Improvement of antigen detection using protein A for the oriented bio-functionalization of integrated photonic biosensor

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The objective of this work is to optimize the detection of antigens with integrated photonic sensing structures by using a protein A layer to promote bio-functionalization with specific antibody probes. Protein A allows the oriented attachment of antibody receptors over the sensor surface (see Fig. 1), since they bind to the protein layer through their Fc section and their Fab sections will be oriented towards the sample to be analyzed, thus enhancing the interaction with the target antigens to be detected [1]. The attention was focused on a well-known antigen typically used as model in experimental development: bovine serum albumin (BSA).

Protein A layer can be created over the sensor simply by physical immobilization, by flowing it over the sensor surface. So the delivery of the protein A and the other fluidic samples to the sensing structure was done by flowing using a simple PDMS-based microchannel. This bio-functionalization technique is quite simple and it allows providing specificity to a photonic sensing structure without the need of using other more complex chemical procedures such as those based on organosilanes. Therefore, it is perfect for the implementation of introductory experiments in the field of integrated photonic biosensors.

The photonic sensing structure used for our experiments is a SOI ring resonator (RR) in add-drop configuration, which was fabricated in CEA-LETI in the frame of the cost-share European nanophotonic fabrication platform ePIXfab (see Fig. 2). The basic structural parameters of the sensing device are: silicon thickness, 220 nm; access waveguide width, 450 nm; ring radius, 20 µm; input coupling gap, 170 nm; output coupling gap, 175 nm; free spectral range (FSR), 4 nm. A broadband superluminescent diode (SLD) was used to excite the ring resonator and the transmission spectrum of its through port was continuously measured using an optical spectrum analyzer (OSA).

The experiment consisted in several fundamental steps (see Fig. 3). First of all protein A was flowed over the ring resonator in order to create an intermediate protein layer on the sensing structure surface. By flowing gelatin, chip's areas not coated with protein A were blocked. From now on, PBS 1x with a small concentration of gelatin was used as buffer in order to keep the blocking of the surface. Anti-BSA, which is the antibody specific to the BSA antigen, was flowed over the chip in order to attach the antibody receptors. Finally, to carry out the specific detection, BSA was flowed. After the experiment, the chip can be regenerated by flowing glycine (note that in the regeneration process the antibodies are detached from the protein, so they will need to be attached again in the next cycle).

In order to test the improvement obtained thanks to the use of the protein A, several concentrations of BSA were flowed over the bio-functionalized sensor. For BSA concentrations of 100 ng/ml, 50 ng/ml and 5 ng/ml a wavelength shift of 140 pm, 70 pm and 10 pm has been detected, respectively. These results confirms the good performance in terms of sensitivity that can be obtained using a very simple bio-functionalization design based on the use of an intermediate layer of protein A.

References:

[1] Zhanhui Wang, Gang Jin, "Feasibility of protein A for the oriented immobilization of immunoglobulin on silicon surface for a biosensor with imaging ellipsometry", J. Biochem. Biophys. Methods 57, pp. 2'3-211, 2003

Figures:

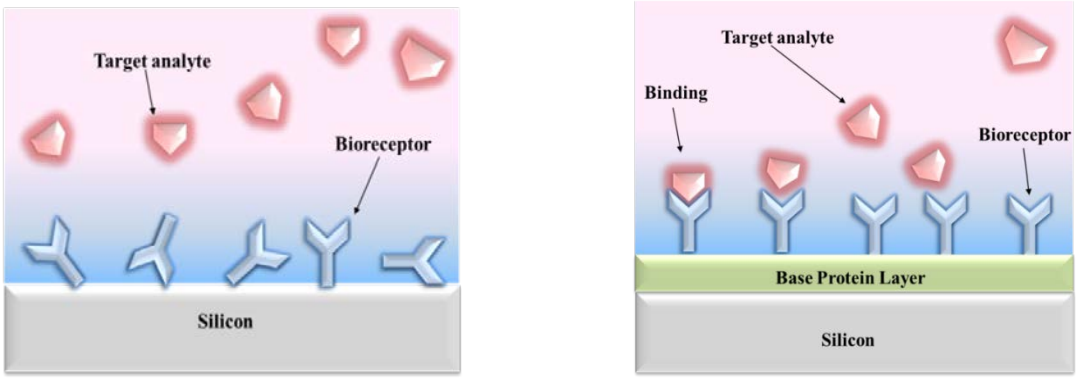

Figure 1. Left: illustration of randomly-oriented antibody receptors on the surface of the sensor; right: illustration of properly-oriented antibody receptors on the surface of the sensor, where an intermediate protein layer has been used.

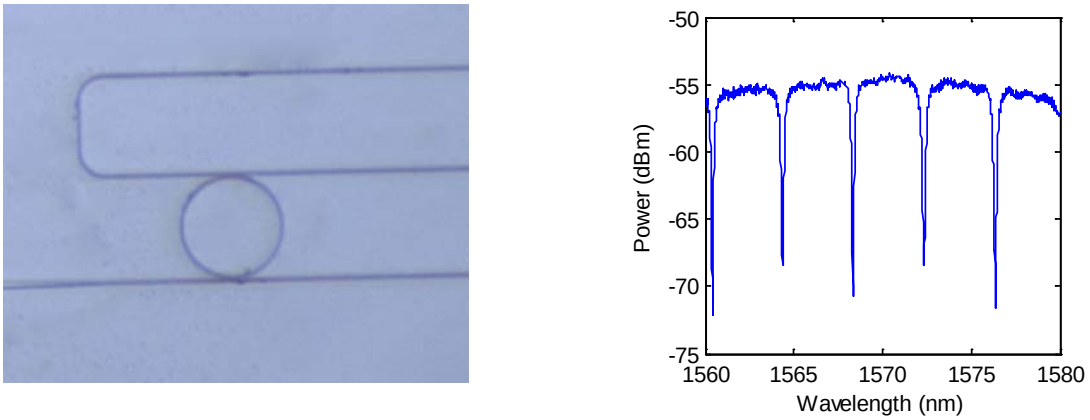

Figura 2. Left: microscope image of the ring resonator used for the experiments; right: transmission spectrum of the through port of the ring resonator.

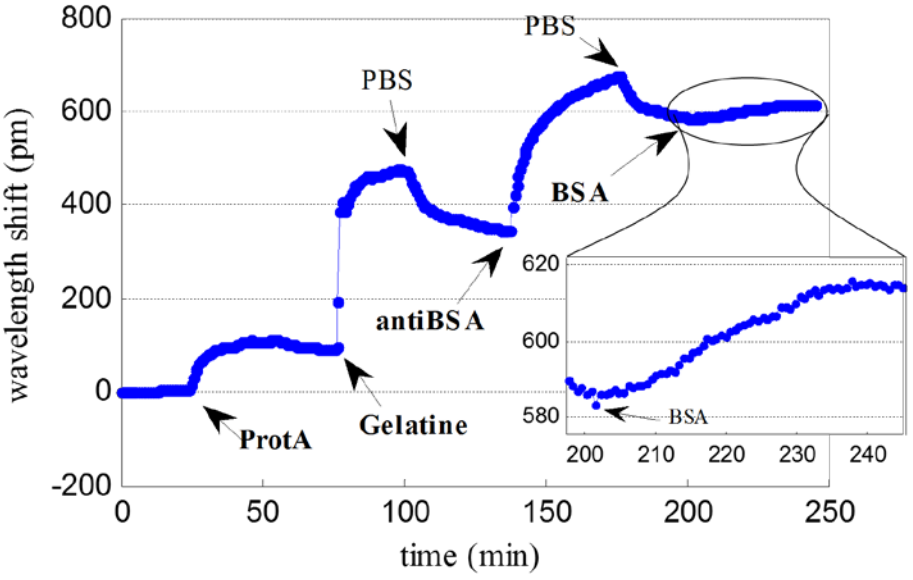

Figura 3. Wavelength shift evolution of the whole experiment; the inset shows a detail of the binding curve for BSA detection.