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Single-Molecule Spectroscopy of Semiconductor Nanocrystals on Plasmonic Nanostructures

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Abstract

In the past several years we have demonstrated the metal-enhanced fluorescence (MEF) and the significant changes in the photophysical properties of fluorophores in the presence of metallic nanostructures and nanoparticles using ensemble spectroscopic studies. Here, in the present study, we explored the new insights of these interactions using single-molecule fluorescence spectroscopy. The single molecule study is expected to provide more information, especially on the heterogeneity in the fluorescence enhancement and decrease in lifetimes associated with fluorophore-metal interactions, which is otherwise not possible to observe using ensemble measurements. For the present study, we considered using CdTe nanocrystals (QDots) prepared using modified Weller method as the fluorophores under investigation. QDots having few nanometer sizes, tunable absorption and fluorescence spectral properties, and high photostabilities are of important class of fluorescent probes. Because of these unique features Qdots are widely used as probes in various fields, including biological labeling and imaging. These CdTe nanocrystals show characteristic spectral features in solution and on the solid substrate. The CdTe nanocrystals dispersed in PVA and spin-casted on SiFs surface show ~5-fold increase in fluorescence intensity and ~3-fold decrease in lifetimes compared to on glass substrate. The data obtained using ensemble and single molecule spectroscopy are complimentary to each other. Additionally as anticipated we have seen increased heterogeneity in the plasmon induced fluorescence modulations. Moreover single molecule spectroscopic study revealed significant reduction in blinking of CdTe nanocrystals on plasmonic nanostructures. Subsequently, we present these important findings on metal-fluorophore interactions of CdTe nanocrystals (QDots) on plasmonic nanostructures.

Keywords

Single molecule spectroscopy; semiconductor nanocrystals; plasmonics; metal enhanced fluorescence; fluorescence; plasmon controlled fluorescence; nanotechnology

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1. INTRODUCTION

Fluorescence detection is the basis of many measurements in biological research. Fluorescence provides numerous measurement opportunities including studies of biological macromolecules, cell imaging and DNA sequencing. Exotic phenomena such as multi-photon excitation and time-resolved microscopy are now routinely performed in many laboratories for cellular imaging. At present use of fluorescence primarily depend on the spontaneous emission of photons occurring nearly isotropically in all directions. Information about the samples is obtained mostly from changes in the non-radiative decay rates, such as collisions of fluorophores with quenchers and fluorescence resonance energy transfer (FRET). The rates of spontaneous emission are not significantly changed in such experiments. In recent papers we began to consider methods to modify the radiative decay rates of fluorophores [1-2]. A review of existing literature revealed that radiative decay rates could be modified by proximity of fluorophores to metallic particles. We use the term metal to describe conducting metallic surfaces and particles, not metal ions. The effects of metals on fluorescence have been subject to prior theoretical reports [3-6], but the practical usefulness of these effects was not explicitly recognized. We refer to the interactions of fluorophores with novel metal particles as radiative decay engineering (RDE) because the radiative decay rates of the fluorophores are modified by placing the fluorophores in close proximity to the metal [7-8]. We observed a variety of favorable effects due to metal particles, such as increased fluorescence intensities, increased photostability and increased distances for fluorescence resonance energy transfer (FRET). We refer to these favorable effects as metal-enhanced fluorescence (MEF).

Single-molecule spectroscopy refers to a set of spectroscopic tools for investigation of single members of an ensemble of molecules, one molecule at a time [9-12]. Detection of single molecules represents the ultimate level of sensitivity and has been a longstanding goal of analytical methods. Because of its high sensitivity and a bright signal appears against a dark background, fluorescence is one obvious choice for single molecule detection. Accordingly, single-molecule fluorescence spectroscopy provides several advantages over ensemble fluorescence measurements. For instance, it eliminates averaging of the spectral properties over all members of ensemble and can reveal fundamental features otherwise masked in ensemble experiments. However, it is difficult to observe the signal from the weakly emissive probes especially using single molecule spectroscopy. On the other hand, the use of metallic structures can improve the brightness of fluorophores due to metal-fluorophore interactions. The value of enhanced fluorophore brightness can be perceived from the instrumental requirements needed to detect the emission from a single molecule above the background signal from the system.

Additionally, using single-molecule fluorescence studies we anticipate that the metal-fluorophore interactions in the MEF studies can be better revealed, especially at single molecule level. For this, we choose CdTe nanocrystals (QDots) as fluorophores. QDots having few nanometer sizes, tunable-absorption and fluorescence spectral properties are widely used as probes in various fields including biological labeling and imaging. Potential applications of QDots are rapidly increasing. Also single QDot fluorescence spectroscopy has been accomplished successfully for several colloidal semiconductor materials [13-15]

and quantum dots fluorescence spectral properties are reported in the presence of metallic nanoparticles at the ensemble level with little details [14-15]. In this article, we present the use of plasmonic metal nanostructures in the form of silver island films (SiFs) to enhance the fluorescence emission of CdTe nanocrystals (QDots). Our single molecule and ensemble steady-state and time-resolved studies show that the CdTe nanocrystals display an appreciable increase in fluorescence emission intensity and decrease in lifetime when they were assembled on the surface of SiFs as compared to glass slides.

2. EXPERIMENTAL SECTION

Materials

All chemicals, $\text{Cd}(\text{ClO}_4)_2 \cdot 6\text{H}_2\text{O}$, 2-mercaptoethylamine, metallic tellurium powder, sodiumborohydride, silver nitrite, Ammonium hydroxide, sodium hydroxide and glucose, were purchased from Aldrich of analytical grade or highest purity available. Polyvinylalcohol (PVA, MW $\sim 13000 - 23000$) purchased from Aldrich and used as received. All the spectroscopic grade solvents were used as received from Fisher or Aldrich. Nanopure water ($>18.0 \text{ M}$) purified using Millipore Milli-Q gradient system, was used in all experiments.

Preparation of CdTe nanocrystals

The synthesis of CdTe nanocrystals was performed using our previously published procedure [8,15]. Briefly, the pH of an aqueous solution of $0.985 \text{ g Cd}(\text{ClO}_4)_2 \cdot 6\text{H}_2\text{O}$ (2.35 mmol) and 0.690 g ligand thioglycolic acid (5.77 mmol) in 125 mL MilliQ-water was adjusted to 11.0 by adding a few drops of diluted NaOH, and then the solution was continuously stirred for 30 min at room temperature with nitrogen purging to eliminate dissolved oxygen. The source of tellurium (NaHTe) was prepared separately, which involved the reduction of metallic tellurium powder (0.192 g , 1.5 mmol) suspended in 10 mL MilliQ-water with sodium borohydride (0.113 g , 3 mmol in 2.5 mL water) at $0-5^\circ \text{C}$. In an about 30 min the suspended tellurium powder completely dissolves in water and the color of solution was changed to pinkish. Under vigorous stirring, the obtained NaHTe solution was injected slowly into the solution of $\text{Cd}(\text{ClO}_4)_2 \cdot 6\text{H}_2\text{O}$ containing thioglycolic acid at room temperature. Subsequently, the reaction mixture was refluxed for 24 hours and then cooled down to room temperature. The resulting dark-brown color solution was used as prepared in the present study. The CdTe nanocrystals are characterized with Transmission Electron Micrographs (TEM), taken with a side-entry Philips electron microscope at 120 keV . Samples for TEM measurements were cast from water solutions onto standard carbon-coated ($200-300 \text{ \AA}$) Formvar films on copper grids (200 mesh) by placing a droplet of a 1 mg/mL aqueous sample solution on grids.

Silver island film (SiF) preparation

The SiFs preparation on the conventional microscope slide ($3'' \times 1''$) used for ensemble experiments and glass coverslips used in single molecule spectroscopy is identical. Both the glass slides and coverslips (Corning, NY) were first cleaned by soaking them for overnight in $10:1 \text{ (v/v)}$ mixture of H_2SO_4 ($95-98\%$) and H_2O_2 (30%), commonly known as piranha. After washing rigorously with MilliQ deionized water, the slides were airdried. SiFs were

deposited on slides using the method reported previously by us [3]. Briefly, about 1.5 mL of freshly prepared 5% NaOH solution was added to a stirring aqueous silver nitrate solution (0.375 g in 45 mL water) in a glass beaker. Subsequently, the resulted dark-brown precipitate was redissolved by slowly adding 1 mL of NH_4OH . The solution was cooled to 5°C in an ice bath and a fresh solution of D-glucose (0.540 g in 11 mL water) was added, followed by four pairs of dried glass slides placed into this solution. The mixture was stirred for 2 min in ice bath and then allowed to warm up to 30°C for the next 5 minutes. As the color of the mixture turned from yellow-greenish to yellow-brown, the color of the slides became greenish. The slides were removed from the beaker, rinsed with Milli-Q water. Excess and non-adhesive silver particles on the glass surface were removed by mild sonication of the SiF coated glass slides for 1 minute. The SiF slides were stored in Milli-Q water until they were used. We used the SiFs in our experiments within two days of forming them. After two days of storage in Milli-Q water we did not see a change in the surface plasmon resonance of the SiFs, both showing an extinction maximum at roughly 460 nm (measured right after formation and after two days of storage in water - data not shown), and hence can negate any detrimental effects of major oxide layer formations on our SiFs. The diameters of the islands are typically 100-500 nm across and some 60 nm high.

Spin coated films on glass and SiF surfaces

For the ensemble experiments, about submillimolar concentrations of CdTe nanocrystals were dispersed in 1 wt% solution of PVA in water and spin coated onto regular glass slides and SiFs coated slides at 3000 rpm. For the single molecule studies, at first, a PVA under layer film on standard glass coverslip and SiFs coated coverslip was deposited using a 0.1 wt% in water solution. Subsequently, after these films were dried at room temperature, a second layer of PVA (0.1 wt% in water) consisting of about nanomolar concentration of CdTe nanocrystal were deposited by spin-casting at 3000 rpm. Accordingly, the blank PVA under layer provides an about 10 nm separation distance between CdTe nanocrystals and SiFs surfaces to avoid quenching of CdTe fluorescence by nearfield interactions of silver nanoparticles.

Absorption and Fluorescence Spectroscopy

Absorption spectra were collected using a Hewlett-Packard 8453 spectrophotometer. Fluorescence spectra were recorded using a Varian Cary Eclipse Fluorescence Spectrophotometer using front face illumination geometry with 470 nm excitation from a Xenon arc lamp. Time-resolved intensity decays were recorded using a PicoQuant Fluotime 100 time-correlated single-photon counting (TCSPC) fluorescence lifetime spectrometer. The excitation at ~ 470 nm was obtained using a pulsed laser diode (PicoQuant PDL800-B) with 20 MHz repetition rate. The Instrument Response Function (IRF) is about 300 ps. The excitation was vertically polarized and the emission was recorded through a polarizer oriented at 54.7° from the vertical position. A bandpass filter at 685 ± 35 nm (Chroma Inc.) was used in the collection path to record the fluorescence from the CdTe nanocrystals.

Data Analysis

The fluorescence intensity decays were analyzed in terms of the multi-exponential model as the sum of individual single exponential decays [16]:

$$I(t) = \sum_{i=1}^n \alpha_i \exp(-t/\tau_i) \quad (1)$$

In this expression τ_i are the decay times and α_i are the amplitudes and $\sum_i \alpha_i = 1.0$. The fractional contribution of each component to the steady-state intensity is described by:

$$f_i = \frac{\alpha_i \tau_i}{\sum_j \alpha_j \tau_j} \quad (2)$$

The average lifetime is represented by:

$$\bar{\tau} = \sum_i f_i \tau_i \quad (3)$$

The values of α_i and τ_i were determined using the PicoQuant Fluofit 3.3 software with the deconvolution of instrument response function and nonlinear least squares fitting. The goodness-of-fit was determined by the χ^2 value.

Single Molecule Spectroscopy

Single CdTe nanocrystal fluorescence measurements were obtained using a scanning confocal microscope (Picoquant MicroTime 200). The excitation laser was reflected by a dichroic mirror to a high numerical aperture (NA) oil objective (100x, NA 1.3) and focused to a diffraction limited spot (~ 300 nm) on the sample surface. CdTe fluorescence was collected by an avalanche photodiode through the dichroic beam splitter and a bandpass (650-720 nm, Chroma) filter. Integration times of 3 msec per pixel were used to obtain 512×512 pixel raster scanned 20×20 μm images. The samples were excited with a typical power density of ~ 55 W/cm² from a 470 nm solid state laser. Intensity-time trajectories and intensity-time decays were obtained by positioning the excitation beam above the individual CdTe. A schematic of the experimental setup and sample geometry for the single molecule measurements are shown in Figure 1.

3. RESULTS AND DISCUSSION

Highly luminescent, water soluble, CdTe nanocrystals that are surface protected with thioglycolic acid were conveniently prepared in large scale using a low-temperature aqueous reaction procedure. Unlike many other synthesis methods reported previously, this is more suitable procedure and is expected to result semiconductor nanocrystals with variety of surface protected ligand. Accordingly, the synthesized carboxyl-terminated CdTe nanocrystals showed a stable dispersion in water. Figure 2 shows the TEM image of a few CdTe nanocrystals. Although the TEM images of CdTe nanoparticles (Figure 2) are not very clear, their average core size could be approximately estimated to be ~ 5 nm.

Figure 3 displays absorption and emission spectra of CdTe nanocrystals dispersed in water. As seen from the figure, the nanocrystals show a characteristic absorption spectrum as observed with other QDots and an emission maximum at 650 nm in aqueous solution when

they were excited at 470 nm. No noticeable change in the emission wavelength and intensity was observed when the CdTe nanocrystals were continuously exposed to the excitation light for 2 h in solution, indicating they were photostable under the current experiment conditions. Fluorescence intensity decay of CdTe nanocrystals in water is shown in Figure 4. The lifetimes of CdTe nanocrystals were measured by time-correlated single-photon counting (TCSPC) and were recovered by fitting the obtained data to non-linear least-squares (NLLS) analysis supplied by PicoQuant Fluofit 3.3 software. The average lifetime of these CdTe nanocrystals in water is of ~ 11 ns. These observed spectral and TEM data are in agreement with the previously published reports.

Subsequently, we first examined the effect of metallic nanostructures on spectral features of the CdTe nanoparticles at ensemble level. The fluorescence spectra of CdTe nanocrystals dispersed in 1 wt% PVA and spin coated on glass and SiFs surface are shown in Figure 5. The CdTe nanocrystals show almost identical emission as that was observed from aqueous solution (Figures 3 and 5). Most importantly, the emission spectra taken from the SiFs surface and that from the glass-surface are completely overlapped on each other with a band maxima at about 660 nm. An intensity normalized spectra is shown in Figure 5 (right panel) to visualize the spectral overlap more clearly. It is apparent from the figure that a significant enhancement (of ~ 5 -fold) in fluorescence intensity is observed from the CdTe nanocrystals on SiF surface as compared to that from the glass surface. This observed increase in fluorescence intensity can be attributed to the so-called MEF effect.

To further understand the effect of metallic nanoparticles on the emission features of CdTe nanocrystals, we measured the fluorescence intensity decays for CdTe nanocrystals dispersed in PVA and spin coated as mentioned before. Representative decay curves obtained from glass and SiFs surface are shown in Figure 6. In contrast to the enhanced fluorescence from CdTe nanocrystals on SiFs surface, faster fluorescence intensity decay with ~ 3 -fold decrease in lifetime is observed. The observed average fluorescence lifetimes are 4.52 ns and 1.45 ns on the glass and SiFs surfaces, respectively. These anomalous fluorescence properties *i.e.* increase in fluorescence intensity and decrease in lifetime of CdTe nanocrystals near metallic surface could be explained based on the stronger excitation rate and modification in the radiative decay rate when the CdTe nanocrystals are in close proximity to the silver nanostructures. A detailed account describing this anomalous behavior that is due to metal-enhanced fluorescence has been reported previously [1-2,7-8].

Figure 7 shows representative scanning confocal images of CdTe nanocrystals on glass and SiF surfaces. Well separated bright spots represent emission from the individual CdTe nanocrystals. The significant differences in the peak intensities of the two images are evident from the intensity Scale bar. For CdTe nanocrystals on the glass and SiF surfaces, the average values of peak intensities are ~ 25 and 125 counts/bin, respectively, indicating single CdTe nanocrystal on SiF surface are ~ 5 -fold brighter than that on the glass surface. This brightness of the spots can be ascribed to MEF. Also it is important to note that the heterogeneity in the spots brightness. On glass surface, the observed heterogeneity in the brightness could be ascribed simply to the size-dependent emission efficiencies of CdTe nanocrystals, which is inherent property of QDots. However the heterogeneity in the spots

brightness observed on SiFs surface is more complex and have been explained in the following section.

In order to more quantitatively compare the collected single molecule count rates and explore the changes in underlying photophysics of individual nanocrystals on glass and SiF surfaces, we monitored the fluorescence intensity of individual nanocrystals as a function of time, while under continuous excitation. Representative intensity-time trajectories collected for CdTe nanocrystals on glass and SiFs surfaces are shown in Figure 8. From these intensity-time trajectories we observe that the fluorescence intensity of individual CdTe nanocrystals on SiFs is several-fold higher than that on the glass substantiating the MEF effect observed in ensemble measurements and from the confocal images shown in Figure 7.

In single molecule experiments probe blinking is a problem with both organic fluorophores and Qdots. Probe blinking is probably due to transitions to the triplet state and a slow rate of return from this state. We also investigated the blinking behavior of single CdTe nanocrystals on both glass and SiFs surfaces (Figure 8). In the absence of silver particles we observed time-dependent changes in intensity, which are probably due to blinking. The changes in intensity near the silver particles are probably due to reorientation and/or movements of the CdTe nanocrystals relative to the metal surface. Importantly, these CdTe nanocrystals did not go into a dark state near silver particles. These results suggest that the CdTe nanocrystals show reduced blinking on SiFs surface.

We measured the lifetimes of single CdTe nanocrystals by time-correlated single-photon counting (TCSPC) and the lifetimes were recovered by non-linear least-squares (NLLS). Figure 9 shows typical intensity decays observed from a single CdTe nanocrystals on glass and SiFs. As can be seen from the figure, the lifetime of CdTe nanocrystals is dramatically shortened on the SiFs surface. This is in agreement with the observed ensemble lifetime changes described in the previous sections.

To quantify and get meaningful insights from the observed single molecule data, over 100 individual quantum dots were examined. Increased intensities and decreased lifetimes were also observed for the individual Qdots. These results show that Qdots are similar to organic fluorophores in terms of their interactions with silver particles. The CdTe fluorescence brightness distribution collected on glass and SiFs surfaces are shown in Figure 10. With in the same experimental conditions, the average fluorescence intensities of single CdTe nanocrystals on glass are ~ 8 kHz and that on SiFs surface are ~ 45 kHz, which imply an about 5-fold increase in fluorescence intensity on SiFs that is similar to that was observed in ensemble measurements (Figure 5). From these histograms, it is clear that the fluorescence intensities of individual CdTe nanocrystals on SiFs surfaces are more heterogeneously distributed than that on glass surfaces. Such a distribution phenomenon is impossible to observe with ensemble measurements. CdTe fluorescence intensity distributions on glass surface show relatively low standard deviation of 16% where as the corresponding value on SiFs is ~ 30 %. This is because, the heterogeneity in the fluorescence intensity on glass surface largely depend on deviation in CdTe nanocrystals size, whereas for SiFs, additional complexity arises from the varied metal-fluorophore interactions that may be due to the heterogeneous surface of SiFs.

We measured the lifetimes of over 100 molecules, on either glass or silver, to determine the range of lifetimes present in each substrate. These histograms (Figure 11) show that the lifetimes are about 5-fold shorter on the SiFs than on glass. The lifetime histogram on the SiF does not show any of longer lifetime components comparable to that on glass. We have also observed a more heterogeneous distribution of lifetimes for the CdTe on SiFs (std dev $\sim 43\%$) compared to that on glass (std dev $\sim 24\%$).

4. CONCLUSION

In this article we have demonstrated the metal enhanced fluorescence from the CdTe nanocrystals spin coated on plasmonic nanostructured substrates (in the form of silver island films (SiFs)) both at the ensemble and single molecule level. We have observed ~ 5 fold increase in fluorescence intensity, and 3-fold decrease in fluorescence lifetimes when the CdTe nanocrystals are on the silver nanostructured substrates. A significant reduction in blinking of CdTe nanocrystals on plasmonic nanostructured substrates were revealed by single molecule fluorescence studies. The single molecule fluorescence results pointed out further insight on the heterogeneity in the enhancement of fluorescence intensity and changes in lifetimes for the CdTe nanocrystals on both glass and plasmonic nanostructured surfaces, which is otherwise not possible to observe using ensemble measurements. Our results demonstrate improved detectability at the single molecule level for CdTe nanocrystals in proximity to the silver nanoparticles due to the near-field metal/fluorophore interactions.

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Figure 1. Schematic design of the single molecule imaging system (Left). Sample configuration for single molecule and ensemble measurements (Right).

Figure 2.
TEM image of CdTe nanocrystals.

Figure 3.

Absorption and emission spectra of CdTe nanocrystals in water. $\lambda_{\text{ex}} = 470$ nm.

Figure 4. Intensity time decay of CdTe nanocrystals in water. Instrument response function is also included.

Figure 5. Ensemble emission spectra of CdTe nanocrystals on glass and SiFs surfaces (Left panel) and the corresponding intensity normalized emission spectra (Right panel). $\lambda_{\text{ex}} = 470$ nm.

Figure 6.

Intensity decays of CdTe nanocrystals on glass and SiF surfaces (dotted line). Instrument response function is also included (gray line). Solid line indicates best fit to the obtained data. $\lambda_{\text{ex}} = 470$ nm.

Figure 7.

Representative scanning confocal fluorescence image ($20 \times 20 \mu\text{m}$) of individual CdTe nanocrystals on the glass and SiFs surfaces. Illumination intensity was 55 W/cm^2 at 470 nm. Sample was imaged with a high NA (1.3) oil objective. Scale bar shows the intensity counts in 3 msec bin.

Figure 8. Representative fluorescence intensity-time traces for CdTe nanocrystals on glass (top panel) and SiFs (bottom panel) surfaces

Figure 9. Representative fluorescence intensity-decays of individual CdTe nanocrystals on glass and SiFs surfaces.

Figure 10. Brightness distribution of CdTe nanocrystals on glass (left panel) and SiFs (right panel) surfaces.

Figure 11.
Emission lifetime histograms of CdTe nanocrystals on glass (left panel) and SiFs (right panel) surfaces.