Published in final edited form as:

J Phys Chem C Nanomater Interfaces. 2008 ; 112(46): 17957–17963. doi:10.1021/jp807025n.

Metal-enhanced Intrinsic Fluorescence of Proteins on Silver Nanostructured Surfaces towards Label-Free Detection

Krishanu Ray, Mustafa H. Chowdhury, Henryk Szmecinski, and Joseph R. Lakowicz

Center for Fluorescence Spectroscopy, Department of Biochemistry and Molecular Biology, University of Maryland School of Medicine, 725 West Lombard St, Baltimore, MD, 21201, USA.

Abstract

In recent years metal-enhanced fluorescence (MEF) using silver particles has been reported for a number of fluorophores emitting at visible wavelengths. However it was generally thought that silver particles would always quench fluorescence at shorter wavelengths. We now report the observation of metal-enhanced fluorescence of the tryptophan analogue N-acetyl-L-tryptophanamide (NATA) on silver nano-structured surfaces. NATA is a model for the intrinsic tryptophan emission from proteins. We have also studied the effects of silver nanostructures on the emission of N-acetyl-L-tyrosinamide (NATA-tyr). In the case of NATA we observed increased emission, decrease in fluorescence lifetimes, and increase in photostability when NATA was embedded in 15 nm thick spin-casted poly(vinyl alcohol) film on silver nanostructured surfaces. We have also investigated the effects of silver nanostructures on the emission from thin poly(vinyl alcohol) films containing NATA-tyr. However, we have observed no increase in fluorescence signal for NATA-tyr on silver nanostructures. To understand these results we performed numerical calculations using the Finite-Difference Time-Domain (FDTD) technique to model a tryptophan-wavelength dipole near a spherical silver particle. Our calculations reveal an enhancement of the power of the radiated emission by the excited-state fluorophore in proximity to a 100 nm silver nanoparticle covering the emission spectra of NATA and NATA-tyr. These calculations show a clear wavelength dependence with the specific spectral region displaying low-enhancement at the shorter NATA-tyr wavelength and higher enhancement at NATA emission wavelength. Our FDTD calculations also reveal that excited fluorophores in the near-field of a 100 nm silver nanoparticle can induce enhancement fields of varying degrees of the intensity of the near-fields around the particle that is dependent on the wavelength of the emission. We believe this enhanced near-fields play a role in our observation of MEF from metal surfaces. The enhanced emission of NATA on silver nanostructures suggests that the extension of MEF to the UV region opens new possibilities to study tryptophan-containing proteins without labeling with longer wavelength fluorophores towards label free detection of biomolecules.

Introduction

Fluorescence detection presently is a central technology in the biosciences. The applications of fluorescence include cell imaging, medical diagnostics and biophysical research. Another growing use of fluorescence is for measurements of a large number of samples as occur on DNA arrays, protein arrays and high throughput screening (HTS). HTS typically includes testing of a large number of small molecules for biological activity, most often drug-receptor interactions. Almost all the applications of fluorescence require the use of labeled drugs and labeled biomolecules, which becomes increasingly inconvenient as the number of compounds

to be tested has increased. The need for labeling of the biomolecules with extrinsic fluorophores results in increased costs and complexity. Because of this added complexity there is a rapidly growing interest in methods which provide label-free detection (LFD), as has been described in recent reviews.¹⁻³ Perhaps the most widely used LFD method is surface plasmon resonance (SPR). SPR detection is based on measurements of small change in refractive index of a sample on a thin gold film.⁴⁻⁵ These changes are detectable because the SPR angle is sensitive to the change in refractive index which occurs when target molecules bind to capture molecules on the gold surface. Because of its general applicability SPR is being extended to high throughput capabilities by the use of SPR imaging.⁶⁻⁸ The importance of label-free detection can be seen from the large number of other methods which are being tested for label-free detection.

Proteins possess three intrinsic fluorophores, phenylalanine (phe), tyrosine (try) and tryptophan (trp). Proteins are highly fluorescent, which is due primarily to tryptophan residues because of its longer excitation and emission wavelengths, good quantum yield, and fluorescence resonance energy transfer (FRET).⁹ Tryptophan residues in proteins occur with a frequency of 1.3% of the amino acid residues and are thus present in almost all proteins. Tryptophan-free proteins are relatively rare. However, since most proteins contain tryptophan, this emission is not specific for proteins of interest in a biological sample. Additionally, proteins are excited near 280 nm and emit near 350 nm. These conditions result in high background fluorescence from most samples. For these reasons intrinsic tryptophan emission from proteins is not used to detect specific proteins. In this paper, we use metallic nanostructures to enhance the fluorescence of the tryptophan analogue N-acetyl-L-tryptophanamide (NATA) near silver nanostructures. NATA contains amide residues in the amino and carboxyl sides of third-carbon and is thus an analogue of tryptophan in proteins. We also studied the tyrosine analogue N-acetyl-L-tyrosinamide (NATA-tyr). The most important fact is that we observe the enhancement of tryptophan fluorescence when NATA is close to the metal nanostructured surfaces. This allows design of surface-based assays with biorecognitive layer that specifically bind the protein of interest and thus enhance its intrinsic fluorescence. Large increases in fluorescence intensity and decreases in lifetime provide the means of direct detection of bound protein without separation from the unbound.

We also used numerical calculations in the form of the Finite-Difference Time-Domain (FDTD) technique to understand our experimental results. FDTD is an implementation of Maxwell's time-dependent curl equations for solving the temporal variation of electromagnetic waves within a finite space that contains a target of arbitrary shape, and has recently become the state-of-the-art method for solving Maxwell's equations for complex geometries.¹⁰⁻¹⁸ A major advantage of FDTD is that it is a direct time and space solution, and hence offers the user a unique insight into a myriad of problems in photonics. More information on the FDTD technique can be found in Refs. 10-18. Our calculations reveal that the enhancement of the power of the radiated emission of an excited-state fluorophore in proximity to a 100 nm silver nanoparticle shows clear wavelength dependence with specific spectral regions displaying high emission enhancements while other regions showing only modest or no enhancements. Our FDTD calculations also reveal that fluorophores in the near-field of a 100 nm silver nanoparticle can induce enhancements of varying degrees of the intensity of the near-fields around the particle that is dependent on the wavelength of the emission. We believe these enhanced fields play a role in our observation of MEF from metal surfaces.

Experimental

Silver island films (SIFs) on quartz slides were prepared as described previously.¹⁹⁻²¹ NATA and low molecular weight polyvinyl alcohol (PVA, MW 13000 - 23000) were purchased from Aldrich chemical company and used as received. N-acetyl-L-tyrosinamide (NATA-tyr) was obtained from Acros Organics. Chemical structures of NATA and NATA-tyr are shown in Fig.

1. NATA and NATA-tyr in solutions of 0.5 wt% polyvinyl alcohol (PVA) was spin coated onto quartz and SIFs slides. A schematic of the sample geometry is presented in Fig. 1.

Absorption spectra were collected using a Hewlett-Packard 8453 spectrophotometer. SIFs displayed the characteristic surface plasmon resonance with an absorption maximum near 450 nm (Figure 1). Fluorescence spectra of probes on solid substrates were recorded using a Varian Cary Eclipse fluorescence spectrophotometer. Both the steady-state and time-domain lifetime measurements were carried out using front face illumination. Time-domain lifetime measurements were obtained on a Pico-Quant lifetime fluorescence spectrophotometer (Fluotime 100).

The fluorescence intensity decays were analyzed in terms of the multi-exponential model⁹:

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

In this expression τ_i are the decay times and α_i are the amplitudes. 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:

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

and the amplitude-weighted lifetime is given by:

$$\langle \tau \rangle = \sum_i \alpha_i \tau_i \quad (4)$$

The values of α_i and τ_i were determined using the PicoQuant Fluofit 4.1 software with the deconvolution of instrument response function and nonlinear least squares fitting. The goodness-of-fit was determined by the reduced χ^2 value.

A portion of the SIFs sample was cut and coated with a thin layer of gold (approx. 5 nm) in a sputter coating system. This step was done to minimize charging effects during scanning electron microscope (SEM) imaging. The sample was then mounted on an Al stub with conductive tape, and observed in a Hitachi SU-70 SEM. Due to the nonconductive substrate (glass), low voltage (3 kV) was employed for high resolution shallow surface observation and imaging using beam deceleration technology. Samples were surveyed at low magnifications to see the general features and the homogeneity. Representative areas were selected for higher magnification investigation. SEM image of the SIFs surface (Figure 1) shows the nanoscale heterogeneity of the silver particles' sizes, shapes and spatial distributions. From this SEM image, we observe the average size of these silver particles is about 100 nm.

FDTD Calculations

3-dimensional FDTD calculations were performed using the program FDTD Solutions (Version 5.0) purchased from Lumerical Solutions, Inc., (Vancouver, Canada). In all of our calculations, it is assumed that the excitation stage of fluorescence has occurred and the

fluorophore is now emitting dipole radiation. A time-windowed dipole source, radiating at a fixed wavelength of 350 nm, was used to mimic the emission of NATA. Similarly, we used a dipole source radiating at 305 nm to mimic the emission of NATA-tyr. This is a soft source, to allow backscattered radiation to pass through it. In order to maintain the accuracy and stability of the FDTD calculations, the smallest spatial grid size to accurately model the prescribed system without being computationally prohibitive was obtained in an iterative fashion. This process is called convergence testing. In our implementation of FDTD, convergence testing was done by starting the first calculation with a grid size of $\lambda_0/20$, where λ_0 is the smallest wavelength expected in the simulation, and then reducing the grid size by half in sequential simulations and comparing the results of the calculations. The reduction of the grid size was stopped when we approached a grid size (Δ) where results closely match with the set of results that are obtained from half of that particular grid size ($\Delta/2$), and that is also computationally feasible.^{10-11,16-18} For our calculations, we employed a grid size of 2 nm. The numerical implementation of Maxwell's equations in the FDTD algorithm requires that the time increment Δt have a specific bound relative to the spatial discretization Δ (as mentioned above) to ensure the stability of the time stepping algorithm.^{10-11,16-18} Typically the durations of our simulations were 400 fs, corresponding to an excess of 200,000 time propagation steps for each calculation. The FDTD package employed has frequency domain monitors that perform discrete Fourier transforms of the time domain fields while the simulation is running. In this manner, continuous wave (CW) information is obtained at any pre-specified wavelengths for the various electric and magnetic field components. All of the calculations were done assuming a background relative dielectric constant of 1.0.

The enhancement in the total radiated power is inferred by integrating the normal flux passing through a closed surface containing the system is given by:

$$P_{\text{rad}}/P_0 \quad (4)$$

where a system is either an isolated dipole (excited-state fluorophore) or a fluorophore in proximity to a silver nanoparticle, P_0 is the radiated power of a classical dipole in a homogeneous background which in our case is air/vacuum, and P_{rad} is the radiated power of the dipole in proximity to the silver nanoparticle. In our calculations, since we use a radiating dipole source to model the excited fluorophore, the power from an isolated dipole P_0 is used for normalization.^{18,22,23} Note that $P_{\text{rad}}/P_0 > 1$ represents enhancement and $P_{\text{rad}}/P_0 < 1$ represents quenching. We used a set of six frequency domain surface monitors to create a box around the system, and measured the total power radiated by the system by integrating the real part of the Poynting vector over all six surfaces. The power was normalized to the analytic expression for the power radiated by a dipole in a homogeneous dielectric (in this case, air/vacuum) to get the relative change in power radiated as described in Eq. 4.

Results and Discussion

Emission spectra of NATA spin coated from PVA solution are shown in Fig. 2 for quartz and silver nanostructured substrates. The emission spectral distribution of NATA measured on metallic nanostructures and quartz is essentially identical and characteristic for tryptophan. Figure 2 shows that the SIFs gives an enhancement of approx. 8-fold when compared to the quartz. The inset of the figure shows the normalized fluorescence emission spectra of the NATA on quartz and SIFs. The normalized spectra have a high degree of overlap thus suggesting that there was no change in the spectral properties of the NATA when it interacts with the silver nanostructures.

We have investigated the lifetimes of 15-nm thick PVA film containing NATA on quartz and SIF substrates (Fig. 2). The solid lines indicate the best fit to the experimental decay curves. As can be noticed from the Figure 2, the intensity decay of NATA on SIFs surface is faster than observed on the quartz control substrate. The intensity-decay of NATA PVA film on quartz could be fitted with a single exponential with a lifetime of 3.2 ns. NATA on SIFs surface could only be fitted with a double-exponential with two lifetimes of 3.2 nsec (19%) and 0.98 nsec (81%). The amplitude-weighted lifetime of NATA on SIFs was 1.4 nsec. Hence, the intensity decays show that the lifetime was decreased to about 2.5-fold (Fig. 2). In the case of NATA on the SIF it is likely that the more complex multi-exponential decay reflects the presence of NATA molecules close to and more distant from the silver surface. This shortening of lifetime on the silver nanostructured substrate supports the notion that the increase in observed fluorescence intensity is due to the radiation from the plasmon-fluorophore complex^{24,25} that results when excited fluorophores interact with silver particles in the near-field. The reduction of lifetime of NATA on SIFs also suggests an increase in the radiative decay rate of NATA due to the silver particles.²⁶⁻²⁸ We note that precise agreement between the increases in intensity and decrease in lifetime is not expected. The lifetime was estimated from a single exponential model. It is well known that time-domain measurements often result in over-weighting of the lifetime by the longer lifetime components in heterogeneous decay, particularly when the decay of the short components overlaps the instrument response function.

Figure 3 shows the emission spectra of spin coated 15-nm PVA film containing NATA-tyr on SIFs and quartz substrates. The emission spectra of NATA-tyr collected through a 300-nm long-pass filter show similar fluorescence intensities for both SIFs and quartz substrates. The emission spectrum of NATA-tyr is slightly blue-shifted on SIFs compared to quartz. Figure 3 (bottom panel) shows the intensity decays of 15-nm PVA film containing NATA-tyr on SIFs and quartz substrates. We observed a slightly faster decay for the NATA-tyr PVA film on SIFs compared to that on quartz. Control measurements on the quartz or SIFs surfaces, without NATA or NATA-tyr, yielded almost no signal when observed through the set of band-pass emission filters used to detect the corresponding emission from those probes.

In general, the detectability of a fluorophore is determined by two factors: the extent of background emission from the sample and the photostability of the fluorophore. We examined the effects of silver island films on the photostability of NATA. Figure 4 shows the photostability of NATA on quartz and SIFS substrates. Using the same incident excitation power, we observed significantly more fluorescence from the silver nanostructured substrates as compared to the quartz control sample (data not shown). In Figure 4, the incident excitation power on the SIFs has been attenuated to give the similar initial emission intensity as observed on the quartz substrate. It is evident from Figure 4 that NATA is more photostable on the SIFs substrate as compared to the quartz substrate. This result is consistent with an increase in the radiative decay rate of NATA in presence of silver nanoparticles, and also with the decreased lifetimes of NATA on SIFs. While a maximum of 8-fold increase in fluorescence intensity of NATA is clearly beneficial, a reduced fluorescence lifetime of probes also enables the system to be cycled faster, as the lifetime of a species determines its cyclic rate. Hence, 8-fold increase in intensity coupled with a 2.5-fold reduction in lifetime of the NATA in proximity to the silver nanostructured surfaces provides a significant increase in detectability. In total, it appears that the detectability of NATA can be increased significantly near silver nano-particles considering the co-operative effects of enhanced fluorescence intensity, reduced lifetime and increased photostability on silver nanostructured surface.

It was surprising to observe metal-enhanced fluorescence at these UV wavelengths. Hence we questioned whether the observed effects were consistent with the known optical properties of silver. We used the FDTD method to calculate the power radiation by a dipole near silver particles, and the wavelength dependence of the effects. Figure 5 shows the radiated power

enhancement for a dipole spaced 8 nm from a 100 nm diameter Ag nanoparticle. The SEM images of the SiFs in Fig. 1 (middle) revealed average particle sizes of approximately 100 nm diameter, and hence we choose this dimension for our calculations. We are aware that the morphology of the actual particles in SiFs is not exactly spherical, but we choose to use the simplest shape for our calculations. In Figure 5, dipoles radiating at different wavelengths from 250-700 nm (in 1 nm intervals) were used to compute the spectra. All the dipoles in this calculation were oriented perpendicular to the surface of the Ag nanoparticle. Our FDTD calculations self-normalizes the radiated power output from a system comprising the Ag nanoparticle and a dipole to the output of an isolated dipole, so any value of radiated power greater than 1 represents an enhancement. The output of an isolated dipole is always 1. It is interesting to note that the normalized radiated power increases rapidly at 350 nm. Also shown in the figure are the emission spectra of NATA and NATA-tyr on SiFs. This figure shows that the NATA emission has a much larger spectral overlap with the radiated power enhancement spectra than the NATA-tyr spectra. This may be a reason why NATA shows good enhancements with SiFs while NATA-tyr does not.

We have also used FDTD to calculate the enhancements in the intensity of the near-fields around the Ag nanoparticles that is induced by the fluorophore. Figure 6(a) is a schematic illustration of the system studied. A spherical, silver nanoparticle with a diameter of 100 nm is placed at the origin. The main objective of the calculations is to investigate whether an excited fluorophore in the near-field of a silver nanoparticle can cause field enhancements around the particle at the UV wavelength region. We believe that any near-field enhancements induced by a fluorophore around the silver nanoparticle plays a significant role in creating the MEF which that we observe experimentally. The fluorophore is oriented with its dipole moment along the x -axis which is normal to the metal surface and placed 8 nm from the surface of the Ag nanoparticle. Figure 6(b) shows the intensity around an isolated fluorophore (or oscillating dipole). We have verified, as might be expected, that this latter intensity is similar to the near-field of a Hertz dipole.²⁹ We have chosen the wavelength of the dipole as 350 nm to match the emission maximum of the NATA. Figure 6(c) shows the electric field intensity in the x - y plane around the 100 nm silver nanoparticle separated 8 nm from the fluorophore (oriented along the x -axis). Figure 6(d) is an image of the near-field enhancement that is generated by dividing the intensity around the fluorophore-nanoparticle complex by the intensity around the isolated fluorophore (i.e. dividing Figure 6 (c) by Figure 6 (b)). All the images are displayed in the logarithmic scale (base 10) for clarity of presentation. The areas in Figure 6(d) which are green, yellow and red in color correspond in the color map to values greater than one are areas where we see strong enhancements in the near-field around the silver particle. It is interesting to observe that the near-field is not enhanced *between* the particle and the dipole, but is distributed in an interesting “wing” shaped pattern around the nanoparticle with the maximum enhancements on the on the central area of this “wing” shape. We also observe appreciable near-field enhancements in the side of the Ag nanoparticle opposite to the fluorophore. Such spatial variations in the near-field enhancements are not easily inferred from experimental observations and thus provide additional insight into the nature of metal enhanced fluorescence.

Figure 7 also presents the near-field intensity distributions of a fluorophore-silver nanoparticle system. However in this case, the wavelength of the dipole chosen is 305 nm which corresponds to the emission maxima of NATA-tyr. The images of Figure 7 were generated in an identical fashion to Figure 6. Figure 7(d) is an image of the near-field enhancement that is generated by dividing the intensity around the 305nm dipole-Ag nanoparticle complex by the intensity around the isolated 305 nm dipole (i.e. dividing Figure 7(c) by Figure 7 (b)). Figure 7(d) shows the enhancement in the near-fields induced around the Ag nanoparticle by the 305 nm emitting dipole is quite modest when compared to the 350 nm emitting dipole (Figure 6 (d)). We again see that the slight near-field enhancements observed are not *between* the particle and the dipole,

but occurs on the side of the Ag nanoparticle opposite to the dipole. Hence a direct comparison of Figure 6 and Figure 7 tells us that the near-field intensity plots agrees qualitatively with our experimental observations where we see a significant enhancement of the NATA emission but not the NATA-tyr emission with SIFs.

Conclusions

The presence of silver nanoparticles significantly increases the brightness of the intrinsic fluorescence of NATA. The lifetimes of the NATA are shorter on silver particles than on quartz substrate. On the other hand, we have not observed any significant effect of silver nanoparticles on the emission of NATA-tyr. We have presented a numerical FDTD study of the effect on the emission of excited fluorophores near a silver nanoparticle and contrasted our results with an isolated fluorophore. In these numerical calculations, we focus only on the emission side of fluorescence. Inspection of intensity patterns reveals how, in the near field, very specific regions around the nanoparticles experience field enhancements and quenching. This type of result is not easily inferred from far-field observations and is relevant to potential applications that would involve spatially resolved molecular spectroscopy or detection using fluorescence. The extension of MEF to the UV region opens new possibilities to study tryptophan-containing proteins without labeling with longer wavelength fluorophores and provides an approach to label-free detection of biomolecules.

Acknowledgment

The present work was supported by the National Institute of Health (NIH), NHGRI (Grant HG002655), and NIBIB (Grant EB006521). The authors thank Dr. Wen-An Chiou of Maryland Nanocenter, University of Maryland, College Park, for SEM measurements. The authors also thank Dr. Alexander Moroz for discussion on metal-fluorophore interaction.

References

1. Ramachandran N, Larson DN, Stark PRH, Hainsworth E, LaBaer J. FEBS Journal 2005;272:5412. [PubMed: 16262683]
2. Yu X, Xu D, Cheng Q. Label-free detection methods for protein microarrays. Proteomics 2006;6:5493. [PubMed: 16991201]
3. Cooper MA. Drug Discov. Today 2006;11:1068. [PubMed: 17129825]
4. Willets KA, Van Duyne RP. Annu. Rev. Phys. Chem 2007;58:267. [PubMed: 17067281]
5. Phillips KS, Cheng Q. Anal. Bioanal. Chem 2007;387:1831. [PubMed: 17203259]
6. Wegner GJ, Wark AW, Lee HJ, Codner E, Saeki T, Fang S, Corn RM. Anal. Chem 2004;76:5677. [PubMed: 15456285]
7. Lee HJ, Wark AW, Corn RM. Langmuir 2006;22:5241. [PubMed: 16732647]
8. Lee HJ, Nedelkov D, Corn RM. Anal. Chem 2006;78:6504. [PubMed: 16970327]
9. Lakowicz, JR. Principles of Fluorescence Spectroscopy. 3rd Edition. New York; Springer: 2006.
10. Taflove, A.; Hagness, SC. Computational Electrodynamics: The Finite-Difference Time-Domain Method. Artech House; Boston: 2000.
11. Sullivan, DM. Electromagnetic simulation using the FDTD method. IEEE Press; New York: 2000.
12. Yang P, Liou NK. J. Opt. Soc. Am. A 1996;13:2072.
13. Gray SK, Kupka T. Phy. Rev. B 2003;68:045415.
14. Chang SH, Gray SK, Schatz GC. Opt. Exp 2005;13:3150.
15. Taflove A, Brodwin ME. IEEE Trans. Microwave Theory and Techniques 1975;23:623.
16. Reference Guide for FDTD Solutions™ Release 5.0. 2007. <http://www.lumerical.com/fdtd>
17. Chowdhury MH, Gray SK, Pond J, Geddes CD, Aslan K, Lakowicz JR. J. Opt. Soc. Am. B 2007;24:2259.
18. Chowdhury MH, Pond J, Gray SK, Lakowicz JR. J. Phys. Chem. C 2008;112:11236.

19. Ray K, Badugu R, Lakowicz JR. J. Phys. Chem. B 2006;110:13499. [PubMed: 16821876]
20. Ray K, Badugu R, Lakowicz JR. J. Phys. Chem. C 2007;111:7091.
21. Ray K, Badugu R, Lakowicz JR. Chem. Mat 2007;19:5902.
22. Kaminski F, Sandoghdar V, Agio MJ. Comp. and Theor. Nanoscience 2007;4:635.
23. Jackson, JD. Classical Electrodynamics. 2nd Ed.. John Wiley & Sons; New York: 1975.
24. Lakowicz JR, Ray K, Chowdhury M, Szmackinski H, Fu Y, Zhang J, Nowaczyk K. Analyst. 2008DOI: 10.1039/b802918k
25. Ray K, Chowdhury M, Lakowicz JR. Anal. Chem 2007;79:6480. [PubMed: 17685553]
26. Lakowicz JR. Anal. Biochem 2001;298:1–24. [PubMed: 11673890]
27. Lakowicz JR. Anal. Biochem 2005;337:171–194. [PubMed: 15691498]
28. Ray K, Badugu R, Lakowicz JR. J. Am. Chem. Soc 2006;128:8998. [PubMed: 16834349]
29. Shadowitz, A. The Electromagnetic Field. Dover; New York: 1988.

Figure 1. Chemical structures of N-acetyl-L-tryptophanamide and N-acetyl-L-tyrosinamide (NATA-tyr). Schematic of the sample geometry (top). FE-SEM image (middle) and absorption spectrum (bottom) of silver island films (SIFs) on quartz.

Figure 2.
(top) Emission spectra of 15 nm PVA film containing NATA on quartz and SIFs. (bottom) Intensity decays of 15 nm PVA film containing NATA on quartz and SIFs.

Figure 3. (top) Emission spectra of 15 nm PVA film containing NATA-tyr on quartz and SIFs. (bottom) Intensity decays of 15 nm PVA film containing NATA on quartz and SIFs.

Figure 4.
Photostability of NATA on quartz and SIFs.

Figure 5.

Radiative power spectrum of 100nm diameter silver nanoparticle separated 8 nm from a radiating dipole calculated by FDTD method. Emission spectra of NATA and NATA-tyr on SIFs are also included.

Figure 6.

(a) Schematic diagram of the model radiating fluorophore/metal nanoparticle system studied using FDTD; Near-field intensity distribution around (b) a 100 nm diameter silver nanoparticle separated 8 nm from a probe radiating at 350 nm and oriented along the x-axis, (c) near-field intensity distribution around the isolated probe and (d) near-field enhancement around the silver nanoparticle. Note all images are displayed in the log scale.

Figure 7.

(a) Schematic diagram of the model radiating fluorophore/metal nanoparticle system studied using FDTD; Near-field intensity distribution around (b) a 100 nm diameter silver nanoparticle separated 8 nm from a probe radiating at 305 nm and oriented along the x-axis, (c) near-field intensity distribution around the isolated probe and (d) near-field enhancement around the silver nanoparticle. Note all images are displayed in the log scale.