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Large enhancement of single molecule fluorescence by coupling to hollow silver nanoshells†

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Abstract

We investigated the effect of plasmon-enhanced fluorescence using hollow silver nanoshells and observed enhancement of a single probe's fluorescence up to a factor of 3 orders of magnitude. The enhancement of the quantum efficiency is attributed to enhanced absorption and increased radiative decay rate.

Fluorescence has broad and significant application in biology, medical imaging, and chemical sensing. The optical properties of fluorophores can be significantly modified by plasmons in adjacent metallic nanostructures.^{1–10} On one hand, the excitation rate of the fluorophore can be altered by a plasmon-induced local electric field. On the other hand, the radiative decay rate of fluorophores can be modified by the near-field fluorophore–metal interactions. The interaction strength depends on several parameters including the geometry, size and composition of the metallic nanostructures, the distance and spectra overlap between the fluorophore's emission or absorption and the metal's plasmonic resonances. The overall interaction can lead to a strong fluorescence enhancement or to a quenching of emission, depending on the specific fluorophore–metal system.

Enhancement of molecular fluorescence is of great interest due to the widespread attractiveness of molecular-fluorescence based measurements and devices. The detection sensitivity of applications such as DNA sequencing, single molecule detection, and cell imaging all rely directly on the intrinsic brightness of the probes. The ability to enhance the emission of fluorescence used in the applications would greatly increase the sensitivity of fluorescence-based techniques. Some extremely large enhancements have been reported only at a few specific configurations required for delicate lithography techniques.⁴ It is significant to scientific applications to increase the enhancement using relatively simplified and more flexible approaches. Recent advancements in the chemical synthesis of metal nanostructures have led to a rise of various shapes such as rods, shells, rings, disks and cubes.^{2,8,11–14} These developments, in addition to lithographic fabrication methods, have provided the tools for realizing experimental studies of plasmon properties of metallic nanostructures.

In this communication, we are considering spherical hollow silver shell nanoparticles with a fluorophore positioned at a distance away from the nanoshell outer surface. The sphere and

†Electronic supplementary information (ESI) available: The experimental details of the preparation of hollow silver nanoshells, DNA tethering procedures, single molecule experiments, additional confocal images, TEM and SEM images of hollow silver nanoshells, and extinction spectra of silver nanoshells.

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nanocavity plasmons are electromagnetic excitons that induce surface plasmons at the inner and outer interfaces of the metal shell. Because of the limited thickness of the shell layer, the sphere and cavity plasmons interact with each other.^{15–17} The hybridization of the plasmons supported by the nanoscale spheres and cavities in the surrounding media leads to large local field enhancement,¹⁷ which can be utilized for various kinds of optoelectronic applications. Smaller values of the dielectric core (*i.e.* high electrical insulation and optical transparency) are more favorable for metal nanoshells to enhance fields. The choice of silver hollow shell particles is based on the well-established synthesis of nanoshells.^{11,13,18}

Silver nanoshells were synthesized on the basis of the nanoshell fabrication chemistry previously reported.^{9,11,18–20} Well-dispersed spherical SiO₂@Ag core–shell nanocomposites were obtained using methods reported¹⁸ previously (ESI[†]). Briefly, silica nanoparticle cores (~20 nm in radius) were synthesized using the slightly modified Stöber methods. Onto these silica nanoparticles, silver shells were coated to form silica core–Ag shell nanoparticles. The etching of SiO₂ was carried out using 4% HF for overnight. The shell thickness of the Ag layer of about 10 nm was estimated based on the size subtraction (ESI[†]). The positioning of single probes was achieved using a DNA oligomer spacer. A double-stranded DNA (dsDNA, 23 bps, ~8 nm in length) oligomer is formed by hybridization with the complementary sequence, being tagged with a single Cy5 probe, and tethered to the silver nanoshell monolayer deposited on amino-coated glass slides as illustrated in Scheme 1. The SEM image confirms the formation of the nanoshell monolayer assembly on the coverslips (ESI[†]).

The free amino groups remaining on the quartz surface were blocked with succinic anhydride. A comparison of two confocal scanning images in Fig. 1a shows qualitative brightness information from single fluorophores deposited on glass and tethered to hollow silver nanoshells. A number of well-separated round spots with diffraction-limited sizes of ~400 nm were observed in individual images. A low number of fluorescence events in such sample regions were observed as expected due to the extremely low incubation concentration of fluorophores and repeated immobilization and washing steps, which could lead to the loss of loosely bound ligands (additional images are presented in ESI[†]). Nevertheless, the significant differences in brightness of emission spots indicate greatly enhanced fluorescence emission in the presence of silver nanoshells. Fluorescence as a function of time is shown in Fig. 1b. Under the same incident excitation power, most of the recorded time trajectories from DNA molecules deposited on glass coverslips illustrate discrete blinking and can be attributed to single molecule's heterogeneous dynamics. Basically many of the observed fluorescent molecules suffered characteristic irreversible one-step photobleaching after exposure to the excitation laser for a period of time. The randomly sampled single molecules in the presence of hollow nanoshells show much higher emission rates, additionally, the emission rate is fairly constant until it drops abruptly to the background level in a single step. Examinations of the statistical distribution of fluorescence brightness were summed up over a sample population of approximately 60 individual molecules in the absence and in the presence of metal nanoshells, respectively (Fig. 2). In order to achieve the desired number of single molecules, many images similar to those in Fig. 1 were recorded. Each spot was observed until single-step photobleaching occurred to ensure that it corresponded to a single molecule. An individual spot has a different brightness as the emitter's dipole moment is randomly oriented with respect to the excitation field. It is obvious from the histogram that the probes on glass are prone to rather weak emission with the brightness less than 2 kHz, while signals in the presence of silver

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nanoshells yield a mean value near 300 kHz. The approximately 300-fold enhancement in the brightness suggests a possible increase in the fluorescence decay rate and also an improvement in photostability as frequently evidenced by the reduced blinking events. The brightness distribution from molecules on silver nanoshells also indicates that there are a number of molecules showing brightness of more than 1000 kHz, which is approximately over 1000-fold enhancement compared to the average emission rate from the single molecules in the absence of silver nanoshells. It is highly likely that the molecule is located near the position of maximum field enhancement, although the exact position and orientation of the molecule is not known. It implies that the estimated enhancement could be up to 3 orders of magnitude.

Since the enhancement of fluorescence in 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 un-enhanced 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 nanoshells could 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 (TCSPC) by plotting a histogram of time lags between the excitation pulses and the detected fluorescence photons. Fig. 3 presents time decay curves from a single molecule on the nanoshells (black) as well as from one on the bare glass (gray). The reference fluorophore showed a mono-exponential decay and yielded an average lifetime of 2.1 ns. In the presence of silver nanoshells, the single molecule exhibited 2-component exponential decays. Clearly, the fluorescence decay of fluorophores in proximity to nanoshells is much faster than that of the free probes on glass. The intensity decay is dominated by a short decay time of around 200 ps. The nearly symmetric lifetime distribution in the absence of nanoshells suggests that most of the molecules deposited on glass are in a relatively homogeneous environment (panel d in Fig. 3). The breadth of the lifetime distribution in the presence of silver nanoshells indicated the presence of a mixture of nanoscale environments in the sample, which could arise from metal nanoshells with various sizes and surface features. In addition, the decay time of individual dye molecules depends on the orientation/position of the dipole moment of the probe molecule relative to the metal.

In summary, metallic nanoparticles can affect the fluorescent emission of nearby molecules in several ways: by enhancing the optical intensity incident on the molecule through near-field interaction, by modifying the radiative decay rate of the molecule, and by increasing the coupling efficiency of the fluorescence emission to the far field emission. Our observations are in accordance with the previously reported metal-enhanced fluorescence phenomenon, where the plasmon-fluorophore interactions result in an increase in the emission rate and a decrease in the lifetime of fluorophore. The effects of metallic nanostructured surfaces on the spectral properties of single fluorophores have been demonstrated through the alternation of the local electromagnetic field. Metallic nanostructures affect both absorption and emission. The large fluorescence enhancement could be attributed to the hybridization plasmon effect of nanocavity and nanoparticles. Large field enhancement for Ag nanoshells has been conceptually reported for the combination of metal shells and dielectric cores, it has also been found that smallest dielectric constants of core media lead to largest field enhancement more than three orders of magnitude.¹⁷ A reduced lifetime enables the emitter to cycle faster, as the intrinsic lifetime determines its cycle rate. Fluorophores will be less prone to optical saturation and can emit more photons per second than equivalent fluorophores with a longer lifetime. A 1000-fold increase in intensity coupled with a 10-fold reduction in the emitter's lifetime provides for an approximately 4-order of magnitude potential increase in terms of

detectability. However, the plasmon-enhanced field of a hollow nanoshell is a sensitive function of the inner and outer radius of the metallic shell, the strength of the interaction between the sphere and cavity plasmon is directly controlled by the thickness of the metal shell layer. Further investigation will open up opportunities for significant and extensive applications of the nanoshell plasmonic nanostructures in imaging and fields.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.

(a) Confocal fluorescence images of $10 \times 10 \mu\text{m}^2$ sample regions recorded for Cy5 immobilized on a bare glass coverslip (left) and on silver nanoshell assembly (right). (b) Representative time trajectories of single fluorophores on bare glass (left) and immobilized on silver hollow nanoshells (right), respectively. A 635 nm diode laser was used to excite fluorescence in a stage-scanning confocal microscope setup (Picoquant MicroTime 200) using incident intensities ($\sim 40 \text{ nW}$) throughout the experiments.

Fig. 2. Histograms of brightness of single fluorophores on bare glass (left) and near silver hollow nanoshells (right). The histograms were constructed from more than 60 single molecules, respectively.

Fig. 3. Typical TCSPC decay curves of single molecules near silver hollow nanoshells (a) and on bare glass coverslips (b). Averaged lifetime histograms of single molecules near silver hollow nanoshells (c) and on bare glass coverslips (d). The histograms were constructed from more than 60 single molecules, respectively.

Scheme 1.

Schematic of tethering a single fluorophore tagged DNA oligomer to hollow silver nanoshell assembly deposited on an aminocoated glass coverslip.