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Enhanced Fluorescence Emission of Me-ADOTA⁺ by Self-Assembled Silver Nanoparticles on a Gold Film

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Abstract

We report a multi-fold enhancement of the fluorescence of methyl-azadioxatriangulenium chloride (Me-ADOTA•Cl) in PVA deposited on a 50 nm thick gold mirror carrying an evaporation induced self-assembly of colloidal silver nanoparticles (Ag-SACs). The average measured increase in fluorescence emission of about 50-fold is accompanied by hot spots with a local enhancement in brightness close to 200. The long lifetime of the dye allows for the first direct determination of the correlation between the enhancement of emission intensity and the decrease in fluorescence lifetime. The Ag-SACs surface preparation and observed enhancements are highly reproducible. We believe that these robust plasmonic surfaces will find use in sensing platforms for ultrasensitive detection.

1. Introduction

1.1 Metal Enhanced Fluorescence

Metal enhanced fluorescence (MEF) has been investigated for a few decades due to inspiration from the field of Surface Enhanced Raman Scattering (SERS) [1–3]. Initial reports on MEF include changes in lifetimes and modest increase in brightness of fluorophores deposited on colloidal surfaces or Silver Island Films (SIFs) [4–6]. The fluorescence enhancement observed on rough silvered surfaces has two origins. First, the enhanced local electromagnetic field from the impinging light provides a higher excitation rate for chromophores localized in the enhancement region. Second, the interaction of excited molecules with nearby silver nanoparticles, results in rapid radiation of the excitation energy into free space. This effect, called radiative decay engineering (RDE) increases the radiative rate which results in lifetime shortening and increased emission quantum yield [7,8]. The total fluorescence enhancement is a product of these two effects. It should be noted that at a close proximity, below 40–50 Å, the excited fluorophores are strongly quenched by the metallic surface. For this reason, the observed fluorescence enhancements on metallic surfaces are much weaker than enhancements

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achieved in SERS. The distance dependence of the fluorescence enhancement has been studied with using Langmuir-Blodgett films [9] and protein multilayer [10]. Both studies found that the strongest enhancements are expected at the distance of about 80–100 Å.

The fluorescence enhancements also depend on the metallic surface morphology. SIFs and colloidal surfaces provide usually enhancements in range of 5 to 15 fold [11]. An electrochemical deposition of silver nanostructures, called fractals, resulted in local enhancements of about 100 fold [12–14]. Recently, we observed that SIFs deposited on silver or gold films can strongly enhance fluorescence, about 50 fold on average [15,16].

Very recently the first report on a nano-wire deposited on mirror surface appeared, it shows that this structure exhibit an enormous Raman enhancement [17]. This report confirms that sharp metallic nanostructures deposited on a metallic film can induce ultra strong local fields.

Here we report that evaporation induced self-assembly of colloidal silver nanoparticles on metallic films (Ag-SACs) form unique, fractal-like surfaces which display even stronger fluorescence enhancement than SIFs on metal mirrors. Furthermore, by use of a long lifetime fluorophore we are able to provide a quantitative account of the correlation between the local MEF effect and change in fluorescence lifetime.

1.2 Long Fluorescence Lifetime Fluorophors

In this work we study the fluorescence enhancement of methyl-azadioxatriangulenium chloride (Me-ADOTA•Cl) on Ag-SACs surface prepared on a gold film. The structure of the molecule and the surface is shown as inserts in Figure 1. Me-ADOTA⁺ belongs to the triangulenium dyes, a class of planar, triangular chromophores of inherently high symmetry. The parent compound is trioxatriangulene (TOTA⁺), first prepared in 1964 by Martin and Smith [18]. The triangulenium dyes were more recently expanded by the aza/oxa triangulenes: azadioxatriangulene (ADOTA⁺) and diazaoxatriangulene (DAOTA⁺) together with the aza analogue of TOTA⁺: triazatriangulene (TATA⁺) [19,20]. They all have the same properties due to high symmetry, but have increased oscillator strength due to the greater donor strength of nitrogen compared to oxygen [21,22]. The triangulenes are highly fluorescent, electron rich cationic dyes, capable of photo electron transfer to most donors and they are excellent DNA intercalators [19,23,24]. Peripheral substitution of hydrogen with nitrogen leads to a different class of triangulenium dyes; amino-trioxatriangulene (A-TOTA⁺) and amino-azadioxatriangulene (A-ADOTA⁺) which effectively are blueshifted symmetric versions of rhodamine and aminoacridine respectively [25,26,27].

The aza/oxa triangulenium dyes have a very long fluorescence lifetime of ~20 ns, uncharacteristic of most organic fluorophors, their high symmetry and low flexibility results in that the natural lifetime of ~50 ns is only reduced by a factor of two even in non-viscous solvent at room temperature [28]. These properties make the aza/oxa triangulenium dyes interesting in various applications in spite of the mid-range absorbance and quantum yields. Thus the Me-ADOTA⁺ is especially suited for studies of MEF. With a lifetime of 20.9 ns in water [29], enhancement of the radiative rate should be directly detectable by the resulting lifetime shortening. In this manuscript we present steady-state and time-resolved study of MEF of Me-ADOTA on Ag-SACs, utilizing the long natural lifetime to measure the lifetime of the MEF.

2. Materials and methods

All compounds, solvents and materials were used as received; water was used directly from a Millipore purification system.

2.1 Silver Colloids Preparation

Silver colloids were prepared as previously described [30]. Briefly: all necessary glassware were soaked in a base bath overnight and washed scrupulously with de-ionized water. The solution of 0.18 mg/ml silver nitrate (200 ml) from Sigma-Aldrich (CAS#7761-88-8) was heated and stirred in a 250 ml Erlenmeyer flask at 95°C. One aliquot (0.5 ml) of 34 mM trisodium citrate (Spectrum, CAS#6132-04-3) solution was added drop wise. The solution was stirred for 20 min and warmed to 96–98°C. Then five aliquots (0.7 ml each) of 34 mM trisodium citrate were added drop wise to the reaction mixture every 15–20 min. Stirring was continued for 25 min until the milky yellow color remained. Then the mixture was cooled in an ice bath for 15 min.

2.2 Preparation of Silver Self-assembled Colloids on Gold Mirror Surfaces (Ag-SACs)

Microscope slides were coated by vapor deposition by EMF Corp. (Ithaca, NY). A 48nm thick layer of gold was deposited on the slide with about 2nm chromium undercoat. Two-dimensional evaporation-mediated self-assembly of colloidal silver nanoparticles was performed as described earlier [31,32]. Briefly, gold mirrors were cleaned and drop coated with silver colloids. The mirrors were air dried. When a liquid containing nanoparticles evaporates different structures are formed [31]. The self-assembly is governed by a combination of different forces such as interactions between nanoparticles, capillary forces, and wetting [30]. The surfaces were rinsed with water after the assembly was completed to remove the loosely bound silver and air dried. The dry gold mirrors with silver self-assembled nanoparticles were stored and used within a month.

2.3 Synthesis of Methyl-Azadioxatriangulenium Chloride (Me-ADOTA•Cl)

Methyl-azadioxatriangulenium chloride (Me-ADOTA•Cl) was prepared from the hexafluorophosphate salt using a Amberlite® IRA-400 ion exchange resin; using a solution of 100 mg of Me-ADOTA•PF₆ in 20 ml of methanol and 1 g of resin. The mixture was stirred for 1 hour and the resin filtered of, cycling the procedure 3 times with fresh resin and removal of the solvent afforded Me-ADOTA•Cl in a quantitative yield. Me-ADOTA•PF₆ was prepared according to literature procedure from 9-(2,6-Dimethoxyphenyl)-1,8-dimethoxy-10-methylacridinium hexafluorophosphate [20,21].

2.4 Microscopic Measurements

Time resolved images were obtained on a confocal MicroTime 200 (Picoquant GmbH, Germany) system coupled with an OLYMPUS IX71 microscope. The samples were prepared from an aqueous solution of Me-ADOTA•Cl in 0.2% PVA, spin-coated on silver colloid nanostructures self assembled on a gold mirror (gold covered glass). The dye-PVA film was placed upside down on a non-fluorescent Menzel-Glaser #1 cover-slip. The prepared samples were located in a piezo-scanner holder in the configuration: Au mirror-PVA film-cover slip-objective. Fluorescence photons were gathered from different places of the sample using 60X water immersed objective (NA 1.2, OLYMPUS). To remove scattered light, a 540-nm long-pass filter, with an additional 583/120 band-pass interference filters (Semrock), were applied for lifetime measurements. As light source, a pulsed laser (470nm -LDH-P-C-470B) with repetition rate of 5MHz was used. Fluorescence photons were collected with the Micro Photon Devices (MPD) PD1CTC single photon sensitive avalanche photodiode (APD) with processing accomplished by the PicoHarp300 time-correlated single photon counting (TCSPC) module. Data analysis was performed using SymPhoTime (v. 5.0) software package.

2.5 Steady State Spectroscopy

The emission spectrum was measured on a Varian Cary Eclipse spectrometer using a true front face setup, employing 505 nm excitation and using a 480–520 nm supporting interference filter for excitation, and a 530 nm long pass filter on the emission path.

2.6 Lifetime Measurements

Fluorescence lifetimes were measured using FluoroTime 200 (PicoQuant, GmbH, Berlin, Germany) equipped with a multichannel plate detector (MCP-PMT from Hamamatsu). The excitation was from a pulsed solid state laser 470nm (65 ps pulse width) driven from PDL800-B driver with repetition rate of 5MHz. The fluorescence decays were analyzed using the FluoFit software package (version 4.2.1). The decay data were fitted by iterative reconvolution with a sum of exponentials:

$$I(t) = \sum_i \alpha_i \exp(-t/\tau_i) \quad (1)$$

where α_i and τ_i are the pre-exponential factor and fluorescence lifetime, respectively.

3. Results and Discussion

The MEF results described here are comparative studies of PVA films doped with a high concentration of Me-ADOTA•Cl deposited on Ag-SACs on a gold mirror and glass, with the latter being the reference sample all results are compared with. Pure PVA samples were used as controls in all measurements.

3.1 Enhancement

The steady state emission spectra of Me-ADOTA⁺ on Ag-SACs and glass are shown in Figure 1 (top). The fluorescence intensity on Ag-SACs is many times stronger than on glass substrate. The chemical structure of chromophore is included as an insert. In the bottom panel of Figure 1 the emission spectra have been normalized. Both spectra are background corrected using the signals from the control samples. The insert in the bottom panel shows the AFM image of the Ag-SACs. In order to estimate the degree of enhancement the integrated emission of the sample and reference was compared, resulting in an enhancement factor of 54 for MeADOTA⁺ on Ag-SACs. Interestingly the emission spectrum of Me-ADOTA⁺ is red shifted by about 40 nm from 560 nm on glass to 600 nm on Ag-SACs. Such a strong shift of the emission spectrum is not common. Therefore we repeated the experiment using a new sample and controls and obtained similar results. It is known that in the strong electromagnetic field the emission of fluorophores can be shifted and the shape of the spectrum deformed [31]. We believe that such spectral shifts can be used as a measure of the strength of local fields.

Next, the samples were investigated under the microscope. Figure 2 shows images with three different intensity and lifetime traces. The correlation of the two parameters is evident, with long fluorescence lifetime in the dark regions and a short lifetime on the bright area of the sample. The dark areas corresponds to the weak intensities on the surface without Ag-SACs. In the bright (hot) spots the intensities are hundreds fold stronger than on dark spots. In order to estimate reliably the lifetime distribution on glass we needed to increase the illumination intensity by an order of magnitude (Figure 3, top).

The distribution of lifetimes in the case of Ag-SACs is strongly shifted to shorter lifetimes indicating a substantial RDE effect.

3.2 Lifetime Effects and Dye Stability

The observed increase in brightness of the emission under the microscope and in steady state spectra as well as the shift in the lifetime histogram on Ag-SACs suggest a drastic decrease in lifetime. Figure 3 shows the lifetime histograms from the inserted images. The average lifetime on glass is about 7 ns, with a wide distribution from 2 to 20 ns. In the sample on Ag-SACs the average lifetime is reduced to about 2.5 ns with significantly narrowed distribution.

The precise lifetime measurements were done with using a FT200 fluorometer equipped with a MCP-PMT detector. The lifetimes was recorded at the maximum of emission in order to measure get the best estimate of average lifetime $\langle\tau\rangle$. The intensity decay on glass can be fitted with two exponents of 11.34 and 1.35 ns and amplitudes 0.39 and 0.61, respectively (Figure 4, top). On Ag-SACs, four exponents are needed to fit the data with lifetimes 11.20, 2.77, 0.52 and 0.018 ns and amplitudes 0.003, 0.008, 0.020 and 0.97, respectively (Figure 4, bottom). The average lifetime was determined to be 5.2 ns on glass, and 0.082 ns on Ag-SACs. It should be noted that on glass the Me-ADOTA⁺ lifetime is reduced compared to the diluted bulk PVA-dye doped samples where a lifetime of 21.6 ns was found [28]. The reduced lifetime is expected to be due to quenching by oxygen, freely diffusing into the thin film, and homo FRET in the highly concentrated sample [28]. The emission in the areas of the PVA film outside the influence of the Ag-SACs are believed to be responsible for the long lifetime component, which are similar in the two samples, the short lifetime component on glass is believed to be from fluorophors in undergoing homo FRET [7]; with a calculated R_0 of 32.6 Å this process is highly likely in the concentrated sample. The two shortest components, accounting for 99 % of the emission originate from MEF. The ratio of average lifetime on glass to the lifetime on Ag-SACs is 63, close to the observed steady state enhancement factor.

Using the natural lifetime of Me-ADOTA⁺ from solution studies (48 ns), the maximum enhancement due to a RDE effect can be determined. The degree of quenching in the thin PVA film can be estimated from the glass sample where the reduced lifetime originates from an increase in k_{nr} ; using this assumption $\phi_f(\text{glass}) = 0.11$ and $k_{nr}(\text{glass}) = 0.17 \text{ ns}^{-1}$. Thus the enhancement from increasing the quantum yield can be no higher than $1/0.11 \approx 9$. This implies that a significant part (6-fold) of the 54 fold enhancement observed is due to an enhanced local field. Using the non-radiative rate from the sample on glass and assuming $\phi_f(\text{Ag-SACs}) = 1.0$ allows for calculation of $k_f(\text{Ag-SACs}) = 1.2 \text{ ns}^{-1}$; which is an 60 fold increase compared to the native $k_f(\text{Me-ADOTA}^+) = 0.02 \text{ ns}^{-1}$. Even though all enhancement can be accounted for by the increase through RDE; the quantum yield, which only increase by a factor of 10, shows effects from single molecule events, also known as blinking, where the molecules make the transition to the triplet state and is unavailable for excitation. Thus the enhancement must be due to a combination of RDE and local field effects.

The reduced lifetime of the excited state of the molecules in the presence of Ag-SACs lowers the probability of bleaching as the excited state reactions leading to photodegradation simply does not have time to happen. Figure 5 shows that this is also the case for Me-ADOTA⁺ even though the excited state still has a relatively long lifetime. The film on glass experiences a degree of bleaching of ~50% in 15 seconds whereas the film on Ag-SACs only loses ~5% of its initial brightness in the same time span.

4. Conclusion

We have found that self-assembled silver colloidal nanostructures are efficient in inducing a metal enhanced fluorescence in a model system consisting of fluorophors in a PVA film. A long fluorescence lifetime fluorophore, Me-ADOTA•Cl was used in order to supply an extra handle in analyzing the MEF, as the average lifetime of the enhanced fluorescence could be reliably determined. The enhancement factor of the Ag-SACs on Me-ADOTA⁺ is over 50 fold

in steady state, which corresponds to the average increase of brightness during laser excitation under the confocal microscope. In the “hot” spots the fluorescence enhancement is about 200, which, to our knowledge, is the strongest reported enhancement with one photon excitation. It should be noted that the fluorescence enhancements observed on silver colloids and islands deposited on glass slides were at least an order of magnitude weaker.

Why is the enhancement effect much stronger in the studied system: gold mirror- Ag-SACs? In short, the metallic particles are positioned very close to metallic surface (1–10 nm). Illumination of the particle layer by light of proper wavelength will excite localized surface plasmons (collective oscillations of free electrons) in the particles. We want to stress that with the form of illumination we are using in this experiments (impinging excitation light from the low refractive index side) we cannot excite surface plasmons in the metal film. However, near field interactions between plasmons in the particle and free electrons in the gold film facilitate excitation of surface plasmons in the film. First, Resonance interactions between plasmons in the particles and surface plasmons in the film dramatically increases local field especially between particle and the film. Second, traveling surface plasmons in the field propagate up to few microns greatly contributing to the dyes excitation in much greater area than the particle alone. In summary the MEF effect is magnified allowing a significant reduction of illumination power, which in turn, reduces the unwanted background.

The increase in brightness relates directly to the measured lifetime, whereas the steady state enhancement factor is too high to be attributed to radiative decay engineering alone; in the steady state measurement the increased light intensity experienced by the fluorophor on the SACs must be a factor of 6. To conclude Ag-SACs have been found to be a platform capable of yielding efficient MEF allowing a reduction of excitation light by a factor of 200. This, in turn, reduces the unwanted background and photo degradation. Additionally it can be concluded that using a long lifetime dye gives valuable new information on the source of the MEF.

We believe, a new sensing devices based on MEF on Ag-SACs will be soon constructed.

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Figure 1.

Emission spectra of methyl-azadioxatriangulenium chloride in PVA on self-assembled Ag colloidal nanoparticles (black) and glass (grey). The top panel shows the background subtracted emission spectra, the lower panel the normalized emission spectra. The inserts show the structure of Me-ADOTA⁺ and a 15×15 μm AFM image of the Ag-SACs, top and bottom respectively.

Figure 2. 30 $\times$ 30 μm confocal fluorescence lifetime microscope intensity images of methyl-azadioxatriangulenium chloride in PVA on self-assembled Ag colloidal nanoparticles (SACs), with three lifetime/intensity traces from the lines indicated by the arrows on the intensity image.

Figure 3. Fluorescence emission lifetime histograms from the inserted 30×30 μm confocal fluorescence lifetime microscope lifetime images of methyl-azadioxatriangulenium chloride in PVA on glass (top) and SACs (bottom).

Figure 4. Fluorescence emission lifetime decays (grey) and fit (black) of methyl-azadioxatriangulenium chloride in PVA on glass (top) and self-assembled Ag colloidal nanoparticles (bottom), recorded at 560 nm and 600 nm respectively. Instrument response functions are included light grey.

Figure 5.

The stability of methyl-azadioxatriangulenium chloride in PVA on self-assembled Ag colloidal nanoparticles (black) and glass (grey) under laser illumination in the confocal fluorescence lifetime microscope.