PROTEIN IMMOBILIZATION METHODS FOR BIOLOGICAL ATOMIC FORCE MICROSCOPY

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Atomic Force Microscopy (AFM) has been increasingly used in biological sciences and it is now established as a versatile tool to address the structure, properties and functions of biosystems. AFM is unique in that it provides 3-D images of biological structures of all kinds in their native environment with nanometer resolution. In the last years has been also applied to measure intermolecular forces using Force Spectroscopy, based on the deflection signal of the cantilever probe caused by the force between the cantilever modified with a molecule of interest and a complementary molecule on a substrate.

A crucial prerequisite for successful, reliable biological AFM is that the samples need to be well attached to a solid flat surface using appropriate, non destructive methods. Our group have designed different procedures for immobilizing proteins for different AFM studies: topography imaging, molecular recognition imaging and Force Spectroscopy. The methods have been optimized to minimize nonspecific adhesion with the tip. We have also developed procedures to control the quantity and functionality of the bound molecules.

In the case of protein-ligand recognition studies, the rupture forces are often obscure by the lack of molecular mobility, nonspecific adhesive bindings or an incorrect orientation of a molecule over the other. Our group has been using a linker molecule [1] to increase the mobility of the molecule at the tip with success and has introduced a correction in the measured force due to the angle that is created between the measured force in the normal direction of the probe and the bond direction caused by the flexibility of the spacer [2].

Typically, the immobilization of the molecules is carried out in a non-oriented manner. In some cases, this could be problematic for imaging, but can be very negative in Force Spectroscopy experiments, where an incorrect orientation of the molecule in the sample over the one at the tip makes the binding does not take place or occurs in a very small percentage of approaches, which is quite common in these experiments. In this work we introduce the factor of protein orientation to measure intermolecular forces in flavoprotein complexes. The enzyme FNR catalyses the transfer of two electrons from two independent Ferredoxin (Fd) or Flavodoxin (Fld) molecules, previously reduced by Photosystem I (PSI), to $NADP^+$ in the photosynthetic chain. This reaction requires formation of a complex that allows the optimal orientation between the redox centers of both molecules for the subsequent electron transfer [3]. We have developed two strategies of oriented immobilization of FNR on mica. On the one hand, we have attached FNR oriented to its redox partners Fd or Fld that will be also oriented bound to the AFM tip. On the other hand, a double histidine mutant in FNR will favour the proper orientation in the surface to the $NADP^+$ substrate that will be attached to the tip.

In summary, we have developed new strategies to control the immobilization of different proteins on substrates and tips in order to optimize imaging and Force Spectroscopy experiments. These procedures could also apply for other uses that require a precise control in immobilization.

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References:

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Figures:

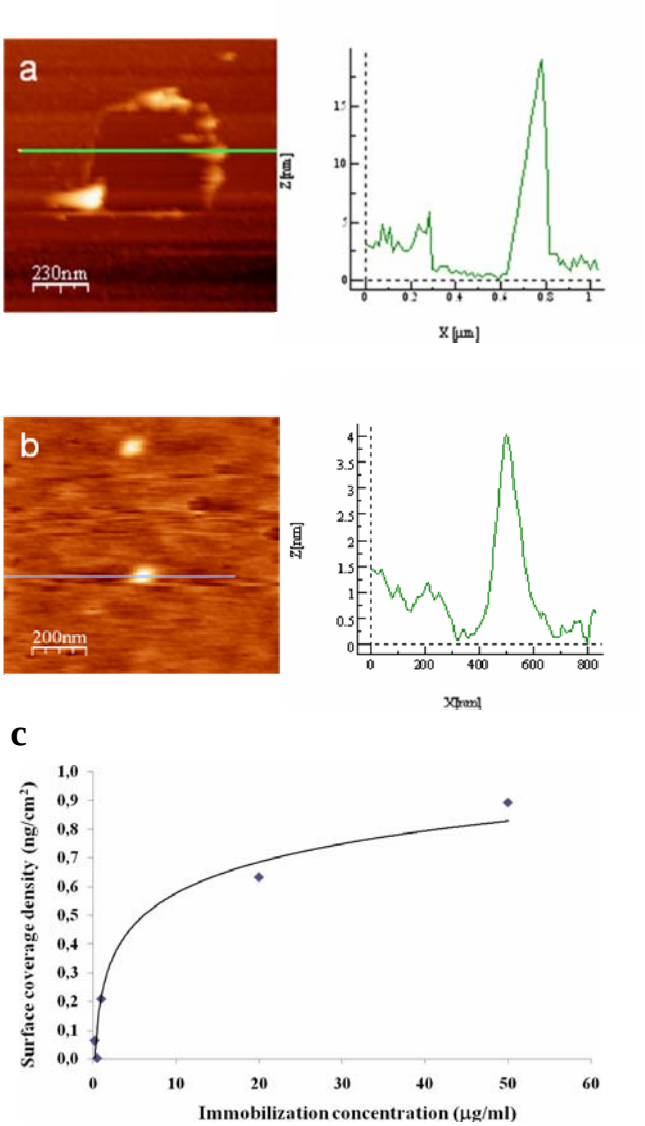

Fig. 1. Streptavidin covalently bound a) to APTES-mica through glutaraldehyde to form a monolayer and b) to thiolated mica to get resolved molecules. Samples were washed with PBST/SDS showing no unspecific tip-sample adhesion. Images taken using Jumping Mode with cantilevers of *k* 30 pN/nm. c) Protein concentration applied for immobilization versus immobilized protein mass for case b), obtained using a specific enzymatic assay with biotin-HRP and TMB as substrate.

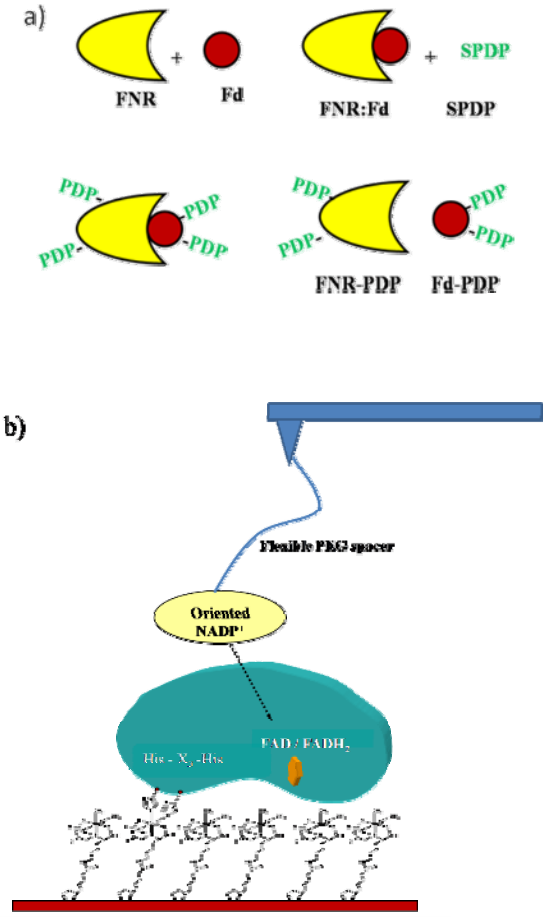

Fig. 2. Orientation strategies in the immobilization of FNR on mica to measure the unbinding force with a) its redox protein partners Fd and Fld and b) its enzymatic substrate $NADP^+$.