Self Assembled and Electrochemically Deposed Layers of Thiols on Gold Compared with Electrochemical Impedance Spectroscopy and Atomic Force Microscopy

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Abstract. Self-Assembling is based on a spontaneous process in which organic molecules (alkanethiols, silanes) are adsorbed on a substrate (gold, glass, silicon). Although the implementation is extremely easy, it shows a big disadvantage in timing, because the solution has to be in contact overnight with the substrate under mild shaking. An alternative method of molecular deposition is the Electrochemically Deposed Multilayers commonly used in our laboratory for further immobilization of biological molecules in order to obtain specific biosensors for several analytes. It consists in applying a constant potential on gold working electrode (1.3 V vs. Ag/AgCl) for driving molecules in proximity of the electrode and allow them to react on the surface and form a layer similar to self assembled ones. Both the layers, self assembled and electrochemically deposed ones, were tested with Electrochemical Impedance Spectroscopy and Atomic Force Microscopy. The substrate electrochemically covered shows a higher and a more homogeneous deposition than self assembled one and the deposition time is extremely reduced from several hours to a few of seconds (50 s).

Keywords: Self assembled monolayer · Electrochemical deposition · Thiols · Biosensors · Sensors

1 Introduction

Self-Assembly is a spontaneous process in which organic molecules (alkanethiols or silanes) are chemisorbed on a 2D substrate (gold, glass, silicon, ceramic materials as well as Al_2O_3, TiO_2, ZnO_2 et cetera) forming a highly ordered monolayer architecture. Self Assembled Monolayers (SAMs) of thiols behave like building elements for which the term “nanoLego” has been coined for similarity with the toys for children. So (bio) sensors could be designed to detect analytes as well as Hg(0), Hg(II), ochratoxin-A, *E. coli*. Figure 1 shows some examples of sensing nano-structures built on gold

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electrodes starting from a thiol precursor ranging from poly-oligonucleotides (poly-thymine), antibodies (anti-ochratoxin A) and specific polymers (limonene).

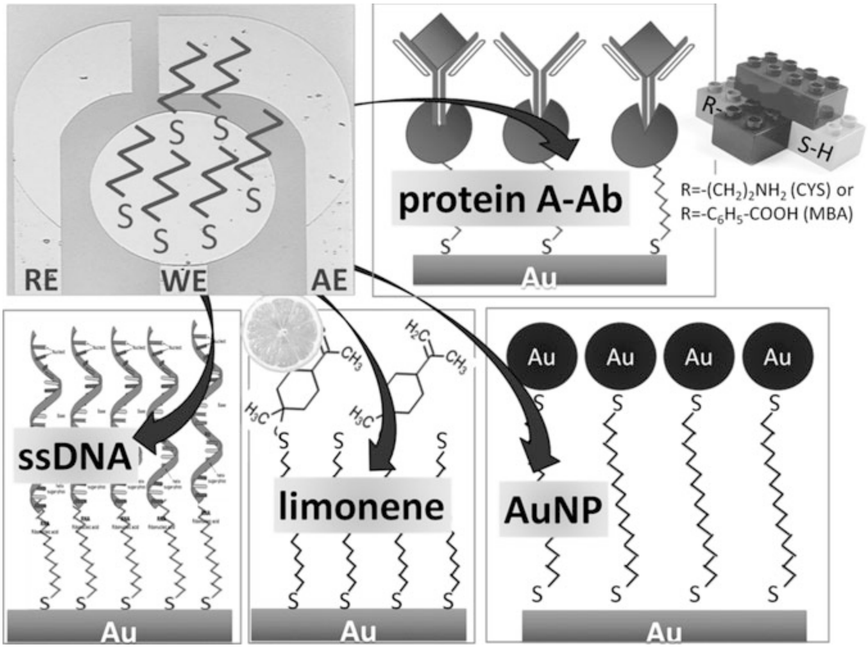

Fig. 1. Sensing nano-structures (oligonucleotides, antibodies, polymers) realized on gold electrodes via thiol precursor

Although the implementation is extremely easy, an overnight treatment after cleaning of the substrate (Au electrodes) with cyclic voltammetry (CV) (inset in Fig. 2) is required. By this way SAMs are deposed not only on working electrode (WE) but also on reference (RE) and auxiliary (AE) ones. Electrochemically Deposed Multilayers (EDMs) are commonly adopted in our lab [1–3] for immobilization of molecular structures in order to obtain (bio)sensors for several analytes [4–7].

2 Materials and Methods

Thiols, as well as Cysteamine (CYS) or 4-mercaptobenzoic acid (MBA), were purchased from Sigma-Aldrich and deposed on a commercial gold three electrodes—Micrux Technologies (Oviedo, Spain). The electrode cleaning, the thiol deposition and the impedimetric analysis were obtained with PalmSens3 (Netherlands), a portable potentiostat/galvanostat and impedance analyzer. At first, the cleaning of Au WE was performed with cyclic voltammetry (CV) at ±1.2 V versus Ag/AgCl pseudo-RE in H_2SO_4, 0.1 M, then Electrochemical Impedance Spectroscopy (EIS) has been performed on the bare electrode in equimolar potassium hexacyanoferrate $K_3[Fe(CN)_6]$/

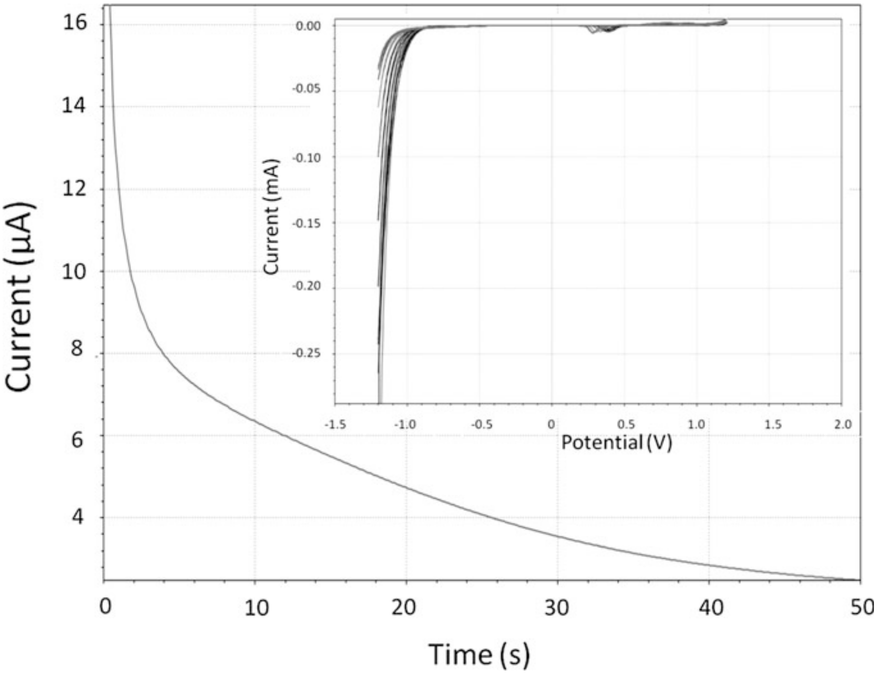

Fig. 2. Current intensity of CYS (20 mM) deposition on Au WE at +1.3 V versus RE. *Inset* Cleaning of AuWE by CV (±1.2 V) in H_2SO_4, 0.1 M, scan rate = 1 V/s, E_{step} = 5 mV

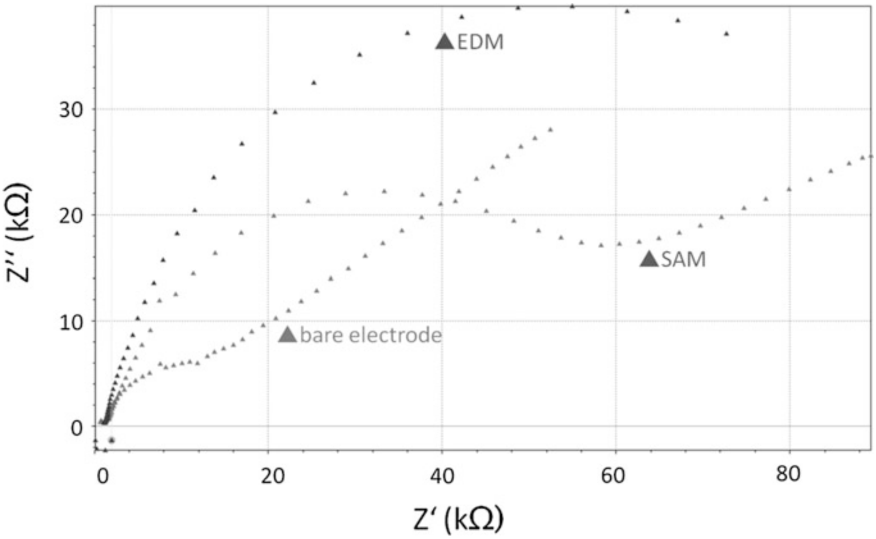

Fig. 3. EIS of bare and cleaned, CYS-SAM, and CYS-EDM AuWEs in 1 mM $K_4/K_3Fe\ (CN)_6$

$K_4([Fe(CN)_6]$ 0.1 M, at 0.0 V versus RE and with an alternate potential of 10 mV exploring a frequency range between 1 Hz and 20 kHz. The thiol deposition was made with a 20 mM solution by self-assembling overnight or by chronoamperometry at +1.3 V versus RE (50 s). Successively, a new EIS of the thiolated electrode was performed. Surface analysis were obtained with Atomic Force Microscopy (AFM, a Nanosurf Flex-ANA).

3 Experimental and Results

Constant potential drives thiols in proximity of a single AuWE (not AE or RE) allowing addressing in an electrode array (Fig. 4) and reaction on the Au surface with formation of an EDM in only 50 s (Fig. 2) instead of hours as needed for SAMs. Thiols as well as cysteamine (CYS) or 4-mercaptobenzoic acid (MBA) have been deposed as SAMs or EDMs on AuWEs.

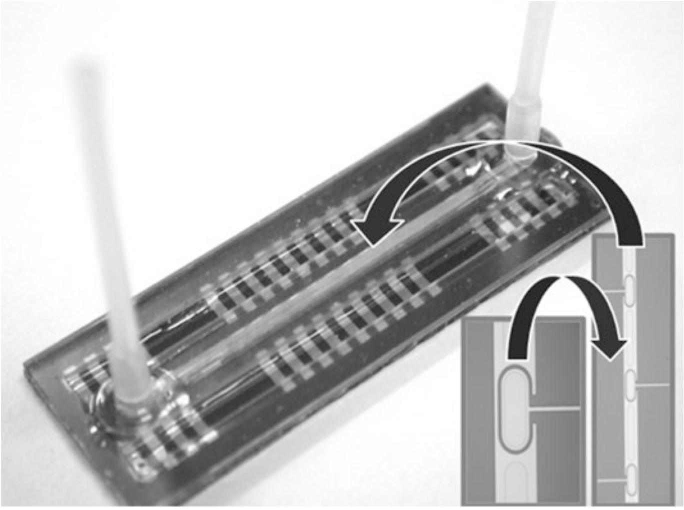

Fig. 4. Multiplexed 16 AuWEs μ-array and μ-flow-cell

Both the layers have been studied with EIS (Fig. 3) and AFM (Fig. 5). By comparison of the impedance spectra for SAMs and EDMs, we can deduce that EDMs shows higher impedance values (Fig. 3), on the other hand, thanks to AFM images, we establish that EDMs is thicker (Fig. 4) than SAMs. SAM shows Au electrodes poorly covered with a thinner film with holes where the bare Au electrode is exposed (poor electrochemical performances of further obtained biosensors). On the contrary the substrate covered with EDMs shows a more homogeneous and smooth surface (Table 1).

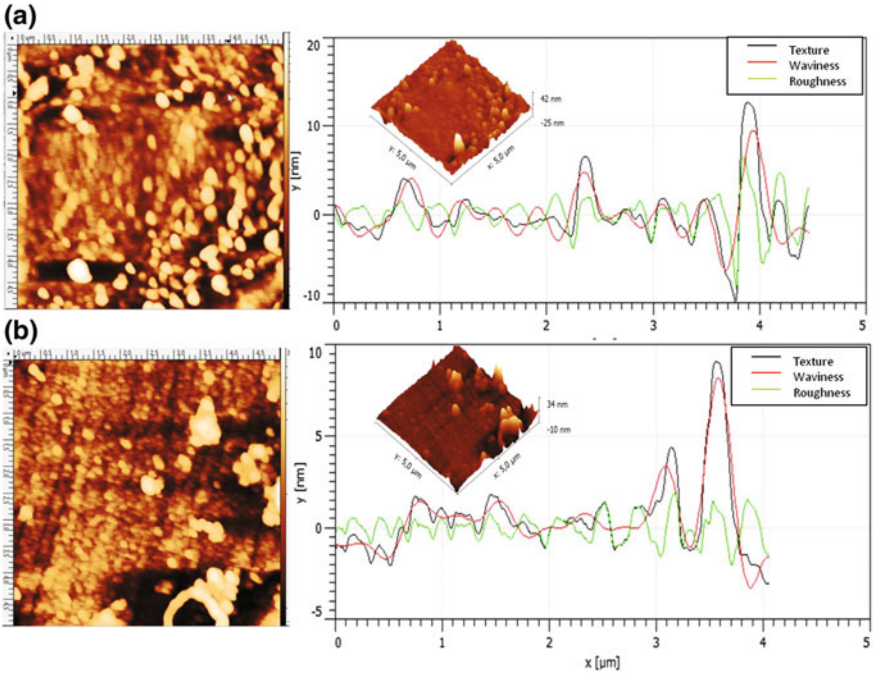

Fig. 5. AFM scans: topographies, 3D views and roughness parameters of SAMs and EDMs on AuWE

Table 1. Comparison between roughness and waviness values for SAM and EDM

	SAM (nm)	EDM (nm)
Ra	1.37	0.56
Rt = Rv + Rp	14.83	3.93
Rv	8.35	2.02
Rp	6.48	1.91

Ra average roughness, *Rp* highest peak, *Rv* deepest valley, *Rt* total roughness

4 Conclusions

EDMs are faster to realize (in 50 s) than SAMs (overnight). They allow molecular addressing on a specific electrode in an array for obtaining multiparametric biosensors. Nano-structures can be easily removed from gold surface at −1.3 V versus RE allowing reversible deposition of new molecules on gold electrode [3]. Additionally, the multilayer offers a higher impedance in EIS although is thicker and more homogeneous than SAMs. Furthermore, the EDMs surface is smoother with a minor number of holes as described by several roughness parameters as Ra, Rt, Rp and Rv.

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