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DNA-directed self-assembly of three-dimensional plasmonic nanostructures for detection by surface-enhanced Raman scattering (SERS)

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ABSTRACT

Surface-enhanced Raman scattering (SERS) is a promising technology owing to its single-molecular sensitivity and molecular specificity. However, producing strong and stable SERS signal from plasmonic nanostructures remains a challenge. Herein, we present a facile generation of SERS-active nanomaterials by organizing metallic nanoparticles onto dye-labeled three-dimensional DNA nanostructures. Stable formations of metal clusters with the dye located in hot spots enabled detection of SERS signals. SERS signals were further regulated via interaction of pyramidal DNA scaffold with target biomolecules. We believe our SERS-active nanomaterials with controllable geometry and reversible SERS effects meet significant requirements for practical SERS-based sensors. By integrating versatile properties of DNA and introducing various Raman dyes into plasmonic nanostructures, the emergence of powerful and multiplexed biosensors should be possible.

1. Introduction

Metallic nanostructures have been recognized for their unique optical properties owing to their ability to support surface plasmons, which are oscillations of free electrons in the metals that are bound by nanoparticle geometry [20,6]. Plasmonic properties sensitively depend on geometric parameters, such as nanoparticle size, shape, crystal face, surface roughness, and interparticle spacing [23,24,13]. These properties have been widely explored in various applications that include surface-enhanced Raman scattering (SERS) [2,5]. Unlike fluorescence-based detection methods, SERS shows high sensitivity (up to single-molecule detection), narrow spectral width, multiplex capability, and insensitivity to quenching [19]. To achieve maximal enhancement of the Raman signal, various SERS-active materials have been developed [1]. High Raman enhancements are generally obtained by increasing the number of "hot spots" at the junctions of particle to particle boundaries. Thus, aggregates of silver or gold nanoparticles have emerged as effective SERS substrates [11]. Aggregations of metal nanoparticles have been conventionally induced by the addition of surface charge altering agents, such as poly-(L-lysine), spermine hydrochloride, and sodium chloride [22]. However, the precise and reproducible formation of metal clusters is not easily achieved, and this interferes with the reproducibility of SERS experiments. There was a lack of control over both the size of resulting aggregates and the gaps between SERS aggregates using these approaches [21,12].

Here, we intended to control the formation of metal clusters using an oligonucleotide-directed assembly. Metal nanoparticles that are conjugated to rationally designed DNA nanostructures can be precisely positioned in three-dimensional spaces [17,16,15,4]. Thus, it is possible to control the SERS nanostructure geometry. Plasmonic nanoparticles that are attached to rigid DNA nanostructures can present distinct structure-dependent optical features [3]. In addition, conformational changes of DNA nanostructures can be triggered upon the interaction with target biomolecules because DNA hybridization is reversible; these changes affect the optical properties. Varying combinations of Raman active molecules that are attached to DNA nanostructures can serve as the specific SERS codes in multiplexed detection methods [7]. There have already been many attempts to develop DNA-programmed plasmonic nanoarchitectures for the purposes of

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diagnostics and biosensor fabrication, but they are often restricted to one- and two-dimensional structures [14,9]. Here we demonstrate a practical usage of discrete three-dimensional DNA nanostructures as SERS based biosensors.

2. Materials and methods

2.1. Materials

All oligonucleotides were purchased from Bioneer Corp. (Daejeon, South Korea). Citrate-stabilized gold nanoparticles (20 nm) were obtained from Sigma–Aldrich. All the other reagents, unless otherwise stated, were purchased from Sigma–Aldrich.

2.2. Assembly of DNA pyramid

For the assembly of the DNA pyramids, stoichiometric quantities of the component DNA strands were mixed in a hybridization buffer (pH 7.6; 20 mM Tris, 12.5 mM $MgCl_2$, 2 mM EDTA) to a total concentration of 0.4 μM. Solutions were heated at 95 °C for 5 min then rapidly cooled to 4 °C using a thermocycler (Bio-Rad). Hybridization between component DNA strands was analyzed with 5% native polyacrylamide gel electrophoresis running in 1× TBE buffer at 70 V for 1 h.

2.3. Preparation of gold-conjugated DNA pyramid

To conjugate gold nanoparticles to DNA, 5′-C_6 S–S modified DNAs were reduced using TCEP solution prior to use. Thiol-modified strands were assembled first, and stoichiometric quantities of gold nanoparticles were then added to the mixture so that each pyramid could recruit one particle at each vertex. The final concentration of DNA in the solution was 1 nM. The gold/DNA mixture was further incubated at room temperature for 2 days with stirring.

2.4. Silver enhancement of gold/DNA pyramid

5 μL of gold/DNA mixture was incubated with 10 μL of 1% poly-*N*-vinyl-2-pyrrolidone (stabilizer), 5 μL of 0.1 M sodium L-ascorbate (reductant), and 7 μL of 1 mM $AgNO_3$ for three hours at room temperature. The formation of silver nanoparticles onto gold/DNA pyramids was investigated by a UV–vis spectrophotometer (Nanodrop, USA).

2.5. Electron microscopy

STEM images and EDX spectra were acquired on a FE-SEM(S-5500) (Hitachi, Japan) operating at 30 keV. Samples were prepared by adding 10 μL of colloidal solution onto a carbon-coated copper TEM grid (200 mesh, Agar scientific, UK) and dried overnight.

2.6. SERS measurement

All the SERS signals from aqueous samples on silica surface were measured using a Raman spectroscope (inVia, Reinshaw, UK) equipped with an argon ion laser. They were obtained with the following condition: 514.5 nm excitation laser, 20 mW laser power, 10 s integration, and 1 s acquisition time.

3. Results and discussions

3.1. Assembly of DNA pyramid and attachment of gold nanoparticles to DNA pyramid

We utilized a DNA pyramid with 7 nm-long edges as the model three-dimensional scaffold. The assembly of a DNA tetrahedron is a well-known system, and it is both mechanically robust and highly efficient [8]. Four-component single-stranded DNAs are partially complementary to each other, such that they wrap around each face of the pyramid and hybridize to form the doubly helical edges. We generated sequences of our pyramids newly using the Tiamat program with slight modifications to that of Turberfield's, where the nicks were rotated to the vertices and restriction sites were removed [10]. As shown in Fig. 1a, our design allows a pyramid with a side length of 7 nm to connect spherical nanoparticles via attachment through its vertices. Gold nanoparticles of 20 nm diameter were chosen as the seeds, and they were further coated with silver to produce gold-silver nanoshell structures. Flexible alkyl-thiol moieties were introduced to 5′ of the component DNAs to connect gold nanoparticles. Strand 4 was internally modified with Cy3, which is a Raman active dye. DNA self-assembly directs Cy3 to be located in the middle of one edge among the four gold clusters assembled along the DNA pyramid.

The DNA nanostructure was formed in a one-pot assembly with rapid cooling, and the assembly was verified by native polyacrylamide gel electrophoresis (PAGE). When all four strands are present, a distinct band with low mobility is present in the native

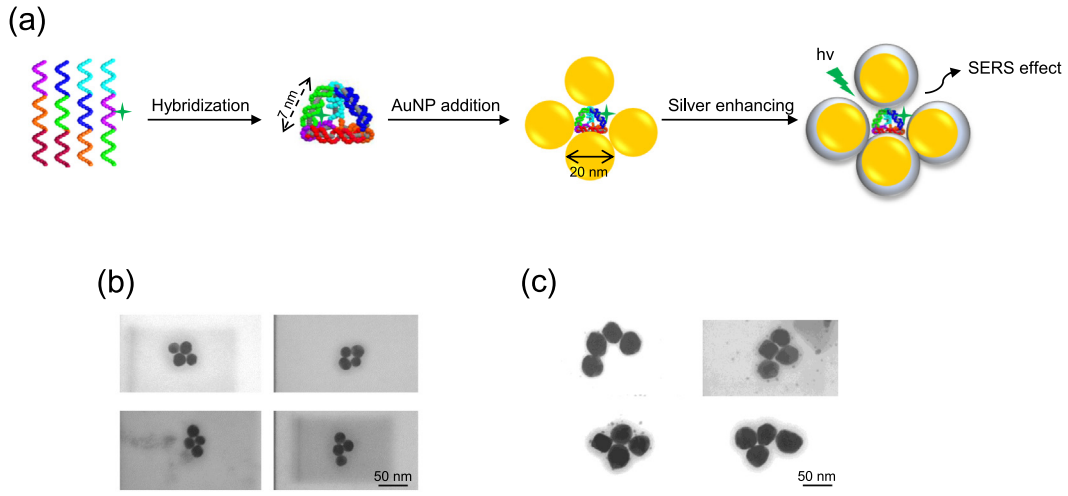

Fig. 1. Strategy to synthesize silver–gold nanoshell clusters using DNA self-assembly. (a) Assembly of the DNA pyramid followed by gold nanoparticle attachment and further silver coating. STEM images of (b) gold nanoparticle-attached DNA pyramid and (c) silver-coated DNA pyramid.

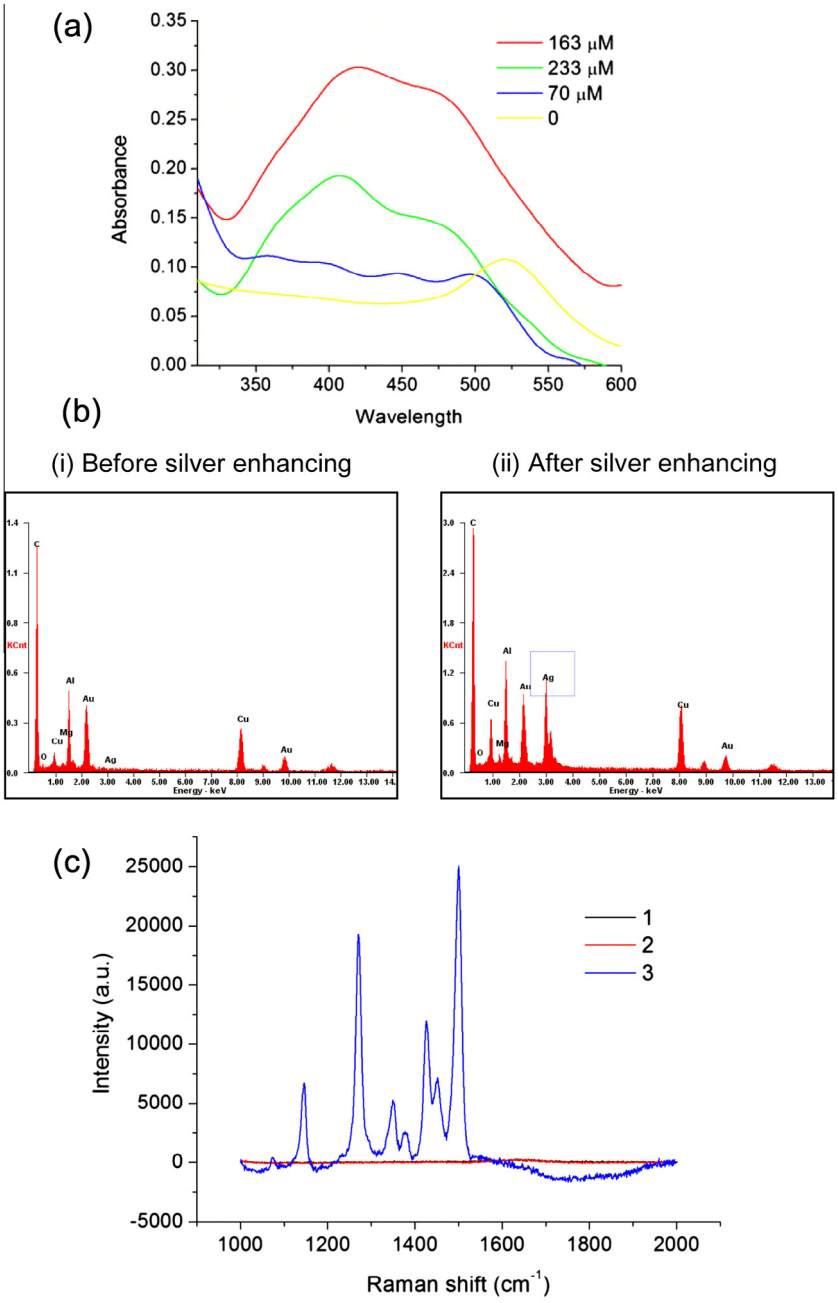

Fig. 2. Characterizations of silver-enhanced gold cluster on DNA pyramid. (a) UV–vis absorbance spectra of silver-enhanced particles with different $AgNO_3$ concentrations. Plasmonic peaks experience a blue shift from 520 nm to 400 nm as the concentrations of $AgNO_3$ increase. (b) EDX analysis of metal cluster (i) before and (ii) after silver enhancement. (c) SERS spectra of (1) Cy3 modified DNA pyramid without silver enhancing, (2) silver enhanced DNA pyramid without Cy3, (3) silver enhanced DNA pyramid in the presence of Cy3. SERS spectra of (1) and (2) were overlapped since they were negligible compared to that of (3). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

PAGE (Fig. S1), which indicates the formation of the intended structure. To obtain STEM (scanning transmission electron microscopy) images (Fig. 1b), four thiol-modified strands were first hybridized to form a DNA pyramid and were then further incubated with citrate-stabilized gold nanoparticles (size of 20 nm). A pyramid can recruit one gold nanoparticle at each vertex, thus a cluster of four gold nanoparticles with close proximity was observed. Due to the high capillary forces upon drying, some structures are stretched and distorted on the grid. Since electron microscopy allows us to view the nanoparticle assemblies two dimensionally, information of three dimensional structure can be lost. Thus, we assembled another DNA pyramid with three strands were thiol-modified and the pyramid was incubated with gold nanoparticles. A cluster formation of three gold nanoparticles was visible (Fig. S2a). These images suggest of tetrahedral geometry of gold nanoparticles in solution. Without DNA pyramid, the mixture showed an irregular distribution of gold nanoparticles (Fig. S2b).

3.2. Silver enhancement of gold/DNA pyramid

The Cy3 and gold nanoparticle-incorporated DNA pyramid was coated with silver to generate Raman active nanomaterials. $AgNO_3$, poly(vinylpyrrolidone) (PVP), and Sodium L-ascorbate were added

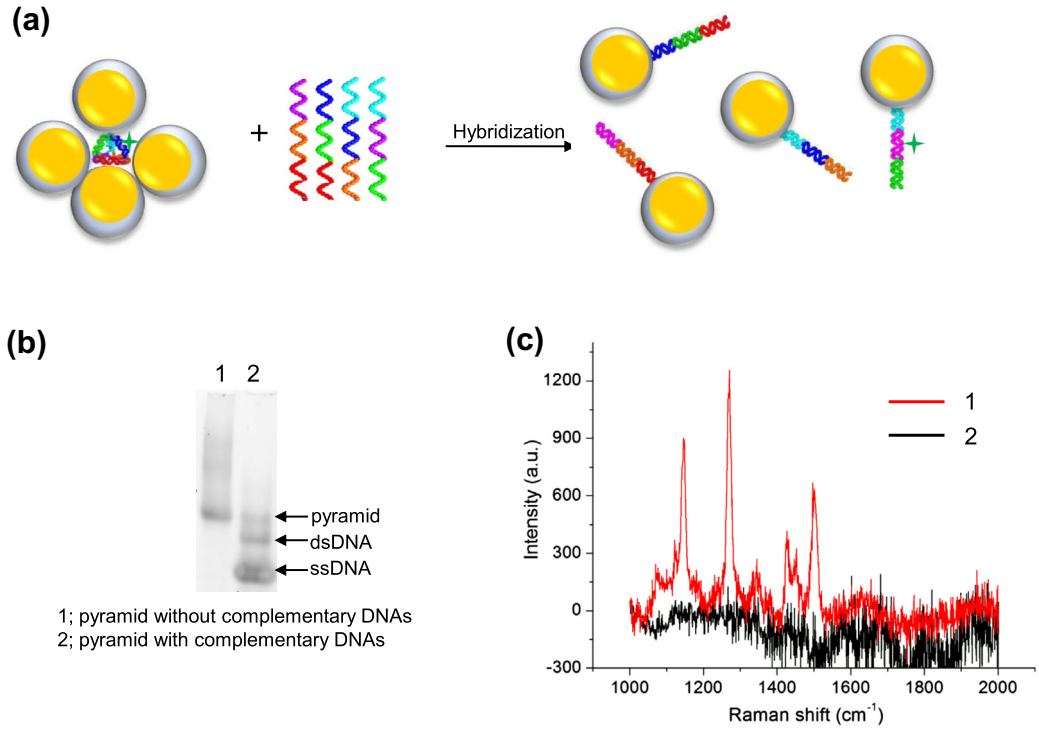

Fig. 3. Conformational change triggered by interaction of DNA pyramid with target DNAs led to switching on or off the SERS signal. (a) Interaction positions silver-enhanced gold nanoparticles close to a DNA pyramid, and the formation of linear structure in the presence of complementary DNAs disrupts this conformation. (b) Native gel analysis of structural changes upon the addition of complementary DNAs. (c) Change of SERS signals upon the addition of complementary DNAs.

to grow a Ag layer on the gold nanoparticle/DNA pyramid surface. Silver-enhanced structures were confirmed by UV–vis spectroscopy, STEM, and EDX (Energy dispersive X-ray) analysis. As shown in Fig. 2a, the plasmonic peak of the nanostructure in the UV–vis spectrum blue shifted from 520 nm to 400 nm as the silver shell grew. The absorbance of the resulting complex was highest using 163 μM of $AgNO_3$, and that concentration was employed for all subsequent experiments. The successful fabrication of a silver shell was also demonstrated in EDX experimentation as shown in Fig. 2b. The presence of Au and Ag after silver coating confirms Ag–Au bimetallic nanostructures. STEM imaging showed the size of particles were increased from 19.3 ± 2.6 nm to 35 ± 2.0 nm after Ag coating, and the particles retained compact structures. When SERS measurements were performed, the intensity of the Cy3 Raman peaks increased in the presence of silver-enhanced Cy3/gold-conjugated pyramid (Fig. 2c). The characteristic Raman peaks appeared at 1426, 1452, and 1500 cm^{-1}. SERS signals were negligible when either silver or Cy3 was excluded from the gold-conjugated pyramid. Since their intensities were very low compared to that of silver enhanced Cy3/gold conjugated pyramid, they overlapped each other. This indicates that the SERS signal was only amplified in the presence of neighboring silver-enhanced gold nanoparticles situated by the hybridization interaction between DNAs. The final concentration of DNA in the solution was 1 nM.

3.3. Reversible SERS effects upon the interaction with target DNAs

The SERS signal of DNA nanostructures can be further regulated by interactions with target molecules. As a proof of concept, pyramidal, silver-enhanced gold-nanoparticle DNA was incubated with other DNA strands. Originally, the DNA pyramid is composed of 4 partially complementary strands. When each component DNA was incubated with its complementary strand, they reorganized to form simple, double-stranded DNAs rather than retaining a pyramidal DNA structure where hydrogen bonds are held among four different partially complementary DNAs (Fig. 3a). The environmental change of attached nanoparticles that results from the structural change of DNA is reflected in SERS signals. To facilitate the efficient transition from a pyramidal structure to a linear form, a four times molar excess of complementary strands were added (2 nM vs. 0.5 nM DNA pyramid), and the DNA solution was heated at 95 °C for 5 min then cooled to 4 °C. Applying heat to the solution thermally denatures the pyramid structure and helps to reassure the formation of new hydrogen bonds among complementary strands. As shown in Fig. 3b, native PAGE indicates that the incubation with complementary strands leads to disassembly of the pyramid to linear double-stranded DNAs or unhybridized single-stranded DNAs. The SERS intensity was decreased in response to the dissociation of DNA pyramid because the distances between nanoparticles and Raman active dye were affected (Fig. 3c). On the basis of a configurational switch between a pyramid and duplex structure, SERS signal effectively switched from the "on" to "off" state following the actions of a biological recognition event.

4. Conclusions

In conclusion, we have demonstrated the generation of silver-coated gold nanoparticles affixed to DNA pyramids for SERS application. The assembly was characterized using electrophoresis, STEM, and Raman spectra. Controlled orientations of the metal clusters with Raman dye located in the hot spot regions were observed, which subsequently led to reproducible intensities in SERS signals. SERS signals in this proof of concept will be further improved by optimizing the particle size, three-dimensional shapes of metal cluster, interparticle gaps, etc. SERS is a powerful tool owing to its high sensitivity, ability to relay detailed chemical and structural information, and high spectral precision. By achieving a large enhancement of signal and tailoring the interactions

between the target molecules and the DNA-based nanostructures, highly sensitive biosensors can be fabricated. Moreover, by incorporating multiple dyes, characteristic fingerprints of each dye in the mixture can be distinguished, allowing simultaneous measurements of multiple analytes in a single assay [18]. In addition to the increased sensitivity, good reproducibility can be achieved by combining SERS detection with DNA-directed assemblies of nanoparticles. DNA structures are not only versatile materials that can be used as scaffolds, but also as biological and genetic materials through the mechanisms of aptamers, DNAzyme, antisense DNAs [5]. Integrating distinct functions of DNAs simultaneously can permit the realization of highly versatile and reproducible DNA-based SERS biosensors.

Conflict of interest

There is no conflict of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.sbsr.2014.06.003.

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