

3D biological architectures based on SWCNT and electropolymerized functionalized polypyrrole films  
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Rapid detection and monitoring in clinical and food diagnostics, environmental and bio-defense monitoring has paved the way for the elaboration of alternative, state-of-the-art analytical devices, known as biosensors (a generic term involving enzymatic sensors, DNA sensors, immunosensors, protein sensors and biochips). With the aim to improve the biosensor sensitivity and the performance of biofuel cell, 3D structures were designed to create a high density of biological entity at the surface of the electrode. In particular, many research efforts have been focused on the combination of carbon nanotubes (CNT) with electrogenerated polymers for designing biosensors [1-4]. Due to the geometry of the carbon nanotubes, this material has an impressive high specific surface of more than 1000 m<sup>2</sup>/g that makes CNT a promising candidate for the construction of highly porous three dimensional nanostructured frameworks. Biosensors based on CNT-polymer composites were commonly prepared by electropolymerization of a monomer in the presence of biomolecules on electrodes already modified by adsorbed CNT coatings. However, the entrapment within the polymer matrix drastically reduced the accessibility to the immobilized biomolecules. This constitutes a major drawback for the development of immunosensors, DNA and protein sensors that requires the positioning of the biomolecule at the polymer-solution interface.

In this context, we report herein the functionalization of single walled carbon nanotube (SWCNT) by electropolymerized films providing affinity interactions. The latter constitute a convenient reagentless approach that provides the biomolecule attachment by a single binding preserving thus its accessibility. One of the most attractive affinity binding systems for the attachment of biomolecules was based on the well-known avidin-biotin procedure that was widely used for ELISA assays. The avidin-biotin strategy is based on the extremely specific and high affinity interactions between four biotins, a vitamin, and the glycoprotein avidin (association constant  $K_a = 10^{15} \text{ M}^{-1}$ ) that lead to strong associations similar to the formation of a covalent binding. We have thus combined the advantages of the high specific conductive surface of SWCNT coatings with those of biotinylated polymer. In particular, the attachment of biotin to the nanotube was carried out by the electropolymerization of a pyrrole biotin derivative onto the SWCNT sidewalls. Thanks to the easy possibility to form avidin bridges between biotinylated biomolecules, and the biotinylated polypyrrole, this film enabled successively natural attachment of avidin and biotinylated probes. The performance of such obtained electrodes for the specific anchoring of biological macromolecules was then investigated with a biotinylated glucose oxidase (GOX) as a model of biotinylated protein. It should be noted that  $J_{\text{max}}$  values (5-15  $\mu\text{Acm}^{-2}$ ) reported for biotinylated poly(pyrrole) films modified by a compact GOX monolayer [[5, 6] were markedly lower than that (178  $\mu\text{Acm}^{-2}$ ) observed with the GOX-biotinylated polymer-SWCNT configuration. The comparison of these values

shows an increase in the amount of immobilized GOX at least by a factor of 12 that demonstrates the 3D character of the bioarchitectures conferred by the SWCNT deposit. Another approach consists in the synthesis and electropolymerization of an adamantane-pyrrole derivative (Figure 1) as a new affinity binding polymer. This affinity system based on the interactions between polymerized adamantane and  $\beta$ -cyclodextrin conjugated molecules is mimicking the biological avidin-biotin interactions. An attractive perspective of this affinity system lies in the removal of the intermediate avidin layer as building block and hence in the suppression of the detrimental effect on the biosensor performance due to the presence of a protein bridge. The construction of the SWCNT- poly(pyrrole adamantane) biosensor was carried out using  $\beta$ -cyclodextrin gold nanoparticles as intermediate layer for the specific anchoring of adamantane-tagged GOX.

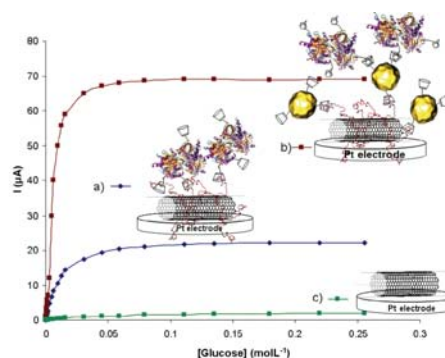


Figure1: Sketch of SWCNT electrodes and related calibration curves for glucose. (c) pure SWCNT coating and SWCNTs covered by poly(pyrrole-adamantane) film post-functionalized by (a)  $\beta$ -cyclodextrin tagged GOX; (b) adamantane-tagged GOX- $\beta$ -cyclodextrin modified gold nanoparticle.

The resulting glucose sensor exhibits the highest  $J_{\text{max}}$  value, (350  $\mu\text{Acm}^{-2}$ ) illustrating the advantage of this design of nanostructured biological architecture [7].

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