

One Pot vs. Two Step Synthesis of Fluorescent Gold Quantum Dots and Capping with Thiolated Organic Linker

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ABSTRACT

The current report demonstrates two methods; one pot and two steps synthesis techniques for the fluorescent gold quantum dots (F-AuQDs) functionalized with thiolated organic ligands using reduction of gold precursor in *N,N'*-dimethylformamide (DMF) with heating for 1h. The F-AuQDs shows fluorescent emission at 425 ± 5 nm upon excitation at 345 ± 5 nm with good water solubility and stability. The F-AuQDs were linked with five different bifunctional ligands comprising of carboxylic acid, alcohol, or aromatic amine. The formation mechanism and functionalization were characterized using UV-vis absorption spectra, UV-vis light, fluorescent emission spectra, pH, TEM, and FTIR. The fluorescent emission of F-AuQDs is greatly dependent on the conjugated thioligand. The functionalized F-AuQDs have wide applications in fluorescent labeling of biomolecules, optical devices, imaging, energy transfer, and biosensing.

Keywords: Gold quantum dots, DMF, bi-functional ligand, Fluorescence, Electron microscopy

1. INTRODUCTION

Gold (Au) and silver (Ag) nanoclusters (NCs) that possess sizes below 2 nm behave as intermediate materials with characteristics between atomic and larger sized nanoparticles [1]. These particles have been given great attention over the past decades for their attractive fundamental properties such as, photoluminescence [2], sensing [3] and bioassay [4]. Comparable to NCs semiconductor quantum dots (QDs) when possessing sizes smaller than the Bohr radius (4-5 nm) show strong quantum-size captivity [5]. Whereas, Au nanoparticles (AuNPs) show a size-dependent plasmon absorption band when their conduction electrons in both the ground and excited states are confined to dimensions smaller than the electron mean free path (ca. 20 nm) [6], but plasmon absorption disappears completely for AuNPs less than 2 nm [7]. This results in molecule-like properties including photoluminescence and size dependent fluorescence [1].

Semiconductor quantum dots (QDs) have been used for cancer targeting and biomedical imaging. However, the heavy metals contained in QDs are toxic, making them unsuitable for *in vivo* clinical application, and may pose risks to human health as well as the environment under certain conditions. In contrast to other QDs, noble metal (Au, Ag) NCs and QDs are highly suitable for bioimaging and biolabeling applications due to their low toxicity as well as ultra fine size.

There have been very few studies reported for the synthesis of fluorescent and luminescent, noble metal NCs and QDs [8, 9]. The advantages of the previously reported methods for preparing fluorescent Au NCs and QDs are good control over both the size and stability. However, disadvantages still remain which include impurities introduced by the use of surfactants and incomplete reduction in the presence of hyper branched ligands. The approach for the formation of fluorescent Au, Ag, and other metallic NCs with various morphologies has been previously demonstrated [10], using DMF as a solvent as well as reducing agent. Using DMF being able to act as a mild reducing agent at high temperature; a special focus has been given to the fabrication of Au QDs. Liu et al., have reported a surfactant-free synthesis pathway for preparing highly fluorescent F-AuQDs [11] using DMF in a two step process taking more than 28 hours of reaction and workup. These F-AuQDs show both good controls over the stability and physical properties.

The present work demonstrates novel approaches for the chemical synthesis of F-AuQDs functionalized with thiolated organic ligands in a one pot and two step reactions that utilize DMF to reduce gold ions at a high temperature. Ligand capped F-AuQD were produced after one hour of heating reaction. This method shows good aqueous solubility, as well as, fluorescence emission stability.

2. EXPERIMENTAL

2.1 Materials and Method

All the chemicals and reagents were purchased from commercial source and used without any further purification. 11-mercaptoundecionic acid (11-MUA), 4-

aminothiophenol (4-ATP), sodium hydroxide, and sodium phosphate monobasic were purchased from Sigma Aldrich, St. Louis, MO, USA. Hydrogen tetrachloroaurate trihydrate ($\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$), *N,N'*-dimethylformamide, 2-mercaptoethanol (TE), 3-mercaptopropionic acid (3-MPA), and methanol were purchased from Acros Organics, NJ, USA. Thiocetic acid (TA) was purchased from Alfa Aesar, Ward Hill, MA, USA. Doubly-distilled de-ionized water (DIW) was collected from Labconco (water Pro PS) water purification system. The 1x phosphate buffered saline (PBS, pH 7.2) was prepared from a 10x autoclaved stock PBS (100 mM phosphate buffer, 1.37M NaCl, 27 mM KCl) in DIW.

2.2 Instruments

UV-visible absorption spectra were recorded using PharmaSpec UV-1700 Shimadzu model and fluorescence emission spectra were acquired with a Fluoromax-3 spectrometer from Jobin Yvon Horiba. TEM images were obtained on JEOL 1200 EX II TEM equipped with LaB6 gun with 0.5 nm resolution. Infrared spectra were recorded using a Mattson ATI Genesis spectrometer with sodium salt plate method.

2.3 One pot synthesis of F-AuNPs conjugated with organic thio-compounds

100 μL of 0.1M aq solution of $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$ and 30 μL of 1.0M thio-compound (TA, TE, 3-MPA, 11-MUA, and 4-ATP) solution were added to 10 mL of DMF, and stirred for 10 minutes at room temperature (RT). The reaction mixture was then heated at 140 °C for 1h with continuous stirring. After 1hr, the reaction changed to pale gold or light brown in color and the reaction was cooled to RT. The reaction mixture was centrifuged for 30 minutes at 11800 rcf., and the supernatant was separated and evaporated under reduced pressure over rotavap. The light brown precipitate was re-suspended in 3 mL of MeOH, aq NaOH solution, Tris-HCl buffer (pH 8.02) and stored at 4 °C.

2.4 Two step synthesis of F-AuNPs

The first part of two step method involved the synthesis of bare F-AuQDs and was followed by purification. The F-AuQDs were then conjugated with thiolated ligands in 24 h reaction during the second the second step. The synthesis of bare and thiolated ligand functionalized F-AuQDs was performed as reported by Liu et al. [11], except that 1h of heating for the reaction was compared to 4h.

3. Results and Discussion

3.1 UV-Vis absorption monitoring of F-AuQDs

The real time monitoring of thio-ligand conjugated F-AuQDs synthesize via the one pot reaction, was performed using UV-vis monitoring of the disappearance of the absorption band of gold ions Au(III). The starting aqueous

solution of $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$ shows an absorption band at 321 ± 3 nm (Fig. 1a and 2a). It was observed that within a short period of reaction time (between 1-1.5h) as the DMF reduces the Au(III) ions into Au(0) ($(\text{Au})_n$; QDs) by heating at 140 °C, the absorption band of 321 ± 3 nm disappears and a weak band at 291 ± 5 appears for bare F-during AuQDs reaction. The longer reactions time (after 1.5 or 2h) this band starts disappearing (Fig.1a). Therefore, the reaction was stopped after 1h of heating, where optimal absorbance is shown. However, in the presence of thiolated ligands, different UV-vis absorption bands appear. Three ligands, were used to functionalized F-AuQDs during the two step synthesis, their absorption bands appear at $255 \pm 5\text{nm}$, $280 \pm 5\text{nm}$ and $325 \pm 5\text{nm}$ for 3-MPA, $255 \pm 5\text{nm}$ and $280 \pm 5\text{nm}$ for 4-ATP, and $280 \pm 5\text{nm}$ for 11-MUA, as shown in Figure 1b.

A total five thiolated ligands (TE, 11-MUA, TA, 3-MPA, and 4-ATP) were conjugated with F-AuQDs using the one pot synthesis method. The absorption bands measured for TE and 11-MUA show two bands at $280 \pm 5\text{nm}$, and $330 \pm 5\text{nm}$, TA and 3-MPA just show bands at $330 \pm 5\text{nm}$, while 4-ATP did not show any clear absorption band as shown in Figure 2. These absorption values are significantly different then the previously reported by Liu et al. [11], however the reproducibility is limited with longer reaction times these a decrease in absorption intensity rather than the reported increase.

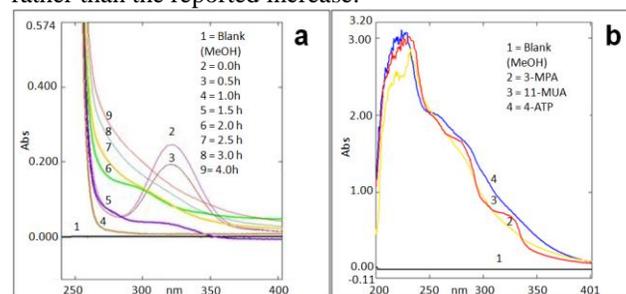


Figure 1: UV-vis absorption spectra (a) kinetic study for F-AuQDs synthesis, (b) Thio-ligand conjugated F-AuQDs using two step method.

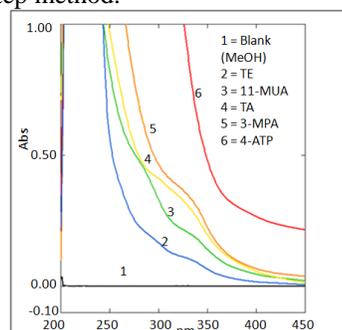


Figure 2: UV-vis absorption spectra of samples synthesized in one pot synthesis; thiolated ligand conjugated F-AuQDs.

A qualitative visual monitoring of bare and functionalized F-AuQDs, synthesized using the one pot method were

characterized using UV-vis light (365nm) as shown in Figure 3. TA conjugates QDs appear pale yellow to gold in color, with 11-MUA appearing light brown, 3-MPA and TE conjugates are pale yellow, 4-ATP conjugated F-AuQDs are intensely brown in color, while bare F-AuQDs are again pale yellow in color. Under UV light, 11-MUA-F-AuQDs and TA-F-AuQDs conjugate shows the highest fluorescence brightness whereas, 4-ATP Functionalized F-AuQDs shows weakest fluorescence brightness.

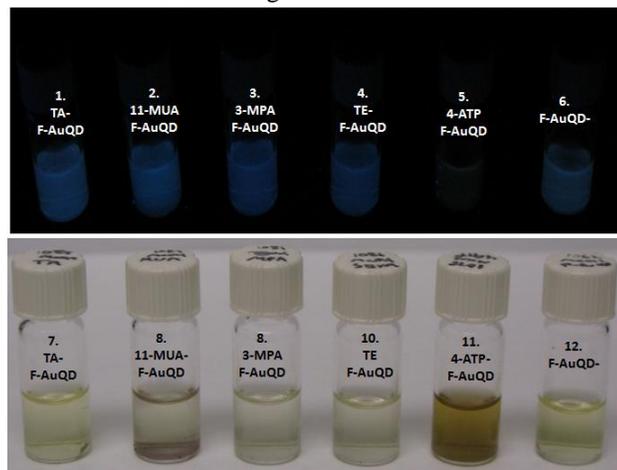


Figure 3: Camera photos of aqueous suspension of thio-ligand F-AuQDs from one pot synthesis. Top: samples (1-6) under UV-vis light (365nm) and bottom: same samples (7-12) under ambient light. Where, 7 and 12 are bare F-AuQDs.

3.2 Fluorescent Emission of F-AuQDs

The fluorescence of gold NCs and QDs is considered to arise from electronic transitions between the filled $5d^{10}$ band and the $6sp^1$ conduction band upon excitation at certain wavelengths of the gold atom. [12] We applied 13 different excitation wavelengths at: 285, 295, 305, 315, 325, 335, 345, 355, 365, 375, 385, 395 and 405 with ± 5 nm of slit to analyze the fluorescence emission intensity (data not shown here). The emission intensity increases from 285 ± 5 nm to 345 ± 5 nm with a gradual red shift of 15nm, thereafter emission intensity constantly decreases until 405 ± 5 nm with a further 10nm red shift. Therefore, an excitation wavelength of 345 ± 5 nm was used for all experiments and a distinct fluorescent emission for bare and functionalized F-AuQDs being observed.

As compared to bare F-AuQDs, the functionalized F-AuQDs synthesized using the two step reaction are not showing any strong fluorescent emission at 425 nm but another blue shifted emission at 330 nm is observed (Figure 4a). Conversely, functionalized F-AuQDs synthesized using the one pot synthesis give only blue fluorescent emission at 425 ± 5 nm in aq suspension except 4-ATP conjugate (412 nm), as shown in Figure 4b. The fluorescent emission intensity is variable and dependent on the conjugated ligands. It is important to point out that recently efforts

have been made in developing biosensors and bioassays based on fluorescent gold NCs and QDs, which have mainly been achieved by using processes that replace or mix ligands. However, the one pot method present here demonstrates that F-AuQDs can be functionalized initially by capping various thio-ligands during the reaction process.

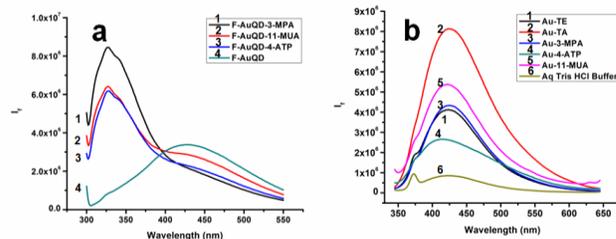


Figure 4: Fluorescence emission spectra of bare and Thio-ligand F-AuQDs (a) using two step reactions and (b) using one step synthesis samples in aqueous suspension pH 8.02. Each sample was excited at 345 ± 5 nm of wavelength.

3.3 TEM Characterization of F-AuQDs

The size of 11-MUA-F-AuQDs and TA-F-AuQDs synthesized using the one pot method are characterized using TEM micrographs as shown in Figure 5a,b. The size of conjugated 11-MUA-F-AuQDs appears about 5 ± 2 nm while TA-F-AuQDs are appearing in the form of 550 ± 50 nm aggregates of sub-nanometer of NCs.

It is well known that the subnanometer atomic NCs synthesized in the absence of any ligand are too small in size to provide sufficient plasmon response to be clearly visible under TEM observation of larger free-electron metal nanoparticles. [13] However, functionalized F-AuQDs provide a good pathway for aggregation, thus the F-AuQDs can appear as a much darker shade under TEM observation, i.e. thus explaining the same effect being seen in the TEM images.

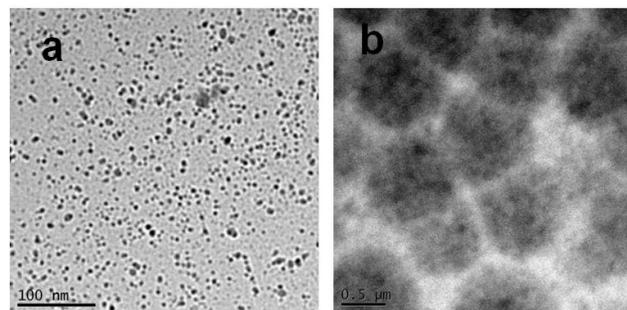


Figure 5: Transmission electron micrographs of samples from one pot synthesis (a) 11-MUA-F-AuQDs and (b) TA-F-AuQDs, where bar scales are 100 nm and 0.5 μ m respectively.

3.4 FT-IR of thio-ligand & Modified F-AuQDs

The linkage between thio-ligand and F-AuQDs was characterized using FT-IR analysis and compared with the unreacted thio-reagent solution in DMF. The spectra were collected by placing a small aliquot of sample on sodium salt plates. The thiol (RS-H) compounds show S-H stretch $\sim 2500\text{ cm}^{-1}$ [14], however after conjugation with F-AuQDs the S-H bond absorption disappears. The 3-MPA-FAuQDs sample was selected for FTIR analysis as shown in Figure 6. This spectrum clearly shows the disappearance of the thiol stretch at 2530.43 cm^{-1} ; thus confirming the linkage with gold QDs.

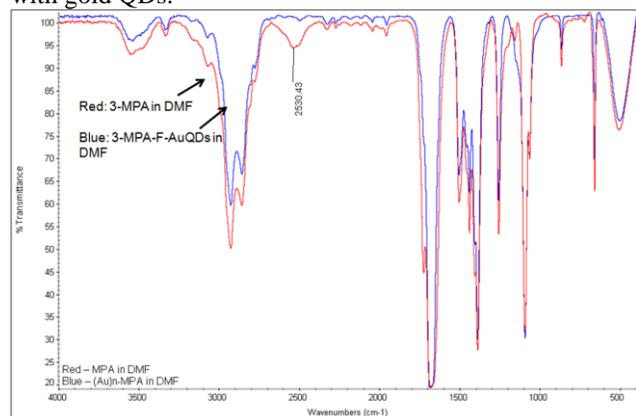


Figure 6: FT-IR spectra of 3-MPA-F-AuQDs conjugates in DMF from one pot synthesis.

4. Conclusion

In this report we have developed novel, facile, and surfactant free synthetic methods for water-soluble highly fluorescent gold quantum dots (F-AuQDs), which can be capped with various bi-functional organic ligands using two steps, or in a one pot reaction. The weak reducing agent DMF, is used to produce functionalized F-AuQDs that, show blue fluorescent emission and nanodiameters of $5\pm 2\text{ nm}$ size. The method does not require complex synthesis, toxic precursors, or difficult capping or solubilization chemistry. The bare and functionalized F-AuQDs provide a better platform to be linked with various biomolecules and for utilization as labeling probes or energy transfer units for developing optical sensor devices and biosensors.

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