ADVANCED OPTICAL MATERIALS
www.advopticalmat.de

Plasmonic Nanoagents in Biophysics and Biomedicine

*Maria-Ana Huergo, Francis Schuknecht, Jinhua Zhang, and Theobald Lohmüller**

The significant rise in implementation and applications of plasmonic nanosystems in biophysics, biochemistry, and medicine has culminated in the emergence of refined plasmonic enabling reagents, or "nanoagents". These are defined as tools that allow researchers to not only investigate, but also actively manipulate biological processes and complex biosystems, such as living cells, on the nanoscale. This development is based on a combination of sensing capabilities, photothermal control, and optical force manipulation offered by metallic nanoparticles. The article reviews the trajectory that plasmonic nanoagents have taken in recent years and highlights seminal recent examples of their application, such as optical sensing both in vitro and in vivo, optical control of biomolecular interactions and protein function, the manipulation of lipid membrane properties, and the possibility of guiding cellular behavior

1. Introduction

The challenge for nanoscience today involves the development and design of nanoscale systems and tools that are capable of sensing and detecting, as well as executing specific tasks on demand. Nanomaterials are in this case no longer defined by mere physical characteristics, such as size, shape, and material properties. Instead, they can be seen as multifunctional enabling reagents, more specifically "nanoagents", that are useful for monitoring and regulating molecular processes and complex operations with high spatio-temporal control and efficiency. Multifunctional nanoagents are widely discussed in the context of theranostics[1] (a portmanteau of the words therapeutics and diagnostics). Here, they have been employed for in vivo diagnostics and subsequent treatment of cancer,[2] and the protection against viral infections.[3] The possibilities for the design of nanoagents are vast.[4] Examples include, but are not limited to multifunctional nano-carriers based on organic molecules and nanoparticles,[5] metal based nanoagents,[6] carbon dots,[7] radio-labeled probes,[8] photo-switchable systems,[9] as well as hybrid bio-active materials and frameworks[10] and smart substrates that interact specifically with living cells.[11] Amongst these many intriguing examples, plasmonic nanoparticles and nanosystems distinguish themselves as nanoagents by three main features that arise from their optical properties: They are highly susceptible environmental sensors, they have superior applicability as nanoscale sources of heat, and they can be moved in a controlled manner by means of light. It is the combination of these properties, that paves the way for the development of plasmonic nanoagents towards applications in biophysics and biomedicine.[12]

The aim of this article is to review a seminal selection of strategies for the design and development of plasmonic nanoagents to manipulate and monitor biological functions. For this, we begin with a brief overview of the physical mechanisms governing plasmonic nanoparticles. We continue with the evolution of plasmonic sensing from single biomolecule detection towards in vivo applications. Here, to keep the article concise, we restrict ourselves to sensing applications that do not involve surface-enhanced Raman scattering (SERS) spectroscopy,[13] although we acknowledge that SERS has been widely applied in biological studies[14] and plays an important role in medical applications such as cancer imaging and treatment, as well.[15] Second, we discuss the potential of light-controlled plasmonic heating, for manipulating biomolecular systems in combination with sensing. Finally, we highlight examples of plasmonic nanosystems that take advantage of a combination of sensing, temperature control as well as optical forces, through which plasmonic particles unfold their full potential as nanoagents. This leads us to an outlook on the future of plasmonic nanoagents as advanced optical materials in biophysics and biomedicine.

2. Main

The optical properties of plasmonic nanoparticles are covered in great detail in numerous review articles[16] and textbooks.[17]

M.-A. Huergo
Instituto de Investigaciones Fisicoquímicas Teóricas y Aplicadas (INIFTA)
Universidad Nacional de La Plata-CONICET
Sucursal 4 Casilla de Correo 16, La Plata 1900, Argentina
F. Schuknecht, J. Zhang, T. Lohmüller
Chair for Photonics and Optoelectronics
Nano-Institute Munich
Department of Physics
Ludwig-Maximilians-Universität (LMU)
Königinstraße 10, 80539 Munich, Germany
E-mail: t.lohmueller@lmu.de
The ORCID identification number(s) for the author(s) of this article can be found under https://doi.org/10.1002/adom.202200572.

DOI: 10.1002/adom.202200572

We will therefore limit ourselves to a brief introduction to the fundamentals on which plasmonic sensing, heating, and force are based and refer to corresponding references. These distinct optical characteristics are founded on collective oscillations of metallic nanoparticle's conduction electrons, termed localized surface plasmons or particle plasmons.[17a] Noble metal particles, made of gold or silver for example, feature large scattering and absorption cross sections, which allows for single nanoparticle imaging and spectroscopy with a dark-field microscope (DFM). Furthermore, the particle polarizability is highly susceptible to the particle's immediate environment.[18] Even a minute change of the surrounding refractive index, can be detected by a shift of the plasmon resonance frequency. The measurement stability is high, since plasmonic properties are not prone to photobleaching and the chemical inertness inherent to most noble metal particles ensures biocompatibility with living cells or small organisms.[19] At the same time, the chemical addressability is straightforward, which allows for functionalization with biomolecules.[20]

Large absorption and scattering cross-sections do not only render plasmonic particles useful for sensing applications but also open up pathways to actively manipulate them with light. Light absorbed by plasmonic particles is converted into heat efficiently and fast. Gold nanoparticles, for example, can feature significant temperature increases within nanoseconds when they are irradiated with light at their plasmon resonance frequency.[21] When using a focused, continuous wave (cw) laser, this grants temperature control over a wide range of up to several hundred degrees Celsius on a single particle level. This thermal energy quickly dissipates into the environment. For a small spherical particle, temperatures fall with the inverse of the distance from its surface.[22]

In recent years, plasmonic heating has been employed in a variety of applications[23] such as control of nanoscale flows,[24] material science,[25] water treatment,[26] biomedicine,[27] and nanochemistry.[28] Some of these examples take advantage of the extremely high temperatures achievable, to the point of particle melting and deformation. For most biological applications, however, high temperatures are often not necessary or desired.[29] Here, heating by a few degrees Celsius and controlled temperature cycles are required, to avoid thermal degradation or irreversible damage to the studied system.[30]

Spatial manipulation of plasmonic particles by optical force is the third concept employed for devising plasmonic nanoagents. There are two kinds of forces that act on a metal nanoparticle in a focused laser beam:[31] A scattering force that originates from momentum transfer of scattered and absorbed photons as well as a gradient force, which is proportional to the intensity gradient of the Gaussian beam. Both forces act on a nanoparticle in different ways. The scattering force is directed along the energy flux of the light beam (in the direction of the Poynting vector), whilst the gradient force (when attractive) pulls the particle towards the beam center. A plasmonic particle can thus be trapped in the focus of a laser beam when the attractive gradient force exceeds the scattering force, which is the principle behind optical tweezers.[32] The contribution of scattering and gradient force can be balanced by the laser wavelength. Optical trapping is best achieved with laser wavelengths that are red shifted far from the plasmon resonance frequency. However, it should be noted that with a laser wavelength blue shifted from the plasmon resonance, the gradient force can also become repulsive.[33] For a laser wavelength closer to the particle's plasmon resonance, the scattering force becomes increasingly strong up to the point where it dominates and the particle is pushed out of the beam focus. Put simply, gold(but also other metallic)-nanoparticles can be trapped or pushed by light, depending on the illumination conditions.[31]

The important aspect to keep in mind is that sensing, heating, and force manipulation can occur at the same time, however with varying significance. These concepts should thus not be considered as strictly parallel modes of operations but are often intertwined. This has both notable benefits, as well as some drawbacks for plasmonic nanoagents, as we will discuss in the following.

2.1. Plasmonic Sensing: A Journey from In Vitro to In Vivo

With the global pandemic in 2020, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), it became apparent that the fast identification of pathogens is imperative for medical self-testing, as well as clinical diagnostics.[34] Optical screening tests, that use plasmonic nanoparticles for reliable detection of specific biomolecular recognition events, have long been used, in this regard.[35] On the most basic level, an assay can be designed where one type of binding partner, that is, specific antigens or single-stranded oligo-nucleic acids, is bound to gold nanoparticles that are either in solution or at a specific location on a diagnostic chip. When exposed to molecular antagonists (e.g., antibodies or the complementary nucleic acid strands) these analytes immobilize the gold particles at the target location via specific molecular recognition (**Figure 1**). The strip-chromatography design has been utilized successfully for the last 6 decades in the form of commercial lateral flow assays (LFA) for biomedicine, agriculture, food, and environmental sciences.[36] The popularity of these platforms relies on their low-cost, rapid diagnostic, and ease of use. These qualities arise from two characteristics of the tests: The sensitivity and specificity derived from biorecognition and the direct readout by the naked eye, originated from the gold nanoparticles' large optical cross sections. Arguably, this rather simple approach is also the most wide-spread commercial application, to date. Especially, as plasmonics-based LFA has played a crucial role in COVID-19 self-diagnostics worldwide.[37]

More sophisticated plasmonic nanoparticle sensors have been designed by following two complementary strategies. As described previously, any change in the particle's dielectric environment leads to a shift of the plasmon resonance, which enables the detection of molecular binding events. This renders plasmonic nanoparticles excellent biosensors, with many review articles[38] and books[39] published on this topic over the years. The first example of plasmonic biosensors was based on bulk solutions of dispersed gold nanoparticles. Almost two decades ago, light scattering spectroscopy of single gold nanoparticles was introduced[40] and the observation of specific binding events on a single particle level became feasible.[41] This greatly improved the sensitivity of such particle plasmonic sensors, as single particle spectra do not suffer from inhomogeneous

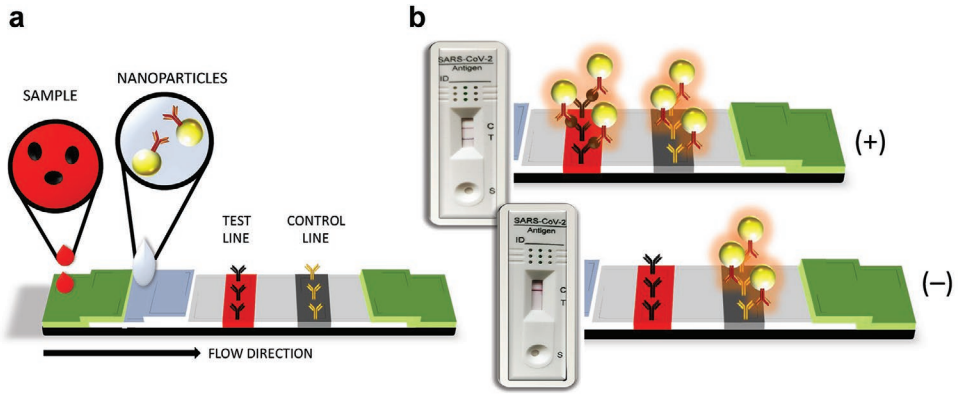

Figure 1. Scheme of a LFA. a) The sample potentially containing relevant analyte flows through the chromatography membrane and mixes with gold nanoparticles functionalized with anti-analyte recognition molecules. The test line contains a second anti-analyte molecule to retain the analyte bound to the nanoparticles. The control line is functionalized to recognize and retain the recognition molecules attached to the nanoparticles and determine the quality of the assay. b) Pictures of a SARS-CoV-2 antigen test with positive (up) and negative (down) results and their corresponding LFA schemes.

broadening (like bulk spectra) and even single nm spectral shifts could be observed in a basic streptavidin/biotin assay (**Figure 2**a).[42] Strategies to improve the sensor design further employed nanoparticles that display sharp resonance peaks and high field enhancement in the vis-NIR range such as gold nanorods,[43] nanotriangles,[44] nanocubes,[45] and nanostars.[46] An exciting development in this area has been the application of plasmonic nanoparticles to obtain enhanced circular dichroism (CD) spectra from chiral molecules.[47] Chirality is ubiquitous in nature and found in almost all biomolecules.[48] CD signals from organic molecules typically appear in the UV spectral region and are weak, which means that large analyte concentrations are required to conduct a measurement. Plasmon-enhanced CD enables a threefold improvement in this regard. Coupling between chiral molecules and the plasmonic near-field yields an enhanced CD excitation as well as signal intensity and can shift optical chirality to visible frequencies. As an example, core-shell nanocubes of gold and silver have been used for ultrasensitive chiral detection.[49] Furthermore, Link and colleagues could reveal by single particle circular differential scattering (CDS)[50] spectroscopy that individual nanorods covered with chiral proteins are CDS-inactive. Instead, aggregates of gold nanorod/BSA that display structural chirality and plasmonic hot-spots are required to obtain a strong CDS signal.[51] These findings pave the way for the design of plasmonic reporter materials that display strong amplification of chirality detection, which is essential for pharmaceutical applications and drug development.

A second strategy for plasmon-based biosensors relies on "radiative decay engineering"[52] via the interaction between

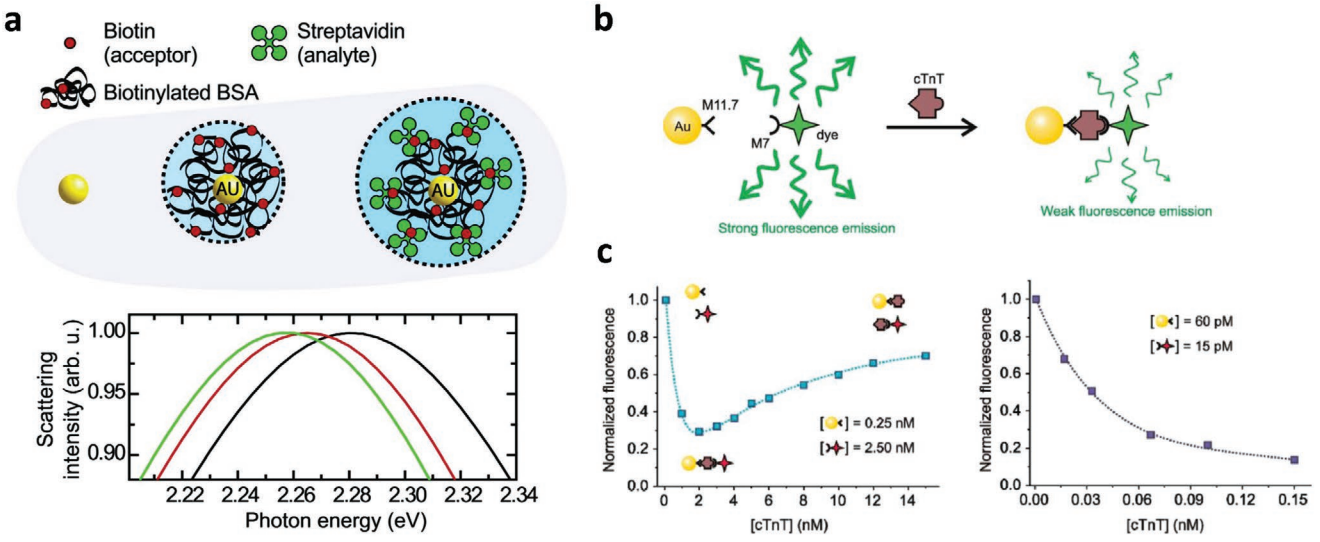

Figure 2. Single plasmonic nanoparticle biosensing. a) Top: Schematic illustration of nanoparticle functionalization with biotinylated BSA and subsequent streptavidin binding. Bottom: Plasmon energy redshift calculations of a single gold nanoparticle from bare to biotin and streptavidin bonding. (Reproduced with permission.[42] Copyright 2003, American Chemical Society.) b) Schematic illustrating the working principle of the cTnT immunoassay. c) The cTnT concentration detection by measuring fluorescence quenching. (Reproduced with permission.[60] Copyright 2003, American Chemical Society.)

plasmonic nanoparticles and fluorescent dyes. Plasmonic nanoparticles manipulate the radiative rate of close fluorophores,[53] which can result in either quenching or enhancement of fluorescence, depending on the separation distance, and the nanoparticle material.[54] Radiative quenching of dyes with small gold particles was shown to be more efficient compared to energy transfer between individual molecules and the distance between fluorescent dyes and a gold nanoparticle can thus be larger than the typical Förster radius.[55] Applications for fluorescence quenching with plasmonic nanoparticles have been demonstrated for oligonucleotides,[56] proteins,[57] small aptamers,[58] and even haptens such as the digitalis glycoside digoxin, a drug used for cardiac arrhythmia treatment that can induce severe side effects upon mis-dosage. As an example, nanoparticle-fluorophore conjugates have been applied to monitor digoxin levels in a nanomolar dynamic range of 0.2–5 ng mL^{-1}.[59] The first immunoassays for the detection of pathogens or biomolecules via fluorescence quenching were a further important step towards more medically relevant diagnostic applications. A seminal example has been the detection of cardiac troponin T (cTnT), a cardio-specific intracellular protein that regulates cell contraction. Plasmatic levels of cTnT are routinely scanned in hospitals, since elevated levels are an indication of heart damage and can be associated with a current or future myocardial infarction. In 2009, a "sandwich assay" was demonstrated by the Feldmann group, where cTnT proteins in solution were trapped between a gold nanoparticle and a fluorescent dye via specific antibodies (Figure 2b,c).[60] The weaker dye emission as a result of the cTnT/antibody-mediated tethering to fluorophores to the gold nanoparticle functioned as a readout for the cTnT concentration in the sample. Nanomolar cTnT concentrations could be reliably detected, which is excellent, even by today's standards. Achieving such high sensitivity renders plasmonic sensors highly useful for applications that involve monitoring of the plasmatic levels of drugs within a narrow therapeutic window. Plasmonic nanosensors have also been employed within competitive hybridization assays for the detection of short, noncoding microRNAs (miRNAs),[61] which have pivotal regulatory functions in a diverse range of biological processes such as gene expression, cell differentiation, and disease development.

Fluorescence enhancement, instead of quenching, can be employed for single molecule detection as well. Anger et al. reported that for a single gold nanoparticle the variation of distance leads to a continuous transition from fluorescence quenching to enhancement, illustrating that both are indeed competitive effects.[62] Large enhancements and the shaping of fluorescence spectra can be obtained with plasmonic resonators, such as nanoparticle dimers[63] and bowtie antennas[64] that create highly localized electric fields in the so-called "hot-spot" region of the particle gap. Strategies to employ single molecule fluorescence detection for diagnostic assays heavily rely on the appropriate methodology to form stable dimers with well-defined spacings and the option of positioning the analyte precisely in the hot-spot region. These challenges can be solved elegantly with DNA-origami nanotechnology (**Figure 3**a).[65] Dynamic biophysical systems, such as lipid membranes, where individual components are in a perpetual state of motion, can be studied by embedding the bilayer within arrays of plasmonic nanoantennas. As an example, bowtie nanoantenna arrays have been employed to investigate the membrane diffusion of single SOS (Son of Sevenless), a guanine nucleotide exchange factor (GEF) enzyme that activates membrane-linked Ras by catalyzing the exchange of Ras-bound GDP for GTP (Figure 3c).[66] This SOS-catalyzed nucleotide exchange in Ras is important for many health-related aspects. Combining bowtie nanoantenna arrays with the supported membrane platform has enabled quantitative studies of the diffusion of single dye-labeled enzymes in a bilayer via fluorescence enhancement, as they pass through individual plasmonic "hot-spots". (Figure 3d).

Not only dimer antennas, but also other particle shapes such as nanocubes and nanorods have been used for label-free monitoring of protein binding events[45,67] and membrane receptor interactions,[68] on lipid bilayer membrane by plasmonic sensing. An example that displays this capability for studying dynamic systems, has been the observation of MinDE pattern formation on supported bilayer membranes. MinDE is a protein system involved in the bacterial cell division process of Escherichia coli.[69] With real-time plasmon monitoring, Sönnichsen and colleagues could analyze fluctuations of local protein concentrations and dynamic Min protein oscillation over hours, and reveal that the oscillation periods were dependent on membrane curvature.[69b] These seminal studies on synthetic lipid membranes pave the way for the ultimate goal of using plasmonic nanosensors for personalized medicine and clinical diagnostics. Towards this objective, Ortega et al. developed a plasmonic islet-on-a-chip (IOC) that mimics the native pancreas host, quantifying insulin levels under different glucose concentrations (**Figure 4**a).[70] This organ-on-chip represents a non-invasive biosensing method for continual bioanalysis of microtissue behaviors. Finally, Kaefer et al., recently described the use of plasmonic tags for diagnostics by demonstrating the application of gold nanoparticle sensors embedded in a tissue-integrating hydrogel scaffold.[71] In this work, the authors avoided some of the inherent problems of diagnostic implants and in vivo biomarker monitoring, by combining the high biocompatibility of hydrogel scaffolding with the long-term optical and chemical stability of gold nanoparticles. Real-time, non-invasive measurements of biomolecule concentrations could be achieved, through the skin of a living rat (Figure 4b). The authors measured the concentration of kanamycin, an antibiotic that was injected into the abdominal skin of the rat's tail, for up to 4 weeks.

These examples highlight the tremendous progress that has been made in the field over the years, culminating in a reliable approach for in vivo measurements. However, one could argue that these plasmonic systems described do not strictly qualify as nanoagents, since the particles are still, for the most part, passive elements. The combination of sensing with temperature control, as outlined in the following chapter, grants a more active influence on biological systems and environments.

2.2. Sensing and Heating

One of the best-known examples for optothermal applications in biomedicine is probably photothermal therapy, where gold nanoparticles are injected into a growing tumor and optically

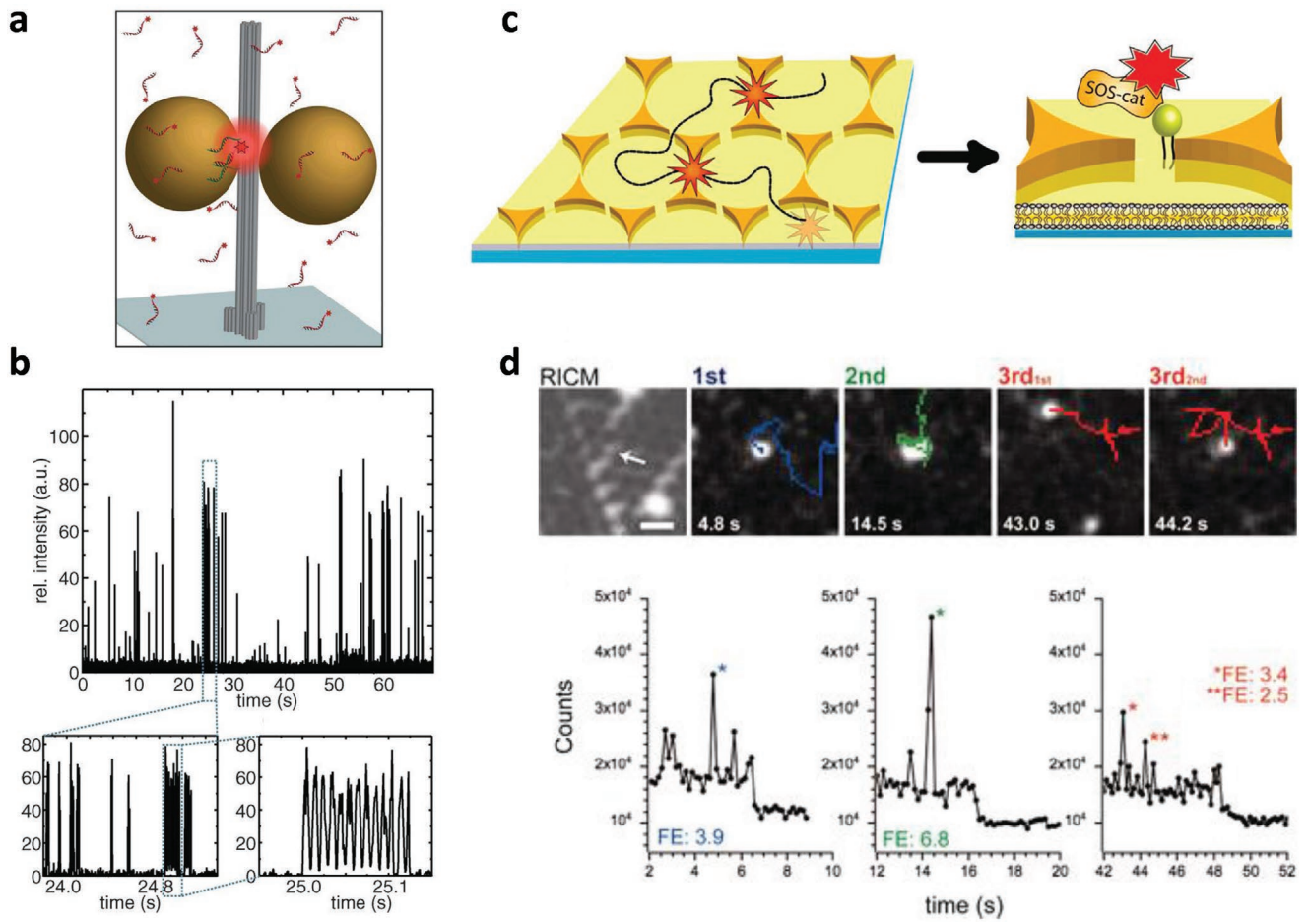

Figure 3. Plasmonic fluorescence enhancement for imaging. a) Scheme of DNA-origami Au-nanoparticle pillar for fluorescence enhancement assembly. b) Fluorescence intensity tracks display transients of binding and unbinding events. (Reproduced with permission.[65b] Copyright 2012, Science.) c) Schematic of single molecule fluorescence enhancement of freely diffusing fluorescently labeled proteins, as they pass through the nanogaps of a plasmonic bowtie nanoantenna array embedded in a bilayer membrane. d) Fluorescence intensity peaks of three molecules, as they diffused through a nanogap (indicated by the white arrow in the reflectance interference contrast microscopy (RICM) image). (Reproduced with permission.[66] Copyright 2012, American Chemical Society.)

heated to destroy malignant tissue in situ.[72] In particular, particles that display strong resonances in the near-infrared are suited for such a purpose, since they can be heated by using tissue penetrating red light. So far, various particle shapes, for example, gold nanorods,[73] nanostars,[74] nanotriangles,[75] and plasmonic core-shell nanoparticles,[76] have been tested as light-to-heat transducers for photothermal therapy.

Thermal cell damage is comparatively easy to accomplish, with the main requirement being that sufficient heat is generated in a specific area. Using temperature to guide or regulate a molecular process or biological function, ideally in a reversible fashion and without inducing permanent damage, requires more precise and sophisticated temperature control. Living cells, for example, are highly complex compartmentalized systems where small temperature variations on the nanoscale can affect local reaction kinetics or molecular transport,[77] which ultimately feeds back to cellular function. Many fundamental properties of cells, including stiffness,[78] membrane permeability,[79] and gene expression levels[80] can be influenced by temperature. Studying these processes requires a strategy to not only apply heat in a controlled way but to also measure local temperature differences. The latter can be achieved by fluorescence methods[81] but also directly by measuring thermally-induced refractive index variations around heated particles.[82] Photothermal microscopy,[83] based on light absorption of gold particles that are only a few nanometers in size, has been used for label-free particle tracking and super-resolution imaging in biological systems.[84] This further adaptation for temperature mapping greatly expands the applicability of plasmonic nanoagents for comprehensive photothermal control.

Temperature dependent biophysical processes, for example, enzyme kinetics, membrane dynamics, or biopolymer assemblies are often studied in reconstituted systems by changing the experimental conditions globally. In contrast, plasmonic materials that combine sensing with optothermal manipulation are capable of addressing biological systems in situ, on the nanoscale. A seminal example for the potential of plasmonic particle heating for controlling biomolecular interactions via temperature has been the introduction of DNA melting[85] and executing ultrafast polymerase chain reactions (PCR).[86]

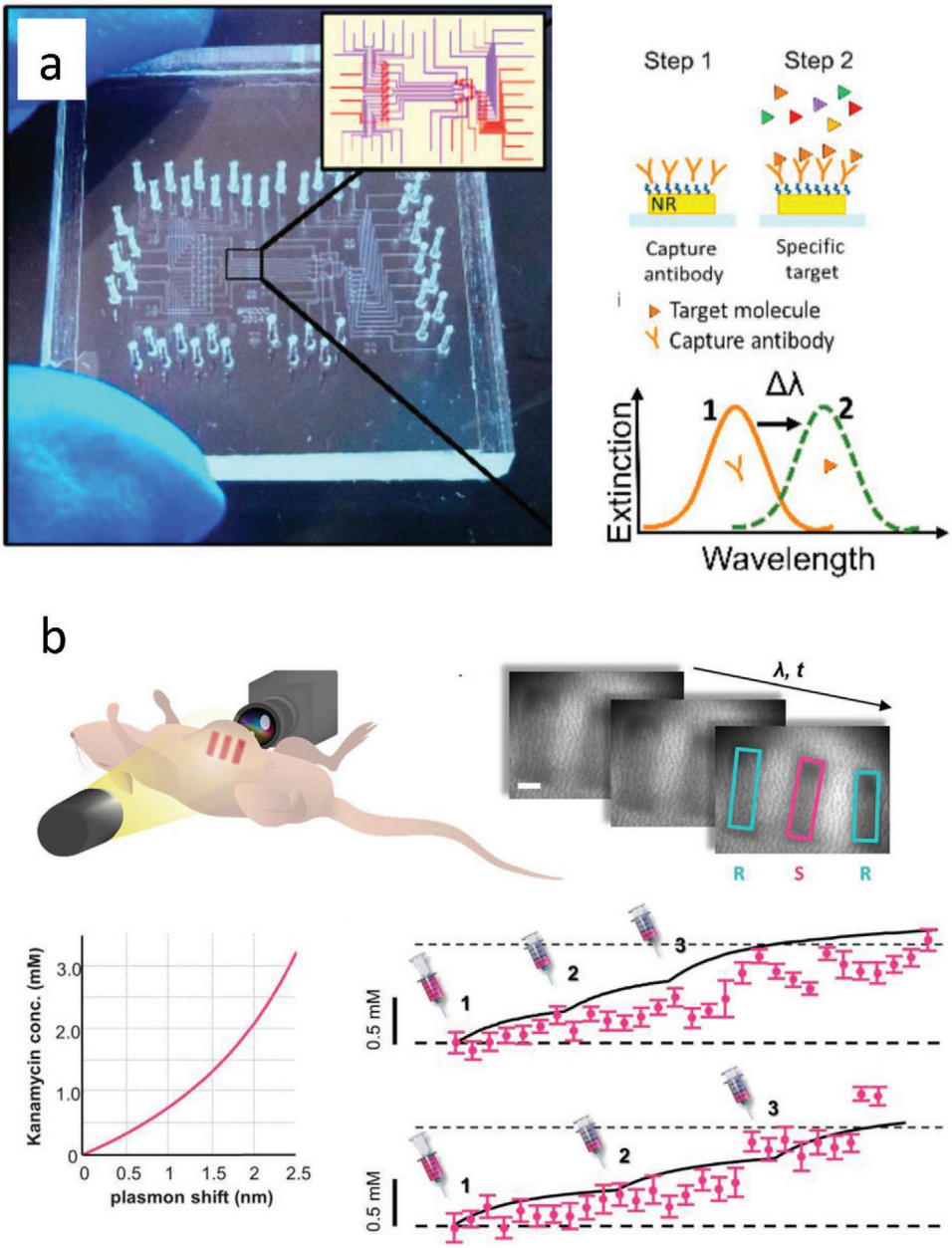

Figure 4. Bio-medical applications of plasmonic sensing. a) Image of organ-on-chip sensing platform and scheme of insulin quantification principle via plasmonic nanoantenna islets. (Reproduced with permission.[70] Copyright 2021, MDPI.) b) Top left: Schematic of the measurement setup through the rat's skin in transmission mode. Top right: Image series skin embedded sensor stripes (R = reference stripe; S = Sensor stripe). Bottom left: In vitro calibration curve: Kanamycin concentration versus plasmon resonance shift. Bottom right: Time-dependent increase of subcutaneous kanamycin concentration after continuous infusion of 3 doses of the antibiotic (pink lines), with modeled predictions (black lines). (Reproduced with permission.[71] Copyright 2010, American Chemical Society.)

Methods for DNA replication and detection, including PCR, rely on thermal cycling to melt DNA double helices and to activate specific DNA replication enzymes. A sample containing the DNA in solution is exposed to temperature ramps that are generated by an external heating element. The processing speed is a limitation in this procedure, since the whole system needs to be heated until a thermal equilibrium is reached and then subsequently cooled down. Typical time scales for DNA melting analysis are therefore in the range of several minutes or even hours. However, in clinical diagnostics, processing time is of the essence. A high sample throughput and a fast analysis of DNA melting and hybridization is therefore desirable. Rather than driving temperature ramps of the whole sample, gold nanoparticles embedded in DNA aggregates have been used as nanoscopic stoves to apply controlled and reversible heating (**Figure 5**a).[87] The heat generated in a nanoparticle/DNA aggregate with a laser pulse is accumulated homogeneously between the particles, which almost instantaneously

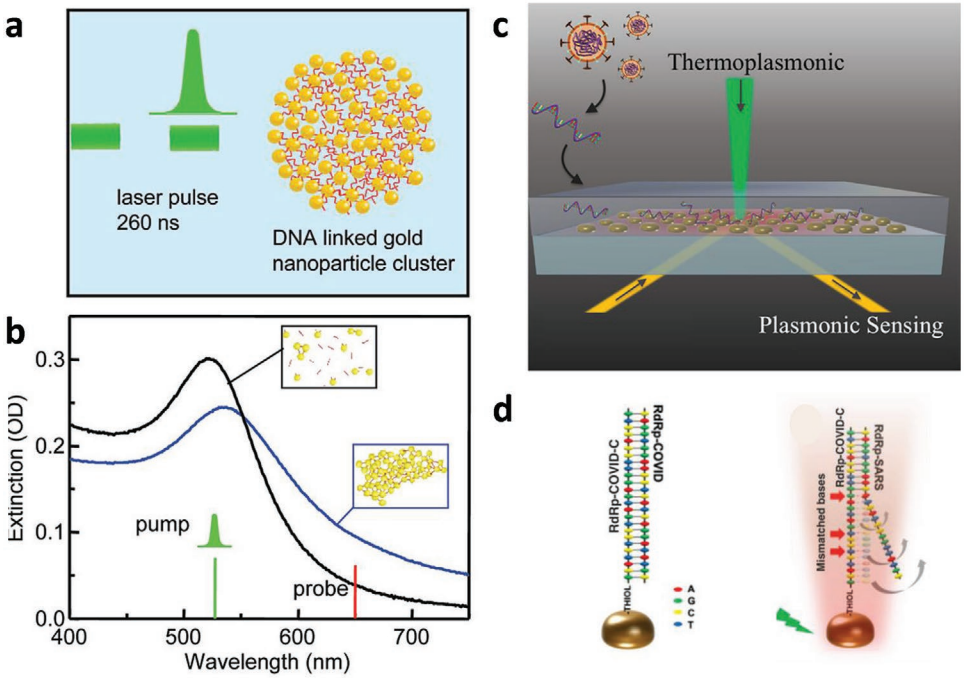

Figure 5. Plasmonic heating for nucleic acid detection. a) Schematic of the DNA nanostove approach for DNA melting analysis. b) Extinction spectra for heating of DNA-linked gold nanoparticle clusters. (Reproduced with permission.[87] Copyright 2010, American Chemical Society.) c) SARS-CoV-2 detection by plasmonic heating and sensing. d) Plasmonic heating and sensing allow for discrimination between complementary (left) and mismatched nucleotide sequences (right). (Reproduced with permission.[88] Copyright 2010, American Chemical Society.)

melts the DNA. Notably, the gold particles not only control temperature ramping, but simultaneously function as spectroscopic reporters of the hybridization state. The extinction spectrum of gold particle aggregates is broader and red-shifted compared to that of dispersed gold nanoparticles. The change from an aggregate to free particles, associated with the melting of the interlinking DNA, is thus detectable by a blue shift of the sample's extinction spectrum. This "nanostove" approach is so sensitive, that it allowed for distinguishing between a perfectly matching target DNA strand, and strands that were mismatched by only a single nucleotide, via slight differences in the dehybridization temperature.[85] With a millisecond observation window and the potential for multiplexing and mutant identification, this approach provides one of the highest turnover rates for DNA melting analysis and has led to the development of ultrafast PCR.[86a] In a recent report, the concept for nucleic acid detection via differences in melting temperatures was further expanded for the realization of a biosensor chip that combined photothermal control with plasmonic sensing for highly accurate SARS-CoV-2 detection (Figure 5c).[88] The sensor chip was designed by coating a substrate with small, separated gold nanoislands that were functionalized with single stranded DNA complementary to the nucleotide target sequence of the coronavirus. The label free detection of a matching virus sequence from solution was monitored by plasmonic sensing upon hybridization with the anchor strand. At the same time, the sample was illuminated through a second channel with laser light matching the plasmon resonance frequency of the gold islands to control the hybridization temperature. This sensing/heating combination allowed to distinguish between the binding of two similar target sequences, one of SARS-CoV-2 and a second target sequence from a different coronavirus, which was slightly mismatched to the anchor sequence. Given this high specificity, it appears feasible that the sensor chip could potentially be designed in such a way, that it would even allow for a fast and reliable distinction between occurring SARS-CoV-2 variants.

Photothermal control is further suited to probe lipid bilayers, cell membranes and associated membrane channels. For example, gold nanoparticles have been attached to the capsaicin transient receptor potential cation channel subfamily V member 1 (TrpV1), a sensitive membrane ion channel that opens at 43 °C and triggers neuron stimulation.[89] This is a typical example for an experiment, where excessive particle heating must be avoided, since temperatures above ≈45–50 °C could lead to irreversible damage and denaturation of the channel protein. Alternatively, the membrane's physical properties can be targeted directly. Moderate plasmonic heating with a cw laser has been used to control gel-fluid phase transitions in synthetic bilayer membranes via temperature gradients on the nanoscale (**Figure 6**a).[90] The increase in bilayer fluidity was observed by an onset of a random particle movement within the bilayer area that was heated above the phase-transition temperature. The temperature profile stemming from single particle heating on a membrane can be visualized with fluorescence microscopy, as shown by Bendix et al.[91] They analyzed the preferential distribution of fluorescently labeled lipid molecules between fluid and gel phase domains in a supported bilayer to determine the boarder and size of the membrane phase transition with respect to the particle temperature. However, local temperature

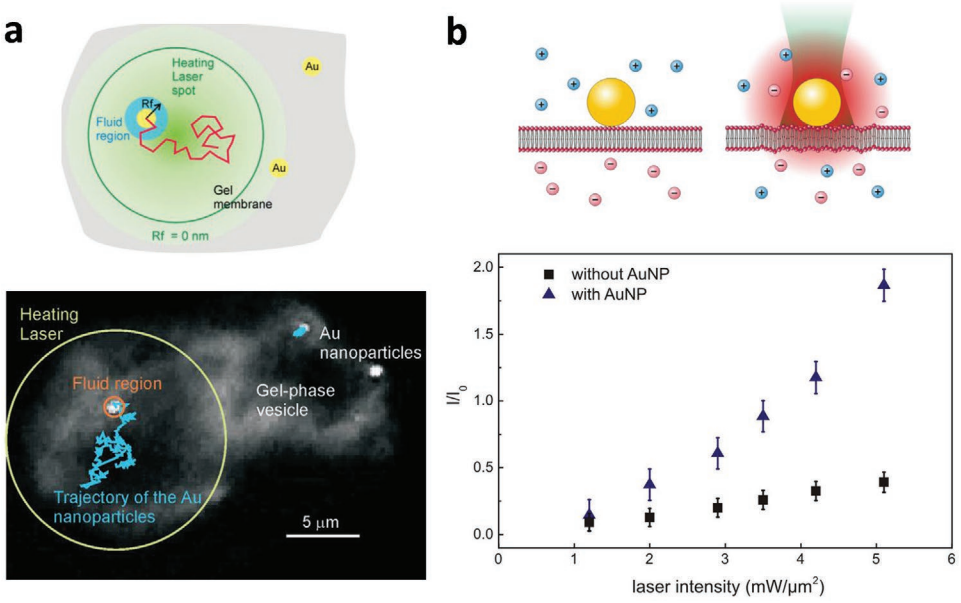

Figure 6. Membrane manipulation via plasmonic heating. a) Top: Local melting of gel-phase lipid membranes is achieved by plasmonic heating of a single gold nanoparticle. The particle temperature controls the region of melting. Bottom: Increased particle diffusion is observed after thermally induced membrane phase transitions. (Reproduced with permission.[90a] Copyright 2009, American Chemical Society.) b) Top: Photothermal control of ion currents across a phospholipid bilayer membrane. Bottom: Trans-membrane current versus laser intensity measured with and without (control) a gold nanoparticle attached to the bilayer. (Reproduced with permission.[92] Copyright 2016, Springer Nature.)

manipulation does not only influence the membrane fluidity, but has also an effect on lipid bilayer permeability and rigidity. Localized nanoparticle heating has been applied to reversibly control ionic currents flowing through a synthetic lipid bilayer, and even cell membranes, in the pA range (Figure 6b).[92] Bendix et al. have further demonstrated that membrane expansion and change in bending stiffness associated with membrane phase transitions can trigger the fusion of synthetic liposomes,[93] and even living cells.[94] Cells are indeed able to adapt their behavior directly with respect to elevated temperatures in their surroundings. Zhou et al. demonstrated that localized heating of nanoparticle arrays can hinder cell migration.[95] They observed the movement of fibroblasts that were seeded onto a linear pattern of gold nanoparticles. The particles were functionalized with a cysteine-terminated linear arginine-glycine-aspartic acid (RGD), a polypeptide that promotes integrin mediated cell adhesion and migration. Heating a spot of the particle pattern to ≈44 °C, put an instant halt to any cell spreading or migration. Notably, the cellular behavior was fully reversible by removing the heat source. The cells did not appear to suffer from irreparable damage during the process. However, in a later report, the Baffou group showed that plasmonic heating of a nanoparticle pattern underneath single cells triggers a heat-shock response.[96] In an elegant approach, the particles were used as a source of heat, while temperature measurements were simultaneously conducted by measuring the temperature dependent wavefront distortions. Very recently, Carrillo-Carron et al. then moved from the extracellular space towards optical control of thermal-driven processes inside living cells. Using gold nanostars encapsulated in metal-organic framework nanoparticles they demonstrated the feasibility of plasmon mediated nucleophilic substitution (i.e., thermocyclization) reactions (**Figure 7**a–c).[97]

Additional work on plasmonic heating of gold nanoparticles includes time-controlled triggering of drug release from liposomes or microcapsules in photopharmacology.[98] In an extension of this idea, Delcea et al. studied the triggered release of intracellular molecules from red blood cells (RBCs) via thermally assisted opto-poration.[99] The authors used laser light to locally heat nanoparticle clusters on the surface of individual RBCs and to thereby induce permeability. This effect was attributed to the formation of transient hydrophilic pores in the cell membrane due to a combination of local phase transitions, the denaturation of membrane glycoproteins and lipid peroxidation via free radicals. If nanoparticle heating exceeds the spinodal decomposition temperature of water, the formation of nanobubbles can be also observed.[29a,100] These fast-expanding bubbles can serve two purposes. First, vapor expansion leads to instantaneous membrane rupture. Second, the vapor surrounding the particles acts as a protective shield due to a much lower thermal conductivity compared to liquid water.[100,101] The transient pores formed by vapor bubbles display a lifetime of only a few μs and cause no further thermal damage to the surrounding area. In a direct comparison, it was shown that vapor nanobubbles greatly outperform other modes of thermally induced photoporation for the transport of molecules in and out of living cells, without introducing any notable cytotoxic effects.[102] The technique was applied for the selective labeling of neurons[103] and targeted drug and gene delivery[104] and the delivery of small interfering RNA to cytotoxic T-cells.[105] These examples have all in common that the molecules and drugs had to pass the photothermally generated membrane pores passively, via diffusion, which is highly dependent on molecule type, the concentration and the pore size. In the following, we will discuss how active delivery

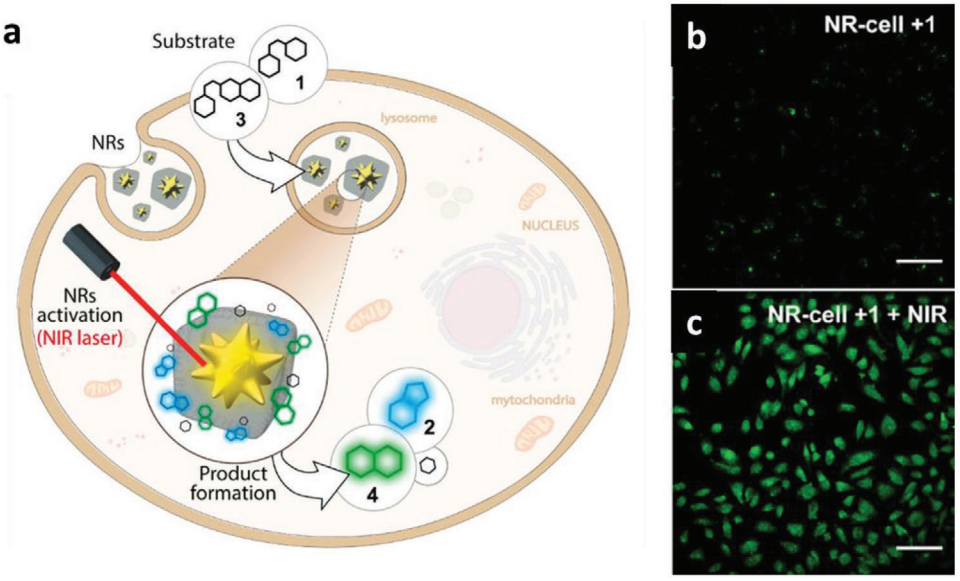

Figure 7. Plasmon assisted control of reactions inside a living cell. a) Gold nanostars encapsulated in a metal-organic framework (MOF) forming a nanoreactor. Plasmonic heating controls photo-thermocyclizaton of thermolabile probes (1 and 3) into fluorophores (2 and 4) inside a living cell. b,c) Confocal microscopy images of nanoreactor pre-loaded cells without (b) and after (c) plasmonic heating with a NIR laser. Scale bars correspond to 20 µm. (Reproduced with permission.[97] Copyright 2021, American Chemical Society.)

of nanosystems into cells can be achieved when additional optical forces are applied.

2.3. Nanoagents Controlled by Optical Force

Balancing of optical gradient and scattering forces allows for controlled trapping and positioning of individual nanoparticles with a single focused laser beam. This adds an important element to the nanoagent toolbox. Small particles immersed in a liquid are subject to Brownian motion. Inside an optical trap this motion is limited by strong gradient forces induced by the focused laser.[106] The trap stiffness and the particle confinement can be tuned by the laser power. Examining the particle motion then allows to characterize the trapping potential and analyze the behavior of molecules interacting with the particle.[107] This basic concept has been employed to detect tiny acoustic vibrations in liquid media. By utilizing a single gold nanoparticle optically trapped in water and processing video sequences of its motion in the frequency domain, it was possible to detect faint acoustic vibrations at sound power levels down to −60 dB; more than six orders of magnitude more sensitive than the human ear.[108] This approach was later expanded upon and employed to monitor the rotation frequency of flagellar bundles of single bacteria cells,[109] the swelling state of red blood cells,[110] and the fitness of aquatic nauplius larvae.[111] Further, by introducing a quasi "lock-in" approach for particle tracking and analyzing the particle motion in the frequency domain, the sensitivity of force measurements with an optically trapped gold particle could be shifted to the fN range.[112] At this point, it should be noted that gold nanoparticles themselves can be used to generate acoustic waves.[113] Gold nanorods, for example, have been used as optoacoustic contrast agents for imaging and diagnostics applications in vivo.[114] Heating particles embedded in biological tissue with a short laser pulse resulted in the thermoelastic expansion of the surrounding matrix and the generation of travelling acoustic waves that were detectable by an acoustic transducer.

Force and temperature can be combined for manipulating binding and interactions between biological molecules. The simultaneous trapping of gold nanoparticles functionalized with complementary strands of DNA, for example, was explored as a tool to fine-tune and control DNA hybridization kinetics in an optical trap.[115] In the experiment, two types of nanoparticles were labeled with nucleotide strands of either adenine or thymine via thiol chemistry. When two complementary particles were trapped by the laser beam at the same time, particle dimerization occurred, which was detectable in situ by a red-shift of the plasmon resonance frequency due to plasmonic coupling. Larger trapping powers led to a stiffer, more stable optical trap, but at the same time, generated higher particle temperatures. This lowered the hybridization rate and hindered the formation of nanoparticle dimers that displayed binding energies lower than the thermal energy.

A precise measurement of intermolecular binding forces between DNA strands was achieved by a combination of heat and force with the aid of plasmonic Janus particles.[116] These particles feature a metallic and a dielectric side. Spherical Janus particles can be prepared by coating one hemisphere of a micron-sized silica particle with a metal layer. When illuminated with light at the resonance wavelength, the metal-coated hemisphere absorbs light directly, and heats up significantly more than the dielectric side. The resulting temperature gradient along the particle axis can drive particle motion via self-thermophoresis.[117] Inside a laser trap, the additional optical forces further determine the orientation and directionality of particle motion. The range of this added optical force control can be adjusted to a certain degree by changing the concentration and type of electrolytes in the surrounding medium.[118]

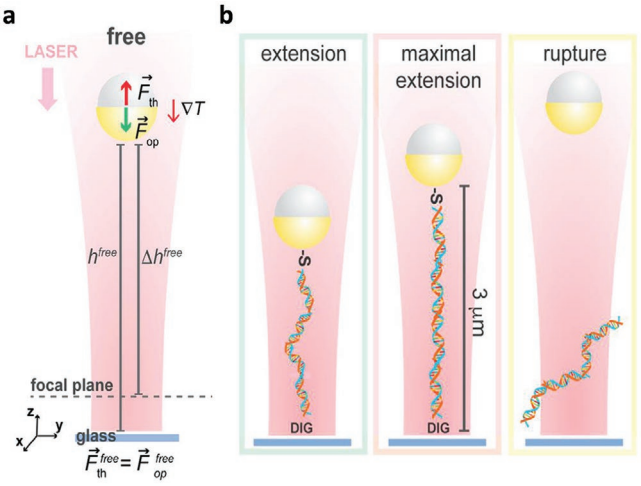

Figure 8. Probing DNA with a thermo-optically controlled Janus microsphere. a) Schematic of a gold-silica Janus sphere illustrating the balance between thermophoretic and optical force acting on the particles in the optical trap. b) Optical manipulation of the DNA-tethered Janus particle. With increasing thermophoretic force, the Janus particle moves upwards in the laser beam and the DNA tether is extended to its maximum contour length. At high laser powers, a rupture of the DNA helix is observed. (Reproduced with permission.[116] Copyright 2017, American Chemical Society.)

Thermophoretic and optical forces in combination result in a laser power-dependent upward or downward movement of the Janus particle with respect to the axis of the optical trap; in analogy to an elevator that is, in this case, driven by light.[119] Thus by modulating the laser power, Janus particles can function as a highly sensitive light-to-force transducer with an additional heating capability. Light-controlled Au–silica microspheres were, for example, used to simultaneously stretch and partially melt a single double-stranded DNA (dsDNA) molecule (**Figure 8**a,b).[116] In this experiment, the optical force acting on the dsDNA molecule was in the pN range, well suited to probe the entropic stretching regime of DNA. At the same time, plasmonic heating induced a measurable partial dehybridization of the DNA strands, in another effective combination of several plasmonic nanoagent features.

By taking advantage of scattering forces, one can also devise a strategy to actively transport nanoagents labeled with molecular cargo. The all-optical injection of nanoscopic objects into living cells is a valuable prospect in the development of novel molecular delivery strategies and intracellular biosensor applications. In the first demonstration of this idea, it was shown that individual gold nanoparticles from the solution can be patterned onto the surface of living cells using a cw laser and later also injected.[101] This was achieved in a two-step process. First, gold particles were optically printed one-by-one onto specific locations on the surface of a chinese hamster ovary (CHO) cell. In a second injection step, short plasmonic heating with a laser matching the plasmon resonance frequency was applied to initiate vapor nanobubble formation around the particles and thus perforate the cell membrane. Strong scattering forces concomitant with the heating step simultaneously injected the particles into the cell. This was confirmed by Rayleigh scattering spectroscopy and dark-field imaging. Although the gold particles reached significant temperatures during injection, a cell survival rate of >70% could be determined for moderate laser power between 5–10 mW. There is, however, a fundamental downside to the approach of using mere gold particles. High temperatures are required to generate nanobubbles, which excludes any labeling of the particles with heat-sensitive biomolecules. Proteins, nucleic acids, or fluorescent tags would be thermally destroyed or desorbed from the nanoparticle surface in the nanobubble formation step.

This obstacle can be avoided with nanocarriers, where only a part of the particle is heated by light. This way, the "cold" part can be functionalized with any molecule of interest, while the "hot" side aids the injection process. The controlled optical injection of genetic material with a plasmonic shuttle into living cells was accomplished with "Janus nanopens", which were composed of a gold nanosphere attached to a dielectric alumina shaft (**Figure 9**a–c).[120] The complete injection process, monitored by DFM, also involved two steps. First, single fluorescently (via ssDNA) labeled Janus nanopens were trapped with a focused NIR laser, red-shifted significantly from the plasmon resonance frequency. Optical trapping of the dielectric rod assisted in this, and also introduced the aforementioned, "plasmonic elevator" height adjustment component to the scheme. The pens could be positioned just above a cell by the laser beam at first and then lowered onto the cell surface by decreasing the power of the trapping laser. The subsequent nanopen injection was then accomplished with a focused green laser, matching the plasmon resonance frequency of the gold particle at the tip. The strong heating neither destroyed the Janus particle, nor the ssDNA label on the alumina shaft. Identifying single Janus nanopens with fluorescence microscopy and DFM inside the cells validated this concept of active biomolecule injection. Hence, using Janus nanopen as light-controlled nanocarriers is a prime example for a nanoagent where applications take full advantage of combined optical sensing, photothermal control, and optical force manipulation.

3. Conclusion & Future Perspectives

In summary, we have explored the development, implementation, and potential applications of plasmonic nanoagents. The use of plasmonic nanomaterials for bio-sensing has been a rapidly expanding field of research for the past two decades. Here, the combination and optimization of effects that arise from light-particle interaction, temperature control, and force manipulation, have allowed them to take on a more active role in biophysical and biomedical research. This progress is perhaps best highlighted by the emergence of commercial applications, such as pathogen detection with LFAs and ultrafast plasmon-assisted PCR. Furthermore, plasmonic nanoagents for in vivo studies have become a reality, and their use for personalized medicine and photo-pharmaceutical applications in humans appears tangible at the time of writing. We believe that upcoming research directions and applications will expand the wider use of plasmonic nanoagents in the future, some of which should be also highlighted as a future perspective.

One exciting concept is the use of plasmonic core-shell nanoreactors to enhance the throughput and sensitivity of

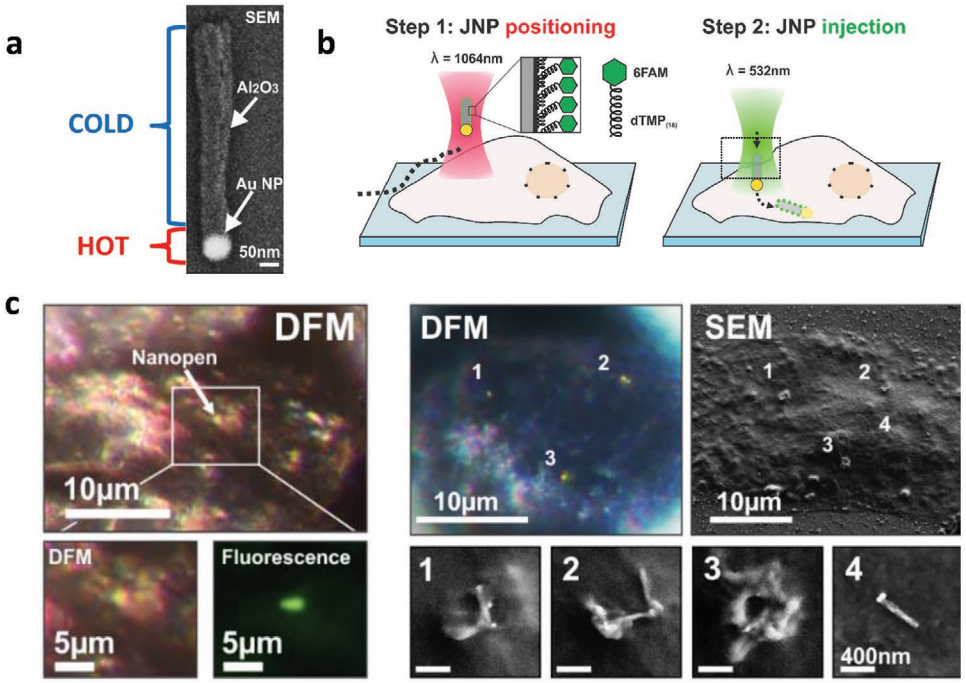

Figure 9. Injection of ssDNA labeled Janus nanopens into living cells. a) Representative SEM image of a nanopen composed of a spherical gold nanoparticle connected to a dielectric alumina shaft. b) Positioning (off-resonant red laser beam) and injection (resonant green laser beam) of a nanopen functionalized with fluorescently labeled ssDNA. c) Left: DFM and fluorescence microscopy image (squared region) of a fluorescently labeled nanopen injected into a living cell. Right: DFM and scanning electron microscopy images of a cell injected with three nanopens. Micrographs 1–3 display SEM images of the nanopen injection sites at a cell membrane. Micrograph 4 shows an un-injected nanopen on the cell surface. (Reproduced with permission.[120] Copyright 2018, American Chemical Society.)

established methods for clinical diagnosis such as mass spectrometry.[121] The strong light-particle interaction enables soft-ionization of the analyte and its efficient ablation from the particle surface at lower laser energies in surface-assisted laser desorption/ionization mass spectrometry (SALDI-MS).[122] The clinical applicability of this strategy has already been demonstrated by in vitro metabolite diagnostics from patient samples.[123] Further improvements, such as varying the shape, size, and composition of the plasmonic matrix materials, will extend their use in biomedicine.

In terms of fundamental research, plasmonic nanoagents already thrive as flexible tools to guide cellular behavior and interactions. Whilst the influence of temperature on cells or cell populations on a macroscopic scale is well investigated, the knowledge on temperature effects on a sub-cellular level is still scarce. A living cell is a highly complex, enclosed environment. It stands to reason that small temperature changes on the nanoscale can have a significant impact on local phenomena. Detecting and manipulating these in further studies, using the described contactless, all-optical approach for the active delivery of nanoobjects into living cells, represents a promising direction for future nanotheranostic strategies. Examples have been reported, where plasmonic nanoparticles enable light-driven control of enzyme activity via temperature.[124] Also, refined hybrid nanoreactors have been used to enhance enzymatic processes and perform metabolite monitoring via SERS in a single assay,[125] emphasizing the idea of plasmonic nanoagents as multifunctional tools to control and monitor a biomolecular process simultaneously.

Beyond photothermal control, the generation of "hot" charge carriers for bio-catalysis is possibly an even more promising future direction.[126] The non-radiative decay of particle plasmons results in the generation of hot carriers with high kinetic energies, which can be employed to drive photo-catalytic reactions on a single particle level. To date, enhancing biochemical reactions with hot charge carriers is only scarcely explored. Examples include the plasmon enhanced generation of molecular radicals and reactive oxygen species (ROS) at laser power densities to induce controlled cell death by mere photochemical effects.[127] The application of plasmonic nanoagents to drive enzymatic and biochemical processes with hot electrons in living cells is an exciting prospect, as it might ultimately grant remote control of specific cell functions.

Finally, high precision manipulation of single biomolecules offered by plasmonic nano-tweezers is an exciting prospect.[128] Dimer antennas made from gold nanospheres[129] or nanorods,[130] as well as nanocavities[131] and nanoapertures[132] in metal films have been used as optical near-field traps to suppress Brownian motion of dielectric spheres beyond the capability of conventional diffraction limited optical tweezers and to monitor the nanoobjects positioning with high accuracy. Further improvements of molecular nano-positioning, manipulation, and sensing combined with the potential of large-scale patterning and the integration of plasmonic nano-tweezers into lab-on-chip devices will be instrumental to future applications.[133] Employing optical arrays for trapping plasmonic nanoparticles with the potential of steering parallelized and automatized nanoagent operations using machine

learning are further examples for future directions of optical manipulation toward scalable applications in biophysical research and biomedicine.

Acknowledgements

The authors first want to express gratitude to many of their colleagues and friends for inspiring discussions and exciting research collaborations on nanoagents. The authors further acknowledge support from the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) – Project-ID 201269156 – SFB 1032 and ANPCyT (PICT 2019–3458) and CONICET (PIP 112-201201-00648). J.Z. is supported by the China Scholarship Council. Support by the German Academic Exchange Service within the framework of the PPP program is acknowledged. The authors thank local research clusters and centers such as the Center of Nanoscience (CeNS) for providing communicative networking structures.

Open access funding enabled and organized by Projekt DEAL.

Conflict of Interest

The authors declare no conflict of interest.

Keywords

optical forces, photothermal properties, plasmonic nanoagents, plasmonic sensing

Received: March 11, 2022
Revised: May 18, 2022
Published online: June 23, 2022

[1] L. Deng, X. Cai, D. Sheng, Y. Yang, E. M. Strohm, Z. Wang, H. Ran, D. Wang, Y. Zheng, P. Li, *Theranostics* **2017**, *7*, 4410.

[2] a) M. Wang, F. Lu, N. Li, W. Pan, B. Tang, *Chem. Commun.* **2022**, *58*, 1554; b) Y. Yang, Y. Liu, D. Tu, M. Chen, Y. Zhang, H. Gao, X. Chen, *Angew. Chem., Int. Ed.* **2022**, *61*, e202116983; c) H. Su, X. Li, L. Huang, J. Cao, M. Zhang, V. Vedarethinam, W. Di, Z. Hu, K. Qian, *Adv. Mater.* **2021**, *33*, 2007978; d) Q. Fu, X. Zhang, J. Song, H. Yang, *View* **2021**, *2*, 20200149.

[3] a) N. Usui, S. Togawa, T. Sumi, Y. Kobayashi, Y. Koyama, Y. Nakamura, M. Kondo, K. Shinoda, H. Kobayashi, S. Shimada, *Front. Med. Technol.* **2021**, *3*, 18; b) M. L. Ermini, V. Voliani, *ACS Nano* **2021**, *15*, 6008.

[4] J. Han, H. Li, J. Yoon, *Nano Today* **2022**, *43*, 101392.

[5] a) X. Song, Q. Chen, Z. Liu, *Nano Res.* **2015**, *8*, 340; b) Z. Zhao, C. Chen, W. Wu, F. Wang, L. Du, X. Zhang, Y. Xiong, X. He, Y. Cai, R. T. K. Kwok, *Nat. Commun.* **2019**, *10*, 768; c) Q. Zhu, Z. Fan, W. Zuo, Y. Chen, Z. Hou, X. Zhu, *ACS Appl. Mater. Interfaces* **2020**, *12*, 51314.

[6] L. Li, L. Cao, X. Xiang, X. Wu, L. Ma, F. Chen, S. Cao, C. Cheng, D. Deng, L. Qiu, *Adv. Funct. Mater.* **2022**, *34*, 2107530.

[7] Y.-F. Wu, H.-C. Wu, C.-H. Kuan, C.-J. Lin, L.-W. Wang, C.-W. Chang, T.-W. Wang, *Sci. Rep.* **2016**, *6*, 21170.

[8] A. B. de Barros, A. Tsourkas, B. Saboury, V. N. Cardoso, A. Alavi, *EJNMMI Res.* **2012**, *2*, 39.

[9] a) K. Hüll, J. Morstein, D. Trauner, *Chem. Rev.* **2018**, *118*, 10710; b) V. R. Cherkasov, E. N. Mochalova, A. V. Babenyshev, A. V. Vasilyeva, P. I. Nikitin, M. P. Nikitin, *ACS Nano* **2020**, *14*, 1792.

[10] a) M. Zhao, Q. Zeng, X. Li, D. Xing, T. Zhang, *Nano Res.* **2022**, *15*, 716; b) Z. Yang, L. Zhang, J. Wei, R. Li, Q. Xu, H. Hu, Z. Xu, J. Ren, C.-Y. Wong, *J. Colloid Interface Sci.* **2021**, *612*, 355.

[11] a) P. Bugga, M. Mrksich, *Curr. Opin. Colloid Interface Sci.* **2018**, *38*, 80; b) M. Arnold, E. A. Cavalcanti-Adam, R. Glass, J. Blümmel, W. Eck, M. Kantlehner, H. Kessler, J. P. Spatz, *ChemPhysChem* **2004**, *5*, 383; c) J. T. Groves, L. K. Mahal, C. R. Bertozzi, *Langmuir* **2001**, *17*, 5129; d) P. J. F. Röttgermann, A. P. Alberola, J. O. Rädler, *Soft Matter* **2014**, *10*, 2397; e) M. Dietrich, H. L. Roy, D. B. Brückner, H. Engelke, R. Zantl, J. O. Rädler, C. P. Broedersz, *Soft Matter* **2018**, *14*, 2816.

[12] a) L. M. Liz-Marzán, *Mater. Today* **2004**, *7*, 26; b) H. Liao, C. L. Nehl, J. H. Hafner, *Nanomedicine* **2006**, *1*, 201; c) P. K. Jain, X. Huang, I. H. El-Sayed, M. A. El-Sayed, *Acc. Chem. Res.* **2008**, *41*, 1578.

[13] P. L. Stiles, J. A. Dieringer, N. C. Shah, R. P. V. Duyne, *Annu. Rev. Anal. Chem.* **2008**, *1*, 601.

[14] a) C. Zong, M. Xu, L.-J. Xu, T. Wei, X. Ma, X.-S. Zheng, R. Hu, B. Ren, *Chem. Rev.* **2018**, *118*, 4946; b) X.-S. Zheng, I. J. Jahn, K. Weber, D. Cialla-May, J. Popp, *Spectrochim. Acta, Part A* **2018**, *197*, 56.

[15] a) J. Lin, O. U. Akakuru, A. Wu, *View* **2021**, *2*, 20200146; b) A. Samanta, K. K. Maiti, K. S. Soh, X. Liao, M. Vendrell, U. S. Dinish, S. W. Yun, R. Bhuvaneswari, H. Kim, S. Rautela, *Angew. Chem.* **2011**, *123*, 6213; c) H. Liu, X. Gao, C. Xu, D. Liu, *Theranostics* **2022**, *12*, 1870.

[16] a) K. L. Kelly, E. Coronado, L. L. Zhao, G. C. Schatz, *J. Phys. Chem. B* **2003**, *107*, 668; b) W. A. Murray, W. L. Barnes, *Adv. Mater.* **2007**, *19*, 3771; c) J. Olson, S. Dominguez-Medina, A. Hoggard, L.-Y. Wang, W.-S. Chang, S. Link, *Chem. Soc. Rev.* **2015**, *44*, 40; d) L. Wang, M. Hasanzadeh Kafshgari, M. Meunier, *Adv. Funct. Mater.* **2020**, *30*, 2005400.

[17] a) S. A. Maier, *Plasmonics: fundamentals and applications*, Springer, New York **2007**; b) M. Quinten, *Optical properties of nanoparticle systems: Mie and beyond*, Wiley-VCH, Weinheim **2010**; c) U. Kreibig, M. Vollmer, *Optical properties of metal clusters*, Springer, New York **1995**.

[18] J. D. Jackson, *Classical electrodynamics*, Wiley, New York **1999**.

[19] L. Gonzalez-Moragas, P. Berto, C. Vilches, R. Quidant, A. Kolovou, R. Santarella-Mellwig, Y. Schwab, S. Stürzenbaum, A. Roig, A. Laromaine, *Acta Biomater.* **2017**, *53*, 598.

[20] a) S. Avvakumova, M. Colombo, P. Tortora, D. Prosperi, *Trends Biotechnol.* **2014**, *32*, 11; b) G. T. Hermanson, *Bioconjugate techniques*, 3rd ed., Academic Press, Cambridge **2013**.

[21] G. Baffou, *Thermoplasmonics : heating metal nanoparticles using light*, Cambridge University Press, Cambridge **2017**.

[22] a) A. O. Govorov, W. Zhang, T. Skeini, H. Richardson, J. Lee, N. A. Kotov, *Nanoscale Res. Lett.* **2006**, *1*, 84; b) G. Baffou, R. Quidant, F. J. García de Abajo, *ACS Nano* **2010**, *4*, 709.

[23] G. Baffou, F. Cichos, R. Quidant, *Nat. Mater.* **2020**, *19*, 946.

[24] J. S. Donner, G. Baffou, D. McCloskey, R. Quidant, *ACS Nano* **2011**, *5*, 5457.

[25] M. Fedoruk, M. Meixner, S. Carretero-Palacios, T. Lohmüller, J. Feldmann, *ACS Nano* **2013**, *7*, 7648.

[26] a) J. Liang, H. Liu, J. Yu, L. Zhou, J. Zhu, *Nanophotonics* **2019**, *8*, 771; b) O. Neumann, A. S. Urban, J. Day, S. Lal, P. Nordlander, N. J. Halas, *ACS Nano* **2013**, *7*, 42.

[27] A. F. Bagley, S. Hill, G. S. Rogers, S. N. Bhatia, *ACS Nano* **2013**, *7*, 8089.

[28] G. Baffou, R. Quidant, *Chem. Soc. Rev.* **2014**, *43*, 3898.

[29] a) E. Lukianova-Hleb, Y. Hu, L. Latterini, L. Tarpani, S. Lee, R. A. Drezek, J. H. Hafner, D. O. Lapotko, *ACS Nano* **2010**, *4*, 2109; b) Y. Liu, J. Lou, M. Ni, C. Song, J. Wu, N. P. Dasgupta, P. Tao, W. Shang, T. Deng, *ACS Appl. Mater. Interfaces* **2016**, *8*, 772; c) S. Linic, U. Aslam, C. Boerigter, M. Morabito, *Nat. Mater.* **2015**, *14*, 567.

[30] R. S. Riley, M. N. Dang, M. M. Billingsley, B. Abraham, L. Gundlach, E. S. Day, *Nano Lett.* **2018**, *18*, 3565.
[31] A. S. Urban, S. Carretero-Palacios, A. A. Lutich, T. Lohmüller, J. Feldmann, F. Jäckel, *Nanoscale* **2014**, *6*, 4458.
[32] P. M. Hansen, V. K. Bhatia, N. Harrit, L. Oddershede, *Nano Lett.* **2005**, *5*, 1937.
[33] M. Dienerowitz, M. Mazilu, K. Dholakia, *J. Nanophotonics* **2008**, *2*, 021875.
[34] a) B. D. Kevadiya, J. Machhi, J. Herskovitz, M. D. Oleynikov, W. R. Blomberg, N. Bajwa, D. Soni, S. Das, M. Hasan, M. Patel, *Nat. Mater.* **2021**, *20*, 593; b) M. A. C. Huergo, N. T. K. Thanh, *Analyst* **2021**, *146*, 382.
[35] A. M. Shrivastav, U. Cvelbar, I. Abdulhalim, *Commun. Biol.* **2021**, *4*, 70.
[36] a) J. H. W. Leuvering, P. Thal, M. v. d. Waart, A. Schuurs, *J. Immunoassay* **1980**, *1*, 77; b) B. G. Andryukov, *AIMS Microbiol.* **2020**, *6*, 280; c) K. M. Koczula, A. Gallotta, *Essays Biochem.* **2016**, *60*, 111.
[37] a) Z. Li, Y. Yi, X. Luo, N. Xiong, Y. Liu, S. Li, R. Sun, Y. Wang, B. Hu, W. Chen, *J. Med. Virol.* **2020**, *92*, 1518; b) P. Mertens, N. De Vos, D. Martiny, C. Jassoy, A. Mirazimi, L. Cuypers, S. Van den Wijngaert, V. Monteil, P. Melin, K. Stoffels, *Front. Med.* **2020**, *7*, 225; c) Y. Zhou, Y. Wu, L. Ding, X. Huang, Y. Xiong, *TrAC, Trends Anal. Chem.* **2021**, *145*, 116452; d) B. Merrick, M. Noronha, R. Batra, S. Douthwaite, G. Nebbia, L. B. Snell, S. Pickering, R. P. Galao, J. Whitfield, A. Jahangeer, *Infect. Prev. Pract.* **2021**, *3*, 100186; e) W.-Y. Hsieh, C.-H. Lin, T.-C. Lin, C.-H. Lin, H.-F. Chang, C.-H. Tsai, H.-T. Wu, C.-S. Lin, *Diagnostics* **2021**, *11*, 1760.
[38] a) X. Guo, *J. Biophotonics* **2012**, *5*, 483; b) E. Petryayeva, U. J. Krull, *Anal. Chim. Acta* **2011**, *706*, 8; c) J. R. Mejía-Salazar, O. N. Oliveira Jr, *Chem. Rev.* **2018**, *118*, 10617.
[39] a) R. B. M. Schasfoort, *Handbook of surface plasmon resonance*, Royal Society of Chemistry, London **2017**; b) Y.-T. Long, C. Jing, *Localized surface plasmon resonance based nanobiosensors*, Springer, Heidelberg **2014**; c) C. Lai-Kwan, H.-T. Chang, *From Bioimaging to Biosensors: Noble Metal Nanoparticles in Biodetection*, Pan Standford Publishing Pte Ltd, Singapore **2012**.
[40] a) T. Klar, M. Perner, S. Grosse, G. Von Plessen, W. Spirkl, J. Feldmann, *Phys. Rev. Lett.* **1998**, *80*, 4249; b) V. Rodríguez-Fajardo, V. Sanz, I. de Miguel, J. Berthelot, S. S. Aćimović, R. Porcar-Guezenec, R. Quidant, *Nanoscale* **2018**, *10*, 4019.
[41] K. M. Mayer, F. Hao, S. Lee, P. Nordlander, J. H. Hafner, *Nanotechnology* **2010**, *21*, 255503.
[42] G. Raschke, S. Kowarik, T. Franzl, C. Sönnichsen, T. A. Klar, J. Feldmann, A. Nichtl, K. Kürzinger, *Nano Lett.* **2003**, *3*, 935.
[43] P. K. Jain, K. S. Lee, I. H. El-Sayed, M. A. El-Sayed, *J. Phys. Chem. B* **2006**, *110*, 7238.
[44] L. Soares, A. Csáki, J. Jatschka, W. Fritzsche, O. Flores, R. Franco, E. Pereira, *Analyst* **2014**, *139*, 4964.
[45] H.-J. Wu, J. Henzie, W.-C. Lin, C. Rhodes, Z. Li, E. Sartorel, J. Thorner, P. Yang, J. T. Groves, *Nat. Methods* **2012**, *9*, 1189.
[46] S. K. Dondapati, T. K. Sau, C. Hrelescu, T. A. Klar, F. D. Stefani, J. Feldmann, *ACS Nano* **2010**, *4*, 6318.
[47] a) I. Lieberman, G. Shemer, T. Fried, E. M. Kosower, G. Markovich, *Angew. Chem.* **2008**, *120*, 4933; b) J. M. Slocik, A. O. Govorov, R. R. Naik, *Nano Lett.* **2011**, *11*, 701.
[48] L. D. Barron, in *Strategies of Life Detection*, (Eds: O. Botta, J. L. Bada, J. Gomez-Elvira, E. Javaux, F. Selsis, R. Summons), Springer US, Boston, MA **2008**.
[49] F. Lu, Y. Tian, M. Liu, D. Su, H. Zhang, A. O. Govorov, O. Gang, *Nano Lett.* **2013**, *13*, 3145.
[50] L.-Y. Wang, K. W. Smith, S. Dominguez-Medina, N. Moody, J. M. Olson, H. Zhang, W.-S. Chang, N. Kotov, S. Link, *ACS Photonics* **2015**, *2*, 1602.
[51] Q. Zhang, T. Hernandez, K. W. Smith, S. A. Hosseini Jebeli, A. X. Dai, L. Warning, R. Baiyasi, L. A. McCarthy, H. Guo, D.-H. Chen, *Science* **2019**, *365*, 1475.
[52] J. R. Lakowicz, *Anal. Biochem.* **2001**, *298*, 1.
[53] a) E. Dulkeith, A. C. Morteani, T. Niedereichholz, T. A. Klar, J. Feldmann, S. A. Levi, F. C. J. M. van Veggel, D. N. Reinhoudt, M. Möller, D. I. Gittins, *Phys. Rev. Lett.* **2002**, *89*, 203002; b) E. Dulkeith, M. Ringler, T. A. Klar, J. Feldmann, A. Munoz Javier, W. J. Parak, *Nano Lett.* **2005**, *5*, 585.
[54] E. G. Matveeva, T. Shtoyko, I. Gryczynski, I. Akopova, Z. Gryczynski, *Chem. Phys. Lett.* **2008**, *454*, 85.
[55] a) C. Chen, N. Hildebrandt, *TrAC, Trends Anal. Chem.* **2020**, *123*, 115748; b) S. Chatterjee, J. B. Lee, N. V. Valappil, D. Luo, V. M. Menon, *Biomed. Opt. Express* **2011**, *2*, 1727.
[56] B. Dubertret, M. Calame, A. J. Libchaber, *Nat. Biotechnol.* **2001**, *19*, 365.
[57] a) L. Shang, J. Yin, J. Li, L. Jin, S. Dong, *Biosens. Bioelectron.* **2009**, *25*, 269; b) S. Pihlasalo, J. Kirjavainen, P. Hänninen, H. Härmä, *Anal. Chem.* **2009**, *81*, 4995.
[58] a) C.-C. Huang, S.-H. Chiu, Y.-F. Huang, H.-T. Chang, *Anal. Chem.* **2007**, *79*, 4798; b) C.-C. Huang, C.-K. Chiang, Z.-H. Lin, K.-H. Lee, H.-T. Chang, *Anal. Chem.* **2008**, *80*, 1497.
[59] S. Mayilo, B. Ehlers, M. Wunderlich, T. A. Klar, H.-P. Josel, D. Heindl, A. Nichtl, K. Kürzinger, J. Feldmann, *Anal. Chim. Acta* **2009**, *646*, 119.
[60] S. Mayilo, M. A. Kloster, M. Wunderlich, A. Lutich, T. A. Klar, A. Nichtl, K. Kürzinger, F. D. Stefani, J. Feldmann, *Nano Lett.* **2009**, *9*, 4558.
[61] W. Wang, T. Kong, D. Zhang, J. Zhang, G. Cheng, *Anal. Chem.* **2015**, *87*, 10822.
[62] P. Anger, P. Bharadwaj, L. Novotny, *Phys. Rev. Lett.* **2006**, *96*, 113002.
[63] A. Bek, R. Jansen, M. Ringler, S. Mayilo, T. A. Klar, J. Feldmann, *Nano Lett.* **2008**, *8*, 485.
[64] A. Kinkhabwala, Z. Yu, S. Fan, Y. Avlasevich, K. Müllen, W. E. Moerner, *Nat. Photonics* **2009**, *3*, 654.
[65] a) K. Trofymchuk, V. Glembockyte, L. Grabenhorst, F. Steiner, C. Vietz, C. Close, M. Pfeiffer, L. Richter, M. L. Schütte, F. Selbach, *Nat. Commun.* **2021**, *12*, 950; b) G. P. Acuna, F. M. Möller, P. Holzmeister, S. Beater, B. Lalkens, P. Tinnefeld, *Science* **2012**, *338*, 506.
[66] T. Lohmuller, L. Iversen, M. Schmidt, C. Rhodes, H. L. Tu, W. C. Lin, J. T. Groves, *Nano Lett.* **2012**, *12*, 1717.
[67] W. J. Galush, S. A. Shelby, M. J. Mulvihill, A. Tao, P. Yang, J. T. Groves, *Nano Lett.* **2009**, *9*, 2077.
[68] R. Ahijado-Guzman, J. Menten, J. Prasad, C. Lambertz, G. Rivas, C. Sönnichsen, *ACS Appl. Mater. Interfaces* **2017**, *9*, 218.
[69] a) C. Lambertz, A. Martos, A. Henkel, A. Neiser, T.-T. Kliesch, A. Janshoff, P. Schwille, C. Sönnichsen, *Nano Lett.* **2016**, *16*, 3540; b) W. Ye, S. Celiksoy, A. Jakab, A. Khmelinskaia, T. Heermann, A. Raso, S. V. Wegner, G. n. Rivas, P. Schwille, R. n. Ahijado-Guzmán, *J. Am. Chem. Soc.* **2018**, *140*, 17901.
[70] M. A. Ortega, J. Rodríguez-Comas, O. Yavas, F. Velasco-Mallorquí, J. Balaguer-Trias, V. Parra, A. Novials, J. M. Servitja, R. Quidant, J. Ramón-Azcón, *Biosensors* **2021**, *11*, 138.
[71] K. Kaefer, K. Krüger, F. Schlapp, H. Uzun, S. Celiksoy, B. Flietel, A. Heimann, T. Schroeder, O. Kempski, C. Sönnichsen, *Nano Lett.* **2021**, *21*, 3325.
[72] a) L. R. Hirsch, R. J. Stafford, J. A. Bankson, S. R. Sershen, B. Rivera, R. E. Price, J. D. Hazle, N. J. Halas, J. L. West, *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 13549; b) X. Huang, I. H. El-Sayed, W. Qian, M. A. El-Sayed, *J. Am. Chem. Soc.* **2006**, *128*, 2115.
[73] J. Morales-Dalmau, C. Vilches, I. de Miguel, V. Sanz, R. Quidant, *Nanoscale* **2018**, *10*, 2632.
[74] C. Song, F. Li, X. Guo, W. Chen, C. Dong, J. Zhang, J. Zhang, L. Wang, *J. Mater. Chem. B* **2019**, *7*, 2001.
[75] M. Ha, S. H. Nam, K. Sim, S.-E. Chong, J. Kim, Y. Kim, Y. Lee, J.-M. Nam, *Nano Lett.* **2020**, *21*, 731.

[76] C. Caro, F. Gámez, P. Quaresma, J. M. Páez-Muñoz, A. Domínguez, J. R. Pearson, M. Pernía Leal, A. M. Beltrán, Y. Fernandez-Afonso, J. M. De la Fuente, *Pharmaceutics* **2021**, *13*, 416.
[77] S. Soh, M. Byrska, K. Kandere-Grzybowska, B. A. Grzybowski, *Angew. Chem., Int. Ed.* **2010**, *49*, 4170.
[78] T. R. Kießling, R. Stange, J. A. Käs, A. W. Fritsch, *New J. Phys.* **2013**, *15*, 045026.
[79] A. Blicher, K. Wodzinska, M. Fidorra, M. Winterhalter, T. Heimburg, *Biophys. J.* **2009**, *96*, 4581.
[80] L. A. Sonna, J. Fujita, S. L. Gaffin, C. M. Lilly, *J. Appl. Physiol.* **2002**, *92*, 1725.
[81] a) O. Zohar, M. Ikeda, H. Shinagawa, H. Inoue, H. Nakamura, D. Elbaum, D. L. Alkon, T. Yoshioka, *Biophys. J.* **1998**, *74*, 82; b) J. S. Donner, S. A. Thompson, M. P. Kreuzer, G. Baffou, R. Quidant, *Nano Lett.* **2012**, *12*, 2107; c) L. Shang, F. Stockmar, N. Azadfar, G. U. Nienhaus, *Angew. Chem., Int. Ed.* **2013**, *52*, 11154.
[82] G. Baffou, P. Bon, J. Savatier, J. Polleux, M. Zhu, M. Merlin, H. Rigneault, S. Monneret, *ACS Nano* **2012**, *6*, 2452.
[83] D. Boyer, P. Tamarat, A. Maali, B. Lounis, M. Orrit, *Science* **2002**, *297*, 1160.
[84] S. Adhikari, P. Spaeth, A. Kar, M. D. Baaske, S. Khatua, M. Orrit, *ACS Nano* **2020**, *14*, 16414.
[85] J. Stehr, C. Hrelescu, R. A. Sperling, G. Raschke, M. Wunderlich, A. Nichtl, D. Heindl, K. Kürzinger, W. J. Parak, T. A. Klar, J. Feldmann, *Nano Lett.* **2008**, *8*, 619.
[86] a) L. Ullerich, S. Campbell, F. Krieg-Schneider, F. Bürsgens, J. Stehr, *Laboratoriumsmedizin* **2017**, *41*, 239; b) E. Vanzha, T. Pylaev, V. Khanadeev, S. Konnova, V. Fedorova, N. Khlebtsov, *RSC Adv.* **2016**, *6*, 110146; c) B.-H. Kang, Y. Lee, E.-S. Yu, H. Na, M. Kang, H. J. Huh, K.-H. Jeong, *ACS Nano* **2021**, *15*, 10194.
[87] C. Hrelescu, J. Stehr, M. Ringler, R. A. Sperling, W. J. Parak, T. A. Klar, J. Feldmann, *J. Phys. Chem. C* **2010**, *114*, 7401.
[88] G. Qiu, Z. Gai, Y. Tao, J. Schmitt, G. A. Kullak-Ublick, J. Wang, *ACS Nano* **2020**, *14*, 5268.
[89] a) H. Ahn, S. Kim, Y. Kim, S. Kim, J.-r. Choi, K. Kim, *Biosens. Bioelectron.* **2021**, *182*, 113150; b) H. Nakatsuji, T. Numata, N. Morone, S. Kaneko, Y. Mori, H. Imahori, T. Murakami, *Angew. Chem., Int. Ed.* **2015**, *54*, 11725.
[90] a) A. S. Urban, M. Fedoruk, M. R. Horton, J. O. Radler, F. D. Stefani, J. Feldmann, *Nano Lett.* **2009**, *9*, 2903; b) T. Nobeyama, K. Shigyou, H. Nakatsuji, H. Sugiyama, N. Komura, H. Ando, T. Hamada, T. Murakami, *Langmuir* **2020**, *36*, 7741.
[91] P. M. Bendix, S. N. S. Reihani, L. B. Oddershede, *ACS Nano* **2010**, *4*, 2256.
[92] P. Urban, S. R. Kirchner, C. Mühlbauer, T. Lohmüller, J. Feldmann, *Sci. Rep.* **2016**, *6*, 22686.
[93] A. Rørvig-Lund, A. Bahadori, S. Semsey, P. M. Bendix, L. B. Oddershede, *Nano Lett.* **2015**, *15*, 4183.
[94] A. Bahadori, L. B. Oddershede, P. M. Bendix, *Nano Res.* **2017**, *10*, 2034.
[95] M. Zhu, G. Baffou, N. Meyerbröker, J. Polleux, *ACS Nano* **2012**, *6*, 7227.
[96] H. M. L. Robert, J. Savatier, S. Vial, J. Verghese, B. Wattellier, H. Rigneault, S. Monneret, J. Polleux, G. Baffou, *Small* **2018**, *14*, 1801910.
[97] C. Carrillo-Carrión, R. Martínez, E. Polo, M. Tomás-Gamasa, P. Destito, M. Ceballos, B. Pelaz, F. López, J. L. Mascareñas, P. d. Pino, *ACS Nano* **2021**, *15*, 16924.
[98] A. S. Angelatos, B. Radt, F. Caruso, *J. Phys. Chem. B* **2005**, *109*, 3071.
[99] M. Delcea, N. Sternberg, A. M. Yashchenok, R. Georgieva, H. Bäumler, H. Möhwald, A. G. Skirtach, *ACS Nano* **2012**, *6*, 4169.
[100] Z. Fang, Y.-R. Zhen, O. Neumann, A. Polman, F. J. García de Abajo, P. Nordlander, N. J. Halas, *Nano Lett.* **2013**, *13*, 1736.
[101] M. Li, T. Lohmuller, J. Feldmann, *Nano Lett.* **2015**, *15*, 770.
[102] R. Xiong, K. Raemdonck, K. Peynshaert, I. Lentacker, I. De Cock, J. Demeester, S. C. De Smedt, A. G. Skirtach, K. Braeckmans, *ACS Nano* **2014**, *8*, 6288.
[103] R. Xiong, P. Verstraelen, J. Demeester, A. G. Skirtach, J.-P. Timmermans, S. C. De Smedt, W. H. De Vos, K. Braeckmans, *Front. Cell. Neurosci.* **2018**, *12*, 80.
[104] L. Raes, S. Stremersch, J. C. Fraire, T. Brans, G. Goetgeluk, S. De Munter, L. Van Hoecke, R. Verbeke, J. Van Hoeck, R. Xiong, *Nano-Micro Lett.* **2020**, *12*, 185.
[105] L. Wayteck, R. Xiong, K. Braeckmans, S. C. De Smedt, K. Raemdonck, *J. Controlled Release* **2017**, *267*, 154.
[106] A. Ashkin, J. M. Dziedzic, J. E. Bjorkholm, S. Chu, *Opt. Lett.* **1986**, *11*, 288.
[107] C. J. Bustamante, Y. R. Chemla, S. Liu, M. D. Wang, *Nat. Rev. Methods Primers* **2021**, *1*, 25.
[108] A. Ohlinger, A. Deak, A. A. Lutich, J. Feldmann, *Phys. Rev. Lett.* **2012**, *108*, 018101.
[109] S. R. Kirchner, S. Nedev, S. Carretero-Palacios, A. Mader, M. Opitz, T. Lohmüller, J. Feldmann, *Appl. Phys. Lett.* **2014**, *104*, 093701.
[110] C. Zensen, I. E. Fernandez, O. Eickelberg, J. Feldmann, T. Lohmüller, *Adv. Sci.* **2017**, *4*, 1600238.
[111] S. R. Kirchner, M. Fedoruk, T. Lohmüller, J. Feldmann, *J. Visualized Exp.* **2014**, *89*, 51502.
[112] C. Zensen, N. Villadsen, F. Winterer, S. R. Keiding, T. Lohmüller, *APL Photonics* **2016**, *1*, 026102.
[113] J. A. Copland, M. Eghtedari, V. L. Popov, N. Kotov, N. Mamedova, M. Motamedi, A. A. Oraevsky, *Mol. Imaging Biol.* **2004**, *6*, 341.
[114] M. Eghtedari, A. Oraevsky, J. A. Copland, N. A. Kotov, A. Conjusteau, M. Motamedi, *Nano Lett.* **2007**, *7*, 1914.
[115] L. Osinkina, S. Carretero-Palacios, J. Stehr, A. A. Lutich, F. Jäckel, J. Feldmann, *Nano Lett.* **2013**, *13*, 3140.
[116] S. Simoncelli, S. Johnson, F. Kriegel, J. Lipfert, J. Feldmann, *ACS Photonics* **2017**, *4*, 2843.
[117] H.-R. Jiang, N. Yoshinaga, M. Sano, *Phys. Rev. Lett.* **2010**, *105*, 268302.
[118] S. Simoncelli, J. Summer, S. Nedev, P. Kühler, J. Feldmann, *Small* **2016**, *12*, 2854.
[119] S. Nedev, S. Carretero-Palacios, P. Kühler, T. Lohmüller, A. S. Urban, L. J. E. Anderson, J. Feldmann, *ACS Photonics* **2015**, *2*, 491.
[120] C. M. Maier, M. A. Huergo, S. Milosevic, C. Pernpeintner, M. Li, D. P. Singh, D. Walker, P. Fischer, J. Feldmann, T. Lohmüller, *Nano Lett.* **2018**, *18*, 7935.
[121] C.-K. Chiang, W.-T. Chen, H.-T. Chang, *Chem. Soc. Rev.* **2011**, *40*, 1269.
[122] L. Huang, J. Wan, X. Wei, Y. Liu, J. Huang, X. Sun, R. Zhang, D. D. Gurav, V. Vedarethinam, Y. Li, *R. Nat. Commun.* **2017**, *8*, 220.
[123] a) W. Shu, Y. Wang, C. Liu, R. Li, C. Pei, W. Lou, S. Lin, W. Di, J. Wan, *Small Methods* **2020**, *4*, 1900469; b) L. Huang, D. D. Gurav, S. Wu, W. Xu, V. Vedarethinam, J. Yang, H. Su, X. Wan, Y. Fang, B. Shen, *Matter* **2019**, *1*, 1669; c) J. Cao, X. Shi, D. D. Gurav, L. Huang, H. Su, K. Li, J. Niu, M. Zhang, Q. Wang, M. Jiang, *Adv. Mater.* **2020**, *32*, 2000906.
[124] a) J. C. Bretschneider, M. Reismann, G. von Plessen, U. Simon, *Small* **2009**, *5*, 2549; b) H. R. de Barros, I. Garcia, C. Kuttner, N. Zeballos, P. H. C. Camargo, S. I. s. C. de Torresi, F. Lopez-Gallego, L. M. Liz-Marzan, *ACS Catal.* **2020**, *11*, 414.
[125] J. Liu, C. Cai, Y. Wang, Y. Liu, L. Huang, T. Tian, Y. Yao, J. Wei, R. Chen, K. Zhang, *Adv. Sci.* **2020**, *7*, 1903730.
[126] K. Marchuk, K. A. Willets, *Chem. Phys.* **2014**, *445*, 95.

[127] a) E. Carrasco, J. C. Stockert, Á. Juarranz, A. Blázquez-Castro, *Front. Chem.* **2020**, *8*, 1160; b) L. Gao, R. Liu, F. Gao, Y. Wang, X. Jiang, X. Gao, *ACS Nano* **2014**, *8*, 7260.
[128] a) M. L. Juan, M. Righini, R. Quidant, *Nat. Photonics* **2011**, *5*, 349; b) M. Samadi, P. Alibeigloo, A. Aqhili, M. A. Khosravi, F. Saeidi, S. Vasini, M. Ghorbanzadeh, S. Darbari, M. K. Moravvej-Farshi, *Opt. Lasers Eng.* **2022**, *154*, 107001.
[129] A. N. Grigorenko, N. W. Roberts, M. R. Dickinson, Y. Zhang, *Nat. Photonics* **2008**, *2*, 365.
[130] M. Righini, P. Ghenuche, S. Cherukulappurath, V. Myroshnychenko, F. J. García de Abajo, R. Quidant, *Nano Lett.* **2009**, *9*, 3387.
[131] R. Sainidou, F. J. G. De Abajo, *Phys. Rev. Lett.* **2008**, *101*, 136802.
[132] M. L. Juan, R. Gordon, Y. Pang, F. Eftekhari, R. Quidant, *Nat. Phys.* **2009**, *5*, 915.
[133] Y. Zhang, C. Min, X. Dou, X. Wang, H. P. Urbach, M. G. Somekh, X. Yuan, *Light: Sci. Appl.* **2021**, *10*, 59.

Maria-Ana Huergo received her M.D. and Ph.D. in Medicine from the National University of La Plata (UNLP, Argentina) and currently works as a permanent staff Researcher at the Theoretical and Applied Physical-Chemical Research Institute (INIFTA), UNLP. Her research interests encompass the synthesis and characterization of nanomaterials with new properties for biomedical applications, with a particular interest in plasmonic nanomaterials for theranostics.

Francis Schuknecht is currently pursuing his Ph.D. in the group of Theo Lohmüller at the Chair for Photonics and Optoelectronics at LMU Munich. His research interests lie in the optothermal manipulation of gold nanorods and surface enhanced Raman scattering spectroscopy with plasmonic DNA origami nanoantennas for bio-molecule sensing.

Jinhua Zhang is a Ph.D. student with Theo Lohmüller at the Chair for Photonics and Optoelectronics at LMU Munich. Her work is focused on photoswitchable lipid membranes, where she studies photosensitization and plasmon-based detection of azobenzene labeled phospholipids.

Theobald Lohmüller is the leader of the Biophotonics Group at the Chair for Photonics and Optoelectronics at LMU Munich. His research aims at developing and applying photonic nano-agents, such as hybrid plasmonic nanosystems, biocompatible fluorescent nanoparticles, and molecular photoswitches, to study, manipulate and control the physical properties of biological systems with light.