Tyrosinase-Gold Nanoparticles Conjugates on Nanostructured Gold Surfaces: Towards a Biosensor of Phenolic Compounds

I. Gomes[1], A. P. Serro[2], A. P. Carapeto[2], P. Paradiso[3], J. Cortez[1], S. Cardoso[4], B. Saramago[2] and R. Franco[1]

[1]REQUIMTE, Departamento de Química, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, 2829-516 Caparica, Portugal

[2]Centro de Química Estrutural, Instituto Superior Técnico, Av. Rovisco Pais, 1049-001 Lisboa, Portugal

[3]Departamento de Engenharia de Materiais, Instituto Superior Técnico, Av. Rovisco Pais, 1049-001 Lisboa, Portugal

[4]Instituto de Engenharia de Sistemas e Computadores - Microsistemas e Nanotecnologias (INESC-MN) and Institute for Nanosciences and Nanotechnologies (IN, Associated Lab.), Rua Alves Redol 9, 1000-029 Lisboa, Portugal

inesgomes@dq.fct.unl.pt

The development of enzymatic biosensors based on the tyrosinase enzyme has attracted great interest for the detection of phenolic compounds (from, *e.g.*, pesticides, pollutants) in ground or wastewaters [1,2]. Our goal is to develop a biosensor based on conjugates of tyrosinase on gold nanoparticles (AuNPs), taking advantage of the high surface areas of AuNPs, its unique electrochemical properties and ideal protein conjugation chemistry afforded by suitable functionalization.

Self-Assembled Monolayers (SAM) of thiolates form nanostructured surfaces with a diversity of functionalities and chemical characteristics that can favor the immobilization of enzymes in gold surfaces [3]. The immobilization of enzymes and bioactive conjugates in this type of nanostructured gold surfaces is a highly suited strategy for the development of biosensors with high activity and specificity.

In the present work, the immobilization of tyrosinase-AuNP conjugates on SAMs of alkanethiols on nanostructured gold surfaces was studied by Quartz Crystal Microbalance (QCM) and Atomic Force Microscopy (AFM). AuNPs were functionalized with mercaptoundecanoic acid (MUA) and conjugated with tyrosinase. Tyrosinase (1.14.18.1) is a copper monooxygenase that catalyzes the *o*-hydroxylation of monophenols and the oxidation of *o*-diphenols to *o*-quinones. It contains a dicopper 2^{+} center and its structure is found in three forms: *met*-tyrosinase, *oxi*-tyrosinase and *deoxy*-tyrosinase [4]. Conjugates were adsorbed on the surface of the piezoelectric quartz crystal coated with nanostructured gold, and the change in mass was measured as a shift in the oscillation frequency [5]. The alkanethiols used to build the different SAMs at the gold crystal surface have terminal groups with different chemical characteristics, namely cationic (11-amino-1-undecanethiol hydrochloride) or anionic (MUA).

The catalytic activity of the enzyme adsorbed on to the gold crystal was measured using a spectrophotometric assay do detect the formation of reaction products.
Preliminary QCM results show that the tyrosinase-gold nanoparticles conjugates have a high adsorption on the surface of the gold crystal (Figure 1) comparatively with the deposition of the gold nanoparticles on the cationic SAM.
The AFM images show a high immobilization of the conjugates on the cationic SAM on gold surface, when compared with the Au surface or cationic SAM on Au surface.

References:

[1] Kim, G. *et al.*, J. Environ. Monit., **10**, (2008), 632-637.
[2] Abdullah, J. *et al*., Sensors and Actuators B, **114**, (2006), 604-609.
[3] Love, JC. *et al*., Chem Rev., **105**, (2005), 1103-70.
[4] Gormally, M. V. *et al*., Langmuir, **25**, (2009), 10014-10019.
[5] Gispert, M.P. *et al*., Surf. Interface Anal., **40**, (2008), 1529-1537.

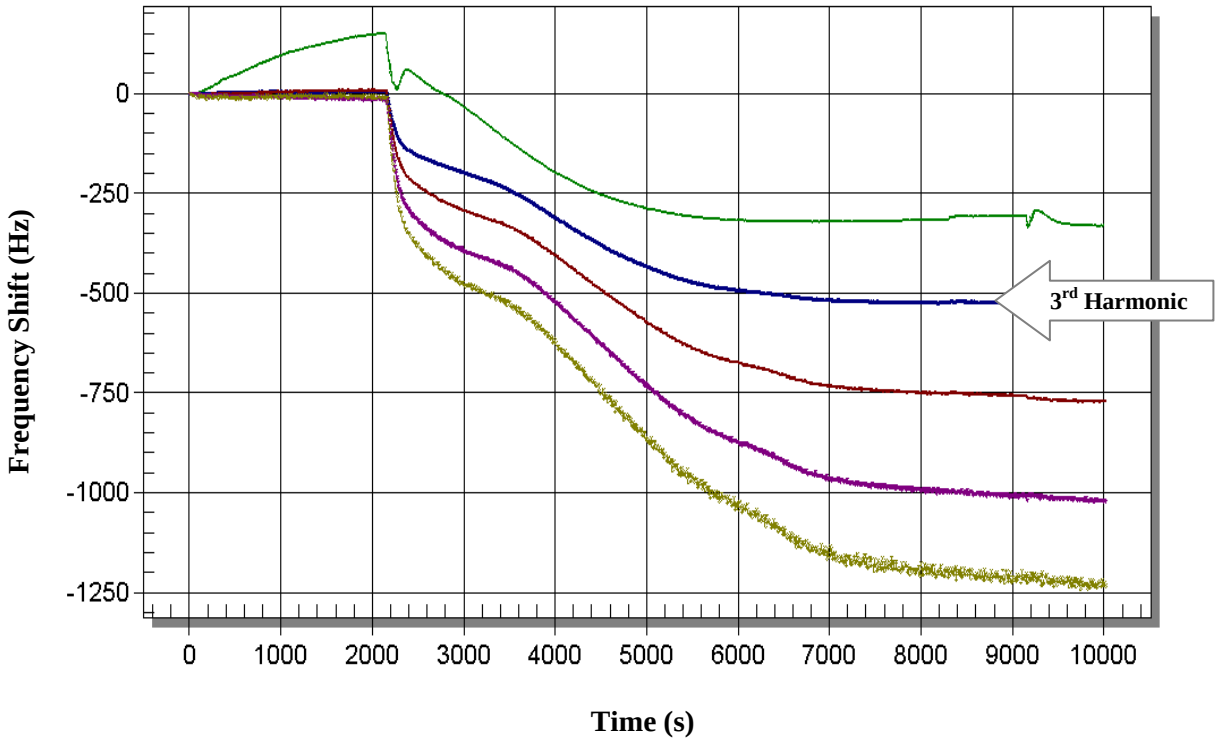

Figure 1. QCM profile for the deposition of the tyrosinase-AuNP conjugates on the cationic SAM on a gold crystal.