Tyrosinase-Gold Nanoparticles Bionanoconjugates on Nanostructured Gold Surfaces: Development of an Enzymatic Biosensor of Phenolic Compounds

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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 [1]. The development of enzymatic biosensors based on the tyrosinase enzyme has attracted great interest for the detection of phenolic compounds (pesticides, pollutants) in ground or wastewaters [2,3]. Our objective is to develop a biosensor based on bionanoconjugates 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 [4]. 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 bionanoconjugates of tyrosinase and gold nanoparticles on nanostructured gold surfaces containing SAMs of alkanethiols, was studied by Quartz Crystal Microbalance (QCM) and Atomic Force Microscopy (AFM). AuNPs were functionalized with mercaptoundecanoic acid (MUA) and second conjugated with tyrosinase, producing bionanoconjugates. The tyrosinase-MUA-AuNP bionanoconjugates were then adsorbed on the surface of the cationic SAM (11-amino-1-undecanethiol hydrochloride) on the piezoelectric quartz crystal coated with gold, and the change in mass was estimated from the shift in the measured oscillation frequency [5]. The catalytic activity of the enzyme adsorbed on to the gold crystal was measured using a spectrophotometric assay to detect the formation of reaction products.

QCM results confirm the high adsorbed amount of tyrosinase-MUA-AuNP bionanoconjugates on the surface of the gold crystal, comparatively with the lower values that were obtained for the deposition of the MUA-AuNP alone on the same type of cationic SAM (Figure 1).
AFM was used as a tool for the visualization of bionanoconjugates on gold surfaces deposited on silicon substrates. The bare surfaces topography enabled the observation of individual bionanoconjugates and provided information on the bionanoconjugates-surface interaction.
AFM images show high immobilization of the conjugates on the cationic SAM (Figure 2), when compared with the gold surface alone or the cationic SAM on gold surface.

References:

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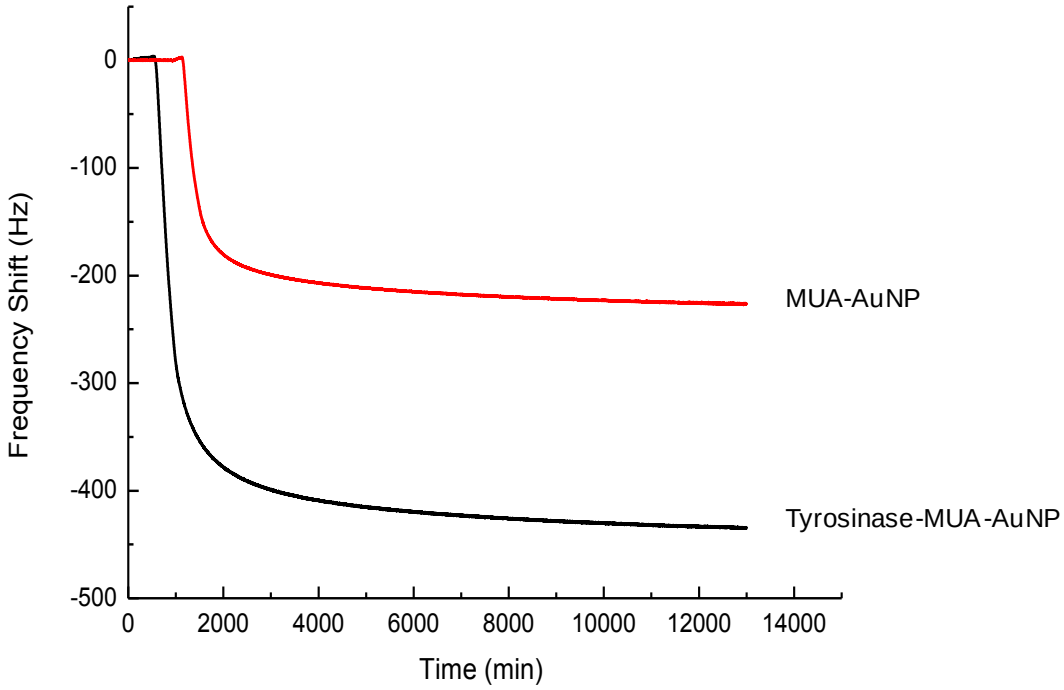

Figure 1. Change of frequency in the 3rd harmonic, obtained by QCM for the deposition of Tyrosinase-MUA-AuNP bionanoconjugates and MUA-AuNP alone on the cationic SAM on a gold coated crystal.

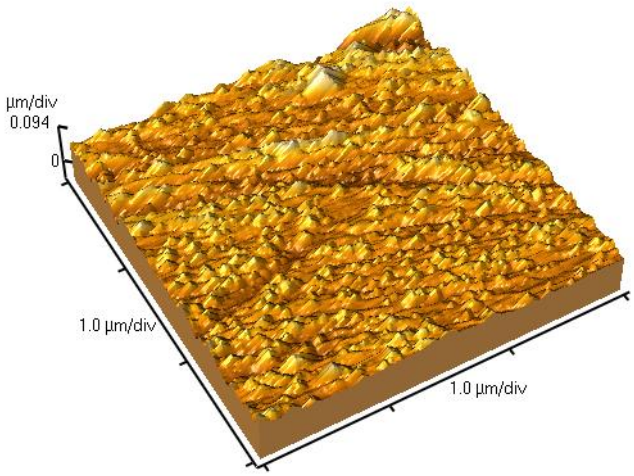

Figure 2. 3D AFM image of the immobilization of Tyrosinase-MUA-AuNP bionanoconjugates adsorbed on cationic SAM over the gold surface.