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Largely Enhanced Single-molecule Fluorescence in Plasmonic Nanogaps formed by Hybrid Silver Nanostructures

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Abstract

It has been suggested that narrow gaps between metallic nanostructures can be practical for producing large field enhancement. We design a hybrid silver nanostructure geometry in which fluorescent emitters are sandwiched between silver nanoparticles and silver island film (SIF). A desired number of polyelectrolyte layers are deposited on the SIF surface before the self-assembly of a second silver nanoparticle layer. Layer-by-layer configuration provides a well-defined dye position. It allows us to study the photophysical behaviors of fluorophores in the resulting gap at the single molecule level. The enhancement factor of a fluorophore located in the gap is much higher than those on silver surfaces alone and on glass. These effects may be used for increased detectability of single molecules bound to surfaces which contain metallic structures for either biophysical studies or high sensitivity assays.

Introduction

Fluorescence enhancement was first observed for fluorophores adsorbed directly onto silver island film (SIF)(1, 2). By placing a fluorophore near a metallic nanostructure, the spectral properties of the fluorophore can be altered, increasing the sensitivity through an increase of quantum yield and photostability of the fluorophore(3, 4). The obtained enhancement of the optical field at and near the metal/dielectric interface can be subsequently used to improve the S/N ratio for the analysis of bio-recognition and interfacial binding events in sensor formats(5-9). When considering the fluorescence detection of molecules near thin metallic films, several factors need to be considered. The emission can be quenched due to radiation energy transfer to the metal as molecules adsorbed directly on the surface. Another consideration is the enhanced fluorescence, which can be understood classically in terms of localization of the optical field intensity, the local intensities can exceed the incident intensity by orders of magnitudes for specific geometries.

Plasmon excitations in metallic nanoparticles allow concentrating optical fields within subwavelength volumes⁽¹⁰⁻¹⁶⁾. In particular, the narrow gap between nanostructured metal surfaces, such as those formed in nanoparticles dimers, can lead to enormous field amplifications that find application in ultrasensitive bioassays⁽¹⁷⁻²⁵⁾. Nanometer-sized gaps provide very large electromagnetic field enhancements, double spheres, and sphere-on-plane geometries possess such gaps with dimensions down to nanometer scale⁽²⁶⁻²⁹⁾. The development of nanojunctions or nanogaps using same approaches developed from Surface enhanced Raman Spectroscopy (SERS) is very promising^(22, 27, 30, 31). Recent work has

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Supporting Information **Available**: TCSPC time decay curves (FigS1). This information is available free of charge via the Internet at <http://pubs.acs.org/>

established more accurate assessments of the electromagnetic fields within nanoscale junctions. A number of computational tools have been developed for modeling the electromagnetic properties of metallic nanostructures^(20, 32-40). Then SERS can be very sensitive tools of trace analysis and very useful in recording Raman spectra of extremely low concentrated biomolecules. The plasmon-enhanced Fluorescence (PCF) technique can become very useful in study of low fluorescent compounds. In contrary to SERS system, PCF has to be specially prepared to avoid quenching of fluorescence i.e. between the metal nanoparticles and fluorophores should exist few nanometers distance layer formed by dielectric or by adsorbed nonfluorescent macromolecules to avoid the fluorescence quenching.

From the pioneering experiments, it could be inferred that the junction between adjacent nanoparticles, occurring for pairs, larger clusters, or even aggregate films of nanoparticles, can give rise to highly intense and localized electromagnetic fields when excited by incident light. There have been tremendous efforts to design and optimize nanostructures in order to obtain the best efficiency to boost light-matter interactions. In spite of good understanding of the involved processes, only limited experimental progress was made with controlled metallic nanostructure fabrication and precise dye positions^(21, 41). In this work, we experimentally introduced a sequential bottom-up sandwiched assembly which appears promising for this purpose. Silver is the most commonly used metal since its plasmon resonance are typically in the visible region. Briefly, in our approach, silver nanoparticles were immobilized onto the silver island films via the use of layer-by-layer (LbL) scheme in which the polyelectrolyte (PE) polymer acts as a coupling agent and a spacer between the particle and the metal film substrate, the fluorophore is sandwiched between the spacer during the spacer buildup. A versatile LbL self-assembly method for modifying nanostructures was adopted via the sequential deposition of polyelectrolytes. Silver nanoparticles carrying negative charges on the surface were deposited on positively charged polyelectrolyte layer. Polyelectrolyte layers offer the necessary affinity to binding to the silver substrate and the silver particle on top, also serves as both a spacer and binding sites for charged fluorophores while being optically inactive⁽⁴²⁾. The fluorophore in the layers can be accordingly placed with nanometer precision in the gap between the metallic structures. Nanogaps between the metal film and metal particles were formed, which were known as “hot spots”, strong local electromagnetic field located at the gaps will be expected,. The gap plasmons obtained by bringing a nanoparticles closed to a metal surface have been used to produced controlled enhancement in surface-enhanced scattering thus enabling single-molecule detection. The field amplification level of “hot spots” in the fabricated nanoscale gaps could be monitored by a single fluorophore probe existed in these fields. As single fluorescent molecules are sensitive to their surrounding environments, profiling individual molecules and probing single molecules including fluorescence intensity time traces, spectra and fluorescence lifetimes can provide unique information about the chemical and physical characteristics of the surrounding nano-environment.

Experimental Section

1. Nanogap Assembly

The layer-by-layer (LbL) deposition of PE film is based on the electrostatic interaction between two types of polyelectrolytes, The fluorescing dye was electrostatically deposited on a negatively charged layer of poly(sodium styrene sulfonate) (PSS). Polydiallyl Dimethyl Ammonium Chloride (PDADMAC, MW: 200,000) was used as positively charged polyelectrolyte, poly(sodium styrene sulfonate) (PSS, MW: 70,000) was used as a negatively charged polyelectrolyte, both were purchased from Sigma and used as received. Cationic fluorophore, 1,1',3,3',3',3' - Hexamethylindodicarbocyanine iodide (DiIC1(5)) was obtained from Invitrogen, this ionic fluorophore species were electronically attached to the

PE layer. DiIC1(5) is a fluorescent lipophilic indocarbocyanine dye, with a 18 carbon-long alkyl hydrocarbon tail, such cationic cyanine dyes have been widely used in single molecule imaging. The approximate excitation and emission peaks of DiIC1(5) are 638 nm and 658 nm.

Silver island films were deposited on cleaned glass coverslip by reduction of silver nitrate as reported previously(4). The formed silver island films are greenish and nonreflective. Only one side of each slide was coated with SIF. The macroscopically smooth SIF consists of particles typically about 70 nm high, covering about 80% of the surface. Coverslips coated with silver island films were obtained using the method reported⁴. The freshly prepared silver island film surface was functionalized by 3-mercaptopropionic acid in 10 ml of water containing 0.05 g of MPA and 0.5 g of NaOH for 1 hour, providing negative charge on the surface due to deprotonated carboxylic group. After washing with water and dried in air, coverslips were sequentially coated with polyelectrolyte layers, briefly, one side of glass was dipped into PDADMAC solution (1mg/ml) for 20 min, rinse with water and dried, then dipped into PSS (1mg/mg) for 20 min and rinsed again. After this step, the slide was immersed into solution containing positively charged DiIC1(5) dye for 2 min, DiIC1(5) is positively charged in aqueous solution and was used in the layer growth process in a PDADMAC (positively charged) step. For single molecule experiment, trace quantity of DiIC1(5) at 10^{-8} - 10^{-9} M was used. The substrate was washed with copious of water, dried and immersed in PDADMAC solution to complete the growth of the positively charged layer. The coating procedures were then repeated by alternating immersion of the sample in the solution of PSS and PDADMAC solutions, respectively, finishing with PDADMAC, and leads to a positively charged surface (Scheme 1).

The negatively charged spherical silver nanoparticles with average particle size of 50 nm were prepared using citrate reducing method as described by Lee and Meisel(43). Briefly, 90 mg of AgNO_3 was dissolved in 500 ml H_2O and brought to a boil. A solution of 1% sodium citrate (10 ml) was added, and the solution was kept boiling for 1h. The resulting Ag nanoparticles were attached on the polyelectrolyte-coated surface by immersing the slides with a colloid solution with a 20-fold dilution for 1h.

2. Single Molecule Experiments

All single molecule studies were performed with a time-resolved confocal microscopy (MicroTime 200, PicoQuant). A single mode pulsed laser diode (635 nm, 100ps, 40 MHz) (PDL800, PicoQuant) was used as excitation light. The collimated laser beam was spectrally filtered by an excitation filter (D637/10, Chroma) before directing into an inverted microscope (Olympus, IX 71). An oil immersion objective (Olympus, 100 $\times$, 1.3NA) was used both for focusing laser light onto sample and collecting fluorescence emission from the sample. The fluorescence that passed a dichroic mirror (Q655LP, Chroma) was focused onto a 75 μm pinhole for spatial filtering to reject out-of-focus signals and then reached the single photon avalanche diode (SPAD). Images were recorded by raster scanning (in a bidirectional fashion) the sample over the focused spot of the incident laser with a pixel integration of 0.6 ms. The excitation power into the microscope was maintained at around 40 nW. Time-dependent fluorescence data were collected with a dwell time of 50 ms. The fluorescence lifetime of single molecules was measured by time-correlated single photon counting (TCSPC) with the TimeHarp 200 PCI-board (PicoQuant). The data was stored in the time-tagged-time-resolved (TTTR) mode, which allows recording every detected photon with its individual timing information. In combination with a pulsed diode laser, total Instrument Response Function (IRF) widths of about 400 ps FWHM can be obtained, which permits the recording of sub-nanosecond fluorescence lifetimes, extendable to less than 100 ps with deconvolution. All measurements were performed in a dark compartment at room

temperature. Fluorescence intensity decay curves were reconvoluted with the instrument response function and analyzed as a sum of exponential terms:

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

Where $I(t)$ is the fluorescence intensity at time t and α_i is a pre-exponential factor representing the fractional contribution to the time-resolved decay of the component with a lifetime τ_i . The contribution of each component to the steady-state intensity is given by

$$f_i = \frac{\alpha_i \tau_i}{\sum_j \alpha_j \tau_j}$$

The averaged lifetime is given by

$$\bar{\tau} = \sum f_i \tau_i$$

3. Spectroscopic Measurements

Absorption measurements were performed using a HP 8453 UV-Vis spectrophotometer. Ensemble emission spectra were collected using a Varian Cary fluorescence spectrophotometer by placing the films on a stationary stage (in front-face geometry) with 635 nm excitation from a xenon lamp. The concentration of the Cy5 sample in the solution was 100 nM.

Scanning electron microscopy (SEM) images were collected with a FEI Quanta 200 SEM instrument.

Results and Discussion

Absorbance and SEM measurement

The adsorption of silver nanoparticles over polyelectrolyte coated SIF film was monitored by UV-vis spectrometry and SEM (Figure 1). The as -prepared SIF film can be considered as a collection of elongated particles heterogeneous in size and shape of roughly 200×500 nm in average. The silver coverage factor in the imaging area is estimated to be about 82%, and such films can be regarded as macroscopically smooth surfaces. The silver plasmon absorption (extinction) spectra of as prepared SIF film typically show a plasmon absorption band around 420 nm and an extended tail towards long wavelength(44, 45). In the sphere over SIF configuration, the extinction spectra are dominated by two peaks. The slightly blue-shift in the plasmon absorption peak around 400 nm observed from sphere over SIF (SOS) configuration can be ascribed to the change in the local dielectric constant around SIF film as a result of adsorption of polyelectrolyte layers onto the SIF film. Additionally, the extinction spectrum shows a noticeable broadening plasmonic resonance band in the longer wavelength region around 600-750 nm, suggesting the dominant plasmon coupling between nanoparticles and the SIF after silver nanoparticle deposition.

Emission Spectra

As an initial experiment we examined the ensemble fluorescence emission spectra obtained on SIF substrate, SOS configuration, and the one on bare coverslips deposited with same PE layers, respectively. In such ensemble experiments, a relatively higher concentration of DiI solutions (10^{-7}M) was used in the immobilized procedures. The fluorescence spectra were collected at the same excitation and detection conditions to allow a direct comparison among different substrates. The immobilized DiI has an emission band maximum of $\sim 660\text{ nm}$ from all three substrates as illustrated in Figure 2. The fluorescence from the bare glass slide is quite weak, whereas the presence of silver nanostructures does improve the fluorescence intensity. We observe a slightly increase in the DiI intensity on surfaces of SIFs. The emission spectra clearly demonstrate that the intensity of the fluorophores immobilized between the Ag nanostructures is enhanced considerably, by a factor of 9, as compared to the spectrum measured for the DiI on a quartz glass. This is contributed to the enhancement of the local electromagnetic field owing to the plasmons excited in metal nanostructures. The effects of metals on fluorophores are due to through-space interaction occurring over distances from about 5 to 50 nm from the metal surface(4). Although electric field enhancements arising from the localized plasmons are very strong at the metal surfaces, the dye layer is only about 4- 5 nm away from the silver surface, it cannot be substantially enhanced by the SIF alone when in close proximity to metals. Additionally, the field enhancement has to take into account of the wavelength dependence of the fluorescence emission for a given plasmon mode, which is not fully understood yet. But it is believed that the maximal effect is expected when the emission peak overlaps closely with the plasmon resonance peak. When the laser line wavelength matches with the plasmon peak wavelength, a maximal local field enhancement and thus a relatively larger excitation enhancement will be achieved. As a result, we observed a rather higher enhancement with SOS configuration, in which the extinction spectra show a dominant plasmon mode around red region.

Single Molecule Experiments

Figure 3 displays typical $10\times 10\text{ }\mu\text{m}$ confocal fluorescence images of individual DiI fluorophores immobilized on glass, SIF and in the nanogaps, respectively. Each pixel has a 0.6 ms dwell time, and the total number of photons counted in that time is displayed in a colorized scale, ranging from dark to bright. The density of well-separated spots on the film increases as incubated concentrations of dye solutions increase, until the spotting saturation of the image is reached at a relatively high concentration, which provides strong proof that the spots are attributed to fluorescence from single DiI immobilized. The fluorescent spots observed from glass substrates show rather uniform brightness, also exhibit occasionally fluorescence intermittencies, indicating the presence of blinking, a typical fluorescent character from a single fluorophore. Obviously, silver nanostructures gave rise to stronger fluorescence enhancements. Furthermore, the brightness was enormously enhanced at the nanogap. Individual fluorescence images show considerable variations of the intensity from spot to spot within an image, especially for the fluorophore immobilized in the silver gap configuration as illustrated in Figure 3. The intensity variations shown initially indicate that dye molecules were located in rather heterogeneous environments in the presence of silver nanostructures compared to those on glass coverslips.

The fluorescence time traces shown in Figure 4 were recorded by focusing the laser focal volume over the bright spots as showed in Figure 3 for a certain period of time. Most of the time traces collected suffer clear one-step photobleaching as evidence by the discrete drop in fluorescence intensity to the background level, corresponding to the typical single-molecule behaviors. The time transients in Figure 4 reveal strikingly different magnitudes of the emission rate levels. The 3 kHz emission rate is the typical fluorescence count rate for a free DiI immobilized on glass coverslips in the absence of any silver nanostructures. On the

contrary, particularly higher emissions were always observed from SOS configuration relative to those on SIF and glass surfaces. The enhancements shown in Figure 4 are estimated to be around over 100-fold for SOS configuration, and 6-fold for SIF alone compared to that on glass surfaces. An examination of the statistical distribution of fluorescence intensity dependence of distance was carried out over a sample population of around 50 individual molecules. During the study, we have selected the molecules in as unbiased a fashion as possible. To be ensuring that each bright spot corresponds to a single molecule and not to a small cluster or to an impurity, all single spots were checked for single-step photobleaching to make certain that the collected emission was from a single molecule. The average emission rate of the population in the absence of metal nanostructures is about 5 kHz and is used as the reference to be compared with the emission rates of other populations in the presence of metal structures. Thus, the average enhancement is about 6-fold on SIF, and 60-fold for SOS, respectively. As statistically depicted in Figure 5, about 25% molecules collected show over 100-fold enhancement in emission, and the maximal of 500-fold is observed. There are in fact lower count rates observed on glass coverslips; most of molecules emit photons at a rate less than 5 kHz as compared to over a few hundred kHz emitted by the molecules existing in the “hot spot” regions. The distribution of fluorescence emission rates measured for DiI on glass substrate is much narrower than those measured with silver nanostructures. The values range from 10 to 90 kHz on SIF substrates as expected, which are similar to previous results(46), indicating the heterogeneity of the silver nanoislands. Remarkably, for DiI immobilized on SOP substrate, the magnitude of the intensity levels is dramatically variable as shown in Figure 5. The emission rates vary from 75 kHz to over 1000 kHz, a span of two orders of magnitudes. In such a configuration, the enhancement of the local electrical field intensity is realized through a spatial distribution of the optical electric field. It is most likely that heterogeneity is due to different molecules locating in various positions in the “hot spot” gap regions, as well as to possible deviation of the inhomogeneity of the Ag nanostructures in size and shape. The skewed brightness distribution is due to the likely existence of two or more different subpopulations of single-molecule species arising from diverse electromagnetic interactions. The low intensities observed in SOS case could also be ascribed to the immobilization of fluorophores in the “void” spaces on the glass substrate (the regions were not occupied by the silver nanoislands). The formation of nanogaps is random, only a fraction of nanosphere-nanoisland pairs was available to make the dominant contributions to strong plasmon field within hot spots. Nevertheless, the large enhancements demonstrate that gap plasmons existing in SOS configuration lead to enormous enhancement in the fluorescence intensity.

Fluorescence lifetime

Fluorescence enhancement is usually expressed in terms of the apparent quantum efficiency, is regarded as a product of terms responsible for enhancement of the absorption rate, enhancement of the emission rate, and change of the decay rate of the fluorescent molecule relative to the non-interacting molecule. Since the enhancement of fluorescence proximity to a metallic surface is well known now to be accompanied by a decrease in the lifetime of excited states, fluorescence lifetime measurements are a useful indicator of plasmon-enhanced fluorescence. The unenhanced molecule has low intrinsic fluorescence quantum efficiency because its intrinsic non-radiative decay rate dominates over the intrinsic radiative decay rate. The presence of silver nanostructures should enhance the quantum efficiency and produce a change in the total decay lifetime. The single molecule fluorescence lifetime measurement is implemented using time-correlated single photon counting by plotting a histogram of time lags between the excitation pulses and the detected fluorescence photons. The analysis of the fluorescence signal of DiI on glass (Panel a in Figure 6) yields an averaged lifetime $\tau = 2.68$ ns. In the contrast, we observed considerable decreases in

lifetimes of single DiI molecules in the presence of silver nanostructures. The decay curves depicted in Figure 6 exhibit reduced lifetimes of 0.98 ns for SIF and 0.31 ns for SOS, obtained by deconvolution with the instrument response emission, respectively. Clearly, the fluorescence decays are faster than that of the free probe on glass. The control sample displays single exponential decay while the decays of dye probes located on silver nanostructures display two clear exponential features. The slow and fast decay components are referred individually as t_s and t_f . These components had values of 2.13 ns (t_s) and 0.65 ns (t_f) for silver island film and 0.86 ns (t_s) and 0.23 ns (t_f) for the probe located in the gaps, respectively. The mean fluorescence lifetimes of DiI in the presence of silver nanostructures are decreased due to the increased radiative decay rates of the fluorophores near metallic nanostructures. The long lifetime component (2.13 ns) for SIF indicates that a fraction of the fluorophore was not interacting strongly with SIF. The fluorophore emission collected through the transparent glass substrate clearly exhibits significant lifetime changes with the presence of silver nanogaps. Both slow and fast components obtained from the decay curve with SOS show greatly reduced lifetimes, indicating the strong coupling effect between the molecule and metals, which also suggest that the molecule is located in the hot spot region inside the plasmon gap. The important effect of the shortened lifetime is a decrease in photobleaching, especially due to a reduced excited state lifetime, where the probe is less prone to photochemistry reactions. As expected, long-surviving fluorophores were observed in the presence of metallic nanostructures (Figure 4).

Generally, the field-enhancement regions are concentrated in close proximity to the surface of plasmonic metal nanostructures as a result of the strong coupling between local surface plasmons (LSPs) of the nanostructures during excitation. Fluorescence enhancement is found to be maximal with a separation of about 5-10 nm between metal nanostructures and molecular emitters. At shorter distances, strong dipole coupling between the molecular oscillator and the plasmonic resonance provides an efficient nonradiative energy transfer pathway from the molecular excited state to the metal that exceeds the molecular emission rate, and the maximum fluorescence enhancement may not be achieved. Consequently, we did not observe an impressive MEF effect with SIF alone in this experiment. When metallic nanostructures are separated by a small distance, the interaction can be viewed as hybridization between LSPs of the nanostructures to form gap plasmons. The gap designed in this experiment was able to achieve enormous enhancement in the electric field as proved by the greatly enhanced single molecule fluorescence observed. It allows a more efficient quantum yield of the fluorescence emission. We noticed that the fluorophores existing in the nanogaps have much faster decay time as well. The observations are in accordance with the previously reported MEF phenomenon, where the plasmon-fluorophore interactions result in an increase in the quantum yield and a decrease in the lifetime of fluorophore. In addition, both greatly decreased exponential decay components could attribute to the coupling effects between the fluorophore and metal nanostructures, which are dissimilar from those observed from SIF substrates. Single molecule brightness distribution reveals the existence of “hot regions” in the junctions between nanoparticles and the SIF substrate, where high electrical field and thus large enhancement occurs. The spatial distribution of the field near the nanoparticles can be taken into consideration for optimizing designs of biosensors utilizing plasmonic response from metallic nanoparticles.

Summary

During the process of noble metallic nanostructures-enhanced fluorescence, plasmons can be induced in metal nanostructures by coupling to incident photons and contributing to the oscillating electric fields, which interact with nearby fluorescent emitters. It has been suggested that narrow gaps between metallic nanostructures such as nanoparticles, pointed corners, edges and tips of metal nanostructures, can be practical for producing large field

enhancement. The effects of metals on fluorescence have been subject to prior theoretical reports, but the practical usefulness of these effects was not explicitly explored. Although individual nanostructures have many fascinating plasmonic properties, controlling their assembly into dimers and larger structures and thus manipulating the collective optical properties has also become an attractive approach to plasmonic engineering. Even the simplest assembly features of nanostructures such as dimer can have novel plasmonic features due to a strong surface plasmon coupling between the nanostructures(47). The gaps or void spaces between metallic nanostructures can concentrate light to exceedingly small volumes and thus create huge near-field enhancements. Experimentally controlling the gaps between nanostructures where the fluorophore molecules are sandwiched between them has been rarely reported(21, 48). We designed a gap of approximate 10nm between the silver nanostructures to achieve strong fluorescence enhancement and provide immobilization sites for housing of probes of interest in hot spot regions. The nanogap was formed by a macroscopically smooth silver substrate and a spherical Nanosphere; single fluorescent probes were used to characterize the plasmon response at the nanogap. To the best of our knowledge, this work constitutes the first experimental single-molecule study of gap plasmon enhanced fluorescence. The single-molecule approach directly enables us to probe the local field enhancement and map the distribution properties in gap. Another significant features of electrostatically driven LBL self-assembly used in our approach is that one can controllably deposit uniform layers of polyelectrolytes with nanometer-thick precision. This strategy has been widely used to coat particles of varying sizes and shapes, since polyelectrolytes adsorb onto a large variety of surfaces and can be modified or performed to introduce a wide variety of functional groups, the LBL approach provides a general route to tailor the surface characteristics of nanoparticles. Briefly, the LbL scheme allows a precise arrangement of nanogap at a defined interparticle distance, an immobilization site for objects of interest in the plasmonic hotspot between metal nanostructures, as well as specific attachment site for surface immobilization. It allows us to study the photophysical behaviors of the dye in the resulting gap at the single molecule level. The results shown provide some promising starting points for further experimental and theoretical investigation. We further note that the work presented here represents only a limited optimization; we anticipate that the use of other active substrates, possibly with defined features of nanofabricated metallic substrates for tunability, will produce controllable enhancements. Controlled assembly technique to direct individual nanoparticles onto predefined surface sites would enable the assembly of nanostructures with unique optical and photovoltaic properties. The geometry holds the promise for implementing highly integrated nanoscale optical sources.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1. (Left) Extinction spectra of a glass coverslip coated with as-prepared silver island film (SIF) and with SOS assembly; (right) SEM images show the surface features of SIF and SOS nanostructures, respectively.

Figure 2. Ensemble fluorescence Emission spectra of DiIC1(5) glass, SIF and sphere-over-SIF (SOS) substrates. Emission spectra were collected in a front-face geometry with 635 nm excitation from a xenon lamp.

Figure 3.

Confocal fluorescence images of $10 \times 10 \mu\text{m}^2$ sample regions recorded for DiI immobilized on glass (a), SIF (b), and SOS (c) at the subnanomolar concentration. Images were recorded by raster scanning the sample over a confocal microscopy (MicroTime 200, PicoQuant). A single mode pulsed laser (635 nm, 100 ps, 40 MHz) (PDL800, PicoQuant) was used as the excitation light. An oil immersion objective (Olympus, 100 $\times$, 1.3 NA) was used.

Figure 4.

Representative fluorescence time trajectories recorded for DiI immobilized on a glass coverslip (green), SIF (blue), and SOS (red) substrates. The excitation power into the microscope was maintained around 40nW.

Figure 5. Histograms of brightness from DiI molecules on glass (gray), SIF (dark gray) and SOS (black) substrates. The Gaussian distributions were represented with red lines.

Figure 6.

Typical normalized TCSPC intensity decay curves of single DiI molecules immobilized on glass (a), SIF (b), and SOS (c). The mean lifetimes were obtained by exponential fitting with the curves. τ_{glass} : 2.68 ns; τ_{SIF} : 0.98 ns (t_s : 2.13 ns, t_f : 0.65 ns); τ_{SOS} : 0.32 ns (t_s : 0.86 ns, t_f : 0.23 ns;). (original decay curves with longer time scales are presented in Figure S1)

Scheme 1.

Molecular structure of DiIC1(5) (left) and the layer-by-layer assembly of nanogap formed between silver nanoparticles and silver island films (right).