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File name:	5._ACTA_Physica_Polonica.vol._12..
File size:	2.21M
Page count:	3
Word count:	2,203
Character count:	11,791
Submission date:	19-Jan-2018 09:19AM (UTC+0700)
Submission ID:	904196790

Vol. 122 (2012) ACTA PHYSICA POLONICA A No. 2

Proceedings of the WELCOME Scientific Meeting on Hybrid Nanostructures, Toruń, Poland, August 28–31, 2011

Spectral Dependence of Fluorescence Enhancement in LH2–Au Nanoparticle Hybrid Nanostructures

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We report on the influence of plasmon resonance in spherical gold nanoparticles on the optical properties of light-harvesting complex LH2 from the purple bacteria *Rhodopseudomonas palustris*. Systematic studies as a function of the excitation energy and the separation distance indicate that metal enhanced fluorescence shows strong dependence upon both of these parameters. We observe substantial increase of the fluorescence from LH2 complex in a hybrid nanostructure with 12 nm silica spacer. On the other hand, the enhancement measured with laser tuned into the plasmon resonance is almost threefold compared to the off-resonance configuration. The enhancement of fluorescence intensity originates in both cases from the increase of carotenoid absorption in the LH2 complex.

PACS: 87.80.Dj, 78.47.D−, 78.67.Qa, 82.35.Np

1. Introduction

Plasmons, free electron oscillations in metallic nanometric materials, enable strong modifications of the electromagnetic field at the nanoscale [1] thus becoming scientific inspiration for many research fields including photovoltaics and biosensors [2, 3]. One of the most spectacular effects associated with plasmon excitations in metallic nanoparticles is metal-enhanced fluorescence [4], i.e. the increase of the radiative rate of a fluorophore due to plasmon coupling. This effect depends among others upon the separation between a fluorophore and metallic nanoparticle as well as their spectral properties [5]. Namely the largest values of fluorescence enhancement have been observed for distances around 10–20 nm and for metallic nanoparticles with plasmon resonances matched spectrally to the emission range of the fluorophore [5]. Analogous consideration is also valid for plasmon-induced increase of absorption rate [6–8], as observed for photosynthetic complexes responsible for absorption of light. When the separation gets shorter, the nonradiative energy transfer from the fluorophore to the metallic nanoparticle takes place leading to efficient quenching of fluorescence.

In this work we study the fluorescence properties of a hybrid nanostructure comprising spherical Au nanoparticles and light-harvesting complex LH2 from purple bacteria excited on- and off-resonance with respect to the plasmon resonance. The distance between them is controlled via SiO_2 spacer, whose thickness varies from 4 to 40 nm. For both excitation wavelengths we observe fluorescence enhancement for the spacer thickness of 12 nm, but the on-resonance case (485 nm) yields three times stronger effect compared to the off-resonance (405 nm). The insensitivity of the fluorescence decay time on the spacer thickness for both excitation wavelengths points towards increase of the absorption rate in the light-harvesting complexes coupled to plasmon excitations in metallic nanoparticles.

2. Materials and methods

The LH2 complexes from *Rps. palustris* were prepared as described elsewhere [9]. The complexes were stored in Tris buffer with 0.1% LDAO. Spherical Au nanoparticles were synthesized by reducing chloroauric acid $HAuCl_4$ with sodium citrate and dispersed in toluene. The average diameter of the gold nanoparticles was 5 nm, which results in plasmon resonance maximum at 530 nm. The hybrid nanostructure was prepared by first spin coating the Au nanoparticles on clean glass substrate. Next the samples were transferred to an e-beam evaporation chamber where silica layers were deposited. The thickness of the SiO_2 layer was varied between 4 and 40 nm. Finally, 10 μL of the light-harvesting complexes dissolved in a polymer (PVA Sigma Aldrich) were spin-coated on top of SiO_2 layer.

Absorption spectra of solutions of the LH2 complexes and Au nanoparticles were obtained using spectrophotometer (Perkin Elmer Lambda 2). Fluorescence measurements of hybrid nanostructures were carried out in a standard optical setup in a backscattering geometry. The laser excitation beams (λ = 405 nm and λ = 485 nm) were focused using a lens with focal length of 30 mm. Lasers can be operated in either continuous-wave or pulsed mode generating 30 ps pulses with 80 MHz repetition rate. Excitation power was controlled using notch filters to obtain 200 μW on the sample surface. The emission was guided through a 150 μm pinhole and focused on a slit of a 0.5 monochromator (Shamrock 500, Andor) coupled with a charge coupled device detector (iDus 420BV, Andor). In order to extract fluorescence of LH2 complexes we used a combination of a longpass filter (Chroma HQ850LP) and a bandpass filter (Chroma D880/40 m). Fluorescence decays were measured with a time-correlated single photon counting technique em-

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