

# Biological Applications of Thermoplasmonics

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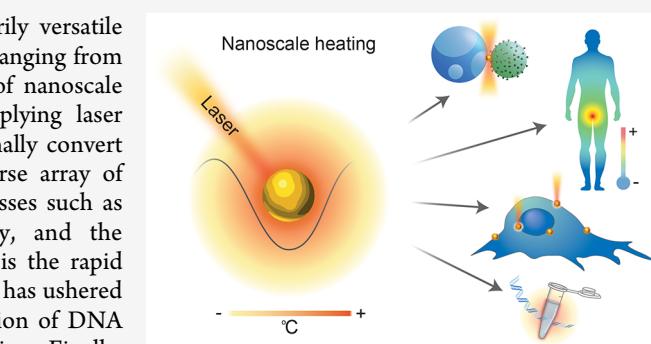
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**ABSTRACT:** Thermoplasmonics has emerged as an extraordinarily versatile tool with profound applications across various biological domains ranging from medical science to cell biology and biophysics. The key feature of nanoscale plasmonic heating involves remote activation of heating by applying laser irradiation to plasmonic nanostructures that are designed to optimally convert light into heat. This unique capability paves the way for a diverse array of applications, facilitating the exploration of critical biological processes such as cell differentiation, repair, signaling, and protein functionality, and the advancement of biosensing techniques. Of particular significance is the rapid heat cycling that can be achieved through thermoplasmonics, which has ushered in remarkable technical innovations such as accelerated amplification of DNA through quantitative reverse transcription polymerase chain reaction. Finally, medical applications of photothermal therapy have recently completed clinical trials with remarkable results in prostate cancer, which will inevitably lead to the implementation of photothermal therapy for a number of diseases in the future. Within this review, we offer a survey of the latest advancements in the burgeoning field of thermoplasmonics, with a keen emphasis on its transformative applications within the realm of biosciences.

**KEYWORDS:** photothermal therapy, thermoplasmonics, plasmonic heating, drug delivery, COVID detection, plasmonic PCR, membrane fusion, cell manipulation, stem cell differentiation

The application of localized heating has unveiled remarkable potential for harnessing control over biological functions and manipulating biomaterials. Through the use of laser irradiation of nanoparticles, often made from noble metals like gold, it has become feasible to generate heat in biological samples, that is highly localized in both space and time. This kind of laser-induced heating was first considered an inadvertent thermal side effect in plasmonics-related applications, but recently, this photoinduced nanoparticle heating has found use in a range of applications in experimental research. The phenomenon, now termed thermoplasmonics or plasmonic heating, has undergone a transition into a promising new field of research that has opened up a vast number of new possible applications within various disciplines spanning chemistry, physics, biology, and medicine.

Light scattering and absorption by nanoparticles are ancient phenomena used for coloring glass paintings, but with the advent of lasers and the synthesis of precisely engineered plasmonic nanoparticles, a new world of opportunities has emerged to harness light and heating at the molecular scale. Together with the sensitivity of biomaterials to heat, thermoplasmonics will allow unprecedented manipulative control over biological processes in living cells and molecular and biomimetic systems.<sup>1</sup>



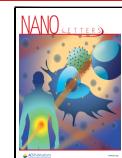
Thermoplasmonics can be applied for different purposes in biological systems. The local heat produced by laser-irradiated nanoparticles can activate cell sensing and has been employed to study temperature-induced changes in transmembrane potentials in neurons with consequent induction of action potentials,<sup>2</sup> activation of heat shock proteins (HSPs),<sup>3</sup> and modulation of cell differentiation.<sup>4</sup> These effects illustrate the potential of manipulating biological functions. In addition, thermoplasmonics allows for precise ablation at the subcellular or cellular level, which has been used for photothermal therapy in cancer<sup>5</sup> and investigation of cellular membrane repair to nanoscopic injuries induced by local heating in living cells.<sup>1</sup> This form of thermal nanosurgery has also proven to be effective in the manipulation of biomimetic systems like fusion of giant unilamellar lipid vesicles (GUVs) for studying membrane–protein interactions<sup>6,7</sup> or selective fusion of cancer cells with immune cells.<sup>8</sup> At the molecular level, thermoplas-

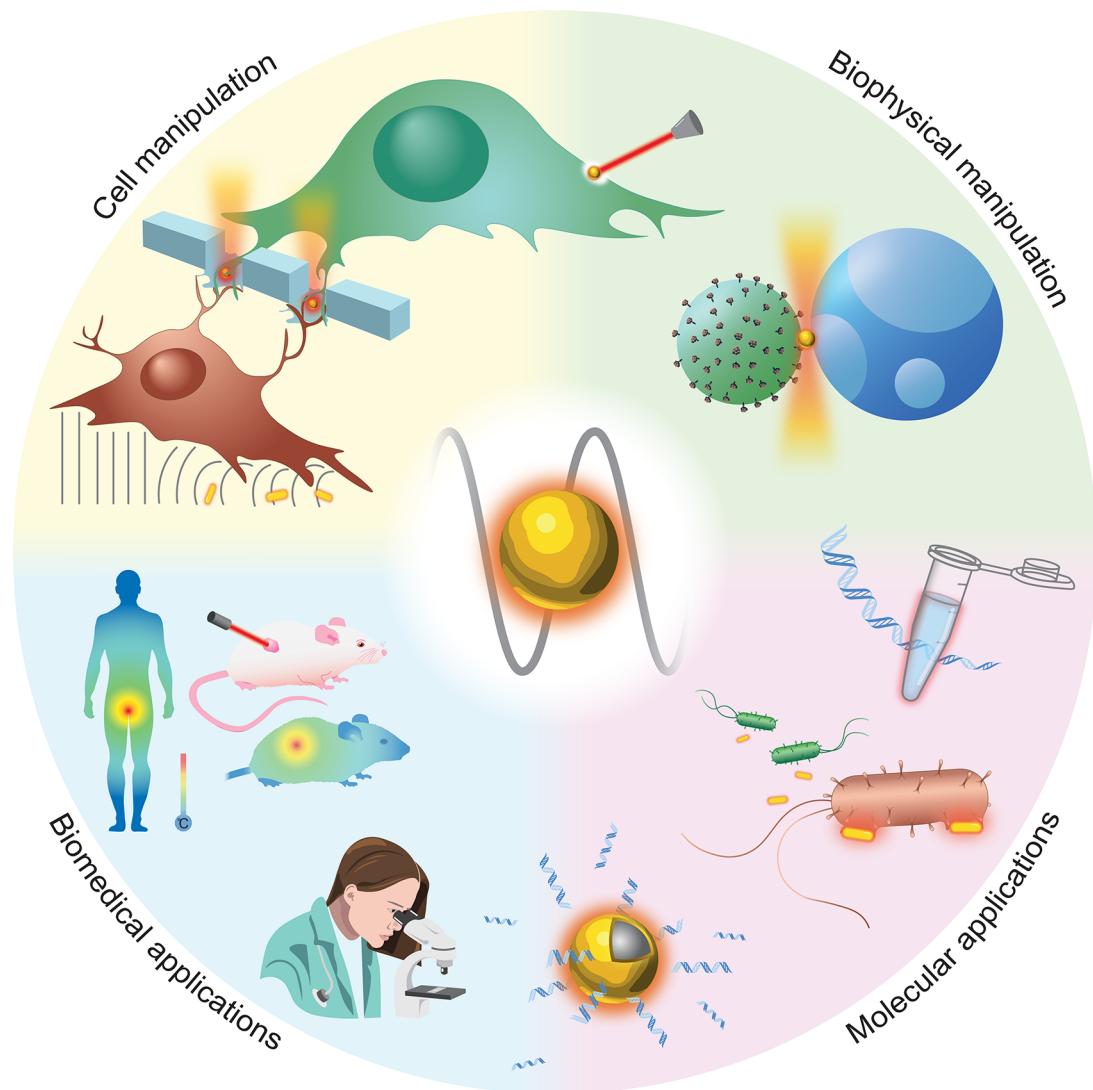
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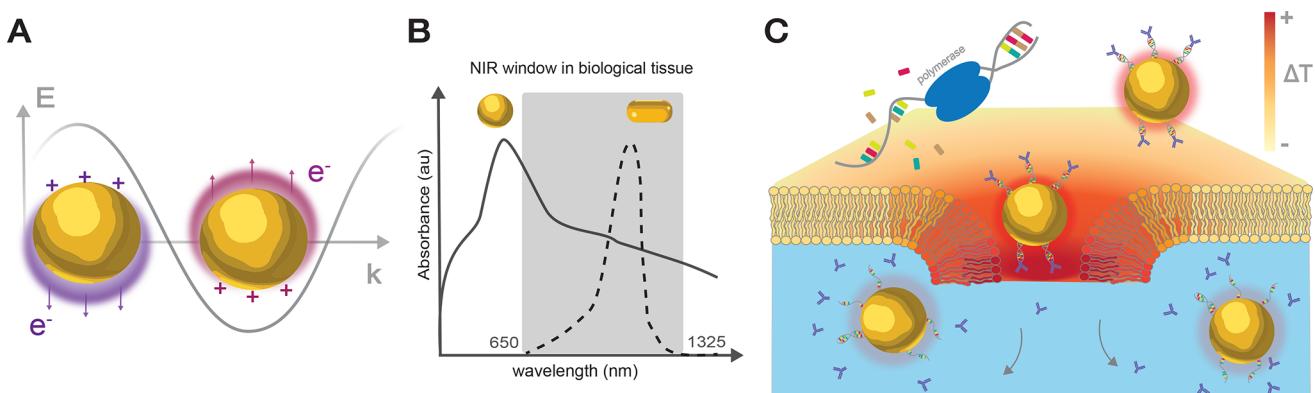
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**Figure 1.** Applications of thermoplasmonics in four biological areas. Each shaded area is discussed as a topic in this review.



**Figure 2.** Plasmonic heating for biological applications. (A) Laser irradiation of plasmonic nanoparticles excites electronic oscillations in the particle, which causes heating. The heating reaches a maximum at a wavelength corresponding to the localized surface plasmon frequency of the particle. (B) Schematic of the spectra of spherical and rod-shaped gold nanoparticles showing how the peak absorption can be tuned by the shape factor to coincide with the wavelengths at which biological tissue is most transparent (shaded region). (C) Local heating for molecular and supramolecular applications. Here, heating of AuNPs results in membrane perforation, release of conjugated antibodies, and polymerase activation for usage in ultrafast PCR.

monics can be used to study and enhance biological processes. Localized heating shows promise in investigations of protein

denaturation effects and thermal control of the hybridization of RNA and DNA nucleotides.<sup>9</sup> These molecular-level manipu-

lations have found applications in drug delivery and other therapeutic strategies.

The concept of thermoplasmonics has been seamlessly integrated into many fields of science. We refer the interested reader to comprehensive reviews on plasmonics with a particular focus on thermoplasmonics in chemistry and physics.<sup>10–15</sup> Here we will first provide a brief introduction to optical heating of plasmonic nanoparticles followed by important and recent applications of thermoplasmonics in several areas of science, as highlighted in Figure 1, with a focus on cellular, biophysical, molecular, and biomedical applications, including photothermal therapy and drug delivery.

Surface plasmon resonances occur when light excites the free electrons on the surface of a metallic material, leading to collective electronic oscillations and charge displacements. In the case of nanoparticles (Figure 2A), this excitation leads to so-called localized plasmon resonance when the free electrons in the metal are spatially confined. When a small spherical metallic nanoparticle is irradiated by light, the oscillating electric field causes the conduction electrons to oscillate coherently, leading to an oscillating charge dipole that resonates with specific wavelengths determined by the particle shape, size, and material property. The absorption of incoming light increases significantly at this resonance, and substantial heat can be generated at the resonance due to the thermal relaxation of the oscillating electron cloud. This property of metallic nanoparticles to convert light to heat is what has generally been termed thermoplasmonics or plasmonic heating. The optical response of a nanoparticle to light can be described through its extinction cross section ( $C_{\text{ext}}$ ), which is defined as the sum of scattering and absorption cross sections ( $C_{\text{ext}} = C_{\text{abs}} + C_{\text{scat}}$ ).

The extinction cross section of a nanoparticle embedded in a dielectric medium with permittivity  $\epsilon_m$  and irradiated at wavelength  $\lambda$  can be described by the optical theorem as<sup>16</sup>

$$C_{\text{ext}} = \kappa \operatorname{Im}(\alpha) \quad (1)$$

where the wavenumber is  $\kappa = 2\pi\sqrt{\epsilon_m}/\lambda$  and for small particle sizes (compared to the wavelength of light used) the scattering is negligible, and we find that the absorption that equals the extinction cross section can be approximated as

$$C_{\text{abs}} \approx \kappa \operatorname{Im}(\alpha) \quad (2)$$

Polarizability  $\alpha$  for spherical particles that are small compared to the wavelength of light is given by

$$\alpha = 3V \frac{\epsilon(\omega) - \epsilon_m}{\epsilon(\omega) + \phi\epsilon_m} \quad (3)$$

where  $V$  is the volume of the particle,  $\epsilon_m$  is the dielectric permittivity of the medium,  $\epsilon$  is the dielectric permittivity of the particle at frequency  $\omega$ , and  $\phi$  is a shape-dependent parameter<sup>17</sup> that for a sphere equals 2.

On the basis of the latter, it is apparent that the localized surface plasmon resonance (i.e., maximum absorption and scattering cross section of the particle) can be tuned on the basis of the shape, size, and composition of the nanoparticle. All of these parameters are being extensively explored by researchers and have led to novel particle designs (shells, rods, stars, cubes, etc.) with peak absorption at specific wavelengths extending into the near-infrared region where biological materials exhibit minimum absorption.

By solving the heat transfer equation,<sup>18</sup> we can calculate the temperature increase in the vicinity of an irradiated nanoparticle through the relation<sup>19</sup>

$$\Delta T(r) = \frac{I}{4\pi Kr} C_{\text{abs}} \quad (4)$$

This expression is valid for only small nanoparticles.  $C_{\text{abs}}$  is the absorption cross section from eq 2, which is defined as the ratio between the power absorbed by the particles and the total incoming laser intensity (power per area).  $I$  is the laser intensity on the particle,  $r$  the distance from the center of the particle, and  $K$  the thermal conductivity of the medium in which the particle is suspended.  $C_{\text{abs}}$  is given above for small particles, but for larger particle having a spherical shape, the absorption cross section can be found using Mie's solutions to the Maxwell equations.<sup>19</sup> For nonspherical particles, the optical cross sections are calculated by using finite-element modeling, which allows the absorption cross section to be computed for any particle shape that has been demonstrated for a number of particle types.<sup>12</sup>

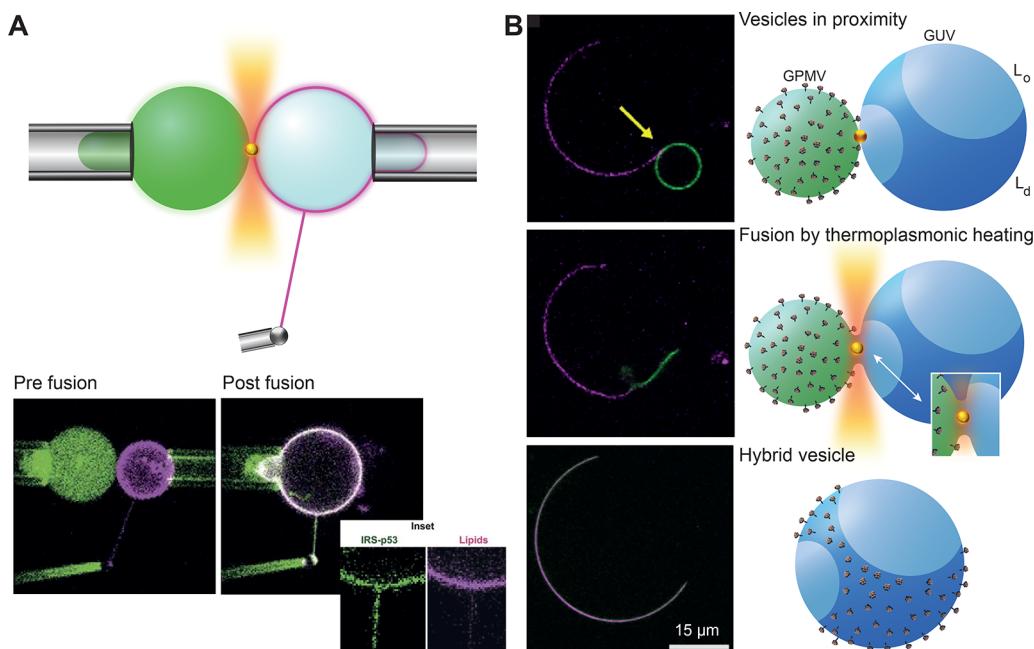
Due to the large diversity of commercially available plasmonic nanoparticles, including gold nanoparticles (AuNPs), gold nanoshells (AuNSs), nanomatryoshkas, nano-stars, nanorods, and many more, it is now possible to choose nanoparticles with a large absorption cross section at practically any wavelength in the optical and near-infrared (NIR) regime (Figure 2B). Other materials used in optical heating applications include platinum<sup>20</sup> and titanium,<sup>21</sup> which have shown great photothermal effects, and nanoparticles made from platinum have also been successfully trapped using optical tweezers.<sup>22</sup> The heating effect of a nanoparticle is highly localized to the vicinity of the particle (Figure 2C), rendering the thermoplasmonic effect of nanoparticles as an extremely versatile and adaptable tool for diverse biological applications,<sup>23</sup> as we will discuss below.

It should be noted here that nanoscale heating exceeding ~240 °C can result in gas bubbles on the particle surface, which drastically changes the heating. Such bubbles have size-dependent lifetimes that are analytically solved in ref 24.

Moreover, we note that collective effects can occur through plasmonic coupling between two particles or a lattice of many particles. This can broaden the parameters of the thermoplasmonic effect. It has been shown recently for thermoplasmonic applications that when identical nanoparticles are arranged in a periodic array, they can support collective modes known as lattice resonances.<sup>25</sup> These resonances produce a much larger temperature increase per particle than for arrays that do not support resonance conditions, and their absorption peak can be tuned to wavelengths where the single nanoparticles cannot.

In the following, we will discuss a number of important applications in thermoplasmonics in which such nanoparticles have been used to answer biological questions and as a tool for the manipulation of biological matter. Most applications to date have used nanoshells and nanorods that can be tuned with respect to absorption resonance wavelengths and hence can also be used in the near-infrared region, but the exploration of new types of particles is an active area of research.

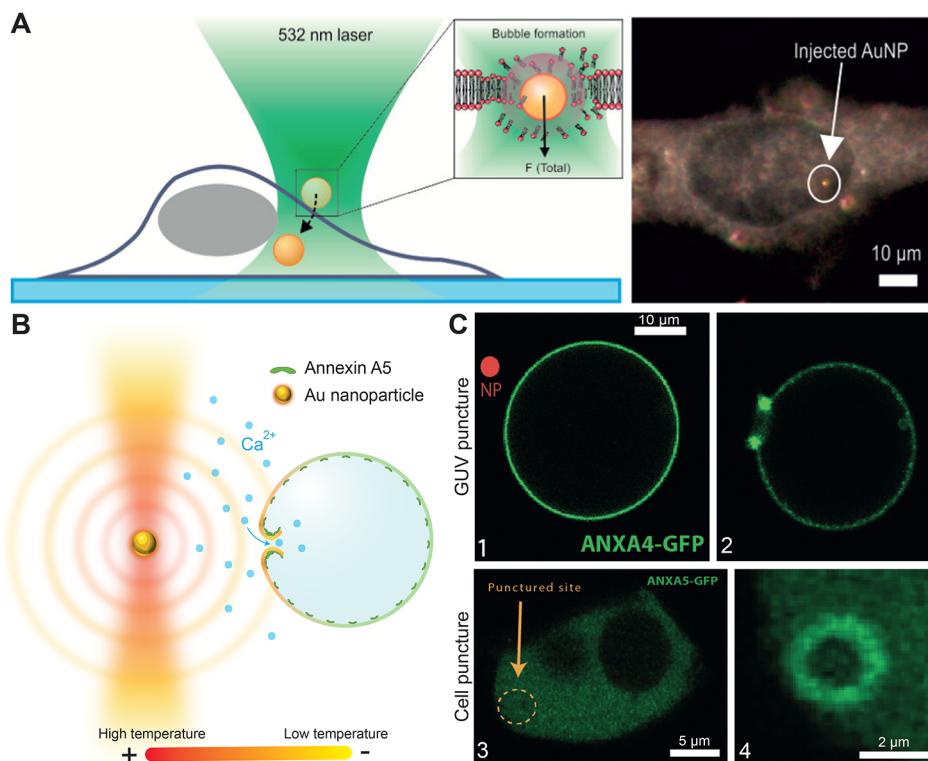
The combination of optical trapping and heating of plasmonic nanoparticles has recently shown great potential in applications, such as the study of protein biophysics. In particular, for the study of interactions of proteins with membranes, thermoplasmonics has offered a unique way to



**Figure 3.** Thermoplasmonically induced remodeling of biomembranes for studying protein dynamics. (A) Via the combination of aspiration using micropipettes with optical trapping of plasmonic nanoparticles, it is possible to fuse two vesicles by locally heating the contact point between the apposing membranes. The bottom panels show an example in which a giant unilamellar lipid vesicle (GUV), containing the I-BAR protein domain of IRS $\beta$ 53 (green) in the lumen, is fused to another GUV that is composed of anionic lipids. The fused vesicle thereby contains anionic lipids that facilitate binding of IRS $\beta$ 53. The bottom right inset shows membrane curvature sensing of IRS $\beta$ 53 within a nanotube pulled from the hybrid GUV. Panel A was adapted with permission from ref 7. Copyright 2019 The Company of Biologists Publishing. (B) Fusion can also be achieved between vesicles resting on a surface, as shown here where a giant plasma membrane vesicle (GPMV) and a GUV are fused to investigate the phase affinity of integral membrane proteins found in influenza virus.<sup>6</sup> First, the GPMV is brought into the proximity of a phase-separated GUV using an optical trap. Fusion takes place after a nanoparticle is trapped at the contact point between the vesicles (top), and upon fusion (middle), the proteins and lipids mix within seconds. After fusion (bottom), the affinity of the proteins for the disordered lipid phase can be detected.<sup>6</sup>

control protein binding and allow both time-resolved imaging of protein binding and monitoring of how membrane geometry affects protein recruitment.<sup>7,26,27</sup> The applicability of thermoplasmonics in this realm lies in the ability of laser-heated nanoparticles to remodel biomembranes via, for example, fusion of membranes or via pore formation. Examples of thermoplasmonic fusion<sup>27</sup> (Figure 3) include studies of membrane binding proteins like ESCRT<sup>26</sup> or the I-BAR domain from IRS $\beta$ 53.<sup>7</sup> These proteins were encapsulated within GUVs made from zwitterionic lipids and subsequently delivered, via thermoplasmonically induced membrane fusion, to other vesicles having a different lipid composition that favored protein binding (Figure 3A). A similar approach was applied in the study of the membrane phase affinity of transmembrane influenza virus proteins.<sup>6</sup> Here giant plasma membrane vesicles (GPMVs) were harvested from cells expressing fluorescently tagged virus proteins followed by fusion of the GPMVs to phase-segregated GUVs, which allowed for investigation of the lateral segregation of proteins (Figure 3B). Such studies offer a unique way to investigate the membrane phase affinity of integral membrane proteins and also membrane-shape remodeling of proteins in real time by mixing biomimetic membranes and natural membranes harvested from cells. This strategy can readily be extended for investigation of any transmembrane/integral protein, including proteins from other viruses like SARS-CoV-1/2. Possible future studies along these lines are numerous and should include the possibility of fusing living cells and biomimetic membranes<sup>28,15</sup> and investigating cellular functions like membrane repair.<sup>1</sup>

The ability of plasmonic nanoparticles to inflict nanoscopic membrane damage has been exploited in the delivery of nanoparticles to vesicles and cells.<sup>29,30</sup> Nanoparticles were optically injected across the membrane in cells<sup>29</sup> and GUVs<sup>30</sup> by using a pulsed laser, which both triggered a light pressure on the particle and softened the membrane by local heating and facilitated delivery across the membrane (Figure 4A). Recently, a NIR continuous wave (CW) laser was also used for membrane perforation to study pore formation in GUVs and cells by locally disrupting the membrane and subsequently monitoring the recruitment of membrane repair proteins to the annular ring of the nanoscopic injury (Figure 4B,C).<sup>1</sup> This approach offers a unique way to study the physical and biological aspects of pore formation in membranes in the presence or absence of proteins. Gentler plasmonic heating can be applied to study local phase behavior by locally heating GUVs existing in an ordered lipid state.<sup>31</sup> This strategy allowed the long-standing question concerning the discrete nature of the melting-induced permeability of membranes<sup>32</sup> to be answered and was also used for quantification of nanoparticle mobility on gel versus fluid lipid membranes.<sup>33</sup> The local effect of heat from a nanoparticle on a membrane was recently modeled using coarse grained simulation of a fluid and a gel membrane interacting with 7 nm particles.<sup>34</sup> These simulations showed that heat affects the two leaflets of the bilayer differently and leads to the bending and wrapping of the membrane around the nanoparticle. A complete understanding of the nanoparticle–membrane interaction remains elusive, and additional work needs to be done to carefully design experiments that can be modeled accurately.



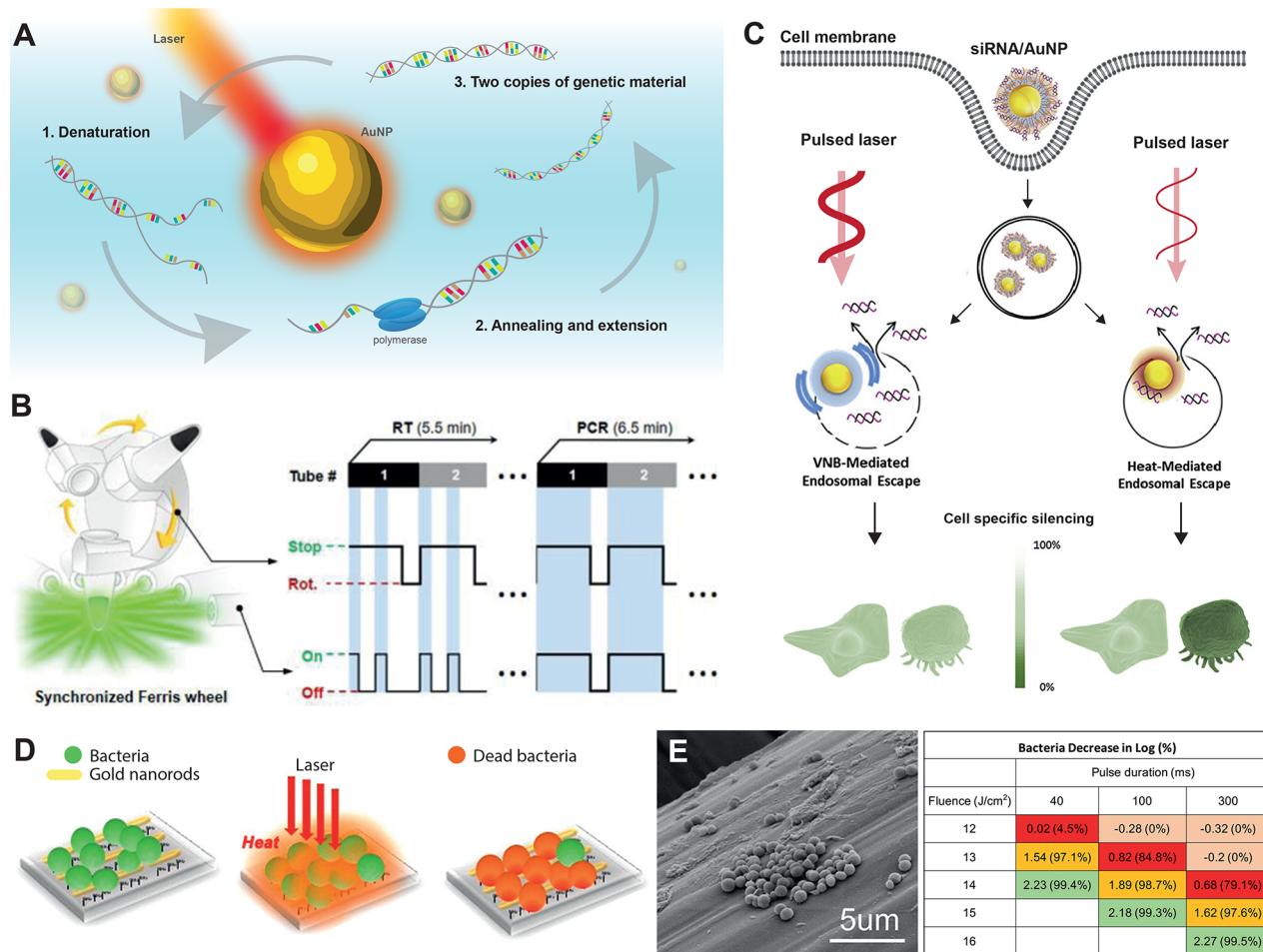
**Figure 4.** Opto-injection and pore formation in biomembranes using thermoplasmonics. (A) Injection of AuNPs using a CW laser with a  $\lambda$  of 532 nm. The AuNP was first immobilized on the cell membrane by using optical forces. Subsequent focusing of the laser beam on the particle facilitated translocation across the membrane due to a local high temperature and optical forces. Panel A was reproduced from ref 29. Copyright 2015 American Chemical Society. (B) Schematic of an experiment for investigation of the response of membrane repair proteins (GFP-labeled annexins, green) to membrane pore formation in GUVs by optically trapping and consequently heating a AuNP in the vicinity of the membrane, thus inducing a pore. Panel B was adapted with permission from ref 1. Copyright 2022 Royal Society of Chemistry. (C) (1) Image showing an intact GUV containing annexin A4 (green). (2) Recruitment of annexin A4 (green) to a membrane pore induced in a GUV results in local enrichment at the pore annulus and curving of the membrane. (3) Living cell expressing GFP-labeled annexin A5. (4) Thermoplasmonically induced puncturing of the cell also results in the recruitment of annexin A5 around the site of injury. Panel C was adapted with permission from ref 1. Copyright 2022 Royal Society of Chemistry.

Proteins respond to heat by changing their binding and folding kinetics. Unfolding of proteins at high temperatures is predicted by Boltzmann's statistics, and studies involving protein function should always raise concerns regarding potential irreversible molecular damage caused by high local temperatures. However, in most applications involving the study of proteins and lipids, the spatial extent of heat exposure has a nanoscopic length scale and levels off quickly beyond a distance corresponding to the particle radius. Also, the brief exposure of <1 s significantly limits molecular damage. Because both cells and GUVs have dimensions of >10  $\mu\text{m}$  and consist of mobile lipids and proteins, which therefore are only transiently exposed to heat, it is estimated that the overall molecular damage can be kept at a minimum.

Alterations in protein structure and even damage are certainly taking place in some applications and can even be a desired effect. Plasmonic nanorods have been found to be efficient in dissolution of protein aggregations formed by  $\text{A}\beta$  amyloids that are abundant in the brains of people with Alzheimer's disease.<sup>35</sup> In vitro studies have confirmed that  $\text{A}\beta$  fibers of various lengths are susceptible to breakage, unfolding, or other structural changes when conjugated to irradiated gold nanorods.<sup>36</sup> Together with improved delivery of nanorods across the blood–brain barrier (BBB),<sup>37</sup> this will allow the therapeutic possibility of treating people with neurodegenerative diseases like Alzheimer's.

Nanoparticles delivered to the bloodstream have a tendency to become decorated with a protein corona, which affects the nanoparticle circulatory lifetime and the conjugation of the particle to cell receptors of interest (targeting). Plasmonic nanoparticles coated with proteins from a commonly used culture medium containing fetal bovine serum (FBS), which resembles the protein composition in the blood, were studied before and after laser irradiation. This study showed that the protein composition was found to be significantly altered upon laser heating because of denaturation and destruction of proteins on the particle surface.<sup>38</sup>

We conclude that biophysical applications of plasmonic nanoparticles have shown great promise as a tool for resolving questions concerning the biological and biophysical functions of proteins and biomembranes. The steep gradient in temperature from irradiated plasmonic nanoparticles makes it possible to manipulate biological systems with nanosurgical precision and keep protein degradation extremely local. The scope of applications is currently in its early stage, and future experiments should be tailored by using cleverly designed nanoparticle shapes and sizes and by introducing novel particles like Janus particles. Janus particles allow generation of anisotropic thermal distributions and have been shown to exhibit very high thermal gradients as high as 40 K/nm in the vicinity of the particle surface.<sup>39</sup>

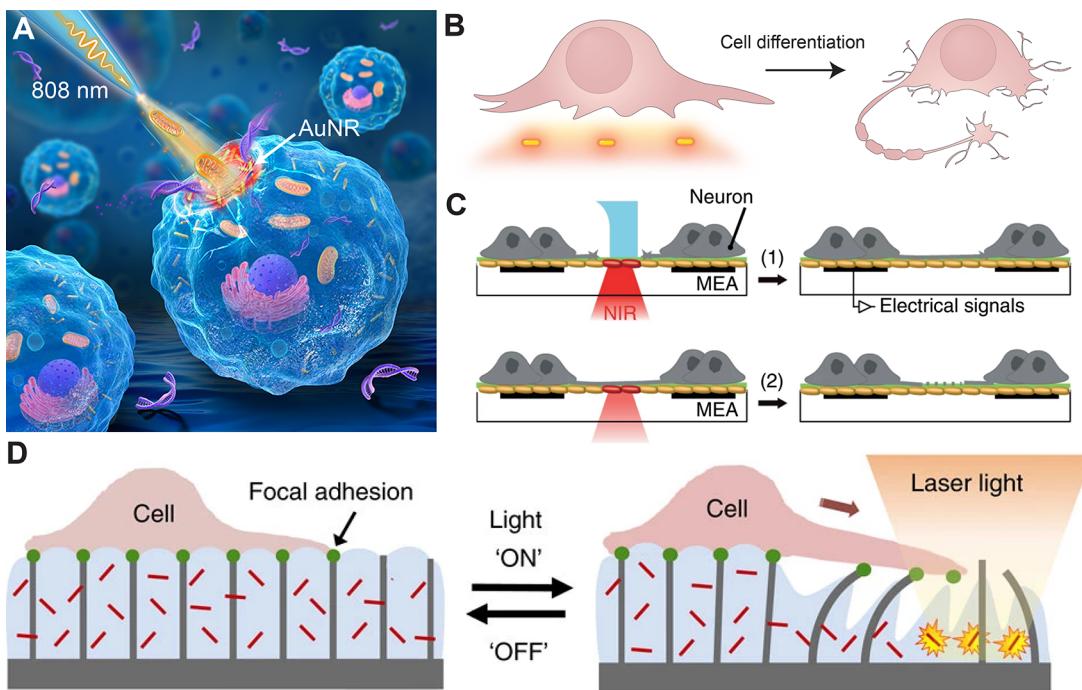


**Figure 5.** Thermoplasmonics in molecular biology and microbiology. (A) Laser heating of plasmonic nanoparticles allows rapid thermal cycling for conducting nano-PCR. (B) Detection of SARS-CoV-2 RNA using high-speed nano-PCR for reverse transcription PCR. A spinning wheel, containing tubes with RNA and nanoparticles, is rotated to allow periodic laser irradiation of the tube content leading to thermal cycling. Panel B was reproduced with permission from ref 9. Copyright 2020 Springer Nature. (C) Mechanism of cellular uptake and intracellular release of nucleotides from AuNPs. Two release mechanisms are proposed on the basis of a thermal mechanism and a nonthermal mechanism. The nonthermal disruption of the endocytic envelope is based on a mechanical effect due to vapor nanobubble formation during pulsed laser irradiation. Laser irradiation with a lower pulse energy results in heating, which depends on the number of particles taken up. Endosomal escape of nucleotides and gene silencing is therefore cell type specific as indicated in the bottom part of the panel. Panel C was modified from ref 56. Copyright 2020 Elsevier. (D) Disinfection of surgical mesh using plasmonic heating of AuNRs. The surgical mesh is functionalized with AuNRs, and subsequent inoculation with *Staphylococcus aureus* leads to biofilm formation. (E) Laser irradiation kills the bacteria as shown by scanning electron microscopy or by cell counting. Panels D and E were reproduced with permission from ref 62. Copyright 2019 American Chemical Society.

The sensitivity of molecules and proteins has led to numerous applications of plasmonic heating in molecular systems. An application, in which plasmonic heating has had a significant impact, is the acceleration of the analysis of DNA using polymerase chain reaction (PCR). PCR is a method of amplifying nucleic acids with high specificity and sensitivity<sup>40</sup> that has been applied in areas ranging from biology to medicine<sup>41</sup> and agriculture.<sup>42</sup> The reaction requires repeated cycles of heating and cooling between temperatures necessary for dehybridization and hybridization of a target sequence. Conventional thermocyclers employ a heating block based on the Peltier element and have a limited rate of heating and cooling so that the entire amplification process is completed within 1–2 h. Because of the desire to rapidly diagnose viral infections caused by viruses like SARS-CoV-2, the use of plasmonic heating offers a promising approach to accelerate DNA or RNA detection.<sup>9</sup>

Rapid heating and cooling can be performed using thermoplasmonics and have recently led to a strategy termed

photonic PCR that was successfully exploited to accelerate PCR.<sup>9,43–46</sup> Both plasmonic nanoparticles and plasmonic substrates can be used for rapid heat cycling. Plasmonic excitation of a Au film resulted in 30 successive heating/cooling cycles within 5 min.<sup>47</sup> A heating rate of  $12.79 \pm 0.93 \text{ }^\circ\text{C s}^{-1}$  was achieved by irradiating a Au film using a light-emitting diode (LED) with a wavelength of 450 nm. When the diode was switched off, the heat dissipated through the Au film, and the reaction mixture cooled at a rate of  $6.6 \pm 0.29 \text{ }^\circ\text{C s}^{-1}$ . A rapid heat cycling using nanoparticles was recently used for the detection of SARS-CoV-2 RNA, via reverse transcription, by performing nano-PCR using a mobile device achieving a detection time of 17 min (Figure 5A,B).<sup>9</sup> Despite the recent advances achieved in accelerating the thermal cycles, it is important to note that the completion of a single PCR cycle still faces a time limit of  $\sim 1$  s, which is set by the kinetic limitations of PCR such as DNA denaturation, primer annealing, and polymerase extension.<sup>48</sup>



**Figure 6.** Optical manipulation of single cells using thermoplasmonics. (A) Perforation of a cell by irradiation of AuNRs near the cell surface. Intracellular manipulations following perforation were made possible by combining optical trapping with a micropipette. Panel A was reproduced from ref 67. Copyright 2021 American Chemical Society. (B) Induction of neurite outgrowth and (C) interrogation of neuronal cell signaling by plasmonic heating. Cell–cell interconnections can be ablated through plasmonic heating or allowed to form by ablation of a barrier consisting of a heat sensitive hydrogel containing plasmonic nanoparticles. Panel C was reproduced from ref 2. Copyright 2020 Springer Nature. (D) Active substrate material for subcellular mechanical actuation of adhered cells. The thermally sensitive hydrogel containing plasmonic nanoparticles contracts upon irradiation and thereby stretches the adhered cell. Panel D was reproduced from ref 68. Copyright 2017 Springer Nature.

Plasmonic heating has been successfully applied in drug delivery primarily to release molecular content conjugated to the plasmonic nanostructures or to facilitate permeation of the plasma membrane to allow the influx of small interfering RNA.<sup>49</sup> In particular, gene silencing, using plasmonic nanostructures conjugated with silencing RNA,<sup>50</sup> can be triggered from laser heating of the nanostructure and has shown great promise in the downregulation of protein synthesis in cell studies.<sup>51,52</sup> As a proof of principle, green fluorescent protein (GFP) is often chosen as the target for interference with gene expression. Other more biologically relevant examples, which have been successfully downregulated using plasmonic heating of gold nanostructures, include HSPs,<sup>53</sup> oncogenes,<sup>54</sup> and proteins controlling transcription like NF- $\kappa$ B.<sup>50</sup>

A major challenge in drug delivery involves the two membrane barriers that have to be penetrated during delivery of the drug of interest. Nanoparticles are excellent for carrying drugs across the cell membrane simply through particle endocytosis.<sup>55</sup> Once endocytosed, the nanoparticles and their molecular content become trapped within the endosome, and here the molecules are in danger of losing their therapeutic effect due to degradation by enzymes and the low pH of the endosomal environment. The release from the endocytic compartment has been shown to be feasible through laser irradiation, which can both release the molecules from the nanoparticle via, e.g., dehybridization of nucleotide strands and disruption of the endosome membrane.<sup>56</sup> However, by testing molecular release using pulsed and CW lasers, it was found that the mechanisms behind these two escape phenomena were more complex than just simple thermally triggered

release. It was found<sup>56</sup> that pulsed lasers were more efficient in silencing gene expression in cells when high-energy pulses were used. The authors discriminated between a thermal mechanism when the pulse energy was low and a nonthermal mechanism when the pulse energy was sufficiently high to cause formation of vapor nanobubbles (VNBs) (Figure 5C). This finding was corroborated by other studies that found pulsed lasers trigger release with reproducibility and efficiency that are both higher than those of CW lasers.<sup>57–59</sup> Release triggered by CW lasers, on the contrary, was found to be dependent on the aggregation status of the NPs within the endosome, which has a great impact on the total light absorption. Wang et al.<sup>60</sup> concluded that the optical extinction of aggregated gold nanoparticles (sizes of  $\sim 30$  nm) increased significantly at NIR wavelengths, which is the relevant range for PTT. Interestingly, the mechanism of molecular release from NPs can also proceed through other nonthermal effects involving electrochemical effects arising from so-called hot electrons<sup>61</sup> or photoinduced free radicals generated during irradiation with resonant light.<sup>59</sup> It is noteworthy that the laser intensities used in CW applications are on the order of  $10 \text{ W cm}^{-2}$ , which are not sufficient to generate any significant heat from a single nanoparticle. However, due to the large number of closely spaced nanoparticles found in endosomes, the extent of heating is increased; however, the degree of endosomal heating is quite uncertain, and the spatial configuration of the nanoparticles is uncontrollable in such an environment. Although these studies provide some insight into the nanoscale mechanisms at play, during nanoparticle heating in cells, we stress the need for further mechanistic studies using, for

example, bioimimetic vesicles as models for the cell and endosomal membrane.

Thermoplasmonically assisted sterilization and disinfection are other emerging fields that utilize plasmonic nanoparticles to produce heat to kill pathogens via molecular targets. Plasmonic nanoparticles can be functionalized to recognize and bind specific targets on the bacterial wall, which allows selective killing of bacteria within a bacterial population by irradiating the sample with laser light.<sup>63</sup> Sterilization of surgical implants has also been suggested by plasmonically assisted overheating. Implant contamination often occurs because of bacterial growth. If the bacterial growth develops further into biofilms, it becomes more resistant to the host immune system and even antimicrobial agents. Via decoration of a surgical polymer mesh with plasmonic gold nanorods, it was possible to eliminate a biofilm of bacteria formed at the surface of the polymer meshes by laser irradiation, thus proposing an alternative disinfection method to the existing biochemical approaches<sup>62</sup> (Figure 5D,E). This photothermal disinfection has recently been extended to create reusable face masks.<sup>64,65</sup> This innovation was driven by the global demand for personal hygiene, including wearing face masks during the COVID-19 pandemic, which quickly led to a shortage of professional face masks. Consequently, using reusable face masks that utilize plasmonic nanoheaters in their structure and take advantage of the same plasmonics principle for pathogen decontamination was proposed.

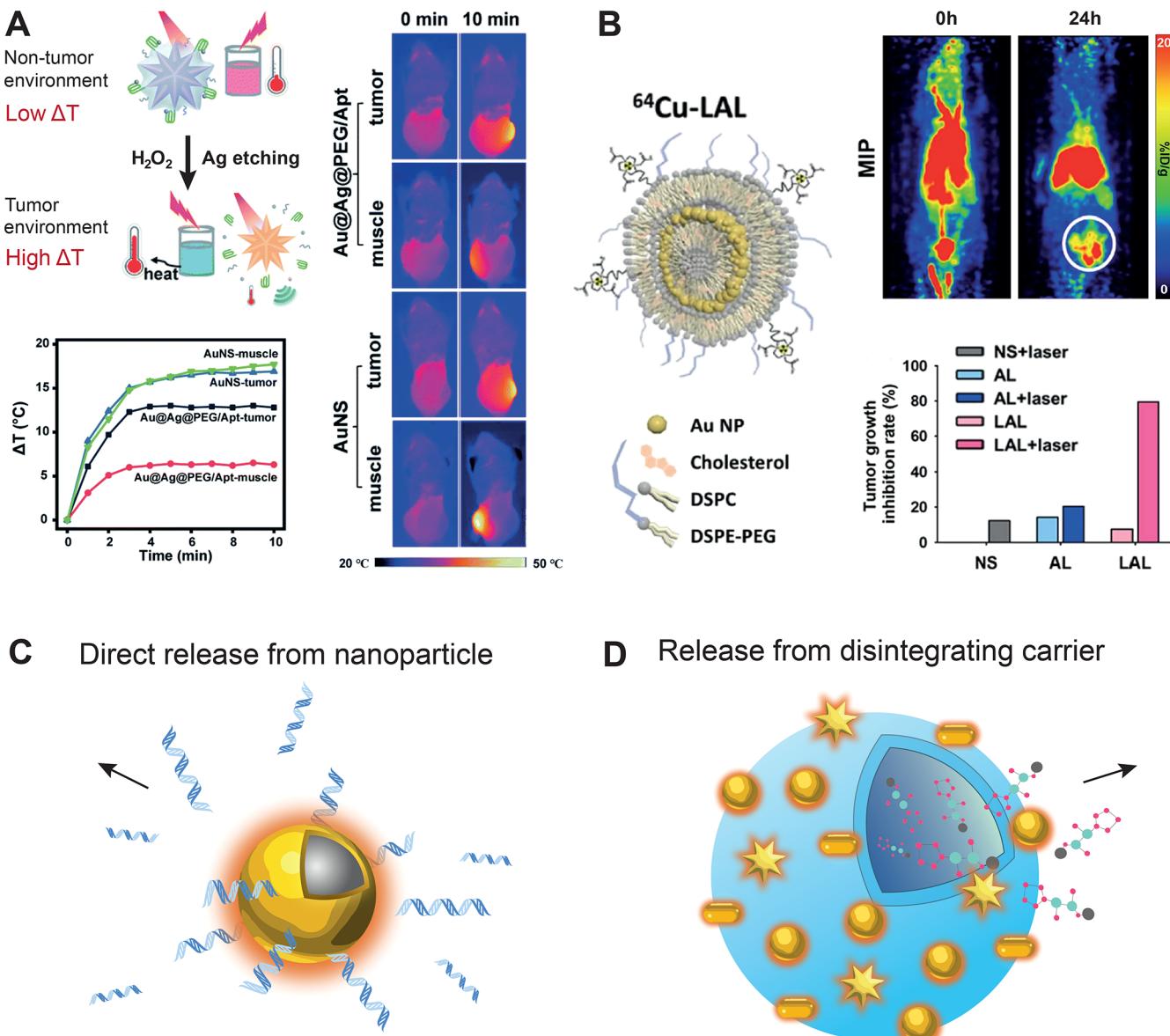
Studying and probing living cells remain challenging despite new developments in micromanipulation techniques. Thermoplasmonic heating provides entirely new opportunities for single-cell interrogation and manipulation and moreover allows investigation of both isolated cells and selective interference of cells within a cell culture while leaving neighboring cells unaffected. Both extra- and intracellular manipulation have been achieved using plasmonic heating, which is made possible by the efficient nanoparticle delivery through endocytosis. In addition to the natural endocytic pathways, thermoplasmonics provides an interesting alternative for nanoparticle delivery by using optically triggered injection. Researchers reported high cellular viability upon effective delivery of gold nanoparticles utilizing plasmonic heating and optical pushing at the membrane site to push the particles swiftly through the lipid bilayer.<sup>29</sup> To avoid the cellular uptake of metallic particles and consequent entrapment of the particles within endosomes, an alternative approach could be to use a plasmonic nanocavity substrate on which cells can be plated to provide high-throughput delivery of very large molecules and possibly nanoparticles upon laser irradiation.<sup>66</sup> Such thermoplasmonic perforation has paved the way for new *in situ* single-cell nano- and microsurgery. Manipulation and extraction of intracellular organelles from a single living cell, through transient light-induced membrane perforations, were achieved using plasmonic heating in combination with optical trapping and micropipette manipulation<sup>67</sup> (Figure 6A). By controlling the optical power, one can achieve both induced membrane perforation and membrane repair by the respective lipid fluidities achieved at high and mild temperatures. This application is an elegant mix of optical heating and optical trapping that pushes the boundaries of what is possible at the single-cell level, thus advancing thermoplasmonic membrane manipulation to another level.

Perforating the cellular membrane using thermoplasmonics is, however, relevant for not only the delivery of molecules but

also intracellular organelle manipulation and extraction. Nanoscopic membrane injury via heating has revealed the dynamics of the subsequent cellular repair response and involved proteins, e.g., how annexin proteins are recruited to injury sites following laser induced puncture of cells<sup>1</sup> (Figure 4C). Annexin proteins are highly upregulated in various cancer cell lines and thus are a target for cancer therapy. This technique can be employed to investigate other membrane-repairing proteins that could play an important role in identifying components in the membrane repair machinery and hence for the development of new therapeutic interventions with the goal of downregulating membrane repair mechanisms in cancer cells.

Transcriptional regulation can also be modulated using thermoplasmonics on single cells, as demonstrated by several studies. Recently, it was demonstrated that thermal heating of nanoparticles can guide the directed differentiation of dental pulp stem cells<sup>69</sup> through regulation of the mitochondrial metabolism. A similar approach using laser heating of copper nanoparticles was used to induce mesenchymal stem cell differentiation into fibroblasts and hence accelerate wound healing.<sup>70</sup> Copper ions have traditionally been used to trigger this differentiation, but by using laser irradiation of copper nanoparticles, it was possible to thermally induce the mesenchymal differentiation and simultaneously weaken the side effects of having free copper ions in the blood. Remote modulation of myotube<sup>71</sup> and neuronal<sup>4,72</sup> cell differentiation was also achieved using plasmonic heating (Figure 6B), and while the mechanism behind the observed biological effects remains poorly understood, it has been shown that several proteins are regulated through thermally altered gene expression, including HSP and other stress sensitive proteins.<sup>50,71</sup> We envisage that plasmonic heating will provide a useful tool in research on stem cell-based therapeutics and tissue engineering by remote heating and wireless stimulation of muscle cells. To gain a more complete understanding of how local hyperthermia works in different cell types, future research should seek to elucidate the underlying mechanisms of how local heat can alter the genetic state of cells.

Plasmonic heating has been extensively used to manipulate cells by thermally triggering changes in the extracellular environment. By embedding cells in thermally sensitive hydrogels, one can turn cellular connections on and off using local heating, allowing for the study of how cellular stimuli propagate in a neuronal cellular network.<sup>2</sup> To this end, neural cells were placed on a layer of gold nanorods, which could be locally heated by laser irradiation. The plated cells were separated by the heat sensitive hydrogel, and hence, the selected connections could be allowed to grow by selective ablation of specific areas of the gel (Figure 6C). Additionally, this platform allowed suppression of neuronal spike activity by selective ablation of existing neural connections like local heat ablation (Figure 6C). Thermally induced contraction of hydrogels has also been used to apply subcellular mechanical stress by embedding a contractile hydrogel between polymeric pillars onto which cells were adhered<sup>68</sup> (Figure 6D). While hydrogels offer a unique manipulation tool for cellular studies, we also emphasize other studies that offer some more flexibility due to the absence of a hydrogel. For example, a micro-patterned plasmonic substrate<sup>72</sup> or a mobile optical fiber, functionalized with gold nanorods at the tip, was successfully used to selectively modulate neuronal signals in cell cultures.<sup>73</sup>



**Figure 7.** Plasmonic heating used in photothermal therapy and for triggering release in drug delivery. (A) Photothermal therapy using gold nanostars coated with silver as a switchable pro-drug agent. Upon entry into the tumor environment, the silver coating is etched off due to the high levels of  $H_2O_2$  in tumor cells. The bare gold nanostars exhibit higher absorption within the near-infrared wavelength region and hence become activated within the tumor environment. The measured difference in heating between bare gold nanostars and silver-coated nanostars is shown in the graph and by thermal imaging for the tumor and muscle tissue. Panel A was modified from ref 83. Copyright 2021 Royal Society of Chemistry. (B) Multilayer theranostic nanoparticles synthesized with a liposomal core, a shell of gold nanoparticles, and an additional outer lipid bilayer. The outer lipid bilayer is labeled with radioisotopes for positron emission tomography (PET) imaging. Data show treatment with PEG lipids containing liposomes and gold (AL) and liposomes, gold, and an outer lipid bilayer (LAL) for improving immune evasiveness and increasing the rate of delivery to tumor site. Laser treatment with LAL showed efficient tumor inhibition compared to laser treatment with normal saline (NS) and also efficient delivery and imaging capabilities as shown by PET imaging. Panel B was reproduced from ref 87. Copyright 2021 Springer Nature. (C and D) General approach for using plasmonic heating in drug delivery. (C) Thermally triggered release of molecular cargo conjugated to the particle surface and (D) thermally triggered permeability changes in temperature sensitive materials (e.g., liposomes or hydrogels) containing drugs.

Here we have highlighted a few applications for studying cell cultures using thermoplasmonics, but it is not hard to imagine endless opportunities for combining new developments in substrate engineering with thermoplasmonics, which will offer many new applications for investigating and manipulating cell–substrate and cell–cell interactions.

Nanoparticles have now been investigated extensively as photothermal agents for cancer therapy often combined with drug delivery<sup>5,74</sup> or in combination with photodynamic therapy (PDT) in which light-absorbing molecules can act as

photosensitizers for the generation of reactive oxygen species.<sup>75,76</sup> During photothermal therapy (PTT), nanoparticles accumulate at tumor sites in tissue by the enhanced permeability and retention (EPR) effect and are irradiated with NIR light to ablate the cancerous cells.<sup>77</sup> After some years with animal testing and lab research, photothermal therapy is these days entering a new era, following some new unpublished data from a recently completed clinical trial. These data show that prostate cancer was successfully treated via laser irradiation with gold nanoshells with remarkably few side effects. Here, we

briefly touch upon these new results and a few recent advances made in this field and point to where the research is heading to improve clinical applications of these smart therapeutic particles.

A challenge in PTT is to design biocompatible nanoparticles that can effectively extravasate into the cancer tumor environment.<sup>78</sup> The leaky vasculature supplying tumors with blood allows the extravasation of NPs (<200 nm) into the tumor tissue. The optimal particle diameter used in the systemic delivery of NPs is approximately 100–200 nm, whereas much smaller NPs are cleared via the renal system.<sup>79,80</sup> Several promising approaches have been adopted to make the nanoparticle evade the immune system by camouflaging nanoparticles with biomimetic coatings, thus increasing their circulation time and their likelihood of reaching their target. At the target site, therapeutic effects are achieved upon irradiation, leading to the efficient treatment of tumors in mice. Such a therapeutic approach has just been finalized in a clinical trial for testing ablation of tumors using gold nanoshells (clinical trial NCT04240639).<sup>5,81</sup> Patients in this trial received gold nanoshells (total diameter of ~150 nm) intravenously. The particle surface was passivated with short polyethylene glycol (molecular weight of 6 kDa) for immune evasion to allow for accumulation in the tumor via the EPR effect. Subsequent irradiation with low-power NIR light (810 nm) resulted in efficient ablation of the tumor with very few side effects observed during a long follow-up observation. Although data from this trial have not been published in final form, preliminary data from this trial can be found in ref 81, which clearly show the convincing effect of PTT on prostate cancer.

Further developments are focused on testing thermoplasmonic particles that function as pro-drugs. These efforts are driving the field toward more clinically safe therapeutics with minimal side effects.<sup>82</sup> For instance, researchers have developed nanoparticles that are plasmonically dormant until activated by the H<sub>2</sub>O<sub>2</sub> environment at tumor sites, inhibiting thermal effects of irradiation in other tissues where the particles may also accumulate<sup>83</sup> (Figure 7A). More recently, to address limitations of selectivity and imaging of therapeutic particles, multimodal systems combining plasmonic and magnetic properties have been proposed.<sup>84–86</sup> A two-layer Au-liposome (LAL) labeled with <sup>64</sup>Cu was recently shown to exhibit a very high level of accumulation in tumors while also being effective for *in vivo* PET imaging and tumor ablation by PTT in mice models<sup>87</sup> (Figure 7B). Such hybrid particles pave the way for the next generation of cancer theranostics by providing a platform that simultaneously serves as a contrast agent for imaging and a source of plasmonically induced hyperthermia.

Plasmonic heating of nanoparticles is also being extensively investigated in the field of drug delivery for light-triggered release of therapeutic molecules, as also discussed above in relation to the delivery of small RNA for genetic interference. There are two generic approaches for using thermoplasmonics in drug delivery: (i) conjugating drugs directly to the surface of nanoparticles, such that the drug will be released when irradiation causes heating of the system (Figure 7C), and (ii) employing a thermosensitive drug container made from, e.g., hydrogels or lipids, to release the content<sup>88,89</sup> (Figure 7D). A hybrid polymeric microcarrier with gold bipyramidal nanoparticles has, e.g., been used to release a therapeutic molecule for diabetic retinopathy upon NIR irradiation.<sup>90</sup> Combination

therapy, in which the discussed thermoplasmonic effects are used for both on-site delivery of drugs or genetic material and simultaneous heat-induced hyperthermia, has been proven to be an effective strategy for combating cancer cells.<sup>91,92</sup> There are several advantages of this approach, including the sensitivity of cancer cells to heat and the stronger therapeutic effect of some drugs at higher temperatures. With early thermoplasmonic particle systems already in clinical trials, innovation on combination therapies involving thermal release and thermal therapy is likely to continue.

Future perspectives in this field include further development of substrate engineering and development of smart materials, which are expected to inspire new applications in thermoplasmonics. Many materials exhibit high thermal sensitivity and hence can act as heat-triggered actuators inside cells or in materials that are interfacing with biological specimens. Also, the existing strategies outlined in this review have other applications. For instance, intracellular or nuclear repair in cells should be possible to investigate using endocytosed nanoparticles that are known, during cellular uptake, to travel from the cell surface toward the nucleus along the endocytic pathway. Also, microchemistry, using thermoplasmonic fusion of GUVs, has great potential beyond what is shown with the biophysical applications presented in this review. Monitoring polymerization or other active molecular events is readily possible by combining reagents in a stepwise manner through fusion and remains to be explored.

Photothermal therapy has now been shown in clinical trials to be efficient in cancer therapy with remarkably few side effects, and future studies should focus on testing PTT on additional types of cancer. Successful outcomes in cancer therapy will also lead to expansion of the same PTT methodology to other diseases like Alzheimer's disease as mentioned in ref 93 or in phage therapy with the aim of killing multiresistant bacteria.<sup>74</sup> Future developments should also focus on molecular mechanisms affected by heating<sup>94</sup> that will strengthen the impact of PTT in combination with other scientific disciplines.

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### Notes

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## REFERENCES

- (1) Moreno-Pescador, G. S.; Aswad, D. S.; Florentsen, C. D.; Bahadori, A.; Arastoo, M. R.; Nielsen, H. M. D.; Heitmann, A. S. B.; Boye, T. L.; Nylandsted, J.; Oddershede, L. B.; Bendix, P. M.; et al. Thermoplasmonic nano-rupture of cells reveals annexin v function in plasma membrane repair. *Nanoscale* **2022**, *14* (21), 7778–7787.
- (2) Hong, N.; Nam, Y. Thermoplasmonic neural chip platform for in situ manipulation of neuronal connections in vitro. *Nat. Commun.* **2020**, *11* (1), 6313.
- (3) Robert, H. M. L.; Savatier, J.; Vial, S.; Verghese, J.; Wattellier, B.; Rigneault, H.; Monneret, S.; Polleux, J.; Baffou, G. Photothermal control of heat-shock protein expression at the single cell level. *Small* **2018**, *14* (32), 1801910.
- (4) Antonova, O. Yu.; Kochetkova, O. Yu.; Kanev, I. L. Light-to-heat converting ecm-mimetic nanofiber scaffolds for neuronal differentiation and neurite outgrowth guidance. *Nanomaterials* **2022**, *12* (13), 2166.
- (5) Li, X.; Lovell, J. F.; Yoon, J.; Chen, X. Clinical development and potential of photothermal and photodynamic therapies for cancer. *Nat. Rev. Clin. Oncol.* **2020**, *17* (11), 657–674.
- (6) Moreno-Pescador, G.; Arastoo, M. R.; Ruhoff, V. T.; Chiantia, S.; Daniels, R.; Bendix, P. M. Thermoplasmonic vesicle fusion reveals membrane phase segregation of influenza spike proteins. *Nano Lett.* **2023**, *23* (8), 3377–3384.
- (7) De Franceschi, N.; Alqabandi, M.; Miguet, N.; Caillat, C.; Mangenot, S.; Weissenhorn, W.; Bassereau, P. The escrt protein chmp2b acts as a diffusion barrier on reconstituted membrane necks. *J. Cell Sci.* **2019**, *132* (4), jcs217968.
- (8) Yeheskely-Hayon, D.; Minai, L.; Golan, L.; Dann, E. J.; Yelin, D. Optically induced cell fusion using bispecific nanoparticles. *Small* **2013**, *9* (22), 3771–3777.
- (9) Cheong, J.; Yu, H.; Lee, C. Y.; Lee, J.-u.; Choi, H.-J.; Lee, J.-H.; Lee, H.; Cheon, J. Fast detection of SARS-CoV-2 RNA via the integration of plasmonic thermocycling and fluorescence detection in a portable device. *Nat. Biomed. Eng.* **2020**, *4*, 1159–1167.
- (10) Baffou, G.; Quidant, R. Thermo-plasmonics: using metallic nanostructures as nano-sources of heat. *Laser Photonics Rev.* **2013**, *7* (2), 171–187.
- (11) Baffou, G.; Cichos, F.; Quidant, R. Applications and challenges of thermoplasmonics. *Nat. Mater.* **2020**, *19* (9), 946–958.
- (12) Jauffred, L.; Samadi, A.; Klingberg, H.; Bendix, P. M.; Oddershede, L. B. Plasmonic heating of nanostructures. *Chem. Rev.* **2019**, *119* (13), 8087–8130.
- (13) Xin, H.; Namgung, B.; Lee, L. P. Nanoplasmonic optical antennas for life sciences and medicine. *Nat. Rev. Mater.* **2018**, *3* (8), 228–243.
- (14) Sharifi, M.; Attar, F.; Saboury, A. A.; Akhtari, K.; Hooshmand, N.; Hasan, A.; El-Sayed, M. A.; Falahati, M. Plasmonic gold nanoparticles: Optical manipulation, imaging, drug delivery and therapy. *J. Controlled Release* **2019**, 311–312, 170–189.
- (15) Bahadori, A.; Moreno-Pescador, G.; Oddershede, L. B.; Bendix, P. M. Remotely controlled fusion of selected vesicles and living cells: a key issue review. *Rep. Prog. Phys.* **2018**, *81* (3), 032602.
- (16) Bohren, C. F.; Huffman, D. R. *Absorption and Scattering of Light by Small Particles*; Wiley-VCH Verlag GmbH & Co. KGaA, 1998.
- (17) Khlebtsov, N. G.; Dykman, L. A. Optical properties and biomedical applications of plasmonic nanoparticles. *J. Quant. Spectrosc. Radiat. Transfer* **2010**, *111* (1), 1–35.
- (18) Govorov, A. O.; Zhang, W.; Skeini, T.; Richardson, H.; Lee, J.; Kotov, N. A. Gold nanoparticle ensembles as heaters and actuators: melting and collective plasmon resonances. *Nanoscale Res. Lett.* **2006**, *1* (1), 84–90.
- (19) Bendix, P. M.; Reihani, S. N. S.; Oddershede, L. B. Direct measurements of heating by electromagnetically trapped gold nanoparticles on supported lipid bilayers. *ACS Nano* **2010**, *4* (4), 2256–2262.
- (20) Samadi, A.; Klingberg, H.; Jauffred, L.; Kjær, A.; Bendix, P. M.; Oddershede, L. B. Platinum nanoparticles: a non-toxic, effective and thermally stable alternative plasmonic material for cancer therapy and bioengineering. *Nanoscale* **2018**, *10* (19), 9097–9107.
- (21) Ifijen, I. H.; Maliki, M. A comprehensive review on the synthesis and photothermal cancer therapy of titanium nitride nanostructures. *Inorg. Nano-Met. Chem.* **2023**, *53* (4), 366–387.
- (22) Samadi, A.; Bendix, P. M.; Oddershede, L. B. Optical manipulation of individual strongly absorbing platinum nanoparticles. *Nanoscale* **2017**, *9* (46), 18449–18455.
- (23) Guglielmelli, A.; Pierini, F.; Tabiryan, N.; Umeton, C.; Bunning, T. J.; De Sio, L. Thermoplasmonics with gold nanoparticles: A new weapon in modern optics and biomedicine. *Adv. Photonics Res.* **2021**, *2* (8), 2000198.
- (24) Baffou, G.; Polleux, J.; Rigneault, H.; Monneret, S. Super-heating and micro-bubble generation around plasmonic nanoparticles under cw illumination. *J. Phys. Chem. C* **2014**, *118* (9), 4890–4898.
- (25) Zundel, L.; Malone, K.; Cerdan, L.; Martinez-Herrero, R.; Manjavacas, A. Lattice Resonances for Thermoplasmonics. *ACS Photonics* **2023**, *10* (1), 274–282.
- (26) Bertin, A.; de Franceschi, N.; de la Mora, E.; Maity, S.; Alqabandi, M.; Miguet, N.; di Cicco, A.; Roos, W. H.; Mangenot, S.; Weissenhorn, W.; Bassereau, P.; et al. Human escrt-iii polymers assemble on positively curved membranes and induce helical membrane tube formation. *Nat. Commun.* **2020**, *11* (1), 2663.
- (27) Rørvig-Lund, A.; Bahadori, A.; Semsey, S.; Bendix, P. M.; Oddershede, L. B. Vesicle fusion triggered by optically heated gold nanoparticles. *Nano Lett.* **2015**, *15* (6), 4183–4188.
- (28) Bahadori, A.; Oddershede, L. B.; Bendix, P. M. Hot-nanoparticle-mediated fusion of selected cells. *Nano Res.* **2017**, *10*, 2034–2045.
- (29) Li, M.; Lohmuller, T.; Feldmann, J. Optical injection of gold nanoparticles into living cells. *Nano Lett.* **2015**, *15* (1), 770–775.
- (30) Urban, A. S.; Pfeiffer, T.; Fedoruk, M.; Lutich, A. A.; Feldmann, J. Single-step injection of gold nanoparticles through phospholipid membranes. *ACS Nano* **2011**, *5* (5), 3585–3590.
- (31) Andersen, T.; Bahadori, A.; Ott, D.; Kyrsting, A.; Reihani, S. N. S.; Bendix, P. M. Nanoscale phase behavior on flat and curved membranes. *Nanotechnology* **2014**, *25* (50), 505101.
- (32) Andersen, T.; Kyrsting, A.; Bendix, P. M. Local and transient permeation events are associated with local melting of giant liposomes. *Soft Matter* **2014**, *10* (24), 4268–4274.
- (33) Urban, A. S.; Fedoruk, M.; Horton, M. R.; Radler, J. O.; Stefan, F. D.; Feldmann, J. Controlled nanometric phase transitions of phospholipid membranes by plasmonic heating of single gold nanoparticles. *Nano Lett.* **2009**, *9* (8), 2903–2908.
- (34) Chen, H.; Dong, X.; Ou, L.; Ma, C.; Yuan, B.; Yang, K. Thermal-controlled cellular uptake of “hot” nanoparticles. *Nanoscale* **2023**, *15* (30), 12718–12727.
- (35) Michaels, T. C. T.; Saric, A.; Curk, S.; Bernfur, K.; Arosio, P.; Meisl, G.; Dear, A. J.; Cohen, S. I. A.; Dobson, C. M.; Vendruscolo, M.; Linse, S.; Knowles, T. P. J. Dynamics of oligomer populations formed during the aggregation of alzheimer’s  $\alpha\beta$ 42 peptide. *Nat. Chem.* **2020**, *12* (5), 445–451.
- (36) Lin, D.; Qian, Z.; Bagnani, M.; Hernandez-Rodriguez, M. A.; Corredoira-Vazquez, J.; Wei, G.; Carlos, L. D.; Mezzenga, R. Probing the protein folding energy landscape: Dissociation of amyloid- $\beta$  fibrils by laser-induced plasmonic heating. *ACS Nano* **2023**, *17* (10), 9429–9441.
- (37) Wang, C.; Wu, B.; Wu, Y.; Song, X.; Zhang, S.; Liu, Z. Camouflaging nanoparticles with brain metastatic tumor cell membranes: a new strategy to traverse blood–brain barrier for

- imaging and therapy of brain tumors. *Adv. Funct. Mater.* **2020**, *30* (14), 1909369.
- (38) Mahmoudi, M.; Lohse, S. E.; Murphy, C. J.; Fathizadeh, A.; Montazeri, A.; Suslick, K. S. Variation of protein corona composition of gold nanoparticles following plasmonic heating. *Nano Lett.* **2014**, *14* (1), 6–12.
- (39) Jiang, M.; Chapman, A.; Olarte-Plata, J. D.; Bresme, F. Controlling local thermal gradients at molecular scales with janus nanoheaters. *Nanoscale* **2023**, *15*, 10264–10276.
- (40) Mullis, K.; Falloona, F.; Scharf, S.; Saiki, R.; Horn, G.; Erlich, H. Specific enzymatic amplification of dna in vitro: The polymerase chain reaction. *Cold Spring Harbor Symposia on Quantitative Biology* **1986**, *51*, 263–273.
- (41) Zhu, H.; Zhang, H.; Xu, Y.; Laššáková, S.; Korabečná, M.; Neužil, P. PCR past, present and future. *BioTechniques* **2020**, *69*, 317–325.
- (42) Postollec, F.; Falentin, H.; Pavan, S.; Combrisson, J.; Sohier, D. Recent advances in quantitative PCR (qPCR) applications in food microbiology. *Food Microbiol.* **2011**, *28*, 848–861.
- (43) Lee, S. H.; Park, S.-m.; Kim, B. N.; Kwon, O. S.; Rho, W.-Y.; Jun, B.-H. Emerging ultrafast nucleic acid amplification technologies for next-generation molecular diagnostics. *Biosens. Bioelectron.* **2019**, *141*, 111448.
- (44) Monshat, H.; Wu, Z.; Pang, J.; Zhang, Q.; Lu, M. Integration of plasmonic heating and on-chip temperature sensor for nucleic acid amplification assays. *J. Biophotonics* **2020**, *13*, 7.
- (45) Kang, B.-H.; Jang, K.-W.; Yu, E.-S.; Na, H.; Lee, Y.-J.; Ko, W.-Y.; Bae, N.; Rho, D.; Jeong, K.-H. Ultrafast plasmonic nucleic acid amplification and real-time quantification for decentralized molecular diagnostics. *ACS Nano* **2023**, *17*, 6507–6518.
- (46) Nabuti, J.; Fath Elbab, A. R.; Abdel-Mawgood, A.; Yoshihisa, M.; Shalaby, H. M.H. Highly efficient photonic PCR system based on plasmonic heating of gold nanofilms. *Biosens. Bioelectron.: X* **2023**, *14*, 100346.
- (47) Son, J. H.; Cho, B.; Hong, S.; Lee, S. H.; Hoxha, O.; Haack, A. J.; Lee, L. P. Ultrafast photonic PCR. *Light: Sci. Appl.* **2015**, *4*, No. e280.
- (48) Millington, A. L.; Houskeeper, J. A.; Quackenbush, J. F.; Trauba, J. M.; Wittwer, C. T. The kinetic requirements of extreme qPCR. *Biomol. Detect. Quantif.* **2019**, *17*, 100081.
- (49) Hasanzadeh Kafshgari, M.; Agiotis, L.; Largilliere, I.; Patkovsky, S.; Meunier, M. Antibody-functionalized gold nanostar-mediated on-resonance picosecond laser optoporation for targeted delivery of rna therapeutics. *Small* **2021**, *17* (19), 2007577.
- (50) Lu, W.; Zhang, G.; Zhang, R.; Flores, L. G.; Huang, Q.; Gelovani, J. G.; Li, C. Tumor site-specific silencing of NF- $\kappa$  B p65 by targeted hollow gold nanosphere-mediated photothermal transfection. *Cancer Res.* **2010**, *70*, 3177–3188.
- (51) Paunovska, K.; Loughrey, D.; Dahlman, J. E. Drug delivery systems for RNA therapeutics. *Nat. Rev. Genet.* **2022**, *23*, 265–280.
- (52) Florentsen, C. D.; West, A.-K. V.; Danielsen, H. M. D.; Semsey, S.; Bendix, P. M.; Oddershede, L. B. Quantification of loading and laser-assisted release of RNA from single gold nanoparticles. *Langmuir* **2018**, *34*, 14891–14898.
- (53) Wang, Z.; Li, S.; Zhang, M.; Ma, Y.; Liu, Y.; Gao, W.; Zhang, J.; Gu, Y. Laser-triggered small interfering rna releasing gold nanoshells against heat shock protein for sensitized photothermal therapy. *Adv. Sci.* **2017**, *4* (2), 1600327.
- (54) Huo, S.; Gong, N.; Jiang, Y.; Chen, F.; Guo, H.; Gan, Y.; Wang, Z.; Herrmann, A.; Liang, X.-J. Gold-dna nanosunflowers for efficient gene silencing with controllable transformation. *Sci. Adv.* **2019**, *5* (10), No. eaaw6264.
- (55) Graczyk, A.; Pawlowska, R.; Jedrzejczyk, D.; Chworus, A. Gold nanoparticles in conjunction with nucleic acids as a modern molecular system for cellular delivery. *Molecules* **2020**, *25* (1), 204.
- (56) Fraire, J. C.; Houthaeve, G.; Liu, J.; Raes, L.; Vermeulen, L.; Stremersch, S.; Brans, T.; Garcia-Diaz Barriga, G.; De Keulenaer, S.; Van Nieuwerburgh, F.; et al. Vapor nanobubble is the more reliable photothermal mechanism for inducing endosomal escape of sirna without disturbing cell homeostasis. *J. Controlled Release* **2020**, *319*, 262–275.
- (57) Huschka, R.; Barhoumi, A.; Liu, Q.; Roth, J. A.; Ji, L.; Halas, N. J. Gene silencing by gold nanoshell-mediated delivery and laser-triggered release of antisense oligonucleotide and sirna. *ACS Nano* **2012**, *6* (9), 7681–7691.
- (58) Braun, G. B.; Pallaoro, A.; Wu, G.; Missirlis, D.; Zasadzinski, J. A.; Tirrell, M.; Reich, N. O. Laser-activated gene silencing via gold nanoshell-sirna conjugates. *ACS Nano* **2009**, *3* (7), 2007–2015.
- (59) Krpetic, Z.; Nativo, P.; See, V.; Prior, I. A.; Brust, M.; Volk, M. Inflicting controlled nonthermal damage to subcellular structures by laser-activated gold nanoparticles. *Nano Lett.* **2010**, *10* (11), 4549–4554.
- (60) Wang, Y.; Gao, Z.; Han, Z.; Liu, Y.; Yang, H.; Akkin, T.; Hogan, C. J.; Bischof, J. C. Aggregation affects optical properties and photothermal heating of gold nanospheres. *Sci. Rep.* **2021**, *11* (1), 898.
- (61) Huschka, R.; Zuloaga, J.; Knight, M. W.; Brown, L. V.; Nordlander, P.; Halas, N. J. Light-induced release of dna from gold nanoparticles: nanoshells and nanorods. *J. Am. Chem. Soc.* **2011**, *133* (31), 12247–12255.
- (62) de Miguel, I.; Prieto, I.; Albornoz, A.; Sanz, V.; Weis, C.; Turon, P.; Quidant, R. Plasmon-based biofilm inhibition on surgical implants. *Nano Lett.* **2019**, *19* (4), 2524–2529.
- (63) Zharov, V. P.; Mercer, K. E.; Galitovskaya, E. N.; Smeltzer, M. S. Photothermal nanotherapeutics and nanodiagnoses for selective killing of bacteria targeted with gold nanoparticles. *Biophys. J.* **2006**, *90*, 619–627.
- (64) Akouibaa, A.; Masrour, R.; Benhamou, M.; Derouiche, A. Thermoplasmonics Decontamination of Respirators Face Masks Using Silver Nanoparticles: A New Weapon in the Fight Against COVID-19 Pandemic. *Plasmonics* **2022**, *17* (6), 2307–2322.
- (65) De Sio, L.; Ding, B.; Focsan, M.; Kogermann, K.; Pascoal-Faria, P.; Petronela, F.; Mitchell, G.; Zussman, E.; Pierini, F. Personalized reusable face masks with smart nano-assisted destruction of pathogens for covid-19: A visionary road. *Chem. - Eur. J.* **2021**, *27* (20), 6112–6130.
- (66) Madrid, M.; Saklayen, N.; Shen, W.; Huber, M.; Vogel, N.; Mazur, E. Laser-activated self-assembled thermoplasmonic nanocavity substrates for intracellular delivery. *ACS Appl. Bio Mater.* **2018**, *1* (6), 1793–1799.
- (67) Zhao, X.; Shi, Y.; Pan, T.; Lu, D.; Xiong, J.; Li, B.; Xin, H. In situ single-cell surgery and intracellular organelle manipulation via thermoplasmonics combined optical trapping. *Nano Lett.* **2022**, *22* (1), 402–410.
- (68) Sutton, A.; Shirman, T.; Timonen, J. V. I.; England, G. T.; Kim, P.; Kolle, M.; Ferrante, T.; Zarzar, L. D.; Strong, E.; Aizenberg, J. Photothermally triggered actuation of hybrid materials as a new platform for in vitro cell manipulation. *Nat. Commun.* **2017**, *8* (1), 14700.
- (69) Wang, J.; Qu, X.; Xu, C.; Zhang, Z.; Qi, G.; Jin, Y. Thermoplasmonic regulation of the mitochondrial metabolic state for promoting directed differentiation of dental pulp stem cells. *Anal. Chem.* **2022**, *94* (27), 9564–9571.
- (70) Xiao, Y.; Peng, J.; Liu, Q.; Chen, L.; Shi, K.; Han, R.; Yang, Q.; Zhong, L.; Zha, R.; Qu, Y.; Qian, Z. Ultrasmall cus@ bsa nanoparticles with mild photothermal conversion synergistically induce mscls-differentiated fibroblast and improve skin regeneration. *Theranostics* **2020**, *10* (4), 1500.
- (71) Marino, A.; Arai, S.; Hou, Y.; Degl'Innocenti, A.; Cappello, V.; Mazzolai, B.; Chang, Y.-T.; Mattoli, V.; Suzuki, M.; Ciofani, G. Gold nanoshell-mediated remote myotube activation. *ACS Nano* **2017**, *11* (3), 2494–2508.
- (72) Andolfi, A.; Arnaldi, P.; Lisa, D. D.; Pepe, S.; Frega, M.; Fassio, A.; Lagazzo, A.; Martinoia, S.; Pastorino, L. A micropatterned thermoplasmonic substrate for neuromodulation of in vitro neuronal networks. *Acta Biomater.* **2023**, *158*, 281–291.

- (73) Kang, H.; Hong, W.; An, Y.; Yoo, S.; Kwon, H.-J.; Nam, Y. Thermoplasmonic optical fiber for localized neural stimulation. *ACS Nano* **2020**, *14* (9), 11406–11419.
- (74) Peng, H.; Borg, R. E.; Dow, L. P.; Pruitt, B. L.; Chen, I. A. Controlled phage therapy by photothermal ablation of specific bacterial species using gold nanorods targeted by chimeric phages. *Proc. Natl. Acad. Sci. U. S. A.* **2020**, *117* (4), 1951–1961.
- (75) Kong, C.; Chen, X. Combined photodynamic and photo-thermal therapy and immunotherapy for cancer treatment: a review. *Int. J. Nanomed.* **2022**, *17*, 6427–6446.
- (76) Guo, S.; Gu, D.; Yang, Y.; Tian, J.; Chen, X. Near-infrared photodynamic and photothermal co-therapy based on organic small molecular dyes. *J. Nanobiotechnol.* **2023**, *21* (1), 348.
- (77) Lv, Z.; He, S.; Wang, Y.; Zhu, X. Noble metal nanomaterials for nir-triggered photothermal therapy in cancer. *Adv. Healthcare Mater.* **2021**, *10* (6), 2001806.
- (78) Otero, C. M.; Simal, G. B.; Scocozza, M. F.; Rubert, A.; Grillo, C. A.; Hannibal, L.; Lavorato, G.; Huergo, M. A.; Murgida, D. H.; Vericat, C. Optimized biocompatible gold nanotriangles with nir absorption for photothermal applications. *ACS Appl. Nano Mater.* **2022**, *5* (1), 341–350.
- (79) Fang, J. Epr effect-based tumor targeted nanomedicine: A promising approach for controlling cancer. *J. Pers. Med.* **2022**, *12* (1), 95.
- (80) Longmire, M.; Choyke, P. L.; Kobayashi, H. Clearance properties of nano-sized particles and molecules as imaging agents: considerations and caveats. *Nanomedicine* **2008**, *3*, 703–717, DOI: 10.2217/17435889.3.5.703.
- (81) Rastinehad, A. R.; Anastos, H.; Wajswol, E.; Winoker, J. S.; Sfakianos, J. P.; Doppalapudi, S. K.; Carrick, M. R.; Knauer, C. J.; Taouli, B.; Lewis, S. C.; et al. Gold nanoshell-localized photothermal ablation of prostate tumors in a clinical pilot device study. *Proc. Natl. Acad. Sci. U. S. A.* **2019**, *116* (37), 18590–18596.
- (82) Ali, M. R. K.; Rahman, M. A.; Wu, Y.; Han, T.; Peng, X.; Mackey, M. A.; Wang, D.; Shin, H. J.; Chen, Z. G.; Xiao, H.; et al. Efficacy, long-term toxicity, and mechanistic studies of gold nanorods photothermal therapy of cancer in xenograft mice. *Proc. Natl. Acad. Sci. U. S. A.* **2017**, *114* (15), E3110–E3118.
- (83) Liu, Y.; Mo, F.; Hu, J.; Jiang, Q.; Wang, X.; Zou, Z.; Zhang, X.-Z.; Pang, D.-W.; Liu, X. Precision photothermal therapy and photoacoustic imaging by in situ activatable thermoplasmonics. *Chem. Sci.* **2021**, *12* (29), 10097–10105.
- (84) Muzzi, B.; Albino, M.; Gabbani, A.; Omelyanchik, A.; Kozenkova, E.; Petrecca, M.; Innocenti, C.; Balica, E.; Lavacchi, A.; Scavone, F.; et al. Star-shaped magnetic-plasmonic au@ fe<sub>3</sub>o<sub>4</sub> nano-heterostructures for photothermal therapy. *ACS Appl. Mater. Interfaces* **2022**, *14* (25), 29087–29098.
- (85) Henderson, L.; Neumann, O.; Kadria-Vili, Y.; Gerislioglu, B.; Bankson, J.; Nordlander, P.; Halas, N. J. Plasmonic gadolinium oxide nanomtryoshkas: Bifunctional magnetic resonance imaging enhancers for photothermal cancer therapy. *PNAS Nexus* **2022**, *1* (4), pgac140.
- (86) Griaznova, O. Yu.; Belyaev, I. B.; Sogomonyan, A. S.; Zelepukin, I. V.; Tikhonowski, G. V.; Popov, A. A.; Komlev, A. S.; Nikitin, P. I.; Gorin, D. A.; Kabashin, A. V.; Deyev, S. M. Laser synthesized core-satellite fe-au nanoparticles for multimodal in vivo imaging and in vitro photothermal therapy. *Pharmaceutics* **2022**, *14* (5), 994.
- (87) Jeon, M.; Kim, G.; Lee, W.; Baek, S.; Jung, H. N.; Im, H.-J. Development of theranostic dual-layered au-liposome for effective tumor targeting and photothermal therapy. *J. Nanobiotechnol.* **2021**, *19*, 262.
- (88) Wang, C.; Vazquez-Gonzalez, M.; Fadeev, M.; Sohn, Y. S.; Nechushtai, R.; Willner, I. Thermoplasmonic-triggered release of loads from dna-modified hydrogel microcapsules functionalized with au nanoparticles or au nanorods. *Small* **2020**, *16* (22), 2000880.
- (89) Chen, R.; Shi, J.; Liu, C.; Li, J.; Cao, S. In situ self-assembly of gold nanorods with thermal-responsive microgel for multi-synergistic remote drug delivery. *Adv. Compos. Hybrid Mater.* **2022**, *5* (3), 2223–2234.
- (90) Stoia, D.; Pop, R.; Campu, A.; Nistor, M.; Astilean, S.; Pintea, A.; Suciu, M.; Rugina, D.; Focsan, M. Hybrid polymeric therapeutic microcarriers for thermoplasmonic-triggered release of resveratrol. *Colloids Surf., B* **2022**, *220*, 112915.
- (91) Zhao, Q.; Yang, Y.; Wang, H.; Lei, W.; Liu, Y.; Wang, S. Gold nanoparticles modified hollow carbon system for dual-responsive release and chemo-photothermal synergistic therapy of tumor. *J. Colloid Interface Sci.* **2019**, *554*, 239–249.
- (92) Tao, W.; Cheng, X.; Sun, D.; Guo, Y.; Wang, N.; Ruan, J.; Hu, Y.; Zhao, M.; Zhao, T.; Feng, H.; et al. Synthesis of multi-branched au nanocomposites with distinct plasmon resonance in nir-ii window and controlled crispr-cas9 delivery for synergistic gene-photothermal therapy. *Biomaterials* **2022**, *287*, 121621.
- (93) Sanati, M.; Khodagholi, F.; Aminyavari, S.; Ghasemi, F.; Gholami, M.; Kebriaeezadeh, A.; Sabzevari, O.; Hajipour, M. J.; Imani, M.; Mahmoudi, M.; Sharifzadeh, M. Impact of gold nanoparticles on amyloid  $\beta$ -induced alzheimer's disease in a rat animal model: Involvement of stim proteins. *ACS Chem. Neurosci.* **2019**, *10* (5), 2299–2309.
- (94) Ali, M. R. K.; Wu, Y.; Tang, Y.; Xiao, H.; Chen, K.; Han, T.; Fang, N.; Wu, R.; El-Sayed, M. A. Targeting cancer cell integrins using gold nanorods in photothermal therapy inhibits migration through affecting cytoskeletal proteins. *Proc. Natl. Acad. Sci. U. S. A.* **2017**, *114* (28), E5655–E5663.