



## Short communication

## Large scale synthesis of surface-enhanced Raman scattering nanoprobe with high reproducibility and long-term stability



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## ABSTRACT

We were successful developed a facile and large-scale synthesis of SERS dots with high reproducibility and long-term stability. Nine kinds of SERS dots with representative and selective Raman bands were fabricated, allowing for simultaneous detection of multiple SERS dots. High sensitivity of SERS dots was demonstrated by analyzing single particles and show sufficient sensitivity to detect a single particle with average enhancement factor in the order of  $1.0 \times 10^5$ . Also, SERS dots were stabilized for 300 h. These features suggest that the SERS dots can be utilized as a targeting agent in practical applications.

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## Introduction

A great deal of scientific and technical attention has been placed on surface-enhanced Raman scattering (SERS) due to its potential utility as an ultrasensitive tool for detecting biological and chemical analytes [1]. When SERS technique is used as a tagging material, it offers several key advantages over other established labeling tools, such as no photo-bleaching, narrow bandwidth, single laser excitation and non-toxicity [2,3]. Raman-labeled nanoparticles (NPs) using the SERS effect (SERS nanoprobe) have been extensively developed for high-throughput multiplex

screening, diagnosis, drug delivery and bio-imaging [4–8]. These SERS nanoprobe typically consist of a SERS substrate for signal amplification, Raman labeling compounds (RLCs) to confer unique optical codes, and an outer protective coating layer [9–11]. The external coating layer has an important role in the SERS nanoprobe: it protects the unique SERS signals and plasmonic structure from the external medium and unpredicted contamination during further modifications, increases dispersion stability and bio-compatibility of the NPs and allows additional sites for bio-functionalization [12–14]. However, the external surface coating process is often limiting for large-scale synthesis associated with uncontrollable aggregation.

Several groups have reported highly reproducible, large-scale synthesis of SERS nanoprobe using gold nanospheres encapsulated with a silica shell [15]. However, these gold nanosphere-based SERS nanoprobe only moderately enhance the SERS signals compared to those having inter-particle junctions or sharp edged nanostructures, so-called “hot spots” [16–20]. We previously reported SERS nanoprobe, named SERS dots, as alternative highly sensitive SERS nanoprobe, consisting of silver NP-embedded silica

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nanospheres (Ag–SiO<sub>2</sub> NPs), various organic RLCs and a silica coating [13,14,21,22]. SERS dots have many advantages, such as easy handling, strong signal generation, and reproducibility of preparation [5,23,24]. Use of Ag–SiO<sub>2</sub> as a SERS substrate could produce good results with excellent size homogeneity and reproducibility during development of the large-scale SERS dots synthetic method but a large-scale silica coating process has not been firmly established. A highly reproducible scaling-up synthetic protocol is required that maintains its optical properties in terms of sensitivity and stability for practical use of SERS nanoprobe.

In this study, we report a scaling-up synthetic method to fabricate ca. 60 mg SERS dots, which is 60 times larger than those reported previously (1 mg per batch scale) [13]. Nine kinds of SERS dots were fabricated on a large scale using various RLCs, and the representative Raman bands for each SERS dot were clearly distinguished without spectral overlap. Moreover, the optical properties of the synthesized SERS dots were evaluated in terms of sensitivity, reproducibility, and long-term stability. The SERS dots exhibited high sensitivity sufficient to detect a single NP with a narrow distribution of SERS enhancement factors (relative standard deviation = 0.69). The SERS dots were showed high stability in deionized water and pH 5, 7, and 9 buffer solutions for 300 h.

## Experimental

### Materials

Tetraethylorthosilicate (TEOS), 3-mercaptopropyl trimethoxysilane (MPTS), 3-aminopropyltriethoxysilane (APTS), ethylene glycol (EG), silver nitrate (AgNO<sub>3</sub>, 99.99%), octylamine (OA), sodium silicate solution, 4-aminobenzenethiol (4-ATP), 2-naphthalenethiol (2-NT), 4-bromobenzenethiol (4-BBT), 4-chlorobenzenethiol (4-CBT), 4-fluorobenzenethiol (4-FBT), 3,4-dichlorobenzenethiol (3,4-DCT), benzenethiol (BT), 3,5-dichlorobenzenethiol (3,5-DCT), and 2-mercapto-6-methylpyridine (2-MMP) were purchased from Sigma-Aldrich (St. Louis, MO, USA) and used without further purification. Ethyl alcohol (EtOH, 99.9% and 95%), and aqueous ammonium hydroxide (NH<sub>4</sub>OH, 27%) was purchased from Daejung (Siheung, Korea).

### Preparation of thiolated silica nanoparticle templates

Approximately 150 nm sized silica nanoparticles were prepared using the Stöber method [25], and 240 mL of 99.9% EtOH, 18 mL NH<sub>4</sub>OH, and 9.6 mL TEOS was added. The solution was stirred vigorously for 20 h at 25 °C. Then, silica colloids were centrifuged and washed several times with 95% EtOH. Silica nanoparticles (500 mg) were dispersed in 10 mL of 95% EtOH, and 500  $\mu$ L MPTS and 100  $\mu$ L NH<sub>4</sub>OH were added to the colloidal solution to obtain thiolated silica nanoparticles. The mixture was stirred vigorously for 6 h at 25 °C. The resulting thiolated silica nanoparticles were obtained with centrifugation, and they were washed several times with EtOH to remove excess reagent.

### Preparation of silver nanoparticles-embedded silica nanoparticles

A 125 mL aliquot of EG was stirred vigorously. Then, 125 mL AgNO<sub>3</sub> solution (30 mM in EG) was added to the silica NPs suspension and thoroughly mixed (final AgNO<sub>3</sub> concentration · 15 mM). A 206.2  $\mu$ L aliquot of OA was added quickly (final OA concentration · 25 mM), and the resulting suspension was stirred for 1 h at 25 °C. The particles were centrifuged and washed several times with EtOH.

### Incorporating Raman tagging materials into the silver-doped silica nanoparticles

The nine kinds of SERS dots were synthesized by using 4-ATP, 2-NT, 4-BBT, 4-CBT, 4-FBT, 3,4-DCT, BT, 3,5-DCT, and 2-MMP as Raman labels. The 5 mL of Raman labeling compounds (10 mM in EtOH) were added to 50 mg of silver-embedded silica nanoparticles. The suspension was stirred vigorously for 30 min at 25 °C. The colloids were centrifuged and washed with EtOH. The washed nanoparticles were dispersed in 75 mL water, and 75  $\mu$ L sodium silicate solution (0.036 wt%) was added to the dispersion and stirred vigorously for 12 h at 25 °C. A 300 mL aliquot of EtOH was added to the resulting colloids for solvent exchange. After 3 h, 200  $\mu$ L TEOS and 1.2 mL NH<sub>4</sub>OH were added and stirred vigorously for 24 h at 25 °C. The resulting colloids SERS nanoprobe were centrifuged, washed several times with EtOH, and dispersed in 99.9% EtOH. The size distribution of SERS dots measured by dynamic light scattering instrument (Zetasizer Helix, Malvern, UK).

### SERS measurement of the SERS dots

To evaluate the sensitivity of synthesized SERS dots, the SERS dots which were spread on the slide glass by drop-casting methods and measured by confocal micro-Raman system (LabRam 300, JY-Horiba, Tokyo, Japan) equipped with an optical microscope (BX41, Olympus, Japan). The SERS signals were collected in a back-scattering geometry using  $\times 100$  objective lens (0.90 NA, Olympus, Japan) and detected by a spectrometer equipped with a thermoelectric cooled CCD detector. As an photo-excitation source, the 532 nm diode-pumped solid-state laser (CL532-100-S, Crystalaser, USA) was used with laser power of 3.3 mW at the sample. The strong Rayleigh scattered light was rejected using a long-pass filter. Selected sites were measured randomly, and all SERS dot spectra were integrated for 1 s.

For calculation of enhancement factor (EF) of the synthesized SERS dots, the SERS<sub>4-FBT</sub> dots labeled by 4-FBT (1 mg/mL in EtOH) solution was dredged on a patterned slide glass to measure the SERS signal from a single SERS dot. These patterned slide glass allowed us to easily find the measured SERS dots by using field-emission scanning electron microscopy (FE-SEM, AURIGA, Carl Zeiss, Germany). The intensity of SERS signals were mapped with a laser power of 2.6 mW, an acquisition time of 1 s, and a 1  $\mu$ m step size using the 532 nm laser line, the resulting SERS intensity maps were overlaid with the corresponding SEM images in order to distinguish the SERS signals come from a single SERS dot.

### Calculation of the SERS enhancement factor

SERS enhancement factors (EF) for a single SERS dot<sub>4-FBT</sub> was estimated using the following equation:  $EF = (I_{\text{SERS}} \times N_{\text{normal}}) / (I_{\text{normal}} \times N_{\text{SERS}})$ , where  $I_{\text{SERS}}$  and  $I_{\text{normal}}$  are the intensity of the bands from SERS and normal Raman scattering, respectively, and  $N_{\text{normal}}$  and  $N_{\text{SERS}}$  are the number of Raman label molecules (4-FBT was used in this study) in pure form and self-assembled on the surface of silver nanoparticle in SERS dots. To estimate the EF, the representative band at 1075 cm<sup>-1</sup> of 4-FBT was used. The Raman signals of both single particles and neat 4-FBT were measured in identical condition involving excitation laser-line, laser power, acquisition time, and objective lens. The scattering volume in normal Raman measurements was estimated 13.6  $\mu$ m<sup>3</sup> by assuming as a cylinder form with a diameter of 1.7  $\mu$ m and a height of 6  $\mu$ m. Since the molecular weight and density of 4-FBT are 125.19 g/mol and 1.203 g/cm<sup>3</sup> respectively, the  $N_{\text{normal}}$  was estimated  $7.7 \times 10^{10}$ . The  $N_{\text{SERS}}$  was calculated by geometrically estimating the particle's surface area and a molecular footprint of

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