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On the application of Surface Enhanced Raman Scattering to study the interaction of DsRed fluorescent proteins with silver nanoparticles embedded in thin silica layers*

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***Abstract*—The interaction of proteins with silver nanoparticles (AgNPs) is of primary importance to uncover silver antimicrobial efficiency and environmental hazard. This interaction can affect silver reactivity, bioavailability and, eventually, silver toxicity towards the environmental media. Detection of the interaction of DsRed fluorescent proteins with AgNPs embedded in thin silica layers is demonstrated using surface enhanced Raman spectroscopy (SERS), but deep analyses require the design and elaboration of dedicated plasmonic substrates giving a high enhancement factor.**

I. Introduction

Current biotechnological achievements offer the possibility to explore chemical, physical and functional properties of proteins, in particular fluorescent proteins, in various applications such as biosensors, bioelectronics, drug delivery systems, *etc.*, [1, 2]. Therefore, they lead to exposure and interactions of proteins with non-biological organic and inorganic solid surfaces. Proteins are also largely involved in the underlying mechanisms of microbial adhesion on dielectric surfaces [3]. The microbial adhesion and biofilm formation cause major complications in the biomedical domain and in the food industry. To inhibit the development of biofilm on a surface, one possibility is to use coatings containing silver nanoparticles (AgNPs), well known for their antimicrobial efficiency. In this context, our purpose is to get a better insight at molecular level of direct or indirect interactions of silver (atoms, ions or NPs) with proteins.

AgNPs are excellent candidates to support simultaneously high antibacterial efficiency and huge enhancement of vibrational and luminescent signals originating from molecules located in their vicinity (antenna effect induced by surface plasmon resonance). Our concept called "spectro-inside" consists of using AgNPs as probes in surface-enhanced Raman scattering (SERS) spectroscopy to amplify and detect optical signals of proteins located in their vicinity and to track interactions of proteins with AgNPs.

II. Experimental part

Considering further development our choice is to go with DsRed protein and to study its interaction with AgNPs embedded in silica (SiO_2) layers. The DsRed is recently cloned from reef coral *Discosoma* sp. Recombinant Red Fluorescent (DsRed) protein [4]. It is tetrameric in nature and expresses excitation and emission maxima at 558 nm and 583 nm, respectively. The results presented here on the application of SERS are obtained for sessile droplets of very small volume (3.8 ± 0.1 μL) containing DsRed proteins of concentration 0.25g/L deposited on plasmonic substrates, after droplet dehydration. The proteins deposition procedure is described elsewhere [5].

Two physical approaches were used to elaborate the plasmonic substrates, containing AgNPs embedded in thin silica layers, necessary to perform SERS. These are: (i) low energy ion beam synthesis (LE-IBS) [6] and (ii) plasma deposition comprising silver sputtering followed by plasma polymerization [7]. The two elaboration techniques are complementary and give the prospect to consider different physical situations. The purpose is to fabricate multifunctional substrates that can be used for both (i) controlled Ag release and biocide action and (ii) efficient plasmonic enhancement [8].

Raman spectra were recorded by using a high resolution Raman spectrometer (Horiba Jobin-Yvon Xplora) equipped with three lasers 532, 632 and 785 nm and a standard confocal microscope. The 532 nm laser was used for this experiment. The laser beam was focused on a uniform area of the DsRed using a x100 objective. To avoid possible degradation of proteins due to laser heating, the intensity and time exposure of the incident beam were limited to 1% of its maximum (about 0.15 mW) and 1s, respectively.

III. Results and discussion

Images obtained by Transmission Electron Microscopy (TEM) of the plasmonic substrates are shown in Fig. 1. A thorough structural characterization of the AgNPs reported in Ref. [8] shows that the selected LE-IBS implanted samples contain spherical AgNPs of mean size 6.5 nm and density 10.1×10^{11} NPs/cm^2, embedded in the SiO_2 layer at 0.5 nm from the surface while for the selected plasma deposited samples the AgNPs are of prolate-spheroid shape with mean

size 18.5 nm and density 1.7×10^{11} NPs/cm^2, and are embedded in the SiO_2 layer at a distance of 5.5 nm from the surface [8].

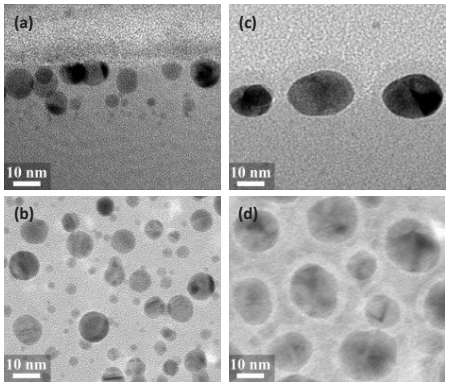


Fig. 1. TEM images of the LE-IBS implanted samples (a, cross-section view) and (b, plane view) and of the plasma deposited samples (c, cross-section view) and (d, plane view).

Fabrication of nanostructured substrates for molecular plasmonics is hampered by the requirement to maintain on large areas, in a reproducible way, a well-defined spacing between metallic nanostructures and molecules: their mutual interaction is indeed governed by the local topography of the electromagnetic field. In that sense, the emission of a fluorescent molecule placed in the vicinity of a metallic nanostructure can be either amplified or quenched, depending on its position and orientation. Hence, the separation between emitting or scattering objects deposited near plasmonic antenna has to remain constant in the range of few nanometers and be easily tunable. In that sense, our nanocomposites samples, with delta-layers of AgNPs at tunable nanometric distances from the silica surface are designed to be efficient SERS substrates.

Figure 2 presents the Raman spectra of DsRed obtained using the two different substrates (LE-IBS implanted sample, Fig. 2(a) and plasma deposited sample, Fig. 2(b)). A theoretical spectrum of DsRed was used as reference to identify the protein signature (black spectrum). The characteristics vibration frequencies of DsRed molecular bonds correspond to the band positions in the Raman spectrum. The average spectrum for each substrate was calculated from the accumulation of 10 acquired spectra. A strong SERS effect of DsRed protein is observed for the plasma deposited sample. In particular, a blinking effect is detected, corresponding to intensity fluctuations of the different Raman peaks. This is due to changes and fluctuations in the molecular orientation and conformation of DsRed protein under the laser excitation (532 nm). On the contrary, the Raman signal is low when the DsRed proteins are deposited on top of the sample elaborated by LE-IBS and the phenomenon of "blinking" is not detected. The difference in the signal enhancement and behavior between these two samples can be ascribed to several factors: (i) higher surface fraction of AgNPs for the plasma deposited sample leading to a high concentration of hot spots, (ii) the effect of the surface waviness which allows trapping of proteins in the dips in between two AgNPs; the size of the DsRed protein (27.6 kDa) being of about 4.2 nm *i.e.*, of the order of the dip width.

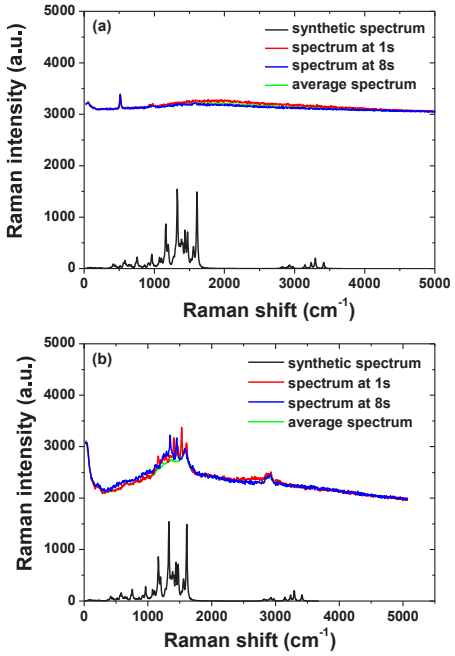


Fig. 2. Recorded series of Raman spectra on (a) the LE-IBS implanted samples and (b) the plasma deposited samples.

IV. Conclusion

The presented results on SERS effect are obtained for DsRed proteins deposited on top of AgNPs based plasmonic substrates. The plasma deposited sample with high surface fraction of AgNPs, located at 5.5 nm from the dielectric conformal surface, shows promising SERS properties to study the interaction of DsRed proteins with AgNPs.

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