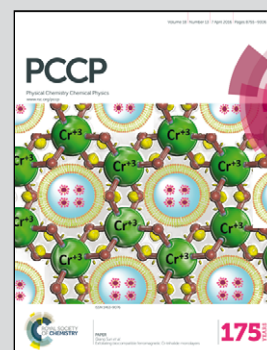


Showcasing research from the Group of Professor Tetsu Yonezawa at Division of Material Science and Engineering, Faculty of Engineering, Hokkaido University, Japan.

Title: Controlling an electrostatic repulsion by oppositely charged surfactants towards positively charged fluorescent gold nanoclusters

This work reports the novel mechanism of controlling an electrostatic repulsion between small cationic thiolate ligands to produce a positively charged fluorescent gold nanocluster. The strategy involves the utilization of thiocholine, the shortest cationic thiolate ligand coupled with anionic surfactant, SDS, to facilitate thiocholine's efficient attachment on gold surface due to the suppression of repulsion among thiocholine ligands during the nucleation, while the absence of anionic surfactant results in the formation of plasmonic particles. This approach will be useful among nanocluster material scientists and have potential benefits in biomedical fields especially in bioimaging, biosensing and biotherapy.

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Controlling an electrostatic repulsion by oppositely charged surfactants towards positively charged fluorescent gold nanoclusters†

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A novel positively charged fluorescent gold nanocluster was successfully synthesized using the shortest cationic thiol, thiocholine. Effective control of electrostatic repulsion by the introduction of an anionic surfactant afforded a nanocluster that showed blue fluorescence emission.

Nanocluster synthesis is an interesting field of study, and various researchers are aiming at manipulating individual atoms, molecules, or groups of molecules to produce novel hybrid materials with unprecedented structures and properties.^{1–8} A typical example is the synthesis of gold nanoclusters with a non-metallic structure, whose distinctive optical properties are derived from the constituent gold nanoparticles, which generally show surface plasmon resonance at relatively larger diameters (> 3 nm). The optical properties of gold nanoclusters are hypothesized to originate from the metallic cluster core, and they can be altered depending on the attached ligands. Unlike the collective oscillation of conduction electrons in the gold nanoparticle, a single electron transition results in molecule-like absorption and emission from the UV to NIR region in a gold nanocluster.^{9–11} Experimentally, it has been determined that the sharp contrast between the optical properties of the gold nanocluster and gold nanoparticles become observable when the particle size is below 2.4 nm and the gold particles no longer exhibit metallic properties.¹²

Among the tunable properties of gold nanoclusters, we focused on their optical properties, particularly photoluminescence. Photoluminescence is an important property because of its potential application in biomedical fields, especially for imaging, sensing, and therapy.¹³ The nanomaterials commonly used for these applications are quantum dots,¹⁴ which contain toxic components such as Cd, Pd, Se, and Te, and are highly discouraged. Hence, many researchers have proposed that gold could be one of the

most viable alternative because of its chemical inertness, photostability, and biocompatibility. Unfortunately, gold in its nanoparticle form shows size-related toxicity,¹⁵ and thus, a synthetic scheme for fluorescent gold nanoclusters is an urgent demand.

There are several reported synthetic routes to fluorescent gold nanoclusters using polymers,¹⁶ dendrimers,¹⁷ DNA,¹⁸ or phosphine¹⁹ as the stabilizing compounds. We focus on gold nanoclusters with thiol stabilizers,²⁰ pioneered by Murray and Whetten. In this regard, there are two commonly exploited methods involving the use of neutral and anionic thiol ligands including alkylthiols,²¹ tiopronin,²² phenylethylthiolate,²³ and thiolate cyclodextrin.²⁴ However, there is no established method for the synthesis of fluorescent gold nanoclusters by means of conventional chemical reduction using cationic thiols. Such nanoclusters are expected to play a significant role in bioimaging²⁵ and sensing as cellular proteins show high affinity for positively charged nanocomposites than for neutral and anionic nanocomposites. In particular, quaternary ammonium-terminated thiols have rarely been used for the preparation of gold nanoparticles.^{26,27} Quaternary ammonium groups are always positively charged under any pH conditions. Thus, we hypothesized that a positively charged gold nanocluster could easily be absorbed inside the cell and potentially be used to study intercellular activities that are imperative in cancer research.¹³ The main limitation of cationic thiolate-protected gold nanoclusters is that the electrostatic repulsion between the cationic ligands on the surface of the nanoparticles hinders the formation of small clusters (*ca.* < 2 nm) during nucleation in solvents. Our previous research showed the formation of a red dispersion of thiocholine bromide-stabilized gold nanoparticles even at a high molar ratio of the thiocholine ligand as compared with the gold ions.²⁶ Thiocholine is the smallest quaternary ammonium terminated mercapto ligand. Moreover, silver,²⁸ palladium,²⁹ and platinum³⁰ nanoparticles can be obtained by chemical reduction using thiocholine as the stabilizing reagent, but these nanoparticles, too, are large. Our objective is the utilization of the smallest cationic thiolate ligand, thiocholine ($\text{HS}-(\text{CH}_2)_2-\text{N}(\text{CH}_3)_3^+$), to synthesize fluorescent gold nanoclusters by chemical reduction.

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Cationic thiolate-protected fluorescent gold nanoparticles or nanoclusters have been obtained only by a physical synthesis method by our group.³¹ This paper reports the first successful synthesis of positively charged nanoclusters by the conventional chemical reduction method.

In this study, the shortest cationic thiolate, thiocholine chloride (TC), was synthesized as described in a previous paper (see ESI†).³¹ Counter-ion exchange was carried out in order to eliminate the heavy metal effect of I^- . The purity of TC was verified by 1H -NMR and FT-IR analyses.

First, we attempted to synthesize gold nanoclusters by the simple reduction of $HAuCl_4(Au)$ with excess $NaBH_4$ in the presence of TC. The Au-to-TC mol ratio was 1:3, 1:5, or 1:7. For our typical synthesis (Au:TC = 1:7 (mol/mol)), we dissolved 0.0218 g of thiocholine chloride in 1 mL of methanol followed by addition of $HAuCl_4$ stock solution (20 mmol dm^{-3} , 1 cm^3). It was then diluted with 8 cm^3 of water-methanol solvent (1:1 vol/vol) and sonicated for 30 min prior to the addition of aqueous $NaBH_4$ (2 mol dm^{-3} , 50 mm^3), the resulting solution was then sonicated for 90 min to ensure complete reduction of Au^{3+} . The black line in Fig. 1 shows the extinction spectrum, including both absorption and scattering from nanoparticles, immediately after the synthesis at the Au:TC = 1:5 (mol/mol). From this result, it could be seen that the samples exhibited plasmon absorption at around 540 nm. Transmission electron microscopy (TEM) images of the representative samples are shown in Fig. S1 (ESI†), where the particle sizes are seen to exceed 4 nm, indicating that gold nanoclusters were not produced. As mentioned above, the strong repulsion between the TC ligands in the “nucleation stage” clusters hinders the formation of nanoclusters; the clusters coalesce spontaneously to form rather large nanoparticles. It is widely known that the surface charges on the gold clusters (mainly Au_{25} clusters³²) could decompose the cluster structure. This would be the critical reason for the formation of rather nanoparticles with TC in the current system. The use of a higher ligand ratio did not solve this problem.

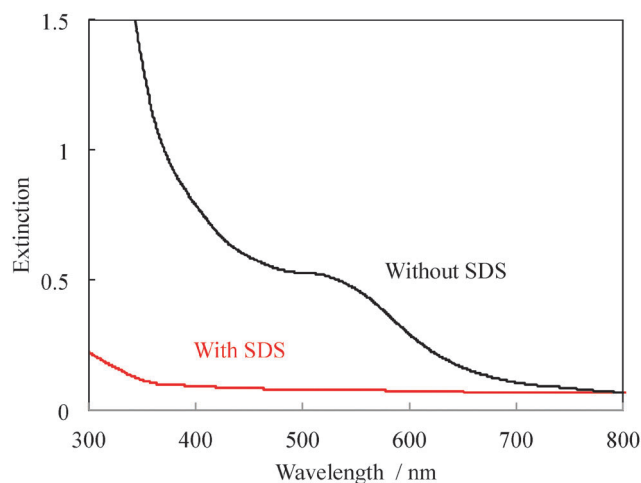


Fig. 1 UV-Vis extinction spectra of Au nanoparticles and nanoclusters synthesized in the absence or presence of SDS (Au:TC:SDS = 1:5:0 and 1:5:5 (mol/mol/mol) for black and red spectra).

In order to suppress the electrostatic repulsion between TC ligands on the surface of gold nanoclusters during the nucleation, we added a negatively charged surfactant, sodium dodecylsulfate (SDS), to form a $Au(I)$ -TC-SDS complex before the reduction, which may neutralize the cation on TC and minimize the repulsion between the TC molecules. Synthesis of Au nanoparticles with SDS alone without thiocholine gave only large plasmonic ones ($\sim 20\text{ nm}$ of diameter, Fig. S4, ESI†). To verify this concept, we synthesized gold nanoclusters at Au:TC:SDS molar ratios of 1:3:3, 1:5:5, and 1:7:7 (mol/mol/mol). In our typical experiment ($HAuCl_4$:TC:SDS = 1:7:7 (mol/mol/mol)), we dissolved 0.0218 g of TC and 0.0404 of SDS in 5 cm^3 of methanol followed by the addition of $HAuCl_4$ stock aqueous solution (20 mmol dm^{-3} , 1 cm^3). The resulting solution was diluted with 3 cm^3 of water and sonicated for 60 min until the solution became white indicating the formation of Au-thiolate complexes. The colour change from yellow to pale yellow/white indicated the reduction of $Au(III)$ to $Au(I)$ by the excess thiol compounds (Fig. S5, ESI†). Aqueous $NaBH_4$ (20 mmol dm^{-3} , 1 cm^3) was then injected and the resulting solution was sonicated for another 60 min. The red line in Fig. 1 shows the UV-Vis extinction spectra of the gold nanoclusters synthesized at the Au:TC:SDS molar ratio of 1:5:5. This result suggested that in the presence of SDS, non-plasmonic particles were formed. We then observed the TEM images to determine the particle sizes (Fig. 2). The sample showing plasmon absorption (Au:TC:SDS = 1:3:3 (mol/mol/mol), see Fig. S2 (ESI†) for the spectrum) had particles with diameters larger than 3 nm. The samples that did not exhibit plasmon absorption (Au:TC:SDS = 1:5:5 and 1:7:7 (mol/mol/mol)), on the other hand, had particles with diameters well below 2 nm, indicating successful gold nanocluster formation. It is difficult to determine the exact diameter of such small clusters ($\sim 1\text{ nm}$ of diameter) by conventional TEM; however, we observed a clear decreasing trend in the particle size with an increase in the mol ratios of TC and SDS.

Judging from the TEM and extinction spectra as well as previous data,^{9,17,33–35} we expected our gold nanoclusters to show fluorescence in the visible region. In order to increase the solubility of obtained gold nanoclusters, we introduced NaOH so that SDS attached to the surface of the TC-stabilized clusters can be removed (*i.e.* de-neutralization, see the detailed procedure outlined in ESI†). As shown in Fig. S3 (ESI†), after NaOH addition, an absorption shoulder appears at around 350 nm, which could be attributed to gold nanoclusters; a similar peak at this position was observed by other researchers, which was attributed to small gold nanoclusters consisting of 11 gold atoms or fewer⁹ (will be discussed later with fluorescence spectrum).

Under irradiation at 300 nm, we observed blue fluorescence, as shown in Fig. 3 (see images of samples under UV irradiation in the inset). The maximum excitation wavelength was around 357 nm, which was in good agreement with that for gold nanoclusters comprising 11 gold atoms and fewer, particularly those reported by Tran¹⁷ and Jin³⁴ for Au_4 ($\sim 313\text{ nm}$) and Au_3 ($\sim 303\text{ nm}$), respectively, and Zheng³⁵ for Au_5 (330 nm) and Au_8 ($\sim 384\text{ nm}$). Moreover, the maximum fluorescence wavelength ($\sim 448\text{ nm}$) agreed well with that of the Au_8 cluster (455 nm).³⁵



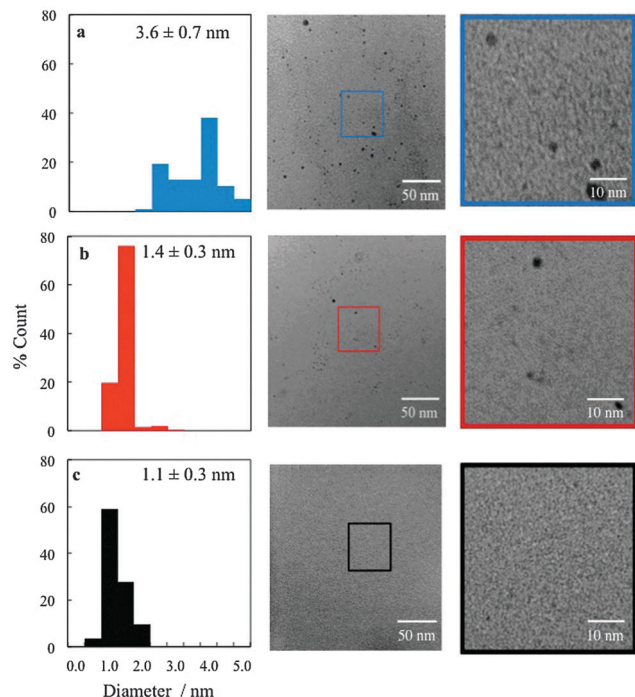


Fig. 2 TEM images and particle size distributions of Au nanoparticles or nanoclusters synthesized at Au : TC : SDS mol ratios of (a) 1 : 3 : 3, (b) 1 : 5 : 5, and (c) 1 : 7 : 7 (mol/mol/mol). For the histograms, 200 particles were counted from several enlarged TEM images.

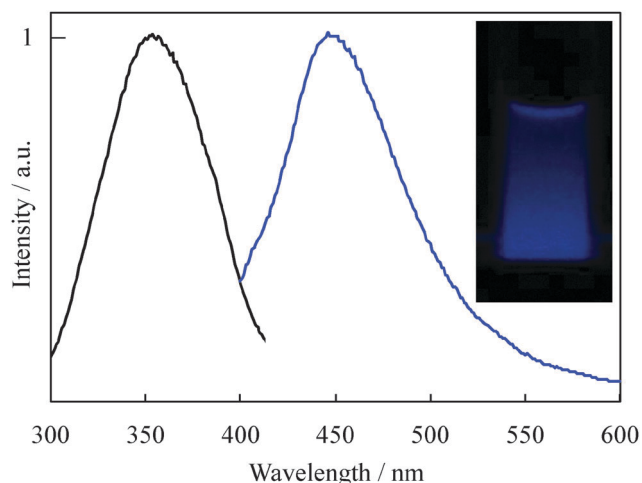


Fig. 3 Fluorescence emission (blue) and excitation (black) spectra of synthesized gold nanoclusters (excitation wavelength = 300 nm, Au : TC : SDS = 1 : 7 : 7 (mol/mol/mol)).

Hence, we hypothesized that the observed emission wavelength mainly originates from the Au₈ nanocluster or a gold nanocluster with a similar size. Other smaller or larger nanoclusters (e.g. Au₁₂, Au₁₀, Au₅, or Au₄ which fluoresce at ~630 nm,³⁶ ~523 nm,³⁷ ~385 nm,³⁵ or ~371 nm,¹⁷ respectively) could be minor contributors to the observed emission, and consequently, our gold nanoclusters showed relatively broad fluorescence.

High-angle annular dark-field (HAADF) images of scanning transmission electron microscopy (STEM) were recorded in

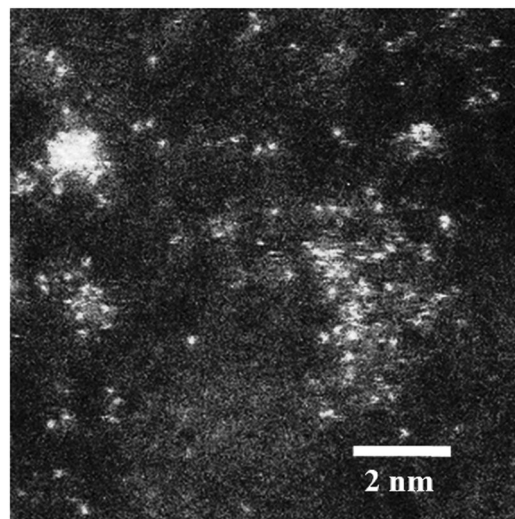


Fig. 4 STEM-HAADF image of obtained Au nanoclusters.

order to confirm the existence of very small nanocluster components (Fig. 4). Small gold nanoclusters are unstable and easily coalesce under strong electron beam irradiation; however, very small clusters composed only of Au atoms were observed in our experiments, consistent with our speculation based on fluorescence measurements.

In summary, fluorescent gold nanoclusters were successfully prepared for the first time using the shortest cationic thiol, TC. By introducing SDS to minimize electrostatic repulsion between the TC ligands on the surface of the gold nanoclusters during nucleation, we could obtain non-plasmonic particles, which showed blue emission at around 448 nm. Without SDS, on the other hand, only large non-fluorescent gold nanoparticles were obtained. The method developed in this study is expected to be beneficial to the field of bioimaging and biosensing and be of interest to scientists pursuing research on nanoclusters.

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