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Distance-dependent intrinsic fluorescence of proteins on aluminum 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. In the represented study, we explored the distance effect on intrinsic fluorescence of proteins adsorbed on our layer-by-layer assembled metallic nanostructures. The study is expected to provide more information on the importance of positioning the proteins at a particular distance for enhanced fluorescence from metallic structures. For the present study, we considered using easy and inexpensive LbL technique to provide well-defined distance from metallic surface. The explored proteins were adsorbed on different numbers of alternate layers of poly(styrene sulfonate) (PSS) and poly(allylamine hydrochloride) (PAH). SA and BSA were electrostatically attached to the positively charged PAH layer. We obtained a maximum of ~11-fold and 9-fold increase in fluorescence intensity from SA and BSA, respectively. And also we observed ~3-fold decrease in BSA lifetime on metallic nanostructures than those on bare control quartz slides. This study reveals the distance dependence of protein fluorescence.

Keywords

Metal enhanced fluorescence; label free detection; fluorescence; plasmon controlled fluorescence; nanotechnology

1. INTRODUCTION

Sensitive fluorescence-based bioassays are extensively used in all aspects of medical research including proteomics, clinical diagnostics, and drug discovery. Most of these assays depend on the use of extrinsic fluorophores in the visible region, which are used to label the biomolecules of interest. While this approach has been successful and target-specific, such a procedure is time-consuming, labor sensitive, prone to contamination, and induces high levels of complexity and cost [1–3]. Label-free detection (LFD) serves a great alternative to overcome the problems mentioned above. The importance of LFD can be seen from the growing attention to develop the large number of methods for LFD. Surface-enhanced Raman scattering (SERS), electrochemical approaches, optical transmission, reflectivity, and surface plasmon resonance are some of them [4–8].

During the past several years our laboratory and others have been developing techniques that use metallic nanostructures for improved fluorescence detection [8–13]. The observed

changes in brightness and/or photostability are the result of interactions of the incident light with the metal and interactions of the excited-state fluorophores with the metal. A subwavelength-sized metallic nanoparticle can interact with the incident light (generally plane waves), which can create enhanced local fields near its surface. This enhanced local field can result in increased rates of excitation of the nearby fluorophores. A second, and perhaps more important, effect is an increase in the radiative-decay rate of fluorophores near metal particles. This increased rate results in higher quantum yields, decreased lifetimes, and improved photostability. We refer to these favorable effects as metal-enhanced fluorescence (MEF).

The efficient use of MEF depends on the understanding of the optimal distance between the fluorophore and the metallic surface for enhanced fluorescence. The MEF phenomenon is based on short-range interactions of fluorophores with metallic nanostructures occurs at distances from 5 to 100 nm [14]. To understand the effect of metal nanostructures on nearby fluorophores, it is desirable to study systems with well-defined metal-fluorophore distances.¹⁸ To locate a probe at a particular distance can be accomplished using easy and simple layer-by-layer (LbL) assembly. LbL assembly is based on the sequential adsorption of polycations and polyanions from dilute aqueous solution onto a solid substrate as a consequence of the electrostatic interaction and complex formation between oppositely charged polyelectrolytes. It is possible to adsorb a variety of charged species ranging from polyelectrolytes, nanoparticles, and ionic dyes to many biological agents such as viruses, proteins, and DNA. Importantly, LbL can be carried out at room temperature with simple apparatus.

In this paper, we represent the distance effect on intrinsic fluorescence emission of streptavidin (SA) and bovine serum albumin (BSA) adsorbed on our Al nanostructured surfaces. We used Mie theory to calculate the cross sections of aluminum nanoparticles. A detailed exploration using steady-state and time-resolved fluorescence spectroscopy has been carried out to reveal the effect of distance on the MEF by aluminum nanostructures. We investigated the potential of layer-by-layer (LbL) assembly to position the proteins at particular distances from nanostructured surfaces. In this LbL assembling process, multilayered structures of poly(styrene sulfonate) (PSS) and poly(allylamine hydrochloride) (PAH) are fabricated in a well-defined and controllable manner. Subsequently, SA and BSA are adsorbed into the topmost positively charged PAH layer. Our results support the importance of the optimal fluorophore-metal distance to get efficient MEF.

2. EXPERIMENTAL SECTION

Materials

Aluminum slugs and silicon monoxide were purchased from Sigma-Aldrich and used as received. Sodium salt of poly(styrene sulfonate) (PSS; MW 70000), poly(allylamine hydrochloride) (PAH; MW 50000–65000), (aminopropyl)triethoxysilane (APS), and phosphate buffer solution pH 7.2 were obtained from Aldrich-Fluka. Bovine serum albumin (BSA), streptavidin (SA), were all obtained from Sigma. Ultrapure water (>18.0 MΩ) purified using a Millipore Milli-Q gradient system was used in preparation of buffers and aqueous solutions.

Preparation of Aluminum substrates

Quartz slides were cleaned with “piranha solution” (35% H₂O₂/H₂SO₄, 1:3) overnight, rinsed with distilled deionized water, and dried with air before thermal vacuum deposition steps. Aluminum was deposited on quartz slides using an Edwards Auto 306 vacuum evaporation chamber under high vacuum (<5 × 10⁻⁷ Torr). In each case, the metal deposition step was followed by the deposition of 5 nm silica via evaporation of silicon

monoxide without breaking the vacuum. The silica layer allowed for comparable surface chemistry as on the control bare quartz substrates. The deposition rate was adjusted by the filament current and the thickness of film was measured with a quartz crystal microbalance. After coating steps, slides were silanized by immersion in an ethyl alcohol solution of 1% of aminopropyl trimethoxysilane (APS).

Preparation of protein adsorbed layer by layer assembled surfaces

PSS and PAH were used to polyelectrolyte layer-by-layer assembly at concentrations of 3 and 2 mg/mL, respectively. Immobilization of PSS/PAH on functionalized quartz or aluminum substrates was carried out manually according to reference 15. Figure 1 shows the schematic representation of protein adsorbed layer-by-layer assembly substrates. 1–3 layers of PSS and PAH were prepared by adsorption of both polyelectrolytes consecutively. SA and BSA solutions (100 µg/mL) in phosphate buffer (pH 7.2) were added onto the completely dried surfaces. PAH terminated slides were used for SA and BSA immobilizations. The same procedure was used for preparation of control samples using bare quartz slides. After incubation with protein solutions, slides were washed with phosphate buffer solution to remove unbound protein solutions.

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 280 nm excitation from a Xenon arc lamp. Special holders were made for bioassay measurements. Time-domain lifetime measurements were obtained on a Pico-Quant lifetime fluorescence spectrophotometer (Fluotime 100). The excitation source was a pulsed laser diode (PicoQuant PDL800-B) with a 20 MHz repetition rate at 280 nm. Intensity decays were measured through a bandpass 320–360 nm filter.

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.

Mie Theory and metallic particles

Mie theory can be used to calculate the optical properties of metal particles. Mie theory provides an analytical solution for the extinction, absorption and scattering properties for spheres but it does not explain fluorophore–metal interactions [16–17]. However, Mie theory is limited to spheres, and the extensions to shells for a few other structures are less frequently used. If the metal particles are spherical then Mie theory provides an exact solution of their optical properties. The extinction (ext), scattering (sca) and absorption (abs) cross-sections (σ) of metal nanoparticles when excited by electromagnetic radiation can be calculated by series expansions of the involved fields into partial waves of different spherical symmetries. Following the notation of Bohren and Huffman[18], the optical cross-sections are given by:

$$\sigma_{ext} = \frac{2\pi}{|k|^2} \sum_{L=1}^{\infty} (2L+1) \text{Re} \{a_L + b_L\} \quad (4)$$

$$\sigma_{ext} = \frac{2\pi}{|k|^2} \sum_{L=1}^{\infty} (2L+1) (|a_L|^2 + |b_L|^2) \quad (5)$$

$$\sigma_{abs} = \sigma_{ext} - \sigma_{sca} \quad (6)$$

where a_L and b_L are the ‘Mie coefficients’ following from the appropriate boundary conditions and can be described as:

$$a_L = \frac{m\psi_L(mx)\psi'_L(x) - \psi'_L(mx)\psi_L(x)}{m\psi_L(mx)\eta'_L(x) - \psi'_L(mx)\eta_L(x)} \quad (7)$$

$$b_L = \frac{\psi_L(mx)\psi'_L(x) - m\psi'_L(mx)\psi_L(x)}{\psi_L(mx)\eta'_L(x) - m\psi'_L(mx)\eta_L(x)} \quad (8)$$

with $m = n/n_m$, where n denotes the complex index of refraction of the particle and n_m the real index of refraction of the surrounding medium; k is the wavevector and $x = |k|R$ the size parameter, and $\psi_L(x)$ and $\eta_L(x)$ are Riccati–Bessel cylindrical functions. The prime indicates differentiation with respect to the argument in parentheses. The summation index L gives the order of the partial wave, where $L = 1$ for a dipole and $L = 2$ for a quadrupole and $L = 3$ for an octapole.

The coefficients a_L and b_L can be used to calculate the optical cross-sections of the particles. The cross-sections for absorption and extinction are given by:

$$C_{sca} = \frac{2\pi}{k^2} \sum_{L=1}^{\infty} (2L+1) (|a_L|^2 + |b_L|^2) \quad (9)$$

$$C_{ext} = \frac{2\pi}{k^2} \sum_{L=1}^{\infty} (2L+1) \text{Re} (|a_L|^2 + |b_L|^2) \quad (10)$$

The cross-section for absorption (C_{abs}) can be calculated from $C_{ext} = C_{abs} + C_{sca}$.

3. RESULTS AND DISCUSSION

As plasmon coupled fluorescence (PCF) deals with the interaction of metallic nanostructures with the fluorophores, theoretical investigation of metal fluorophore interactions has great importance to lighten the nature of PCF. A sound theoretical understanding of metal-fluorophore interactions can help in predicting the parameters that needed to be optimized to design the most efficient metallic nanostructures as well as their interaction with the fluorophores. In this study we used Mie theory to obtain the cross section values of aluminum particles. The expressions for calculation of the cross-sections are rather complex. For this reason we use a commercially available program, MieCalc [19]. Figure 2 shows calculations of the cross-sections for two aluminum particles. For both particles the total extinction is mostly due to scattering. In this case, wavevector matching can occur at the metal interface and the energy radiates to the far-field. We obtained one band with band maxima at ~250 nm for smaller metal particle. The larger aluminum particle showed also a broad band at ~400 nm beside the same band observed from smaller aluminum particle. We believe that the difference between the extinction and scattering of the different size particles is the reason that small metal particles often have no effect or quench fluorescence and larger metal particles can enhance fluorescence.

The SEM image of 10 nm aluminum film indicates that the film is not continuous and aluminum nanoparticles are formed at various shapes and sizes by evaporating the aluminum on the quartz substrate. Average particle size of aluminum particles are approximately 50 nm by a rough estimate.

To investigate the distance-dependence of protein fluorescence, we measured their intrinsic fluorescence emissions on PSS/PAH nanocomposites assembled on both Al and quartz surfaces. Protein distance is varied from 5 to 11 nm in these experimental sets. Figure 4 shows the fluorescence emission spectra from protein-adsorbed PSS/PAH layers with maximum intensities at varied protein distances from the metallic surface. The fluorescence emission intensities of proteins show a significant dependence of distance from the Al surfaces. The fluorescence spectra and emission intensities from the protein-adsorbed PSS/PAH nanocomposites at different distances from the quartz surfaces are similar.

The largest enhancement of 11-fold is observed for the SA-adsorbed PSS/PAH nanocomposite with the probe distance of ~9 nm from the Al surface. Approximately ~9-fold increase in fluorescence intensity of BSA on metallic nanocomposites was observed with the same probe distance. Above 9 nm the intensity relative to the quartz surfaces decreases progressively with an increase in the number of PSS/PAH layers. We have also observed a reduction in the fluorescence intensity (from 11-fold to 4-fold for SA and from 9-fold to 2-fold for BSA) with a decrease in the probe distance from 9 to 5 nm. The present study shows that the MEF of proteins are distance-dependent and the distance is one of the important parameters for maximal fluorescence enhancement. The decrease in the fluorescence enhancement below 9 nm distances from the metalized surfaces could be related to the usual quenching of emission when the fluorophores are in close proximity to the metals.

Time-resolved fluorescence can open up to view to effects of interactions of proteins with their environments. The lifetimes of proteins on PSS/PAH nanocomposites on quartz and aluminum surfaces provide more understanding on distance-dependent metal-fluorophore interactions. We investigated the fluorescence intensity decays of BSA-adsorbed PSS/PAH nanocomposites assembled on both Al and quartz surfaces. Fluorescence intensity decays of BSA on aluminum and quartz are showed in Figure 5a. The intensity decays of tryptophan residues in BSA are faster on the aluminum substrate compared to that on bare quartz. The

average lifetimes of BSA adsorbed at different distances on aluminum nanocomposite surfaces are shown in Figure 5b. The amplitude-weighted average lifetime of BSA represents ~3-fold decrease on aluminum. The short lifetime on the aluminum nanoparticles/nanostructures supports the notion that at least part of the increased in observed fluorescence intensity is due to the plasmonic structures.

4. CONCLUSION

In this article we have demonstrated the intrinsic metal enhanced fluorescence from SA and BSA adsorbed metallic nanostructures. We also used Mie theory to get extinction properties of aluminum nanoparticles. We made calculations for 25 and 50 nm diameter aluminum particles. Results revealed that the most of the total extinction is due to scattering for both particle sizes. We use PAH and PSS polyelectrolyte layers to locate the proteins at a well-defined distances from aluminum surfaces. We have observed ~11-fold increase in fluorescence intensity of SA at ~9 nm distance. Approximately 9-fold increase in fluorescence intensity, and 3-fold decrease in fluorescence lifetime were obtained from BSA adsorbed LbL assembled nanostructures. The experimental results pointed out that optimal distance is important to get efficient MEF.

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Figure 1.
Schematic representation immobilization of proteins on of layer-by-layer assembled substrates.

Figure 2. Extinction, scattering and absorption cross-section of 25 nm Al particle (left) and 320 nm Ag colloid (right) calculated using Mie theory.

Figure 3.
SEM photomicrographs of 10 nm aluminum substrate.

Figure 4.
Effect of Al distance on the fluorescence intensity of SA (a) and BSA (b).

Figure 5. Intensity decays of BSA on quartz, Al and PSS/PAH nanocomposites (a); and the ratio of amplitude-weighted average lifetimes of BSA on quartz to on Al nanostructures (b).