

[Electronic Supplementary Information]

**The controlled synthesis of plasmonic nanoparticle clusters for efficient
surface-enhanced Raman scattering platforms**

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1. Experimental and calculation details

Chemicals and materials. AgNO₃ (Sigma-Aldrich), poly(vinyl pyrrolidone) (PVP, M_w = 55,000, Sigma-Aldrich), trisodium citrate dihydrate (Aldrich), HAuCl₄·3H₂O (Sigma-Aldrich), H₂O₂ (30 wt%, Daejung Chemicals & Metals Co.), NaBH₄ (Sigma-Aldrich), KI (Sigma-Aldrich), ascorbic acid (AA, Daejung Chemicals & Metals Co.), NaBr (Sigma-Aldrich), citric acid (Acros), 1,4-phenylene diisocyanide (1,4-PDI, Aldrich), 4-nitrobenzenethiol (4-NBT, TCI), rhodamine 6G (R6G, TCI), crystal violet (CV, Sigma-Aldrich), Nile blue (NB, Sigma-Aldrich), and phosphate-buffered saline (10× concentrate, Sigma-Aldrich) were used as received. Other chemicals, unless specified, were reagent grade and highly purified water with a resistivity of greater than 18.0 MΩ·cm was used in the preparation of aqueous solutions.

Synthesis of Ag nanoprisms and Ag nanooblates. In a typical synthesis of Ag nanoprisms, 25.5 mL of deionized water and 0.6 mL of AgNO₃ solution (5 mM) were mixed in a 100 mL Erlenmeyer flask. To this solution were added 1.8 mL of PVP solution (5 mg/mL) and 1.8 mL of sodium citrate solution (30 mM) with vigorous stirring at room temperature. After 3 min, 60 μL of H₂O₂ and 300 μL of NaBH₄ solution (100 mM) were injected quickly into the reaction solution. Ag nanoprisms were formed after 30 min. Prepared Ag nanoprisms could be used for 2 months. Ag nanooblates were prepared by adding 0.2 mL of NaBr solution (50mM) to 3.8 mL of Ag nanoprism solution.^{S1}

Synthesis of large size Ag nanoprisms. Large size Ag nanoprisms were prepared by growing pre-formed Ag nanoprisms.^{S2} To prepare seed solution, Ag nanoprisms was collected by centrifugation of 25 mL of Ag nanoprism solution (4,300 rpm for 40 min) and then re-dispersed in 40 mL of deionized water. Into a 100 mL Erlenmeyer flask, 10 mL of seed solution, 0.375 mL of AA (100 mM), 0.125 mL of trisodium citrate (75 mM) were added sequentially with stirring (600 rpm). Then, a separate solution prepared by mixing 20 mL of 1 mM AgNO₃, 0.125 mL of 0.1 M citric acid, and 0.1 mL of 1.5 mM sodium citrate was injected into the reaction mixture by using a syringe pump at a rate of 0.2 mL/min.

Synthesis of AuNPCs. In the standard synthesis of AuNPCs, 1 mL of sodium citrate (60 mM), 1 mL of deionized water, and 2 mL of 10 mg/mL PVP were mixed in a 10 mL glass vial at room temperature. To this solution, 0.1 mL of 10 mM HAuCl₄·3H₂O and 20 μL of KI (50 mM) were added. And then 0.2 mL of 200 mM H₂O₂ and 0.1 mL of 100 mM AA were added into the solution. Into this reaction mixture, 0.2 mL of Ag nanoprism solution (aged for 3 days) was quickly injected. The formed AuNPCs were collected and purified by a repeated washing with de-ionized water by centrifugation (3,400 rpm, 10 min, three times).

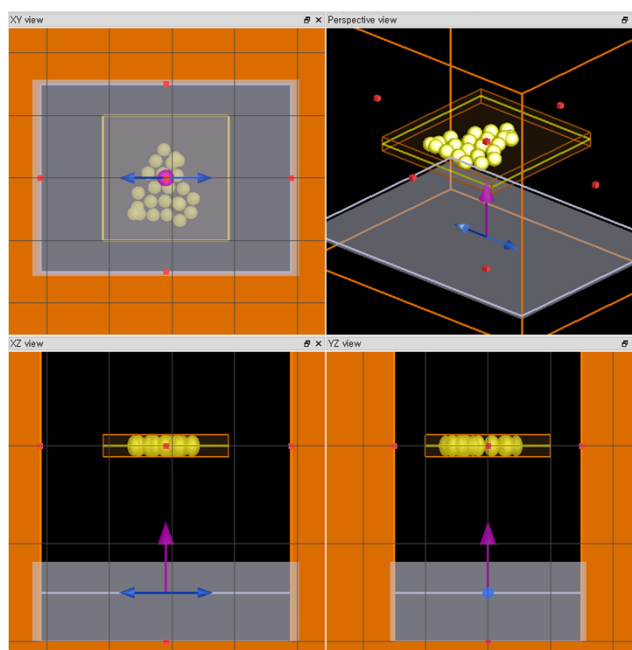
Synthesis of AuNPs. Au NPs were prepared by following the literature with a slight modification.^{S3} In a 250 mL round-bottom flask equipped with a condenser, 100 mL of 1 mM HAuCl₄·3H₂O was heated at 100 °C with vigorous stirring in an oil bath. After the rapid addition of 10 mL of 38.8 mM sodium citrate to the solution, the color of the solution

changed from pale-yellow to red-brown. Heating was continued for 10 min, and then oil bath was removed. The solution was further stirred for 15 min. For surface-enhanced Raman scattering (SERS) measurements, 6 mL of AuNP solution and 19.28 mL of 5.68 mg/mL PVP were mixed in a 30 mL glass vial and shaken overnight to exchange the surfactant. Before mixing with Raman dye, AuNPs were purified by centrifugation (12,000 rpm for 10 min) and washing with deionized water three times.

Characterization. The extinction spectra were recorded using an UV-vis-NIR absorption spectrometer (Shimadzu UV-3600). Scanning electron microscopy (SEM) images were obtained with a field-emission scanning electron microscope (FESEM, FEI-INSPECT F50). Transmission electron microscopy (TEM) images were taken using a JEOL JEM-2010 transmission electron microscope operating at 200 kV or a FEI Tecnai G2 F30 Super-Twin transmission electron microscope operating at 300 kV after placing a drop of the hydrosol on a carbon-coated Cu grid (200 mesh). The composition of AuNPCs was determined by inductively coupled plasma-atomic emission spectroscopy (ICP-AES, OPTIMA 3300DV). The size of AuNPCs in solution was measured by dynamic light scattering (DLS) method using a Malvern Zetasizer (Nano ZS). Raman spectra were obtained on a Raman microscope equipped with an integral microscope (Olympus BX 43), a spectrometer (Acton Spectra Pro 2300i), and a thermoelectrically cooled 1024×127 pixel charge coupled device (CCD) detector (ANDOR, DV401A-BV). The 632.8 nm line of an air-cooled He/Ne laser was used as an excitation source. A glass capillary tube with an inner and outer diameter of 1.1-1.2 and 1.5-1.6 mm, respectively, was used for obtaining Raman spectra. The laser beam (2 mW) was focused inside the glass capillary with an objective lens ($\times 50$, NA = 0.55). The holographic grating (1200 grooves/mm) and the slit allowed the spectral resolution to be 1 cm^{-1} . The Raman bands of indene at 1018.3, 1205.6, and 1610.2 cm^{-1} were used to calibrate the spectrometer. To compare the SERS efficacies of the prepared AuNPCs and AuNPs, the particle concentration (the definition of “particle” in the case of the AuNPCs is the individual particles that make up the clusters) was adjusted to a similar value for each sample, i.e., 3.98×10^{13} colloids/ cm^3 , which was estimated based on the total Au content measured by ICP-AES and their average particle size. This could be achieved through adjusting the concentration of Au precursor for the preparation of AuNPCs and AuNPs to the same value, 0.216 mM. Since the average particle sizes of the prepared AuNPs and the NPs that make up the AuNPCs were almost same and the yields of two samples were $\sim 100\%$, adjusting the concentration of Au precursor to the same value for both samples should give a similar particle concentration and thus a similar surface area for each sample.

Calculation of the analytical SERS enhancement factor (EF). The analytical EF of the AuNPCs was calculated by using the following equation: $EF = (I_{\text{SERS}}/I_{\text{Raman}})/(C_{\text{SERS}}/C_{\text{Raman}})$,^{S4,S5} where I_{SERS} and I_{Raman} are the SERS intensity of the Raman dyes measured with the AuNPCs (Fig. 2a-d) and the Raman scattering intensity of the Raman dyes in solution, respectively, and C_{SERS} and C_{Raman} are the concentration of the Raman dyes to obtain the SERS and Raman spectra, respectively. I_{SERS} and I_{Raman} were measured at 612, 1329, and 1615 cm^{-1} for R6G, 4-NBT, and CV respectively.

Finite-difference time domain (FDTD) simulations. The simulated electromagnetic field distributions of the AuNP and AuNPC were calculated by using the three-dimensional FDTD method^{S6} with the commercial FDTD-LumericalTM simulation package. The frequency-dependent dielectric constants of Au have been taken from the literature.^{S7} The electromagnetic field distribution was then acquired with the mesh size of 0.2 nm at the excitation wavelength of $\lambda_{\text{ex}} = 632.8$ nm. The geometric model for AuNPC was constructed based on the experimentally observed structure of AuNPCs (Fig. 1b) (see the following schematic).



[The geometric model for AuNPC]

References

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- (S2) Q. Zhang, Y. Hu, S. Guo, J. Goebel and Y. Yin, *Nano Lett.*, 2010, **10**, 5037.
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2. Supplemental experiment results

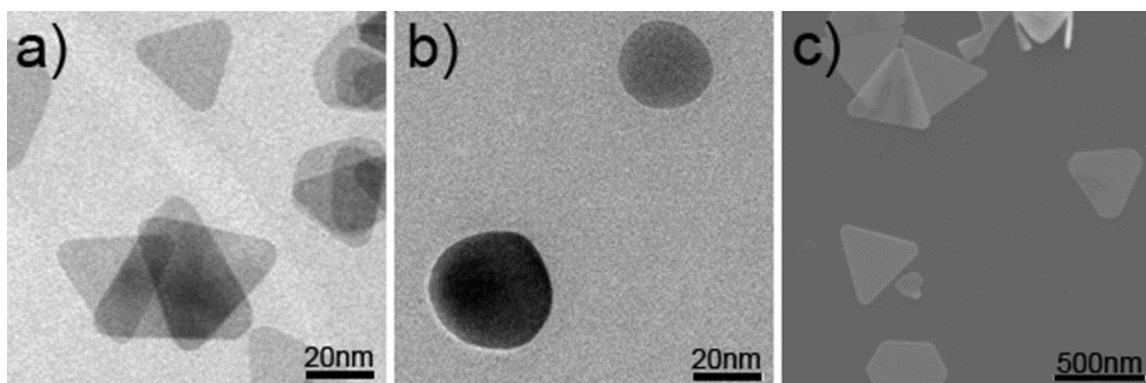


Fig. S1 TEM images of (a) Ag nanoprisms (average edge length = 35 ± 8 nm) and (b) oblate-shaped AgNPs (average diameter = 22 ± 5 nm). (c) SEM image of large Ag nanoprisms (average edge length = 522 ± 64 nm).

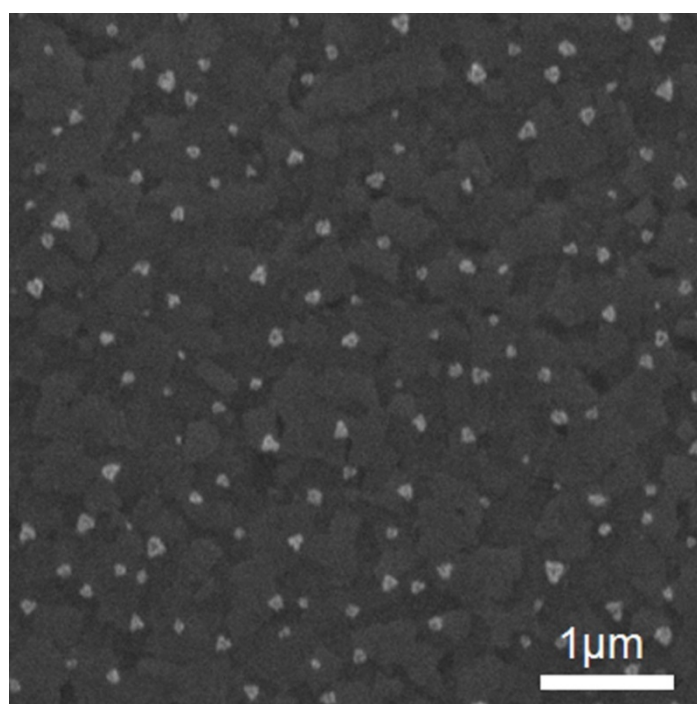


Fig. S2 Low-magnification SEM image of the AuNPCs. The observed polydispersity in size of the AuNPCs originated from that of the sacrificial Ag nanoprism templates. Some structures that look like small fragments are small-sized nanoparticle clusters.

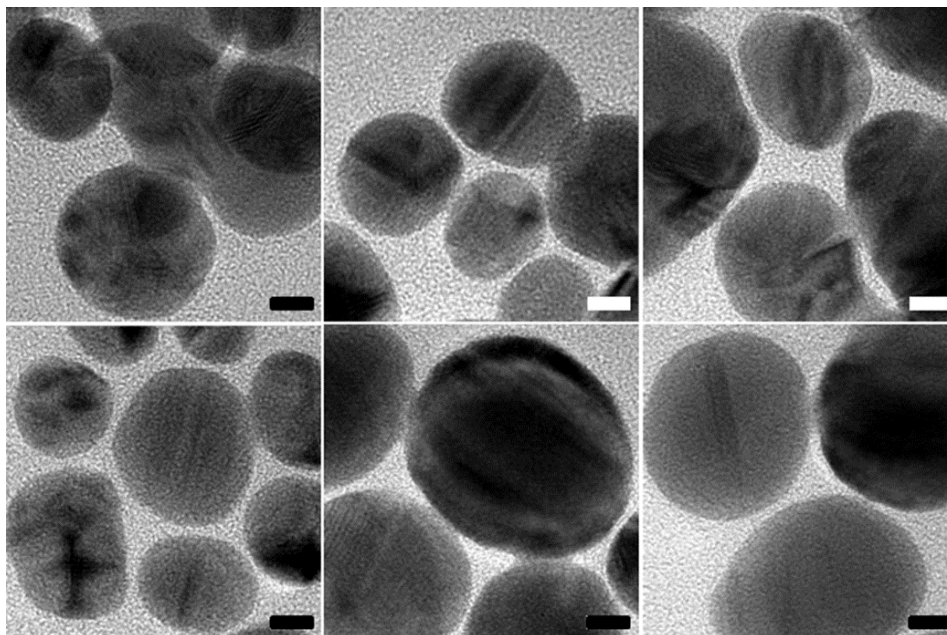


Fig. S3 High-magnification TEM images of the junction points between particles within the AuNPCs. The scale bars indicate 5 nm. Although some of the constituent particles contact with their neighboring particles, most of the particles within the clusters maintain the interparticle gaps between them.

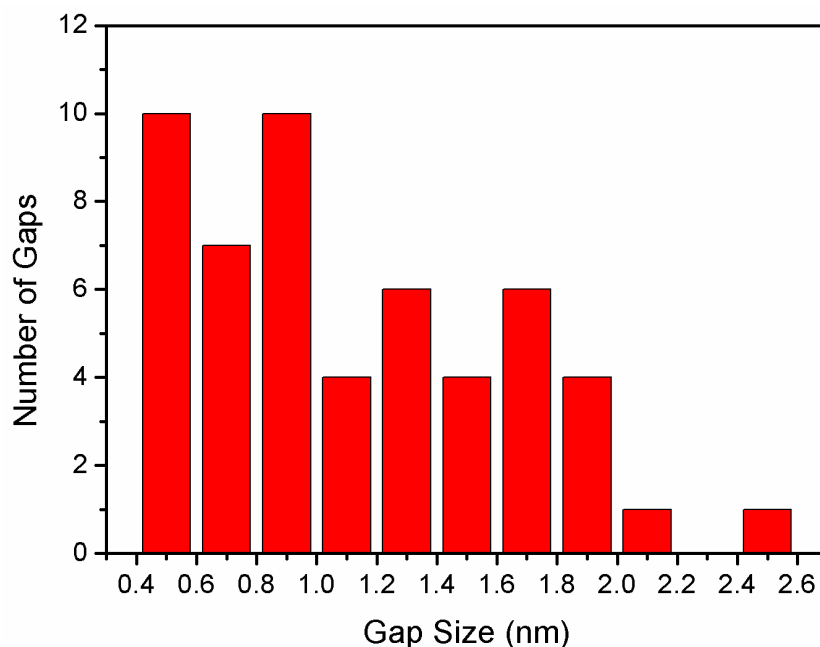


Fig. S4 The distribution of gap sizes between NPs in the prepared AuNPCs, which was determined by TEM. The average gap size is 1.1 nm.

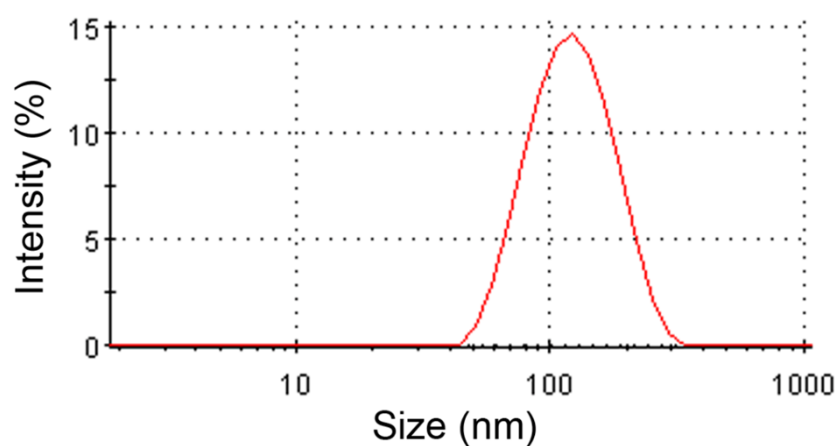


Fig. S5 The dynamic light scattering (DLS) data of the AuNPC solution. The Z-average diameter of the AuNPCs is 106.7 nm with a polydispersity index (PDI) of 0.166, indicating that the structures are assembled in solution and not during deposition.

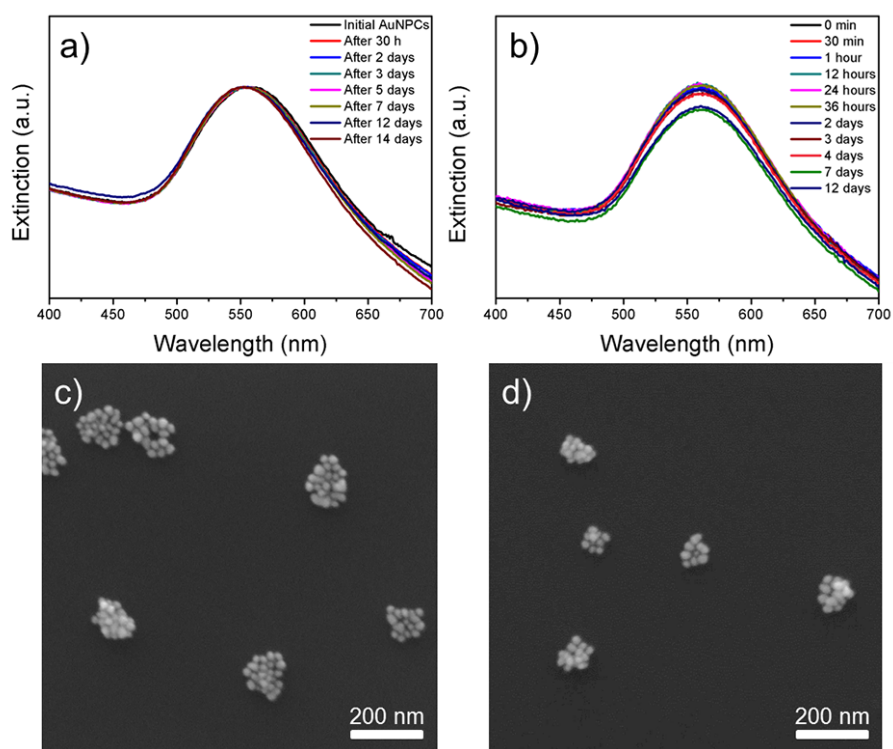


Fig. S6 Time-dependent UV-vis extinction spectra of the AuNPCs in (a) water and (b) PBS solution. SEM images of the AuNPCs after (c) 14 days in water and (d) 12 days in PBS solution.

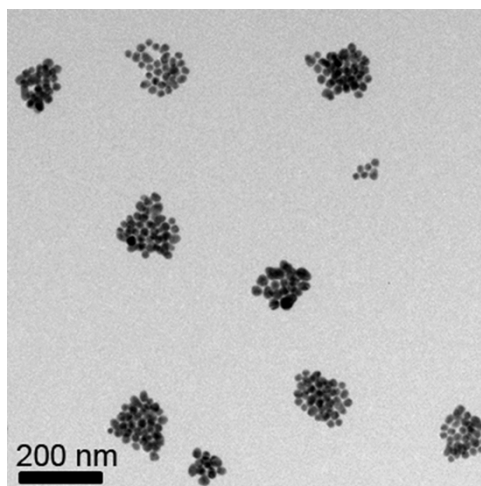


Fig. S7 TEM image of the AuNPCs after the sonication for 5 h with an ultrasonic cleaner (JAC Ultrasonic 4020, frequency = 40 kHz, power = 320 W).

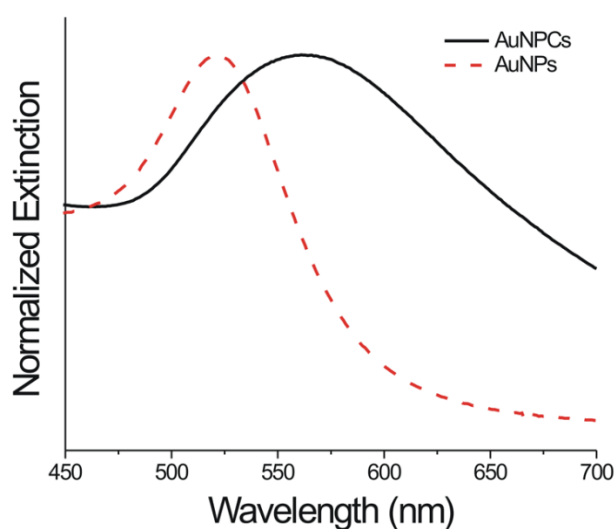


Fig. S8 Normalized UV-vis extinction spectra of AuNPCs and AuNPs with a similar average particle size (20 nm, Fig. S18) to that of the particles in the AuNPCs.

The maximum SPR peak positions of the AuNPCs and AuNPs are 561 and 521 nm, respectively. The SPR peak of the AuNPCs was broadened and red-shifted in comparison to that of the AuNPs. This can be attributed to the coupling of dipole plasmon resonance of constituent AuNPs. The extent of SPR peak shift of the AuNPCs compared to the AuNPs was smaller than that observed in the previous report (ref. 10). This can be attributed to the difference in dielectric medium, substrate effect, and difference in the size of nanoparticle assembly (refs.: G. C. Schatz, *J. Mol. Struct. (THEOCHEM)*, 2001, **573**, 73; J. J. Storhoff, A. A. Lazarides, R. C. Mucic, C. A. Mirkin, R. L. Letsinger and G. C. Schatz, *J. Am. Chem. Soc.*, 2000, **122**, 4640).

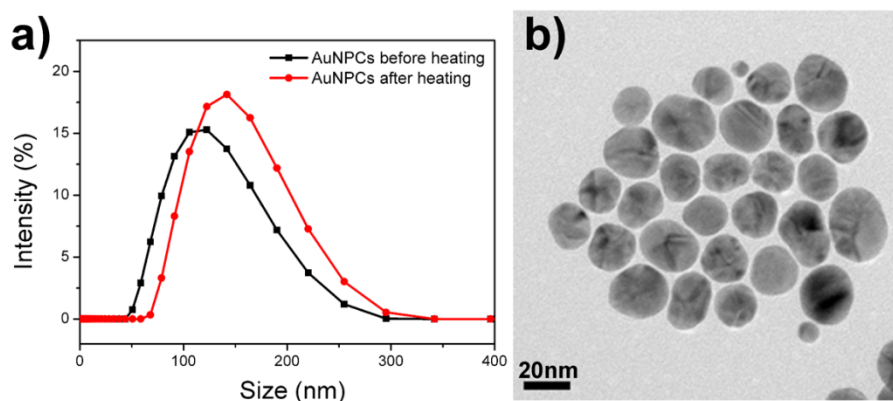


Fig. S9 (a) DLS data and (b) TEM image of the AuNPCs after the heat treatment at 85 °C for 12 h. When the AuNPC solution was heated at 85 °C for 12 h, the DLS-determined Z-average diameter of the AuNPCs in solution was increased to 134 nm, while their cluster structure was maintained overall. The TEM image of the AuNPCs after the heat treatment unambiguously demonstrates that the AuNPCs are composed of isolated AuNPs.

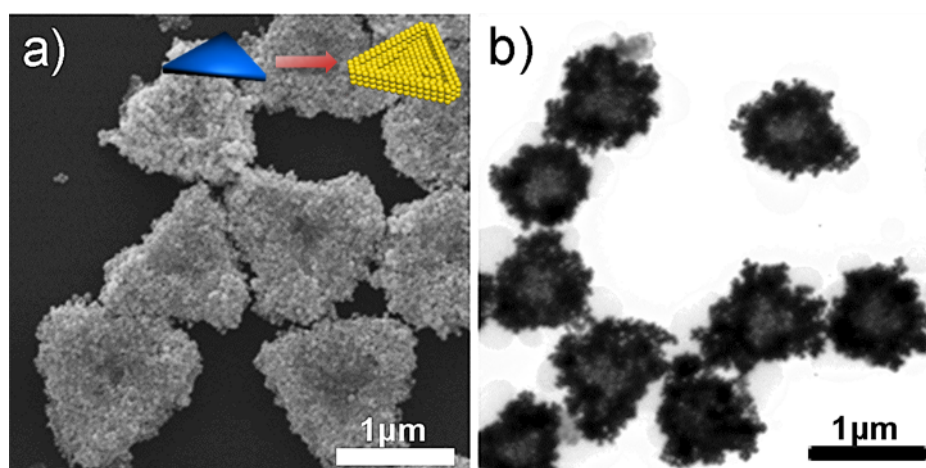


Fig. S10 (a) SEM and (b) TEM images of AuNPCs prepared from large Ag nanoprisms.

As shown in the above images, when large Ag nanoprisms (average edge length = 522 ± 64 nm, Fig. S1c) were used as sacrificial templates under otherwise similar synthesis conditions with the standard procedure, large triangular AuNPCs (average cluster size = 841 ± 112 nm, average AuNP size = 37 ± 6 nm) were produced. Interestingly, a concave feature was observed in the AuNPCs formed from the large Ag nanoprisms. This can be attributed to the fact that assembled layers of NPs at the center region of AuNPCs are thinner than those at the edges of AuNPCs. In the case of large Ag nanoprisms, the relative amount of Au precursors to Ag was smaller than those in other AgNP cases. Therefore, the opportunity of forming AuNPs at the highly energetic corners and edges of Ag nanoprisms is higher than that at the center, thus leading to the observed difference in the thickness of assembled layers of NPs.

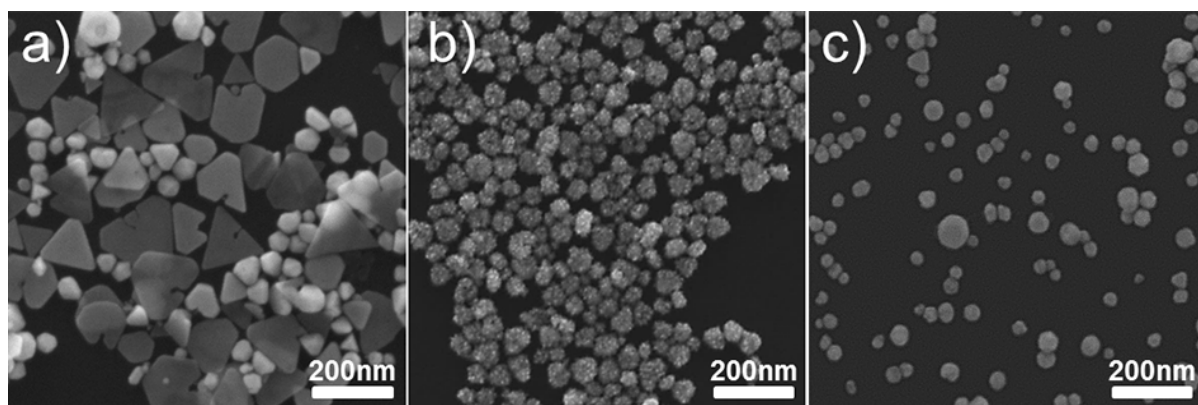


Fig. S11 SEM images of Au nanostructures prepared in the absence of (a) KI and AA, (b) KI, and (c) AA.

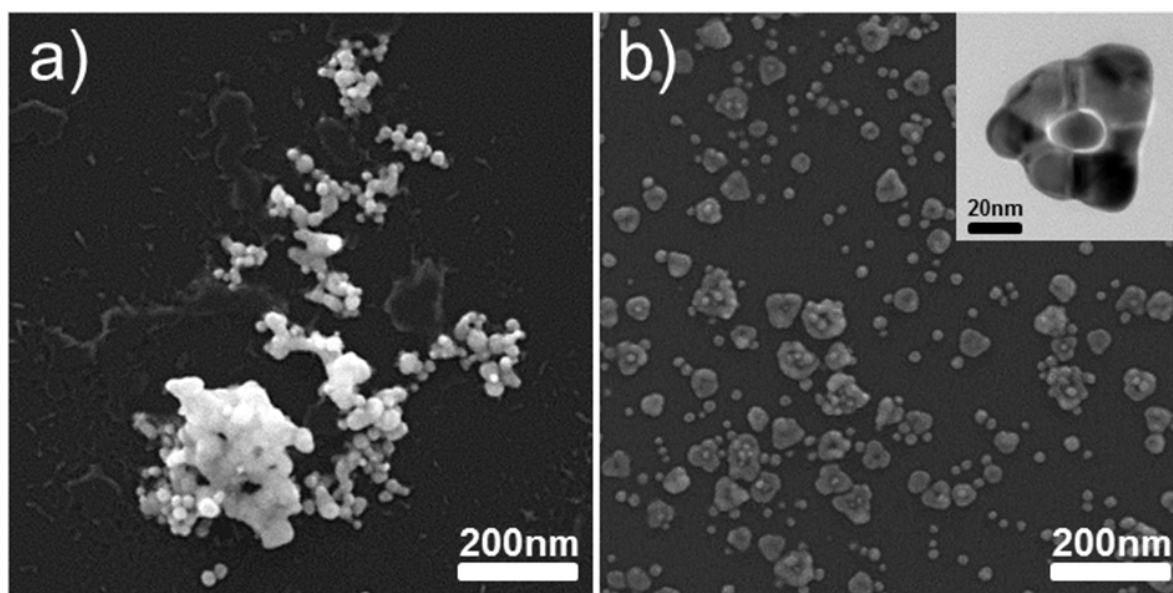


Fig. S12 SEM images of Au nanostructures prepared in the absence of (a) PVP and (b) citrate. High-magnification TEM image of a single Au nanostructure prepared in the absence of citrate is shown in the inset of (b).

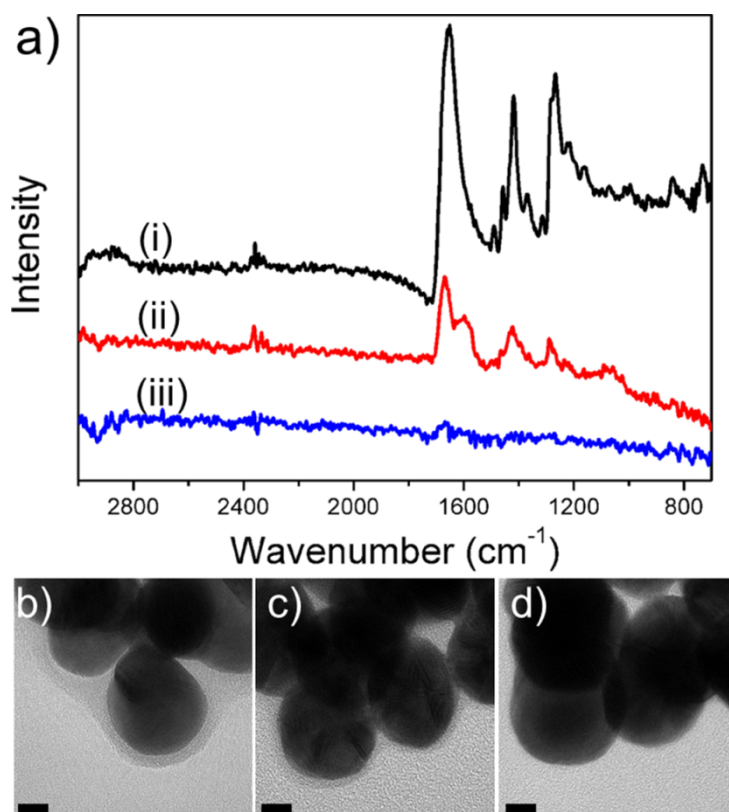


Fig. S13 (a) FTIR spectra of (i) PVP and AuNPCs after the removal of PVP by (ii) one and (iii) three times washing with de-ionized water by centrifugation (3,400 rpm, 10 min). TEM images of AuNPCs after the (b) one, (c) two, and (d) three times washing. The scale bars indicate 5 nm.

The above FTIR spectra and TEM images of AuNPCs clearly show that stabilizing agents could be effectively removed by a repeated washing with de-ionized water by centrifugation, while the assembly structure of AuNPCs was almost maintained.

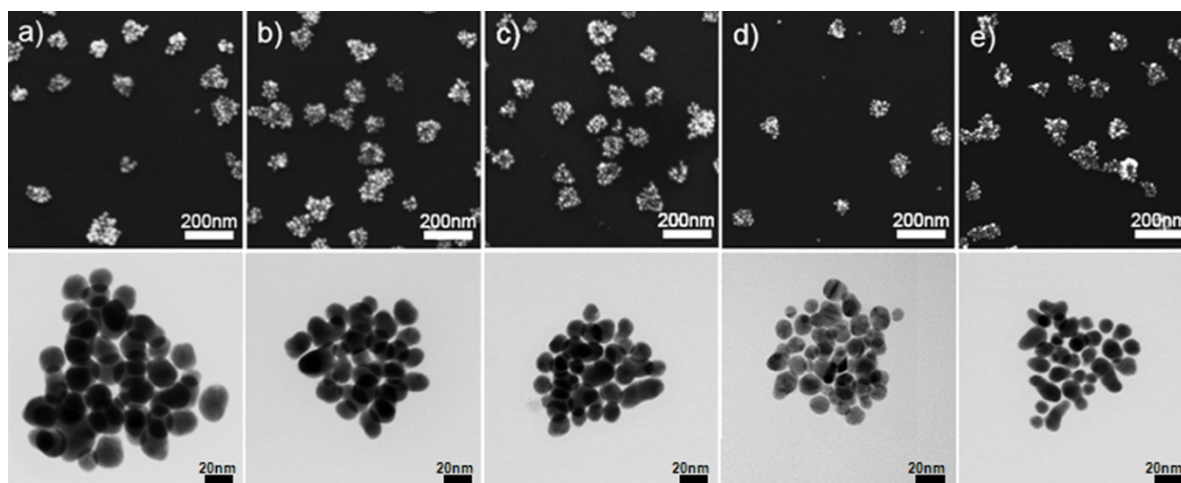


Fig. S14 SEM (top panel) and TEM (bottom panel) images of NPCs prepared with the Au:Ag ratios of (a) 50:1, (b) 25:1, (c) 16.7:1, (d) 12.5:1, and (e) 10:1. The average cluster sizes are (a) 98.7 ± 24 , (b) 88.3 ± 19 , (c) 86.5 ± 20 , (d) 84.4 ± 17 , and (e) 76.5 ± 17 nm. The average NP sizes are (a) 21.1 ± 3 , (b) 20.2 ± 3.2 , (c) 19.7 ± 3.5 , (d) 17.1 ± 3.4 , and (e) 15.7 ± 3.6 nm. The ICP-AES-determined Ag molar ratios in AuNPCs are (a) 1.8, (b) 3, (c) 4.8, (d) 6.5, and (e) 16.8%.

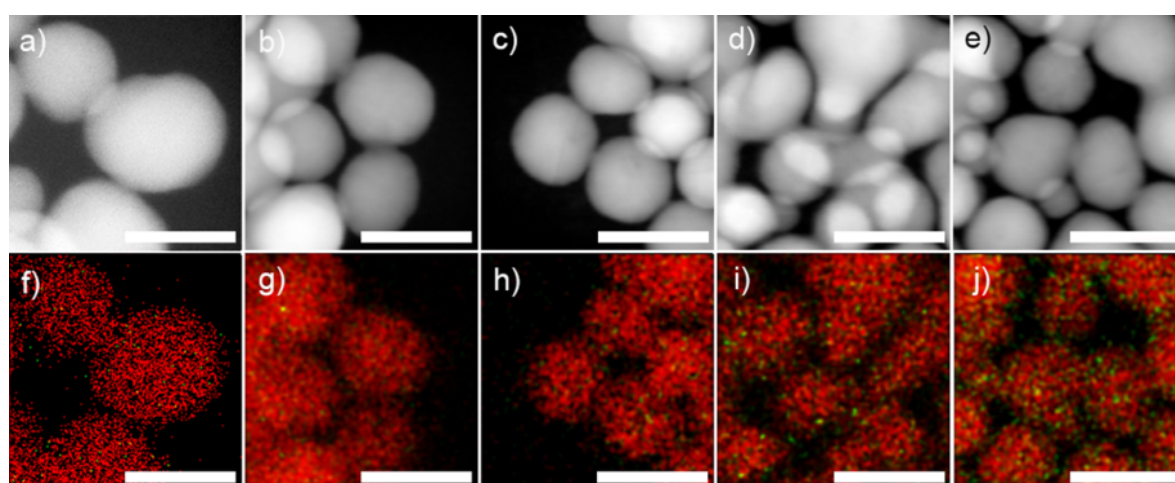


Fig. S15 (a-e) High-angle annular dark-field scanning TEM images and (f-j) corresponding energy dispersive X-ray spectroscopy (EDS) elemental mapping images (Au: red, Ag: green) of NPCs prepared with the Au:Ag ratios of (a,f) 50:1, (b,g) 25:1, (c,h) 16.7:1, (d,i) 12.5:1, and (e,j) 10:1. The scale bars indicate 20 nm.

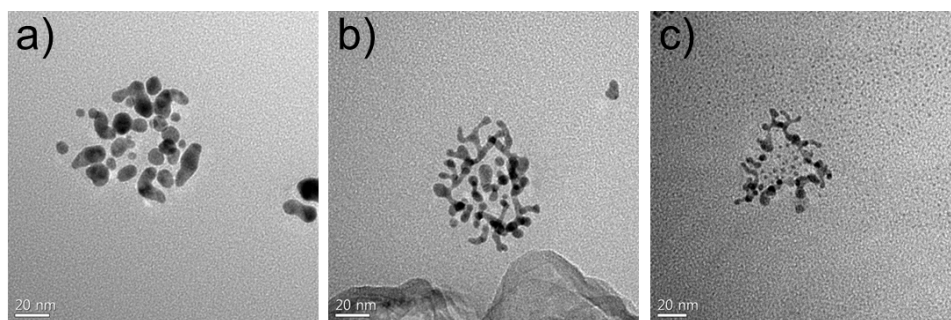


Fig. S16 TEM images of Au nanostructures prepared with the Au:Ag ratios of (a) 5:1, (b) 2.5:1, and (c) 1:2.

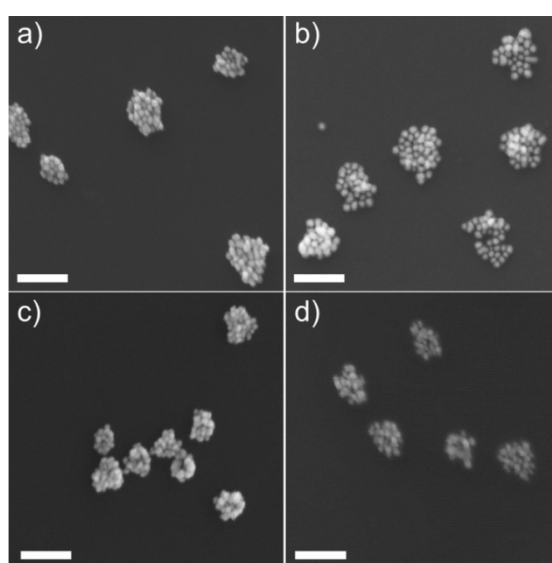


Fig. S17 SEM images of AuNPCs prepared by type II method with (a) R6G, (b) 4-NBT, (c) CV, and (d) NB. The scale bars indicate 200 nm.

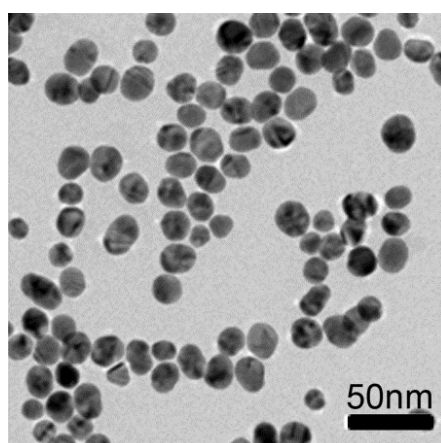


Fig. S18 TEM image of AuNPs with an average particle size of 20 nm.

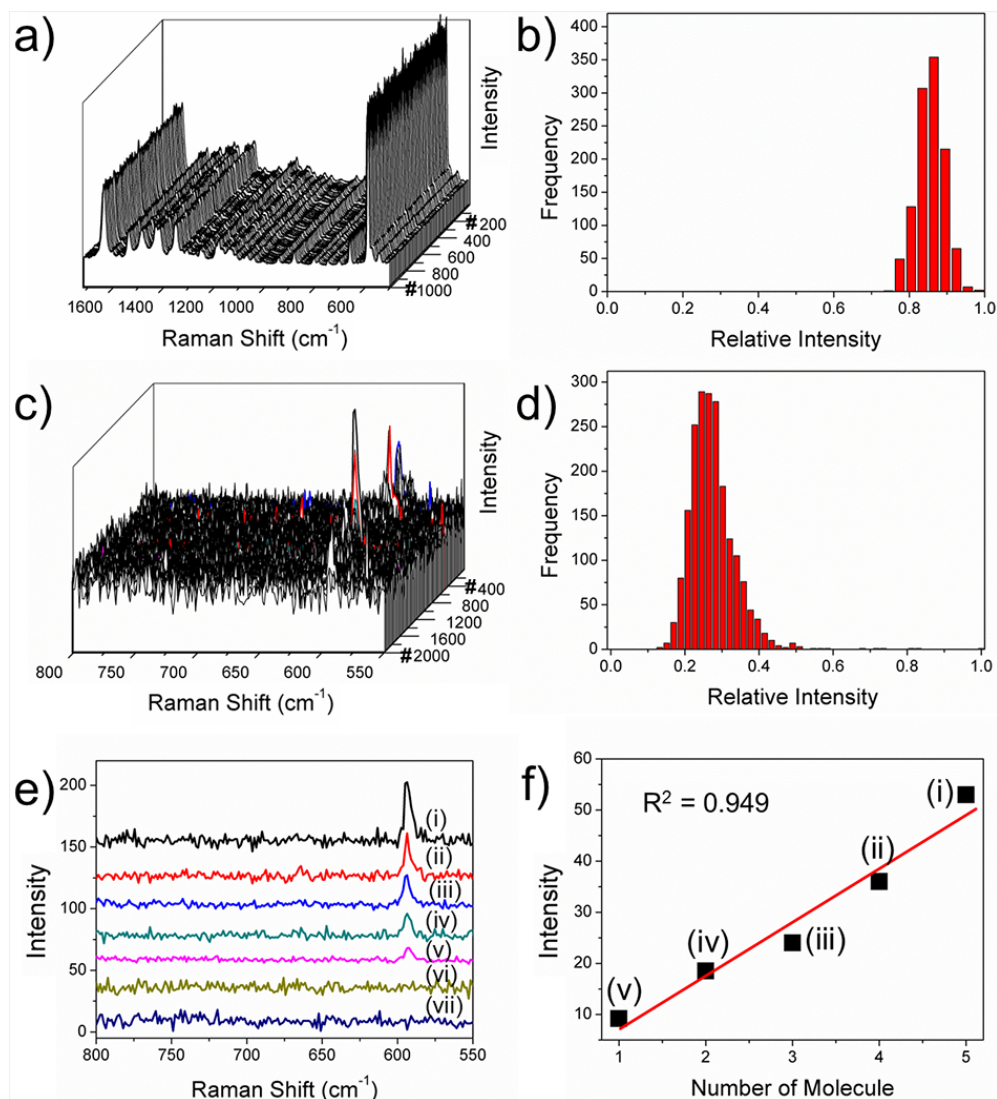


Fig. S19 Time-dependent SERS spectra of (a) 1×10^{-7} and (c) 5×10^{-11} M NB obtained with the AuNPCs (acquisition time = 100 ms, time interval = 120 ms). Statistical analyses of SERS intensities at 592 cm^{-1} for (b) 1×10^{-7} and (d) 5×10^{-11} M NB. (e) Representative SERS spectra of 5×10^{-11} M NB taken from (c): (i) # 1370, (ii) # 505, (iii) # 278, (iv) # 523, and (v) # 1612 spectrum. Spectra of SERS blinking and AuNPCs without NB are shown in (vi) and (vii), respectively. (f) SERS intensities as a function of the number of NB molecules. Intensities were measured from the spectra shown in (e).

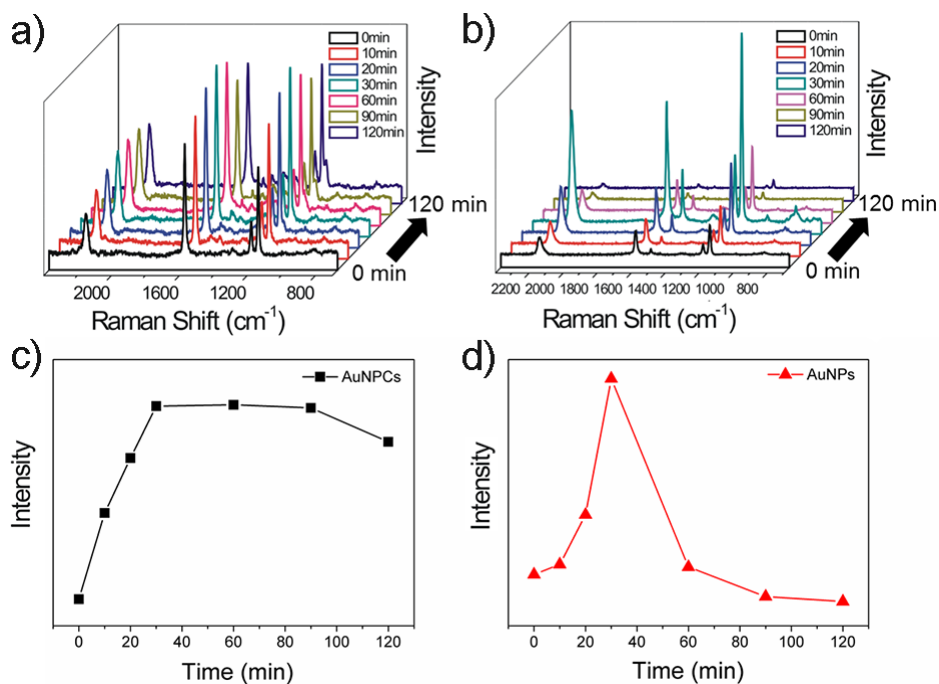


Fig. S20 Time-dependent SERS spectra of 1,4-phenylene diisocyanide (1,4-PDI, 1×10^{-4} M) in aqueous solution obtained with the colloidal (a) AuNPCs (type I) and (b) AuNPs. The normalized peak intensities of the $\nu(\text{NC})$ mode monitored as a function of time for (c) AuNPCs (2182 cm^{-1}) and (d) AuNPs (2170 cm^{-1}).

As shown in (a) and (c), SERS signal intensity from AuNPCs increased gradually until 30 min, which might be associated with the induction time required for adsorbing 1,4-PDI. Notably, there is no significant change in the signal intensity after 30 min, implying that AuNPCs are very stable SERS platforms even for analytes with multiple binding sites. In sharp contrast, AuNPs exhibited a volcano-type relationship between the SERS activity and time, where the SERS intensity reached the maximum value at 30 min, and then decayed drastically (d). This indicates that severe particle agglomeration occurred after 30 min in the case of AuNPs due to the 1,4-PDI. This was also evidenced by the formation of black precipitate in AuNP solution. In addition, the peak position of $\nu(\text{NC})$ mode in the case of AuNPs (2170 cm^{-1}) is highly correlated with that of 1,4-PDI adsorbed on Au surface by using two NC groups (ref.: K. Kim, D. Shin, K. L. Kim and K. S. Shin, *J. Raman Spectrosc.*, 2012, **43**, 1427).

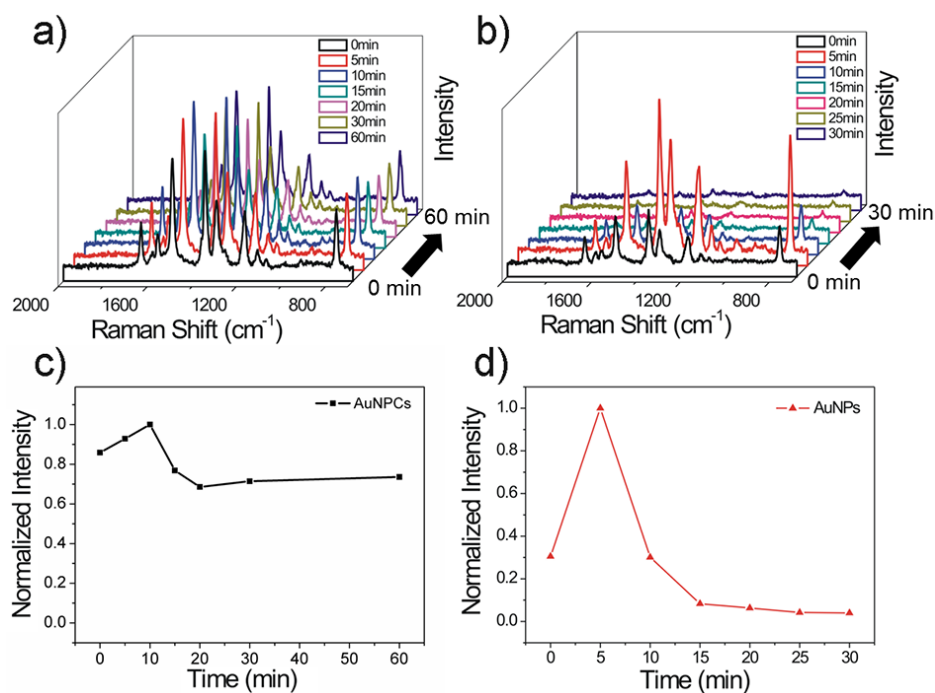


Fig. S21 Time-dependent SERS spectra of 1×10^{-5} M R6G in PBS solution obtained with the colloidal (a) AuNPCs (type I) and (b) AuNPs. The normalized peak intensity of the C-H out-of-plane stretching mode at 776 cm^{-1} monitored as a function of time for (c) AuNPCs and (d) AuNPs.

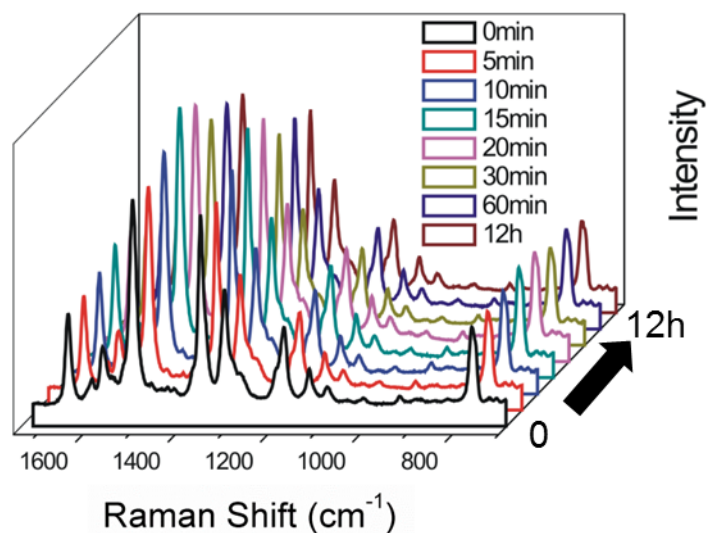


Fig. S22 Time-dependent SERS spectra of 5×10^{-6} M R6G in PBS solution obtained with the colloidal AuNPCs (type II).