Protein A Denaturation Sensing Through Surface Enhanced InfraRed Absorption (SEIRA) Spectroscopy

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Abstract. A pixeled plasmonic metasurface (MS) has been developed as a surface-enhanced infrared absorption (SEIRA) spectroscopy substrate to monitor the denaturation process of a protein A (PA) monolayer. The different pixels of the MS have been properly engineered to monitor different regions of the electromagnetic spectrum. Specifically, these pixels have their plasmonic resonances placed in the range 1500–2000 cm^{-1}, well matched with Amide I and Amide II vibrational bands. In particular, the SEIRA reflectance spectra of the native PA and the denatured PA have been compared, observing a redshift of about 10 cm^{-1} for both Amide I and Amide II groups. Moreover, the evaluated enhancement factor (up to 7×10^4) allows to reveal both the presence and the denaturation process occurring to a very low amount of PA molecules (about 3 fmoles).

Keywords: SEIRA spectroscopy · Plasmonic metasurfaces · Pixeled sensing platform · Nanoplasmonics · Biosensors

1 Introduction

Biological samples sensing can be performed with several optical techniques [1–4]. Among these, mid-infrared (mid-IR) spectroscopy allows to powerfully solve the structural determination of molecules, since it enables the identification of the unique vibrational signatures of the chemical bonds occurring to the target analyte [5]. Among the mid-IR possible spectroscopic techniques, Fourier-Transform IR (FTIR) is one of the most routinely employed for the structural analysis of chemical and biological molecules, due to its capabilities to provide structural information on the target analyte, together with its dynamics, in a non-invasive and univocal way [6]. However, owing to the very low molecular absorption cross-section of IR radiations ($\sigma_{abs} \approx 10^{-20}$ cm^2) [7], a large

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amount of material is required for accurate determinations. Surface-enhanced IR absorption (SEIRA) spectroscopy allows to overthrow this constraint thanks to the strong near-field enhancement offered by 2-D arrays of metallic nanoantennas (NAs) sustaining plasmonic resonances [8]. By varying the shape, size, and metal film thickness of each NA, as well as the array periodicity, it is possible to engineer the optical response of this metasurface (MS) through the whole electromagnetic spectrum [9]. This, in turn, enables spectroscopic analysis with a very huge sensitivity thanks to the high amplification of the vibrational modes of the molecules localized near the NAs [10–12].

In this study, we have monitored the denaturation process occurring to a protein A (PA) monolayer, by using a plasmonic MS (consisting of cross-shaped gold NAs fabricated on a silicon chip) as the substrate for SEIRA spectroscopy. Thanks to a multiresonant pixeled design, it has been possible to analyze the entire Amide spectral region ($1500 \div 2000\ \mathrm{cm}^{-1}$). First, the identification of the PA layer binding has been performed, through the identification of the typical Amide I and Amide II vibrational bands. Then, the PA denaturation process has been induced and then monitored, observing a wavenumber red-shift of about $10\ \mathrm{cm}^{-1}$ for both Amide I and Amide II vibrational bands. The SEIRA enhancement factor has been estimated around 7×10^4, thereby enabling the detection of spectral variation in the vibrational bands even for a very small number of molecules (3 fmol in our experiment).

2 Material and Methods

The proposed plasmonic MS has been developed by following the subsequent research line, consisting of the device design, its fabrication, the functionalization and then the optical characterization.

2.1 Device Design

The modeling of the proposed platform has been carried out through the commercial software COMSOL Multiphysics v.5.1 [13]. Due to the polarization insensitivity offered by cross-shaped NAs and the high near field enhancement [14, 15], this geometry has been arranged in a 2-D periodic array positioned on a silicon substrate, as schematically shown in Fig. 1a. By taking fixed the NAs thickness t to 50 nm while varying the NAs geometrical parameters (cross arm length L, cross arm width W and the array period P), it has been possible to identify the parameters (summarized in Table 1) that locate the plasmonic resonance in the desired wavenumber range, i.e. $1500–2000\ \mathrm{cm}^{-1}$, as shown in Fig. 1b.

Table 1. NAs geometrical parameters, together with their numerical and experimental resonances.

	Geometrical parameters			$\nu_{num}(cm^{-1})$	$\nu_{exp}(cm^{-1})$
	L [μm]	W [μm]	P [μm]		
Pixel 1	1.12	0.2	2.0	1500	1475
Pixel 2	1.25	0.27	1.5	1600	1601
Pixel 3	1.03	0.27	1.5	1737	1835
Pixel 4	0.84	0.25	1.5	2032	1945

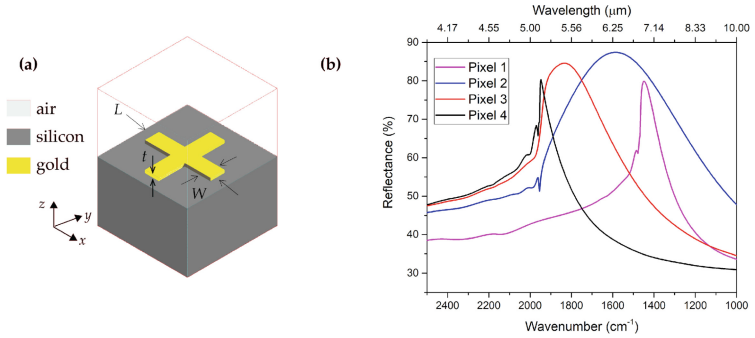

Fig. 1. Device modeling. (a) shows the schematization of the single unit cell. (b) shows the numerical optical responses of four pixels, whose parameters have been presented in Table 1.

2.2 Device Fabrication

The fabrication process of the proposed MS has been carried out through Electron Beam Lithography (EBL) and lift-off process. Indeed, to achieve a precise tuning of the plasmonic resonance to the desired range, the fabrication process should ensure an accurate control on NAs geometry. EBL offers high flexibility of use, high reproducibility and high reliability, therefore resulting as one of the most employed fabrication techniques for ordered arrays of NAs [16, 17]. The pixels, having an area of 0.5 × 0.5 mm^2, have been fabricated on a 1 × 1 cm^2 area float-zone silicon chip, as schematically shown in Fig. 2.

2.3 Device Functionalization

The developed MS functionalization protocol consists of the following steps: (i) chip surface cleaning and incubation with 2 mg/ml of dithiobis (succinimidiyl propionate) (DSP) in dimethyl sulfoxide (DMSO) for 1 h at room temperature; (ii) chip washing and incubation with PA (concentration 56.5 μM in phosphate buffered saline (PBS) solution) at 4 °C over night.

After the optical detection subsequent to the PA binding, a denaturation process has been performed, by inducing a pH jump by immersing the MS in a HCl solution (0.1 M) for 1 h, at room temperature.

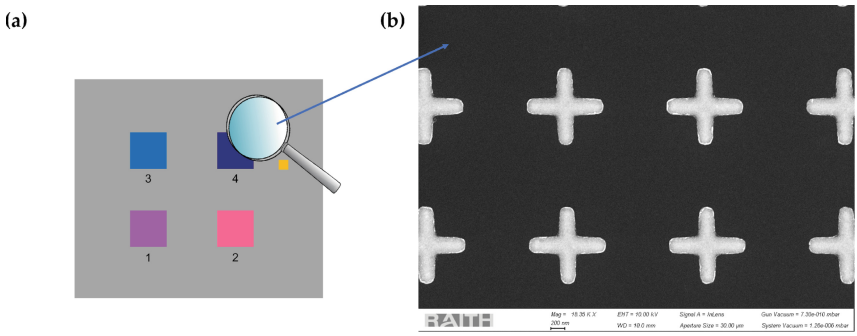

Fig. 2. (a) Schematization of the fabricated MS. (b) SEM image of a representative pixel.

3 Experimental Results

The proposed MS has been optically characterized at each step of the proposed experiment. The optical measurements have been performed with a Thermo-Nicholet NEXUS Continuum XL FTIR spectrometer, by collecting the reflection spectra of each pixel with a 4 cm^{-1} resolution over the range 4000–600 cm^{-1} for 128 scans.

The first characterization has been performed in air, to validate the numerical model. As reported in Table 1, a good agreement between the numerical and the experimental wavenumber resonance has been achieved, with a maximum discrepancy of about 6%, allowing an optical detection into the desired wavenumber range.

Once the validity of the numerical model has been confirmed, the MS has been functionalized with PA according to the protocol described in Sect. 2.3. After the PA binding, both a resonance peak wavenumber redshift of 8 cm^{-1} and the typical Amide I and Amide II vibrational bands appear, as shown in Fig. 3. In particular, Amide I bands, mainly attributable to the C = O stretching vibrations, are placed in the spectral range 1700–1620 cm^{-1} [18]. On the other hand, Amide II vibrational modes are mainly due to the C-N stretching vibrations and to the in-plane N-H bend; these bands are placed in the range 1560–1500 cm^{-1} [19].

In order to validate the possibility to monitor conformational changes of a target analyte present on the proposed MS, the denaturation of the PA monolayer has been induced through a pH jump, as described in Sect. 2.3. As shown in Fig. 4, it is possible to observe a 9 cm^{-1} redshift for the Amide I band, and a redshift of 10 cm^{-1} for the Amide II band. These redshifts are attributable to the protein α-helical structures structural modifications and to the breaking of the hydrogen bonds that produce the unfolding of the protein chains [20].

The enhancement factor (EF) [10] of the proposed MS has also been estimated; in particular, EF values up to 7×10^4 have been evaluated, thus ensuring the capabilities to monitor the vibrational modes present even for extremely low amounts of molecules (3 fmoles in the proposed experiment).

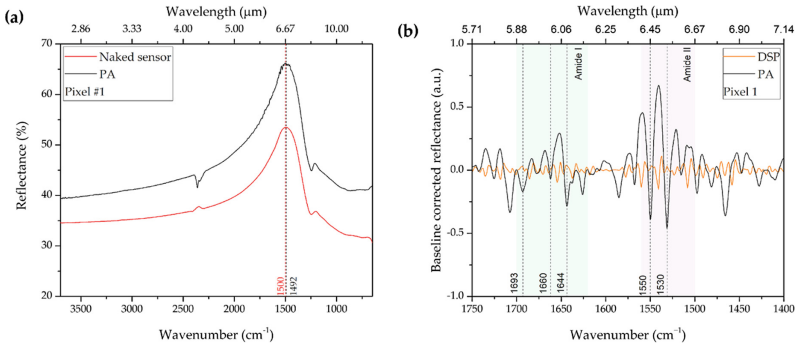

Fig. 3. Optical characterization of the MS after the PA binding. (a) shows an 8 cm^{-1} redshift of the plasmonic resonance peak. Red curve refers to the spectrum acquired with no molecules adsorbed on the MS, while black curve refers to the reflectance spectrum acquired after the PA binding. (b) shows the typical Amide I and Amide II vibrational bands appearing after the PA functionalization protocol. Orange curve refers to the reflectance spectrum acquired after the DSP incubation, while black curve refers to the reflectance response after the PA layer binding.

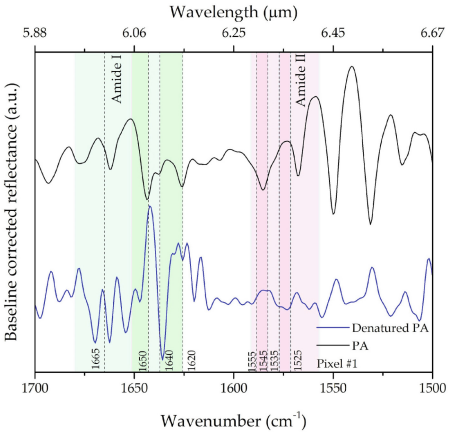

Fig. 4. Comparison between the baseline corrected SEIRA spectrum of native PA (black curve) and of the denatured PA (blue curve). A redshift of both Amide I bands (green area) and Amide II bands (pink area) appears.

4 Conclusions

In this work, a plasmonic MS suitable for SEIRA spectroscopy sensing has been employed to observe the denaturation process occurring to a PA monolayer. The proposed MS shows a pixeled design, with four different pixels able to cover the spectral region between 1500–2000 cm^{-1}. The MS is able to identify the typical Amide I and Amide II vibrational bands appearing after the PA layer binding, and also to monitor its conformational changes when a pH jump has been induced. This pH change has induced the denaturation of the PA monolayer, which can be observed in the SEIRA spectra as a

redshift of about 10 cm^{-1} for both Amide I and Amide II bands. Moreover, an EF up to 7×10^4 has been estimated, thus ensuring the SEIRA capabilities to monitor chemical changes in the target analyte even for small quantities of molecules.

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