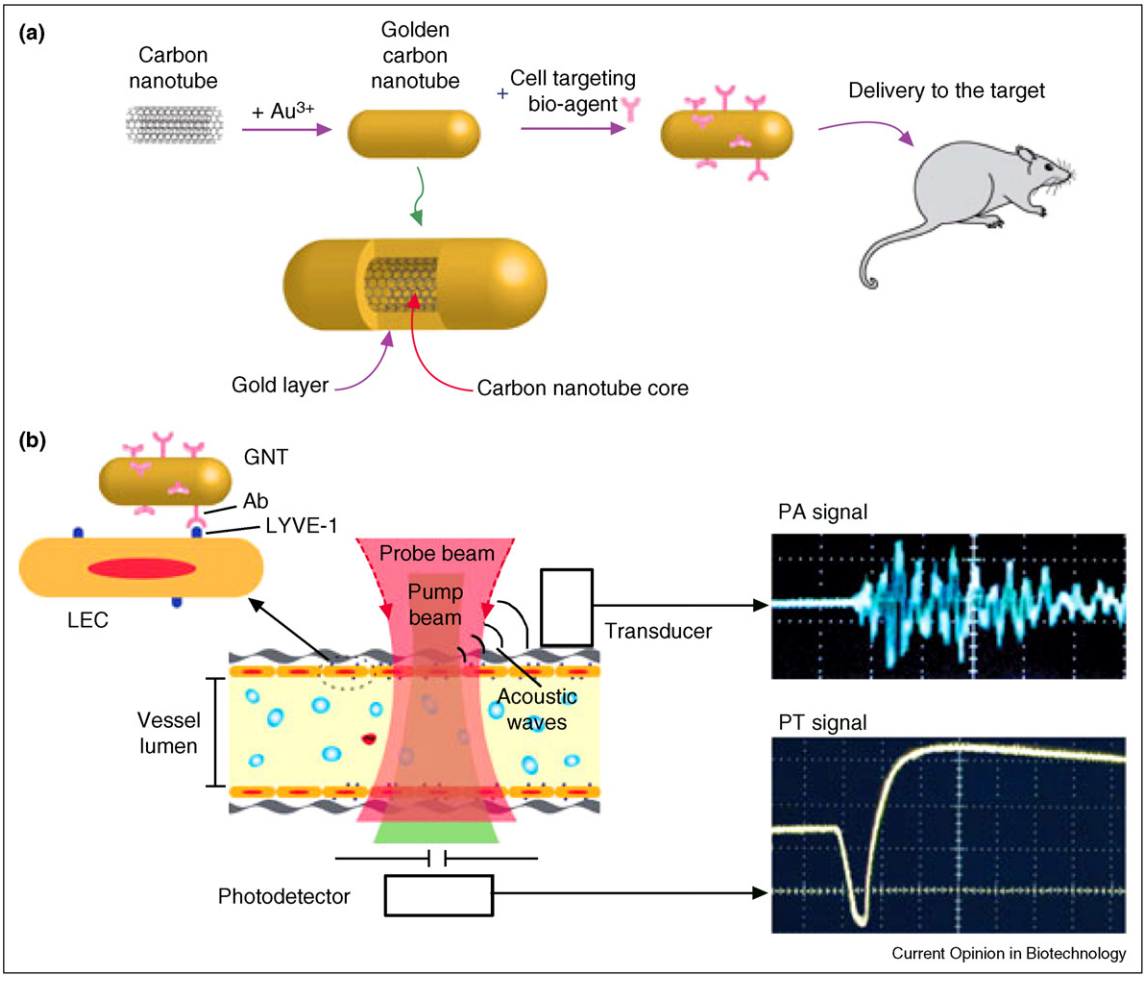
information of the source area. As contrast agents, carbon nanotubes can be used for integrated photoacoustic–photothermal imaging of biological systems. Since carbon nanotubes are limited by (1) low absorption displayed by carbon nanotubes at NIR wavelengths and also (2) toxicity, a creative solution was demonstrated to overcome these problems by developing golden carbon nanotubes [18••] by coating carbon nanotubes with a thin layer of gold. As integrated photoacoustic–photothermal contrast agents, golden carbon nanotubes have minimal toxicity and enhanced near-infrared contrast ($10^2$-fold).

To demonstrate *in vivo* imaging capabilities, antibody-conjugated golden nanotubes have been used to target lymphatic vessels in a mouse model (Figure 5). As a result, strong photothermal and photoacoustical signals resulted that were preferentially located at the lymphatic wall in the mouse model. In addition to imaging, real-time tracking of golden carbon nanotubes in vasculatures can lead to detection and potential treatment in metastatic cancers.

## Conclusions

Here, the creative designs of nanoplasmonic optical antennae for quantitative biology and nanomedicine have been discussed. Functioning as nanoplasmonic gene switches, nanoplasmonic optical antennae enable on-demand and precise intracellular regulation of genetic activity. Functioning as label-free biosensors, nanoplasmonic optical antennae enable PRET-based and SERS-based biosensing and molecular imaging of living cells as well as *in vitro* molecular detection. Nanoplasmonic molecular rulers for label-free measurement of DNA length and real-time kinetic studies of nuclease activity have also been reviewed. Equipped with new multifunctional nanoplasmonic optical antennae to directly manipulate and image the intracellular environment, quantitative approaches should capture dynamic 'snapshots' of the

**Figure 5**




Golden carbon nanotubes for integrated photoacoustic–photothermal imaging. **(a)** Carbon nanotubes are coated with a thin layer of gold, forming golden carbon nanotubes. Antibodies are conjugated to the surface of gold carbon nanotubes and introduced into a mouse model. **(b)** Detection of photoacoustic and photothermal signals at specific locations in the lymphatic vessel (Ref. [18••]).

intracellular biochemical distribution of living systems that were otherwise previously impossible to detect using conventional methods.

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- •• of outstanding interest

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This paper is a demonstration of nanoplasmonic gene induction of exogenous foreign genes using plasmid DNA in living cells. Thiol-modified, linearized EGFP-N1 plasmid DNA were covalently bound to gold nanorods and internalized in human HeLa cervical cancer cells. Pulsed illumination was used to photothermally melt gold nanorods into spheres and release plasmids. Nanoplasmonic gene induction of EGFP expression was qualitatively demonstrated using immunofluorescence imaging.

5. •• Liu GL, Long Y, Choi Y, Kang T, Lee LP: **Quantized plasmon quenching dips nanospectroscopy via plasmon resonance energy transfer**. *Nat Methods* 2007, **4**:1015-1017.

In this work, plasmon resonance energy transfer (PRET) was discovered for the first time. When the plasmon resonance spectrum of a nanoplasmonic optical antenna was intentionally matched to the absorption spectrum of the biomolecules, energy transfer by PRET resulted in wavelength-specific quenching in the Rayleigh scattering spectrum. In the case of cytochrome *c*, PRET biosensing of cytochrome *c* resulted in wavelength-specific spectral quenching in the Raleigh scattering spectrum, where the quenching positions exactly corresponded to the absorbance peaks of cytochrome *c* at 525 nm (FWHM $\sim$ 20 nm) and 550 nm (FWHM $\sim$ 12 nm).

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In this article, highly sensitive and selective metal ion sensing was enabled by PRET spectroscopy. When the transition metal ion (blue) binded with the matching ligand (red), d orbitals split, generating a new absorption band of the metal–ligand complex in the visible range. Owing to this new absorption band, Rayleigh scattering energy from the biosensor was transferred to the metal–ligand complex. There was no spectral overlap between ligands and the biosensor in the absence of the metal ion. When the electronic absorption frequency of the metal–ligand complex matched with the Rayleigh scattering frequency, the selective energy transfer was induced by this spectral overlap and distinguishable resonant quenching in the resonant Rayleigh scattering spectrum was observed.

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Nuclease enzymatic kinetics, at the surface of a single gold, DNA-tethered nanoparticle, were studied in real-time. An average wavelength shift of approximately 1. 24 nm is observed per DNA base pair. By taking advantage of the high quantum efficiency of Rayleigh scattering in comparison to fluorescence or Raman scattering, the time resolution of this nanoplasmonic molecular ruler was shown to be as high as one spectrum per second. Kinetic reactions can potentially be measured on the timescale of seconds using this method.

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