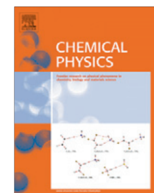
Chemical Physics 406 (2012) 15–20

Contents lists available at SciVerse ScienceDirect

Chemical Physics

journal homepage: www.elsevier.com/locate/chemphys



Plasmonic interactions of photosystem I with Fischer patterns made of Gold and Silver

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ARTICLE INFO

Article history:
Available online 15 May 2012

Keywords:
Plasmonics
Fischer pattern
Single-molecule spectroscopy
Light-harvesting
Signal enhancement

ABSTRACT

Single-molecule spectroscopy has been used to investigate the plasmonic interaction effects of nanometer-sized hexagonal arrays of Au- and Ag-triangles on the fluorescence properties of photosystem I (PSI) – a key component of the photosynthetic apparatus. The fluorescence emission of PSI is intensified upon coupling with these nanostructures. For single PSI trimers, enhancement factors of up to 10.5 (Au) and 15 (Ag) were observed. The average enhancement is 2.2/5.7 for Au/Ag, respectively. Comparison of the emission spectra shows that the enhancement depends on the wavelength. This wavelength dependence can be explained by the multichromophore composition of PSI. Furthermore, plasmonic interaction increases the fluorescence emission of spectral components, which are barely visible under natural conditions. The metal nanostructures produced via nanosphere lithography turned out to be beneficial tools for signal enhancement due to the very low intensity of their autoluminescence signal.

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1. Introduction

Metallic nanostructures in combination with proteins connected to their surface provide one of the most adaptable architectures for design and implementation of biofunctionality at the nanoscale [1,2]. One field of research concerns the electrical field enhancement of nanostructures (surface plasmon resonance (SPR)) and its use as optical antennas for light-harvesting and signal enhancement of fluorophores. The increased sensitivity of fluorescence-based assays finds its application in identifying new medications, cellular fluorescence imaging, high-throughput screenings, and in the design of solar cells [2–8]. Such applications depend on the interaction of fluorophores with surface plasmons of the nanostructures. Using plasmonic nanostructures in biophysical applications requires exact design since the interaction between proteins and nanostructures can lead to mutual modification of their properties [9,10]: molecular adsorbates on the nanoparticle surfaces can strongly shift SPRs as they may change the dielectric constant of the surrounding [11]. Likewise, plasmonic nanostructures can influence the optical properties of proteins, including changes of the fluorescence quantum yields and lifetimes, Raman scattering cross sections, Förster radii, Förster rates, and excitation energy transfer pathways in multichromophor assemblies [3,12–15]. The interaction between fluorophores and plasmonic active particles depends strongly on their distance from one another and their relative orientation. Fluorescence can be either enhanced or quenched depending on these sorts of geometrical conditions [16,17].

Detection of fluorophores at the single-molecule level is often limited by low signal intensities due to restricted photostablity [18]. Signal enhancement by plasmonic nanostructures proves especially beneficial since the coupling to SPR reduces optical saturation and dramatically decreases fluorescence lifetimes thus improving emission rates [3].

This work presents the effects of coupling photosystem I (PSI) to ordered nanostructures consisting of periodic hexagonal patterns of Au- or Ag-nanoparticles. Such metallic nanostructures are also known as Fischer patterns and are produced by nanosphere shadow lithography [19–21]. This method allows easy tuning of the SPR frequency since all parameters determining this frequency – such as structure size and shape, lattice constant, and material composition of the Fischer patterns – are readily accessible and variable over large ranges (see Fig. 1) [22].

PSI is a biological light harvesting system that absorbs across a wide spectral range covering ~45% of the solar radiation at the Earth's surface. Light harvesting by these proteins is so efficient that a single monolayer of photosynthetic proteins is sufficient to absorb up to 20% of the incident light (for review see Ref. [23]). After absorption, the energy is transferred to the site where energy conversion into an electric gradient usable by subsequent proteins is processed. This in combination with a unique quantum yield of ≈1 for charge separation, makes PSI an attractive component in electrochemical approaches for the conversion of light energy into electrical or chemical energy [12,24–31]. Almost 100 chlorophyll

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http://dx.doi.org/10.1016/j.chemphys.2012.05.005

molecules per PSI monomer are involved in light harvesting, excitation energy transfer, and charge separation (Fig. 2(a)) [32–34]. The chlorophyll molecules' specific electronic transition energies depend on their binding conditions inside the protein scaffold, providing a set of states allowing for fast, robust, and efficient exciton transfer to the reaction center (P700), a chlorophyll dimer that absorbs at 700 nm [34,35]. Charge separation is initiated at P700, leading to a charge-separated state across the thylakoid membrane. Astonishingly, PSI contains a few strongly coupled chlorophyll dimers and one trimer with site energies lower than the reaction center, which can be observed as additional absorption bands at wavelengths above 700 nm (Fig. 2(d)) [35–40]. Although energy transfer via these low-energy chlorophylls requires a subsequent uphill energy transfer towards P700, they are shown to be involved in the exciton transfer [38]. Due to this extra-ordinary behavior, the so-called red chlorophylls are of great interest and their function is still under debate. Energy transfer from the red chlorophylls to P700 is blocked at low temperatures. The energy is then trapped in these states and partially relaxes through fluorescence emission [40]. This fluorescence is detectable down to the single-molecule level [41–44,18,45], there the contributions of the red chlorophylls can be resolved (Fig. 2(e)). Their emission can be used e.g. as indicators for different stages of the energy transfer in PSI [46]. The composition of the PSI emission spectra is extremely dependent upon the interplay of the protein-bound chlorophylls that determine the energy transfer pathways. These interplays can be modulated by changes on the inside or the outside of the protein complex [47]. Such changes can be as small as proton fluctuations close to chromophore binding sites [48,46].

In the described experiments, the fluorescence of the red chlorophylls of PSI deposited on plasmonic active surfaces was detected. Single-molecule fluorescence detection below the temperature of liquid helium on a reasonable number of isolated PSI complexes allows for an accurate investigation of the influence of plasmonic structures on PSI's fluorescence properties. Based on such spectroscopic results, the influence of the interaction with plasmonic surfaces on the function of PSI can be discussed.

2. Material and methods

Sample preparation: Nanostructures were produced by nanosphere shadow lithography as described in [19]. Polystyrol nanospheres (Microparticles GmbH Berlin) with a diameter of 470 nm self-assemble into a 2D hexagonally ordered crystal that was deposited on quartz substrate (Herasil). These samples were covered with 2 nm Cr and 20 nm Au (or Ag) by vapor deposition. The polystyrol spheres were subsequently removed.

Solution of isolated and purified PSI trimers from *Th. elongatus* (procedure described in Ref. [49]) were diluted in buffer solution (pH 7.5) containing 20 mM Tricine, 25 mM $MgCl_2$, and 0.4 mM β-DM as detergent, to obtain a chlorophyll *a* concentration of ~20 μM. This amount of detergent is adequate for the critical solubilization concentration for a PSI trimer concentration of 0.5 μM to avoid PSI aggregation [50]. To obtain a concentration suitable for single-molecule experiments, the samples were further diluted with a buffer solution containing 20 mM Tricine, 25 mM $MgCl_2$ 0.4 mM β-DM, and 5 mM sodium ascorbate in milli-Q water. The sodium ascorbate was added for pre-reduction of P700. After stepwise dilution, the final PSI trimer concentration was ~3 pM. Less than 1 μL of this solution was placed on bare quartz or nanostructured surfaces, after which a coverslip was placed on top.

Spectroscopic measurements: Experiments were carried out using a home-built confocal microscope operating at 1.3 to 1.4 K. A piezo tip tilt module (Physik Instrumente PI S-334.2SL) was used to deflect the beam for the imaging of single molecules. The excitation source was a diode laser (680 nm, Schäfter and Kirchhoff). The fluorescence emission was detected using either an avalanche photodiode (Perkin–Elmer SPCM-AQR-15, <50 dark counts/s) for fast integral fluorescence detection or an Acton Research 300 mm spectrograph (Acton SpectraPro308) equipped with a back-illuminated CCD camera (Roper Scientific Spec-10:100BR/LN) for recording fluorescence spectra. Stray laser light was blocked by a Raman long-pass filter (AHF HQ695LP). The microscope objective (60×, N.A. 0.8 JIS, Edmund Optics) was immersed in liquid helium (cyrostat, Janis Research) for illumination and collection. A laser intensity of ~100 μW measured behind the scanning module was used for excitation. The usual exposure time for each spectrum was 1 s in a sequence of spectra resulting in a typical S/N-Ration of >6 for single PSI trimers on bare quartz at the given excitation power.

The absorption spectra of the nanostructures were captured on a home-built spectrometer using a standard lock-in technique with intensity modulation; an empty substrate was used as reference.

3. Results

Fig. 1(a) shows an AFM image of Ag-nanostructures as used for the described experiments. Fig. 1(b) presents a height profile

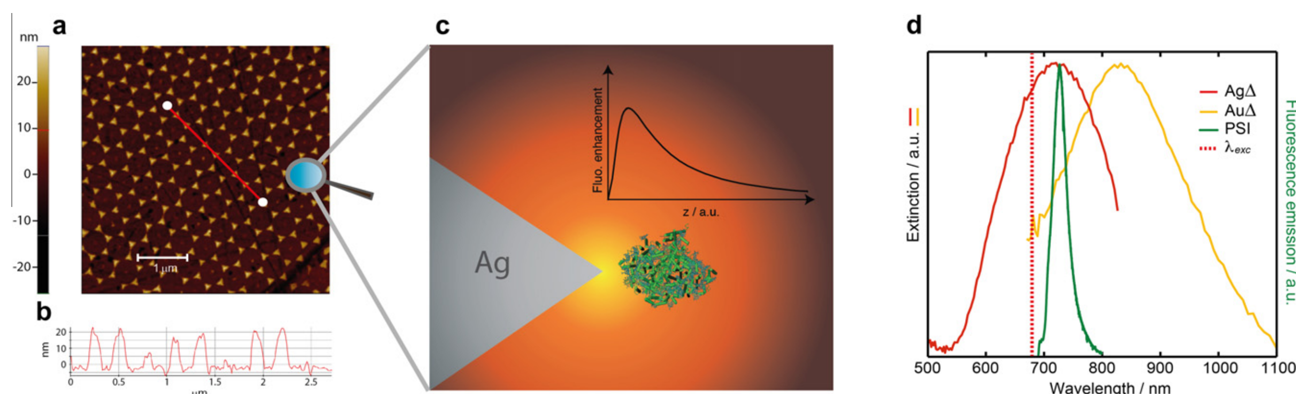

Fig. 1. (a) AFM image of an AgΔ Fischer pattern. (b) Cross section along the red line as indicated in the AFM image above. (c) Scheme of a single PSI complex in the electric field produced by an AgΔ. The region of the electric field enhancement produced by a nanoparticle depends on its shape and the material. In general, however, the penetration depth of these fields is larger than the dimension of a PSI complex [60], but the enhancement factor for the fluorescence emission varies remarkably over the dimension of a single PSI complex [17]. (d) The extinction spectra of the Fischer patterns used (data were scaled to similar magnitude). The low temperature fluorescence of PSI is also shown in green. The dotted red line is the wavelength position of the excitation source used for the fluorescence excitation in the following experiments. The wavelength is close to the maximum absorption of PSI (see also Fig. 2(d)). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

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