Custom system for single molecule force spectroscopy

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Introduction

Atomic Force Microscopy (AFM) is nowadays a widely distributed imaging technique, able to achieve nanometric resolution on a broad range of samples, from innovative materials to living biological systems. The physical principle underlying the AFM technology is based on the measurement of the interaction force between a sharp tip and the sample, with picoNewton sensitivity. This aspect of AFM was more recently exploited to perform force spectroscopy experiments on single molecules, paving the way for the study of mechanical properties of single proteins and peptides [1].

This type of measurement has two major requirements: great stability and precision in terms of vertical displacement and force sensing, and the ability to acquire a large, statistically relevant, set of repeated measurements.

Apart from the stability and precision point, with commercial systems it is not always easy to gather the needed amount of curves for the analysis, since this ideally requires the instrument to be able to perform multiple curves on a number of different spots on the sample surface. To overcome this limitation, researchers have to customize their instruments and/or the control software that comes with them, spending time and also money on already expensive systems.

By tailoring the hardware and using an open source software architecture that could allow easy and fast modifications and also a complete redesign of the control software, we managed to create a system that addresses the problem cited above, without losses on the performance side.

Materials and Methods

The system consists of the following parts: a custom AFM head, with a single axis (Z) piezoelectric actuator; two piezoelectric step motors for XY movements, connected to the client PC via a USB controller; a PC running Ubuntu 10.04 with a real-time kernel patch; a National Instruments data acquisition board (NI PCI-6259), on the Linux PC to communicate with the AFM; a Windows 7 PC with .Net 4.5 for the client software.

On the software side we used an already existing open source architecture developed as part of a project called RTAI-XML [2]. The main advantage of this architecture is the separation between the hardware interface and the user one. This allows to implement both interfaces focusing on their specific features without any constraint or bond between them.

While the hardware interface and the communication server, which exchange information between the instrument and the user, run on a modified Linux pc, the client software has been developed for Windows. The reason behind this choice is that Windows is the most widely spread OS in the world and so the majority of the users won't have to get familiar with an unknown environment.

To test the performances of the system, we performed a series of experiments on the bond strength between streptavidin and biotin.

For these tests, we functionalized the cantilever chip (MSNL-10 Bruker) with streptavidin and adsorbed biotin-BSA on a borosilicate glass slide following the procedure described by Lo et al. [4], adding a further UV irradiation for both the chip and the slide for ensuring the exposure of the OH groups.

Results and Conclusions

The various tests conducted on the stability and the performances of the system showed us that, apart from the obvious tunings and improvements that characterize every prototype, the system possesses all of the characteristics that we had fixed as our objective.

The software provides all the features needed to perform experiments and allows for easy and fast modifications when needed.

On the hardware side, we managed to satisfy the key features required by the measurements in terms of stability and precision. The curve report-

ed in Figure 1A is a good example of the system performances.

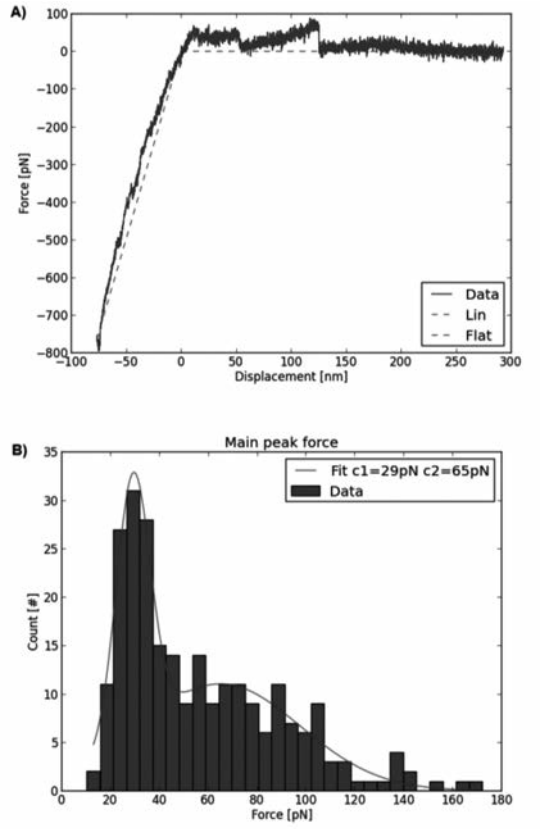

Figure 1. A) Streptavidin *vs* biotin sample FD curve; B) Main peak force histogram fitted with a sum of two Gaussian functions

Regarding the ability of gathering a large amount of curves for the sake of statistical analysis, the histogram shown in Figure 1.B has a trend that can be fitted with the sum of two Gaussian functions. The first peak is associated to non specific interaction events occurring near the contact point, at lower forces.. The second one contains the relevant information about the system under investigation: the force peak corresponds to the rupture of the bond between the streptavidin on the tip and the biotin on the glass substrate. This result shows how the remarkable quantity of curves that can be taken by the system for each experiment provides a stable statistics and as such a reliable way to distinguish positive results from the ones to be discarded, without any complex data filtering.

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