

THE USE OF X-RAY PHOTOELECTRON SPECTROSCOPY (XPS) IN THE CHARACTERIZATION OF THE BIOTRANSDUCER NANOSTRUCTURED INTERFACE.

[A.Azioune¹](#), [P.Louette¹](#), [B.Lindner²](#), [A.Neubauer²](#), [S.Muyldermans³](#) & [J-J.Pireaux¹](#)

¹*Laboratoire Interdisciplinaire de Spectroscopie Electronique (LISE) – FuNDP 61, rue de Bruxelles B5000 Namur (Belgium).* ²*TSP Nanoengineering, Hettenkofergasse 13/46 A-1160 Wien (Austria).*

³*Department of Immunology, Parasitology and Ultrastructure, VUB, Brussels (Belgium).*

INTRODUCTION: In this work, we show the use and the importance of X-ray photoelectron spectroscopy (XPS) in the characterization of a nanostructured biotransducer interface. Indeed, the gold substrate has been decorated by the highly nanostructured S-layer protein layer before the immobilization of the Camel antibody (cAb) which is the responsible of the prostate specific antigen (PSA) recognition. The homogeneity of the crystallized S-layer onto gold substrate has been checked before the immobilization of cAbs. In order to take a finger print of the immobilized cAbs, we have labelled it with iodine, one of the most sensitive element detectable by XPS.

METHODS: S-layer protein was crystallized onto [IMEC](#) gold substrates.

The cAb-PSA-N7 antibody was prepared at the institute of biotechnology at VUB (Brussels). A volume of this solution was labelled with cold iodine, according to the following protocol: 100 µl of the antibody was incubated with 4 µl of KI under stirring with a rate of 800 t/min, at RT during 1 h. The resulting yellow colour product was dialyzed three times in PBS during 90 minutes until it became uncoloured.

The S-layer/Au substrates were subsequently incubated at pH 4.75, during 1 h at RT with water soluble carbodiimide (EDC). Finally, different concentrations of cAb-PSA-N7 were incubated overnight with S-layer in PBS 10 mM, pH 9 and RT. The unbound proteins were removed by washing the samples three times with 2% of Tween 20 in PBS (150 mM), pH 7.4; three times with PBS 10 mM and three times with pure water. The plates were blown dried with a stream of pure argon, before analysis. The fresh samples were analyzed by XPS using an SSX100 spectrometer. The monochromatic AlKα X-ray source (1486.6 eV) was used. The X-ray spot size was 1000 and 300 µm for the acquisition of the survey and narrow scan regions, respectively.

RESULTS: Table 1 reports the measured composition of S-layer, expressed in atomic percent.

Table 1. Surface atomic composition of S-layer – coated IMEC gold substrate

Au	C	N	O	C/N	O/N
5.3	60.9	13.5	20.4	4.5	1.5

The C/N and O/N ratios are in good agreement with those calculated for the pure S-layer. These ratios may also be taken as a fingerprint of the complete S-layer to measure its lateral homogeneity and stability with time on the solid substrates. For this purpose, we have analyzed different positions of the samples using the facility of the XPS small-spot focalization. The thickness of the layer is about 3 nm as determined by ARXPS.

The immobilized cAb onto S-layer was studied through its iodine marker. The detection of the iodine was possible from 1 µg/ml initial concentration. The immobilization of cAb was also followed from the XPS survey spectra. Indeed, the shape of the background of gold spectrum (between the Au signal at 84 eV and the carbon one at 285 eV) changed when a “thick” layer is deposited. This is more clear at high cAb immobilized amount.

DISCUSSION & CONCLUSIONS: S-layer is very complete and homogeneous on the gold substrates; it is 3 nm thick; the detection of immobilized cAb through its iodine marker is possible at high initial concentration.

The mechanism of the interaction between S-layer and cAb may be obtained from the XPS survey spectra. cAb may be attached covalently via its amino groups with the carboxyl groups of S-layer which are exposed at the extreme surface; then, it may intercalate between the pores of the S-layer (since carboxyl groups exist also in the pores).

ACKNOWLEDGEMENTS: The work is performed with the financial support of the EU project “PAMELA”(IST-1999-13478).2000-2003. <http://www.imec.be/PAMELA/>