Functionalization of electrode surfaces with three-dimensional networks of electropolymerized gold nanoparticles for biosensor design

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Abstract

Nowaday, the construction of new electrochemical biosensors with improved bioanalytical performance and robustness is directly linked to the development of novel strategies for tailor-made design of electrode surfaces. Such strategies should provide electrical interfaces with nano- or microsized tree-dimensional topology that allow the successful and stable immobilisation of the biomolecules without affecting their biological function, but also favouring the fast and efficient occurrence of the electrochemical processes involved in the analytical reaction on such interface.

During last recent years, nanosized materials have been exhaustively employed in the modification of electrodes for biosensing purposes [1]. In general, the development of methodologies for nanostructuring electrode surfaces with such purpose should consider the following points: i) the type of surface to be modified, ii) the nanomaterial to be used, iii) other compounds/materials that could be also employed, iv) the physical/chemical method for surface modification, v) the biomolecule to be immobilized and the method to do that, and vi) the electrochemical reaction to take place on the functionalized electrode surface.

The present work describes an original approach for nanostructuring electrodes surfaces, based on the electrochemical preparation of a three-dimensional matrix of gold nanoparticles through the formation of bisaniline-cross-linked networks. These nanostructured networks were further employed as supports for the immobilization of redox enzymes for the construction of electrochemical biosensors.

The rationale of our strategy is based on the design and synthesis of small polyfunctionalized gold nanoparticles (2.5-5.7 nm diameter), specifically capped with three different thiol derivatives: *p*-aminothiophenol as polymerizable unit, 2-mercaptoethanesulfonic acid as solubilising moiety and a third thiol ligand which should be employed for enzyme immobilization (Fig. 1). In the present work, we used as third capping ligands the following thiol derivatives:

A) 3-Mercaptophenyl boronic acid for the oriented immobilization of the glycoenzyme horseradish peroxidase [2].
B) Cysteamine core polyamidoamine (PAMAM) G-4 dendron for the multipoint covalent immobilization of tyrosinase using glutaraldehyde as cross-linking agent [3].
C) 1-Adamantanethiol for the supramolecular immobilization of a cyclodextrin-xanthine oxidase neoglycoconjugate through host-guest interactions.

The nanoparticles capped with the ligands described in A) and B) were electropolymerized on gold electrode surfaces, previously coated with a monolayer of *p*-aminothiophenol. Horseradish peroxidase was further immobilized on the boronic acid-modified nanostructured matrix (Fig. 1A), yielding a wired enzyme electrode which was employed to construct a third generation amperometric biosensor toward H_2O_2. This biosensor showed excellent analytical characteristics, with a linear behavior in the range between 5 μM and 1.1 mM of H_2O_2, a high sensitivity of 498 $\mu A \cdot M^{-1} \cdot cm^{-2}$, and a low detection limit of 1.5 μM.

On the other hand, tyrosinase was covalently cross-linked on the electrode surfaces covered with the PAMAM dendron-coated Au nanoparticle networks (Fig. 1B). The biosensor constructed with these electrodes showed a very low detection limit of 20 nM toward cathecol, with a linear response from 50 nM to 10 μM of this analyte and a sensitivity of 1.94 $A \cdot M^{-1} \cdot cm^{-2}$.

Au nanoparticles coated with 1-adamantanethiol moieties were finally electropolymerized on glassy carbon electrodes previously coated with single walled carbon nanotubes (Fig. 1C). This nanostructured surface was used for the supramolecular immobilization of a β-cyclodextrin-xanthine oxidase neoglycoconjugate, for the design of a biosensor device toward xanthine. This

biosensor showed an excellent electroanalytical behavior, with a low detection limit of 30 nM and a sensitivity of 543 $mA \cdot M^{-1} \cdot cm^{-2}$.
All enzyme biosensors prepared also showed high reproducibility, selectivity and stability. Taking into account these results achieved in this research, it can be predicted that the use of a bisaniline-cross-linked nanostructured network of metal nanoparticles constitutes an excellent nanoelectrochemical strategy to construct scaffolds for the successful immobilization of redox enzymes in order to prepare amperometric biosensors with improved analytical characteristics.

References

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Figures

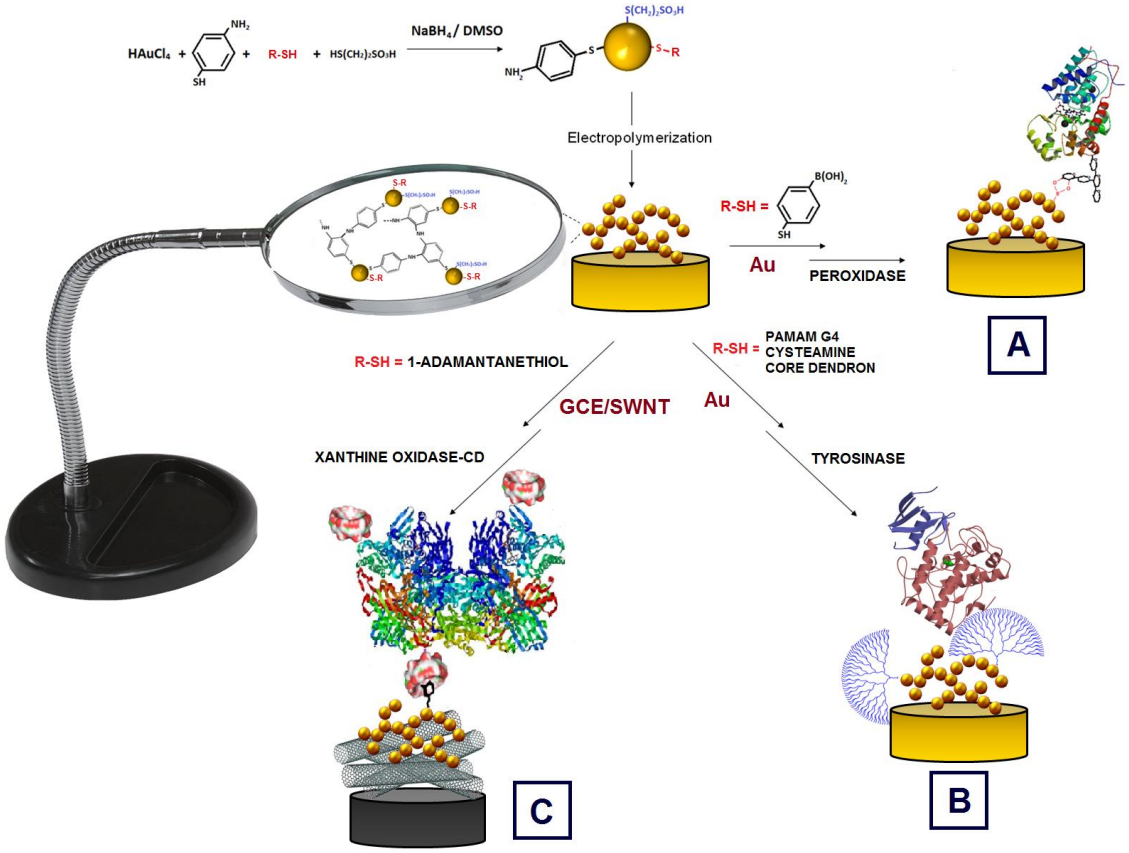

Figure 1. Preparation of the electrodes modified with electropolymerized Au nanoparticles-based networks for the detection of H_2O_2 (A), cathecol (B) y xanthine (C).