Published in final edited form as:

Proc SPIE. 2012 September 2; 8234: 82340M-. doi:10.1117/12.916177.

Plasmon-controlled fluorescence and single DNA strand sequencing

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Abstract

During the past several years we have studied the effects of metallic surfaces and nanostructures with fluorophores. 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. These studies have shown dramatic increases in brightness and photostability, especially for low quantum yield fluorophores. Much of this work was performed using visible or NIR fluorophores. In the present study, we have extended our studies to UV wavelengths and have shown that aluminum and platinum particles can enhance the emission of UV fluorophores including intrinsic protein fluorescence from 300 to 420 nm. We used the finite-difference timedomain (FDTD) method to calculate the effects of aluminum nanoparticles on nearby fluorophores that emit in the UV. And also we performed experiments to investigate the effect of metallic nanoparticles on fluorescence intensity of DNA bases and DNA G-quadruplex. We observed increase in fluorescence intensities of DNA bases varied range changing from 20 to 3-fold in steady-state fluorescence emission measurements. We obtained ~5-fold increase in fluorescence intensity of DNA G-quadruplex on both Al and Pt metallic substrates when compared with control quartz substrates.

Keywords

DNA sequencing; plasmonics; metal enhanced fluorescence; fluorescence; plasmon controlled fluorescence; nanotechnology

1. INTRODUCTION

Fluorescence detection has been a valuable tool in the biosciences for over 40 years [1–6]. Many of these studies have taken advantage of the intrinsic fluorescence from proteins, which is due primarily to the tryptophan residues. However, in contrast to proteins, nucleic acids and the nucleotides are almost non-fluorescent in water. As a result DNA sequencing and genetic analysis relies almost exclusively on the use of extrinsic fluorophores which are used to label DNA or the bases. While this approach has been successful there is a growing interest in label-free detection to avoid the need for labeling reactions, which become increasingly costly and complicated as the number of biomarkers continues to increase. In recent years the availability of high time-resolution, such as up-conversion methods, has resulted in a renewed interest in the photophysics of DNA [7–9].

During the past several years, there have been significant efforts in utilizing the metallic nanostructures or nanoparticles for improved detection of fluorescence [10–14]. This approach represents a fundamental change in fluorescence technology because the fluorophores can be excited by the near fields created by plasmons on the metallic structures, rather than freely propagating light. Additionally, the metallic structure can substantially modify the rates of spontaneous emission and the directionality of the emission. It has been shown that the fluorescence intensity of a number of probes can be increased by proximity to metal island films or nanoparticles. We referred to this phenomenon as metal-enhanced fluorescence (MEF). MEF occurs due to a short-range interaction of fluorophores with metallic particles and roughened surfaces, which depending on the metal geometry; take place at distances from 5 to 30 nm [15–16]. It is the short-range distance of MEF that can facilitate the detection of nucleic acid bases from their intrinsic fluorescence emission.

In this study our goal is investigation of new metallic nanostructures which would allow DNA sequencing without using of extrinsic fluorophores. In this paper, we use finite-difference time domain (FDTD) calculations to explore the possibility of enhancing the intrinsic fluorescence of DNA bases with aluminum nanoparticles of various sizes and configurations. We use the FDTD calculations to show that aluminum nanoparticles are very promising substrates for enhancing the emission of fluorophores in the UV region for DNA bases. We also present experimental results showing effect of aluminum and platinum nanostructures on fluorescence intensity of DNA G-quadruplex and DNA bases. Our results showed aluminum and platinum metallic nanoparticles are highly promising in determination of nucleic acids from their intrinsic fluorescence intensity.

2. EXPERIMENTAL SECTION

Materials

Aluminum and platinum slugs and silicon monoxide were purchased from Sigma-Aldrich and used as received. 3-(aminopropyl)triethoxysilane (APS), and phosphate buffer solution pH 7.2 were obtained from Aldrich-Fluka. Guanosine 3',5'-cyclic monophosphate sodium salt (GMP), thymidine 5'-monophosphate (TMP), cytidine 5'-monophosphate (CMP) and uracil 5'-monophosphate were obtained from Sigma-Aldrich. G-quadruplex DNA was obtained from Integrated DNA Technology (IDT). Ultrapure water (>18.0 M Ω) purified using a Millipore Milli-Q gradient system was used in preparation of buffers and aqueous solutions.

Preparation of Aluminum and Platinum nanostructures

Quartz slides were cleaned with “piranha solution” (30% 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 ($<5 \times 10^{-7}$ 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 for both the Al-coated and bare glass region of the 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).

10 nm of platinum were deposited on quartz slides after cleaning with piranha solution as previously described. A sputtering system was used for deposition of platinum on quartz substrates. The metal deposition step was followed by the deposition of 5 nm of silica via evaporation without breaking vacuum.

Spin coated films on glass and Aluminum surfaces

An aliquot of approximately 500 μL of 0.5 wt % aqueous solution of low molecular weight PVA containing separately dissolved GMP, CMP and TMP was spin coated at 3000 rpm (Specialty coating system Inc., Speedline Technologies, Indiana) on the surface of the aluminum and quartz substrates respectively. This composition of PVA forms ~15 nm thick films. We verified the thickness of the spin-coated PVA films using a technique that has been reported previously. The concentration of the bases in the PVA solution (before spin-coating) was approximately 1 mM.

Preparation of G-quadruplex and DNA bases immobilized surfaces

After silanization steps with APS, DNA G-quadruplex, GMP, TMP, CMP and UMP solutions (0.1 mg/mL) in phosphate buffer (pH 7.2) were added onto the dried surfaces. The same procedure was used for preparation of control samples using bare quartz slides. After overnight incubation with DNA bases and G-quadruplex solution, slides were washed with phosphate buffer solution to remove unbound nucleotides. Figure 1 shows the schematic of preparation steps of nucleic acid immobilized metallic substrates.

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.

FDTD Computational Details

Three-dimensional FDTD simulations are performed using the *FDTD Solutions* package from Lumerical Solutions, Inc. (Vancouver, Canada) [17–19]. The FDTD code was implemented in the parallel option on a Dell Precision PWS690 Workstation with the following components: Dual Quad-Core Intel Xeon E5320 processors at 1.86 GHz, and 8 GB RAM. Additional postprocessing of the FDTD data were performed using *MATLAB* (version 7.0) from Mathworks (Natick, MA), and OriginPro 7 from Originlab Corporation (Northampton, MA). For calculations involving fluorophores, it is assumed that excitation of the fluorophore has already occurred and the fluorophore is now emitting dipole radiation. Hence the fluorophore is modeled as a time-windowed, oscillating point dipole source for the electric field, with frequency content spanning the spectral range (100–700 nm) of interest and polarization perpendicular to the metal nanoparticle surface. The FDTD calculations were done for a fluorophore near one aluminum nanoparticle (monomer) or between two aluminum nanoparticles (dimer).

2. RESULTS AND DISCUSSION

We performed FDTD calculations for a 350 nm dipole near a 60 nm diameter aluminum particle. To ensure the correctness of the calculations we used the FDTD method to calculate the extinction, scattering and absorption spectra of the Al particle (Figure 2 left top). These calculated spectra are in excellent agreement with those calculated analytically using Mie theory (not shown). Figure 2 (bottom) shows the effect of wavelength and distance on the power radiated by a nearby fluorophore. As explained in Part D the radiated power reflects the radiative decay rate Γ of the fluorophore. The FDTD calculations show that the radiative decay rate of a nearby fluorophore can be increased 20-fold when near the aluminum particle (Figure 2 left bottom). Enhancements occur at all wavelengths from 300 to 400 nm where the bases emit. It is interesting to note that the largest increase in radiated power occurs slightly to the red side of the Al plasmon resonance. This effect has been reported for longer wavelength fluorophores and similar structures [20–22], which supports the validity

of our calculations. The radiated power calculations also show some enhancement at wavelengths up to 700 nm. This effect occurs because fluorophores in the near-field can interact with metal structures even if they do not display interactions with far-field illumination above 500 nm. Figure 2 right top shows that the peaks and spectral widths of the power enhancements can be tuned over a large range by changes in particle size, and that these wavelengths overlap with intrinsic base fluorescence.

We modeled a base as an oscillating dipole near an Al colloid or between two Al colloids with different sizes and distances. In order to model the broad emission from the DNA bases we integrated the power radiated from 300 to 420 nm and compared this to an isolated dipole (no metal) over the same range of wavelengths. Figure 2 right bottom shows the radiative rate enhancements for a base near a monomer (M) or dimer (D) at various distances from the Al surfaces. The radiative rates are much larger for a base between the dimers than near the monomers (log scale on y-axis). The radiative rates can increase about 1000-fold for a 4 nm dimer spacing, and up to 3500-fold for a 2 nm dimer spacing. We note that these calculations are of the radiative power and thus include the effects of quenching at short distances. These FDTD calculations show that we can obtain the large increases in the radiative decay rate needed for detection the DNA bases.

We also performed experiments to corroborate the theoretical predictions above concerning the efficiency of aluminum nanostructures to detect unlabeled nucleotides using their intrinsic emission. The emission from AMP and GMP are distinct, and the emission spectra of TMP and CMP are similar. In total these absorption and emission spectra show that the four bases could be identified using a combination of selected excitation and emission wavelengths if it is possible to detect an adequate number of photons above the background emission. As discussed in the Experimental Section, we spin coated a 15 nm layer of polyvinyl alcohol (PVA) containing separately the dissolved DNA nucleotides CMP, GMP and TMP on a thin film of aluminum on a quartz slide and compared its emission intensity with an identical sample on a control quartz substrate. Al substrates were prepared by evaporation of 5 nm silica on 10 nm the aluminum film without breaking vacuum which serves to protect the metal surface as well as adds a spacer layer between the metal surface and DNA bases. Finally an approximately 15 nm thick layer of PVA containing separately dissolved GMP, CMP, and TMP was spin coated on the surface of the silica layers for the aluminum samples and directly on the quartz substrates for the control samples. TMP showed approximately 4-fold increase in fluorescence intensity when compared to the quartz control. We observed approximately 11 and 5-fold increase in the emission intensities of the CMP and GMP on aluminum, respectively. At present we have not yet obtained enhancements larger than 2-fold for AMP. In our study, the thermally evaporated aluminum particles are smaller than the wavelength of the excitation and emission, so the 10 nm thick aluminum films essentially behave like a particulate surface. In this configuration we believe that the excited state molecule couples to the plasmons on the surface of the aluminum particles and the combined excited fluorophore-metal complex acts as a unified system, which then radiates the emission observed in the experiments. The experimental results showed in Figure 3 corroborate the validity of our theoretical predictions that aluminum nanoparticles can be used to efficiently enhance the intrinsic emission of various DNA bases (in the UV region), and so creating the possibility of DNA sequencing based on the intrinsic base fluorescence, thus potentially avoiding the pitfalls involved in additional external labeling steps that are currently involved in DNA sequencing reactions.

In an effort to identify other metals for label free detection of nucleic acids which would allow DNA sequencing without use of extrinsic fluorophores we examined metallic platinum. Effect of Pt particles on DNA G-quadruplex and DNA bases were investigated on 10 nm metallic substrates. We prepared nucleotide or G-quadruplex DNA adsorbed

substrates described method in experimental section. G-quadruplex DNA and three different nucleotides thymine monophosphate (TMP), uracil monophosphate (UMP), and guanine monophosphate (GMP) showed significant increases in fluorescence intensity when compared to a control quartz substrate. Cytidine monophosphate (CMP) showed moderate increase in fluorescence intensity according to other three nucleotides. Approximately ~20, 9 and 5-fold increase in fluorescence intensities of GMP, UMP and TMP on metallic nanostructures were observed, respectively. Approximately 3-fold increment in fluorescence intensity of CMP was obtained on platinum nanostructures.

We investigated effects of Al and Pt metal particles on fluorescence intensity of DNA G-quadruplex. DNA bases showed significant enhancement varied between 20 and 3-fold on platinum substrates. Approximately 5-fold increase in fluorescence intensity of DNA G-quadruplex is observed on both metallic substrates when compared to a control quartz substrate. Figure 4 shows G-quadruplex DNA steady-state fluorescence emission on Al substrate (left) and Pt substrate (right).

3. CONCLUSION

In this paper, we present computational and experimental studies showing the possibility of determination of DNA nucleotides from their intrinsic fluorescence by using aluminum and platinum nanostructures. We can say that such metallic nanostructures hold potential in the future for designing high-throughput, low-cost DNA sequencing studies using the intrinsic fluorescence of DNA bases in ultraviolet region. In our FDTD calculations, the excited fluorophore was modeled as an oscillating dipole source, and a variety of spherical nanoparticle sizes, and fluorophore-nanoparticle distances relative to the aluminum surface were studied. The peak enhancement wavelength is a function of the nanoparticle size, with larger nanoparticles showing enhancement peaks at longer wavelengths. Additionally, the degree of enhancement of the radiated power increased significantly when the fluorophore is placed in between two aluminum nanoparticles of a dimer system. In the nanoparticle systems investigated in this study, we observe maximum enhancements of over 800-fold when the fluorophore is located between two $d = 60$ nm aluminum nanoparticles spaced $2s = 4$ nm apart.

Also our experimental results represent that thin aluminum and platinum films can significantly increase the fluorescence intensity of nucleotides when compared to quartz substrates. Platinum and aluminum substrates are highly promising for determination of DNA G-quadruplex, GMP, TMP, UMP and CMP from their intrinsic fluorescence emission. These results support our theoretical predictions about that aluminum nanoparticles are efficient substrates for enhancing the intrinsic fluorescence of the low quantum yield DNA nucleotides that emit in the UV. Platinum substrates also make a promise to design low-cost high-throughput DNA sequencing devices using the intrinsic fluorescence of bases.

Acknowledgments

F131 This work was supported by the National Institutes of Health (NIH) - Grant Nos. NHGRI RO1 HG002655, R21 HG005090 and NIAID K25 AI087968.

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Figure 1.
Schematic of preparation steps of nucleic acid immobilized metallic substrates

Figure 2.

Left top: FDTD calculations of the absorption, scattering and extinction spectra of a 60 nm diameter aluminum particle. Left bottom: FDTD calculations of the effect of a 60 nm diameter aluminum particle on the power radiated by a nearby fluorophore. Right top: FDTD calculations of the aluminum particle size on power radiated by a nearby dipole. Right bottom: Power radiated from 300 to 420 nm for a dipole near 80 nm Al particles, normalized to the same dipole without metal. M, monomer. D, dimer. The x-axis numbers are the distance in nm.

Figure 3. Fluorescence spectra of a 15 nm thick PVA film on top of a 10 nm thick aluminum film, and on a quartz control that contain the DNA nucleotides (left) CMP, and (right) GMP.

Figure 4. Fluorescence spectra of DNA G-quadruplex on top of a 10 nm thick aluminum film(left), and on top of a 10 nm thick platinum film (right).