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# Colloidal Nanoplasmonics: From Building Blocks to Sensing Devices

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**ABSTRACT:** Nanoplasmonics is a rapidly developing field of research and technology that is based on the ability of small metal particles to interact strongly with light of wavelength significantly larger than their size. The development of nanoplasmonics has been closely associated with the application of colloid science to the controlled growth of metal nanocrystals in solution and to directing the self-assembly of such nanocrystals into organized arrays with enhanced collective properties. Engineering the morphology and the assembly of metal nanoparticles is a key step toward the fabrication of devices with great potential in detection and diagnosis as well as in a wide variety of other fields. In this Feature Article, we provide an overview of the recent work in our laboratory, which in our view somehow reflects the evolution of the field itself and provides guidelines for future research.

## INTRODUCTION

Nanoplasmonics can be defined as the science that deals with the manipulation of light using materials that are much smaller than the wavelength of such light.[1] This is typically achieved using nanostructured metals because they can very efficiently absorb and scatter light as a result of their ability to support coherent oscillations of free (conduction) electrons, so they can act as nanoantennas.[2] Although the remarkable optical response of "finely divided" metals has been well known for more than 150 years, the recent development of sophisticated characterization techniques and modeling methods[3] has dramatically reactivated the field. Another extremely important pillar on which the development of nanoplasmonics has been based on the great advancement of fabrication methods, which provide us with exquisite control over the composition and morphology of nanostructured metals. Both lithography and colloid chemistry have seen a tremendous improvement in the control that can be achieved, to a degree that seemed impossible only a decade ago. A wide variety of lithographic and other physical methods have been devised that provide a perfect morphological definition and reproducibility, even down to the nanometer scale.[4] However, colloid chemistry has the advantage of simplicity and larger-scale production while offering a number of parameters that can be used as handles to direct not only nanoparticle morphology but also surface properties and subsequent processing.[5]

This Feature Article aims to show that colloidal nanoplasmonics is now sufficiently mature to bridge the basic (wet chemistry) fabrication of nanoplasmonic building blocks, all the way to devices that can be used for real applications. On the basis of examples taken principally from recent work in our laboratory, the main message is that, although we have all

necessary tools at hand and can make use of them to demonstrate the usefulness of these materials, a basic understanding of the structure, surface chemistry, and colloidal (phase) behavior is crucial to reaching this target.

## COLLOIDAL SYNTHESIS OF PLASMONIC BUILDING BLOCKS

The presence of well-defined localized surface plasmon resonances (LSPRs) in the visible and NIR is dictated by the complex dielectric function of the material comprising the nanostructures and has been typically restricted to a few metals, namely, gold, silver, and copper. (New materials such as copper chalcogenides[6] are currently being explored, but we shall not enter this discussion here.) Although silver possesses dielectric properties yielding the most efficient plasmonic response, gold is still of major interest because of its greater chemical inertness. Copper is far less often employed because it is both less stable and less efficient, though its performance can be improved by optimizing the nanoparticle morphology.[7] The use of colloid chemistry in preparing metal nanoparticle dispersions has a long history, but research in this direction has increased during the last 20 years. Major advances in understanding the role of the various reaction parameters have allowed tight control over size and shape monodispersity, which has been essential in understanding the correlation between nanoparticle morphology and optical response but is also a determining factor in the
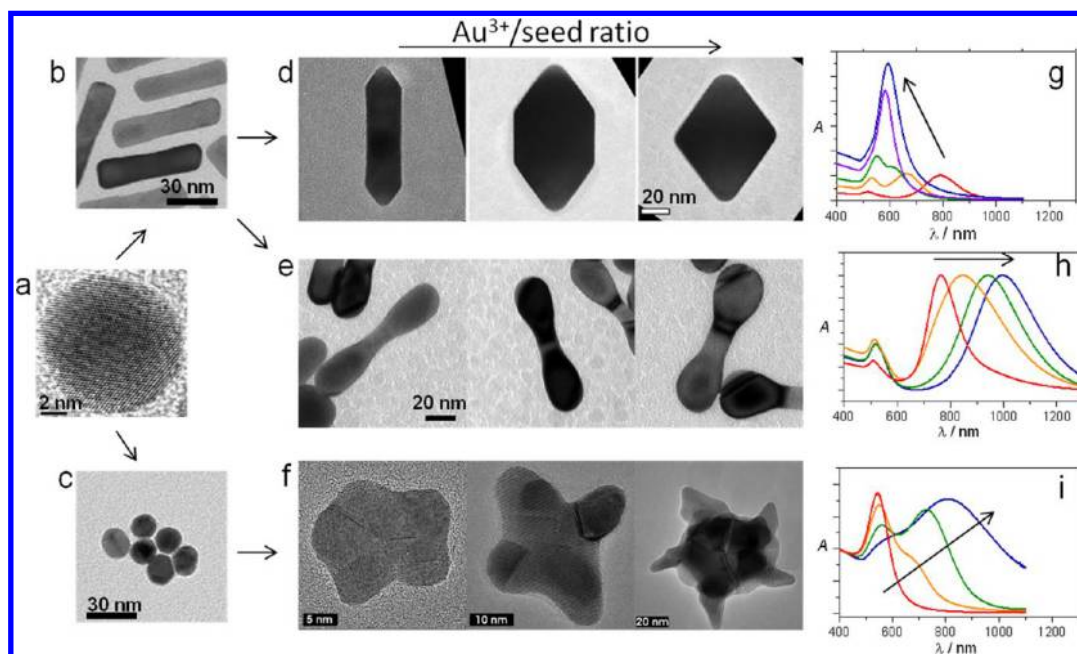
**Figure 1.** Seeded growth method as an example of the autocatalytic reduction of metal ions on preformed seeds. The ratio between metal ions and seed determines the final particle size. The growth of gold nanorods can be tuned to achieve platonic-shaped particles (octahedrons)[13] or more exotic shapes (e.g., dumbbells). Even faster reduction of the metal ions on seeds promotes the formation of branched structures, so-called nanostars. Reproduced with permission from refs 13, 14, and 17. Copyright Wiley-VCH (d, e) and American Chemical Society (f).

fabrication of nanoparticle supracrystals of high quality and crystallinity.

One major step toward morphology control has been the separation of nucleation and growth processes, which is the basis of the so-often quoted seeded growth methods. The concept of seeded growth is based on the autocatalytic reduction of the metal ions, exclusively on the seed particles, which thus act as both catalysts and nucleation points. In such nucleation-free reduction, the final concentration of particles is determined by the initial number of seeds, and the final particle size is controlled by the ratio between metal ions and seed. However, under certain conditions, the growth is not isotropic, with some crystalline facets growing faster than others and therefore leading to the formation of anisotropic nanoparticles. A clear example of such anisotropic growth is the synthesis of gold nanorods in aqueous surfactant solutions, which has been the paradigm of the application of the seeded growth method.[8,9] However, the same example can be used to show the complexity of such growth processes because the morphology of the particles is strongly affected by slight variations of several synthesis parameters, such as the seed concentration, surfactant composition, and temperature, but the concentration of silver ions or other additives such as salicylic acid also plays an important role.[10] Importantly, the crystallinity of the initial seeds is another crucial factor determining the final morphology, and thus nanorods with a pentagonal cross section are obtained from pentatwinned seeds, whereas single-crystal seeds lead to rods with an octagonal cross section. Similar observations were made for the seeded growth of the same seeds in *N,N*-dimethylformamide (DMF) in the presence of poly(vinylpyrrolidone) (PVP), where pentatwinned seeds could be uniformly grown into regular decahedra and single-crystal seeds resulted in perfectly defined monocrystalline octahedra.[11] Much has been discussed regarding the influence of the various parameters, and concepts such as the preferential adsorption of surfactants or polymers on selected crystalline facets have been repeatedly quoted. However, it turns out that often these conclusions arise from poorly established findings. An example can be found in the seeded growth of gold nanorods through the mild reduction of HAuCl$_4$ in DMF/PVP solutions. Despite the high anisotropy of the initial seeds (nanorods), it has been found that the progressive reduction of gold salt leads to increasingly more isotropic particles, which has been explained on the basis of the faster growth of crystalline facets with higher surface energy. Although this was found to make perfect sense for pentatwinned nanorods,[12] the morphological evolution of monocrystalline rods did not agree with this model because it seemed to indicate that [100] facets were less stable than [110] facets, in disagreement with surface energy data.[13] However, subsequent work demonstrated that this apparent disagreement was likely due to a wrong assignment of the crystalline indexes for the lateral facets of the starting single-crystalline nanorods. Still, this process can be used to tailor the morphology of the particles because not only can the number and type of facets be modulated, but this also has a strong impact on the LSPR-based optical response of the particles. During this specific growth process, the longitudinal LSPR band gradually blue shifted whereas the transverse LSPR red shifted, eventually merging into a single band with considerably increased intensity, as expected for the final quasi-isotropic octahedral particles (Figure 1).

Although the overgrowth of gold nanorods in the presence of silver ions and cetyltrimethylammonium bromide (CTAB) leads to a decrease in their aspect ratio and to a corresponding blue shift of the plasmon band, the manipulation of the plasmonic response of gold nanorods through anisotropic growth may also lead to LSPR shifts in the opposite direction (lower energies). Understanding and controlling the LSPR energy has serious implications for many potential applications but specifically in the biomedical field, where gold nanorods have been proposed as one of the most promising nanoma-
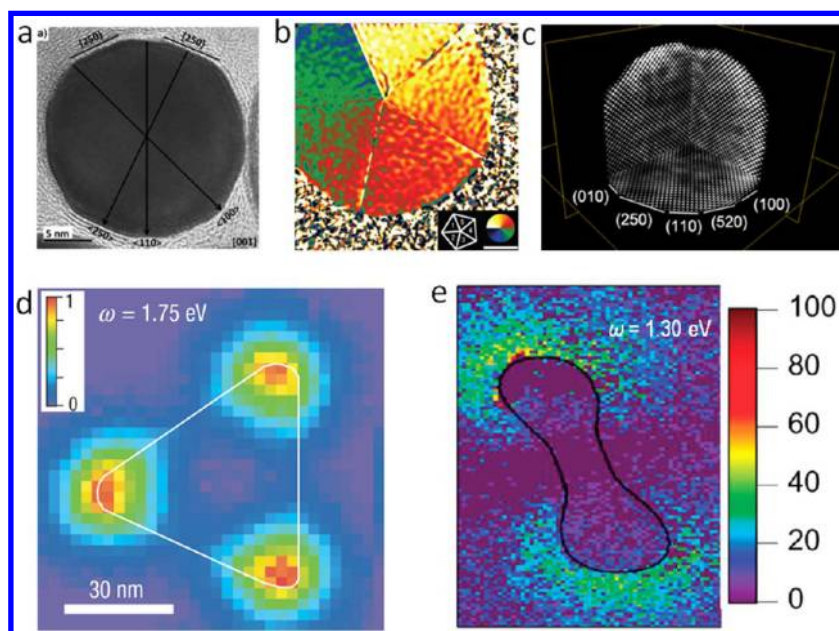
**Figure 2.** Advanced microscopy techniques become crucial in understanding the structure of plasmonic nanoparticles. (a) HRTEM image of a standing gold nanorod, showing the main crystallographic directions and the indexes of the facets.[18] (b) Inhomogeneous strain distribution in a decahedral gold nanoparticle.[22] (c) 3D atomic-resolution electron tomography of a gold nanorod.[21] (d, e) Single-particle EELS surface plasmon mapping of a silver nanoprism[24] and a gold nanodumbbell.[26] Reproduced with permission from refs 18, 22, 21, 24, and 26. Copyright Wiley-VCH (a), Nature Publishing Group (b−d), and American Chemical Society (e).

terials. In these applications, the plasmon band should be located in the NIR spectral range (i.e., in the biological transparency window). We have recently reported that the addition of small numbers of iodide ions prior to the seeded growth of standard CTAB nanorods leads to unusual morphological changes, which are optically equivalent to an increase in the aspect ratio of the gold nanorods.[14] The effect of growing the rods in the presence of a small amount of iodide is the formation of dumbbell-like nanoparticles, meaning that the reduction of the gold salt takes place preferentially on the tips while the lateral facets remain unchanged. Interestingly, the specific concentration of iodide and nanorods determines the extent of tip growth and accordingly the magnitude of the LSPR shift. The mechanism that explains preferential reduction on the tips relates again to the surface chemistry of the nanoparticles. The as-prepared gold nanorods contain bromide on their surface (arising from the surfactant used during synthesis, CTAB), which can be readily replaced by iodide to produce AgI preferentially on the tips. Silver iodide can decrease the surface redox potential of gold nanorods, leading to more favorable deposition of the metal on the tips. Therefore, changing the number of seeds while maintaining a constant iodide/gold ratio leads to the formation of larger particles but still with well-defined dumbbell-like shapes (Figure 1). Because research on gold nanorods is still extremely active, new and more efficient ways to tune the optical response are to be expected in the near future.

Apart from nanorods, gold and silver nanoparticles with other morphologies have received much attention with respect to their optical properties, among which probably nanocubes and nanostars are the most frequently chosen ones. Gold nanostars in particular have been intensively studied during the past 5 years,[15] mainly because sharp spikes can accommodate large electric fields at their tips, which makes them extremely interesting for a number of plasmonic applications. Among the

various synthesis methods that have been reported so far for Au nanostars, one of them illustrates again the complexity and sensitivity of these seeded growth processes in the context of the discussion above. The method, reported by Kumar et al. in 2008,[16] is based on the seeded growth of gold nanoparticle seeds in a solution of PVP in DMF, just like the formation of decahedra and octahedra except that the concentration of PVP is increased ca. 5-fold. Such a dramatic change in the growth fashion has been assigned to the reducing ability of PVP, so the reduction rate would be increased in the autocatalytic process, resulting in such multipod Au nanoparticles (nanostars).[16] Although one might argue that the polycrystallinity of the seeds might be responsible for the growth of multiple spikes, it has been demonstrated that similar growth can be induced on single-crystal seeds, nanowires, or nanorods. Careful HRTEM analysis of particles at different growth steps indicates that twinning gradually increases during growth,[17] but the precise mechanism is still to be disclosed. It is also important to stress that this faster reduction was achieved with no use of external energy sources such as temperature or light. For nanostars, two LSPR modes are predicted (and found experimentally), one localized at the central core and another at the tips, which are usually hybridized. Whereas the core mode changes very little with the nanostar morphology, the tip modes can be tuned through the length and sharpness of the tips, which is usually achieved through variations in the molar ratio between Au salt and seed (Figure 1).

## ■ ADVANCED TECHNIQUES HELP IN UNDERSTANDING NANOPARTICLES ULTRASTRUCTURE

Although colloidal nanofabrication often boils down to relatively simple chemistry, we have already shown that its mechanistic understanding requires complex and multidisciplinary knowledge and characterization. The combination of

crystallography, electrochemistry, soft matter, and colloid chemistry allows us to picture general scenarios for nanoparticle growth, but still within the framework of experimental limitations. For instance, although there is general agreement on the growth mechanism of gold nanorods using a $CTA^+$-type surfactant as a stabilizer, no reports are available on the synthesis of these particles using other types of molecules, which could be explained by means of a similar mechanism. It is therefore still too early to predict the outcome of a specific synthesis path, even if all conditions are fully described, and often only a detailed postsynthesis characterization of the nanoparticles aids our understanding and helps us to propose suitable mechanisms. This approach requires going down to the single-particle level in order to describe the surface structure or even the spatial distribution of the atoms within the nanocrystals.

Electron microscopy has been the most useful tool for the characterization of nanoparticles. Advanced electron microscopy techniques allow a complete investigation of the composition, crystalline structure, surface morphology, and even lattice strain at the single-particle level. Such detailed studies have recently allowed a revision of the crystallographic structure of single-crystalline gold nanorods, prepared by silver-assisted seeded growth (Figure 2a).[18] An analysis of high-resolution images of standing nanorods showed that particles are enclosed by eight identical high-index crystalline facets of the [250] type rather than by alternating [100] and [110] facets, as previously proposed by Wang et al.[19] Similar findings have been reported on the basis of electron tomography.[20] The identification of the crystalline facets in different parts of the nanoparticles is especially relevant with respect to our understanding not only of the surface energy and stability but also of the adsorption of different types of molecules during particle growth and postsynthesis functionalization.

An additional turn of the screw in electron microscopy characterization can be provided by aberration-corrected electron microscopy. The technical quality of this technique renders it the ideal method for studying nanomaterials with atomic resolution, but (until very recently) with the limitation imposed by the two-dimensional (2D) nature of the resulting images, which are thus projections of 3D nano-objects and therefore result in incomplete characterization. Recent developments, however, have allowed the implementation of atomic-resolution electron tomography as a technique capable of registering the 3D atomic structure of free-standing nanoparticles. By means of advanced 3D reconstruction algorithms, the 3D atomic lattice of gold nanorods has been recently fully determined on the basis of a small number of projection images. From the reconstruction images, the surface facets, surface relaxation, and imperfections on the atomic scale could be characterized.[21] Although this technique was initially applied to gold nanorods, it can find application in the characterization of a variety of nanostructures.

Aberration-corrected HRTEM has also been recently exploited to analyze the internal structure of decahedral gold nanoparticles.[22] Such 5-fold-twinned nanoparticles are of special interest because of their unique morphology, which endows them with intrinsic internal strain. A decahedron is made by joining five tetrahedrons to each other, which leaves a solid-angle deficiency that can be compensated only by strain or defects. There has been much discussion regarding the strain model behind this morphology, and two competing theoretical models were proposed that required experimental verification.

The synthesis of gold decahedrons of controlled size allowed the experimental determination of the local plane strain, indicating that the defect-free decahedral nanoparticles exhibit an inhomogeneous strain distribution that agrees with the predictions of a disclination model (Figure 2b). The development of more sophisticated 3D reconstruction algorithms, together with the continuous improvement in the quality of the instruments, provides reasonable expectations regarding the evaluation of strain in 3D for very small particles.

We have thus shown that modern TEM techniques provide important information about the structural data of metal nanoparticles. This information can be additionally supplemented by chemical information achieved by, for example, electron energy-loss spectroscopy (EELS). The EELS technique involves the analysis of the energy loss by electrons in TEM after they have been inelastically scattered by the sample. A typical EELS spectrum can be divided into three regimes: a zero loss regime (the electrons that passed through the sample without interactions), a valence regime (dominated by collective electron excitation modes), and an ionization edge (which originates from the excitation of core electrons into an unoccupied electron state). In the context of nanoplasmonic studies, the valence regime is of great interest because it provides similar information to that provided by optical spectroscopy. Therefore, the combination of the subnanometer resolution of HRTEM with EELS analysis makes it possible to identify surface plasmon modes in individual metal nanoparticles. However, until recently the limitation of the EELS technique was the partial coverage of the valence regime (1−5 eV) by the tail of zero-loss peaks. Thus, the measurements were limited to the UV spectral range, which is far from the commonly observed plasmon modes in plasmonic nanoparticles (in the visible and the NIR). Such difficulties have been recently overcome by the application of a deconvolution scheme that improves the signal-to-background ratio for low-energy resonances.[23] A great advantage of EELS plasmon characterization is that the spatial distribution of the loss probability, which is closely related to the electric near-field enhancement distribution for the same plasmon mode, can be mapped. This technique has been applied to the characterization of the various plasmon modes corresponding to particles with a variety of shapes.[24−27] A landmark in this field has been the unequivocal mapping of the spatial distribution of surface plasmon modes in individual triangular silver nanoprisms (80 nm edge length) in the near-infrared and the visible ranges.[24] The characteristic LSPR modes of the nanoprisms were identified through the analysis of EEL spectra measured at different spots (1−4 nm apart) using a narrow electron beam (1 nm diameter) that was rastered through the sample. Once the LSPR energies were determined, mapping based on monochromatic EELS was used to plot the distribution of each LSPR mode (1.9, 2.9, and 3.4 eV), showing confinement at the corners, the middle of the edges, and the center, respectively. By making possible the detection of peaks in the near-IR range, this approach provides a powerful tool in the development of nanometer-level optics. In fact, the application of this method in the study of nanoparticles with more exotic shapes has contributed to revealing the presence of plasmon modes that were undetectable by other techniques. For example, the identification of bright and dark modes has been discussed for bare and silver-coated gold nanodumbbells.[26] It is important to stress that the interpretation of EELS spectra requires proper knowledge of the morphology and the
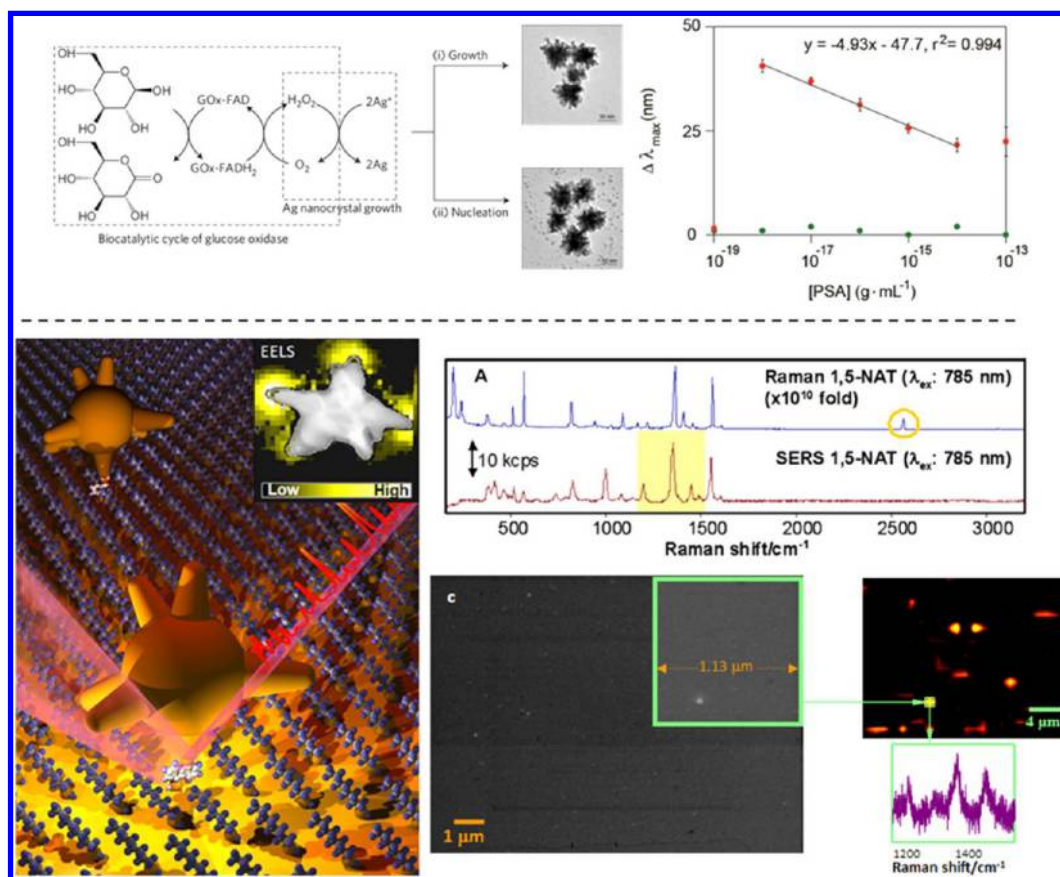
**Figure 3.** Upper panel: Reverse sensitivity mechanism of enzyme-modified plasmonic nanosensors. Enzymatically generated hydrogen peroxide acts as a reducing agent to form metallic silver. For low antigen concentration, silver ions are slowly reduced exclusively on the nanostar surface, resulting in a large LSPR shift. Oppositely, for larger amounts of antigen and thus hydrogen peroxide, silver is reduced quickly and nanoparticles are formed in solution. Adapted with permission from ref 42. Copyright 2012, Nature Publishing Group. Lower panel: Sketch of the deposition of nanostars onto single aromatic molecules through thiol binding. The inset shows an STEM image overlapped with EELS intensity mapping. Raman spectrum of a concentrated analyte solution and SERS spectrum of the analyte molecule located in the gap between the nanostar and gold film. SEM images and single-particle SERS mapping recorded from a sample with less than 1 particle/mm^{2}. Adapted with permission from ref 25. Copyright 2009, American Chemical Society.

position of the irradiation spot, which can be realized in situ during the same experiment.

Another important question that colloidal (nano)science is facing is the exact distribution or quantification of the stabilizing molecules on the surface of nanoparticles. This topic is of great importance, in particular when dealing with particle growth mechanisms or with postsynthesis ligand exchange for biological applications. It has been shown that the toxicity of CTAB-stabilized gold nanorods is due to free surfactant molecules in the solution.[28] Therefore, to replace toxic CTAB molecules precisely with nontoxic capping agents, one first needs to know the distribution of these molecules on the gold surface. In the case of gold nanorods, CTAB molecules adsorb onto the gold surface through the complexation of the quaternary ammonium headgroup, so the long hexadecyl chains should be facing the solvent. This is in contrast to the high colloidal stability of nanorods in aqueous solution, and thus it has been proposed that surfactant molecules self-organize on the particle surfaces in the form of a bilayer[28] in which the hydrophobic alkyl chains interdigitate and the positively charged ammonium headgroups of the outer layer provide electrostatic stability. However, although several techniques were previously used to characterize CTAB bilayers on microscopic surfaces, it has not been until very recently that direct measurements were carried out directly on nanorod dispersions. The presence of the surfactant bilayer on the surface of gold nanorods was thus confirmed by using a combination of small-angle X-ray scattering (SAXS) and small-angle neutron scattering (SANS).[29] SAXS relies on electron density contrast and can provide information on the dimensions of the nanorod metallic cores, and SANS is based on elastic neutron scattering and the contrast can be varied through changes in the composition of the solvent, thereby allowing a separation between the scattering from the core and that from the shell. In this way, precise values for the thickness of the CTAB bilayer could be determined to be 3.2 ± 0.2 nm, in agreement with a model considering the partial interdigitation of the alkyl chains, because the obtained thickness is smaller than twice the extended alkyl chain length. It should also be taken into account that such scattering measurements were performed in solution and thus provide an average over millions of particles, in contrast to the limited number of particles probed in electron microscopy measurements.

## ◼ ISOLATED NANOPARTICLES AS PLASMONIC SENSORS

One of the most interesting aspects of LSPRs in metal nanoparticles is their extreme sensitivity to environmental

changes, which renders them excellent transducers for sensing devices. Although much has been published regarding LSPR sensing,[30−32] the field is under continuous development and even new concepts are still regularly introduced. Historically, perhaps the first biosensing application of gold nanoparticles was the pregnancy test, in which the functionalization of the nanoparticles with specific antibodies provided their anchoring at the test window and their extremely high extinction coefficient provided sufficient signal in the colorimetric detection of the human gonadotropic hormone.[30] DNA detection has been greatly simplified through a method based on the spectral changes derived from the aggregation of two different ssDNA-coated gold nanoparticles in solution, in the presence of a target oligonucleotide, complementary to both ssDNA sequences in the nanoparticles.[33] This concept was later extended to the detection of a wide variety of analytes, from proteins[34] to heavy metal ions.[35]

A different concept in LSPR is refractive index sensing,[30] where the transducer is a spectral shift in the LSPR band upon variations in the local refractive index near the metal nanoparticle surface. This is also an old concept that can be readily predicted even by using the dipolar approximation in Mie theory and has been applied in biosensing[36] because the local refractive index may change significantly when a target analyte binds to a biomolecular receptor that has been previously bound to the nanoparticles.[37] However, the recent advances in the synthesis of metal nanoparticles with controlled morphologies and the development of single-particle spectroscopy methods, such as dark-field microspectroscopy,[38] have allowed us to improve the sensitivity and the understanding of such processes drastically. On one hand, the magnitude of the plasmon shift upon changing the local refractive index strongly depends on the morphology of the nanoparticles, so the sensitivity can be optimized by tailoring the particle shape. Particularly attractive are anisotropic particles, with sharp edges or narrow spikes, that showed enhanced sensitivity toward changes in the medium refractive index as compared to those in spherical or smooth particles.[17,39]

However, other conceptually different methods exist in which biosensing relies on using plasmonic particles as signal transducers. Pavlov et al. have demonstrated that the conjugation of particle growth with enzymatic reactions can be exploited for sensitive biodetection. In essence, an enzyme can selectively react with an analyte, thereby producing a reducing species, which leads to the autocatalytic growth of nanoparticles present in solution. Interestingly, the extent of the process can be modulated by the presence of a specific inhibitor. In such a model, the concentration of the biological moieties (substrate, product, enzyme, and inhibitor) can be directly translated into the optical response of the final product after the reduction of the metal.[40] Recently, a plasmonic version of enzyme-linked immunesorbent assay (ELISA) has been reported in which the generation of colored solutions with characteristic tonality was utilized to detect a target molecule with the naked eye.[41] In the proposed method, an enzyme label controlled the growth of gold nanoparticles and generated blue or red solutions in the presence or absence of the analyte, respectively. However, a significant drawback in all of the methods mentioned so far is that the magnitude of the transduced signal increases with the amount of target substance, so they necessarily have an intrinsic limitation in the lower limit of detection. At the level of ultralow concentrations of the analyte, the variations in the physical properties of the

nanoparticle sensor are tiny and therefore difficult to detect. In this context, a completely novel concept has been recently conceived, which in fact may seem counterintuitive and has been termed reverse sensitivity. In this new concept, the transducer signal becomes larger as the concentration of the target molecule is decreased.[42] Such reverse sensitivity was achieved by using the enzyme glucose oxidase (GOx), which in the presence of glucose generates hydrogen peroxide. When GOx is immobilized on gold nanostars, the production of hydrogen peroxide can promote the reduction of silver ions present in solution (Figure 3). It was found that GOx could regulate the rate of silver reduction to favor either the nucleation of small particles (at high GOx concentration) or the deposition of silver on the nanostars surface, forming a shell (at low GOx) and thereby leading to a strong blue shift in the LSPR band of the nanostars. In fact, a linear relationship (with negative slope) was found between the GOx concentration and LSPR position. This phenomenon was exploited to detect the cancer biomarker prostate-specific antigen (PSA), for which the nanostars were modified with polyclonal antibodies against PSA. Upon PSA binding, this cancer biomarker was detected with monoclonal antibodies and labeled with secondary antibodies bound to GOx, which could promote silver reduction. Again, the spectral shift was inversely proportional to the PSA concentration, with a reported detection limit as low as $10^{-18}$ g mL$^{-1}$ in whole serum (Figure 3a). Certainly, this concept opens up new possibilities for biosensing when the rich chemistry of crystal growth is merged with the outstanding physical properties of plasmonic nanosensors.

We conclude this section by making reference to another plasmonic sensing technique that offers the highest chemical specificity, namely, surface-enhanced Raman scattering (SERS) spectroscopy. In SERS, the high electric fields created at nanostructured metals when irradiated under their LSPR condition are able to promote an enhancement of the efficiency of the Raman scattering by molecules adsorbed on their surface (or within a few nanometers from it). Raman spectroscopy can provide a specific vibrational fingerprint of the probed molecules, thus becoming one of the most efficient molecular identification techniques. Therefore, SERS has been identified as a powerful analytical technique that allows ultrasensitive chemical or biochemical analysis.[43−46] However, the efficiency of SERS enhancement by isolated nanoparticles also depends on their specific morphology because this determines the concentration of light at specific locations, either at the nanoparticle's surface or at gaps near it.[47] Again, the most efficient morphologies have been found to be those containing high anisotropy, such as nanorods, or sharp tips and edges, such as nanostars. The outstanding efficiency of nanostars has been demonstrated through an elegant experiment[25] in which aromatic dithiols were uniformly distributed on a flat gold surface, where the spacing was provided by aliphatic thiols (with a low Raman cross section). This configuration (Figure 3) allowed single gold nanostars to bind to the dithiols through one of their tips, and SERS mapping of the substrate showed that a single nanostar was able to provide sufficient enhancement for the detection of a single molecule (the calculated enhancement factor was $10^{10}$). The extraordinary enhancing properties of gold nanostars have been shown to allow a variety of SERS-based applications, such as the fabrication of encoded particles,[48] avoiding the need to create hot spots by the controlled aggregation of nanoparticles. However, to accomplish the promising future perspectives of SERS in biosensing,
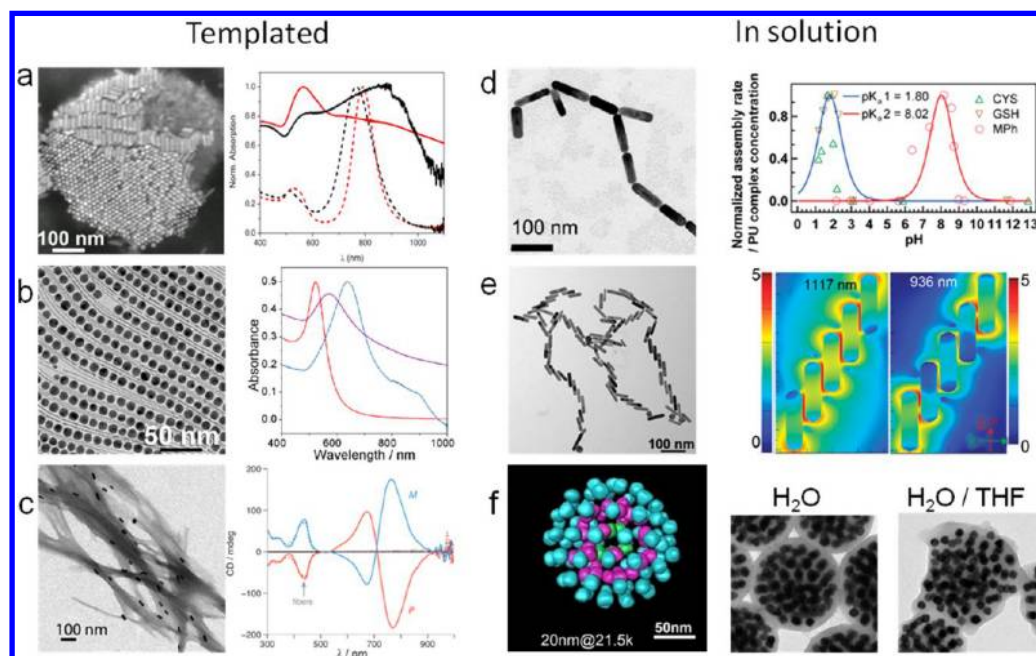
**Figure 4.** Self-assembly of gold nanoparticles on templates (a−c) and in the liquid phase (d−f). (a) Superlattice of standing gemini-stabilized gold nanorods; UV−vis−NIR spectra of gemini (red) and CTAB (black) nanorods in solution (dashed lines) and on glass (solid lines). Adapted with permission from ref 56, copyright 2009 Wiley-VCH. (b) Binary arrays of nanospheres and nanowires; UV−vis−NIR spectra of spheres in solution (1), in a packed assembly (2), and spaced by wires (3). Adapted with permission from ref 58, copyright 2010 Wiley-VCH. (c) Chiral arrays of gold nanorods and circular dichroism (CD) spectra showing plasmonic chirality. Adapted with permission from ref 60, copyright 2011 Wiley-VCH. (d) Hydrogen-bonding-mediated assembly of gold nanorods. The maximum interaction was found at pH values close to the p$K_a$ of the linking molecule. Adapted with permission from ref 62, copyright 2010 American Chemical Society. (e) Ladderlike assembly of nanorods leading to antibonding plasmon modes in solution. Adapted with permission from ref 65, copyright 2012 American Chemical Society. (f) Electron tomography reconstruction of Au nanoparticle clusters with sensitivity to solvent composition. Adapted with permission from ref 67, copyright 2012 American Chemical Society.

including integration into mobile devices for personal use, for example, the design of suitable and highly reproducible plasmonic substrates remains a bottleneck. Although a huge effort is being put into colloidal nanofabrication for the development of plasmonic substrates, either in solution or for in vivo application,[49] the uniformity of the substrates remains an issue.

## SELF-ASSEMBLY OF PLASMONIC NANOPARTICLES

Although the above examples clearly show that isolated particles can serve as (bio)sensors, theoretical studies have shown that multiparticle systems can strongly improve the sensitivity by increasing the absorption cross section of the plasmonic transducer or by creating new properties that cannot be present in the single particles. A clear example of collective effects is the plasmon coupling and subsequent enhancement of the electric near field at the gaps between particles, which can affect the chemical and optical properties of molecules in their proximity.[47] This is the core concept of a new field known as hotspot engineering. However, precise control over the hotspot distribution requires chemical tools that allow the spatial organization of the particles with well-defined interparticle distances and mutual orientations. Therefore, a currently active area of research is related to the self-assembly of plasmonic particles toward producing effective platforms for biosensing (or other applications).[50] Among the large number of recent contributions to this field, we have selected here a few examples related to the self-assembly of plasmonic nanoparticles and the corresponding optical effects.

Historically, colloidal self-assembly originated through studies of phase transitions in hydrosols of silica or polymer (latex) spheres, where the formation of metastable arrays know as colloidal crystals was observed. The so-called coffee-ring effect (i.e., how a spilled drop of coffee dries on a solid substrate) has also long been studied. These apparently independent questions opened up the field of colloidal self-assembly, which provided theoretical models that are still valid for the self-assembly of nanoparticle building blocks, even for nonspherical shapes in which anisotropic interactions are highly relevant. Notwithstanding, we can distinguish two kinds of assembly processes. The first one is the irreversible and spontaneous organization of building blocks, referred to as self-assembly. The second kind would involve energy-requiring processes that lead to long-lived dynamic structures and has been termed directed self-assembly.[51] In the context of nanotechnology, directed self-assembly has become a dominant concept in the fabrication of novel dynamic nanomaterials that organize and function when displaced from thermodynamic equilibrium.[52] By following such a division, we discuss below recently reported examples of both assembly processes.

Because nanoparticle self-assembly leads to the formation of structures with dimensions much larger than those of the building blocks, it often requires the presence of a template that facilitates their organization or even that gets incorporated and becomes a part of the newly formed heterostructure.[51,53] Thus, the template can be a simple piece of glass (bulk substrate) or a colloid itself (e.g., carbon nanotube, polymeric bead, or supramolecular fiber). In a typical self-assembly process on a solid substrate, capillary and convective flows of solvent

molecules drive the nanoparticles into specific areas of the drying droplet. This area-specific increase in the local nanoparticle concentration invokes the gradual formation of superstructures made of nanoparticles. However, interparticle distances or mutual orientations strongly depend on the chemical nature of the capping agents present on the surface of the nanoparticles. Short, rigid stabilizing molecules (e.g., alkyl chains) can interdigitate upon increased concentration of the particles, leading to closely packed monolayers[54] or 3D superstructures.[55] For example, Gemini-type surfactants, which possess two alkyl chains linked to each other through their corresponding ammonium headgroups, have been shown to facilitate the side-to-side assembly of gold nanorods much more efficiently than standard surfactant CTAB, which possesses a single hydrophobic alkyl chain and an ammonium head (Figure 4a).[56] This observation was also confirmed by spectroscopic measurements that revealed that directional optical properties were observed only for supercrystals made of gemini surfactant-capped gold nanorods. Such directional properties are related to the selective excitation of the transverse LSPR mode as a result of the orientation of the nanorods, parallel to the illumination direction. Additional structural and functional diversity in plasmonic nanoparticle arrays can be achieved by using multicomponent colloidal mixtures.[57] A particularly interesting case is that where the components of the mixture display very different sizes and especially aspect ratios. This could lead to a scenario in which one kind of particle induces the spatial distribution of the other. For example, highly anisotropic gold nanowires can drive the oriented assembly of spherical nanoparticles into extended ordered arrays (Figure 4b).[58] The presence (or absence) of nanowires in the nanoparticle colloid affected the distance between spherical particles upon drying, which in turn drastically changed the collective optical response of the monolayer film in such a way that it could be observed by the naked eye.

Interestingly, templates can also induce the achievement of unconventional optical properties that emerge from particular orientations between the nanoparticle building blocks, such as chiral geometries that enable the observation of circular dichroism effects in the visible and NIR spectral ranges.[59] Recent studies resulted in a novel class of nanocomposites derived from the use of a self-assembly strategy in which spherical plasmonic nanoparticles were adsorbed onto chiral polymeric scaffolds (DNA, peptides, or supramolecular fibers). In the same line of thinking, the assembly of gold nanorods on helical fibers yielded record values of optical activity across the visible and NIR (Figure 4c).[60] The combination of nanorods with 3D chiral structures paves the way for a new realm of applications of circular dichroism, including nonlinear optics or negative refraction.

The directed self-assembly of nanoparticles is often carried out in the absence of a template (i.e., directly from the nanoparticle components in the liquid phase). Of course, this is a more challenging task because subtle chemical changes in the colloidal system can invoke strong forces between the particles, which in turn can lead to uncontrolled aggregation. In the examples below, we describe the directed self-assembly of gold nanoparticles by pH-regulated hydrogen bonding, light-induced cross-linking of the capping agent, and solvent-induced nonspecific hydrophobic interactions.

Dynamic aggregation of the nanoparticles into functional structures at different pH requires a careful interplay between attractive and repulsive interactions.[61] The key factor in such an approach is the chemical nature of the molecules adsorbed on the metal surface. They should provide sufficient charge (repulsion) and feasibility for effective hydrogen bonding (attractions) in the desired pH range. Particularly interesting is the self-assembly of 1D nanoparticles, such as gold nanorods, that possess tips with high curvature whereas their sides are basically flat, thereby enabling site-specific functionalization with small molecules. Therefore, the pH-dependent self-assembly of the nanorods leads to preferential tip-to-tip arrangements. However, there has been a discussion of whether rods self-assembling purely via hydrogen bonding or other nonspecific interactions, originating from the presence of CTAB molecules on the lateral faces, are involved in the process. It was recently shown that both experimental results and theoretical calculations support the concept of a hydrogen-bonding-directed assembly mechanism of gold nanorods in the presence of bifunctional linking molecules such as cysteine and glutathione.[62] The assembly rate peaked at pH values close to the $pK_a$ of the linking molecule, and the calculations show that hydrogen bonding preferentially occurs between monomers bearing different numbers of protons (Figure 4d). Thus, the proper experimental design of nanoparticle self-assembly can be successfully explained by commonly accepted theories in molecular science.

Light-induced nanoparticle self-assembly has also attracted growing interest.[63,64] The advantage of using light as the driving force for the self-assembly of nanoparticles relies on non-invasive and specific chemical modifications of either stabilizer or solvent molecules, which alter the equilibrium of colloidal stability. In a recent report, UV light was used to induce the self-assembly of PVP-stabilized gold nanorods dispersed in $N$-methyl-2-pyrrolidone (NMP), yielding an unexpected ladder-like conformation (Figure 4e). The UV irradiation induces the formation of radicals in NMP solvent molecules, which then promote the cross-linking of PVP chains on the surface of adjacent nanorods.[65] In situ monitoring of the optical response upon assembly and detailed theoretical modeling allowed the detection of antibonding plasmon modes directly in solution, which resulted from asymmetric plasmon coupling.

Even though colloidal interactions have been studied both theoretically and experimentally for many decades, certain processes seem to escape the reasoning based on traditional theories. Thus, certain types of molecular interactions remain poorly understood, which are of much relevance in the field of self-assembly. An example is hydrophobic interactions, which are important nonspecific interactions in biological systems and are responsible for the creation of lipid bilayers. They emerge when water molecules rearrange as two hydrophobic species come close to each other.[66] In the field of colloidal self-assembly, hydrophobic interactions between the nanoparticles have often been invoked by solvent composition (induced by a nonsolvent), but their poor understanding, on the experimental and theoretical level, has caused the overuse of the concept of hydrophobic effects. A general methodology has been recently proposed for the solvent-induced self-assembly of hydrophobic gold nanoparticles into 3D clusters, when water was added and thus the dielectric constant of the solvent was varied. A simple theoretical description of the process confirmed that hydrophobic interactions are the main driving force behind nanoparticle aggregation.[67] Because the clusters were held together by noncovalent interactions, their sensitivity to solvent composition could be expressed in terms of cyclic assembly and
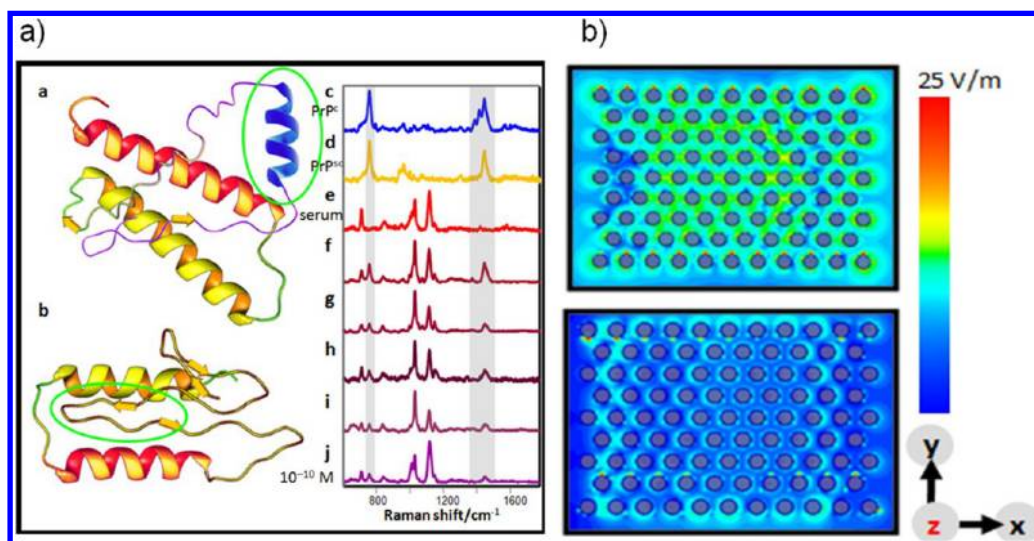
**Figure 5.** Detection of scrambled prions by SERS on gold nanorod supercrystal islands. (a) Scheme showing the prion mutation and detection limits for the scrambled version in bovine serum. (b) Electric field enhancement maps calculated for the top part of a three-layer rod stacked supercrystal. Adapted with permission from ref 70. Copyright 2011, National Academy of Sciences.

disassembly (Figure 4f). Interestingly, the application of the same strategy to the self-assembly of nanoparticles with specifically selected shapes, such as dumbbells, was shown to lead to the formation of unusual mutual orientations, crosses in this case, that may be relevant for plasmon mode engineering.[68]

## NANOPARTICLE SUPERLATTICES AS SENSORS

Even though SERS spectroscopy is extremely sensitive to molecular detection and can work under biological conditions, practical applications require a high reproducibility and uniformity of the substrates. This is particularly important when the enhancement is mainly due to the formation of hotspots because slight variations in their geometry can strongly affect their efficiency. Many examples have been reported of such substrates, including lithographic methods, colloidal self-assembly, or even combinations of both.[69] Currently, the bottom-up approach for the fabrication of highly organized photonic structures from nanoparticles as building blocks is considered to be an appealing solution for providing high electromagnetic field enhancement with reproducible geometries. In this respect, the additional benefits of anisotropic nanoparticle shapes can be combined with their oriented assembly (see above) so that extended nanoantenna effects can show up, providing extremely high enhancement factors and hotspot uniformity. This concept has been recently proven to have the potential to become a significant technological development for resolving biomedical problems, such as the presymphthomatic detection of scrambled prions directly in biological fluids. Therefore, gold nanorod super-crystals were fabricated and used as substrates to develop a SERS-based protocol not only for sensing prions but also for discriminating between functional and scrambled prions (Figure 5).[70] The direct analysis of spiked blood allowed the detection of scrambled prions in the attomolar regime. Importantly, the mechanical robustness of the supercrystal islands allowed multiple uses of the substrates upon plasma etching for removal of the previous analyte. This protocol and the use of supercrystal substrates show great promise for the fast presymphthomatic detection of prions and other bio-molecules in biological fluids such as blood and urine.

## CONCLUSIONS AND OUTLOOK

The main take-home message for the readers of this Feature Article should be that the development of nanoplasmonics-based sensing (or other) devices strongly relies on the fundamental understanding of a large number of processes, from the growth of metal nanocrystals with the desired composition, morphology, size, and surface properties through the interaction forces involved in the organization and assembly of such nanocrystals so that the final properties of both the individual, isolated nanoparticles and their assemblies can be characterized and optimally used for their targeted function. We have shown that directed nanocrystal growth is based on the preferential deposition of metal atoms on certain (more energetic) facets, which can be influenced by the capping molecules used during synthesis to maintain colloidal stability and prevent nanoparticle agglomeration, or the addition of other moieties, such as iodide ions, that can affect surface reactivity. The characterization of such processes is currently possible thanks to the extraordinary improvement of many experimental techniques, in particular, those based on electron microscopy. Modern electron microscopes are able to provide not only morphological and chemical information but also detailed information regarding the energy and localization of surface plasmon resonance modes within the nanoparticles. Many examples of the application of metal nanoparticles for plasmonic detection have been reported, but novel concepts are still regularly presented, which result in dramatic improvements in detection sensitivity. Although isolated particles have been demonstrated to be extremely helpful for such sensing techniques, devices will ultimately rely on well-defined assemblies of such nanocrystals, which can be designed through directed self-assembly techniques and are constantly improved thanks to the modeling of interparticle interactions and their modulation by the ability to tailor surface properties.

With such advances at hand, we can expect even greater achievements in the near future, where issues such as SERS substrate uniformity, test reproducibility, and the selective trapping and detection of analytes with arbitrary composition will be fully addressed and plasmonic sensing devices will become a reality.

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