Notes

Controlled Clustering of Gold Nanoparticles using Solid-support for Surface-enhanced Raman Spectroscopic Probes[†]Hyejin Chang,[‡] Jinjoo Chae,[‡] Homan Kang,[§] Yoon-Sik Lee,^{§,*,*} and Dae Hong Jeong^{‡,§,*}[‡]Department of Chemistry Education, [§]Interdisciplinary Program in Nano-Science and Technology,[#]School of Chemical and Biological Engineering, Seoul National University, Seoul 151-742, Republic of Korea

*E-mail: yslee@smu.ac.kr (Y.-S. Lee); jeongdh@smu.ac.kr (D. H. Jeong)

Received July 9, 2013, Accepted August 4, 2013

Key Words : SERS, Clustering control, Gold nanoparticle, Solid support, Optical bio-probe

Single molecule-level sensitivity of surface-enhanced Raman scattering (SERS) was known approximately fifteen years ago.¹ Ever since there have been many different applications benefiting from the ultra-high sensitivity such as single molecule detection,² chemical sensing³ and bio-molecular probes.⁴⁻⁶ Especially, SERS has drawn much attention in bio-multiplexing probes owing to its unique optical characteristics claiming extremely narrow bandwidth, high sensitivity of light signals, and non-bleaching feature.

Various SERS probes have been reported for bio-molecular detection. Single gold nanoparticle-based probes have been widely used by many researchers.⁴ Successful aggregation of silver nanoparticles (COINs) was reported and successfully applied to multiplexing in bio-systems such as tissue sample.⁵ Ensemble-averaged strategy on silica core (SERS dots) was demonstrated and evolved diversely by incorporating various functionalities such as fluorescence and magnetism within the probe particles.⁶ One of ideal structures for SERS probe is conjoined dimer of nanoparticles or small cluster of nanoparticles for SERS hot spot generation. However, dimerization or aggregation control of metal nanoparticles is inherently difficult because clustering of nanoparticles is followed by destabilization of nanoparticles resulting into extensive aggregation in solution phase. In this regards, an assembly method for asymmetric core-satellite plasmonic nanostructures employing glass substrates was reported recently, which is very efficient for constructing and separating the nanoassemblies.⁷

In this note, we demonstrate controlled clustering of gold nanoparticles (Au NPs) for synthesizing small gold nanoclusters as SERS probes by using solid-supported aggregation of Au NPs in order to minimize extensive aggregation. Fabricated dimers or small nanoclusters were homogenous in cluster size and had a narrow distribution of SERS intensity, which means these nanoclusters have potential for

Scheme 1. Solid-supported clustering of Au NPs. (a) Formation of mixed self-assembled monolayer on the silicon surface using a mixed solution of 3-aminopropyltrimethoxysilane and ethoxytrimethylsilane, (b) linking additional spacers with 3-nm long BEBEBE amino-acid chains where E = ϵ -aminocaproic acid and B = β -alanine, (c) attaching primary Au NPs to the substrate by electrostatic forces between amine group of the linker chemical and Au NPs, (d) introduction of 4-aminobenzenethiol (4-ABT), acting as a Raman reporter and also as the linker molecule for introducing of secondary Au NPs, (e) attachment of secondary Au NPs onto the 4-ABT adsorbed primary Au NP resulting that the 4-ABT is placed in the SERS hot spot, and (f) detachment of clusters from substrate into solutions phase by ultrasonication.

utilization as bio-probes.

Fabrication of controlled clustering was performed by two-step immobilization of gold nanoparticles on solid substrate followed by detachment the clusters from the substrate. The experimental processes are illustrated in Scheme 1. The mixed self-assembled monolayer (SAM) was formed by dipping the pretreated Si wafer in a mixed solution of 3-aminopropyltrimethoxysilane (3-APDMS) and ethoxytrimethylsilane (ETMS). Additional spacing linkers composed of β -alanine (β -Ala; B) and ϵ -aminocaproic acid (ϵ -ACA; E) pairs were used for easier immobilization of gold nanoparticles from solution to substrate. Fmoc- β -alanine (Fmoc- β -Ala) and Fmoc-6-aminocaproic acid (Fmoc- ϵ -ACA) were coupled three times in turn to the Si surface through the

[†]This paper is to commemorate Professor Myung Soo Kim's honourable retirement.

Figure 1. (a) AFM images of the primary layer of Au NPs on Si wafer. Attached primary Au NPs on BEBEBE spacers of *ca.* 3-nm height exhibited *ca.* 16-nm heights. SEM images of (b) the primary layer of Au NPs on Si wafer, **A**, and (c) the Au nano-clusters on Si wafer after secondary assembly of Au NPs, **B**.

Table 1. The number of particles in $1 \mu\text{m}^2$

	Single particles	Clusters
After primary assembly of Au NPs (A)	14.5 ± 2.4	0.5 ± 0.5
After secondary assembly of Au NPs (B)	37.7 ± 10.2	19.9 ± 6.3

Fmoc strategy to introduce an ϵ -ACA- β -Ala- ϵ -ACA- β -Ala- ϵ -ACA- β -Ala (BEBEBE) spacer.

The BEBEBE spacer-grafted Si wafer was submerged in a gold colloidal solution for attaching primary Au NPs (~ 19 nm) to the surface of the Si wafer. And then the Si wafer was immersed in a solution of 4-aminobenzenethiol (4-ABT) in methanol, which acts as a Raman reporter and also a linker between Au NPs (**A** in Scheme 1). After that, the Si wafer was submerged into a gold colloidal solution again to introduce secondary Au NPs (~ 19 nm). The substrate (**B** in Scheme 1) was washed sufficiently by DI water and dried with blowing nitrogen gas. Finally, these gold clusters were detached by ultrasonication for 30 minutes in DI water.

Atomic force microscopy (AFM) image was obtained after primary assembly of Au NPs (Figure 1(a)). The structures of *ca.* 3-nm height correspond to BEBEBE spacer according to the report by Kim *et al.*⁸ and those of *ca.* 19-nm height could

be identified as Au NPs assembled on the BEBEBE spacers. This result confirms that the processes for grafting BEBEBE spacer to amine-functional group of the silane linker and immobilizing primary Au NPs onto the end of these BEBEBE spacers were successfully performed as intended. The SEM image shows distributions of primary Au NPs and clusters of Au NPs on Si wafer in Figure 1(b) and 1(c), respectively. The latter has higher density of particles and larger particles than the former.

The number densities of the single particles and clusters assembled on the substrate were calculated from analysis of SEM images (Table 1). After primary assembly of Au NPs, almost no clusters were observed and the number of single Au NPs was 14.5 in $1 \mu\text{m}^2$, indicating that the single particles were *ca.* 300 nm away from one another on average. In order to immobilize Au NPs homogeneously and separately one another, mixture of silane coupling reagents containing amine-functionality (3-APDMS) and non-amine functionality (ETMS) were used with an appropriate ratio (1:30), which could keep the proper distance between primary Au NPs acting as the core of the probe. After secondary assembly of Au NPs, the number of single Au NPs were 37.7 in $1 \mu\text{m}^2$. The increased density of particles is caused by additional assembly of Au NPs on the BEBEBE spacers that are still left unbound in the first assembly process. Number of clusters was 19.9 in $1 \mu\text{m}^2$ substrate on average, indicating most of primary Au NPs became clusters after secondary assembly.

After secondary assembly of Au NPs, 65.4% particles existed in monomers, and clusters were 34.6% (Figure 2). These clusters have 4-ABT molecules on their surface while single Au NPs adsorbed on the substrate during the secondary assembly do not. Among clusters fabricated on the substrate, dimers account for 88% and the clusters consisting of over 4 particles were merely about 2%. The Au nano-clusters are well separated and distributed evenly without forming extensively large clusters. This result could be achieved by formation of mixed monolayer of amine-functionality and non-amine functionality of silane linker on Si wafer and introduction of BEBEBE spacers to expose amine functional groups to Au NPs.

The dimer or clusters of Au NPs detached from solid substrate can be used as SERS probe for bio-multiplexed applications. Optical characterization of the SERS probe can be performed using the clustered Au NPs on Si wafer prior to detachment. An SERS intensity maps were obtained by measuring SERS spectra of 4-ABT molecule for $1.8 \mu\text{m} \times 1.8 \mu\text{m}$ area of the primary Au NPs-assembled substrate and the secondary Au NPs-assembled substrate. The intensity maps were constructed for the 1085-cm^{-1} band of 4-ABT as shown in Figure 3(a) and 3(b). Map analysis exhibited the average SERS intensity of the 1085-cm^{-1} band after secondary assembly was increased to 30.0 ± 9.2 (a.u.) from 1.8 ± 1.8 (a.u.). Figure 3(c) shows that the clusters have homogeneous SERS intensities. We could measure similar SERS intensity map in a larger area ($10 \mu\text{m} \times 10 \mu\text{m}$) with $1 \mu\text{m}$ step size (Figure S2). These results show that the solid sub-

Figure 2. Histogram of cluster size in collected area ($14 \mu\text{m}^2$).

Figure 3. (a) SERS intensity map for the 1085-cm⁻¹ band of 4-ABT adsorbed on the primary assembled Au NPs, (b) SERS intensity map for the 1085-cm⁻¹ band of 4-ABT adsorbed after secondary assembly of Au NPs, (c) histogram of SERS intensity at each spot in the SERS intensity maps (a) and (b), and (d) the bottom and top spectra, corresponding to (a) and (b), respectively are averaged over the scan area. The mapping area is 1.8 μm × 1.8 μm.

Figure 4. Effective area within 2-nm gap between Au NPs.

strates facilitated fabricating homogeneous and enhanced SERS probe. Average SERS intensity was increased by 17 times after clustering. This could be explained only due to SERS ‘hot spot’ generation by clustering NPs. Since Raman reporter molecules (4-ABT) were introduced before formation of secondary assembly, thus they were placed on primary Au NPs. Although the numbers of single Au NPs were increased with formation of clusters after secondary assembly, the single particles additionally adsorbed on the substrate in that step did not have 4-ABT molecules on their surface and hence had no contribution to SERS intensity.

To examine these clusters as SERS probe, the enhancement factor (EF) of fabricated clusters was measured experimentally. The EF value could be calculated as following expression:

$$EF = (I_{\text{SERS}}/N_{\text{SERS}})/(I_{\text{NRS}}/N_{\text{NRS}})$$

where the I_{SERS} and I_{NRS} correspond to intensity of a vibrational mode in the SERS spectrum and normal Raman scattering (NRS) spectrum, respectively. The N_{SERS} and N_{NRS} correspond to number of molecules that are laser-illuminated in the measurement of SERS and NRS spectra. I_{NRS} and I_{SERS} were 260 and 30 (a.u.) respectively. Since the number

Figure 5. TEM images of (a) single Au NPs, (b) dimer, and (c) clusters after detached into solution by ultrasonication of solid substrate. Scale bar is 50 nm.

of 4-ABT molecules illuminated by the laser light in bulk sample (N_{NRS}) and the number of 4-ABT molecules adsorbed on Au NPs within the laser light (N_{SERS}) were calculated to be 3.2×10^{10} and 1.0×10^5 respectively, the EF value was determined to 3.6×10^4 . Especially when N_{SERS} was determined by calculation of number of 4-ABT molecules adsorbed on effective area within 2-nm gap junction between Au NPs as shown in Figure 4, so called ‘hot spot’ site, the EF value was calculated approximately to be of the order of 10^6 .

Finally, we could obtain these clustered SERS probes in solution phase by ultrasonication as shown in Figure 5. Single Au NPs, dimers, and larger clusters were observed in TEM images, however, further heavy aggregations were not shown. Thus, successfully controlled clustering and purification for homogeneous SERS probes was confirmed benefiting from the solid substrate.

In summary, we fabricated small clusters of gold nanoparticles by using solid-supported aggregation of gold nanoparticles. The fabricated Au nanoclusters consisting mainly of dimers showed homogeneous characteristics in cluster size and SERS intensity. The SERS enhancement of 4-ABT molecules in an effective area within 2-nm gap appeared to be approximately 10^6 . Detachment process by ultrasonication was successively carried out in order to use the nanoclusters as SERS probes. The possibility of these clusters as SERS probe was proved in terms of signal and cluster size.

Experimental

Synthesis of Gold Nanoparticles. A 50 mg of HAuCl_4 was dissolved in DI water (50 mL) followed by adding 1 mL of citrate aqueous (1 w/w%). The temperature during the reaction was maintained at 100 °C for 30 min, and then the colloidal solution was kept in room temperature for 24 h with stirring, followed by storage at 4 °C for long term stability.

Pretreatment of Si Wafer. Silicon wafers were ultrasonicated for 20 minutes in acetone and in DI water serially to clean the surface. And the surface of the Si wafer was activated by soaking in piranha solution (a mixture of H_2SO_4 and 30 v/v% H_2O_2 (3:1)) for 15 minutes, and then rinsed with DI water several times, and then dried by blowing nitrogen gas. The mixed self-assembled monolayer (SAM) was formed by dipping the pretreated Si wafer in a mixed solution of 3-aminopropyltrimethylethoxysilane (3-APDMS)

and ethoxytrimethylsilane (ETMS) in anhydrous ethanol (total 1 v/v%) with the ratio of 1:30 for 2 h. The excess chemicals were removed by ultrasonication of the Si wafer in ethanol for 20 minutes followed by rinsing with ethanol and DI water several times, and then the substrate was dried with nitrogen gas. Additional spacing linkers composed of β -alanine (β -Ala; B) and ϵ -aminocaproic acid (ϵ -ACA; E) pairs were used for easier immobilization of gold nanoparticles from solution to substrate. Fmoc- β -alanine (Fmoc- β -Ala) and Fmoc- ϵ -aminocaproic acid (Fmoc- ϵ -ACA) were coupled three times in turn to the Si surface through the Fmoc strategy to introduce an ϵ -ACA- β -Ala- ϵ -ACA- β -Ala- ϵ -ACA- β -Ala (BEBEBE) spacer.⁸ Briefly, the silane-coated Si wafer was immersed into the preactivated solution (with 3 equiv. of 1-hydroxybenzotriazole (HOBt), benzotriazol-1-yloxy-tris(dimethylamino) phosphonium hexafluorophosphate (BOP), diisopropylethylamine (DIPEA)) of each Fmoc-amino acid in N-methylpyrrolidone (NMP) for 3 h. And then Si wafer was washed by immersing in NMP with shaking for 15 minutes and then rinsed with NMP. Fmoc-deprotection to reveal the amine-functionality was performed with 20 v/v% piperidine in NMP for 30 min at 25 °C.

Fabrication of Gold Nanoclusters on Si Wafer. The BEBEBE spacer-grafted Si wafer was submerged in a gold colloidal solution for attaching Au NPs (~19 nm) to the surface of the Si wafer for 90 minutes at room temperature. And then the Si wafer was immersed in a solution of 4-ABT in methanol (1 mM) for 1 h at 25 °C followed by 90 minutes dipping the Si wafer into a gold colloidal solution again for formation of Au NP clusters. The substrate was washed sufficiently by the solvent used in that step and dried with blowing nitrogen gas after the each processes.

Measurements and Characterization. UV-Visible extinction spectrum of gold colloid was measured with a UV-Vis spectrometer (Varian, Cary 300 Bio). Atomic Force Microscope (Park systems, XE-100) was utilized to confirm the self-assembly of additional spacer and gold nanoparticles. Nano-scale images also were obtained with field-emission scanning electron microscope (Carl Zeiss, SUPRA 55VP) operated at 2.0 kV and transmission electron microscope (JEOL, JEM1010) operated at 80.0 kV. For the Raman measurement, a micro-Raman system (JY-Horiba, LabRam 300) was utilized to characterize the SERS activity of the prepared gold nanoclusters. The signal was collected by a $\times 100$ objective lens (Olympus, 0.90 NA) with the back-scattering

geometry equipped with a thermoelectrically cooled (−70 °C) CCD detector. The 647 nm laser line from Kr ion laser (Coherent, Innova 300C) was used for an excitation source. The laser spot diameter and penetration depth of the focused laser beam were ~1.1 μ m and ~6 μ m, respectively. At each measurement, acquisition time was 1 s, and the sample power was about 2 mW.

Acknowledgments. This research was supported by the Pioneer Research Center Program through the National Research Foundation of Korea funded by the Ministry of Science, ICT & Future Planning (Grant Number 2012-0000460).

References

- (a) Kneipp, K.; Wang, Y.; Kneipp, H.; Perelman, L. T.; Itzkan, I.; Dasari, R.; Feld, M. S. *Phys. Rev. Lett.* **1997**, 78, 1667. (b) Nie, S.; Emory, S. R. *Science* **1997**, 275, 1102.
- Lim, D. K.; Jeon, K. S.; Kim, H. M.; Nam, J. M.; Suh, Y. D. *Nat. Mater.* **2010**, 9, 60.
- (a) Shafer-Peltier, K. E.; Haynes, C. L.; Glucksberg, M. R.; Van Duyne, R. P. *J. Am. Chem. Soc.* **2003**, 125, 588. (b) Li, J. F.; Huang, Y. F.; Ding, Y.; Yang, Z. L.; Li, S. B.; Zhou, X. S.; Fan, F. R.; Zhang, W.; Zhou, Z. Y.; Wu de, Y.; Ren, B.; Wang, Z. L.; Tian, Z. Q. *Nature* **2010**, 464, 392.
- (a) Grubisha, D. S.; Lipert, R. J.; Park, H. Y.; Driskell, J.; Porter, M. D. *Anal. Chem.* **2003**, 75, 5936. (b) Qian, X.; Peng, X. H.; Ansari, D. O.; Yin-Goen, Q.; Chen, G. Z.; Shin, D. M.; Yang, L.; Young, A. N.; Wang, M. D.; Nie, S. *Nat. Biotechnol.* **2008**, 26, 83.
- Su, X.; Zhang, J.; Sun, L.; Koo, T. W.; Chan, S.; Sundararajan, N.; Yamakawa, M.; Berlin, A. A. *Nano Lett.* **2005**, 5, 49.
- (a) Kim, J. H.; Kim, J. S.; Choi, H.; Lee, S. M.; Jun, B. H.; Yu, K. N.; Kuk, E.; Kim, Y. K.; Jeong, D. H.; Cho, M. H.; Lee, Y. S. *Anal. Chem.* **2006**, 78, 6967. (b) Yu, K. N.; Lee, S. M.; Han, J. Y.; Park, H.; Woo, M. A.; Noh, M. S.; Hwang, S. K.; Kwon, J. T.; Jin, H.; Kim, Y. K.; Hergenrother, P. J.; Jeong, D. H.; Lee, Y. S.; Cho, M. H. *Bioconjugate Chem.* **2007**, 18, 1155. (c) Jun, B. H.; Noh, M. S.; Kim, J.; Kim, G.; Kang, H.; Kim, M. S.; Seo, Y. T.; Baek, J.; Kim, J. H.; Park, J.; Kim, S.; Kim, Y. K.; Hyeon, T.; Cho, M. H.; Jeong, D. H.; Lee, Y. S. *Small* **2010**, 6, 119. (d) Kang, H.; Jeong, S.; Park, Y.; Yim, J.; Jun, B. H.; Kyeong, S.; Yang, J. K.; Kim, G.; Hong, S.; Lee, L. P.; Kim, J. H.; Lee, H. Y.; Jeong, D. H.; Lee, Y. S. *Adv. Funct. Mater.* **2013**, 23, 3719.
- Yoon, J. H.; Lim, J.; Yoon, S. *ACS Nano* **2012**, 6, 7199.
- Kim, D. H.; Shin, D. S.; Lee, Y. S. *J. Pept. Sci.* **2007**, 3, 625.
- (a) Fang, Y.; Seong, N. H.; Dlott, D. D. *Science* **2008**, 321, 388. (b) Li, W.; Camargo, P. H.; Lu, X.; Xia, Y. *Nano Lett.* **2009**, 9, 485.