### Aptamer Based Surface Enhanced Raman Scattering Detection of Adenosine Using Various Core Sizes of Au-Ag Core-Shell Nanoparticles

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The combination of the varied sizes of Au NPs and seeding growth approach can be exploited to control the sizes of Au-Ag core-shell NPs. The silicon substrate with self-assembled Au-Ag core-shell NPs can be used to detect adenosine by a structures-switch aptamer.
Aptamer based surface enhanced Raman scattering detection of adenosine using various core sizes of Au-Ag core-shell nanoparticles

Fu-Hsiang Ko\textsuperscript{a}, Yu-Cheng Chang\textsuperscript{b,}\textsuperscript{*}

The present study synthesizes the different sizes of Au nanoparticles (NPs) by adjusting the concentration of citrate. This method produces Au NPs with uniform sizes that can be used to grow Au-Ag core-shell NPs by the seeding growth method. We report the fabrication of monolayer Au NPs or Au-Ag core-shell NPs through the self-assembly of NPs on a 3-aminopropyltriethoxysilane (APTM)-modified silicon substrate. Results indicate that the self-assembly time of Au NPs or Au-Ag core-shell NPs plays a crucial role in determining the surface coverage of NPs on the silicon substrate. The appropriate sizes of Au NPs or Au-Ag core-shell NPs were optimized to yield the greatest SERS effect in the rhodamine B molecule. The substrates with self-assembled Au-Ag core-shell NPs can also be used to detect adenosine by a structures-switch aptamer. The combination of Au-Ag core-shell NPs and DNA-based adenosine sensors provide a facile, high enhancement, low detection limit (0.1 nM) and low cost fabrication, which shall be of significant value for practical applications of other aptamer sensing systems.

1. Introduction

Surface enhanced Raman spectroscopy (SERS) is a powerful vibrational spectroscopy technique with a highly sensitive and selective application that allows for detection of chemical or biological molecules in very low concentration, and provides rich structural information.\textsuperscript{1, 2} The enhancement effect is normally described by two enhancement mechanisms. One is a long-range electromagnetic (EM) effect that results from the enhancement of the local electromagnetic field due to surface plasmon resonance of nanoscale surface roughness features in the 10-200 nm range.\textsuperscript{3, 4} The enhancement intensity is determined by many factors, such as sizes, shapes, composition, and analytic molecules adsorbed on the substrates.\textsuperscript{5, 6} The other mechanism is a chemical enhancement effect that results from the charge-transfer excitation of chemisorbed molecules.\textsuperscript{1, 7} Since its discovery, SERS has been employed as a powerful tool in the non-destructive and ultrasensitive detection of proteins, DNA, nucleic acids, glucose, and small molecules.\textsuperscript{8, 9}

Aptamers are oligonucleic acid or peptide molecules that can bind to a wide range of target molecules (proteins, drugs, and amino acids, etc.) with high specificity and selectivity.\textsuperscript{10, 11} Due to their high selectivity, stability, versatile target binding, and easy regeneration capabilities over traditional antibodies, aptamers have received considerable research attention as recognition ligands in biological detections.\textsuperscript{12, 15} Nanomaterials have recently emerged as ideal candidates enabling efficient signal transduction and amplification in aptasensors. The nanomaterial-assisted aptasensors display unprecedented advantages in sensing applications and have attracted significant consideration in other multidisciplinary studies.\textsuperscript{12, 16} Optical detection methods such as colorimetry, fluorescence, and SERS are most widely adopted for signal harvesting in aptasensors because of typical and easy approach techniques.\textsuperscript{17} SERS provides a high sensitivity that is comparable with fluorescence detection. SERS has several intrinsic advantages over fluorometry such as fingerprints of analyses, excitation at any wavelength, alleviated photo-bleaching, narrow peak widths, and reproducibility.\textsuperscript{1, 7} The combination of SERS and nanomaterial-assisted aptasensors open up a whole new area for the application of biosensors.

The ability to develop SERS substrates with strong enhancement factors, low cost, good stability, and high reproducibility is still an important challenge.\textsuperscript{18} Various approaches have been developed to fabricate SERS substrates, but with limited success.\textsuperscript{19} The success of SERS is highly dependent on the interaction between adsorbed molecules and the surface of plasmonic nanostructures. SERS substrates commonly used gold (Au), silver (Ag), or copper (Cu).\textsuperscript{1, 20} In general, Au and Ag are most often used as SERS substrates because they are air-stable materials rather than Cu.\textsuperscript{18} Compared to Ag, Au has lower enhancement factors in visible light, but it is easier to prepare as monodisperse sols in a variety of diameters.\textsuperscript{21} By combining these two metals into a single entity, the catalytic properties can be further enhanced and the localized surface plasmon absorption can be varied continuously between the limits of the monometallic Au and Ag nanoparticles (NPs).\textsuperscript{22, 23} Recently, a strategy for extracting optical constants of the core and/or the shell material of bimetallic Au-Ag NPs from their measured surface plasmon extinction spectra was reported.\textsuperscript{24} SERS enhancement as a function of the layered Au-Ag NP composition and the state of their aggregation was investigated using pyridine and other types of probe adsorbates.\textsuperscript{25} However, understanding the effect of core sizes on SERS signals and the synthesis of large sizes of Au-Ag core-shell NPs without aggregation is still unclear.
The present study has successfully synthesized the different sizes of Au NPs by a modified Turkevich reaction system. The concentration of citrate plays an important role in growing the different sizes of Au NPs. The Au-Ag core-shell NPs with different sizes have been synthesized by the seeding growth method. The appropriate self-assembly time can be used to control the similar surface coverage of Au NPs or Au-Ag core-shell NPs on a 3-aminopropyltrimethoxysilane (APMTMS)-modified silicon substrate. Au-Ag core-shell NPs can provide a greater SERS effect on the rhodamine B molecule rather than Au NPs. The silicon substrate with self-assembled Au-Ag core-shell NPs can also be used to detect adenosine by a structures-switch aptamer. The fabrication of DNA-based adenosine sensors is facile, of high reproducibility, and low detection limit (0.1 nM), which shall be advantageous in applications for other aptamer sensing systems.

2. Experimental

2.1 Chemicals

Hydrogen tetrachloroaurate(III) trihydride, ethanol, hydrogen peroxide, adenosine, and phosphate buffered saline (PBS) were purchased from SIGMA. Silver nitrate, trisodium citrate dihydride, and sulfuric acid were purchased from J. T. Baker. 3-amino propyltrimethoxysilane (APMTMS), rhodamine B, and 3-Mercapto-1-hexanol (MCH) were purchased from Alfa Aesar. The three types of deoxyribonucleic acid (DNA) were synthesized by MDBio, Inc. All chemicals and materials were of analytical grade and used as received. De-ionized water (>18 MΩ/cm) was used throughout the experiments.

2.2 Characterization

The morphology of nanostructures was examined with a field emission scanning electron microscope (FESEM) using a JEOL JSM-6700F SEM operating at 10 kV accelerating voltage. A JEOL-529, 524, 521, and 519 nm, respectively. The wavelength of 632.8 nm. The maximum absorbance was decreased with the increase in concentration. The maximum absorbance was decreased with the increase in concentration of trisodium citrate, as shown in Figure 2b. The inset photos are depicted from left to right with the color change from dark wine to light wine, and the concentrations of citrate at 15.5, 19.4, 25.9, and 38.8 mM, respectively. The blue shift is ascribed to the quantum confinement effect arising from the reduced sizes of the Au NPs.

2.3 Preparation of Au NPs

The different sizes of Au NPs were synthesized by adding 1 mL of a different concentration of trisodium citrate to 10 mL aqueous solution of boiling 1 mM hydrogen tetrachloroaurate(III) trihydride under vigorous stirring. After the appearance of a wine color, boiling and stirring were continued under refluxing for 10 min and then cooled at room temperature.

2.4 Preparation of Au-Ag core-shell NPs

The 2.5 mL Au NPs with different sizes were diluted to 8 mL with de-ionized water and reheated to boil, followed by adding 1 mL of a different concentration trisodium citrate under vigorous stirring. Next, 2 mL of 5 mM silver nitrate was added, and the boiling was continued for 10 min. The Au-Ag core-shell NPs solution was cooled and stored at room temperature.

2.5 Preparation of substrate

Si (100) wafers were cleaned in a boiling piranha solution (H₂O₂-concentrated H₂SO₄, 3:7, v/v) for 10 min, and then rinsed with de-ionized water and ethanol. The substrate was immersed into an ethanol solution containing 5 mM APTMS and then heated under reflux for 2 h. Next, the substrate was rinsed with ethanol and de-ionized water, and then dried under a N₂ purge. The Au NPs or Au-Ag core-shell NPs were deposited onto the amino-terminated bonding by using an immersion method. After immersing the substrates in a solution of Au NPs or Au-Ag core-shell NPs for an appropriate time, the substrates were immediately rinsed with de-ionized water and dried under a N₂ purge.

2.6 Detection of adenosine

Figure 1 depicts schematically the method used to fabricate the adenosine sensing platform. All the solutions were prepared with a PBS buffer. First, the hybridization of adenosine solution with DNA (1) (5'-AGA GAA CCT GGG GGA GTA TTG CCG AGG AAG GT-3') and DNA (2) (3'-TAMRA-TCT CTT GGA CCC-5', TMR-labellled DNA): the 500 µL of 1 µM DNA (1) was mixed with 500 µL of 1 µM DNA (2) and heated in a water bath at 95 °C for 10 min. This solution was cooled at room temperature and stored at 4 °C. Second, the thiolated DNA (3) (5'-SH-A GAA GAA CCT GGG-3') was modified to substrate according to the literature: 20 µL of 1 µM DNA (3) was dropped onto the prepared substrate for 12 h at 4 °C. After rinsing with PBS, the substrate was immersed under 1 mM MCH for 5 min and thoroughly rinsed. Third, the adenosine solution was diluted to various concentrations ranging from 0.1 nM to 1 µM with PBS, and then mixed in an adenosine solution with DNA (2) double-stranded DNA (1) with a 1:1 ratio. 20 µL of mixture was then dropped onto the prepared SERS substrate for 2 h. Finally, the sensing platform was washed with a PBS buffer and dried under a N₂ purge. The signal intensity of the band at 1648 cm⁻¹ was recorded versus different concentrations of adenosine.
The role of the citrate in the growth process of Au NPs is not only reducing Au\(^{3+}\) to Au, but also controlling the sizes of the Au NPs. If the concentration of citrate is lower than 15.5 mM, the sizes of Au NPs appear non-uniform. The UV-vis spectra (Figure S1) exhibit a broad peak, which is attributed to the lower concentration of citrate being unable to provide for the inhibition of aggregation. The appropriate concentration of citrate can be exploited to control the different sizes of Au NPs. The sizes of Au NPs were determined by transmission electron microscopy. The variation of concentration of citrate was found to affect the sizes of Au NPs. Figures 2c-f show TEM images of Au NPs with spherical geometry, which were grown in citrate concentrations of 15.5, 19.4, 25.9, and 38.8 mM, respectively. The diameters of the Au NPs are 29.5, 23.4, 18.7, and 13.1 nm, respectively. The high concentration of citrate may induce the reduction in the sizes of Au NPs at the same Au precursors. On the other hand, varying the concentration of citrate led to significant changes in the sizes of Au NPs. The chemical composition of the NPs was analyzed by energy-dispersive spectroscopy (EDS). The EDS spectrum (Figure S2) reveals the composition of Au NP with only one gold component (Cu is from the TEM grid). Appropriate amounts of citrate led to the growth of Au NPs with different sizes.

**Figure 2.** (a) UV-vis spectra of Au NPs solutions synthesized in different concentrations of citrate. (b) The wavelength of Au NPs maximum absorbance as a function of the concentration of citrate. The inset image is the photo of (a). TEM images (scale bar = 10 nm) of an Au NP grown with c) 15.5 mM, d) 19.4 mM, e) 25.9 mM, and f) 38.8 mM citrate solution, respectively.

### 3.2 Evaluation of the different core sizes of Au-Ag core-shell NPs

Recently, the seeding growth approach based on the temporal separation of nucleation and growth processes is considered to be a very efficient method in controlling the bimetal core-shell NPs size and shape precisely.\(^{28}\) Herein, the seeding growth method can be used to synthesize Au-Ag core-shell NPs on the different sizes of Au NPs. Figure 3a shows the UV-vis spectra of Au-Ag core-shell NPs solution with a maximum peak at the 405, 411, 416, and 420 nm, respectively. The diameters of Au NPs are 13.1, 18.7, 23.4, and 29.5 nm, respectively. The wavelength of maximum absorbance increased with the increase in the sizes of Au NPs, as shown in Figure 3b. The inset photos are depicted from left to right with the color change from yellow to yellow brown. The red shift is also ascribed to the quantum confinement effect arising from the increased sizes of the Au-Ag core-shell NPs. When the sizes of Au NPs solution were higher than 18.7 nm, the shoulder band of Au-Ag core-shell NPs solution became relatively evidence. The shoulder in the SPR band can be observed at around 490 nm, which seems to increase in relative intensity as compared with the sizes of Au NPs. The phenomenon is caused by the LSPR of the large Au core.

**Figure 3.** (a) UV-vis spectra of Au-Ag core-shell NPs solutions synthesized on the different sizes of Au NPs. (b) The wavelength of Au-Ag core-shell NPs maximum absorbance as a function of the sizes of Au NPs. The inset image is the photo of (a). TEM images (scale bar = 10 nm) of an Au-Ag core-shell NP synthesized on the different sizes of Au NP: (c) 13.1 nm, (d) 18.7 nm, (e) 23.4 nm, and (f) 29.5 nm.

### 3.3 Evaluation of self-Assembly Au NPs and Au-Ag core-shell NPs

The self-assembly method, which is an essential part of nanotechnology, is one of the best non-physical deposition techniques for building 2D or 3D nanostructures.\(^{29, 30}\) Previous studies have employed the self-assembly technique utilizing the chemical interaction between NPs and the substrate or structure surface. For instance, organic molecules containing thiol (–SH) or amine (–NH\(_2\)) can be adsorbed onto the gold or silver surface to form a well-organized self-assembly monolayer.\(^{31}\) In our previous works, the fabrication of monolayer Au NPs was carried out using immersion or the spin coating method on a 3-aminopropyltrimethoxysilane (APTMS)-modified silicon substrate.\(^{32}\) Figure 4a illustrates the procedure used to fabricate Au NPs or Au-Ag core-shell NPs onto the silicon substrate. The fabrication processes can be briefly divided into two steps: (1) APTMS-functionalization of the silicon substrate and (2) self-assembly of Au NPs or Au-Ag core-shell NPs on APTMS. The first step deposits a large-scale monolayer APTMS on the silicon substrate with a thin native oxide layer. The second step deposits the Au NPs or Au-Ag core-shell NPs onto the amino-terminated bonding using an immersion method. After immersing the substrate in a solution of Au NPs or Au-Ag core-shell NPs for the desired time duration, the substrate is immediately rinsed with de-ionized water and dried under a N\(_2\) purge.
Figures 4a-e show top-view SEM images of the different sizes of Au NPs self-assembled on the Si substrate. The average diameters of Au NPs are 12.2±1.8, 17.5±2.7, 20.1±3.5, and 26.8±5.1 nm, respectively. The uniform sizes of Au NPs can be effectively controlled with the appropriate concentration of citrate as well as the growth condition. The densities and self-assembly time of Au NPs are 2356(15), 1164(30), 723(60), and 396(100) per micron² (min), respectively. In addition, the surface coverage of Au NPs can be calculated from SEM images at 27.5, 24.9, 22.9, and 22.3 %, respectively. The average diameters and self-assembly conditions for the different sizes of Au NPs are listed in Table S1. Appropriate images at 27.5, 24.9, 22.9, and 22.3 %, respectively. The surface coverage of Au NPs can be calculated from SEM images at 27.6, 25.2, 24.0, and 22.4 %, respectively. The average diameters and self-assembly conditions for the different core sizes of Au core-shell NPs were optimized to yield the greatest SERS effect. Au core-shell NPs: (a) 13.1, (b) 18.7, (c) 23.4, and (d) 29.5 nm. The SEM images of the different sizes of Au-Ag core-shell NPs were deposited on the Si substrate: (f) 24.8, (g) 32.9, (h) 43.5, and (i) 52.9 nm.

The different sizes of Au-Ag core-shell NPs can also be self-assembled on the Si substrate, as shown in Figures 4f-i. The average diameters of Au-Ag core-shell NPs are 23.4±3.3, 30.9±5.5, 38.4±5.6, and 52.1±8.2 nm, respectively. Compared to the average diameters of Au NPs, the average shell thicknesses of Au-Ag core-shell NPs are 5.6, 6.7, 9.2, and 12.7 nm, respectively. The shell thicknesses of Au-Ag core-shell NPs increase with an increase in sizes of Au NPs. This phenomenon is attributed to the decrease in the concentration of Au NPs solution. On the other hand, the shell thicknesses of Au-Ag core-shell NPs can also be controlled by the different sizes of Au NPs. The densities and self-assembly time of Au-Ag core-shell NPs are 641(45), 336(75), 207(120), and 105(180) per micron² (min), respectively. The surface coverage of Au-Ag core-shell NPs can be calculated from SEM images at 27.6, 25.2, 24.0, and 22.4 %, respectively. The average diameters and self-assembly conditions for the different core sizes of Au-Ag core-shell NPs are listed in Table S2. In order to achieve the similar surface coverage of NPs, Au-Ag core-shell NPs require a longer self-assembly time. When the self-assembly time was longer than 180 min, the Au-Ag core-shell NPs appeared the aggregation and precipitation. This phenomenon is attributed to the occurrence of aggregation during the long self-assembly time of NPs. These results confirm that the self-assembly time plays a crucial role in determining the different sizes of Au NPs and Au-Ag core-shell NPs with similar surface coverage on the silicon substrate. The different sizes of Au NPs and Au-Ag core-shell NPs shall be beneficial in fabricating SERS substrates.

3.4 SERS-based detection of rhodamine B

SERS effect is very sensitive to the roughness of metal surface.1, 7 Herein, the different sizes of Au NPs and Au-Ag core-shell NPs were used as substrate to examine the SERS effect. Rhodamine B was chosen as the probe molecule owing to its well-established vibrational features.33, 34 Figures 5 a-d show the SERS spectra of the rhodamine B solution (1×10⁻⁶ M) dispersed on the different sizes of Au NPs. The strong and medium-strong Raman bands at 1198, 1279, 1357, 1509, and 1646 cm⁻¹ are observed on the nanostructure surface. The strong peaks at 1279, 1357, 1509, and 1646 cm⁻¹ are assigned to the stretching modes of aromatic C-C. The band at 1198 cm⁻¹ is assigned to aromatic C-H bending.33, 34 An obvious trend shows that the intensity of the Raman signals at 1646 cm⁻¹ (Figure S5) increase gradually with an increase in the sizes of Au NPs, and the maximum SERS signals are at the sizes of 29.5 nm. The SERS spectra of the rhodamine B solution (1×10⁻⁶ M) are dispersed on the different sizes of Au-Ag core-shell NPs, as shown in Figures 5 e-h. For the different sizes of Au-Ag core-shell NPs, the intensity of the Raman signal at 1646 cm⁻¹ also reveals similar characteristics. The SERS signals at 1646 cm⁻¹ (Figure S5) are indeed found to increase with an increase in the sizes of Au-Ag core-shell NPs. Compared to the SERS signals at 1646 cm⁻¹, the thin Ag shell can provide for more than twice the SERS enhancement in the different sizes of Au NPs. Herein, the substrates are deposited Au NPs or Au-Ag core-shell NPs with similar surface coverage (Figure 4). It appears that the sizes and shell thicknesses of Au-Ag core-shell NPs are the main factors affecting SERS enhancement activity at the same surface coverage. The appropriate sizes of Au-Ag core-shell NPs were optimized to yield the greatest SERS effect in the rhodamine B molecule. We believe that not only the high SERS activity of this substrate contributes to its high detection sensitivity, but also to the sorption effect of the large sizes of Au-Ag core-shell NPs, which helps to improve the detection sensitivity.

3.5 SERS-based detection of adenosine

The present study uses SERS based on a structure-switching aptamer for the detection of adenosine (Figure 1). First, a DNA duplex is formed through the hybridization between an expanding anti-
adenosine aptamer (DNA (1)) and a TMR-labelled DNA (DNA (2)). As the DNA (1)/adenosine complex is more stable than the DNA (1)/DNA (2) duplex, the DNA (1) prefer to form the DNA (1)/adenosine complex rather than the DNA (1)/DNA (2) duplex and trigger the release of DNA (2) from DNA (1)/DNA (2) duplex. Second, the silicon substrate with self-assembled the varied sizes of Au NPs or Au-Ag core-shell NPs is prepared as SERS substrate and modified with thiolated capture DNA (DNA (3)). Finally, the released DNA (2) is easily hybridized with DNA (3) on the SERS substrate, resulting in an increase in SERS signals.

Figures 6a-d depict the SERS spectra obtained by using the aptameric sensor on the silicon substrate with self-assembled the different sizes of Au-Ag core-shell NPs at 1×10⁻⁴ M adenosine. SERS signals were observed with five typical Raman bands appearing in the region from 1100 to 1700 cm⁻¹. The peaks of these Raman bands are located at 1215, 1351, 1510, 1533, and 1648. The strong peaks at 1215, 1351, 1533, and 1648 cm⁻¹ are assigned to the stretching modes of aromatic C-C. The band at 1215 cm⁻¹ is assigned to aromatic C-H bending. Although the overall SERS signals of TMR show a similar increase in the response to the target, the band at 1648 cm⁻¹ was chosen as the marker band for TMR due to its ideal peak profile. The Raman intensity at 1648 cm⁻¹ (Figure S6) obtained from the different sizes of Au-Ag core-shell NPs. It is clearly observed that the signals increase with an increase in the sizes of the Au-Ag core-shell NPs. The largest Au-Ag core-shell NPs have the maximum SERS signals. The SERS enhancement factor (EF) of the largest Au-Ag core-shell NPs is about 5.4 times compared to the smallest. This indicates that the Au-Ag core-shell NPs as SERS substrates provided strong Raman signals of TMR.

The SERS spectra (Figure S7) obtained by using the aptameric sensor on the silicon substrate with the different sizes of Au NPs at 1×10⁻⁴ M adenosine. The SERS spectra reveal no Raman peak at 1648 cm⁻¹. The Au NPs detection schemes were limited by the complex sample preparation and relatively low enhancement factors compared Ag NPs. Results indicate that the Ag shell of Au-Ag core-shell NPs plays a crucial role in aptameric sensor. Figures 7a-e depict the SERS spectra obtained by using the aptameric sensor on the silicon substrate with self-assembled the largest sizes of Au-Ag core-shell NPs at the different concentration of adenosine. For the different concentration of adenosine, the intensity of the Raman signal at 1648 cm⁻¹ reveals similar characteristics. The SERS signals at 1648 cm⁻¹ (Figure S8) are indeed found to increase with an increase in the concentration of adenosine. The peak remains clearly observable in the adenosine solution with concentrations as low as 0.1 nM. This indicates that the Au-Ag core-shell NPs as SERS substrates provided strong Raman signals of TMR. The fabrication of DNA-based adenosine sensors is facile, of high reproducibility, and a low detection limit (0.1 nM), which shall be advantageous in applications for other aptamer sensing systems.

4. Conclusions

In summary, the Au NPs with the different sizes can be synthesized by adjusting the concentration of citrate in an aqueous solution. The different sizes of Au NPs can also be used to grow Au-Ag core-shell NPs by the seeding growth method. The appropriate self-assembly time can be used to control the densities of Au and Au-Ag core-shell NPs with similar surface coverage on an APTMS-modified silicon substrate. The largest Au-Ag core-shell NPs are beneficial in yielding the greatest SERS effect of the rhodamine B molecule on the similar surface coverage. The optimizing substrate also exhibits the best enhancement for the detection of adenosine by a structures-switch aptamer. This aptameric SERS sensor is simple and can detect adenosine as low as 0.1 nM. The SERS-based sensing system continues to have great potential for multiplex and sensitive determination in biochemical and biomedical studies.

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Notes
biggest core sizes of AuMAg coreMshell NPs at the different concentration of adenosine. See DOI: 10.1039/b000000x/

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