Optical and biological sensing capabilities of Au$_2$S/AuAgS coated gold nanorods

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A R T I C L E  I N F O

Article history:
Received 23 April 2009
Accepted 26 June 2009
Available online 21 July 2009

Keywords:
Biological sensing
Nanorods
Functionalization
Au$_2$S/AuAgS coated gold nanorods
Core–shell nanostructure
Optical properties

A B S T R A C T

Gold nanorods coated with a multiplex component, namely Au$_2$S/AuAgS coated gold nanorods, are produced without precipitation and aggregation among the nanorods. Both the thickness of the shell and size of the core can be readily controlled by this technique allowing one to tune the plasmon resonance of the nanocomposites over a range of several hundred nanometers. These Au$_2$S/AuAgS coated gold nanorods exhibit interesting optical properties and are suitable for many biological sensing applications. Functionalization of the Au$_2$S/AuAgS coated gold nanorods is achieved by manipulating the affinity between the Au$_2$S/AuAgS and thiol compounds. Biomolecules can be covalently attached via the NH$_2$ bond of the antibodies to the NHS-terminated nanorods. The longitudinal peaks of the Au$_2$S/AuAgS coated gold nanorods are extremely sensitive to the refractive index changes induced by target binding, suggesting that they are excellent sensors for target-specific binding events and have the potential to achieve single-molecule sensitivity in microspectroscopy.

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1. Introduction

In recent years, there has been significant interest in nanocomposite materials consisting of assembled molecules or nanosized inorganic objects in biomaterials research [1–3]. In particular, hybrid structures based on inorganic nanocrystals are becoming increasingly attractive due to the strong dependence of their optoelectronic, magnetic, catalytic, and sensing properties on the size, shape, and composition. Recent advances in colloidal syntheses have revealed the possibility of producing gram-scale amounts of nanoparticles with elaborate shapes to serve as the building blocks of future materials and nanodevices [4–6]. Incorporation of different functional units into the same nanoparticle structure is expected to result in novel or amplified physical–chemical properties paving the way for the development of nanosized entities that are able to perform multiple technological tasks such as those required by biomedical engineering, diagnostics, pharmaceutics, and biosensing [7–11].

Gold nanorods (GNRs) are elongated nanoparticles with distinctive optical properties that depend on their shape. Owing to the structural geometry, these structures are useful in biological sensing, imaging, and therapy. However, the use of GNRs as biosensors for analytes has not yet been widely pursued. This is because the cetyltrimethylammonium bromide (CTAB) layer on the GNR surface cannot be easily displaced by the biomolecules of interest [12,13]. It is thus necessary to develop new surface chemistry that will render them amenable to surface functionalization, bioconjugation, and assembly. There are currently two different heterostructure design methodologies. One strategy involves careful selection of the molecules that can provide the suitable functional groups to preferentially or selectively bind to the desired nanoparticle type, which leads to the spontaneous formation of assemblies with varying degrees of spatial order [14–16]. The second approach relies on epitaxial growth of different materials by delicate control of parameters such as surface reactivity, interfacial energy, and crystal solubility on the nanoscale [17–20]. However, regardless of the technique, synthesis of core–shell structures by conventional physical blending or chemical precipitation followed by surface adsorption usually produces insoluble materials. Hence, precise control of the size, morphology, and dispersion of the metal component are inherently difficult. Furthermore, conventional protocols usually involve many elaborate steps thereby making accurate control even more arduous. Consequently, reduced conversion yields and/or loss of colloidal stability ensue and preparation of metal heterostructures in a controllable fashion are quite challenging. In this paper, we report a novel concept to fabricate core–shell structures consisting of gold nanorods coated...
2. Materials and methods

2.1. Materials

Hydrogen tetrachloroaurate (H[AuCl₄] 3H₂O; ≥99.99%), AgNO₃, NaBH₄, Na₂SO₃, N,N-dimethylacetamide (DMA), and N,N-dimethylformamide (DMF) were purchased from Sigma-Aldrich (Milwaukee, USA). Other reagents included N-hydroxysuccinimide (NHS) from ACROS (New Jersey, USA), 4-(dimethylaminopropyl)carbodiimide (EDC) from Avocado Research Chemicals Ltd (Lancashire, UK), mercaptopentanoic acid (MUA) from Aldrich (Milwaukee, USA). The bovine serum albumin, goat IgG, and rabbit anti-goat IgG were obtained from Biodie Biotechnology Co., Ltd (Beijing, China). Doubly distilled water was used in all the experiments.

2.2. Synthesis of gold nanorods

The gold nanorod seed solution was prepared according to previous methods with slight modification [21,22]. The CTAB solution (1.5 mL, 0.1 M) was mixed with 100 µL of 0.02 M HAuCl₄ and 100 µL of ice-cold 0.01 M NaBH₄ was added while being stirred resulting in the formation of a brownish yellow solution. Rigorous stirring of the seed solution continued for 2 min. The seed solution was kept at room temperature (25 °C) and used at least 2 h after its preparation.

To grow the gold nanorods, 1.5 mL of 0.02 M HAuCl₄ and 0.8 mL of 0.01 M AgNO₃ were added into 30 mL of 0.1 M CTAB, followed by the addition of 0.8 mL 0.08 M ascorbic acid (slightly in excess). Ascorbic acid worked as a mild reducing agent and changed the growth solution from dark yellow to colorless. Finally, 70 µL of the seed solution was added to the solution. The color of the solution gradually changed within 15 min.

2.3. Fabrication of Au₂S/AuAgS coated gold nanorods

The gold nanorods were synthesized by the seedling growth method in an aqueous CTAB solution. 50 µL of a 1 M Na₂S solution was added into 3 mL of the as-synthesized gold nanorods at room temperature, heated, and kept at 50 °C. Absorption spectroscopy was conducted on the resulting nanorods on the Lambda 35 (Perkin Elmer). Because of the longitudinal plasmon wavelength (LPW) of the resulting nanorods red shifted with increasing reaction time, various Au₂S/AuAgS coated gold nanorods were acquired at different reaction time intervals during which the mixture of gold nanorods and Na₂S solution was taken out, cooled to room temperature rapidly in ice water, centrifuged, and re-dispersed in an aqueous solution.

2.4. Functionalization of Au₂S/AuAgS coated gold nanorods and immunochemistry

The MUA monolayers were formed on the Au₂S/AuAgS coated gold nanorods in the presence of 0.1 M ethanol solution for at least 18 h, followed by thorough rinsing with ethanol and water. The modified nanorods were diluted to NHS by immersing in an aqueous solution of 75 µM EDC and 15 mM NHS for 30 min. In order to attach biological macromolecules on the surface, the NHS-terminated nanorods were incubated in a goat IgG solution (0.01 g/L) and BSA solution (0.01 g/L). After reacting for about 30 min, the nanorods were rinsed with PBS and then blocked by 1 M ethanolamine (pH = 8.5) for 10 min.

2.5. Electron microscopy

Transmission electron microscopy (TEM) was carried out on a JEM-2010 transmission electron microscope at 80 kV. X-ray photoelectron spectroscopy (XPS) was conducted using the ESCALab201i-VL spectrometer (VG Scientific Ltd., East Grinstead, Sussex, UK). The base pressure during acquisition was about 3 × 10⁻⁷ Pa and the C₁s line (284.6 eV) was used for energy calibration. The X-ray diffraction (XRD) data were obtained on a Siemens D5000 diffractometer equipped with the Cu Kα₁ (λ = 1.54056 Å) X-ray source and analyzed using Traces Software. Energy-dispersive X-ray spectroscopy was carried out at 25 kV using a JEOL JSM-6380LV field-emission scanning electron microscope (FEI Co.). The extinction spectra were obtained using the Lambda 35 (Perkin Elmer) and the resonance light scattering spectra were acquired on the F-4500 fluorescence spectrometer (Hitachi, Japan).

3. Results and discussion

3.1. Fabrication and characterization of Au₂S/AuAgS coated gold nanorods

Several parameters such as the amount of seed can be altered in this method to adjust the geometry of the particles and consequently the optical response. In order to fabricate the formation of the core-shell nanocomposite, an excess of 1 M freshly prepared Na₂S aqueous solution was mixed with the GNRs. The mixture was stirred in a vessel exposed to air towards the end of the reaction and the color of the solution changed gradually from wine red to pale green. Fig. 1 shows the temporal evolution of the GNRs reacting with Na₂S at a constant temperature of 60 °C. The GNRs initially exhibit one band around 520 nm attributable to the transverse plasmon wavelength (TPW) and another band at a longer wavelength corresponding to the longitudinal plasmon wavelength (LPW). A chalcogenide layer is probably formed on the GNR surface in the reaction. The resulting nanoparticles are characterized by transmission electron microscopy (TEM). Fig. 2 shows the micrographs of the as-synthesized GNRs and resulting nanocomposites. The two different phases can be ascribed to the chalcogenide layer covering the GNR and reduced GNR core. The metal chalcogenide layer is a narrow band gap semiconductor with a large refractive index at typical optical frequencies. The LPW should produce red shifts if the larger refractive index chalcogenide layer covers the GNRs. As expected, a continuous red shift in the LPW accompanied by reduced intensity is observed with longer reaction time as shown in Fig. 1. The final LPW of the nanorods depends on the reactant concentration, temperature, and reaction time.

This protocol can be readily scaled up to prepare gram-scale chalcogenide coated gold nanorods. In order to confirm the components and structure of the nanocomposite, X-ray photoelectron spectroscopy (XPS) is performed and the results are shown in Fig. 3. In addition to Au and S, elemental Ag is found in the nanorods. The small quantity of Ag arises from the addition of AgNO₃ which allows better control of the shape of the synthesized GNRs, as shown in Fig. 4. Fig. 5 compares the XRD spectra obtained from the as-synthesized GNRs, and nanocomposite. The Au₂S and AuAgS can be identified according to previous reports [23–28]. The unlabelled peaks may originate from the CTAB remaining on the nanocomposite, and the shell enshrouding the GNR consists of multiplex components including Au₂S and AuAgS. According to our experimental data, the shell appears to consist of primarily Au₂S together with a small fraction of AuAgS. Here, this nanocomposite structure is referred to as Au₂S/AuAgS coated gold nanorods. Formation of this core–shell structure is a result of the as-synthesized GNRs being corroded gradually by Na₂S from the outside. This is different from the conventional formation mechanism of a core–shell structure by gradually coating the shell onto the unaltered starting nanorods. These GNRs are capped by CTAB micelles and
few CTAB molecules on the ends in the CTAB solution, and the configuration facilitates selective modification of the GNRs [29,30]. During the formation of the Au$_2$S/AuAgS coated gold nanorods, there is no evidence of selective corrosion in the CTAB solution, and the shell is formed along the transverse and longitudinal directions at almost the same rate. Therefore, the nanorods do not evolve proportionally. Resonance light scattering (RLS) which can be used to monitor the changes of size and shape is used to characterize the nanocomposite and the results are depicted in Fig. 6. The GNRs display two RLS peaks centered at 450 and 570 nm attributable to the transverse and longitudinal surface plasmon resonance, respectively. The relative intensity of the scattering peaks from the nanorods with various LPWs discloses one important observation. The longer the LPW of the nanocomposite, the higher is the intensity of their first scattering band and the lower is the intensity of the second scattering band. Eventually, the second band disappears leaving only one observable band.

During the formation of the core–shell structure, the nanocomposite and thickness of the shell are controllable. Fig. 2 also shows the evolution of the starting GNRs and resulting nanocomposites. The starting GNRs exhibit a narrow size distribution as illustrated in Fig. 2a. In the first stage, the sulfide ions in the solution react with Au and Ag on the surface of the GNRs in the CTAB medium and the formed nano-shell covers the remaining GNR as shown in Fig. 2b. The thickness of the chalcogenide layer is controlled by the reaction time. The longer the reaction time, the thicker is the chalcogenide layer. Therefore, a further red shift in the LPW occurs until the excess Na$_2$S is removed from the solution. Fig. 2c and d illustrates that the Au$_2$S/AuAgS coated gold nanorods formed from the Au$_2$S/AuAgS coated gold nanorods shown in Fig. 2b further react with Na$_2$S. It indicates further formation of the chalcogenide layer inside the original shell along with reduction of the starting GNRs. It should be noted that this is different from that accomplished by conventional methods in which the formed layer

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**Fig. 2.** TEM images obtained from the starting GNRs and the resulting gold nanorods coated with multiplex components: (a) Starting GNRs with LPW of 796 nm, (b,c,d) corresponding nanocomposite with LPW of 847, 989 and 1090 nm and the high resolution TEM images (inset figures).
covers the shell. This means that the structure of the formed chalcogenide shell is looser allowing sulfide ions to penetrate through this layer. Thus, the reagent reacts with Au and Ag successively on the surface of the GNRs inside the layer and chalcogenide is generated subsequently. Formation of the chalcogenide layer by Na$_2$S occurs via reactions (1) and (2) in the CTAB medium as shown below. It is important for the solution to be in contact with air [31], as exemplified by the following reactions:

\[
4\text{Au} + 2\text{S}^{2-} + \text{O}_2 + 2\text{H}_2\text{O} = 2\text{Au}_2\text{S} + 4\text{OH}^- \quad (1)
\]

\[
2\text{Au} + 2\text{Ag} + 2\text{S}^{2-} + \text{O}_2 + 2\text{H}_2\text{O} = 2\text{AuAgS} + 4\text{OH}^- \quad (2)
\]

As aforementioned, the degree of red shift observed from the resulting nanorods depends on the reactant concentration, temperature, and reaction time. Although previous work reported that smaller concentration of Na$_2$S might stabilize GNRs in room temperature [32], in our experiments, under certain reactant concentrations, the red shift in the LPW is observed to be significantly enhanced by raising the temperature in the first stage and

![XPS spectrum acquired from the gold nanorods coated with the multiplex components.](image1)

![Energy dispersive X-ray (EDS) spectrum of the as-synthesized GNRs.](image2)
there is no unwanted interference in the formation of Au$_2$S/AuAgS experimental procedures. Our experimental results elucidate that nanorods. Fig. 6. Resonance light scattering spectra of GNRs reacting with Na$_2$S gradually, (a) 80/C$_{14}$ can be observed even though the reaction proceeds for 5 min at the rate of the red shift is linear with temperature. Upon further heating, the rate diminishes significantly and no distinct red shift can be observed even though the reaction proceeds for 5 min at 80 °C. However, a larger red shift will occur when this mixture is left for one day under room temperature because the sulfide ions can slowly penetrate the thicker chalcogenide layer to react with the remaining GNR inside the shell. In this work, a slightly excessive amount of ascorbic acid was added during the synthesis of gold nanorods. In order to eliminate possible interferences, pure gold amount of ascorbic acid was added during the synthesis of gold nanorods. In order to eliminate possible interferences, pure gold nanorods resulting from resuspending the centrifuged as-synthesized gold nanorods into 0.1 M CTAB were subjected to similar experimental procedures. Our experimental results elucidate that there is no unwanted interference in the formation of Au$_2$S/AuAgS coated gold nanorods. The final LPW of the formed nanocomposite depends on the aspect ratio of the remaining GNR and thickness of the shell. On the one hand, a red shift in the LPW occurs and the absorption intensity is attenuated as the chalcogenide layer is formed. This can be explained by the larger refractive indices of Au$_2$S and AuAgS thereby screening the incident electromagnetic field and changing the refractive index in the immediate vicinity of the GNRs. The EDS spectra of the Au$_2$S/AuAgS coated gold nanorods at various stages shown in Fig. 7 reveal that the amounts of the elemental S increase with thicker Au$_2$S/AuAgS layers, further demonstrating that the increasing shell results form the remaining GNRs reacting successively with sulfide ions penetrating through the formed chalcogenide layer. On the other hand, the generated chalcogenide shell in return reduces the GNR in all directions. The same corrosion rate in the transverse and longitudinal directions leads to an increase in the aspect ratio of the GNRs inducing the red shift from the starting nanorods. As illustrated in Fig. 2, an increase in the average aspect ratio of these remaining GNRs in the nanocomposites leading to red shifts in the LPW is observable at 3.8, 3.9, 5.1, and 5.5. Therefore, the degree of the red shift in the LPW of the Au$_2$S/AuAgS coated gold nanorods can be finely tuned over a larger range and indeed, our experiments show that LPW red shifts up to as much as 400 nm can be accomplished. In addition, the TPW red shift with increasing LPW is observed (Fig. 1), although the degree of the red shift in the TPW is less than that in the LPW. This method therefore renders a way by which the TPW can also be fine tuned thus boding well for potential optoelectronic and sensing applications. As discussed above, Au$_2$S/AuAgS coated gold nanorods are produced from the as-synthesized with the addition of Na$_2$S in the CTAB solution, and red shifts are observed in the LPW. However, no red shift in the LPW can be observed if CTAB is removed. This simple reaction described here is exciting because of the following reasons. Firstly, this method introduces a novel concept to fabricate complex core–shell nanostructures. Secondly, this method provides high yield because the formation of core–shell structures from the starting gold nanoparticles proceed completely without any precipitation and aggregation among the nanoparticles. Thirdly, the process is very simple compared to conventional methods such as creation of a core with subsequent growth of a shell. It is particularly advantageous if the shell consists of multiplex components because other methods can be difficult and time consuming. Last but not least, both the thickness of the shell and size of the core can be readily controlled.

3.2. Optic properties of Au$_2$S/AuAgS coated gold nanorods

The properties associated with localized surface plasmon resonance from the nanocomposite are evaluated from the perspective of biological sensing. Firstly, the effects of different solvents on the GNRs and Au$_2$S/AuAgS coated gold nanorods are investigated. Three types of GNRs are studied: (1) LPW of 872 nm, (2) LPW of 850 nm and (3) LPW of Au$_2$S/AuAgS coated gold nanorods of 860 nm. Ethanol is added to the three types of nanorod suspensions to a volume ratio of water to ethanol of 1:1. A red shift in the LPWs of the nanorods is observed, namely 45 nm plasmon wavelength shift observed from the Au$_2$S/AuAgS coated gold nanorods. Similarly, 21 nm and 35 nm plasmon wavelength shifts corresponding to gold nanorods with LPWs of 872 nm and 850 nm are observed. The changes in the LPW indicate that the Au$_2$S/AuAgS coated gold nanorods are more sensitive to the changes in the environment and surrounding medium. Fig. 8 depicts the extinction spectra of the three types of nanorods dispersed in water and water/ethanol, respectively. The Au$_2$S/AuAgS coated gold nanorods are investigated from the perspective of biosensors. Fig. 9 depicts the extinction spectra after the gradual addition of glucose to the GNRs and Au$_2$S/AuAgS coated gold nanorods. The two types of nanorods exhibit similar behavior.
Fig. 7. EDS spectra of gold nanorods coated with different thicknesses of Au$_2$S/AuAgS showing (A) LPW 847, (B) 989, and (C) 1090 nm corresponding to Fig. 2.
The LPW red shifts but the absorption intensity decreases due to the reduced nanorod concentration as a result of addition of glucose.

3.3. Functionalization of Au$_2$S/AuAgS coated gold nanorods and detection of biological targets

Functionalization of the Au$_2$S/AuAgS coated gold nanorods takes advantage of the affinity between the Au$_2$S/AuAgS and thiol compounds. It should be noted that the formation of alkanethiol SAM introduces a red shift in the plasmon peaks on account of changes in the refractive index in the vicinity of the Au$_2$S/AuAgS coated gold nanorods. Fig. 10 displays the spectra of the nanorod suspension before and after alkanethiol (mercaptoundecanoic acid, MUA) attachment. A red shift of 7 nm in the longitudinal peak is clearly identified indicating formation of alkanethiol SAM. Repeated measurements performed on the same nanorod sample over 2 days yields a maximum peak drift of 0.5 nm, indicating that the 7 nm red shift can only be caused by SAM formation.

After the SAM of MUA is formed, biomolecules can be covalently attached via the NH$_2$ bond of the antibodies to the NHS-terminated nanorods. A further red shift of the plasmon peak can be observed. The same nanorods after the goat IgG attachment show a significant shift (of up to 20 nm) compared to the unmodified nanorods. The sensitivity of the plasmon spectra of the nanorods to the attachment of layers of molecules forms the basis of molecular biosensors using single particles. Fig. 10 also depicts the detection of rabbit anti-goat IgG in the solution binding to goat IgG on the Au$_2$S/AuAgS coated gold nanorod optical biosensor.
Control experiments were carried out in order to further prove the high sensitivity of $\text{Au}_2\text{S}/\text{AuAgS}$ coated gold nanorods. Two types of nanorods, gold nanorods with LPW 769 nm and $\text{Au}_2\text{S}/\text{AuAgS}$ coated gold nanorods with LPW 770 nm were selected to investigate, respectively. Significant red shift of plasmon peaks of the absorption curves corresponding to the two types of nanorods attached with biological molecule can be observed, as shown in Fig. 11. From this figure, it can be seen that the $\text{Au}_2\text{S}/\text{AuAgS}$ coated gold nanorods display 18 nm wavelength shifts while gold nanorods 12 nm after the attachment of BSA. Apparently, $\text{Au}_2\text{S}/\text{AuAgS}$ coated gold nanorods show larger plasmon wavelength shift than the gold nanorods under the same experimental conditions. Therefore, comparing to the common gold nanorods, these results show the $\text{Au}_2\text{S}/\text{AuAgS}$ coated gold nanorods exhibit higher sensitivity.

4. Conclusion

$\text{Au}_2\text{S}/\text{AuAgS}$ coated gold nanorods are produced with the addition of $\text{Na}_2\text{S}$ in the CTAB solution, and red shifts are observed in the LPW. However, no red shift in the LPW can be observed if CTAB is removed. This technique can be used to conveniently fabricate complex core–shell nanostructures with a high yield because the formation of core–shell structures with controllable shell thickness and core size proceed completely without any precipitation and aggregation among the nanoparticles. Hence, the plasmon resonance of the nanocomposite can be tuned over a range of several hundred nanometers. These $\text{Au}_2\text{S}/\text{AuAgS}$ coated gold nanorods are more sensitive to changes of the environment and medium compared to other common GNRs. Our experimental results demonstrate that $\text{Au}_2\text{S}/\text{AuAgS}$ coated gold nanorods have excellent properties in sensing specific target binding events since significant changes occur in the plasmon spectra in response to a change in the refractive index in the vicinity of nanocomposite. The materials are thus useful in many emerging applications including biosensors, chemical sensing, surface enhanced Raman scattering, and so on.

Acknowledgements

This work was jointly supported by Scientific Research Fund of Hunan Provincial Education Department (06B028), National Basic Research Fund (2005CB623901), National Natural Science Foundation of China (30700170, 20772027, 20803020), Shanghai Science and Technology R&D Fund (07QH14016, 07JC14057), Innovation Fund of SICCAS (SCX0606), and Hong Kong Research Grants Council (RGC) General Research Funds (GRF) No. CityU 112307.

Appendix

Figures with essential colour discrimination. Most of the figures in this article have parts that are difficult to interpret in black and white. The full colour images can be found in the on-line version, at doi:10.1016/j.biomaterials.2009.06.060.

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