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Metal enhanced fluorescence of flavin mononucleotide using new plasmonic platform

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ABSTRACT

New plasmonic platform was successfully obtained to investigate the increase of fluorescence intensity of a fluorophore in the presence of silver nanoparticles. A flavin mononucleotide, was selected by us as a fluorophore for this study as a very important biological compound playing a key role in many biochemical process. Plasmonic platforms were characterized by means of luminescence spectroscopy. Flavin mononucleotide deposited on plasmonic platform exhibits dramatic emission enhancements in presence of silver nanoparticles deposited on gold mirror.

1. Introduction

Over the last decades silver nanoparticles have been applied in many different fields from catalysis through optics and electronics and many other areas due to their unique size-dependent optical, electrical and magnetic properties [1–3]. Currently, most of the applications of silver nanoparticles are related to light harvesting and enhanced optical spectroscopy including metal-enhanced fluorescence (MEF) and surface-enhanced Raman scattering (SERS) [4–6]. It is known that silver nanoparticles can influence the fluorescence properties of an organic fluorophore on account of the localized surface plasmon resonance. Moreover, fluorophores close to noble metallic films or nanostructures show exciting and desired properties like higher brightness and increased photostability.

This work shows the results of enhanced fluorescence intensity of a flavin mononucleotide (FMN) on especially designed and prepared plasmonic platform. The plasmonic platform consists of silver nanoparticles deposited and arranged on semitransparent gold mirrors. Light-induced localized plasmons in assemblies of nanoparticles interact with traveling plasmons in a conductive surface and create extremely strong local electric fields. Fluorescent molecules deposited on the platform surface are exposed to the enhanced local fields and are excited with higher rate constant. In addition, excited molecules interact with surface plasmons in metallic nanostructures resulting in the enhanced radiation of the excitation energy. Obviously, the brightness of fluorophores is then also greatly enhanced and their lifetime is significantly shortened. A shorter lifetime of fluorophore prevents photobleaching due to reduced excited state residence time as photodegradation occurs predominantly in the excited state [7–10].

A flavin mononucleotide, which was selected by us as a fluorophore for this study, is a very important biological compound as it plays a key role in many biochemical processes [11,12]. FMN and other flavins can for example participate in the process of destroying pathogens and inactivation of many microorganisms [13]. Some studies indicate also that flavins exhibit anti-cancer activity and therapeutic properties in the treatment of several other diseases [14]. Furthermore they participate in many signaling events occurring in the cell membranes, mitochondria, or cytoplasm. They also act as photoreceptors of blue light in plants (for example phototropism) [15–17]. Spectroscopic properties of FMN and its biological actions depend strongly on its local environment, concentration and temperature [18,19]. Therefore, the detection of FMN enhanced fluorescence and the introductory recognition of FMN spectroscopic properties in the presence of metal nanostructures is an important task which herein is undertaken.

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2. Experimental part

All materials used to prepare the plasmonic platform were purchased from commercial sources (analytical grade) and used without further treatment. Silver nitrate, $AgNO_3$ (99.99%); trisodium citrate dihydrate, $(OH)C_3H_4(COONa)_3{\cdot}2H_2O$ (99%) were purchased from Aldrich Company. Flavin mononucleotide (riboflavin-5′-mono phosphate sodium salt $C_{17}H_{20}N_4NaO_9P{\cdot}H_2O$) (FMN) analytically pure was purchased from Lancaster. For the preparation of all the samples deionized water was used which was obtained from Hydrolab system installed in our laboratory.

2.1. Preparing of silver nanoparticles

The silver nanoparticles used in this work were obtained by the reduction of silver nitrate in aqueous solution in the presence of trisodium citrate according to the developed procedure presented earlier [20]. First, the sodium citrate (2 ml at 34 mM) was added dropwise to a stirred solution of $AgNO_3$ (100 ml at 1 mM). During the process, all solutions were mixed vigorously and heated to 90–95 °C for 15 min or until change of color was evident (yellow). Then the mixture was incubated in an ice bath for 15–20 min. Finally, the silver nanoparticles were purified by centrifugation at 3500 rpm for 8 min and the precipitate was then suspended in 1 ml of 1 mM trisodium citrate.

2.2. Preparation of plasmonic platform

The plasmonic platforms were obtained as follows. First, gold mirrors (Thin Metals Films Ltd. (UK)) were cleaned and drop coated with silver nanoparticles. Then, the mirrors were dried in air. When a liquid containing silver nanoparticles was evaporated, different structures were formed. In the final stage, FMN in water ($C = 10^{-3}$ M) was applied on gold mirror with Ag nanoparticles by spin-coating method. The spin coating was done at 300 rpm for 60 s to disperse the solution. After that, the thin films were allowed to dry in a dark room and in air atmosphere for 24 h.

For comparison and control experiment, FMN was deposited on the gold mirror and a microscopic glass without silver nanoparticles. Microscopic glasses used were kept in a mixture of 33% H_2O_2 and H_2SO_4 in molar ratio 2:1 over 24 h and rinsed with deionized water. The spin coating was done at 300 rpm for 60 s. After that, the thin films were allowed to dry in a dark room and in air atmosphere for 24 h. The graphical idea of the platform is shown in Fig. 1.

2.3. Apparatus

Steady-state fluorescence intensity measurements of all the samples were carried out using a Carry Eclipse spectrofluorometer (Varian Inc., Australia) with the front face geometrical format described previously in detail [21]. The emission was scanned from 480 nm to 750 nm following a 470 nm excitation and using 495 nm long pass filter on the emission side.

Fluorescence lifetimes of all the samples were measured using FluoTime 200 (PicoQuant, GmbH, Berlin, Germany) Fluorescence Lifetime Spectrometer. It contains a microchannel plate detector (Hamamatsu, Japan) and a 470 nm laser diode was used as the excitation source. The front face setup was used for these measurements as well. To minimize polarization effects the fluorescence intensity decays were collected from the front side upon so called magic angle (54.7° between the excitation beam and the normal to the sample surface) excitation. Data was analyzed with FluoFit 4.0 version software using multiexponential intensity decay model as follows:

$$I(t) = \sum_i \alpha_i e^{-t/\tau_i} \tag{1}$$

where α_i is the amplitude of the fluorescence intensity decay of the i-th component at time t and τ_i is the lifetime of the i-th component. The intensity weighted average lifetime (τ_{Avg}) was calculated using following equation:

$$\tau_{Avg} = \sum_i f_i \tau_i \text{ where } f_i = \frac{\alpha_i \tau_i}{\sum_i \alpha_i \tau_i} \tag{2}$$

The morphology of the samples was investigated by Schottky field Emission Scanning Electron Microscopy (FEI Quanta FEG 250) with an ET secondary electron detector. The beam accelerating voltage was kept at 10 kV.

A confocal MicroTime 200 (PicoQuant GmbH, Germany) system coupled with an Olympus IX71 microscope was used to obtain time resolved images. Fluorescence photons were gathered from different places on the sample using 60× water immersed objective (N.A 1.2, Olympus). A 500-nm long-pass filter with additional two 488 R long-pass filters (Shemrock) were applied to remove scattered light. A pulsed laser (470 nm-LDH-P-C470B) with repetition rate 20 MHz was used as a light source. Fluorescence photons were collected with a photon counting module (SPCM = AQR-14, Perkin Elmer) with processing accomplished by the PicoHarp300 time correlated single photon counting (TCSPC) module. Data analysis was performed using a SymPhoTime (5.2.4) software package.

3. Results and discussion

Electron microscopy was done in order to study the morphology and size distribution of silver nanoparticles. Representative SEM

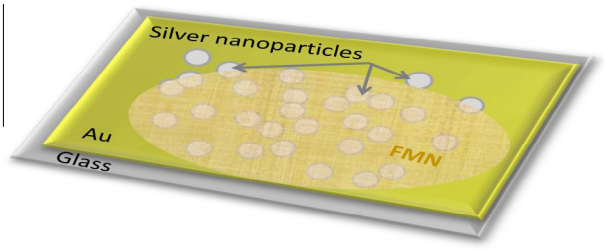

Fig. 1. Scheme of plasmonic platform.

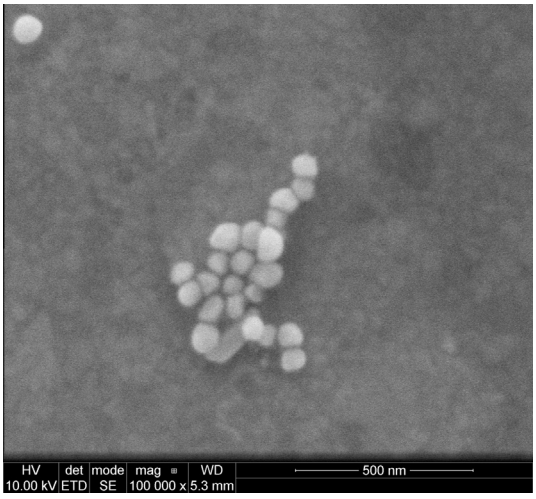

Fig. 2a. SEM image of the silver nanoparticles.