Single fluorescent gold nanoclusters

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Abstract: Both ensemble and single-molecule measurements were performed to explore the fluorescence properties of Au nanoclusters (NCs). Photoinduced fluorescence enhancement was observed for ensemble NCs in solution, but photobleaching was found at ambient environments. At the single-molecule level, fluorescence blinking and single-step photobleaching were observed. Furthermore, their time-resolved fluorescence shows a single exponential decay with a lifetime of ~7 ns and is insensitive to changes in fluorescence intensity. The lifetime distribution is more homogeneous within ensemble Au NCs as compared to CdSe QDs. Therefore, Au NCs have potential applications as nontoxic fluorescent labels for lifetime-based imaging microscopy. However, their low quantum yields and poor photostability are disadvantageous factors, which require further improvement.

OCIS codes: (330.0130) Fluorescence; (170.6280) Spectroscopy; Fluorescence blinking; Metal nanoclusters.

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1. Introduction

Recently, nanomaterials including metals and semiconductors have attracted much attention due to their unique optical properties for fundamental research and potential applications in biological labeling [1]. Noble-metal nanoparticles (NPs) with sizes ranging from 10 nm to 100 nm have been investigated extensively. They exhibit strong optical absorption and scattering in the visible range as caused by collective oscillation of free electrons within the NPs (called localized surface plasmon resonance, LSPR) [2]. Such strong LSPR effects offer many potential applications, including biological markers and sensors, even down to the single-particle levels [3,4]. In this case, Au NPs were commonly used because of their biocompatibility, non-toxicity, and photostability. In general, to probe single-particle LSPR effects, one general way is to detect the scattering light by dark-field microscopy [5]. However, the intensity of scattered light varies as the sixth power of the NP sizes. Consequently, the scattered intensity would be decreased considerably with NP sizes down to ~10 nm. In contrast, the absorption cross-section varies only with the third power of the NP sizes. Therefore, fluorescence could be an alternative method to probe smaller NPs.

Actually, fluorescence from metal materials has been observed for more than thirty years [6] but has not attracted much attention due to very low quantum yields (QYs) of ~10^{-10}. Recently, fluorescence with much higher QYs (>10^{-5}) was observed in small metal NPs (< 100 nm) [7,8] and metal NCs (< 2 nm) [9,10]. For Au NPs, the origin of fluorescence was assigned to radiative emission of excited LSPR due to similar characteristics [7,8]. For fluorescent Au NCs, their LSPR features disappeared due to a small number of free electrons. Therefore, the fluorescence could be due to electronic state transitions, and the actual mechanism is still under investigation. In addition to biological applications, Au NCs have been incorporated into solar cells to enhance conversion efficiency [11].

Greener, fluorescent and small-sized Au NCs are expected to be the promising candidates for future biological applications [12]. Although colloidal CdSe QDs exhibit excellent fluorescence properties, such as high QYs and good photostability [13], the use of toxic precursor is a fatal drawback for their practical applications in life science. Thus, it is important for us to understand the fluorescence properties for Au NCs, in particular, down to single-NC levels. In this study of fluorescence properties of Au NCs, we report our ensemble and single-particle measurements.

2. Sample synthesis and experiments

We synthesized Au NCs according to the reported method and the details could be found in ref. 12. Briefly speaking, Au NCs were formed based on precursor-induced fragmentation. Initially, Au NPs stabilized by didodecyldimethylammonium bromide (DDAB) were

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synthesized in toluene [14]. The resulting solution exhibits red-translucent appearance due to a typical plasmonic absorption peak. From TEM analysis, the mean size of Au NPs is 5.5 ± 0.7 nm. Subsequently, continually adding Au precursors and dodecanethiol (DDT) into original NP solution can cause the solution to be colorless due to disappearance of plasmonic absorption [12]. Finally, ligand exchange with dihydrolipoic acid (DHLA) was used to obtain water-soluble Au NCs, which dispersed in alkaline buffer solution and with the sizes of 1.5 ± 0.3 nm, as shown in Fig. 1(b). On the other hand, colloidal QDs at ~600 nm emission were purchased from Evident Technologies.

To measure single NCs, extremely dilute colloidal solution (10^{-9} M) was first prepared and then dispersed onto a pre-clean glass coverslip by spin coating technique. In this case, the mean separation between NCs was larger than excitation laser spots, thus individual NCs can be monitored by far-field laser scanning confocal microscopy (MicroTime 200, PicoQuant). For excitation, a pulsed diode laser at ~460 nm was focused to a diffraction limited spot by an oil-immersion objective (Olympus, N.A.=1.4). The fluorescence was collected by the same objective and guided to a confocal pinhole to reject out-of-focus light. After a pinhole, the fluorescence was detected by a single-photon avalanche photon diode. Furthermore, to obtain temporal information, such as fluorescence lifetime, fluorescence intensity time traces, we also performed time-tagged, time-resolved measurements [15] (TTTR, Time-Harp 300, PicoQuant) during the courses of single-NC measurements. Based on the TTTR modes, the relative time interval between the pulsed laser excitation and the photon emission (time-resolved modes) and the absolute time between the experimental start and the photon emission (time-tagged modes) can be obtained simultaneously. Fluorescence lifetime images were obtained by constructing all lifetime information pixel by pixel. Fluorescence lifetimes were extracted from the histograms of the relative time of all photons (time-resolved modes) within the pixel. The time resolutions for the time-tagged modes, the time-resolved modes, and the instrument response functions are 100 ns, 40 ps, and 350 ps, respectively.

3. Results and discussion

Figures 1(a) and 1(b) show the absorption and the fluorescence spectra of ensemble Au NCs in solution. Obviously, no LSPR feature was found in the visible range, instead a broad absorption band was observed in the ultraviolet region. It implies that fluorescence is not attributed to radiative emission by excitation of LSPR. The origin of this fluorescence is likely due to the transition of molecule-like electronic levels. The mechanism is still under debate [10]. The fluorescence spectrum has an asymmetric line shape and a broad line width. This broad line width originates from the size distribution within the ensemble NCs, as evidenced by the TEM image, which was shown in the inset of Fig. 1(b). Moreover, a Stoke-shift of more than 200 nm was found, which is significantly larger than that of the conventional fluorescent dye molecules.

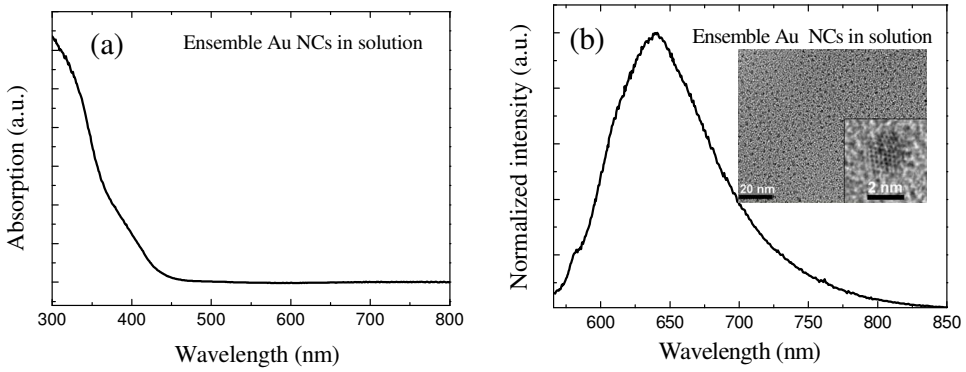

Fig. 1. (a) Absorption and (b) fluorescence spectra of ensemble Au NCs in solution along with TEM images in the inset.

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In order to estimate the photostability of Au NCs, fluorescence intensity as a function of illumination time was measured based on TTTR acquisition modes. Figure 2(a) and 2(b) shows the variations in fluorescence intensity with illumination times for ensemble Au NCs in solution and at ambient environments (evaporation of solvent). Ensemble NCs in solution are photostable, moreover photoinduced fluorescence enhancement was observed for long-time illumination. This photoinduced fluorescence enhancement has been observed for colloidal CdSe QDs [16-18] and silver nanoparticles [19], likely due to photoinduced surface passivation. However, when the solvent was evaporated, fluorescence intensity dropped rapidly with illumination time. This photobleaching behavior only occurred at ambient environments but did not appear in solution.

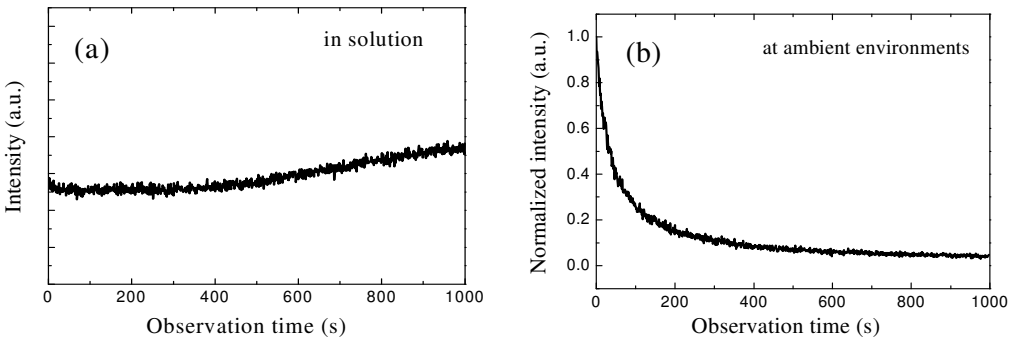

Fig. 2. Fluorescence intensity variations with illumination time in solution (a) and at ambient environments (b).

To gain more insight into the fluorescence properties, the single-molecule detection (SMD) technique was employed to probe individual Au NCs within the ensemble samples. Based on the SMD technique, single-NC behavior could be highlighted and inter-NC interaction was diminished. Thus, intrinsic properties could be obtained. Figure 3 displays the fluorescence intensity images of Au NCs. Diffraction-limited spots with streaky patterns were found for some NCs due to fluorescence blinking. In addition, some of the spots exhibit an incomplete stripe shape due to fast photobleaching during the scanning processes. Figure 4(a) shows the representative fluorescence time traces of a single Au NC with a 10 ms bin time for total the measured time. At the beginning, reversible on/off blinking behavior was observed clearly as shown in Fig. 4(b), and then irreversible single-step photobleaching occurred. This single-step photobleaching is another evidence for detection of single NCs. Note that, not all NCs exhibited blinking behavior, and some of them suffered from very fast photobleaching.

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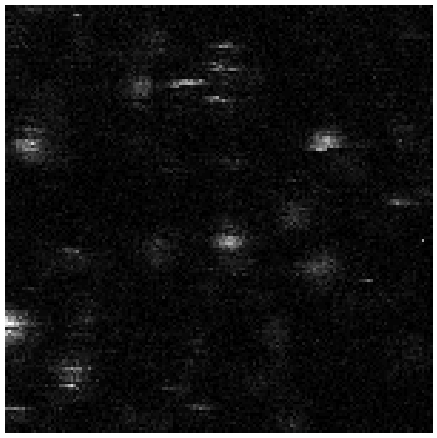

Fig. 3. Fluorescence intensity images of $4 \times 4 \mu m^2$ for single Au NCs.

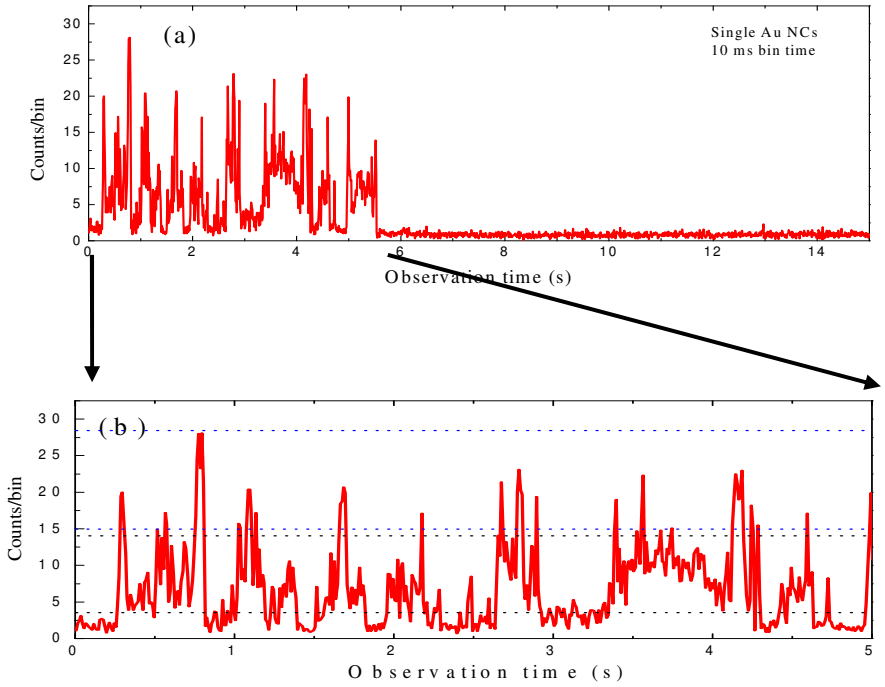

Fig. 4. Fluorescence time traces for single Au NCs for total measured time (a) and the initial time window of 5 s (b).

To obtain quantitative information about the blinking statistics, we adopted the conventional method for colloidal CdSe QDs by selecting a proper threshold intensity (measured background noise + 3-fold standard deviation) to separate on/off time events. Thus, the blinking statistics of on/off times can be constructed. Figures 5(a) and 5(b) show the blinking statistics of on (a) and off times (b) with log-log plot by compiling ~20 individual NCs. Due to suffering from fast photobleaching, we can only construct the on/off time distribution with about one decade. Therefore, we could not get enough data to judge whether the blinking statistics follows a power-law or not. Here, we used a linear function to fit the on/off times distributions within this one decade. Unlike colloidal QDs with better

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photostability, the on/off time distribution could extend many decades to display unambiguously a power-law distribution [20-24].

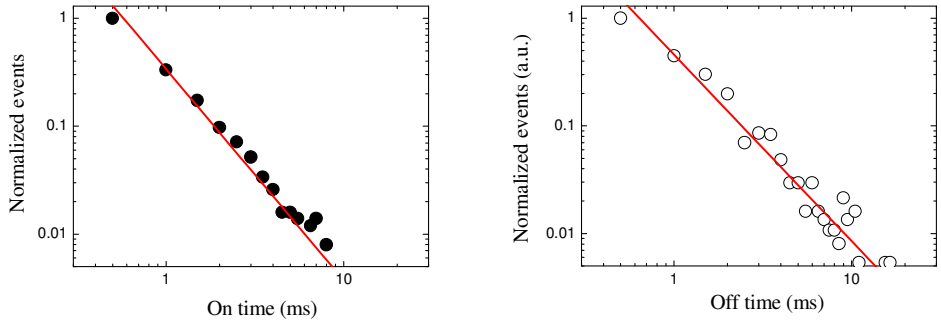

Fig. 5. Blinking statistics of the on-times (a) and the off-times (b).

Furthermore, based on the TTTR measurements, we also recorded the fluorescence decay profiles for single Au NCs. As shown in Fig. 6(a), the fluorescence decay profile of single Au NCs exhibits a pure single exponential curve with ~7 ns fluorescence lifetime upon subtracting background contribution. This fluorescence lifetime is longer than common auto-fluorescence from most of the biological systems (~2 ns) [25]. In addition, this fluorescence lifetime is not sensitive to fluorescence intensity. As shown in Fig. 6(b), fluorescence decay curves are similar for high and low intensity regimes, as marked by the dotted line in Fig. 4(b). In contrast, fluorescence lifetimes for colloidal CdSe QDs are sensitive to fluorescence intensity due to fluctuation of nonradiative decay rates [26]. Therefore, the fluorescence lifetime could be a useful parameter in image applications.

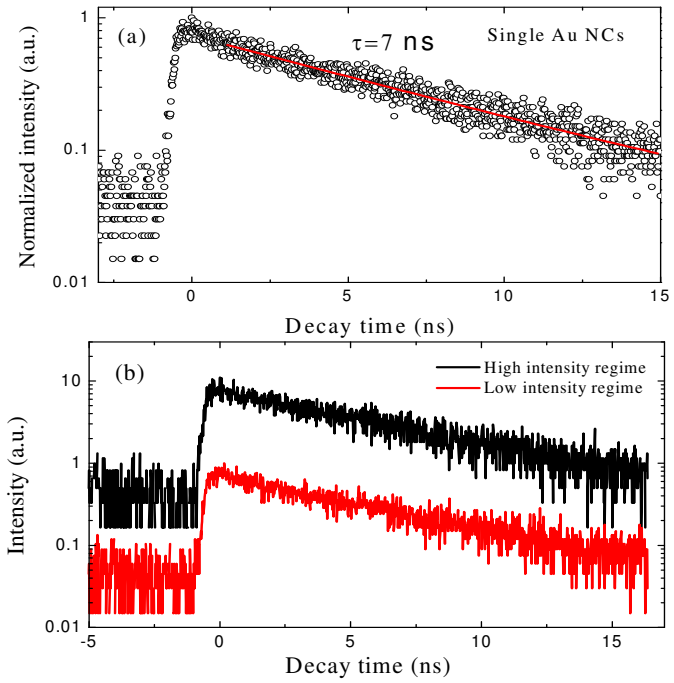

Fig. 6. (a) Fluorescence decay profile of single Au NCs, showing a single exponential decay. (b) Fluorescence decay profiles for high and low intensity regimes.

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In general, fluorescence lifetime is sensitive to variations of local environment and can provide local information about their immediate environments. Therefore, lifetime can be a useful parameter for biological applications, such as fluorescence lifetime image microscopy (FLIM). In contrast to intensity-based microscopy, lifetime-based microscopy is insensitive to label concentration. In this case, a fluorescence image is constructed by fluorescence lifetimes within every pixel instead of fluorescence intensity. By integrating all photons within every pixel and fitting by a single exponential decay function, we can extract fluorescence lifetime information for every pixel. For colloidal CdSe QDs, they possess longer lifetimes but often exhibit multi-exponential decay behavior [25,26]. Therefore, Au NCs could be a better candidate in lifetime imaging applications. However, their low QYs and poor photostability are the major drawbacks. Therefore, enhancement of the QYs and the photostability are needed for practical applications.

As illustrated in Fig. 7(a), pure color and high contrast lifetime images can be obtained by means of single Au NCs. For comparison, a fluorescence lifetime image for single CdSe/ZnS QDs is also shown in Fig. 7(b), which exhibited a multicolor image due to lifetime variations [26]. The comparison clearly demonstrates the advantages for Au NCs. Shown in Fig. 8(a) is the lifetime distribution of all pixels within FLIM images for Au NCs and CdSe QDs, and a selected single nanoparticle in Fig. 8(b). The lifetime distribution of Au NCs is relatively narrower and the FWHM of an individual NC is ~2.3 ns. Shown in Fig. 8(c) and 8(d) are the lifetime distributions for four Au NCs and CdSe QDs, respectively. Obviously, Au NCs exhibit a more homogeneous lifetime distribution. Further FLIM studies of Au NCs within biological cells are underway.

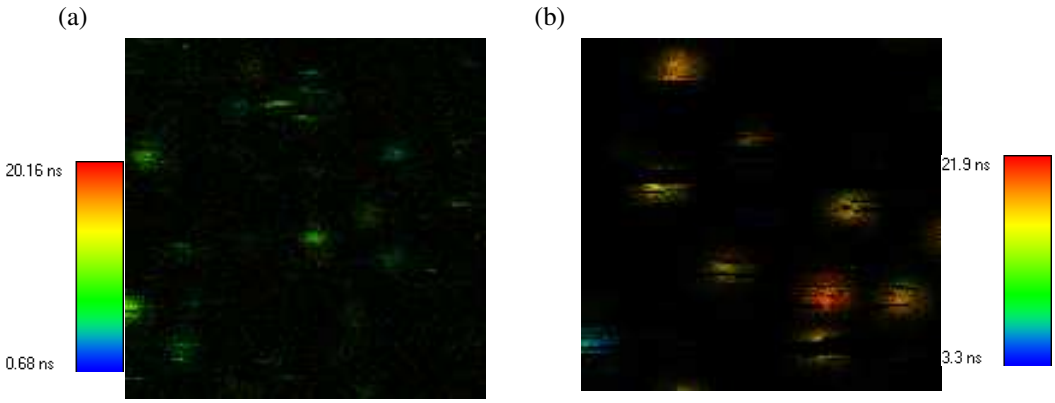

Fig. 7. Fluorescence lifetime images for single Au NCs (a) and single CdSe QDs (b).

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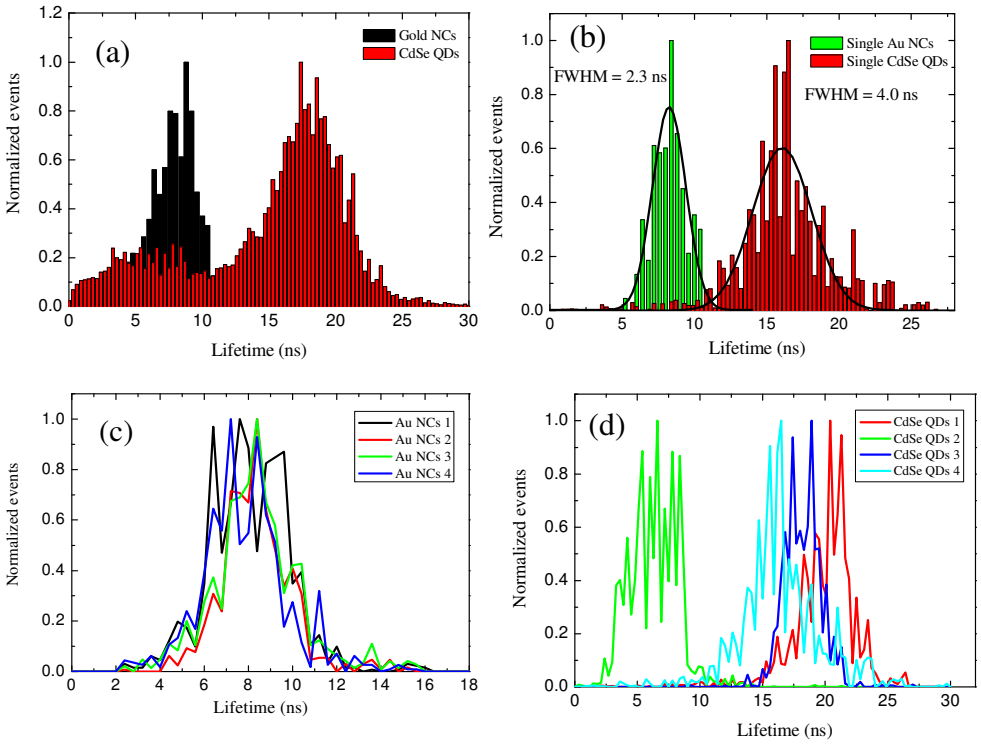

Fig. 8. Fluorescence lifetime distributions of all imaging pixel for Au NCs and CdSe QDs (a); for a selected single Au NCs and single CdSe QDs (b); for four Au NCs (c); and for four CdSe QDs (d), respectively.

4. Conclusions

In this study, we investigated fluorescence properties of Au NCs in an ensemble system as well as on a single-particle level. Unlike large Au particles which are not fluorescent, these small NCs become fluorescent. Photoinduced fluorescence enhancement was found for ensemble Au NCs in solution, but these NCs exhibited photobleaching at ambient environments. On the single-NC level, fluorescence blinking and single-step photobleaching were observed. Moreover, the fluorescence decay profile for single NCs shows a monoexponential decay behavior with a lifetime of ~7 ns. These Au NCs appear to exhibit a similar fluorescence lifetime distribution for different NCs. Other than non-toxicity, the fluorescence property and the uniform lifetime distribution of Au NCs offer potential applications as labels in fluorescence lifetime imaging. Further improvements of their QYs and photostability are needed to make Au NCs a better alternative than other materials. Such an effort is underway.

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