

Synthesis of DNA-templated fluorescent gold nanoclusters

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Abstract Water-soluble and red-emitting gold nanoclusters (Au NCs) were synthesized with single-stranded DNA as a promising biotemplate and dimethylamine borane as a mild reductant. The fluorescent Au NCs can be formed in a weakly acidic aqueous solution that is free from the simultaneous formation of large nanoparticles. The cluster feature of the formed Au species has been revealed by fluorescence spectra, absorption spectra, and transmission electron microscopy. Additionally, DNA sequences could be used to tune the Au NCs' emissions. The as-prepared Au NCs display high stability at physiological pH condition, and thus, wide potential applications are anticipated for the biocompatible fluorescent Au NCs serving as nanoprobe in bioimaging and related fields.

Keywords Gold nanoclusters · Fluorescence · DNA · Dimethylamine borane · Template

Introduction

Decreasing the size of noble metal nanostructures (mainly Au and Ag) down to less than 2 nm will produce nanoclusters (NCs) and restrict the motion of their free electrons in a very confined space that results in discrete electronic band structures. When the discrete band energies become

larger than thermal energies, the NCs will behave like molecules in respect of optical properties such as light absorption and emission. Au NCs have emerged as novel fluorescent nanomaterials because of their better performance in many aspects like biocompatibility, photostability, and non-toxicity relative to organic dyes and semiconductor quantum dots [1–4].

Fluorescent Au NCs have been prepared mainly in a bottom-up manner by the reduction of gold precursors in the presence of various templates such as macromolecules (dendrimers [5–9], proteins [10–24], poly-butadiene [25]), small molecules (histidine [26], carbohydrate [27], thiols [28–33], *N,N*-dimethylformamide [34, 35], penicillamine [36]), and even solid functional organisms (eggshell membrane [37]). Alternatively, top-down etching of preformed large nanoparticles down to desired NC sizes has received much attention due to many available synthetic strategies for the large nanoparticles. In this aspect, polyethylenimine [38], dihydrolipoic acid [39, 40], thiols [41–43], Good's buffers [44], cyclodextrins [45], and even hydrochloric acid [46] have been employed as effective etchants. Recently, large nanoparticles have been reported to be even fluorescent after being sensitized by thiols [47, 48].

It is widely accepted that the formation of stable Au NCs is controlled by a slow thermodynamic process for a narrow size distribution following their relative rapid formation [49] or by a cyclic process of growth and etching reactions around the most stable cluster species to form nearly monodisperse product distributions [46, 50]. In addition, the optical properties of the Au NCs are related to the ligands that protect them from aggregations [51] and redox state of the gold core [52, 53], or the gold core geometry tuned by the oxidation states [53]. On the basis of this mechanism understanding, many applications, for example, detections of Hg^{2+} [12–15, 37, 47], Cu^{2+} [19, 20, 32], CN^- [17], H_2O_2

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[18], glucose [24], and dopamine [21], and cell labeling or imaging [13, 23, 40, 48], have been achieved with Au NCs as reporters by direct or indirect reaction of the NCs' protecting ligands or gold cores with the species of interest. However, in comparison to the fruitful strategies for the DNA-templated synthesis and optical tunability of silver nanoclusters (Ag NCs) [54, 55], there are fewer reports for the successful synthesis of Au NCs with DNA as template. Recently, atomically monodisperse fluorescent Au NCs were obtained by etching gold particles (either spheres or rods) with the assistance of DNA under sonication in water [56]. Due to the photosensitivity of Ag species, the most prominent advantage of DNA-templated Au NCs over Ag NCs in biocompatible applications would be the Au NCs' favorable stability. In this work, single-stranded DNA was first employed as an alternative template during reduction of Au precursor to produce Au NCs (see Fig. 1).

Experimental

Synthesis of fluorescent Au NCs

Twenty-three-mer DNAs with the sequences of 5'-GAGGCGCTGCCYCCACCATGAGC-3' (named **23-Ys**, Y = C, A, G, and T) were synthesized by TaKaRa Biotechnology Co., Ltd. (Dalian, China). All the DNA samples were HPLC purified by the manufacturer. Other reagents were of analytical grade and used without further purification. Nanopure water (18.2 mΩ; Millipore Co., USA) was used in all experiments. In a typical experiment, chloroauric acid (HAuCl₄, Sigma Chemical Co., St. Louis, USA) solution was added to the single-stranded DNA solution in 20 mM phosphate containing 1 mM magnesium acetate (PBS) by an appropriate HAuCl₄/DNA concentration ratio. After being thoroughly mixed, the solution was aged at room temperature for 10 h to allow for the completion of the interaction of HAuCl₄ with DNA. Then, the freshly prepared dimethylamine borane (DMAB, Sigma Chemical Co., St. Louis, USA) solution was added to the aged HAuCl₄/DNA solution, which was followed by another 36-h reaction at room temperature in the dark to produce fluorescent Au NCs. The resulting solutions were examined at room temperature (22±1 °C). For control experiments, sodium borohydride was used as the reductant to replace DMAB.

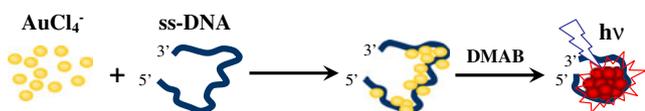


Fig. 1 Schematic illustration for the formation of Au NCs templated by DNA

Characterization of fluorescent Au NCs

Fluorescence spectra were acquired with a FLSP920 spectrofluorometer (Edinburgh Instruments Ltd., UK) at 22±1 °C, equipped with a temperature-controlled circulator (Julabo, Germany). UV/vis absorption spectra were determined with a UV2550 spectrophotometer (Shimadzu Corp., Japan). Transmission electron microscopy (TEM) images were acquired on a JEOL 2010F transmission electron microscope at the acceleration voltage of 200 kV. The TEM samples were prepared by dropping a dispersion of the as-prepared Au NCs onto a Cu grid covered by a holey carbon film.

Results and discussion

Fluorescent Au NCs have been widely synthesized in a bottom-up manner by reduction of gold precursors that are associated with various biotemplates [10] such as bovine serum albumin [11], horseradish peroxidase [18], lysozyme [12], and transferrin protein [20]. Nevertheless, synthesis of Au NCs templated by DNA has rarely been reported maybe because of the weak association between the negatively charged DNA and commonly used precursor AuCl₄⁻. Here, we tried to qualify the right conditions to synthesize fluorescent Au NCs in the presence of DNA (Fig. 1). Twenty-three-mer single-stranded DNAs with the sequences of 5'-GAGGCGCTGCCYCCACCATGAGC-3' (named **23-Ys**, Y = C, A, G, and T) were employed in this work. These sequences are stable in aqueous solution free from any secondary structure at room temperature. In an optimized experiment, the concentration ratio 1:15:75 of DNA/HAuCl₄/DMAB was used to produce fluorescent Au NCs in PBS at pH 4.4 (Fig. S1 and S2 in the Supporting information). As shown in Fig. 2, the red fluorescent Au NCs can be prepared in aqueous solution by reducing the gold salt with DMAB using single-stranded 23-C as the template. The DNA-

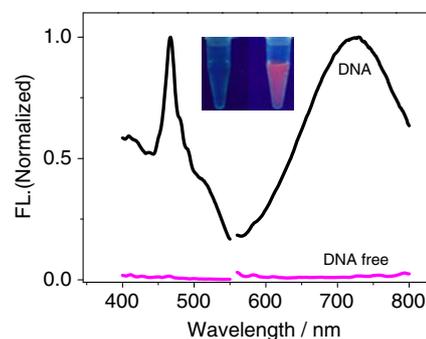
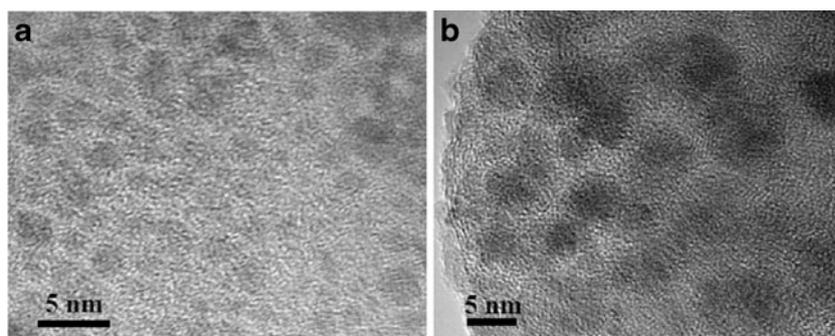


Fig. 2 Fluorescence excitation (measured at 725 nm) and emission (excited at 467 nm) spectra of 20 mM PBS (pH 4.4) containing 75 μM HAuCl₄ and 375 μM DMAB in the absence and presence of 5 μM 23-C. *Inset*: photographs of the solutions in the absence and presence of DNA (from left to right) under UV illumination

Fig. 3 TEM images of Au nanomaterials prepared in PBS at pH 4.4 for NCs (a) and pH 7.0 for larger nanoparticles (b)

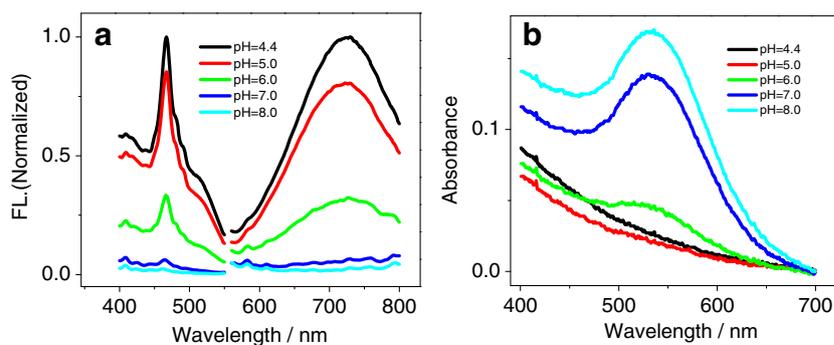


templated Au NCs display excitation and emission bands at 467 and 725 nm, respectively. However, reducing the HAuCl_4 solution by DMAB in the absence of 23-C induces a light pink sample without any noticeable emission, confirming the crucial role of DNA for the formation of fluorescent Au NCs. Under UV illumination, a bright red emission from the as-prepared Au NC solution can be clearly distinguished from that of the solution without 23-C by the naked eye, indicating that highly fluorescent Au NCs are formed in the presence of DNA. Previously, Dickson et al. [5] have explained their experimental results with the spherical Jellium model for predicting the size of Au NCs by fitting the Au NCs' emission energy with the scaling relation of $E_{\text{Fermi}}/N^{1/3}$, where E_{Fermi} is the Fermi level of gold element and N is the number of Au atoms composed of Au NCs. From the observed emission energy of 1.71 eV in our experiment for the DNA-templated Au NCs, we roughly estimate that the number of gold atoms composed of the Au NCs is about 21. As revealed by the TEM analysis (Fig. 3a), it is difficult to accurately determine the diameter of the fluorescent Au NCs due to the low TEM contrast with the background for such small-sized materials, which is in good agreement with the cluster dimension predicted by the Jellium model. However, the cluster profile can be easily seen from the TEM image.

We found that many factors strongly affected the formation of fluorescent Au NCs. As shown in Fig. 4a, the solution pH plays a key role in modulating the emissions of Au NCs templated by 23-C. By comparison to the emission from the solution prepared in PBS at pH 4.4, the resulting solutions prepared in PBS at pH 5.0 and 6.0 exhibit

1.2- and 3.1-fold decreases in the fluorescence intensities, respectively. However, there is almost unnoticeable fluorescence emission for those prepared in PBS at pH 7.0 and 8.0. Therefore, acidic solution conditions seem to facilitate the creation of fluorescent Au NCs. Absorption spectra were then followed to further confirm the influence of the solution pH on the formation of fluorescent Au NCs. As shown in Fig. 4b, the solutions prepared at pH above 6.0 accordingly exhibit clear absorption peaks located at about 525 nm, which suggests the formation of larger gold nanoparticles with characteristic surface plasmon resonance absorption. As an example, the production of such gold nanoparticles at pH 7.0 is thus evidenced by TEM analysis with diameter larger than 5 nm (Fig. 3b). By contrast, featureless absorption spectra are observed for the solutions prepared at lower pH values. This fact indicates that the fluorescent Au NCs produced at the weakly acidic conditions should be smaller than 2 nm in diameter [57, 58], which is in agreement with the TEM results and the Jellium model-based prediction. Previously, the similar absorption spectra with such featureless behaviors were observed for fluorescent Au NCs by their intensities decaying roughly exponentially toward the visible region from the UV region [14, 36, 38, 39]. Therefore, the production of fluorescent Au NCs is free from the simultaneous formation of large gold nanoparticles at the acidic conditions. On the basis of these observations, our method would expand the potential applications of fluorescent Au NCs with DNA as the biotemplate because the previously reported protein-based synthesis of fluorescent Au NCs was mostly carried out at strong alkaline conditions ($\text{pH} \geq 12$) [10].

Fig. 4 Effects of solution pH on the formation of fluorescent Au NCs: **a** fluorescence spectra, **b** absorption spectra



However, the immediate addition of DMAB into the freshly mixed DNA–HAuCl₄ solution prepared at whatever pH results in the prompt formation of pink samples without any fluorescence response to be observed. Consequently, we speculate that the first crucial step for the creation of fluorescent Au NCs is the formation of an Au(III)–DNA complex, which occurs by replacing the Cl[−] ligands in AuCl₄[−] with DNA bases before the reduction. To follow the reaction between AuCl₄[−] and DNA, we monitored the time evolution of the DNA absorption spectra at 260 nm after the addition of HAuCl₄. As shown in Fig. 5, an abrupt decrease in the absorption is evidenced after aging the sample prepared at pH 4.4 for 10 h, whereas there is no distinct change in the absorption for the sample prepared at pH 7.0 even with the reaction time extending up to 50 h. Although the exact interaction mechanism of the DNA base with HAuCl₄ is not yet clear, the coordination and chelation between gold and both the ring and amino nitrogens of the nucleic acid bases [59] should contribute to this process. At an acidic solution, cytosine in DNA should be partially protonated [60] to facilitate its interaction with the negatively charged AuCl₄[−], while at neutral and alkaline conditions, there is a relative large repulsion force to prevent the negatively charged DNA from approaching the negatively charged AuCl₄[−]. The possible protonation of cytosine in DNA would induce less base stacking, which is reflected by the higher absorption at pH 4.4 than that at pH 7.0 as observed at the initial stage of AuCl₄[−] addition (Fig. 5). Nevertheless, the at-least 10-h aging time for the production of fluorescent Au NCs at the weakly acidic condition shows that the specific interaction between AuCl₄[−] and DNA is still a slow process. Thus, without the aging step prior to reduction, Au(III) mainly in the form of AuCl₄[−] free in water can be directly reduced by DMAB into large gold nanoparticles. Further works will be expected in this laboratory to identify the interaction mode of DNA bases with the fluorescent Au NCs by, for example, infrared and circular dichroism spectra.

It is well known that different DNA sequences and lengths can be used to modulate the emissions of DNA-

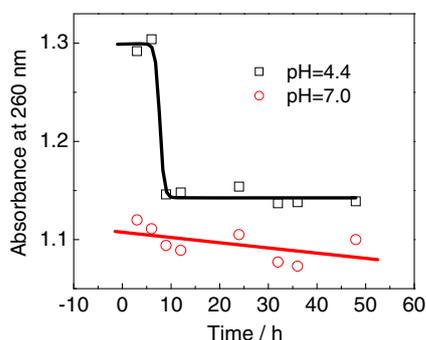


Fig. 5 Time evolutions of the corresponding absorbances at 260 nm for the HAuCl₄–DNA solutions at pH 4.4 and 7.0 before DMAB addition

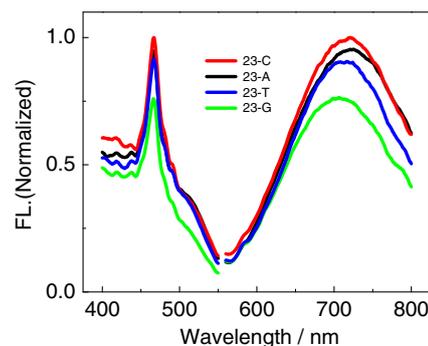


Fig. 6 Dependences of DNA sequences on the fluorescence spectra of the as-prepared Au NCs

templated silver nanoclusters [54]. Thus, it is expected that the DNA sequences could be also used to tune the Au NCs' emissions. To examine the impact of DNA sequences, we only changed the central base in 23-C from cytosine to adenine (23-A), guanine (23-G), and thymine (23-T) and kept the other reaction conditions unchanged. As shown in Fig. 6, the Au NCs' emissions are dependent on the DNA sequences with the intensities decreasing in the order of 23-C > 23-A > 23-T > 23-G. The emission maxima are also blue shifted in the same order. Due to the one-base alteration for all the used DNAs at the same length, small changes in Au NCs' emissions could be imaged as observed here. Therefore, we believe that it is feasible to synthesize Au NCs with different emission behaviors by DNA sequence alterations.

We found that the used reductant had a profound effect on the formation of fluorescent Au NCs. For example, replacement of DMAB with NaBH₄, a common reductant in the synthesis of noble metal nanoclusters [54], mainly resulted in prompt production of large nanoparticles with barely faint fluorescence even though the aging procedure was still carried out, which is in agreement with the previous observation that NaBH₄ was an ineffective reductant for the production of fluorescent Au NCs [36]. By comparison to

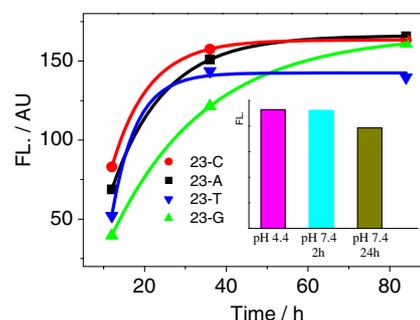


Fig. 7 Time evolutions of the Au NCs' fluorescence emissions after the addition of DMAB. *Inset:* the emission intensities of the preformed Au NCs templated by 23-C at pH 4.4 and then after 2 and 24 h of adjusting the solution pH to 7.4

the strong reduction capacity related to NaBH_4 , DMAB was a weak reductant [61] and proved to be a fine candidate to reduce the DNA-bound gold species to fluorescent Au NCs. As shown in Fig. 7, an incubation time of 36 h after the addition of DMAB is needed to get the stable emissions on account of the weak reducing capacity of DMAB at the weakly acidic condition. The formed Au NCs are stable enough to keep their emissions for more than 2 days. Thus, we reasonably conclude that a slow reduction process of the DNA-bound gold species is crucial to prevent the preformed Au NCs from aggregating into large nanoparticles.

Lastly, we tested the stability of fluorescent Au NCs at the solution with different pH from that for their preparation. As shown in the inset of Fig. 7, the fluorescence intensities of the preformed Au NCs at pH 4.4 decrease only 1 and 15.6 % after 2 and 24 h of adjusting the solution pH value to 7.4, indicating that the preformed Au NCs' emission is not seriously affected by electrolyte's pH. Accordingly, we expect that although the fluorescent Au NCs can be formed only at the weakly acidic conditions, the high stability of the preformed Au NCs at the physiological pH condition would greatly facilitate their potential use in bioimaging applications due to biocompatibility of the used DNA template.

Conclusion

In summary, we presented a new approach for the synthesis of water-soluble, red fluorescent Au NCs templated by DNA. Investigations by fluorescence, TEM, and absorption spectra convince that the fluorescent Au NCs can be formed by reducing the Au precursor with DMAB at weakly acidic pH conditions. During this process, the aging time for completing the interaction of DNA with HAuCl_4 before reduction is critical to form the fluorescent Au NCs. In addition, the Au NCs' emissions could be tuned by DNA sequences. The high stability of the preformed Au NCs at the physiological pH condition and the biocompatibility of the used DNA template would support their wide applications as novel nanoprobe.

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