Composite polymer colloids for SERS-based applications

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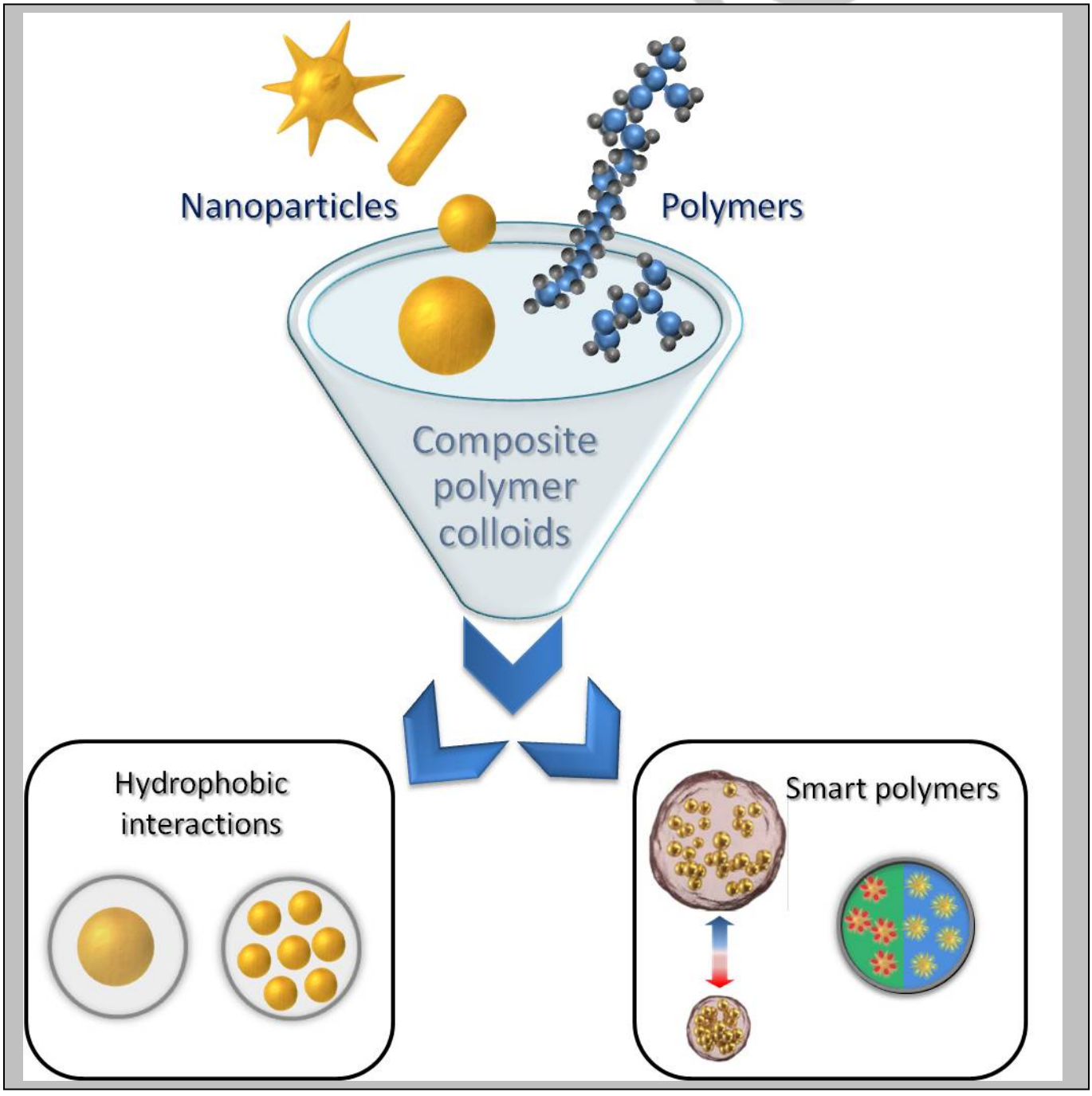
Dedicated to Prof. Eduardo Ruiz-Hitzky



Abstract: Polymers and nanoparticles can be combined into different materials with applications in various fields like catalysis, biotechnology, or drug delivery, to cite just a few. Colloidal composites may vary significantly, ranging from a single nanoparticle stabilized by a polymer shell through a polymeric carrier decorated with hundreds of particles. We review here composite colloids comprising gold nanoparticles, with an emphasis in systems with potential application in surface enhanced Raman scattering (SERS). The focus is on selected strategies for synthesis and functionalization, such as: encapsulation of gold nanoparticles by amphiphilic polymers, polymeric matrices as nanoparticle carriers and smart polymer based composites. We stress the benefits derived from the combination of polymers and metal particles toward SERS, such as chemical and colloidal stabilization in complex environments, and collective optical effects through hot spot generation for optimized SERS enhancement or improved imaging tags.

1. Introduction

Composite materials comprise the combination of various components with distinct compositions and properties, typically with the aim to achieve enhanced or even novel functionalities. In the most common configuration, one of the materials acts as a matrix in which one or more other components are embedded. Even though most polymer-particle composite materials involve a polymeric bulk matrix containing dispersed nanosized particles, a particular class can be defined as “colloidal composites” or “composite colloids”, in which the colloidal matrix containing embedded nanoparticles is dispersed in a continuous dispersion medium (solvent). In such composite colloids, the nanoparticles provide the physical properties of interest, such as optical absorption, fluorescence or scattering, magnetic response or mechanical strength, for example. The matrix on the other hand, may provide not only colloidal stability, but also further functionalization through suitable surface chemistry, controlled porosity, or responsiveness toward external stimuli. Among the wide variety of materials combinations that can be used for the fabrication of composite colloids, we restrict ourselves in this account to those involving plasmonic gold nanoparticles (AuNPs), i.e. “gold based polymer colloids”, which have been proposed for applications in fields like sensing, catalysis, energy conversion or stimulated drug delivery.[1]

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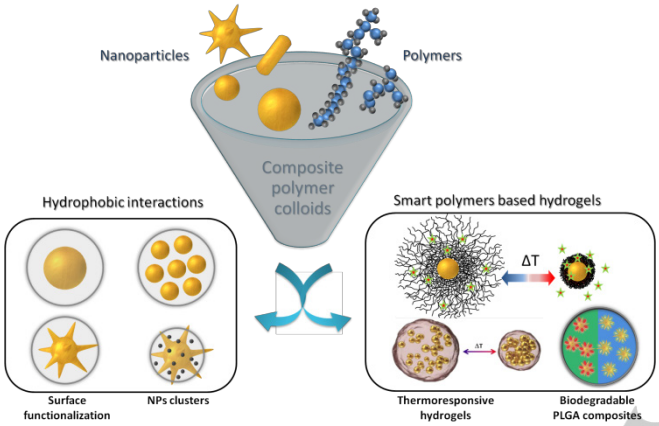
The synthesis of gold nanoparticles has seen huge progress since the well-known report by Turkevich,[2] in which citrate was used as reducing and capping agent. Although this method is still widely employed, scientists have achieved a high degree of control and understanding of the reaction conditions, such that an extensive library of AuNPs is currently available, in a variety of shapes and sizes.[3] In some cases, AuNPs can be directly used after synthesis for the selected application, but often additional elements are required, such as tailored NP assemblies or combination with materials that can impart additional functionalities and achieve unique properties.[4] Among the large choice of materials to be used, polymers offer a wide variety of possibilities for combination with AuNPs in many different configurations, resulting in improved hybrid materials –

including composite colloids – for specific applications. The implementation of functional groups with a strong affinity toward gold, the fine control over the chemical composition and the stability that polymers can provide, render them a versatile tool for implementation of hybrid composites. Scheme 1 illustrates the most common types of gold nanoparticle – polymer composites, based on the encapsulation of NPs due to hydrophobic interactions, using polymeric matrices as AuNP carriers or templates (based on electrostatic interactions) and smart polymer based composites. These composite colloids can be made of a single NP covered with a polymeric shell or of a combination of multiple NPs embedded into a polymeric matrix.



Scheme 1. Gold nanoparticle based composite polymer colloids, with potential application in SERS-based sensing and imaging.

AuNPs contribute unique optical properties to the composite, in the form of localized surface plasmon resonances (LSPR), which are related to collective oscillations of the electron density, as a response to an external electromagnetic field (a beam of light in the visible or near IR). Spherical AuNPs typically display intense colors due to absorption and scattering in the visible, which is accompanied by a significant enhancement of the electric field at the NP surface, with a fast decay within just a few nm away from the surface. Such enhanced electric fields largely affect the polarizability of molecules adsorbed on the nanoparticles, resulting for example in huge enhancements (by many orders of magnitude) of Raman scattering signals, which is known as surface enhanced Raman scattering (SERS). For a detailed description of the theory and nature of LSPRs and SERS, we refer the reader to recent excellent reviews.[5]

A major aspect for successful SERS measurements is the creation of so-called “hot spots”, i.e. regions close to metal nanoparticle surfaces where the electric field is enhanced due to the excitation of surface plasmons at nanoparticles or their assemblies, which are particularly strong at nm-sized gaps between nanoparticles.[6] A simple way to create “hot spots” comprises aggregation of the nanoparticles, by simply increasing the ionic strength in the colloidal solution. Even though this approach is simple and effective, it suffers from a lack of control over the process, and consequently poor reproducibility of the SERS signal. Therefore, the ability to control or even direct the aggregation of particles is of high interest and polymers can play a key role in this endeavor.

Anisotropic nanoparticles displaying sharp edges, corners or tips, also exhibit high electric field enhancement at specific regions, rendering them excellent substrates for SERS, with no need for induced aggregation. A popular example are gold nanostars (AuNSs), which comprise a large number of branches with sharp tips, thereby showing excellent SERS characteristics as individual, non-aggregated particles, overperforming nanospheres or nanorods.[7] These particles however require additional stabilization efforts, as they may slowly reshape into a less energetic spherical shape, which can be achieved by advanced polymer surface functionalization.[8]

In both cases, i.e. single particle SERS measurements and controlled aggregation, the target molecule must be located at the hot spot, i.e. inside an interparticle gap or in close proximity to the nanoparticle surface. This can be challenging in many real systems, since the analyte molecules should display chemical affinity for the Au surface and compete with adsorbed surfactants or polymers, meaning that a suitable compromise between stabilization and SERS signal intensity should be achieved. This can however be a benefit toward the preparation of SERS imaging probes. In this case the nanoparticles can be directly coated with a SERS active molecule, which would act as both surfactant and Raman reporter, leading to high SERS intensity. These nanoparticles however require protection with an external shell to avoid leakage, while enhancing stability and biocompatibility [9]

Alternatively, nanoparticles can be deposited on colloidal particle carriers, such as polymeric or inorganic microbeads. This is typically achieved by exploiting electrostatic interactions or other molecular recognition tools, including DNA hybridization or peptide bonds. These techniques result in composite structures that retain the properties of colloidal systems, and can thus be applied e.g. as drug delivery systems,[10] to create artificial organelles[11] or to improve the intracellular SERS imaging.[12]

Special attention is devoted to smart polymers, which are expected add special features to composite colloids. Smart polymers are sensitive to external stimuli, including temperature, light, pH, ionic strength, etc.[13] We focus here on temperature responsive and biodegradable microgels, which can be used to create unique AuNP-polymer composites, with properties that can hardly be matched by other materials. The three-dimensional microgel network around AuNPs not only provides protection but also offers the ability to entrap analyte molecules close to the AuNP surface, resulting in a generalized sensing material that can be used for molecules with no affinity for metal surfaces.[14]

With few exceptions, most applications presented in this article will be related to SERS imaging. Ultrasensitive sensing applications usually require that the surface is accessible to the analyte, which may require the design of more complex systems, especially when using AuNPs in solution, as naked NP surfaces often lead to uncontrolled aggregation. Therefore, most sensing applications are based on nanoparticles deposited on solid substrates, where aggregation and colloidal stability are not an issue. In these systems, cleaning (e.g. by plasma etching) can

be carried out after synthesis to render the AuNP surface accessible, while avoiding SERS signal contamination from residual impurities. Further reading on SERS substrates as sensing devices is recommended from the existing literature.[15]
In brief, polymers can be used in various ways to improve and provide new properties to plasmonic nanoparticles, through controlled assembly, surface protection, colloidal support, as well as imparting a wide variety of properties and functionalities. We base this account in selected contributions by our group, in the context of other contributions in the field. We focus mainly on AuNP-polymer composites formed by hydrophobic interactions, electrostatic adsorption, as well as composites based on smart polymers.

2. Encapsulation in amphiphilic polymers

The encapsulation of nanoparticles by amphiphilic polymers is based on hydrophobic interactions between a polymeric ligand shell and a suitable block-copolymer. The resulting self-assembled composite colloids are usually stable water because the hydrophilic block of the amphiphilic block-copolymer faces the aqueous solvent. A similar approach can be applied to the encapsulation of nanoparticle clusters, which offers multiple opportunities to tune the optical properties, as well as the incorporation of further functionalities. In both cases, the interfacial properties of the resulting hybrid material are determined by the polymer shell, while AuNPs are confined in the interior. These materials have a great potential for applications, which we highlight here in the context of SERS-based detection and bio-imaging.

2.1. Polymer wrapping of single nanoparticles

The significant progress in the synthesis of AuNPs has provided us with reliable approaches, not only to control particle size and shape, but also surface chemistry, facilitating dispersion in various solvents. Whereas small spheres (< 20 nm) can be directly synthesized in organic solvents, larger spheres and anisotropic AuNPs are commonly obtained in water. Most AuNPs comprise an inorganic core surrounded by an organic layer (ligands or surfactants), which acts as a stabilizer to prevent agglomeration in solvents with suitable polarity.[16] While NPs synthesized in non-polar solvents usually carry hydrophobic molecules, e.g. fatty acids[17] or alkanethiols,[18] ligands for aqueous synthesis contain polar head groups, typical examples being sodium citrate or cetyltrimethylammonium bromide (CTAB).[19] These ligands provide high stability during the synthesis, but further applications may be limited due to issues like CTAB toxicity, poor stability in biofluids and lack of specificity. Polymers can be used to replace weakly bound surfactants or ligands after synthesis, enhancing colloidal stability and/or new functionalities. This is commonly known as ligand exchange and requires a higher affinity by the new ligand/polymer toward the particle surface than the initial one. Polymers containing a thiol group are commonly used to coat AuNPs because of the strong binding of thiols on gold surfaces, so that they can readily replace ligands containing carboxylic or amino functional groups. In cases where the surfactant is difficult to desorb or if a specific molecule should be in close contact to the AuNP surface, a second polymer layer can be adsorbed, exploiting hydrophobic interactions. Often, the amphiphilic polymer poly(isobutylene-alt-maleic anhydride (PMA) is used for this purpose,[20] which comprises a hydrophilic backbone made of maleic anhydride rings, onto which hydrophobic side chains are covalently attached *via* amide bonds. This method works for hydrophobic NPs, which in particular for anisotropic particles requires phase transfer prior to functionalization.[21] Upon ligand exchange, the NPs can be further coated with PMA, so that the hydrophilic backbone of the polymer determines the external surface chemistry of the NPs. Upon transfer back into water, the anhydride rings get hydrated, resulting in a high density of carboxylic groups at the NP surface. This encapsulation method provides the possibility to incorporate different molecular species within the polymer shell, resulting in particles with functionalities related to both the inorganic core (e.g. plasmonic properties from AuNPs) and to the bound molecules.[22]

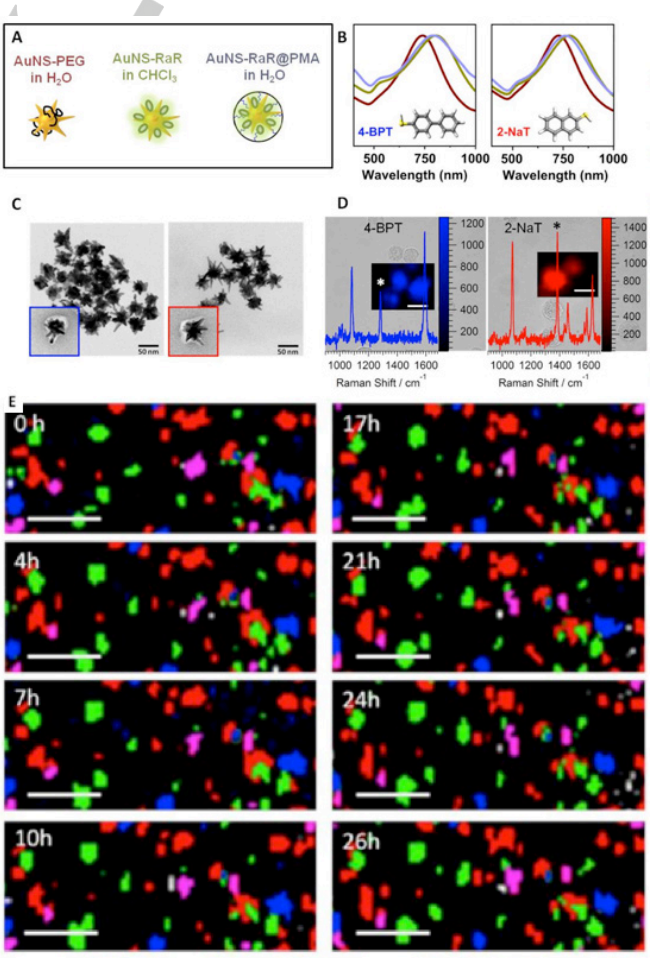


Figure 1. (A) Scheme of the preparation of SERS-encoded AuNSs labeled with RaR molecules and coated with PMA. (B) Vis−NIR spectra of AuNSs (red), after coating (green) with Raman active molecules (4-BPT and 2-NaT), and after PMA encapsulation (blue). (C) TEM images of encoded AuNSs.. (D)

Optical images overlaid with colored SERS maps, showing J774 macrophages labeled with 4-BPT and 2 NaT SERS tags. SERS spectra are also plotted on each image; the signals marked with * indicate specific signals used for SERS mapping; scale bars correspond to 20 µm. (E) Time-lapse SERS images for a constant area of 0.08 mm^2 from a *quintuple* cell culture over a time span of 29 h. The white scale bar corresponds to 100 µm. Lateral scanning step size was 5.5 µm. Reproduced with permission from ref [9b]. Copyright 2016 American Chemical Society

Interestingly, the high colloidal stability provided by the polymer shell prevents NP agglomeration, even in solvents with high ionic strength such as cell culture media.[9b,23] A recent example of SERS-related applications is the demonstration of multiplexed cell discrimination using SERS imaging. Au nanostars were used as metal cores, coated with hydrophobic Raman active molecules (RaRs) and subsequently encapsulated with PMA.[9b] The hydrophobic RaR molecules act as capping agents, when densely packed on the AuNSs surface, while providing intense, stable and reliable SERS signals. The PMA coating allows dispersion of such SERS encoded AuNSs in cell culture media and incubated with cells (see Figure 1). The polymeric coating serves multiple purposes, as it enhances the colloidal stability of AuNSs in cell media, prevents AuNSs from reshaping, rendering them suitable for biological applications, such as SERS bioimaging (Figure 1D,E). Most importantly, leaching of RaR molecules from the AuNS surface is prevented, thus preserving a stable SERS signal over extended periods of time. Depending on the nature of the RaR incorporated in the shell, a different SERS spectral fingerprint will be obtained, so different cell lines can be distinguished from each other, i.e. we obtained multiplex bioimaging, as shown in Figure 1D. Long term stability of the SERS codes was demonstrated by specific imaging of breast cancer cells from five different cell lines, within a quintuple co-culture over time periods over 24 h (Figure 1E).

2.2. Polymer coating of nanoparticle clusters

Even though the methods used for encapsulation of single nanoparticles can be applied to the incorporation of a controlled number of NPs within a polymer bead or vesicle, it is difficult to control the precise number of NPs to be encapsulated. Suitable conditions must be identified, concerning not only NP surface chemistry, but also ligand and solvent composition, etc., before NPs can be trapped into monodisperse polymeric micelles, containing a similar number of particles.[24] Upon controlled aggregation, encapsulation by block copolymers would mimic amphiphilicity of lipids and can thus lead to self-assembly into micelles or vesicles. Inspired by the work by Kumacheva and co-workers,[66–68] our group has developed a general method by which polystyrene-coated AuNPs can be encapsulated by a di-block copolymer containing a (hydrophobic) polystyrene block and a (hydrophilic) polyacrylic acid block (PS-b-PAA).[25] This has been demonstrated for hydrophobic AuNPs in toluene/DMF, which slowly aggregate as solvent polarity is increased by gradual addition of water (20 wt%).[26] PS-b-PAA can be added during the aggregation to suppress further growth and stabilize the AuNP clusters. PS chains in PS-b-PAA are thought to interdigitate with PS brushes at the external AuNP layer, facilitating confinement of the particles in the hydrophobic core and leaving the PAA block as the surface chemical functionality. The amphiphilic character of PS-b-PAA and in particular the hydrophilic character of the PAA block, provide colloidal stability in polar solvents, including water. The same strategy can be applied to a wide variety of nanoparticle systems, as exemplified in Figure 2.[27]

This encapsulation method results in organized clusters when using spherical AuNPs, with a structure of concentric layers (Figure 2-A.2).[28] Encapsulation of anisotropic particles such as dumbbells allows formation of cross-like assemblies, which display circular dichroism related to plasmonic chirality.[29] From the perspective of SERS, this may however not be the ideal system, as polymer encapsulation may hinder molecular access to the AuNP surface, and encapsulated particles are well separated from each other, which hinders hot spot formation. Whereas interparticle distance can be tuned by the molecular weight of the polymer, the interparticle distances are still too long. Alternatively, SERS sensing with this kind of materials can be obtained by using anisotropic AuNPs displaying intrinsic hot spots with large electric field enhancement, such as sharp tips in AuNSs. Encapsulation of AuNSs with long arms can result in colloidal composites where the AuNS tips protrude out from the polymer shell (Figure 2C), so they are accessible to analyte molecules diffusing from solution.[27b] With this feature in mind, AuNSs were co-encapsulated with magnetic iron oxide nanoparticles, so that an external magnetic field induced accumulation of the hybrid clusters at a spot, where the recorded SERS signals were largely enhanced, as compared to those in solution. Such multifunctional hybrid materials offer additional possibilities, e.g. in ultrasensitive sensing and multimodal imaging.

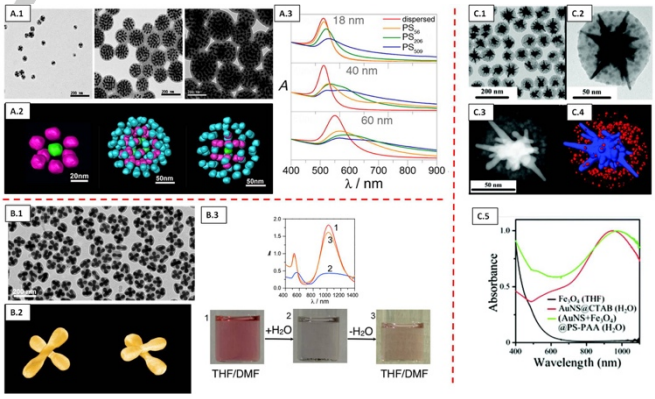


Figure 2. (A) Clusters of different sizes, comprising 20 nm AuNPs capped by PS of different molecular weights (left to right 5.8, 21.5 and 53 kg/mol). Electron tomography reconstructions in A.2 show a concentric layer structure. Interparticle distances increase with PS chain length. A.3 UV–vis–NIR spectra of different clusters formed with 18, 40, and 60 nm gold nanoparticles and different PS length. Reproduced with permission from ref.[26] Copyright 2012, American Chemical Society. (B) Gold nanodumbbells encapsulated in polymeric micelles as cross-like clusters. 3D reconstructions in B.2 correspond to the same dimer, in two different orientations. Reversibility of self-assembled dumbbells is shown in the UV-vis spectra (B.3). Reproduced with permission from ref.[27a] Copyright 2012, American Chemical Society. (C) Hybrid colloidal clusters of Au nanostars and iron oxide nanoparticles. C.3 shows an HAADF-

STEM image of an individual composite; C.4 shows a 3D reconstruction. C.5 UV-Vis spectra of AuNSs in water (red), iron oxide nanoparticles stabilized in THF (black) and composite clusters in water (green). Reproduced with permission from ref.[27b] Copyright 2015, The Royal Society of Chemistry.

An alternative direction toward improving SERS efficiency may be a modification of the conditions for cluster formation, so as to achieve shorter interparticle distances. Examples can be found e.g. in the work by Weller and co-workers,[30] who used partial ligand exchange on the NPs with a multidentate polyolefinic amine ligand, poly(isoprene)-diethylentriamine (PI-N3). In this case, the molecular weight of PI-N3 is significantly smaller (Mw~1300 Da) than that of the smallest PS-SH (Mw~5800 Da) in Figure 2A, likely facilitating encapsulation of AuNPs at shorter distances, and in turn leading to plasmon coupling and hot spot formation. RaR molecules can be introduced at such hot spots, resulting in efficient SERS imaging probes. Micellar encapsulation was achieved in this example by using an amphiphilic polyisoprene-b-polyethyleneglycol (PI-b-PEG) diblock copolymer, in which the PI chains intercalate within the PI-N3 prepolymer. Using different molecular weights of PI-b-PEG, different overall sizes and packing degree of the NPs in the clusters were obtained. Cross-linking of adjacent alkenyl bonds leads to robust and stable polymer shells, yielding water-soluble NPs which virtually retain the original physical properties. The same group developed a microfluidic mixing route to obtain a higher degree of control and reproducibility of the clusters, which has been applied to make hybrid systems, including quantum dots/quantum rods, magnetic nanoparticles and metal nanoparticles, so further development and demonstration of applications are to be expected.[31]

3. Smart polymer composites

The term *smart polymers* has been coined to describe polymeric materials that change their structure as a response to external stimuli or environmental changes. Biodegradable polymers for example feature polymer chains that can be hydrolytically or enzymatically cleaved, resulting in soluble degradation products. Thermoresponsive polymers on the other hand respond to temperature changes. Other smart polymers can be manipulated by pH changes, light, chemical reactions, etc. We focus here on composites comprising gold nanoparticles and thermoresponsive or biodegradable polymers, on the basis of recent examples.

3.1. Thermoresponsive plasmonic microgels

Thermoresponsive polymers are characterized by a phase transition at a specific temperature, so that they can undergo reversible structural changes upon thermal stimuli. When formulated as sub-micron particles, so-called microgels, they can be dispersed in water but the phase transition leads to a sharp change in hydrophilicity upon heating. Most thermoresponsive polymers become more hydrophobic above the so-called lower critical solution temperature (LCST), which depends on polymer composition.[32] In the case of microgels, a sharp decrease in particle size occurs above the LCST, i.e. a transition from a swollen to a collapsed state. The most popular thermosensitive polymer is poly(N-isopropylacrylamide) (pNIPAM), which displays an LCST that is mostly independent of cross-linker concentration and other environmental parameters, rendering it very attractive for various applications.[33] Apart from pNIPAM and its derivatives, other compositions exist, such as those made of poly ethylene glycol methacrylate (PEGMA),[34] with increased biocompatibility and the possibility to finely tune the LCST between 26 °C up to 90 °C.[95] Another common polymer for the synthesis of microgels is vinyl caprolactam, which displays biodegradability.[35]

Thermoresponsive microgels are usually synthesized by radical precipitation polymerization.[36] Above the LCST, the monomers will be dissolved whereas the newly formed polymers precipitate and at suitable crosslinker and surfactant concentrations, uniform and monodisperse microgels are formed. Several procedures have been reported for the growth of crosslinked hydrogels from plasmonic nanoparticles, as well as their applications, ranging from catalysis, to drug delivery and sensing.[37] We restrict ourselves here to the preparation of Au@pNIPAM core-shell systems, and their application for SERS detection and imaging. The preparation of a well-defined core-shell structure containing a single AuNP covered by a uniform pNIPAM shell, comprises a multi-step synthetic method, in which AuNPs are first synthesized with the appropriate size and shape, and subsequently functionalized so that they can be used as seeds for the uniform polymerization of the hydrogel around the AuNP core. Alternative methods in which nucleation and growth of AuNPs are carried out in situ, inside pre-made microgels, rarely result in the formation of a single nanoparticle per microgel, but almost invariably in multiple NPs with a certain size distribution and lack of morphology control. These systems can however still be of interest for certain applications, as discussed in Section 3.1.2 below.

3.1.1. Encapsulation of single AuNPs in microgels

The preparation of single NPs covered with a uniform thermoresponsive polymer shell requires deposition of a primer layer on the surface of NPs, so that polymerization and shell growth can be successfully implemented. As schematically shown in Figure 3, various approaches have been used for this initial step. Typically, CTAB stabilized AuNPs are first covered with a polystyrene layer, followed by pNIPAM polymerization and encapsulation of the core particles.[38] Styrene and divinylbenzene are polymerized into polystyrene within the hydrophobic CTAB bilayer, providing anchor points for polymerization of the pNIPAM shell (Figure 3B).

Alternative, more versatile approaches have been implemented, in which butenoic acid or polyelectrolytes are used to surround AuNPs with the required vinyl anchor groups (Figure 3A,C). TEM images in Figure 3D-G illustrate the successful encapsulation of gold spheres, octahedra, nanorods and nanostars, synthesized under different conditions and using

different stabilizing agents, prior to the polyelectrolyte-based encapsulation method.[37a]

Upon surface modification of AuNPs, the polymerization of NIPAM is carried out following the precipitation polymerization method, at a temperature above the LCST. Detailed studies showed that pNIPAM shell thickness and pore size can be varied through the crosslinker concentration or adding surfactants to the reaction mixture.[38] The thermoresponsive shell around the AuNP core offers interesting properties which have been demonstrated for a variety of applications. First of all, the diffusion of small molecules toward the AuNP surface can be controlled by temperature, so that in the collapsed state (T>LCST) dissolved molecules can be either hindered to reach the metal core or trapped and retained close to it. This is of particular interest for the detection of molecules with low affinity towards gold surfaces, which are usually impossible to detect by SERS, for which close contact between the analyte molecule and the metal surface is required. In the landmark publication on this topic, detection of 1-naphtol was demonstrated as an example.[14] When Au@pNIPAM particles were mixed with 1-naphthol solution at 4 °C, molecular diffusion occurred through the open structure of the swollen microgel, yielding a noisy SERS spectrum, but when the temperature was raised up to 60 °C (above the LCST), the polymer structure collapsed, analyte molecules were trapped and the SERS signal was enhanced. Interestingly, upon cooling back to 4 °C, the signal was lost, indicating diffusion of 1-naphthol out of the composite microgel. This proof of concept experiment demonstrated the possibility of using hybrid colloidal materials as substrates for generalized SERS detection in aqueous solution.

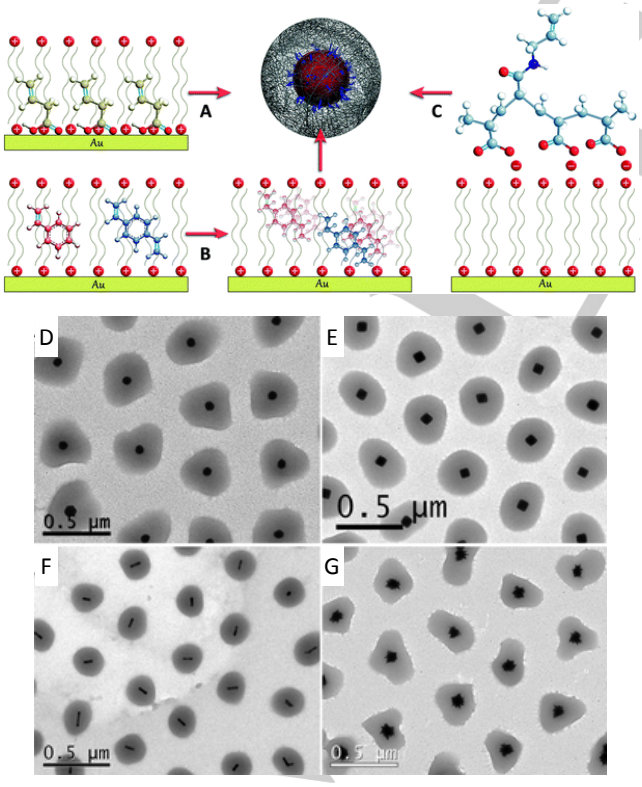


Figure 3. Upper panel: Scheme of the surface functionalization with vinyl groups: butenoic acid (A), polystyrene and vinylbenzene (B) and allylamine (C). Lower panel: TEM images of Au spheres (D), octahedra (E), nanorods (F) and nanostars (G), coated with pNIPAM. Reproduced with permission from ref.[37a] Copyright 2013, Royal Society of Chemistry.

This encapsulation strategy opened up interesting opportunities for additional applications of Au@pNIPAM particles, such as encoded tags for bioimaging and cell discrimination. This application requires loading of Au@pNIPAM microgels with a Raman reporter (RaR) molecule, which must remain inside the shell and close to the metal core for high signal stability. This has been achieved by layer-by-layer assembly of polyelectrolytes, to seal the openings of the gel matrix and prevent RaR leakage. The outmost polyelectrolyte layer can additionally be used to attach biorecognition elements, such as antibodies. In a recent study,[39] this system has been demonstrated using three different RaRs (Astra Blue, Nile Blue and Malachite Green), which show molecular absorption in resonance with the excitation laser wavelength (633 nm), thereby resulting in increased signal intensity and potential for simultaneous detection of their clearly distinguishable spectra. Each of the three encoded microgels was functionalized with a different antibody, as depicted in Figure 4A-C. A tumor cell line (human epithelial carcinoma A431) and a non-tumor cell line (3T3 2.2 fibroblasts) were co-cultured and incubated in the presence of a mixture of all three encoded nanoparticles. Differentiation of the two cell lines was achieved on the basis of their different ability to express the complementary receptors to the selected antibodies, so that the experiment can be used as a proof of concept to demonstrate cell discrimination using SERS. The cancer cells express all three selected membrane receptors and hence the signals for all three RaRs are identified in the corresponding SERS spectra, whereas fibroblasts only express CD44, resulting in the detection of malachite green but not the other RaR codes. Figure 4D-H shows a fluorescence microscopy image, next to SERS mapping of a small group of cells, showing signals from all three RaR codes in an A431 tumor cell, whereas the other three cells in the selected area only show the signal of CD44 functionalized particles, as expected for 3T3 fibroblasts. This assignment is in agreement with the fluorescent staining for EGFR, thereby validating the initial hypothesis for SERS-based cell discrimination.

The diffusion of small molecules can be extended to metal precursors, which has been applied e.g. to coat gold cores with a different metal that provides an additional functionality. For example, upon diffusion of metal salts through the porous polymeric shell, both Au/Ni@pNIPAM (with plasmonic-magnetic properties) and Au/Ag@pNIPAM (with improved SERS detection properties) have been successfully prepared.[40]

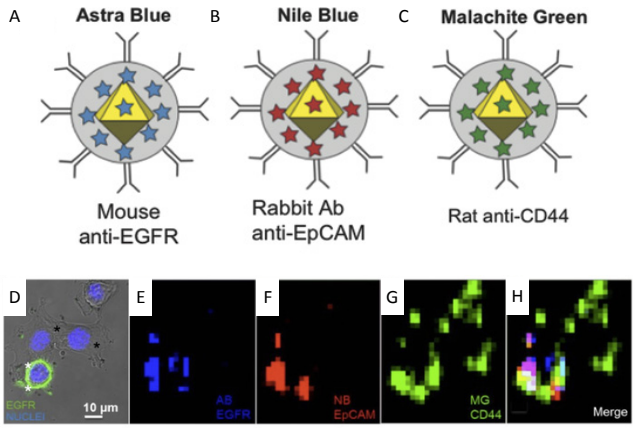


Figure 4. (A-C) Schematic representation of Au@pNIPAM particles containing different RaR and antibody combinations. (D) Bright field image with additional EGFR fluorescence staining shows the presence of one A431 (EGFR positive) and three 3T3 (EGFR negative) cells. (E-F) Correlated SERS mappings using the characteristic peaks of Astra Blue (E), Nile Blue (F) and Malachite Green (G). The merged image of the three mappings in H shows the overlap of all signals in the A431 cell and only the Malachite Green signal in the 3T3 cells. Reproduced with permission from ref. [39] Copyright 2015, Wiley-VCH.

3.1.2. Thermoresponsive hydrogel AuNP carriers

We highlight here recent work regarding the use of thermoresponsive microgels as carriers for multiple AuNPs. An interesting approximation involves the deposition of AuNRs on the surface of pNIPAM microgels,[41] copolymerized with a monomer containing carboxylic groups, to obtain an overall negative charge on the hydrogel. Positively charged AuNRs can then adsorb by electrostatic interactions, as exemplarily shown in Figure 5A.

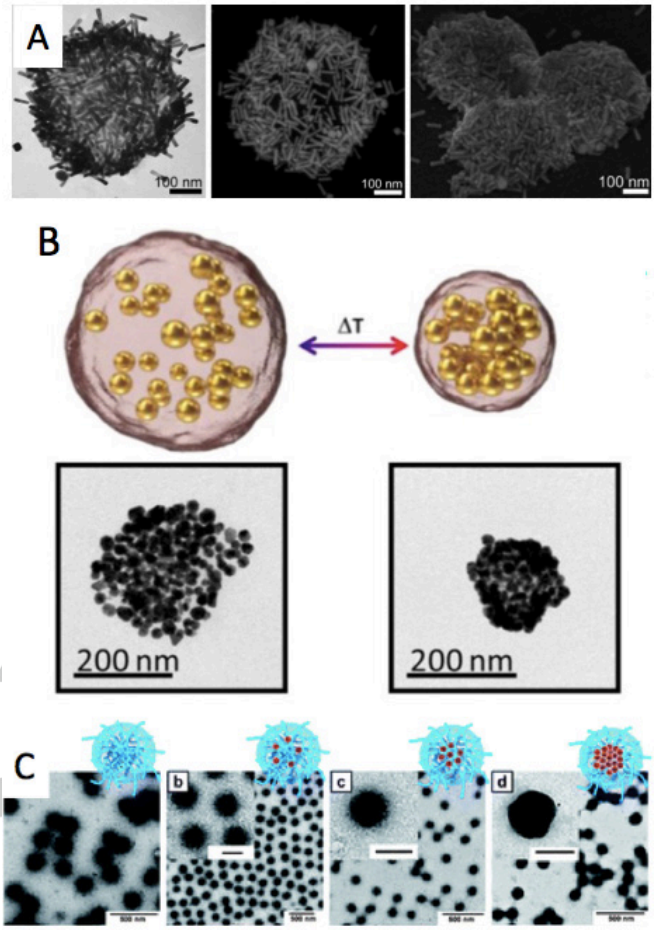


Figure 5. (A) Transmission electron micrographs of AuNRs assembled on pNIPAM microgels. (B) Scheme and TEM images of in situ grown AuNPs in *PEGMA microgels, below and above the LCST. (C) Gold nanoparticles grown* inside microgels of vinyl caprolactam copolymerized with acetoacetoxyethyl methacrylate, which acts as in situ without reducing agent. A reproduced with permission from ref.[41a] Copyright 2009, American Chemical Society. B reproduced with permission from ref.[42] Copyright 2017, American Chemical Society. C reproduced with permission from ref.[43] Copyright 2013, The Royal Society of Chemistry.

The thermoresponsive behavior of these hydrogels can be used to alter the optical properties of the hybrid material. Recently Fernández-López et al. carried out an experimental and theoretical investigation of the SERS performance of pNIPAM microgels decorated with AuNRs.[44] Upon heating, a red shift of the LSPR occurs which is related to the decrease of interparticle distance between the AuNRs. This resulted in the formation of hot spots and thus a further enhancement of the Raman signal. Similar results were also shown for Ag nanoparticles.[45]

Apart from pNIPAM, other systems have been reported, e.g. for in situ grown AuNPs in PEGMA microgels.[42] Small gold seeds were formed by fast $HAuCl_4$ reduction $NaBH_4$, followed by slow overgrowth with formaldehyde at high pH (Figure 5B). Although In situ growth implies poorer control over AuNP size distribution and morphology, surfactants can be avoided as required for biological applications. This system was used to attach

doxorubicin, a chemotherapeutic anticancer drug, which was selectively delivered upon surfactant replacement by glutathione inside cells.

Another interesting and different approach for an in situ growth of AuNPs[43] involved vinyl caprolactam copolymerized with acetoacetoxyethyl methacrylate (AAEM) and acetylic acid. Due to the faster conversion of AAEM, a core-shell microgel was formed, with AAEM mainly located in the core. Gold could then be slowly reduced inside the microgel without an additional reducing agent. Figure 5C shows TEM images of the particles grown for different Au concentrations.

The above described AuNP-polymer composites comprise a three-dimensional network of the thermoresponsive polymer, derived from a free radical polymerization process. These synthesis methods are straightforward but can still accommodate slight modifications regarding particle size and incorporation of functional groups. Milder but more sophisticated polymerization processes such as atom transfer radical polymerization (ATRP) or reversible addition−fragmentation chain transfer (RAFT), more complex structured composites can be synthesized.[46] Reaction conditions are milder and thus more delicate monomers, e.g. biodegradable crosslinkers can be used.[47] In spite of the great potential of this field, little has been published on the combination of complex polymeric structures and synthesis methods to create NP-loaded thermoresponsive microgel composites.

3.2. Biodegradable PLGA-AuNP composites

Poly(lactic-co-glycolic acid) (PLGA) is likely the most widely used polymer for drug delivery, due to its high biocompatibility and biodegradability, being FDA approved.[48] Biomedical applications of PLGA are not restricted to drug delivery but include e.g. cell culture scaffolds. These applications usually require labeling, typically with organic dyes, but also with quantum dots or AuNPs.[49] Various routes have been reported for the synthesis of NP-PLGA structures, but often with insufficient loading density and poor NP distribution in the polymeric matrix.[50] Electrohydrodynamic (EHD) co-jetting has been shown to allow high loading and versatile functionalization by formation of multiple compartments, leading to better diversity for applications.[51]

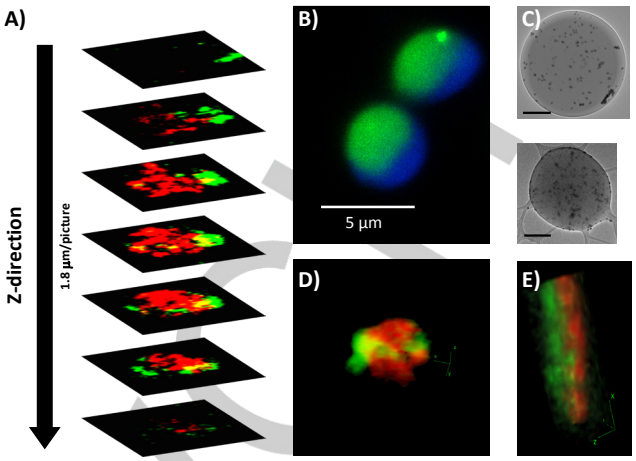


Figure 6. (A) Z-stack images of a bicompartmental microgel obtained by 3D SERS microscopy. Red color corresponds to 4-BPT, green to 2-NAT. (B) Fluorescence images of the microgels showing the two compartments. c) TEM images of two different microgels. Scale bars are 1 µm (D,E) 3D reconstruction of spherical microgels (D) and fibers (E), using confocal SERS microscopy. Reproduced with permission from ref. [52] Copyright 2017, Wiley-VCH.

We recently reported the incorporation of AuNPs in PLGA microstructures, demonstrating high loading with SERS-encoded AuNSs.[52] The polymeric structures retain their functionality and separated compartments, upon EHD co-jetting, e.g. into bicompartmental microstructures simultaneously loaded with fluorescent dyes and SERS encoded nanoparticles. The method can be applied to form spherical particles (Figure 6C), but also rods and fibers. The compartments are clearly distinguishable by both fluorescence and SERS microscopy. The size and structural composition of the particles allowed the application of confocal microscopy for 3D SERS imaging, using the signal from AuNS tags. Shown in Figure 6A are intensity maps of both Raman dyes for different Z-heights and the resulting 3D reconstruction (Figure 6D). Similar results were also obtained for fibers labelled with SERS tags, with excellent resolution of both compartments (Figure 6E).

The main advantage of such biodegradable polymer-AuNP hybrid systems is their potential use for several applications, including efficient imaging probes when long imaging periods are needed (the SERS signal does not suffer from photobleaching), or as selective drug delivery systems, among others.

4. Outlook

We have discussed different designs for the preparation and application of composite polymer colloids, mainly focusing on their optical properties and SERS-based sensing. We showed recent advances related to the encapsulation of AuNPs using amphiphilic polymers (based on hydrophobic interactions), the use of polymer beds as AuNP carriers (by electrostatic interactions) and composites comprising smart polymers, as

examples of the recent progress of the field. All these polymer structures show unique properties that derive in novel optical and structural features, which allow their use for SERS sensing in combination with other applications such as drug delivery, imaging or catalysis.

A particular attention has been paid to the development of bright contrast agents for SERS (bio)imaging, which offer advantages such as long term stability, true multiplexing or response in the near-IR (biological transparency windows), which we propose will lead to breakthroughs in biomedical imaging and understanding of complex biological systems such as tumors or the brain. Combination of this extraordinary sensing ability with the stimuli-dependent response of smart polymers is likely to lead to the development of true theranostic systems, with application in personalized medicine. Our example of PLGA-AuNP composites can also be considered to construct cell culture scaffolds that can be monitored over time, which is promising thanks to the long lifetime of the SERS tags and the improved penetration depth of the excitation wavelength in the NIR.

One major task remains however, regarding the reproducibility of SERS measurements, as well as the difficulty to derive quantitative data. In our opinion this can be overcome by a deeper understanding and an accurate control over synthesis and functionalization, which will keep us motivated toward further improvement and solving these challenges.

Acknowledgements

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Keywords: composite colloids • polymer colloids • SERS • gold nanoparticles • bioimaging

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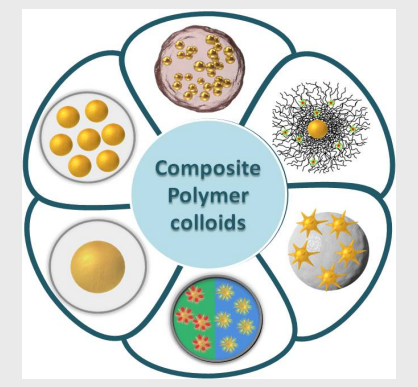
Entry for the Table of Contents (Please choose one layout)

Layout 1:

PERSONAL ACCOUNT

We review recent progress on the synthesis of gold/polymer composite colloids. The focus lies on composites derived from hydrophobic interactions and those containing smart polymers. Synthesis strategies are discussed and the main features and advantages derived from the combination of polymers and gold nanoparticles are discussed, with a special emphasis towards applications based on SERS.



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