Yeast cytochrome c on gold electrode: a robust hybrid system for bio-nanodevices

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***Abstract* — Yeast cytochrome c has been directly self-chemisorbed on bare gold electrodes via its native free sulphur-containing group Cys102. Topological, spectroscopic and electron transfer properties of immobilised molecules have been investigated by *in situ* Scanning Probe Microscopy and Cyclic Voltammetry. Atomic Force and Scanning Tunnelling Microscopy reveal individual protein molecules adsorbed on the gold substrate, with no evidence of aggregates. The electrical conductivity has been investigated also by Conductive Atomic Force Microscopy. The adsorbed proteins appear firmly bound to gold and display dimensions in good agreement with crystallographic data. Cyclic Voltammetry analysis shows that the electrode surface is functionalised with electro-active proteins, at high coverage, with a measured redox midpoint potential in good agreement with the formal potential. Additionally, the vibrational features of the cytochrome adsorbed on gold have been studied by Surface Enhancement Raman Spectroscopy. Our results clearly indicate that this variant of cytochrome c adsorbs on bare gold electrodes preserving morphological properties and redox functionality.**

***Index Terms* — Yeast Cytochrome c, Scanning probe microscopy, SERS, single molecule.**

I. Introduction

The integration of biomaterials with electronic transducers, such as electrodes, field-effect-transistors and piezoelectric crystals is a crucial issue toward the development of low dissipation, highly sensitive, bioelectronic nanodevices [1,2]. In this perspective, redox metalloproteins have attracted much attention, thanks to their low dimension and electron transfer (ET) properties which make them a good candidate in view of applications in hybrid submicrometer-sized electronic components [3]. A key requirement for such applications is the ability to connect functional redox proteins to the electrode, preferably via chemical bond, to achieve good electrical contact between the molecule and the conducting substrate [4].

In this framework, we have focused on self-chemisorbed yeast cytochrome c (YCC) on bare gold electrode. The presence in this species, differently from other cytochromes, of an additional sulfur containing group (Cys102) not directly bound to the heme and close to the protein surface, makes this protein interesting for direct immobilization on gold electrodes [5,6]. Morphological properties and functionality of YCC molecules chemisorbed on bare Au(111) have been deeply investigated at the single molecule level by high-resolution microscopy techniques, such as Scanning Probe Microscopy (SPM), and Surface Enhanced Raman Spectroscopy (SERS), also integrated with Cyclic Voltammetry (CV).

II. Materials and methods

YCC, purchased from Sigma Chemical Co., has been used without further purification and dissolved in 1mM TRIS buffer (pH 8.0) to a concentration of 2.6 μM. Substrates (from Arrandee^{TM}) consist of a vacuum evaporated thin gold film (thickness 200 nm) on borosilicate glass. These substrates have been flame-annealed to obtain recrystallized Au(111) terraces. Afterwards, they have been directly incubated with the protein solution at 4ºC for times ranging between 30 minutes and few hours, both for SPM and Cyclic Voltammetry experiments. Tapping mode Atomic Force Microscopy (TMAFM) images have been acquired by a NanoscopeIIIa/Multimode scanning probe microscope (Digital Instruments) equipped with a 12-μm scanner.

The Scanning Tunnelling Microscopy (STM) measurements have been performed with a PicoSPM (Molecular Imaging, Co) equipped with a Teflon cell and a PicoSTAT bipotentiostat/galvanostat. STM images have been acquired either in ambient conditions or under electrochemical control. Conductive AFM measurements have been performed by using a PicoSPM (Molecular Imaging, Co) equipped with a current sensing module

with a sensitivity of 1V/nA. Experiments have been done under nitrogen atmosphere.

CV on YCC monolayer adsorbed on Au(111) has been performed with a PicoSTAT bipotentiostat (Molecular Imaging Co).

The SERS spectra of YCC on gold have been recorded through a Jobin-Yvon Labram confocal system by exciting with the 514.5 nm radiation, equipped with a nitrogen cooled CCD detector. The spectral resolution is lower than 5 cm^{-1}. The laser spot size is about 1 μm^2 and the incident power 15 mW.

III. RESULTS AND DISCUSSION

TMAFM experiments revealed homogeneous and stable adsorption of YCC molecules onto Au(111) substrates, where individual YCC molecules can be clearly discerned [5]. The high quality of single protein images, even after repetitive scans, is indicative of a strong binding to gold, as expected for stable covalent immobilization (see Fig.1).

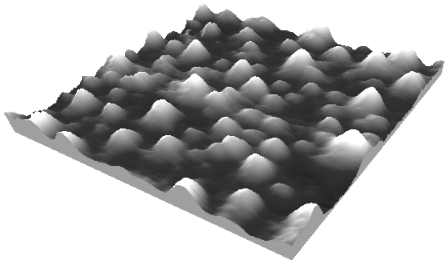

Fig. 1. Topographic image of YCC molecules adsorbed on Au(111) as measured by TMAFM in buffer solution. Scan area: 200 x 200 nm^2. Vertical range: 6 nm.

Protein lateral dimension, and height above gold substrate, as obtained after deconvolution with the tip radius curvature, well agree with crystallographic data, consistently with a non-denaturing adsorption.

The redox functionality of YCC molecules upon immobilization on the gold electrode has been assessed by CV experiments (see Fig.2).

The redox potential indicates a preserved redox functionality of adsorbed molecules; a coverage up to 84% of the Au (111) electrode substrate having been obtained.

YCC molecules adsorbed on Au(111) substrates, have been imaged by STM in ambient conditions. A typical STM image recorded in air from a sample incubated for about one hour with the protein solution is shown in Figure 3.

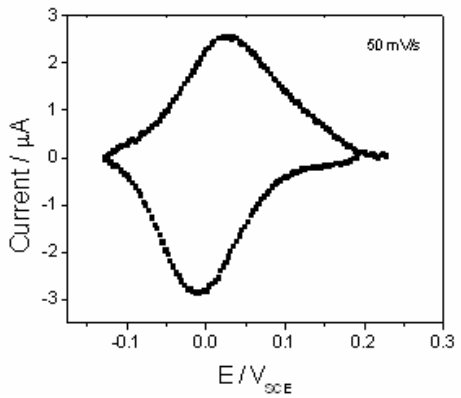

Fig. 2. Cyclic voltammogram of YCC on Au(111) electrode after subtracting the faradaic current of the bare substrate. Data are recorded in buffer solution at a potential scan rate of 50 mV/s.

Self-chemisorbed proteins are stable and give reproducible images during repetitive scans. To verify the robustness of the single protein-gold bond, the same area has been consecutively imaged at different tunnelling currents, from 50 pA up to 1 nA and bias fixed at -0.3 V. In such a way, the tip-protein distance has been reduced, i.e. the local tip-molecule interaction has been increased. Despite this procedure, none of the imaged YCC isolated proteins has been swept away, confirming a robust binding of YCC to gold.

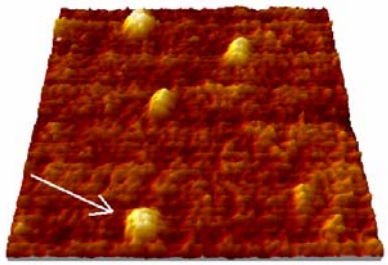

Fig. 3. STM image of YCC in ambient conditions. Scan area: 80 x 80 nm^2. Tunnelling current 100 pA, V_{bias} -0.300 V (tip positive), scan rate 1.5 Hz.

Conductive AFM experiments according to the configuration shown in Fig.4, has allowed gaining deeper insights into the ET properties of adsorbed single molecules. In order to minimize any contribution to the current signal from the water molecules surrounding the bio-molecules, the relative humidity has been reduced by purging the measuring chamber with nitrogen.

To avoid any damaging of the metal coating at the tip and possible contamination, the monolayer of YCC on

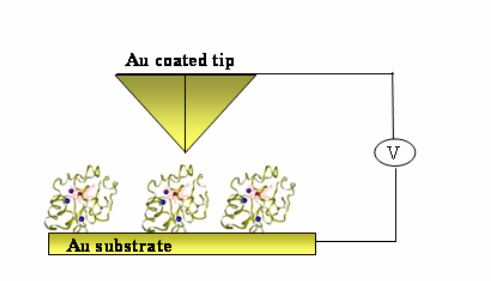

Fig.4. Au coated tip in contact with cytochrome c monolayer immobilized on Au(111) substrates through a thiol group opposite to the heme functional group.

gold has been electrically investigated by local I-V spectroscopy. From the slope of the I-V plots in the ohmic region, the resistance has been obtained as a function of the force exercised by the conductive AFM probe. Figure 5 shows the typical response obtained on a monolayer of YCC molecules sandwiched between the Au(111) substrate and the gold coated tip. In the range of forces explored, resistance shows almost three different regimes. Such a complex dependence of the resistance on the applied force is very difficult to be described in term of simple transport mechanism. An increase in the contact area between tip and YCC monolayer may explain the exponential decrease of resistance up to 6.3 nN. The overcoming of a sort of contact resistance at the tip/YCC interface may justify the jump at 7 nN. Finally, an inner structural reorganization of YCC may cause the discontinuity at 14 nN.

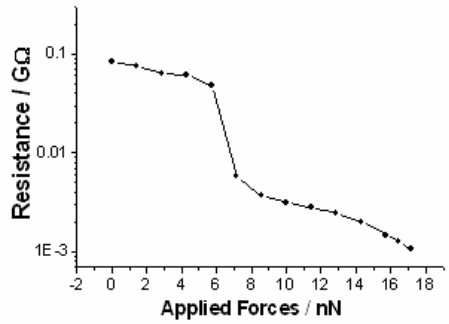

Fig.5. Resistance vs. applied force on a monolayer of YCC molecules chemisorbed on Au(111) as measured by conductive AFM.

A spectroscopic characterization at level of single molecule of YCC immobilized on gold substrate has been obtained by SERS investigation. From the analysis of SERS spectrum, shown in Fig.6, it comes out that YCC molecules still maintain their vibrational features, once adsorbed on bare gold substrate. Furthermore, the analysis of specific Raman modes provides some insight on the heme orientation with respect to the metallic surface.

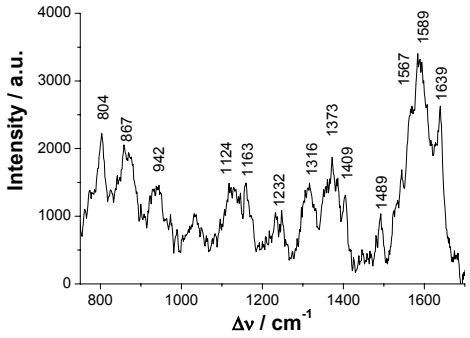

Fig.6. SERS spectra of a monolayer of YCC molecules chemisorbed on Au(111); the main peaks being labeled.

IV. Conclusion

We have shown that it is possible to successfully adsorb on metal electrodes, a high concentration of cytochrome c, with preserved redox properties and morphological characteristics. This deserves a remarkable importance in view of applications in biosensors and biocatalytic devices.

Acknowledgement

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