Surface-enhanced Raman scattering inside Au@Ag core/shell nanorods

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ABSTRACT

The design and synthesis of plasmonic nanoparticles with Raman-active molecules embedded inside them are of significant interest for sensing and imaging applications. However, direct synthesis of such nanostructures with controllable shape, size, and plasmonic properties remains extremely challenging. Here we report on the preparation of uniform Au@Ag core/shell nanorods with controllable Ag shells of 1 to 25 nm in thickness. 1,4-Aminothiophenol (4-ATP) molecules, used as the Raman reporters, were located between the Au core and the Ag shell. Successful embedding of reporter molecules inside the core/shell nanoparticles was confirmed by the absence of selective oxidation of the amino groups, as measured by Raman spectroscopy. The dependence of Raman intensity on the location of the reporter molecules in the inside and outside of the nanorods was studied. The molecules in the interior showed strong and uniform Raman intensity, at least an order of magnitude higher than that of the molecules on the nanoparticle surface. In contrast to the usual surface-functionalized Raman tags, aggregation and clustering of nanoparticles with embedded molecules decreased the surface-enhanced Raman scattering (SERS) signal. The findings from this study provide the basis for a novel detection technique of low analyte concentration utilizing the high SERS response of molecules inside the core/shell metal nanostructures. As an example, we show robust SERS detection of thiram fungicide as low as 10⁻⁹ M in solutions.

1 Introduction

The potential of surface-enhanced Raman scattering (SERS) as a sensitive detection technique with high molecular specificity has been known for many years [1, 2]. Recently, SERS has been used to design SERS tags [3], demonstrating optical labeling efficiency comparable to that of chromophores such as organic dyes and quantum dots [4, 5]. Usually, an SERS tag consists of a plasmonic nanoparticle with Raman-active
molecules adsorbed on its surface. These tags have become promising candidates for cellular and molecular imaging because of absence of photobleaching and self-quenching effects, as well as because of the advantageous use of a single excitation source for multiple species and multiplexing [6–8]. For practical applications, SERS tags should induce high signal enhancement, generate reproducible and uniform responses, possess stable half-lives, and be easy to fabricate. Individual nanoparticles have moderate field enhancement around their surfaces. For example, with nanostars, Vo-Dinh et al. reported an enhancement factor of about $4 \times 10^4$ [9, 10], which is much lower than that of the best SERS substrates. Aggregation allows higher SERS to be obtained from “hot spots” but it also results in a larger distribution of the particle sizes and a lower reproducibility of the signal [11, 12].

Recently, a novel type of SERS tag, based on multilayered noble metal particles (nanomatroshkas, NM) with a nanometer-sized interior gap between the metal core and the shell, was suggested as an effective Raman label [13–17]. Fabrication of such NMs consists of three steps: synthesis of a plasmonic core, functionalization with dye molecules and spacers, and overgrowth of a secondary metal shell. In this way, the Raman-active components are successfully located in the nanogap junction. With these built-in hot spots, the SERS tags are suitable for single label detection, providing a strong and uniform Raman signal with enhancement factors of up to $10^6$ [14]. Because the Raman molecules are confined in the nanoparticle interior, the NMs avoid typical fluctuations of the SERS response caused by desorption of Raman molecules, effects of the external medium, and non-controllable variations in the number and intensity of electromagnetic hot spots.

Designing a nanometer-scale gap between the metal layers has been achieved with macromolecular spacers such as DNA [14, 15, 18–21], polyelectrolytes [22, 23], and amphiphilic block copolymers [16]. Thiolated Raman-active molecules such as benzenedithiol (BDT) can themselves form a gap inside a core/shell particle [13]. In this case, the gap size is relatively uniform, about 0.62 nm wide as found by transmission electron microscopy (TEM), and somewhat smaller than the expected thickness of a BDT monolayer (0.8 nm) [13].

Sometimes, however, it is not possible to visualize the gap inside a multilayered particle, e.g., a Ag-coated Au nanostructure, even under high-resolution TEM (HRTEM) imaging [10, 22–25]. Kang et al. reported on the invisibility of the nanogap between Au core and Au shell separated by T10 spacers [18].

This effect, together with a strong SERS signal, was attributed to the formation of a very narrow intranogap. Despite the recent progress in the synthesis of multilayered nanoparticles with embedded Raman reporters, all previous studies have focused only on the high SERS response from these nanostructures. Usually, spherical Au particles are used as the core, and the thickness of the metal shell is distributed and uncontrolled, resulting in broad plasmonic peaks, near 550 nm for Ag@Au particles and near 450 nm for Au@Ag particles. On the other hand, the use of Au nanorods as the core can shift the plasmon resonance of the resultant nanoparticles to the near-infrared (NIR) region, which is thought to be more efficient for biological imaging because of overlap with the tissue transparency window. Additionally, existing protocols of nanorod overgrowth enable tuning of a narrow plasmon resonance peak in a wide wavelength range, with an accuracy of up to 1 nm [26, 27].

In this work, monodisperse Au@Ag nanorods (“anisotropic nanomatroshkas”) with controllable shell thickness, and Raman-active molecules embedded between the core and shell were synthesized. We demonstrate that plasmonic resonances and SERS responses can be finely tuned simultaneously by varying the Ag shell thickness. Successful embedding of 1,4-aminobenzenophenol (4-ATP) inside the core/shell particles was directly confirmed by the absence of selective oxidation of the amino groups during incubation with hydrogen peroxide. Furthermore, in contrast to the usual surface-functionalized Raman tags, aggregation and clustering of nanoparticles with embedded molecules decreased the SERS signal. The dye molecules inside the particles showed a strong and uniform Raman intensity, at least an order of magnitude higher than that of the molecules on the nanoparticle surface. We suggest using the high SERS response of molecules inside the plasmonic core/shell particles as a novel sensing platform for detecting low analyte concentrations.
2 Experimental

2.1 Materials

All chemicals were obtained from commercial suppliers and were used without further purification. Cetyltrimethylammonium bromide (CTAB, >98%), cetyltrimethylammonium chloride (CTAC, 25% solution in water; >98%), sodium oleate (NaOL, technical grade; >82% fatty acid), l-ascorbic acid (AA, >99.9%), hydrochloric acid (HCl, 37 wt.% in water), 1,4-aminothiophenol (4-ATP, 97%), hydrogen peroxide (H₂O₂, 37%), and sodium borohydride (NaBH₄, 99%) were purchased from Sigma-Aldrich. Thiram fungicide solution (250 mg/mL) was from Avgust Co. (Russia). Hydrogen tetrachloroaurate trihydrate (HAuCl₄·3H₂O) and silver nitrate (AgNO₃, >99%) were from Alfa Aesar. Ultrapure water obtained from a Milli-Q Integral 5 system was used in all experiments.

2.2 Preparation and study of core/shell nanorods

2.2.1 Au nanorod synthesis

A high-yield nanorods solution was prepared in a binary surfactant mixture as described elsewhere [26, 28]. The reagent concentrations were adjusted to produce nanorods with widths of about 12 nm and lengths of about 72 nm. After the synthesis, the nanorods were centrifuged (8,500 g, 20 min) and double concentrated (final Au concentration, 1 mM).

2.2.2 Synthesis of Au@Ag nanorods with different Ag shell thicknesses and with embedded 4-ATP molecules

First, the nanorods were functionalized with 4-ATP Raman reporter molecules. To this end, 30 μL of 0.2 mM 4-ATP solution in ethanol was added to 30 mL of Au nanorods, and the mixture was incubated for 30 min, and then subjected to triple rounds of centrifugation and re-suspension in 30 mL of 20 mM CTAC. In the second step, the functionalized nanorods were used as seeds, which grew into core/shell nanoparticles. Briefly, 9 mL of 20 mM CTAC and an appropriate volume of 100 mM AgNO₃ were added to 3 mL of 4-ATP-modified nanorods. Subsequently, aqueous AA solution was added at a four-fold molar excess and the mixture was incubated, unstirred, at 70 °C for 3 h. Next, the solution was centrifuged at 8,500g for 15 min, the supernatant liquid was decanted, and the sediment was re-suspended in 3 mL of water. A series of Au@Ag nanocuboids with different Ag shell thicknesses were synthesized by changing the amount of 100 mM aqueous AgNO₃ used, as shown in Table S1 in the Electronic Supplementary Material (ESM). The 4-ATP-embedded Au@Ag nanorod samples were designated as Au@ATP@Ag1 to Au@ATP@Ag7 (Table S1, ESM).

For the synthesis of Au@Ag nanorods without the Raman reporters inside, the same protocol was followed except that the as-prepared Au nanorods were used as seeds. The surface of the core/shell nanoparticles was further functionalized by adding 3 μL of a 2 mM 4-ATP solution to 3 mL of the nanoparticles and incubating the mixture for 1 h. Finally, the nanoparticles were washed by centrifugation and re-suspended in water. The reagent concentrations used are listed in Table S2 (in the ESM). The Au@Ag nanorod samples adsorbed with 4-ATP molecules were designated as Au@Ag@ATP1 to Au@Ag@ATP7 (Table S2, ESM). Note that the final concentration of 4-ATP in these samples is ten-fold higher than that of the core/shell nanorods with embedded 4-ATP. The reasons for such a difference are: (a) Au@Ag nanorods have larger surface areas than Au-only nanorods do, and (b) increasing the Raman reporter concentration afforded SERS signals of the same order of magnitude for both the Au@Ag@ATP and Au@ATP@Ag samples.

2.2.3 Nanoparticle characterization and SERS measurements

Extinction spectra were measured with a Specord 250 spectrophotometer (Analytik, Jena, Germany). TEM images were recorded with a Libra-120 transmission electron microscope (Carl Zeiss, Jena, Germany) at the Simbioz Center for the collective use of research equipment in the field of physical–chemical biology and nanobiotechnology, IBPPM RAS, Saratov. For TEM measurements, 10 μL of as-prepared core/shell nanorods was deposited onto a microscopic grid. HRTEM images and energy-dispersive X-ray spectra (EDS) were recorded with Tecnai G2 200 kV electron microscope (FEI, USA).

SERS spectra of the Au@Ag@ATP and Au@ATP@Ag colloidal solutions were acquired with a Peak Seeker
Pro 785 Raman spectrometer (Ocean Optics) in 1 cm quartz cuvettes under 785 nm irradiation (10 mW). The influence of nanoparticle absorption (the inner-filter effect) was excluded by focusing the laser beam on the near cuvette wall. The acquisition interval was 30 s, and all SERS spectra were averaged over 10 independent runs. Normal Raman spectra of 4-ATP in solution (1 M) were collected by using the same optical device, acquisition interval, and focusing conditions under 785 nm laser irradiation. The background SERS signal of ethanol was subtracted after normalization.

2.2.4 SERS detection of thiram

First, a series of ethanolic solutions containing $10^{-3}$–$10^{-7}$ M thiram were prepared. Au nanorods were functionalized by mixing 30 μL of each fungicide solution with 3 mL of as-prepared nanorods, followed by incubation of the mixture at room temperature for 15 min. The final concentration of thiram ranged from $10^{-5}$ to $10^{-9}$ M. Ag shells were then grown on the surface of the thiram-functionalized nanorods by using the same reagents at concentrations described before for the Ag@ATP@Ag5 sample. Finally, the nanoparticles were centrifuged at 8,500 g for 15 min, the supernatant liquid was decanted, and the sediment was re-suspended in 3 mL of water, giving rise to core/shell nanorods with partially embedded thiram molecules. Surface-functionalized Au@Ag nanorods were used for comparative study. These were prepared by mixing 3 mL of Au@Ag core/shell nanorods, obtained as described above, with 30 μL of $10^{-3}$–$10^{-7}$ M thiram solutions.

2.2.5 Oxidation of the 4-ATP amino groups on the nanoparticle surface

This was performed with hydrogen peroxide as reported previously [29]. A 1 mL portion of concentrated (37%) hydrogen peroxide was added to 3 mL of the Au@Ag@ATP7 and Au@ATP@Ag7 samples. The mixtures were incubated at 50 °C for 1 h. The nanoparticles were centrifuged at 2,600 g for 5 min, the supernatant liquid was decanted, and the sediment was re-suspended in 3 mL of water. Partial oxidation of the amines and their conversion to nitro compounds were monitored by SERS measurements.

3 Results and discussion

3.1 Nanoparticle synthesis and characterization

The initial Au nanorod suspension was prepared in a binary surfactant mixture as described elsewhere [26, 28]. From an analysis of 300 TEM images (Fig. S1(a), ESM), the average nanoparticle width was 12 ± 1 nm and the thickness was 72 ± 7 nm. The axial ratio of the nanorods was 6 ± 0.5. For the as-prepared uncoated nanorods, the longitudinal and transverse resonances were located near 984 and 507 nm, respectively (Fig. S1(b), curve 1, ESM). These resonances are characteristic of the Au nanorods with diameters of 10 to 15 nm, and an aspect ratio of about 6. The ratio of the major and minor resonance maxima was 7.7, indicating that the percentage of by-product particles was small [30]. The full width at half-maximum (FWHM) was about 150 nm, which is typical of Au nanorods of low polydispersity in length and width [31].

Functionalization of the nanorods with 4-ATP and three successive cycles of centrifugation and re-suspension in CTAC resulted only in a small red shift of the plasmon peak and a slight increase in extinction (Fig. S1(b), curve 2, ESM). The high axial-to-baseline ratio of the extinction spectrum for the functionalized nanorods is indicative of the absence of particle aggregation. The high monodispersity of the Au nanorods, which act as seeds, and the absence of nanorod aggregation during functionalization are undoubtedly key prerequisites for the synthesis of monodisperse Au@ATP@Ag nanorods. Monodisperse Au@ATP@Ag nanorods with Raman reporter molecules, confined between the Au and Ag layers, are synthesized by a modified process of Ag overgrowth on the functionalized nanorod cores. This process involves using Ag chloride particles, which appear rapidly when Ag nitrate is added to CTAC. These particles serve as a Ag-ion source to obtain Ag shells [32]. Analogous to the preparation of un-functionalized nanorods [33], the Ag shell thickness in our case could be controlled by adjusting the Au/Ag molar ratios that ranged from 10/3 to 1/10, and together with the use of functionalized nanorods as seeds, we were able to simultaneously tune the plasmonic properties of the nanoparticles and the SERS response.
Figures 1(a)–1(g) show TEM images of Au@ATP@Ag nanoparticles grown from different Au/Ag molar ratios. At relatively high concentrations of AgNO₃, the Au@Ag nanorods were observed as semi-transparent layers, in agreement with previous observations of Au@Ag core/shell nanoparticles [27].

The formation of Ag shell depends on the surface density of 4-ATP molecules adsorbed on Au nanorod core. The threshold concentration of 4-ATP required to form a dense 4-ATP monolayer can be evaluated as follows. From the average geometrical parameters of Au nanorods, we calculated their average surface area (2.94 × 10³ nm²) and volume (8.14 × 10³ nm³). Considering 1 mM as the concentration of Au, and 1.57 × 10⁻¹⁶ g as the mass of a single rod, we obtain the number concentration of Au rods as 1.25 × 10¹²/mL. The topological polar surface area of a 4-ATP molecule is 0.27 and 0.2 nm² according to Refs. [34, 35], respectively. Using an average area of 0.23 nm², we estimate the average number of 4-ATP molecules per rod to be about 1.28 × 10⁴, thus giving the critical threshold concentration of 4-ATP, C̄ₜₐₚ = 2.7 × 10⁻⁵ M. For our standard experimental conditions, the 4-ATP concentration used was about two orders lower, Cₐₚ = 2.0 × 10⁻⁷ M, i.e., due to the sub-threshold concentration regime, the number of adsorbed molecules was not enough to form a dense monolayer of 4-ATP.

In the case of over-the-threshold concentration, we increased the 4-ATP concentration by 100 times to Cₐₚ = 2.0 × 10⁻⁵ M, which is about the threshold concentration. In this case, the dense monolayer of 4-ATP disturbed the formation of regular Ag layer, and gave rise to irregular nanoparticles with ill-defined geometrical and plasmonic properties; see Figs. 2(c) and 2(d), and Figs. S2 and S6 in the ESM. Since, our aim was to establish precise protocols for controlling the geometrical and plasmonic properties of the 4-ATP-modified nanorods, further work was done only using a low, sub-threshold, concentration of 4-ATP.

Note that a distinct gap between Au and Ag layers, which is usually seen in Au plasmonic NMs embedded with thiolated molecules inside [36], is absent from our TEM images. Furthermore, no distinct gaps were observed in HRTEM images shown in Fig. 2.

For the low 4-ATP concentration (Cₐₚ = 2.0 × 10⁻⁷ M), the Ag shell grows symmetrically about the Au nanorod core (Figs. 2(a) and 2(b)), whereas for the high concentration (Cₐₚ = 2.0 × 10⁻⁵ M), the Ag shell grows in a nonsymmetrical manner. For instance, the

![Figure 1](image-url)  
**Figure 1** TEM images of Au@ATP@Ag(1–7) nanorods ((a)–(g)). Scale bar is 100 nm for ((a) and (b)) and 200 nm for (c)–(g). (h) Dependence of the core/shell nanorod length (1) and width (2) on the Au/Ag molar ratio.
The formation of Ag shells on Au cores was independently confirmed by EDS (Fig. 3). EDS spectra were recorded with Tecnai G2 electron microscope by using the same TEM electron-microscopy grids with samples applied. Because the grids undergo fast heating and slight displacements due to the electron beam, we were not able to collect the spectral EDS counts for a sufficiently long time during electron beam scanning. That is why we decided to record the EDS spectra from a selection of points containing only Ag atoms in the shell, or both Ag and Au atoms of the shell and core. First, in the scanning transmission electron microscope (STEM) mode of Tecnai G2 electron microscope, the points were chosen, as indicated in Fig. 3(a). Then, for each of the selected points, the EDS spectrum was recorded. For clarity, only the major peaks of the metals are marked in the EDS spectra. The original EDS spectra contained several minor peaks for both the Ag and Au atoms (Fig. S3, ESM).

The absence of a gap between the Au core and Ag shell, similar to other types of Au@Ag nanoparticles, e.g., functionalized Au nanospheres [22, 23], and nanostars [24] could be due to two reasons. First, we believe that the molecules between Au and Ag layers cannot be resolved by TEM or even HRTEM imaging because of the low contrast of the Ag layer. The other reason could be the low concentration of reporter molecules on the Au nanorod surface. As reported by Gandra et al. [13], when a sub-threshold concentration of benzenedithiol (0.5 μM) was used to modify the cores of the multilayered Au nanoparticles, no interstitial gap was seen between the core and the shell, instead, a uniform 75 nm thick layer of rhombic dodecahedron structure was observed. In this study,
we used 30 μL of a 200 μM 4-ATP solution to functionalize 30 mL of a nanorod colloid with a Au concentration of 1 mM. TEM images in Fig. 1 show the transformation of Au nanorods into cuboid-like Au@Ag nanoparticles with increasing molar ratio of Ag/Au. Preliminary functionalization of the seeds with a sub-threshold concentration of the Raman reporter did not affect the Ag shell growth, and the nanoparticle morphology was in line with that reported previously for un-functionalized nanorods [32]. It should be noted that growth of the Ag shells was anisotropic. As shown in Fig. 1(h), the Ag shells in the transverse direction were thicker than those in the longitudinal direction.

The extinction spectra of the Au@ATP@Ag nanoparticles are shown in Fig. 4(a). In the phase of shell formation, the longitudinal plasmon band of the Au nanorods at around 950 nm shifted to the shorter wavelength region. After a thick shell of Ag had materialized, three bands at around 320, 375, and 450 nm also appeared. The presence of these three strong plasmon peaks in the wavelength range 300–450 nm indicated cubic shell formation. In the case of irregular particles, for all Au/Ag ratios, no such peaks in the short wavelength range had appeared. Recently, we examined the origin of longitudinal (L) and transverse (T1–T3) plasmonic modes in the Au@Ag cuboids [37]. The position of the longitudinal plasmon resonance is determined by the axial ratio of the resultant nanoparticles, whereas the transverse plasmonic mode depends mostly on the shape. The evolution of the peak positions during Ag shell growth is shown in Fig. 4(b). Varying the Ag concentration is an effective way to tune the plasmon peaks in a wide

Figure 3  (a) STEM image of a Au@ATP@Ag5 nanoparticle. The circles 1 and 2 indicate the points for which EDS spectra (b) and (c) were collected, respectively. For clarity, only major Au and Ag peaks are marked. Clearly, the spectrum from the point 1 contains both Au and Ag bands, whereas no Au bands were observed for the spectrum (c) from the point 2.

Figure 4  (a) Extinction spectra of the Au@ATP@Ag(1–7) samples with different Au/Ag molar ratios. (b) Dependence of the major extinction peak position on the added Ag.
wavelength range.

The physical nature of the high-order multipole resonances has attracted significant attention at both the ensemble [33, 37, 38] and single-particle levels [39]. Related discussion and citations can be found in Ref. [37]. In short, a small plasmonic cube can support six fundamental modes as described by Fushs [40]. For the thick Ag layer, the influence of Au core on the electromagnetic response of Au@Ag nanocuboids is suppressed [37], and so the extinction spectra of such particles are similar to that for silver nanocuboids. It has been shown [38, 41] that the Au@Ag nanocuboids can support seven plasmonic modes, four of which can be excited by longitudinal electric field, and the other three transversal modes can be excited at specific perpendicular polarization of the incident electric field. However, all these fundamental modes can be observed only at specific orientation-polarization configurations. For randomly oriented particles, with round edges and corners, some cubic modes become smoothened and cannot be resolved in the extinction spectra of the colloids [37]. In general, from the electromagnetic simulations [37–39, 41], one can attribute the short wavelength plasmonic peaks of the Au@Ag cuboid to a superposition of the longitudinal modes of a silver rod and the multipole transversal modes of a silver cube [37].

For comparison, we performed experiments on Au@Ag core/shell nanorods synthesized using the same set of reagents, except that the Au nanorod seeds were un-functionalized. Their geometrical and optical parameters are summarized in Table S2 in the ESM, and the TEM images and extinction spectra are shown in Fig. S4 (ESM). After the synthesis, the surfaces of these nanoparticles were functionalized with the same 4-ATP Raman reporters. In general, no large differences in size, morphology, or optical properties were observed between the Au@ATP@Ag nanorods with embedded reporter molecules and Au@Ag@ATP nanorods with external surface-adsorbed reporter molecules. Both the types of nanoparticles were further tested for SERS response to calculate the enhancement factors for the molecules inside and outside the core/shell nanorods, as well as the dependence of SERS response on the Ag shell thickness.

3.2 SERS study

For examining the optical enhancement properties of the Raman-active molecules, SERS spectra were acquired from the 4-ATP molecules embedded either inside or adsorbed on the surface of Au@Ag nanorods. All measuring parameters were same for the colloidal samples except for the surface functionalization, which required a ten-fold increase in the concentration of 4-ATP. A normal Raman spectrum of 1 M 4-ATP served as the benchmark data. This spectrum, after normalization and subtraction of the ethanol background, is shown in Fig. S8 (ESM). In close agreement with previous findings [42], the spectra were dominated by ring stretching (1,589 cm$^{-1}$), C–H bending (1,080 and 1,034 cm$^{-1}$), out-of-plane bending (968 and 822 cm$^{-1}$), ring deformation (664 cm$^{-1}$), and C–S stretching (389 cm$^{-1}$) modes.

A typical metric of the SERS response is the fundamental enhancement factor (EF), which is calculated as a ratio of the SERS intensity to the normal Raman intensity normalized by the number of excited molecules [43]. The main challenges are calculation of the number of excited molecules, and measurement of the Raman intensity with the same laser power and accumulation time as used for SERS measurements. Since our experiments employed a relatively low, sub-monolayer concentration of 4-ATP, we assumed that all the molecules were involved in scattering. In this case, the number-concentration of excited molecules is proportional to their molar concentration in solution.

Figure 5 shows the SERS spectra of 4-ATP in the Au@ATP@Ag and Au@Ag@ATP samples. For a minimal Ag shell thickness, the SERS peaks could only be resolved for the samples with embedded 4-ATP. Despite the lower Raman reporter concentration, the SERS peak intensity was higher for the 4-ATP-embedded samples. For EF calculations, we used the most intense SERS peak, at 1,078 cm$^{-1}$, and the most intense normal Raman peak, at 1,080 cm$^{-1}$, for a 1 M 4-ATP solution. All input data and calculated EFs are listed in Table 1.

The calculated EF values for the samples with embedded 4-ATP were at least an order of magnitude higher than that for the samples with surface-adsorbed
4-ATP. For example, the EF of Au@ATP@Ag5 was thirty two times higher than that of Au@Ag@ATP5. This result is in line with previous observations for nanoparticles with spherical [24] and star-like morphologies [25]. Fine control over the plasmonic properties of the anisotropic NMs led us to the important finding that the EF depends on the plasmon resonance. For the surface-functionalized nanoparticles, a higher SERS response was obtained for the Au@ATP@Ag sample, with an extinction plasmon peak at 780 nm. In general, the high SERS response of these colloidal particles was predictable because the laser wavelength overlapped better with the longitudinal plasmon resonance in our SERS experiment. It is well known that EFs are greater for substrates that have plasmon band overlap with the excitation source.

Table 1  Enhancement factors calculated for the Au@ATP@Ag and Au@Ag@ATP samples. The LPR plasmonic peaks are also indicated

<table>
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<tr>
<th>Sample</th>
<th>ATP (M)</th>
<th>$I_{1078}$ (counts)</th>
<th>EF</th>
<th>LPR (nm)</th>
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<td>650</td>
<td>1</td>
<td>N/D</td>
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Figure 5  SERS spectra for Au@ATP@Ag nanorods with embedded 4-ATP (a), and for Au@Ag@ATP nanorods with surface-functionalized 4-ATP (b). Curves 1–7 are shown for different Ag shell thicknesses according to Tables S1 and S2 in the ESM. Panel (c) shows the SERS intensity $I_{1078}$ for samples Au@ATP@Ag(1–7) (magenta columns) and Au@Ag@ATP(1–7) (green columns), respectively.
than for substrates whose plasmon bands do not [44]. However, the situation changed dramatically for 4-ATP molecules embedded inside core/shell nanorods. The highest SERS response was observed for nanoparticles with plasmon resonances in the range of 625–635 nm, quite far from the laser source wavelength. From these data, different mechanisms of electromagnetic field enhancement seem to be operating for the two types of nanoparticle functionalization.

Since the question of positions of the 4-ATP molecules in/on Au@Ag nanorods is a key point of our study, and because of the elusiveness of TEM visualization of the gap inside the nanoparticles, an additional experiment was conducted to confirm 4-ATP was embedded successfully between Au and Ag layers. To this end, the 4-ATP amino groups on the nanoparticle surface were oxidized with hydrogen peroxide [29]. The thickest Ag shell was chosen to provide the best protection of the embedded molecules. The SERS spectra before and after oxidation are shown in Fig. 6. Similar results were obtained for the thin Ag layer samples of Au@Ag@ATP2 and Au@ATP@Ag2 (Fig. S9, ESM).

One important note is in order here. Our oxidation experiments were performed with hydrogen peroxide, reported previously by Bach et al. [29]. Addition of hydrogen peroxide resulted in aggregation of particles, and a change of the plasmonic extinction spectrum to neutral gray. Yet, the nanoparticles did not sediment and we were able to record the Raman spectrum. Because of aggregation, the Raman intensity of the Au@ATP@Ag cuboids decreased owing to the change in excitation conditions, including the number of excited molecules, the internal filter effect, etc. However, all these effects have no importance in relation to our main question: Would oxidation change the SERS spectrum of the ATP molecules that are assumed to be located on the Au@Ag@ATP or inside the Au@ATP@Ag particles? The Raman spectra in Fig. 6 provide univocal evidence for the internal location of ATP molecules in Au@ATP@Ag particles.

For the samples with surface-adsorbed 4-ATP, the total SERS intensity increased and additional peaks appeared (marked with asterisks in Fig. 6). In close agreement with the data reported by Oo et al. [45], the origin of these peaks can be associated with the C–H out-of-plane bending (869 cm⁻¹), NO₂ asymmetric stretching (1,020 cm⁻¹), and C–H in-plane bending (1,341 cm⁻¹) modes in nitrobenzene. Therefore, it can be concluded that the 4-ATP amino groups underwent partial oxidation to nitro compounds. By contrast, incubation of the Au@ATP@Ag7 sample with concentrated hydrogen peroxide decreased the SERS signal without the appearance of additional spectral lines. This fact strongly confirmed that the 4-ATP molecules were embedded inside the nanoparticles, with the Ag layer protecting them from oxidation.

The origin of the Raman scattering enhancement for molecules inside plasmonic nanostructures is a subject of intense discussion. This phenomenon has been well

![Figure 6](image_url)

**Figure 6**  SERS spectra of the Au@Ag@ATP7 (a) and Au@ATP@Ag7 (b) samples before and after oxidation of the amino groups with hydrogen peroxide. The stars indicate the appearance of new peaks after oxidation of the surface molecules on Au@Ag@ATP7 particles. No new intense peaks were observed for 4-ATP inside Au@ATP@Ag7 nanoparticles. For clarity, the spectrum 2 in panel (a) and spectrum 1 in panel (b) are shifted up by 1 kcounts.
explained by classical electromagnetic theory, which predicts strong electric field enhancements in the region of nanogaps formed by reporter molecules [46, 47] (see also Fig. S10 in the ESM). We performed extensive finite-difference time-domain (FDTD) simulations of the near-field and internal field distributions for Au@Ag nanoparticles with and without internal gaps, and for different geometrical particle parameters (data not shown). The main result is as follows: Strong electromagnetic field is not observed inside nanostructures without distinct nanogaps (see, for example, Fig. S10 in the ESM). In such a case, classical electromagnetic theory fails to describe plasmonic behavior, and, perhaps, quantum mechanical effects, related to tunneling and nonlocal screening, which may play an increasingly important role [48, 49].

Very recently, Lim et al. described plasmonic modes in benzenedithiol-embedded NMs by using a quantum-corrected model that accounts for electron tunneling across the gap containing reporter molecules [36]. We believe that such effects may be involved in the strong Raman scattering enhancement inside gap-free core/shell plasmonic nanostructures.

It follows from the above discussion that the precise physico-chemical mechanism related to the observed phenomena is still unclear. For instance, Huang et al. [50] pointed out that at high laser power density, the transformation of 4-ATP to 4,4’-dimercaptoazobenzene (DMAB) can contribute to SERS spectra measured with Ag substrates. However, such a mechanism would work in a similar way for both Au@Ag@ATP and Au@ATP@Ag particles, which is not the case. It should be emphasized that we compared SERS responses from the internal and outer molecules of Au@ATP@Ag and Au@Ag@ATP particles synthesized using the same quantity and concentration of AgNO₃, or, in other words, at the same Ag layer thickness. Accordingly, if the basic mechanism behind the measured SERS signals had been the laser-induced transformation of 4-ATP to DMAB, then the observed SERS responses would have been similar for both the particle types. It follows from Table 1, however, that SERS intensity increased for the six samples of Au@ATP@Ag1–6, but decreased for the five samples of Au@Ag@ATP3–7, and for the sample of Au@ATP@Ag7. These observations seem to remain unexplained by the contributions from DMAB. Another point is that we did not use sharply focused laser beam in our SERS measurements. Specifically, the maximal power density was less than 10⁵ mW/cm². This value is two to three orders lower than the power densities capable of transforming the 4-ATP to DMAB [50].

Another principal difference between the Au@Ag@ATP and Au@ATP@Ag particles manifests from aggregation experiments. It is well known that aggregation or clustering produces strong SERS responses from the electromagnetic “hot spots” in the core–shell gaps [11, 12]. Since all previous studies have been focused only on nanoparticles with surface-adsorbed reporter molecules, it is still unclear how the SERS response changes during aggregation of SERS tags with embedded reporters. To elucidate this point, we measured the SERS spectra of the colloidal and aggregated Au@ATP@Ag5 and Au@Ag@ATP5 samples. Aggregation was induced by addition of 100 μL of 1 M NaCl to 3 mL of nanoparticles, causing the solution color to change from green to gray. SERS spectra were collected from the colloids and all measurement conditions including laser focusing, power, and the integration time were kept same for the non-aggregated and aggregated colloids. All spectra were corrected to the base line. As aggregation is a time-dependent process, we measured SERS spectra 20 min after addition of the salt, when the extinction spectra had reached a steady state shape and the aggregates were not too large to sediment quickly.

Figure 7 shows that the spectra have changed dramatically after the aggregation of Au@ATP@Ag5 and Au@Ag@ATP5: The SERS intensity increased in the case of surface functionalized sample, whereas it decreased in the sample with embedded 4-ATP. Obviously, additional “hot spots” of the aggregated particles give no additional electromagnetic field enhancement inside the Au@ATP@Ag nanoparticles. Thus, the aggregation of Au@ATP@Ag particles does not enhance their SERS response. This gives substantial evidence that the signal was measured from the molecules inside the core/shell nanorods, and the electromagnetic enhancement mechanisms were possibly different from that of the surface-located Raman reporters.

At first sight, the decrease of SERS signal from the
clustered Au@ATP@Ag particles appears somewhat unusual. However, this effect can be explained by several factors. First, clustering of particles results in electromagnetic coupling and a subsequent decrease of the internal excited field. Exact simulations for spherical clusters by the multiparticle Mie solution or cluster T-matrix method confirm this possibility (see Ref. [5] and references therein). On the other hand, aggregation can decrease the number of particles and, correspondingly, the number of excited molecules. In the case of externally adsorbed 4-ATP molecules, however, aggregation of particles results in the formation of electromagnetic hot spots and, accordingly, in the notable increase of the Raman intensity according to the well known E4 law [51, 52]. Such a behavior is typical for Raman measurements before and after aggregation of particles [52].

### 3.3 Improved SERS detection of thiram

In all the previous studies, plasmonic nanoparticles with embedded Raman-active molecules served as tags for SERS sensing and imaging [15, 18]. However, the same nanostructures can also be important for the direct detection of analytes owing to the higher SERS response from the embedded molecules. In such a case, the molecules of interest should be placed between the metal layers. Following this strategy, we propose a novel approach based on the adsorption of an analyte on Au nanorods before a Ag layer is overgrown. This should lead to partial inclusion of the molecules of interest inside the resultant core/shell nanoparticles. We expect that the detection limit of our modified technique should be much lower than that of the traditional ones, which are based on the adsorption of analytes on SERS-active substrates. In our experiments, we used thiram fungicide as a model analyte molecule. Thiram is moderately toxic if ingested but is highly toxic if inhaled [53]. On the other hand, thiram has a disulfide bond, which breaks spontaneously upon exposure to the Au surface, and binds to Au nanorods through the Au–S bond. Several studies have addressed the low-level detection of thiram using Ag nanoshells [54] and Au nanorods [55] as SERS platforms, achieving detection limits of about $10^{-7}$ M. In this work, we compared the SERS detection limits of two sample types, partially embedded thiram vs. surface adsorbed thiram, by using the fungicide at concentrations of $10^{-9}$ to $10^{-5}$ M. The concentrations of the other reagents were same as the ones used for the Au@ATP@Ag5 sample.

Figure 8 shows the representative raw SERS spectra from the samples, for the different final thiram concentrations that were added to the Au nanorods, before (panel (a)) and after (panel (b)) Ag shell growth. Several characteristic peaks are present in the 300–1,900 cm$^{-1}$ shift range. In good agreement with the previously reported data [55, 56], the major peak is at 1,387 cm$^{-1}$, which is attributed to the modes of C–N stretching and symmetric CH$_3$ deformation. In the nanomolar concentration range ($10^{-8}$–$10^{-5}$ M), the SERS peak can be resolved only for the thiram-embedded samples. Therefore, the detection limit for the thiram-
embedded samples can be roughly estimated as $10^{-9}$ M, whereas for the thiram-capped samples is higher by two orders of magnitude ($10^{-7}$ M). This example demonstrates the advantages of including analyte molecules inside the core/shell AuNRs over traditional SERS detection of molecules on the nanoparticle surface.

Figure S7 in the ESM shows HRTEM images of Au@thiram@Ag particles. As in the case of internalized 4-ATP Raman molecules, no evident gap junctions were observed, except for some inhomogeneous structures between Au and Ag layers (Fig. S7(d) in the ESM). Thus, the SERS spectra of the thiram molecules inside the Au@Ag nanocuboids still need to be explained.

4 Conclusions

Uniform Au@Ag core/shell nanorods of controllable Ag shell thickness, and Raman-active molecules entrapped between the Au and Ag surfaces have been synthesized for the first time. The ability to finely control the geometrical and plasmonic properties of the nanorods has allowed us to differentiate the SERS responses of the molecules in the interior, from those on the surface of identical anisotropic nanoparticles. Raman scattering from the embedded molecules has been found to be at least an order of magnitude higher in intensity than their surface-functionalized counterparts. In addition, Raman scattering from the embedded molecules displayed an unusual behavior, viz., (1) maximal SERS response was observed for nanostructures at the plasmon resonance located far from the excitation laser wavelength, and (2) aggregation and clustering decreased the SERS signal. Based on the high SERS response from the embedded molecules, we have demonstrated a dramatic improvement of the detection limit of thiram fungicide molecules in the solution phase.

It follows from the discussion in section 3.2 that the precise physicochemical mechanism underlying the superior SERS enhancement of Au@ATP@Ag particles remains unclear. In particular, our FDTD and multilayer Mie simulations (data not shown) indicate that electromagnetic mechanisms alone cannot explain the observed SERS enhancements. Thus, due to the absence of evidence for gaps from HRTEM images, we believe that future work is needed to elucidate the possible mechanisms behind the observed SERS spectra of the 4-ATP molecules embedded inside anisotropic nanocuboids.

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Electronic Supplementary Material: Supplementary material (the ESM file contains tables with reagent concentrations used for nanoparticle synthesis (Tables S1 and S2), TEM and UV−vis spectra of the initial nanorods and the Au@Ag core/shell nanorods (Fig. S1), TEM
and UV–vis spectra for core/shell nanorods obtained by using high concentrations of 4-ATP (Fig. S2), STEM images and original EDS spectra collected from selected points (Fig. S3), TEM images of the Au@Ag@ATP nanorods and extinction spectra of seven Au@ATP@Ag samples with different Au/Ag molar ratios (Fig. S4), HRTEM images of Au@ATP@Ag5 particles (Fig. S5), Au@ATP@Ag nanorods at a high ATP concentration (20 μM, Fig. S6), and Au@thiram@Ag nanorods (Fig. S7), a normal Raman spectrum of a 1 M 4-ATP solution with a reference 4-ATP spectrum (Fig. S8), SERS spectra of the Au@Ag@ATP2 and Au@ATP@Ag2 samples before and after oxidation of the amino groups with hydrogen peroxide (Fig. S9) and examples of FDTD simulations (Fig. S10) is available in the online version of this article at http://dx.doi.org/10.1007/s12274-016-1117-7.

References


