AC and DC electrical imaging of biosamples at the nanoscale by Atomic Force Microscopy

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Abstract. We present a new tool and measurement protocol based on Atomic Force Microscopy for the AC and DC electrical characterization of biological samples at the nanoscale. The new developments allow performing a variety of electrical measurements (DC conductivity, AC small signal impedance) both in spectroscopic mode (i.e. in a single point) as well as in imaging mode (i.e. by providing a map of the electrical properties with nanometric spatial resolution) with minimum sample damage. Successful test measurements have been carried out on Purple Membrane.

1. Introduction

The development of biosensors with active areas in the nanoscale as well as the understanding of the fundamental electronic transport properties of biosamples (membranes, proteins, etc.) makes necessary the development of new tools and measurement protocols for the electrical characterization of biological samples at the nanoscale. One of the tools that is currently being adopted for these purposes is the Atomic Force Microscopy (AFM). AFM has nanoscale spatial resolution, it is able to work under liquid physiological environment, it can be adapted to perform a variety of electrical and electrochemical measurements and it allows controlling the force applied to the biosamples.

In spite of some recent efforts, very few results are available in the current literature on the electrical properties of biological samples at the nanoscale. In fact, until now only spectroscopic (i.e. single point) electrical DC current-voltage characteristics on a few biological samples have been reported; on DNA [1], on azurin [2], on ferritin [3], and photosynthetic complexes [4]. To our knowledge, neither AC spectroscopic measurements nor electrical images (DC or AC) have been reported so far.

The reason for the absence of AC measurements is related to the requirement of a very sensitive instrument able to measure for instance capacitance of a few ~attoFarads. Moreover, the absence of electrical images (both DC and AC) is related to the difficulties in minimizing shear forces during a scan in contact mode. These shear forces are usually high enough to irreversibly damage most biological samples under study.
In the present work we extend the current state of the art by presenting a new instrument and protocol able to perform DC and AC electrical measurements on Purple Membrane in both spectroscopic mode (in a single point) and in imaging mode with nanometric spatial resolution.

2. Materials and Methods

2.1. Atomic Force Microscope set up

To perform the measurements we have adapted a commercial Atomic Force Microscope (Nanotec Electronica, S.L.) by connecting it to a fully-customised low-noise wide-bandwidth current amplifier [5] in a lock-in detection scheme. The new set up allows carrying out simultaneous measurements of the sample topography and a number of electrical signals (DC current and AC impedance components, namely imaginary $Z_{im}$ and real part $Z_{re}$) with nanoscale spatial resolution. The specifications of the new electronic set up are summarized in Table 1.

Using the described set up, we have shown that the instrument is able to resolve the electrical properties of interest, notably the local capacitance, with nanometric spatial resolution in the three dimensions Fumagalli et al., in press.

The measurements have been performed under nitrogen $N_2$(g) atmosphere in order to avoid the contribution from any water layer. Conductive boron doped diamond coated tips have been used throughout the measurements.

| Table 1 |
|------------------|------------------|
| **DC performance** | **AC performance** |
| Bandwidth | BW $\sim [0.60]$ Hz |
| Minimum current | $\sim 0.3\text{pA}$ |
| Maximum current | $\sim 10\text{nA}$ |
| Bandwidth | BW $\sim [60,1M]$ Hz |
| Minimum capacitance* | $\sim 1\text{aF@10KHz}$ |
| Maximum resistance* | $\sim 10\text{T}\Omega \text{ @10KHz}$ |

*measurement time of 1s, AC voltage applied of 1V

2.2. Measurement protocols

In order to perform the electrical measurements in spectroscopic mode (i.e. in a single point of the sample) we have combined dynamic mode with a stepwise approach towards the sample. In a first stage, dynamic mode is used to locate and to position the AFM tip over the area of interest. In a second stage, the cantilever oscillation is stopped and the AFM tip is approached stepwise towards the sample. At each step the applied bias is scanned in the forward and backward directions in open feedback conditions. The use of stiff cantilevers allows minimizing the influence of electrostatic forces in cantilever motion, therefore measurements over a broad range of applied bias can be performed while causing minimal sample deformation. If a 40N/m spring constant cantilever is used, cantilever deformations are kept below 1nm even at applied bias up to 9V. It is important to be able to apply high enough voltages so the whole range of electrical behaviour can be fully characterized.

DC and AC imaging require the application of a bias between sample and tip. At DC imaging the bias is kept constant whereas for AC imaging the bias is a sinusoidal sweep in a small range of voltages ($<1$V). The electrostatic forces on the cantilever are constant in DC imaging. In the case of AC
imaging the electrostatic forces on the cantilever are two orders of magnitude smaller than in the spectroscopic mode commented before. Both at DC and AC imaging, cantilevers of low spring constant can be used. Typically, cantilevers with spring constant around 0.2 N/m are selected for these modes.

2.3. Biological samples

The equipment and methodology developed have been tested in Purple Membrane (PM) which is a two dimensional crystalline membrane lattice composed by membrane protein bacteriorhodopsin (bR) in 75% and lipid molecules in 25%. bR is a photosynthetic protein and belongs to the 7 TransMembrane superfamily, being responsible for the solar energy harnessing at Halobacterium.

PM was extracted from Halobacterium, which was exposed to an anaerobic and highly salted environment. Once purified, the PM comes out as patches whose thickness is of 5 nm and whose lateral dimensions are in the range of some microns.

PM samples were deposited on a substrate of gold evaporated on mica (Scientec S.L.). The method followed was to place a drop of suspension containing the PM or the nano-liposomes on top of the gold substrate and it was let to rest for 10 to 15 minutes and removed by using an air gun.

The studies shown herein have been performed in N₂(g) atmosphere in order to avoid the formation of a water layer on top of the sample [6]; in this way the AFM tip is able to have a direct electrical contact with the sample.

3. Results

Purple membrane was imaged using the Jumping Mode. Simultaneous topographic and DC conduction imaging of a patch of PM deposited on top of the gold substrate was performed, Fig. 1. DC images show strong contrast between the membrane and the underlying gold substrate indicating an insulating nature of the purple membrane with respect to electron transport.

A further insight into PM conduction mechanisms was obtained by spectroscopic mode characterization of a single point of the PM. Results allowed to characterize the electrical properties of the PM under different degrees of deformation. In Fig. 2 the evolution of the current-voltage curves during the experiment is shown. The block of data recorded is able to provide the evolution of the current with distance for a single bias as well as the evolution of the current with bias for a single tip-substrate distance. The analysis of the curves demonstrate that current flow takes place exclusively by means of quantum mechanical tunneling and that different conduction regimes are present depending on the applied bias, as will be detailed elsewhere.

A study of the dielectric properties of the PM was performed using AC imaging in jumping mode. The Jumping mode succeeded in causing no sample damage. Fig 3 shows the topography and capacity images obtained on the PM. The area showed consists in two PM patches piled up one on top of the other. The patch above is of smaller area than the lower one, thus producing a step over the patch underneath. This location is where the image shown was taken.

As can be seen, the capacitance image locally follows locally the topographic variations of the membrane thickness, the change in the clearance between the AFM tip and the gold substrate being reflected in a change in capacitance. As the number of PM layers between the tip and substrate goes from two to one, an increase in the capacitance of about 10 aF is produced.
The 10 aF value can be translated through the use of theoretical algorithm previously developed [6][7][8] into the value of dielectric constant of the PM which comes out to have a relative value to the vacuum dielectric constant of 4.

Figure 1. 2.2 x 2.2 µm² topographic and DC current imaging of a Purple Membrane patch in jumping mode. A bias of 1.5 V was applied.

Figure 2. Current-voltage data acquired at different depths at a single point of the Purple Membrane. A current voltage curve is acquired each 0.1 nm. In (A) a false color image of the complete set of data is shown. In (B) the current-voltage curves for three different heights are shown in detail.

Figure 3. 600 x 600 nm² topography and capacitance characterization of a stack of two patches of Purple Membrane performed in Jumping mode. The capacitance image has been subjected to a Gaussian averaging. The top patch is smaller than the lower one and the step that the border of the top one is visible in the figure 3A. The variation in the clearance between the AFM tip and the substrate creates variation in the capacitance which is clearly observable in figure 3B. The insets show the profiles signaled in the images. The profile of figure A rather than the height variation shows the total clearance between the tip apex and the substrate. From the capacitance variation it is calculated a dielectric constant of 4 for the Purple Membrane in N2(g) atmosphere.

4. Discussion

In the previous section we have presented DC/AC electrical measurements both in spectroscopic and imaging mode on biological samples with nanometric spatial resolution by Atomic Force Microscopy. From the results obtained there are different comments which are significant. The measurements performed have revealed that purple membrane behaves as an excellent insulator with respect to electronic transport while nano-liposomes display an effective dielectric constant lower than expected for biological membranes.

From the results obtained there are different comments which are:

(i) Concerning the AFM set up it is worth remarking some relevant aspects. The electronic set up specifications detailed in Table 1 have shown to be the adequate ones to get track of the electric properties of biological samples for both DC and AC measurements at the nanoscale. To this result,
several improvements of the AFM set up has been necessary in order to minimize spurious noise and shielding, which turned to be as important as the performance of the custom amplifier

(ii) Concerning the measuring protocol, we have verified that jumping mode is the appropriate imaging mode in order to perform electrical measurements on biological samples.

(iii) Finally, concerning the measurements performed on purple membrane two unexpected results have been obtained. On the one hand, we have verified at the nanoscale that biological membranes are excellent insulators with respect to electron transport, comparable to the best inorganic insulators. On the other hand, the dielectric constant measured is somewhat smaller than the expected values. Investigations are on the way to further understand these results.

5. Conclusions

We have presented a new tool and measurement protocol based on Atomic Force Microscopy for the AC and DC electrical characterization of biological samples at the nanoscale. The new developments allow performing a variety of electrical measurements (DC conductivity, AC small signal impedance) both in spectroscopic mode (i.e. in a single point) as well as in imaging mode (i.e. by providing a map of the electrical properties with nanometric spatial resolution) with minimum sample damage. Successful test measurements have been performed on purple membrane and nano-liposomes. Present results open new possibilities on the electrical characterization of biological samples with nanometric spatial resolution.

6. References

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