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J. Grzelak, A. Krajewska, B. Krajnik, D. Jamiola, J. Choma, B. Jankiewicz, D. Piątkowski, P. Nyga, S. Mackowski*

Hybrid silica-gold core-shell nanoparticles for fluorescence enhancement

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Abstract: We demonstrate that SiO_2 nanoparticles coated with a gold island film (GIF) provide an efficient plasmonic platform for enhancing fluorescence intensity of chlorophyll-containing photosynthetic complexes. Fluorescence images obtained for single SiO_2-Au core-shell nanoparticles mixed with photosynthetic complexes reveal very uniform emission patterns of a circular shape, similarly as observed for bare SiO_2 nanoparticles. The fluorescence enhancement of chlorophyll emission for SiO_2-Au nanostructures is up to four-fold compared to bare SiO_2 nanoparticles and shortening of fluorescence decay indicates its plasmonic origin. For doublets or triplets of core-shell SiO_2-Au nanoparticles, the intensity of emission is further increased as a result of hot-spot formation at the interfaces of such assemblies.

Keywords: core-shell nanoparticles, plasmonics, fluorescence enhancement, hot-spot formation.

1 Introduction

Devising plasmonic substrates for applications in fluorescence- or Raman-based sensing has become one of the key aspects of current research. The inclusion of plasmon interactions to architectures based on biophysical or biochemical recognition [1], may frequently result in significant increase of detected signal intensity, improving sensitivity of the technique. Alternative – more sophisticated – approaches focus on inducing and observing minute changes of local dielectric properties by monitoring scattering of individual metallic nanoparticles [2]. Typical configurations consist in this case of nanostructured metal, either in the form of nanoparticles or corrugated films and emitters that are either specifically attached to the metallic nanostructures or deposited directly thereon. When properly designed, metal–enhanced fluorescence (MEF) can occur in such structures due to interactions between dipole moments of fluorophores and plasmonic excitations in metallic nanostructures [3]. Besides matching the spectral properties of emitters and metallic nanostructures, it is necessary to avoid the quenching of the emission via non-radiative energy transfer from the emitter to the metallic nanostructure, which can be achieved by applying dielectric shells or spacers [4-6].

The concept of coupling fluorescent molecules with metallic nanoparticles has been extended to other nanostructures, such as conjugated polymers, semiconductor nanocrystals, and biologically functional complexes [7]. Observation of strong increase of fluorescence intensity for all these diverse emitters points toward universality of applying metallic, plasmonically active substrates as sensing platforms. From substantial experimental and theoretical data it is obvious that the fluorescence enhancement depends on both the optical properties of metallic nanoparticles defined through particular material selection or morphology, and separation between metallic nanoparticles and emitters, including complex biomolecules. This superb sensitivity renders plasmonic nanostructures as efficient and flexible materials for sensoric applications.

On the other hand, dielectric nanoparticles have also been studied in the context of modifying the optical properties [8-10] or improving collection efficiency in the fluorescence imaging experiment [8]. Such structures are

***Corresponding author S. Mackowski,** Institute of Physics, Faculty of Physics, Astronomy and Informatics, Nicolaus Copernicus University, Grudziadzka, 5, 87-100 Torun, Poland, E-mail: mackowski@fizyka.umk.pl
J. Grzelak, B. Krajnik, D. Piątkowski, Institute of Physics, Faculty of Physics, Astronomy and Informatics, Nicolaus Copernicus University, Grudziadzka, 5, 87-100 Torun, Poland
A. Krajewska, B. Jankiewicz, P. Nyga, Institute of Optoelectronics, Military University of Technology, Warsaw, Poland
D. Jamiola, J. Choma, Institute of Chemistry, Military University of Technology, Warsaw, Poland

characterized by strong magnetic resonances and can be used for controlling the emission of molecules that feature not only magnetic but also electric dipole moment [11]. Application of such nanoparticles has been discussed in the light of enhancing optical response in the infrared as well as in the visible spectral ranges. It has been shown that placing a single molecule onto a surface of a dielectric nanosphere can yield 2- to 3-fold enhancement of the fluorescence intensity [8]. This effect was attributed to strong confinement of the electromagnetic field near the nanosphere. In addition, it has been shown that such nanoparticles can be coated with metallic islands for enhanced Raman scattering [12]. Recently we have shown that SiO_2 spherical nanoparticles when mixed with chlorophyll- containing biomolecules [10], can act as sources of local collection efficiency improvement.

In this work we expand the concept of fabricating hybrid nanostructures based on photosynthetic complex and dielectric nanoparticles by introducing a shell of plasmonically active structures at the surface of dielectric, silica nanoparticles. It is achieved by coating SiO_2 nanoparticles with a gold island film (GIF). For a structure where photosynthetic complexes are deposited on such SiO_2-Au core-shell nanostructures we find large MEF effect with overall enhancements larger than 10-fold. Plasmonic origin of this fluorescence enhancement is demonstrated by shortening of the fluorescence lifetime. The results, combined with large surface of the nanoparticles and their nanometric sizes, render SiO_2-Au core-shell nanostructures as attractive plasmonically active building blocks for optoelectronic and sensing applications.

2 Materials and Methods

Peridinin-chlorophyll-protein (PCP) photosynthetic complexes were obtained according to protocol developed by Miller et al. [13,14]. Briefly, PCP apoprotein in 50 mM Tris-HCl pH 8.0 solution was added to 25 mM Tricine and 10 mM KCl (pH 7.6), mixed with a stoichiometric amount of PCP pigments dissolved in ethanol. The sample was hold in 4°C for 72 h. Reconstituted samples were equilibrated to 5 mM Tricine with 2 mM KCl (pH 7.6) and next removed with 5 mM Tricine with 2 mM KCl (pH 7.6) containing 0.06 M NaCl. The protein solution was characterized using absorption and fluorescence spectroscopy.

The silica–gold core–shell particles were synthesized in the way described in [12]. Shortly, 600 nm diameter silica particles (POCH, Poland) were functionalized with amine groups using 3-aminopropyl-trimethoxysilane (APTMS) [15]. Gold shell was fabricated on the functionalized silica particles through direct reduction of tetrachloroauric acid using formaldehyde as reducing agent. The core-shell particles were stabilized with *polyvinylpyrrolidone* (PVP). Morphology of SiO_2-Au core-shell nanostructures was studied using LEO 1530 (Zeiss) scanning electron microscope (SEM).

The samples for fluorescence imaging and spectroscopy were prepared by spin-coating the solution of SiO_2-Au core-shell nanostructures onto a clean microscope coverslip. The concentration of the sample was adjusted in order to obtain images, where the nanostructures were separated from each other and did not form extended aggregates. Next, a solution of the PCP complexes was deposited on the nanostructures. Alternative approach of mixing both solutions prior the spin-coating was used and the results were qualitatively the same.

Absorbance spectra were recorded on a Varian-Cary 50 UV-visible spectrophotometer. Steady-state fluorescence measurements were performed using the FluoroLog 3 spectrofluorometer (Jobin Yvon) equipped with a Xenon lamp for excitation and a photomultiplier for detection.

Fluorescence intensity maps of layered samples were measured with a Nikon Eclipse Ti inverted wide-field microscope equipped with Andor iXon Du-888 EMCCD detector. The excitation was provided by a LED illuminator with a central wavelength of 480 nm and illumination power of 60 μW. The beam was reflected with a dichroic beam splitter (Chroma 505DCXR) to the microscope objective (Plan Apo, 100x, NA=1.4, oil immersion, Nikon). Fluorescence of PCP was extracted with a band-pass filter (Chroma HQ675-20). The spatial resolution of the microscopy imaging system is about 300 nm, therefore it is less than the diameter of SiO_2-Au core-shell nanostructures used in the experiment.

Fluorescence spectra and decays were measured using our home – built scanning confocal fluorescence microscope based on the Olympus long working distance microscope objective LMPlan 50x, NA 0.5 [16]. The sample was excited at a repetition rate of 50 MHz with a picosecond pulsed laser at 480 nm (excitation power of 60 μW). This wavelength corresponds to the maximum of PCP absorption and also matches the high energy tail of the absorption band of the SiO_2-Au core-shell nanostructures. The spectra were measured by dispersing the emission using an Amici prism and detecting the spectrum with a CCD detector (Andor iDus DV 420A-BV). Fluorescence decays were obtained using time-correlated single photon counting technique (SP-150, Becker & Hickl), with fast avalanche photodiode as the detector with the emission of PCP complexes extracted with a band-pass filter HQ675-20. The experimental sequence was as follows: First, by

raster-canning the sample, we determined positions of the SiO_2-Au core-shell nanostructures on the surface, and then series of fluorescence spectra and decays were acquired for these locations. For the reference, we also measured similar sets of data for PCP complexes off the nanostructures.

3 Results and discussion

Figure 1a shows the SEM image of the SiO_2 nanoparticles with a nominal diameter of 600 nm coated with Au nanoislands. The sample is quite homogeneous, with only few nanoparticles featuring different sizes. The structural and optical properties of bare SiO_2 nanoparticles were discussed previously [10]. As can be seen in Fig. 1a, the coverage of the SiO_2 surface with Au islands is very uniform, and the lateral size of these metallic nanoparticles ranges from 50 to 100 nm. The extinction spectrum of SiO_2-Au core-shell nanostructures is shown in Fig. 1b and it is compared with the absorption of the PCP solution. The characteristic feature of the SiO_2-Au core-shell nanostructures is strong absorption around 550 nm which we attribute to plasmon excitations in the Au islands on the surface of SiO_2. The major absorption of the PCP complexes band spans from 400 nm to 550 nm and is attributed predominantly to the absorption of peridinins [17]. The chlorophylls in PCP absorb around 440 nm and 660 nm. The emission spectrum of the PCP complexes features a single band at 670 nm [17], which is due to excited state recombination of chlorophylls. The comparison shows that the absorption and emission of the PCP complexes overlap to some degree with the plasmon resonance of the SiO_2-Au core-shell nanostructures.

The method used for sample preparation is relatively simple and it results with the PCP complexes being either very close to the nanostructures or completely away. In this way we can determine the fluorescence intensity for both sets of the PCP complexes in the same sample. Typical fluorescence image of the PCP complexes mixed with SiO_2-Au core-shell nanostructures with a diameter of 600 nm is shown in Fig. 2. The 90x90 mm image obtained using wide-field microscopy technique features many almost identical ring-shaped structures, with only a few exceptions. Such a high uniformity indicates – in accord with the structural data – high homogeneity of the Au/SiO_2 nanoparticles used for preparing the hybrid nanostructure. Many of the nanostructures are connected together, however, uniform intensities of the PCP emission suggest that they form a sub-monolayer on the coverslip surface. The observed rings are due to the PCP complexes that are close to the SiO_2-Au core-shell nanostructures. The emission from such complexes exhibit considerably higher intensity as compared to the PCP complexes that are far away from the nanoparticles. As shown for bare SiO_2 nanoparticles [10], such an increase of the fluorescence intensity may be due to enhanced collection efficiency due to higher refractive index of the silica sphere. Therefore, at this point we cannot attribute this enhanced emission to the plasmonic interaction between GIF and PCP complexes. However, as shown in the inset to Fig. 2 when two or more nanoparticles aggregate, the intensity of fluorescence between them is considerably higher as on isolated nanoparticles.

In order to demonstrate the difference in fluorescence intensity between doublets of bare and Au-coated SiO_2 nanoparticles, we plot cross sections of the PCP emission for single nanoparticles and for doublets, as shown in Fig. 3. In the case of bare SiO_2 nanoparticles the intensities measured at the edges of the nanoparticle as well as in

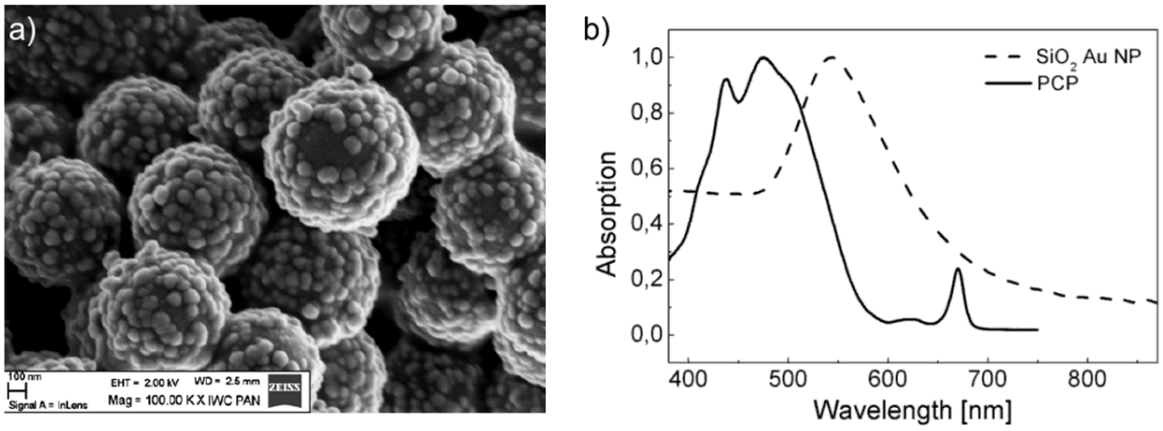

Figure 1. (a) Scanning electron microscopy image of the silica nanoparticles coated with nanostructured Au film. (b) Absorbance spectra of SiO_2-Au core-shell nanostructures (dashed line) and PCP complexes (solid line).

the middle between the two nanoparticles are essentially identical, pointing towards purely optical origin of the observed enhancement of the emission. In a clear contrast, for SiO_2-Au core-shell nanostructures two effects can be observed. On the one hand, the fluorescence intensity of the PCP complexes at the edges of SiO_2-Au core-shell nanostructures is considerably higher than for bare silica nanoparticles. On average this increase reaches a factor of 3. Furthermore, for the PCP complexes placed in between the SiO_2-Au core-shell nanostructures the intensity of the emission is further increased, presumably as a result of a hot-spot formation at the interface between the two nanoparticles. This qualitative observation strongly suggests that coating SiO_2 nanoparticles with nanostructured Au layer strongly enhances the emission of the PCP complexes placed in the vicinity of the nanoparticles and that this effect presumably is of plasmonic origin, similarly as in the case of hot-spots between two spherical or elongated nanoparticles [18].

An important assumption behind the prior discussion concerns assigning the emission observed using a wide-field microscope indeed to the PCP complexes. This can be verified using confocal fluorescence spectroscopy. However, since the experimental setup used for confocal imaging has lower resolution than the wide-field microscope, of about 1.2 microns, collected images contain bright spots superimposed on otherwise uniform background. In other words we had no possibility to observe ring-like structures similar to the ones shown in Fig. 2. We attribute the spots to the emission of the PCP complexes close to either SiO_2 and SiO_2-Au core-shell nanostructures, while the background originates from the PCP complexes placed far away from the nanoparticles. Nevertheless, by locating a laser spot on a particular place on a sample we

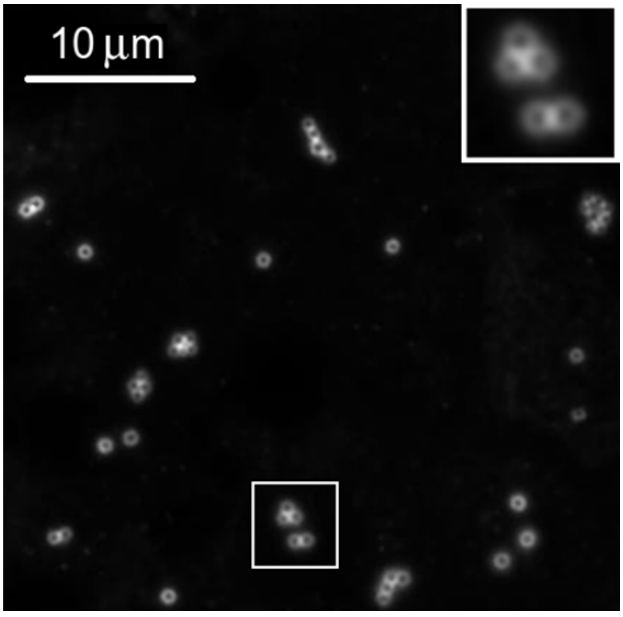

Figure 2. Wide-field fluorescence image of the PCP complexes deposited on SiO_2-Au core-shell nanostructures. Excitation wavelength was 480 nm.

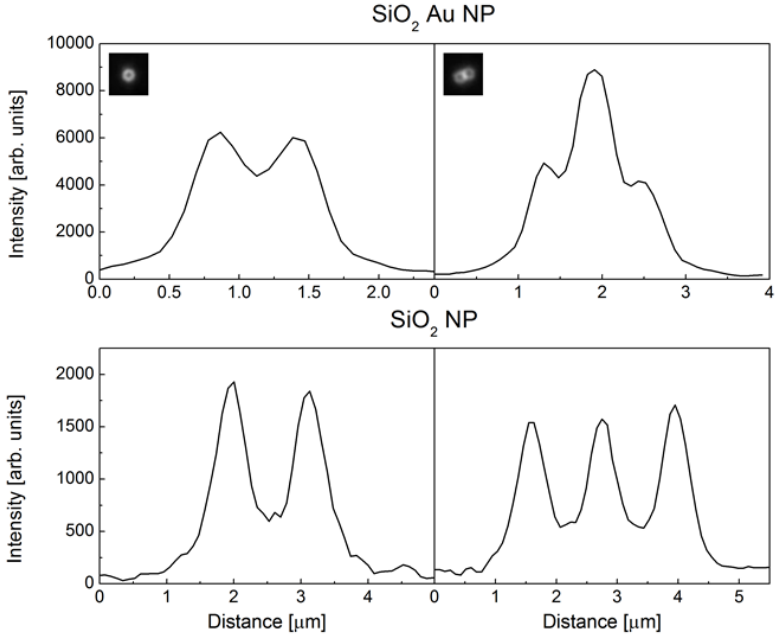

Figure 3. Cross-sections of the fluorescence intensity of PCP obtained for a single and two SiO_2-Au core-shell nanostructures (upper row) compared with analogous data obtained for SiO_2 nanoparticles.

can probe the properties of the PCP complexes on and off the nanoparticles. Thus, after collecting a confocal image with approximate size of 100x100 microns, we measured spectra and decays for several tens of bright spots in order to compare the result with the data obtained for the areas free of the nanoparticles.

In Fig. 4 we compare typical fluorescence spectra obtained for a sample where PCP complexes were mixed with SiO_2 (dotted line) or SiO_2-Au core-shell nanostructures (solid line) to the reference, which in our case are PCP complexes located off the nanoparticles. The spectrum indeed corresponds to the PCP complexes, it is identical to published data [19]. First of all, the comparison of the fluorescence spectra measured for the PCP complexes on and off the nanoparticles indicates that the coupling with the nanoparticles, both Au/SiO_2 and SiO_2, leaves no effect upon the spectral shape of the emission. The only difference concerns the fluorescence intensity. While in accord with previous work [10], the intensity of the PCP emission on SiO_2 nanoparticles is by approximately 2-3-fold increased as compared to the reference, coating the SiO_2 nanoparticles with Au nanostructured film results in further, quite substantial increase of the emission intensity. We note that analogous measurements carried out on a sample containing only SiO_2-Au core-shell nanostructures on the surface yield no measureable florescence under our experimental conditions.

Plasmonic origin of this enhancement is proven by the results of time-resolved fluorescence spectroscopy. As shown in Fig. 5, where we compare normalized decay curves measured for PCP complexes on both SiO_2-Au core-shell nanostructures and SiO_2 nanoparticles with the reference, the fluorescence decay is significantly shorter for the PCP complexes on SiO_2-Au core-shell nanostructures. This effect can be quantified by fitting the curves shown in Fig. 5 with decay times. The approach used for extracting the decay parameters was to fit the transient for the reference first and then use the obtained parameters to fit the other two curves, measured for PCP complexes on SiO_2 and SiO_2-Au core-shell nanostructures. The reference data can be approximated with a biexponential decay, with the decay constants of 0.47 ns and 3 ns. While the latter is typical of PCP complexes [10], the shorter one can be due to some unspecific effects associated with interaction with ambient conditions, as the sample was not prepared using any polymer matrix. Importantly, the transient behavior of the fluorescence intensity is identical for the PCP complexes placed on and off the silica nanoparticles as the decay times obtained are essentially the same in both cases (0.45 nm and 3.1 nm, respectively). Identical

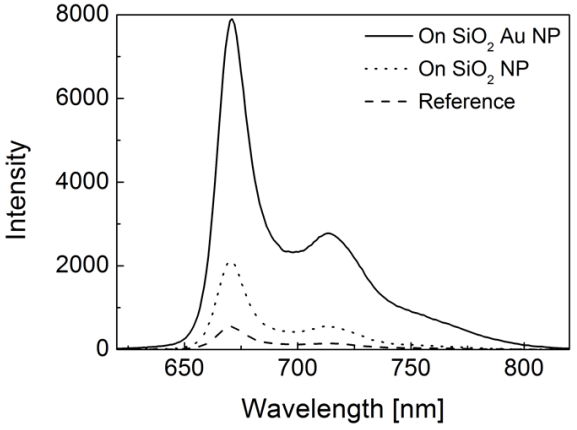

Figure 4. Fluorescence spectra obtained for PCP complexes on an SiO_2-Au core-shell nanostructure (solid line), on an SiO_2 nanoparticle (dotted line), and the reference – off nanoparticles (dashed line). Excitation wavelength of 480 nm was used.

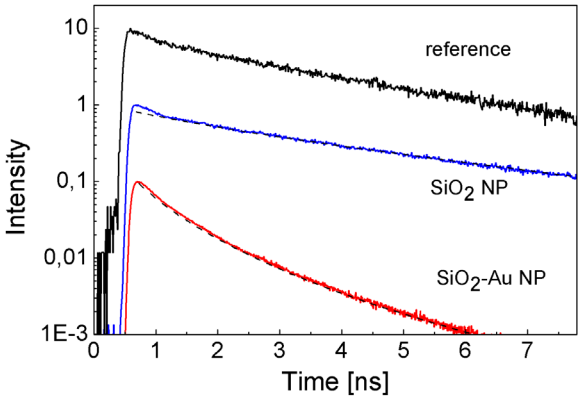

Figure 5. Fluorescence decays collected for PCP on an Au/SiO_2 nanoparticles (red), on an SiO_2 nanoparticle (blue), and off nanoparticles (black). Dashed lines represent fits, as described in text. Excitation wavelength of 480 nm was used.

lifetimes indicate that the interaction between the nanoparticles and the photosynthetic complexes induces no changes in the radiative properties of the chlorophyll molecules that are responsible for the fluorescence emission. In contrast, in order to account for plasmonic effects associated with the presence of Au, it is necessary to include a third decay constant, equal to 1.3 ns, to the pair of these previously used to simulate fluorescence decay for the reference and for the PCP complexes on SiO_2 nanoparticles. The obtained value corresponds rather well with the estimation obtained by comparing fluorescence spectra. This result confirms that in addition to purely optical effect, associated with scattering of electromagnetic radiation by SiO_2 nanoparticles, coating them with metallic layer leads to further improvement of fluorescence properties due to plasmonic interactions.

4 Conclusions

Fluorescence imaging and spectroscopy was applied to study the influence of SiO_2-Au core-shell nanostructures on the optical properties of light-harvesting complexes, PCP. We find that PCP complexes exhibit enhanced fluorescence intensity when deposited on SiO_2-Au core-shell nanostructures. Part of this enhancement is associated with pure optical effect due to higher refractive index of silica, but shortening of the fluorescence decay time proves that plasmon excitations in Au island shell increase radiative rate of chlorophyll emission.

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