

Synthesis and Modeling of Fluorescent Gold Nanoclusters

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Fluorescent metallic nanoclusters (e.g., Au and Ag) with small numbers of metal atoms have great potential in bio-labeling and single molecule detection as fluorescent tags due to their extremely small sizes. Fluorescent Au and Ag nanoclusters are primarily produced inside large molecule templates with molecular weights of hundreds to thousands of Daltons. These templates not only increase the overall size of the nanocluster complexes, but also make it difficult to identify the cluster core sizes and nanocluster-ligand interfaces. Here, we report the successful production of fluorescent Au nanoclusters using two small (< 500 Daltons) biological molecules, L-ascorbic acid and 2-(N-Morpholino)ethanesulfonic acid (MES). We investigated how the synthetic parameters such as reaction temperature, concentration of the molecules, and pH effect the fluorescent emission of the Au nanoclusters. Further, we used electronic structure calculations to model and predict the geometric structure and HOMO-LUMO gaps of the nanocluster complexes.

Introduction

Fluorescence sensing and imaging techniques remain as the two primary methods for *in vitro* detection of molecules in solution and *in vivo* imaging of cells and cellular processes [7]. Unfortunately, the performance and sensitivity of these fluorescence techniques are greatly limited by the fluorescent tags attached to the biological or cellular components. Organic dyes are the most commonly used fluorescent labels, but their poor photostability leads to reduced sensitivity and decreased tracking lifetime of the targets. Their small Stokes shifts, difference between the absorption and emission wavelengths, also limit their use in multi-colored imaging [8]. In addition, most organic dye molecules cause aggregation in biological environments due to the hydrophobicity and salt effect [13]. Relative to organic dyes, semiconductor quantum dots have shown great promise in bio-labeling due to their valuable photophysical properties (i.e., size-tunable narrow emissions, large Stokes shifts, and minimal photobleaching) [5]. Unfortunately, the uses of quantum dots are limited by their harsh synthetic conditions, tedious surface passivation steps [6], and uncertain cytotoxicity *in vivo* [3].

The development of fluorescent metallic nanoclusters provides alternative labels for biological application [11,14]. One of the major advantages of these fluorescent tags is their extremely small size

(< 1 nm), which does not disturb the biological functions of the labeled bio-entities. Currently, large molecules are mainly used as templates for the synthesis of fluorescent nanoclusters, such as polymers [1,15] or proteins [12]. These templates not only increase the overall size of the nanocluster complexes, but also make it difficult to identify the cluster core sizes and the details of the nanocluster-ligand interfaces. The tunable fluorescence emissions of the nanoclusters are mainly attributed to their size variation, according to the spherical Jellium model. This model was originally derived for gas phase alkali metal nanoclusters, stating that the transition energy is a function of the cluster size [9]. Unlike in gas phases, nanoclusters in solution are stabilized with ligands, the role of which is totally overlooked when using this model.

Here, we report the successful production of Au nanoclusters using small biological molecules such as L-ascorbic acid and 2-(N-Morpholino)ethanesulfonic acid (MES). L-Ascorbic acid is the scientific name of vitamin C, and MES is a biological buffer, commonly used for biological conjugation and assays. We found that the fluorescent emission of Au nanoclusters strongly depended on the concentration of the molecule in solution, and the optimized yield was related to the reaction temperature. Our simulated models of L-ascorbic acid stabilized Au nanoclusters suggested a three-atom nanocluster core.

Experimental

Chemicals

The biological buffer, MES (2-(*N*-Morpholino)-ethanesulfonic acid, $pK_a=6.15$) was purchased from Agros. The biological molecules, L-ascorbic acid and gold chloride, were purchased from Sigma Aldrich.

Synthesis of Au nanoclusters

Fluorescent Au nanoclusters were synthesized by first mixing a gold chloride aqueous solution with a biological molecule aqueous solution at room temperature. The concentration of the biomolecules was kept at 100 mM. The biomolecule to Au ratios were set to be 100:1, 10:1, or 1:1. The well-mixed reaction solution was then kept at a different temperature (25, 37, or 45 °C) in a shaking incubator. After incubation, a dark red solution was obtained, which contained a mixture of Au nanoparticles and fluorescent Au nanoclusters. The Au nanoparticles were removed by centrifugation (15,000 rpm), resulting in a clear Au nanocluster solution. The fluorescent emission of Au nanoclusters was studied as a function of molecule concentration and temperature.

Characterization of Au nanoclusters

The bulk fluorescence property of the Au nanocluster solution was analyzed on a Varian Cary Eclipse Fluorescence Spectrometer. Generally, both emission and excitation spectra were recorded to provide the optimum emission and absorption. All spectra were recorded using preset conditions (medium scan rate, medium detector PMT, and using a 3 mL volume cuvette).

Modeling of Au nanoclusters

Electronic structure calculations were performed on ascorbic acid stabilized Au nanoclusters, using the Gaussia03 software package [4]. The optimized geometry and the HOMO-LUMO gap of the nanocluster complexes were predicted using all-electron density-functional theory (DFT) with the generalized gradient approximation of the Becke three-parameter Lee-Yang-Parr (B3LYP) hybrid exchange-correlation functional [10,2].

Results and Discussion

Nanocluster emission analysis

Two typical fluorescence emission spectra of Au nanocluster solutions after centrifugation are shown in Figure 1. Gold nanoclusters stabilized with ascorbic acid exhibited maximum absorption/emission at 370 nm/450 nm, while MES stabilized clusters yielded maximum absorption/emission at 420 nm/495 nm. The ascorbic acid stabilized nanoclusters exhibited a bluish color under 365 nm UV light irradiation, and MES stabilized clusters showed a greenish color under mercury light irritation with a 405 ± 20 nm filter (Figure 1 inserts).

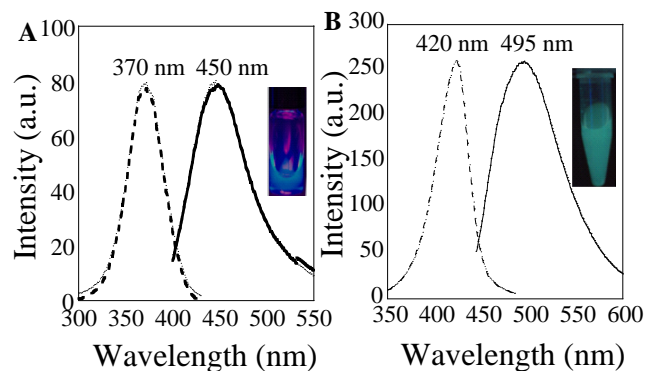


Figure 1. Fluorescence excitation (dashed line) and emission (solid line) spectra of Au nanoclusters, stabilized with biological molecules: (A) ascorbic acid and (B) MES. Insert photography was taken using a 365 nm UV lamp or mercury arc lamp with an emission filter of 405 ± 20 nm.

Simulation of nanoclusters

In the simulations, two optimized structures of the Au_3 -ascorbic acid complexes were obtained with either 2 or 6 ascorbic acid molecules attached to the nanoclusters (Figure 2). The shortest binding distance between the Au_3 nanocluster and the ascorbic acid in $Au_3(C_6H_8O_6)_2$ was $d_{Au-O} = 2.37$ Å.

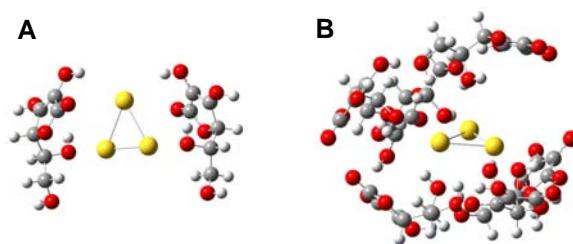


Figure 2. Geometry of ascorbic acid stabilized Au nanoclusters. (A) $Au_3(C_6H_8O_6)_2$ and (B) $Au_3(C_6H_8O_6)_6$.

In contrast, for $\text{Au}_3(\text{C}_6\text{H}_8\text{O}_6)_6$, a cage-like structure with six ascorbic acid molecules was predicted, mainly due to the formation of the intermolecular hydrogen bonds between the hydroxyl ($-\text{OH}$) groups ($d_{\text{O}-\text{H}\cdots\text{O}} = 2.73 \text{ \AA}$). This ascorbic acid cage structure was also proposed to contribute to the stabilization of the Au_3 nanoclusters ($d_{\text{Au}-\text{O}} = 2.29 \text{ \AA}$), suggesting that the H-bonds play a key role in stabilizing the Au_3 nanoclusters.

The calculated HOMO-LUMO gaps for the complexes, $\text{Au}_3(\text{C}_6\text{H}_8\text{O}_6)_2$ and $\text{Au}_3(\text{C}_6\text{H}_8\text{O}_6)_6$, were 2.68 eV (462 nm) and 1.60 eV (775 nm), respectively. These numbers are significantly different from the HOMO-LUMO gap (1.29 eV) of bare Au_3 , which indicates that the ligands indeed influenced the fluorescent emissions of the metallic nanoclusters. The HOMO-LUMO gap of $\text{Au}_3(\text{C}_6\text{H}_8\text{O}_6)_2$ was very close to our experimentally observed blue emission of 450 nm.

To experimentally confirm the formation of the hydrogen-bonded cage structure, we modified our experiments by increasing the ascorbic acid concentration and lowering the reaction temperature. Interestingly, we observed a red shift in the emission spectrum, as shown in Figure 3. Along with the blue fluorescent emission (465 nm), a broad shoulder around 560 nm (Figure 3 arrow) was observed, indicating the likelihood of the more cage-like structure at higher concentrations. The broader emission spectrum suggests the presence of multiple emissive species.

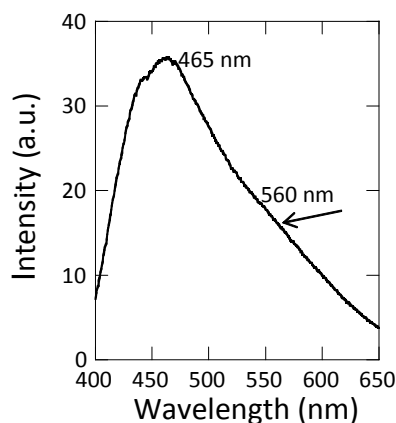


Figure 3. Fluorescent emission of ascorbic acid stabilized Au nanoclusters produced at high molecule concentration and low temperature.

Synthetic parameters

Using MES stabilized Au nanoclusters as a model system, we studied the effects of the synthetic conditions on the fluorescent emissions. Our experimental results show that the fluorescent emission intensity of the MES stabilized Au nanoclusters increased with the reaction temperature, suggesting a higher nanocluster yield (Figure 4A).

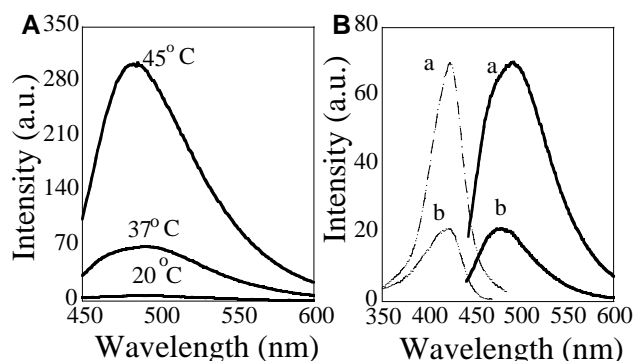


Figure 4. Fluorescent emission of MES stabilized Au nanoclusters at (A) different temperature and (B) different Au concentration at 37 °C.

Temperatures higher than 45°C were not pursued. For the targeted biological applications, the temperature is normally below 45°C. At the same reaction temperature, the yield of the nanoclusters increased with the Au concentration. Figure 4B shows the two emission spectra of Au nanoclusters synthesized at 37 °C for biological molecule to Au ratios of 10:1 (a) and 100:1 (b). Further increasing the Au concentration resulted in significant increases in the production of non-fluorescent Au nanoparticles, which significantly reduced the yield of fluorescent nanoclusters.

Conclusion

In summary, we report a new method for preparing fluorescent and water-soluble Au nanoclusters using small molecules as reducing agents and stabilizing ligands. The yield of the nanoclusters was strongly affected by the reaction conditions, such as temperature and concentration. Our simulation models for the different arrangements of molecules indicate that a mixture of species may have been present, resulting in different fluorescent emissions.

This study provides a starting point towards understanding the formation and stabilization of small size nanoclusters. Furthermore, our work may represent an inexpensive method for producing fluorescent species from bulk colloidal gold.

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