Microwave-Assisted Synthesis of Photoluminescent Glutathione-Capped Au/Ag Nanoclusters: A Unique Sensor-on-a-Nanoparticle for Metal Ions, Anions, and Small Molecules

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Highly photoluminescent glutathione-capped Au/Ag nanoclusters were rapidly synthesized in a microwave-assisted manner. Instead of acting as a sensor for one single target, the present nanoclusters respond to metal ions, anions, and small molecules, functioning as a unique sensor-on-a-nanoparticle (see picture).

Provide the authors’ webside if possible.
Prof. Shu-Hong Yu, http://staff.ustc.edu.cn/~yulab/
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ABSTRACT
Even though great advances have been achieved in the synthesis of luminescent metal nanoclusters, it is still challenging to develop metal nanoclusters with high quantum efficiency as well as multiple sensing functionalities. Herein we demonstrate the rapid preparation of glutathione-capped Au/Ag nanoclusters (GS-Au/Ag NCs) by microwave irradiation and its unique sensing capacities. Compared to bare GS-Au NCs, the doped Au/Ag NCs possess much enhanced quantum yield (7.8% compared to 2.2% for GS-Au NCs). A panel of characterization techniques was used to elucidate its atomic composition, particulate character, and electronic structure. According to the X-ray photoelectron spectroscopy (XPS) and X-ray absorption near-edge structure (XANES) spectra, a significant part of Au exists in the oxidized state as Au(I) and Ag atoms are positively charged. In contrast to those nanoclusters which detect only one analyte, the GS-Au/Ag NCs can be used as a versatile sensor for metal ion, anions, and small molecules. In this manner, the NCs can be regarded as a unique sensor-on-a-nanoparticle.

1. Introduction

Noble metal nanoparticles with core sizes less than 2 nm have now been recognized as metal nanoclusters (or clusters) [1]. After a decade's efforts, the library of metal clusters with varied...
sizes and compositions has been profoundly enriched. Compared with their larger counterparts, metal clusters behave like molecules due to the electron confinement effects, possess unique optical and charging properties, and exhibit enhanced catalytic activity and luminescence [2-4]. One typical example is the surface plasmon resonance band characteristic of metal nanocrystals is replaced by a series of fingerprint optical absorption spectra for metal nano-clusters [5-8], especially those using thiolates as ligands, which are generally called thiolated metal clusters. The thiolate-stabilized metal clusters serve as the bridge between metal-thiolate complexes and thiolate-stabilized metal nanocrystals; and understanding this, the preparation of thiolated metal clusters can be rationalized via two strategies: the bottom-up reduction of metal-thiolate complexes by reducing agents, and the top-down decomposition of aggregated metal-thiolate complexes or large metal nanocrystals. One of the intrinsic properties of thiolated metal clusters (gold clusters in particular) that has been most studied is luminescence. Whetten and coworkers first observed near-infrared luminescence from 1.1 and 1.7 nm dodecanethiol-passivated gold clusters with quantum yield of $(4.4 \pm 1.5) \times 10^{-5}$, five orders of magnitude greater than that of bulk gold [9]. After that, they observed emissions over visible to infrared spectral regions from glutathione-capped gold clusters with improved quantum yield $(3.5 \pm 1.0) \times 10^{-3}$ [10]. Inspired by these works, researchers synthesized a variety of metal clusters using different thiolate molecules with distinctive luminescent emissions [11-16]. Nevertheless, the preparation of highly luminescent thiolated metal clusters, especially in a relatively large scale, is still challenging.

Microwave-assisted heating technique has established its fundamental position in the modern preparation of inorganic nanoparticles and organics [17]. Compared with conventional convective heating, the use of microwave irradiation as energy source takes advantage of several dominant merits. First, it allows high reaction rate due to rapid elevation and exquisite control of temperature and pressure. Second, it favors for homogeneous heating and thus uniform product formation because of reduced thermal gradient effect. Third, primarily for organics, reaction selectivity can be improved. Finally, the microwave method shows high reproducibility and can be readily scaled up to large reaction volumes. Until now, some metal nanoclusters were successfully synthesized by microwave irradiation and utilized for metal ions sensing and bioimaging. Lin et al. observed enhanced luminescence quantum yield for dendrimer-entrapped gold clusters that were generated by microwave irradiation for 30 min [18]. Shang et al. synthesized dihydrolipoic acid-capped fluorescent gold nanoclusters by microwave strategy whose quantum yield was five times higher than that of product produced by conventional heating [16]. Generally, it takes from several minutes to a couple of hours to obtain these metal clusters with enhanced quantum yields. Despite those outstanding advantages, the microwave-assisted synthesis of metal clusters with desired luminescent properties is far less than expected.

Compared to nanoclusters composed of mono-metallic atoms, those bimetallic clusters have recently attracted special research interest owing to the fact that doping with foreign atoms may bring new properties to the subject being doped. Related studies have revealed that doping Au$_{25}$(SC$_{12}$H$_{25}$)$_{18}$ clusters with palladium atom increases cluster stability [19] while doping same clusters with copper atom reduces cluster stability [20]; doping Au$_{25}$(SC$_{2}$H$_{4}$Ph)$_{18}$ clusters with a single platinum atom greatly enhances cluster stability against oxidation treatment and
its catalytic activity [21] whereas doping Au₅₅(SC₅H₅)₁₈ clusters with silver atoms makes shifts in the optical spectra and fluorescence peaks by modulating the electronic structure [22]. Of such bimetallic clusters, Au/Ag alloy clusters [23-26] are especially fascinated because they often manifest much enhanced luminescence, which is beneficial for sensing and bioimaging.

Aimed to develop brighter metal clusters in a time-efficient and macroscale manner, herein we report on the rapid synthesis of highly luminescent Au/Ag nanoclusters by microwave-assisted technique. By incorporating silver atoms at a proper molar ratio of Ag:Au, the highest quantum yield of 7.8% is obtained for the optimized Au/Ag clusters relative to the quantum yield of 2.2% for the Au clusters counterpart. The bimetallic nanoparticle is then used to be sensor for the detection of metal ion, anions, and biological molecules. Although a lot of metal clusters have been used to detect metal ions [13, 16], anions [27, 28], or small molecules [29, 30], this is the first time to employ single nanoclusters as the sensor for three different kinds of targets, which behaves like sensor-on-a-nanoparticle.

2. Results and discussion

The glutathione-capped Au/Ag nanoclusters are formed by the dissociation of Au(I)-SG polymer complex in the presence of different concentrations of silver ion. Keeping the loading concentration of HAuCl₄ constant, the Ag:Au molar ratio increased from 0 to 1 as gradual increasing the amount of AgNO₃. Figure 1 shows the optical absorption spectra and photoluminescent (PL) spectra of the GS-Au/Ag NCs. All of the absorption spectra exhibit onsets at 500 nm and shoulder peaks at ~400 nm. Neither stepwise multiple bands nor localized surface plasmon resonance (LSPR) bands characteristic of plasmonic gold or silver nanocrystals appear in the absorption spectra, suggesting that the obtained clusters are not conventional (AuAg)₉(SR)₄ [22, 26, 31] clusters and besides, the core sizes in metallic regime are less than 2 nm [32]. Corresponding to the optical bands, the photoluminescent spectra show well-defined emission peaks between 610 and 620 nm, when excited at 380 nm. Table 1 lists the emission wavelength (λem) and quantum yield (QY) of the clusters. It clearly shows that the Ag-doped clusters outperform the bare Au clusters in QY and the largest QY, three times more than that of bare Au clusters, is obtained for clusters at Ag: Au molar ratio of 0.2. Besides, the λem shows small bathochromic shifts for the Ag-doped clusters compared to the bare Au clusters. Quantum yield boost in the photoluminescence is featured of Ag-doped Au clusters [23, 24, 26, 33, 34]; however, red shift in the emission wavelength was rarely reported [35]. We postulate that the shift in the λem for Ag/Au clusters is determined by the surface ligand and the way of Ag doping, but the exact reason is not known right now. Since the highest quantum efficiency was obtained for Au/Ag NCs at Ag/Au molar ratio of 0.2, the clusters was chosen for the following studies.

Table 1. The photoemission wavelength and quantum yield of the GS-Au/Ag NCs with varied Ag: Au molar ratio. The quantum yield was obtained with quinine sulfate (in 0.5 M H₂SO₄, 54%) as the reference.

<table>
<thead>
<tr>
<th>Ag/Au</th>
<th>0</th>
<th>0.1</th>
<th>0.2</th>
<th>0.5</th>
<th>1.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>λem / nm</td>
<td>610</td>
<td>614</td>
<td>618</td>
<td>614</td>
<td>614</td>
</tr>
<tr>
<td>QY / %</td>
<td>2.2</td>
<td>4.6</td>
<td>7.8</td>
<td>4.3</td>
<td>4.3</td>
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We used TEM and DLS to identify the generation of nanoclusters. Figure 2a shows a representative TEM image of the GS-Au/Ag NCs. Counting 80 particles gives a mean diameter of 2.3 nm, which is a little smaller than the average hydrodiameter (3.2 nm) by DLS measurement, as observed in Figure 2c. The difference of 0.9 nm reflects that the single layer of GSH coating measures 0.45 nm, similar with the calculation (0.4 nm) of the GS-Au NCs [36]. A high-resolution TEM image in Figure 2b clearly manifests lattice fringe separated by about 0.23 nm, corresponding to the (111) lattice spacing of the face-centered cubic (fcc) Au. An attempt to localize the distribution of Ag atom within the NCs by STEM-based elemental mapping was not successful, owing to the extremely small size. Shang et al. also observed lattice fringe spacing of 0.235 nm in the Au clusters [16, 37]. Albeit this, the clusters are different from the metallic fcc nanocrystals in the following aspects. Contrary to the LSPR band at ~520 nm for spherical metallic particles, there is only a shoulder peak at ~400 nm for the clusters. The absence of LSPR band in the absorption spectrum for the clusters with such apparent size (2.3 nm) suggests that a significant part of Au exists in the oxidized state as Au(I) valence [38]. Moreover, this Au/Ag clusters do not adopt the fcc structure of gold or silver, because the diffraction angles in the XRD pattern (Figure 2d) exhibit appreciable deviation from those for the Au (JCPDS 04-1784) and Ag (JCPDS 04-0783) of fcc structure. The diffraction angle at 38.2° corresponding to (111) plane for the fcc structure of bulk Au is shifted to 32.7° for the clusters, and besides, that at 51.4° is not present for the fcc structure. This is in agreement with many other literatures [39-41]. All the evidence confirms the clusters rather than metallic particles, and more importantly, the appearance of lattice fringes featured by fcc Au may suggest some kind of connection between nanoclusters and nanocrystals. EDX analysis (data not shown) identifies the existence of Au and S in the clusters whereas fails to find Ag. This may be caused by the low doping of silver. The concentrations of Au and Ag in the clusters were measured to be 276.0 and 16.80 µg/mL, respectively, according to the ICP-OES result, and thus the Ag/Au atomic ratio is 0.11, below the initial molar ratio of Ag/Au (0.2) in the starting material. It is expected since the amount of Ag atoms is reduced by the formation of AgCl precipitate. Moreover, the production yield based on the conversion of HAuCl₄ to Au was measured to be 98%.

Figure 2. (a) TEM and (b) high-resolution TEM images of the GS-Au/Ag NCs. (c) Size distributions of GS-Au/Ag NCs by TEM and DLS. (d) XRD pattern of the NCs. The red and yellow sticks represent the standard diffraction angles for bulk Au and Ag of fcc structure, respectively.

We first used XPS to verify the exact valence state of elements in the GS-Au/Ag NCs. The survey spectrum (Figure 3a) reveals the elements of C, N, O, S, Au, and Ag expected in the clusters. The Au 4f and Ag 3d spectra in Figure 3b, c show that the Au
4f7/2 and Ag 3d5/2 peaks are centered at 84.0 and 367.6 eV, respectively; and meanwhile, the S 2p spectrum in Figure 3d contains 2p3/2 and 2p1/2 components at 162.4 and 163.6 eV, respectively, which compares well with the typical values for chemisorbed thiolated forms [42]. Besides, the atomic ratio of Ag/Au (0.65%/6.62%) is determined to be 0.098, consistent with the ICP result. The Au 4f7/2 peak of the Au/Ag NCs is on the lower energy side relative to that of bare GS-Au NCs (84.3 eV) (Electronic Supplementary Material Figure S1), while still slightly higher than that of bulk Au (83.8 eV) [43], implying that a part of Au exists in the oxidized state as Au(I). On the other hand, the lower energy of the Ag 3d5/2 peak relative to that of bulk Ag (367.9 eV) [43] indicates that the Ag atoms are positively charged, based on the counterintuitive fact that a negative shift in Ag 3d binding energy is consistent with Ag oxidation [44]. To support the XPS results, we further probe the electronic state of Au and Ag by X-ray absorption near-edge structure (XANES). Complementary to XPS, XANES is a particularly useful and effective technique for probing the valence state of atoms, especially those hard differentiated by XPS, such as Ag. Referenced by a Au foil, the XANES spectrum of GS-Au/Ag NCs shows a shift of edge absorption to higher energy and an apparently more intense white-line (Figure 3e), indicating a significant contribution of Au(I) electronic character [45]. This is consistent with the XPS result. Meanwhile, the Ag K-edge XANES spectrum is different from that for Ag foil (Figure 3f), showing characteristics of oxidized state of Ag [46]. Since the Au/Ag NCs possess a lower 4f7/2 binding energy than that of bare Au NCs, we believe that this is caused by the Au-Ag charge redistribution occurred within the clusters which balances the electron-withdrawing effects of the thiolate ligand. This phenomenon has been observed for these Au25-xAgx clusters [22, 47].

The Au/Ag NCs solution appears in bright yellow color under visible light and emits intense orange luminescence under UV excitation (Figure 4a, b). The generation of luminescent GS-Au NCs from the dissociation of Au(I)-SG complexes was early studied by Zheng et al. [38] whereas they performed the synthesis for more than 2 weeks due to the extremely slow reaction kinetics at room temperature. Afterwards, Xie et al. [48] shortened the reaction time to 24 h via a heat-facilitated approach, proposed a synthesis scheme, and unraveled the origin of luminescence. The GS-Au/Ag NCs possess comparable or even better physicochemical properties than those of the GS-Au NCs, but the reaction only takes 3 h, demonstrating the prominent advantages of the microwave-assisted method. The benefit of our method also lies in the scalability of synthesis as well as high reproducibility. At most eight reaction vessels are to be used in a single synthetic
procedure with each vessel containing at least 40 mL of solution (Electronic Supplementary Material Figure S2), and the relative standard deviations for the quantum yield and emission wavelength of the products are 3.3% and 0.23%, respectively. Similar with the GS-Au NCs [48], the Au/Ag NCs display excellent stability in both solution and solid states, with minor change in quantum yield after storage at 4 °C for two months. The agarose gel electrophoresis (Electronic Supplementary Material Figure S3) shows that the clusters carry negative charge, in accordance with the \( \zeta \)-potential of -28 (± 5) mV. The photoluminescent spectra (Figure 4c) show that the emission intensity decreases gradually with the increase of excitation wavelength (\( \lambda_e \)) from 360 to 440 nm, while the peak maximum remains at 618 nm. The large Stokes shift infers that the emission is mainly due to phosphorescence. The clusters show prominent stability against continuous photo-excitation, as observed in the time-dependent emission intensity (Figure 4d). In addition, we found that the emission intensity decreased linearly when the temperature elevated (Figure 4e). Such phenomenon has recently been observed for lipoic acid-capped AuNCs [49]. Moreover, the emission intensity varied a little between pH 5 and 9 (Electronic Supplementary Material Figure S4). Taken together, the GS-Au/Ag NCs are of high potential to be used in intracellular imaging only if it can be tested with minimal toxicity and capable of entering into cells.

We investigated the reactivity of the Au/Ag NCs with other substances by following the PL intensity. It reveals that apart from the response to metal ions, the clusters also respond to small anions and molecules (Figure 5a-c). As observed in Figure 5d, the cupric ion quenched the luminescence completely among the metal ions, and the luminescence was much attenuated in the presence of iodide, sulfide, cysteine, and glutathione, compared to those incubated with other anions and amino acids. Therefore, the Au/Ag NCs can be seen as a versatile sensor for the five analytes. Until now, numerous metal clusters have been developed as luminescent probe to detect metal ions [13, 16, 25], anions [27, 28], and small molecule [29, 30], but none was designed by purpose to be a sensor for three different kinds of matters. Figure 6 shows the detection of cupric ion, iodide/sulfide, and cysteine/glutathione. The PL intensity decreased stepwise with the increased concentration of Cu\(^{2+}\) ion, and correlation of the \( I/I_0 \) ratio with the Cu\(^{2+}\) concentration obtained a calibration curve that can be fitted linearly from 0 to 100 nM (\( y = 1 - 0.00467x, R^2 = 0.996 \)). The limit of detection (LOD) was determined to be 2 nM (S/N = 3). Similar luminescence quenching was also observed for iodide/sulfide, and cysteine/glutathione, but with much less sensitivity. The calibration curve of sulfide can be fitted linearly from 0 to 0.2 \( \mu \)M (\( y = 1 - 1.87x, R^2 = 0.999 \)), while that of iodide be fitted with two linear lines (\( y_1 = 1 - 1.53x_1, 0 < x_1 < 0.2 \mu \)M, \( R^2 = 0.999 \) and \( y_2 = 0.777 - 0.292x_2, 0 < x_2 < 1.0 \mu \)M, \( R^2 = 0.996 \)). On the other hand, utilizing \( I_0/I \) as the variable, the calibration curves of cysteine can be fitted linearly from 0 to 1 \( \mu \)M (\( y_1 = 0.765x_1, R^2 = 0.988 \)) and 1 to 5 \( \mu \)M (\( y_2 = 0.389 + 0.344x_2, R^2 = 0.988 \)), while that of glutathione be fitted linearly from 0 to 0.8 \( \mu \)M (\( y = 1.074x, R^2 = 0.994 \)). The LODs of sulfide, iodide, cysteine, and glutathione were 5, 5, 10, and 9 nM, respectively, comparable with or better than those of reported clusters sensors [28, 30, 50, 51].

![Figure 4](image-url)

**Figure 4.** Digital photographs of the GS-Au/Ag NCs solution under (a) room light and (b) UV light (365 nm). (c) The optical spectra and photoluminescent spectra under different excitation wavelength of the GS-Au/Ag NCs solution. (d) Time-dependent photo-irradiation stability study. (e) The correlation of photoluminescent intensity with temperature.
Figure 5. Photoluminescent responses for the interaction of GS-Au/Ag NCs with (a) metal ions, (b) anions, and (c) amino acids. (d) A digital photograph of the clusters solution incubated with different substances in a 96-well plate under UV excitation (365 nm). For a-c, the concentration of GS-Au/Ag NCs was 1.38 µg/mL and the concentrations of metal ions, anions, and amino acids were 1, 10, and 10 µM, respectively. For d, the concentrations of GS-Au/Ag NCs and all substances were 20-fold than those in a-c. The substances in d are as follows: Ca²⁺, Al³⁺, Fe³⁺, Co²⁺, Ni²⁺, Cu²⁺ (B1-G1); Zn²⁺, Cr³⁺, Mn²⁺, Cd²⁺, Pb²⁺, Hg²⁺ (B2-G2); F, Cl, Br, I, S²⁻, SCN⁻ (B4-G4); NO₃⁻, SO₄²⁻, CO₃²⁻, H₂PO₄⁻, Cr₂O₇²⁻, P₂O₇⁴⁻ (B5-G5); Ala, Arg, Asn, Asp, Glu, Gly (B7-G7); His, Ile, Leu, Lys, Met, Phe (B8-G8); Pro, Ser, Thr, Trp, Tyr, Val (B9-G9); Gln, Cys, GSH (B10-D10).

Figure 6. (a) The PL spectra of GS-Au/Ag NCs solution (1.38 µg/mL) with incubation of varied cupric concentrations and (b) the corresponding calibration curve. (c-f) The calibration curves for the detection of sulfide, iodide, cysteine, and glutathione, respectively. HEPES-NaOH (pH = 7.4, 10 mM) was used as buffer. The excitation wavelength was set at 380 nm, the emission intensity was quantified at 618 nm, and the widths of excitation and emission slit were both 10 nm.
Cu²⁺ ion is known to be an efficient quencher for various fluorophores, including GS-Au NCs [52]. Meanwhile, sulfide, iodide, and cysteine have been reported to quench the luminescence of Au or Ag clusters through their strong interaction with metal surface [30, 50, 51]. The quenching mechanism was probed by the optical absorption spectra and time-resolved photoluminescent lifetime spectra, exemplified with Cu²⁺ ion, sulfide, and cysteine. We observed little change in the absorption spectrum of NCs with respect to Cu²⁺ ion, whereas appreciable changes for sulfide and cysteine (Electronic Supplementary Material Figure S5a). This implies that the NCs interact with sulfide and cysteine but not with Cu²⁺ ion. On the other hand, dramatic decreases of PL lifetime were observed for all the targets (Electronic Supplementary Material Figure S5b and Table S1), indicating the presence of dynamic quenching contributions. Because the origin of luminescence for the GS-Au NCs is due to aggregation of Au(I)-SG complexes on a Au(0) core [48], and moreover, the improved luminescent intensity after the addition of Ag⁺ ions is probably induced by the linkage of those Au(I)-SG complexes [35]. Therefore, we suspect that the PL quenching in the presence of sulfide (or iodide) and cysteine (or glutathione) is caused by their reaction with Ag⁺ ions to form insoluble silver salts on the surface shell of clusters [53] or detach the Ag⁺ ions from the surface shell of clusters [35]. This is supported by that no PL responses could be observed for bare GS-Au NCs incubated with sulfide, iodide, cysteine, and glutathione (Electronic Supplementary Material Figure S6).

3. Conclusions

In summary, we observed improved photoluminescent properties for GS-Au NCs doped with Ag⁺ ions. On the basis of this observation, we develop a rapid method to prepare GS-Au/Ag NCs with optimal quantum yield of 7.8% by microwave-assisted decomposition of Au(I)-SG complexes in the presence of Ag⁺ ions. XPS and XANES spectra demonstrated that silver exists in the oxidized state and Au-Ag charge redistribution occurs within the clusters to balance the charge-withdrawing effects of thiolate. The PL intensity of Au/Ag NCs solution changes appreciably in the presence of cupric ion, sulfide, iodide, cysteine, and glutathione compared to other metal ions, anions, and amino acids, thus making the NCs as a fascinating kind of sensor-on-a-nanoparticle. This work further demonstrates the advantages of microwave irradiation in the synthesis of luminescent metal clusters with versatile sensing capabilities.

4. Experimental Section

4.1 Materials. HAuCl₄·3H₂O and AgNO₃ were purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). Glutathione in the reduced form (GSH) and L-amino acids were purchased from Sangon Biotech Ltd. (Shanghai, China). All other compounds were commercially available and used without further purification. Ultrapure water (18.2 MΩ cm) was used throughout.

4.2 Microwave synthesis of luminescent GS-Au/Ag NCs. In a typical procedure, freshly prepared aqueous solutions of GSH (10 mL, 7.5 mM) were mixed with mixtures of HAuCl₄ (10 mL, 5 mM) and AgNO₃ (1 mL) of different concentrations at specific Ag/Au molar ratios. Under gentle stirring for 5 min, the solutions were transferred into the specific microwave vessels and sealed. The reaction temperature was set at 90 °C, which took 3 min to reach from room temperature, and the irradiation time was set for 3 h. After cooling down, the solutions were centrifugated to rid the white sediment of AgCl and then stored at 4 °C for at least 24 h before further treatment. The NCs were finally precipitated by adding ethanol and NaCl (1 M) by centrifugation (10,000 rpm), dispersed in 35 mL HEPES-NaOH buffer (10 mM, pH = 7.4), and stored at 4 °C for use.

4.3 Detection of metal ion, anions, and small
molecules by the GS-Au/Ag NCs. The NCs solution (1.38 $\mu$g/mL) in HEPES-NaOH buffer (10 mM, pH = 7.4) was spiked with varied concentrations of cupric ion, sulfide, iodide, cysteine, or glutathione, individually, and the PL intensity was measured within 5 min, using 380 nm as the excitation wavelength.

4.4 Characterization. The microwave reaction was conducted with a MDS-6G microwave digestion system (Sineo, China). UV-vis optical spectra were recorded with a Lambda 25 UV-vis spectrophotometer (PerkinElmer). Steady state photoluminescence (PL) spectra were recorded with a Hitachi F-4600 fluorescence spectrophotometer, and time-resolved PL lifetimes were measured by time-correlated single photo counting (TCSPC) on a Horiba JY Fluorolog-3-Tou spectrophotometer with 380 and 615 nm as the excitation and emission wavelength, individually. Transmission electron microscopy (TEM) equipped with energy dispersive X-ray spectrometer (EDX) were obtained on a JEOL-2100F TEM instrument operating at 200 kV. The hydrodiameter distribution by dynamic light scattering (DLS) and zeta potential of the clusters were examined on a Malvern Zetasizer instrument. The product yield with Au atom basis was calculated from inductively coupled plasma-optical emission spectrometry (ICP-OES) result by an Optima 7300DV (PerkinElmer). X-ray photoelectron spectroscopy (XPS) study was performed on an ESCALAB 250 spectrometer (VG Scientific). Au L-edge and Ag K-edge X-ray absorption near-edge structures (XANES) spectra of the sample were recorded at room temperature in the fluorescent mode with silicon drift fluorescence detector at beamline BL14W1 of the Shanghai Synchrotron Radiation Faculty (SSRF), China. The station was operated with a Si(111) double crystal monochromator. The storage ring was operated at 3.5 GeV with injection currents of 100 mA. The photo energy was calibrated with the first injection point of Au L-edge and Ag K-edge in Au metal foil and Ag metal foil, respectively. The raw data were background-corrected and normalized using the IFEFFIT software. X-ray diffraction (XRD) pattern was obtained using a D/Max-$\gamma$A rotating-anode X-ray diffractometer (Rigaku). Agarose gel electrophoresis was run by 2% agarose gel in 0.5×TBE buffer (Tris, 44.5 mM; EDTA, 1 mM; and boric acid, 44.5 mM, pH 8.0) for 45 min at 10 V/cm at 4 °C.

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Electronic Supplementary Material: Supplementary figures are available in the online version of this article at (automatically inserted by the publisher).

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