Certain metal nanoparticles exhibit the effect of localized surface plasmon resonance when interacting with light, based on collective oscillations of their conduction electrons. The interaction of this effect with molecules is of great interest for a variety of research disciplines, both in optics and in the life sciences. This paper attempts to describe and structure this emerging field of molecular plasmonics, situated between the molecular world and plasmonic effects in metal nanostructures, and demonstrates the potential of these developments for a variety of applications.

Keywords: molecular plasmonics; metal nanostructures; gold nanoparticles; localized surface plasmon resonance

1. Introduction

The detection and quantification of minute amounts of (bio)molecules are key requirements in a wide variety of applications in medicine and the life sciences, environmental science, food production and other areas. The development of novel sensor technology that fulfils this purpose even outside of specialized laboratories and/or with significantly decreased costs is the driving force for research in this field. Other typical requirements for such technologies are the parallelization of several assays into one measurement and the reduction of the sample volume—both points demanding a miniaturization of the whole measurement set-up. Besides the further development of established technologies in this direction, also novel approaches may emerge that could fulfil these requirements. A joint feature of many of these developments is high sensitivity towards just a few molecules, which is usually achieved by miniaturization of the sensor’s core part, the transducer itself. In the case of affinity sensors, which are often used in molecular analytics due to their high specificity, the sensitivity is increased by smaller sensors that more easily react to the presence of analyte molecules.

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Typical examples are nanowire sensors, where the electrical conductivity of very thin structures is influenced by the binding of molecules. This influence of analyte molecules on the mobility of electrons is somehow comparable with the effect that is used in the case of localized surface plasmon sensors, especially when working with a few or even single particles.

The phenomenon of localized surface plasmon resonance (LSPR) is based on the interaction of the conduction electrons of metal nanostructures with incoming light [1]. The electromagnetic waves induce an oscillation of these electrons, and resonances can be observed. These resonances depend on parameters such as the kind of metal and its composition (in the case of a mixture), the geometry (size, shape) and the immediate environment. The last is the basis for its potential use as a sensor: for example, by using an affinity layer, certain molecules bind to the surface of such a structure, influencing and shifting the resonance, which can be observed and used as the sensed signal for readout.

Molecular plasmonics represents the field that deals with localized surface (also called particle) plasmon resonance effects in interactions with molecular components (usually bound to the surface of metal nanostructures). The observed effects can be used for novel conjugates for nano-optics (here the molecules act as a tool) as well as for biomolecular analytics aimed at molecular analytes.

This paper gives an overview of the typical approaches to prepare such structures for use as sensors, to functionalize them in order to achieve the required specificity, to use them in bioassays and possibly to integrate them into analytical systems.

2. Synthesis and (bio)conjugation of nanoparticles

Molecular plasmonics requires metal nanostructures. They are accessible in principle by two approaches, either starting from larger structures using lithographic techniques (the ‘top-down’ strategy) or by assembly of rather small units, e.g. atoms and molecules (the ‘bottom-up’ strategy). These two approaches exhibit differences regarding the required equipment, the throughput (parallelization), the crystallinity (and thereby quality) of single structures as well as the ability for integration into a technical environment.

Since the days of Faraday [2], wet chemical synthesis of metal (especially gold) nanoparticles has been used. Therefore, metal (gold) salts are reduced by reducing agents in solution. Lately, citrate is probably the most common reducing agent [3], because these ions act at the same time to stabilize the synthesized particles. In the synthesis, the final size of the particles is usually determined by the ratio of metal salt and reducing agent. The first part of the reaction includes the formation of seeds, which later grow. For a fixed amount of metal salt, the number of seeds determines the final size, because the more seeds that exist, the less metal is accessible for further growth for each particle. These reactions lead to an ensemble of particles of approximately the same size with a quite narrow size distribution. Other bottom-up approaches are less common, such as particle preparation in the gas [4,5] or the solid [6,7] (polymer, glass) phases.

Instead of chemical means, as in the bottom-up approach, the top-down technology is based on physical technologies similar to the ones used in the microelectronics industry, e.g. for integrated circuit production. It is based
on photolithography, where light is used to pattern a light-sensitive layer (photoresist) on a thin layer of metal, for example, and this resist pattern is then transferred into the metal. For nanostructures as required for the LSPR effect, light is usually replaced by an electron beam (e-beam) in order to realize smaller structures in the lower nanometre range. This step extends the accessible structure size. However, it also replaces the rather parallel photolithography (an area is irradiated in one exposure) by the serial e-beam, which in principle writes structures point by point. This slows down the fabrication significantly and makes it rather low throughput and thereby expensive. On the other hand, this technology is compatible with standard microfabrication, so e-beam structures can be positioned into chip layouts at desired locations, which is not easily possible for chemically synthesized particles. The potential of this technology is further enhanced by emerging replication technologies, which use methods like stamping or imprinting in order to replicate nanoscale features created by e-beam technology, thereby leading to a much higher throughput and significantly decreased costs. Other emerging technologies that replace the complicated e-beam technology are nanosphere lithography and scanning probe microscopy (SPM) techniques. Nanosphere lithography [8–10] actually relies mostly on microspheres (polymer beads of several hundred nanometres up to about some micrometres diameter), which are densely packed in a monolayer and used as a sputter mask. Then material (e.g. metal) is deposited on top of the monolayer, and would only deposit as islands on the surface in between the particles. These islands are preserved in a subsequent step of polymer bead removal, and represent the desired nanostructures. In their simplest set-up, they show a triangular shape with sharp corners. Other nanosphere approaches use individual spheres for anisotropic sputtering (usually using a low angle) in order to produce crescent structures, or for lift-off masks in the sputtering of a metal layer, leading to nanometre holes in a metal film. So nanosphere lithography is a rather parallel approach, in contrast to SPM. These techniques are based on a probe that is raster-scanned over the sample surface, and used to probe local properties with an extreme lateral resolution (comparable with that of the electron microscope) but under ambient conditions (or even in liquids). The first development was the scanning tunnelling microscope, probing the tunnelling current between probe and sample surface [11]; the next main success was the scanning force microscope (also called atomic force microscope, AFM) [12]. The main features of this family are not only the high (sub-nanometre!) precision of probe positioning, but also the possible visualization in between manipulation steps in one and the same instrument. Nanomanipulation of the surface can be by voltage pulses (inducing, e.g., local oxidation), mechanically (`scratching’), transfer of organic films (self-assembled monolayer of thiolated molecules) or optically (analogous to photolithography, but with a resolution below 50 nm). Although these techniques are quite flexible and the basic instrumentation is increasingly to be found in nanotechnology laboratories, it is still not an established standard procedure and it is quite laborious to fabricate a plasmonic structure of defined properties by this approach.

In order to arrange particles produced by bottom-up technologies, several methods for an integration step are under consideration. If a regular arrangement is needed, one can densely pack the particles in a monolayer, leading to a hexagonal pattern [13,14]. If a certain distance is needed in between the particles,
then techniques based on block polymers [15], phase interfaces [16] or (sequential) adsorption [17] are possible. For an arrangement in a defined pattern, templated adsorption is possible, where the lateral positions have to be somehow predefined by chemical and/or topographical modifications of the substrate [18,19], and this arrangement can be transferred by stamping [20]. Single structures made of nanoparticles are possible by electrostatic [21] or dielectrophoretic [22] trapping. However, templates based on two-dimensional arrangements of biomolecules such as crystalline bacterial cell surface layers [23] or DNA [24–26] have also been used for the defined positioning of metal nanoparticles.

3. Post-processing and biofunctionalization

Particles can be further modified after fabrication—e.g. by additional metal layers or by light-induced reshaping—prior to biofunctionalization. These steps can be used to further tune the resonance band of the particles and/or for stabilization purposes. Often a silver deposition step is applied, based on a reaction similar to wet chemical particle synthesis: the reduction of metal salt in solution. This reaction occurs preferentially on noble metal surfaces, so that silver deposits specifically onto the gold nanoparticles that are usually used as seeds [27]. As a result, the gold core is sequentially covered by a silver shell, which changes the optical properties of the particles, and thereby the solution changes its colour gradually from reddish to yellow [28]. This step increases the diameter [29] and thereby the optical contrast, allowing for a simple single-particle detection in bright field optical microscopy [30] or by electrical means when a continuous silver layer is formed between prestructured electrodes [31]. Even several shells (such as Au/Ag/Au) have been demonstrated [32]. Other typical modification steps are possible, for example, a silica shell, which stabilizes the whole system by isolating the metal core (with its affinity to certain proteins) from the surrounding solution. Such a passivation makes sense when molecular components (dyes) attached to the metal core, as in the case of Raman markers [33,34], should have minimal interactions with molecules in the solution surrounding the particle. Moreover, it separates the plasmonic functional unit from the bioaffinity (such as antibodies) part. On the other hand, a silica shell opens the way to using the well-established siloxane surface chemistry (known from the DNA microarray field) for biofunctionalization of these particles.

In order to combine the plasmonic nanostructures with the molecular world, techniques for the defined attachment of (bio)molecules to plasmonic structures are required. Probably the most used system is the one of thiol–gold, based on the high affinity of sulphur (such as in a thiol group) to gold (or silver) surfaces. It was well studied about three decades ago for self-assembled monolayers of alkane thiols using SPM [35], and has since been extended using thiolated derivatives of various molecules (such as DNA) for attachment to plasmonic nanostructures. Even older are techniques to attach proteins to gold nanoparticles for electron microscopy, which are usually based on the same sulphur–gold affinity [35]. Besides thiol, amino groups also show a certain affinity to gold surfaces and have been used to attach biomolecules, such as DNA, to gold nanoparticles [36,37]. A silica shell, as already discussed, allows the use of conjugation chemistry, widely (also commercially) established in the field of DNA and protein microarrays, where even ready-prepared substrates can be ordered to realize reproducible results.
4. Life science applications of localized surface plasmon resonance

Plasmonic nanostructures can be used for labelling purposes, as affinity biosensors or for light-controlled thermal manipulation of biological material (figure 1).

The use of plasmonic nanostructures as labels is based on the significant scattering efficiency of metal nanostructures, which allows for a rather simple detection of metal nanoparticles even by optical means [38–40]. By attachment of targeting molecules onto the nanoparticle, it can be directed towards the location of interest, such as on a tissue section or in cells. Additionally, local field enhancement at metal nanostructures allows also for the use as tip-enhanced Raman scattering (TERS) tips [41] or surface-enhanced Raman scattering (SERS) labels by attaching Raman-active dye molecules onto such nanostructures [34]. In contrast to the rather wide bands of fluorescence labels, the narrow Raman bands should allow a significantly higher multiplexing.

For affinity biosensors, specificity of plasmonic nanostructure sensors is usually realized using capture molecules, which specifically bind to the analyte of interest. In the case of proteinaceous analytes, antibodies or molecules with antibody-like properties are used; for DNA detection, DNA (or analogous molecules such as peptide nucleic acid) with a complementary base sequence is used. These capture molecules have to be conjugated to the sensor structure (for plasmonic sensors, usually metal nanostructures). Figure 2 gives an overview of possible schemes for molecular detection based on sensing refractive index change. The signal could be induced by the approach of a second particle in the vicinity (distance in the range of the diameter), so that particle–particle interactions determine the signal, as depicted in the left part of the scheme. The simplest approach (which was also the starting point of the plasmonic nanostructure sensing field) is to detect molecules that bridge biofunctionalized particles leading to aggregation (figure 2a) [42]. Thereby, the resonances typical for separated particles (such as about 520 nm for gold particles) shift dramatically (and even observable to the naked eye) to the blue. This process can be reversed by a respective design of the bioassay, so, for example, one can detect molecules that restrict (cut) certain molecules bridging particles, leading to a dissociation of particle aggregates (figure 2b) [43]. Again, the significant colour change can be followed easily even in small droplets.
Bioassays based on aggregation or dissociation of metal nanoparticles are based on the endpoints of nanoparticle approach: they are either close together or (in a first approximation) rather distant from each other. The intermediate states can be used in order to determine distances in the lower nanometre range even in the far field, by measuring the resonance band of such a nanoparticle pair. This approach is comparable with the application of fluorescence (or Förster) resonance energy transfer, where a fluorescence donor and an acceptor are positioned on two points of a molecule and the distance is determined by the measured energy transfer (the smaller the distance, the larger the transfer). In the case of metal nanoparticles, the approach leads to a shift of the resonance band (as observed already in the case of aggregation); for gold, it would be from the red to the blue region. For particles of known plasmonic properties, the measured position of the resonance band can be used to calculate the distance in between this particle pair (figure 2c). Based on the same effect, but quite different from the applications, are strain sensors (e.g. for cell growth substrates) based on embedded particles, which change their interparticle distance due to the applied strain. Using spectroscopic readout, the distance change can be determined (figure 2d).

In addition, not only does the approach of a second particle change the resonance in an observed particle, but also even small changes in the vicinity as caused by the adsorption of a molecule resulting in a molecular (sub)monolayer (figure 2e) or the subsequent attachment of a second layer specifically bound to the first one (figure 2f). The latter process is the basis for the specificity of biosensors, when the first layer consists of capture molecules (specifically binding a given analyte).

5. Single nanoparticle applications

(a) Biosensor

The process of molecular binding results in changes in the spectroscopic properties of the particles, which can be measured not only by ensemble but also by single-particle measurements. The miniaturization of bioanalytical assays down
Figure 3. Microspectroscopy of single particles—instrumentation and simulations. (a) Set-up with dark field illumination and pinhole that collects light just from a single particle (cf. inset: particle in the centre of the field of view). (b,c) Simulation (using MiePlot v4108) of the spectroscopic effect of protein adsorption (IgG with refractive index of 1.53 and a 15 nm thickness) onto a 80 nm spherical gold nanoparticle resulting in various (0, 20, 40, 60, 80 and 100%) coverages for measurements in (b) air and (c) water. With increasing protein coverage, a shift of the peak can be observed from 508 to 514 nm (air) and from 536 to 540 nm (water). (Online version in colour.)
to single-particle sensors holds great promise for miniaturization and also for ultrasensitivity, with implications for a parallelization as required for many potential applications. So the demonstration of successful measurements of molecular binding events on this level marked an important step in the field of molecular plasmonics [44,45]. How is this single-particle microspectroscopy realized? Typically, a scattering (dark field excitation) set-up with dark field illumination is used, as introduced by Siedentopf and shown in figure 3 [46,47]. The light comes from the side at an angle, and reaches the objective only when scattered. Owing to the very high scattering efficiency of metal nanoparticles, particles of sub-wavelength dimensions can be detected in this microscopic set-up and spectroscopically characterized separately from the surrounding particles using a pinhole that collects light only from a certain region (marked in the field of view). The collected light is then guided by a glass fibre to a spectrometer. Typically, particles are visible in such set-ups down to diameters of approximately 30 and 15 nm for gold and silver, respectively. However, this technique is not limited to noble metal structures—other nanostructures such as individual Si nanowires can also be detected and characterized by it [48].

What are the expected results from measurements using such a single-particle microspectroscopy set-up? Figure 3b,c shows simulations for the effect of protein adsorption of the peak shift of gold nanoparticles (80 nm spheres). The increasing adsorption leads to a shift in the range of a few nanometres; the shift is more pronounced when measured in air compared with water. Because the resolution of such spectroscopic set-ups is usually around or even below 1 nm, these shifts should be detectable in the experiment.

An experiment demonstrating detection of proteins on a single nanoparticle level is presented in figure 4. A single gold nanoparticle was deposited on a glass substrate. In the first step, this nanoparticle was spectroscopically as well as topologically characterized. The size of the nanoparticle was 78 nm as determined by an AFM height measurement. The nanoparticle exhibited a clear plasmon resonance peak at 546 nm. In the next step, antibodies were attached to the nanoparticle, which caused a shift of the plasmon peak to 552 nm. In the last step, the nanoparticle sensor with immobilized antibodies was immersed in a test solution with antigen. This led to the specific binding of the antigen to the nanoparticles and it caused a further shift of the plasmon resonance to 569 nm.

The experimental value of the plasmon shift of around 6 nm caused by adsorption of a layer of antibodies to the nanoparticle (first layer in figure 4) is in good agreement with the calculated value in figure 3b. The calculation also correctly predicts the increase in the intensity of the plasmon peak upon binding of molecules (as also apparent in the dark field image of the particle after every step in figure 3b), which is because of the higher polarizability of the attached molecules. The discrepancy in the position of the plasmon peak is caused by the simplicity of the model, in which the effect of a substrate was not considered.

These single-particle spectroscopy demonstrations show the potential for nanosensors, which can be envisioned as intracellular sensors, comparable with the fluorescence approaches in this field sometimes already incorporating gold cores [49]. Such sensors could detect the presence of certain analytes of interest inside cells or sense certain physicochemical parameters such as pH with a sub-wavelength lateral resolution.
A third field of life science applications of LSPR effects is using the nanoantenna character of particles: under femtosecond laser irradiation, they can collect the incoming energy and convert it into heat and/or destruction. This process can be highly selective, so that the surrounding area is not influenced at all. That light influences the behaviour of metal nanoparticles is well known: laser light can trap particles and fix them on the substrate surface [50], but can (as pulses) also change the size and shape [51], leading to approaches for tailoring the size by light [52]. On the other hand, the temperature increase induced by laser irradiation of particles can be used to induce changes in polymeric structures in the vicinity or to release attached molecules, which could, for example, be used for drug release [53] or to release RNA or DNA in order to control gene activity [54–56]. This manipulation ability can be extended to tissue [57], cells [58] and even subcellular structures (such as chromosomes) [59] or protein aggregates [60]. An important point in all applications is the parallel approach: the laser does not have to be focused down to illuminate only a single particle, but the whole sample is irradiated either by beam widening or by scanning the beam over the substrate. The extreme (nanometre-scale) localization of the damaging effects is realized by the antenna effect—significant energy conversion only occurs at the nanoparticles, and is limited to this size scale. This could be nicely demonstrated by the limited size of the holes (comparable with the size of the nanoantenna particle) that were introduced by laser pulses onto nanoparticles sequence-specifically attached to metaphase chromosomes by in situ hybridization [59].

Our goal is the manipulation of individual DNA molecules using this approach. We would like to combine the potential of DNA-guided specific binding of particles [36,37,61] with the nanoantenna effect. Therefore, we studied the laser light effect

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Figure 5. Manipulation of single DNA molecules using the antenna effect on plasmonic particles. (a) Control sample. Pulsed laser light is able to induce damage limited to the immediate surrounding of metal nanoparticles. (b) Particle on a polymer (PMMA) film before (top) and after (bottom) laser irradiation. The light-induced, particle-mediated damage (hole) is clearly visible. This effect was used to manipulate DNA molecules (on PMMA layer) by positioning silver particles on such molecules. (c) Laser irradiation damaged the particle (arrow), and thereby the DNA (arrow heads). Height scale: (a, b) 40 nm and (c) 20 nm. (Online version in colour.)

on metal nanoparticles, also in combination with DNA. The laser effect could be visualized by immobilization of particles onto polymer-coated substrates [62]. Poly(methyl methacrylate) (PMMA) was chosen as polymer material, owing to its established layer fabrication technique (using spin coating). AFM imaging was used to follow possible structural changes of metal nanoparticles induced by the laser pulses. Therefore, the particles were imaged before laser irradiation (figure 5, upper row), to get the original state. Then a defined (number of pulses, pulse length and amplitude) irradiation was applied, prior to further AFM imaging in order to determine possible topographical changes (figure 5, lower row). In figure 5b, the laser effect on an Ag nanoparticle is studied: compared with the particle before irradiation (top), after laser manipulation the particle disappeared and a hole with approximately the particle dimensions is observed (bottom). Now, this technology was applied to nanoparticles attached to DNA, which is visible as a rod-like structure and marked by arrow heads (figure 5c). The irradiation leads to a highly localized destruction of the particle as well as the material (such as DNA) in the vicinity, demonstrating the realization of molecular manipulation using optical control and the nanoantenna effect on plasmonic metal nanoparticles. Under certain conditions, transport of this discussed laser-based excitation of nanoparticles can be observed along DNA bundles over distances of several micrometres. This effect is documented in nanometre-deep trenches along the original position of the DNA bundles, these bundles themselves having disappeared after irradiation [63].
6. Conclusion and outlook

Plasmonic effects based on localized surface (or particle) plasmon resonance and directed towards molecules and molecular conjugates represent an emerging field between nano-optics and the life sciences, with a great potential for applications especially in diagnostics and therapy. Further progress will be supported by even more interdisciplinary approaches, including fields such as microfluidics for both sample pre-processing and separation, as well as for the core bioassay realization itself, or the combination of these plasmonic functional structures with other optical components like special glass fibres (such as microstructured optical fibres). These fibres represent a promising platform technology for fully integrated next-generation plasmonic devices; and the combination with plasmonic nanoparticles (cf. figure 6) will open novel applications especially in applications for use as sensors.

In the future, we will certainly witness a growing number of life science applications established around a core process combining LSPR effects with molecular components. ‘Molecular plasmonics’ has had its first showing and thereby marked its great potential for future developments. Fuelled by the exploding interest in ultrasensitive bioanalytics and nano-optics, the future looks bright for this young and promising field.

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