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Progress in Physics (49)

Cantilever based Biophysics

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Biophysics is the branch of physics that specifically addresses the challenges posed by the biological matter at large and aims at quantitatively describing biological phenomena. It is difficult to precisely define which are the domains of biophysics, but we can say that it concerns all elements of the biological matter starting at the level of the atom up to the behavior of flocks of birds or fishes: the length and time scales involve many orders of magnitude. Length scales span approximately 10 orders of magnitude from the Ångström up to the meter, while the time scales are even more excessive, namely they go from molecular times up to years spanning from picoseconds up to the 100-1000 years (the life span of certain living organisms): approximately 22 orders of magnitude. Biophysics applies the general principles of physics in order to understand the biological matter, experimentally as well as theoretically. From the preceding lines, it is clear that biophysics has to encompass a large set of techniques and theoretical tools, phenomena and of course all the variety of biological objects.

In the present article we will limit our excursion into biophysics to the *application of levers (or micromechanical devices) in biophysics*, although it is clear that *all* other fields of biophysics have substantially progressed because of new theoretical and experimental tools or because of newly available biological techniques.

Local probe microscopy was born in 1981 (Scanning Tunneling Microscope) and 1986 (Atomic Force Microscope). We would like to circumscribe the present article to the field of imaging, of manipulating at the level of single molecules or the use of micromechanical devices. The IBM Laboratory in Rüschlikon has made the most fundamental contributions in the invention of these two instruments and related techniques like the scanning near-field optical microscope. And Switzerland, through the laboratories in different institutions (to mention IBM Rüschlikon and University of Basel), has over-proportionally contributed to the rise of these techniques. The characteristics of the AFM allow interrogating biological matter at the single molecule level.

Why are single molecule experiments relevant for the understanding of the basic biological processes? Let's divide the science world in two parts by the trade of their actors: on the one side "sociologists", on the other side "psychiatrists". "Sociologists" will collect data on the whole society and study the collective behaviors. "Psychiatrists" interrogate one single individual for the sake of understanding his/her behavior and his/her inner functioning. The latter way of investigation is what single molecule biophysicists are aiming at. From the realm of the millions, billions copies of a biological molecule, one is selected and followed over an entire cycle of its activity. Many of these molecules are studied and a general behavior is extracted with the intended goal

of extrapolating from this few hundreds of detailed traces to the general behavior of billions of molecules, but with the advantage to have insight into the exact functioning from the single molecule interrogation. Bridges between the detailed behavior and structural information at the atomic level are then built and a model of the functioning of a biological molecule is then proposed. On the other side, solution experiments on bulk samples are analogous to "sociologists" who try to understand from the average the behavior of a collection of individuals, but only average quantities are collected. Both camps have their importance in biophysics.

We will present here some applications coming from our laboratory in which levers, for example in an Atomic Force Microscope, are used. We are very well aware that many other laboratories have made important contributions. The imaging and single molecule capabilities of the AFM microscope are exceptionally versatile and very well adapted to biology since the instrument can image biological samples with molecular resolution (nanometer or better) in physiological fluids.

Binnig et al. [1] developed the atomic force microscope in 1986 to image non-conducting sample's surfaces at high lateral and vertical resolution (see Figure 1). The instrument basically consists of a sharp tip fixed at the end of a very soft cantilever. The tip is brought close to the sample and the deflection of the cantilever, caused by the interaction forces between tip and surface, is detected by a laser beam reflected from the cantilever and impinging on a photodiode.

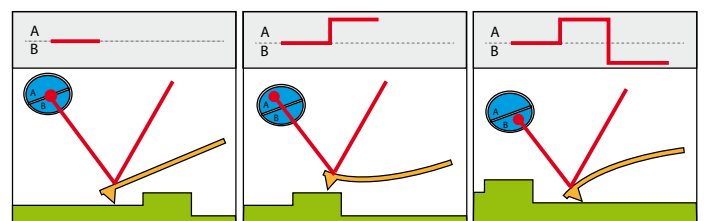


Figure 1: The AFM tip scans the surface of the sample and the deflection of the lever corresponds to the topography of the sample. An image of the sample can be reconstructed when the tip is scanned over the entire sample's surface. The calibrated photodiode response (upper panels) is used to determine the cantilever's deformation.

Imaging with the AFM

In order to obtain an image of a sample's surface, the tip scans over the surface as illustrated in Figure 1. The minute deflections of the cantilever are recorded and used to build a topographical image of the sample's surface as depicted in Figure 2. The resolution of the instrument is astonishing high, up to 0.1 Å in the axis perpendicular to the surface. In addition the instrument can indifferently image samples

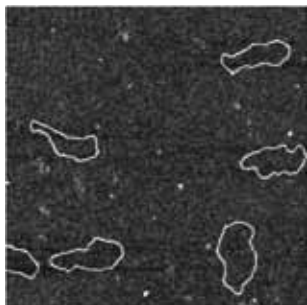


Figure 2: Topographical height image of a circular double stranded DNA on a mica surface. Scan size $1,5 \times 1,5 \mu\text{m}$. (Courtesy G. Witz)

in air, vacuum or liquid media. This last possibility made the instrument very popular among biophysicists since it permits the observation of living samples in nearly physiological conditions. The possibility to exchange liquids in the analysis chamber during observation paves the way to explore dynamical phenomena involving proteins, cells, etc. Novel ultra-rapid AFMs permit even high speed imaging of single molecules and proteins with rates up to 100 frames per second [6]. The instrument can also be used to explore the mechanical properties of the sample at almost a nanometric resolution. The AFM tip is pushed into the sample and by monitoring the deformation of the cantilever one can obtain an indentation curve from which elastic properties of the sample can be deduced. The schematic of such a measurement is depicted in Figure 3.

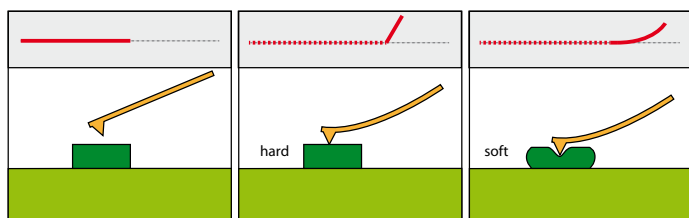


Figure 3: Measurement of the mechanical properties of a sample by AFM. If the sample is soft, indentation occurs and the shape of the force curve is can be used to compute the sample's Young modulus.

By repeating this indentation procedure all over the sample's surface one obtains an elastic map that can be used to "paint" the 3D images obtained by the classical imaging mode of the microscope.

Figure 4 shows such a picture. In this case a rat hippocampal nerve cell was grown on a glass substrate and indented all-over its surface by the AFM tip. The calculated elastic properties were eventually coded in false colors that overlay the topography of the cell.

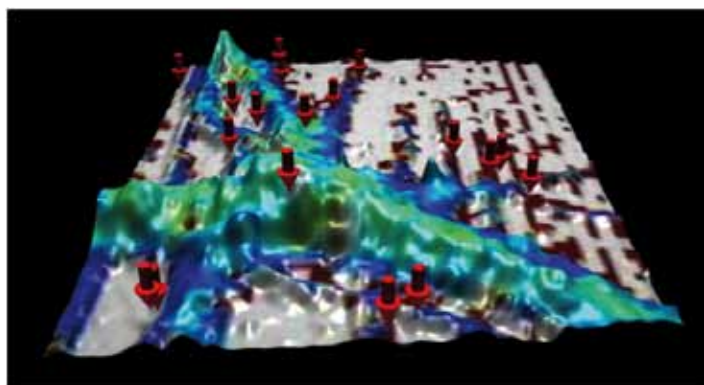


Figure 4: Axon of a mouse hippocampal nerve cell as seen by AFM. The stiffness of the cell as calculated after AFM tip indentation into the sample (white color represent the stiff substrate, the green color represents low elastic modulus parts of the cell body). The arrows indicate spots where binding-unbinding events were recorded (see below in the text). (Courtesy C. Roduit)

It was also demonstrated that the AFM could be used to assess the affinity between single molecules [2]. In this type of measurements one molecular species is attached onto the tip and another one onto the substrate. By lowering the AFM tip close to the substrate, the two molecular species can interact, while during the retraction of the tip the two molecules will be put under tension. If the retraction process continues the bond between the two molecules ruptures and the rupture force can be determined. Such an experiment is schematized in Figure 5.

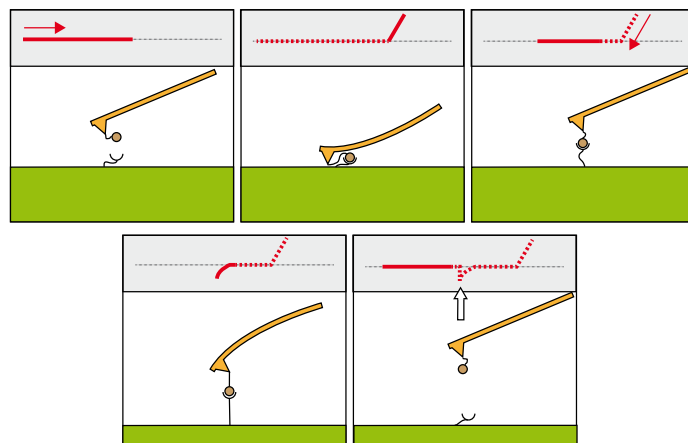


Figure 5: By attaching one molecular species onto the tip and another one onto the sample it becomes possible to determine their interaction forces. The kink (white arrow) that appears in the force curve (upper panels), indicates an unbinding event between the two molecules.

Multi-parametric experiments exploit the fact that the AFM lever is a force sensor. For example, Figure 4 was acquired with a protein (aerolysine) attached onto the AFM tip. The spots where protein-protein interactions occurred (i.e. where aerolysine interacted with some specific protein bound to the hippocampal nerve cell membrane) unbinding events (like in Figure 5) were detected and labeled on the figure with red arrows. This case illustrates the AFMs capacity to simultaneously record topography, mechanical properties as well as specific protein-protein interactions.

A novel application of the AFM consists in depositing the sample to be explored directly onto the cantilever. Gerber and collaborators have exploited this to construct for example an electronic nose [3].

Our group has recently demonstrated that by randomly depositing living organisms such as bacteria or single mammalian cells onto AFM cantilevers one can assess their metabolic state simply by observing the cantilever dynamic deflection that they induce while alive. As soon the sample dies, the oscillations stop. The origin of these oscillations is still unclear, however we could evidence the relationship between the metabolism and the observed oscillations [4].

Since all the bacterial species that we have tested up to now (about 20) induce cantilever oscillations, we recently suggested that this technique could be very useful to explore bacterial resistance to antibiotics. The method is incomparably faster than the traditional technique since it gives results in a timeframe of minutes instead of days or weeks (see Figure 6).

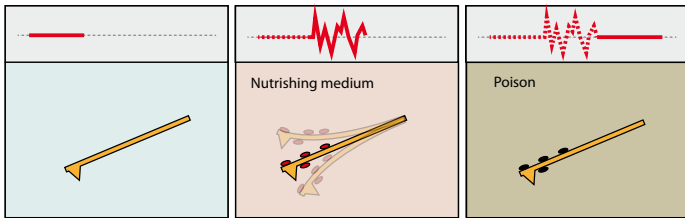


Figure 6: A cantilever with no attached organisms onto its surface does not oscillate (upper panels show the cantilever deflection as a function of time). As soon as living organisms (bacteria or mammalian cells, in red on the lower middle panel) are attached onto its surface and the analysis chamber is immersed into a nourishing medium, nanometric scale cantilever oscillations can be detected. Replacing the nourishing medium with a poison (e.g. antibiotic) immediately inhibits the oscillations.

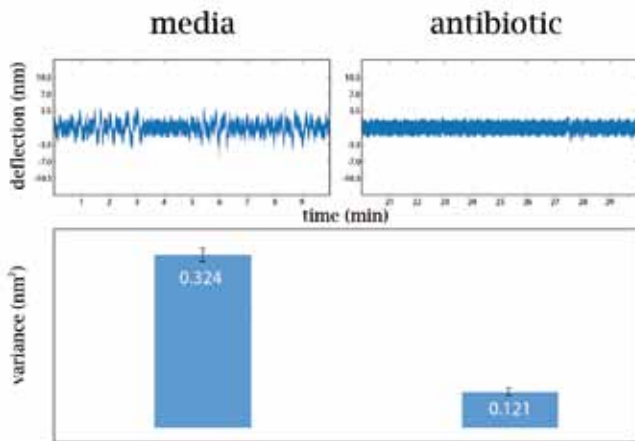


Figure 7: Typical measurement for the bacterium *E. coli* attached to the lever. Top panels: trace of the cantilever's deflection, total elapsed time 8 min; left before and right after the injection of an antibiotics; bottom panel: variances of the above signals. Scale: +/- 6 nm, time total 8 min. (Courtesy P. Stupar)

Figure 7 depicts the results of a typical experiment in which bacteria were deposited on a cantilever and its oscillations were recorded as a function of time and chemicals to which the microorganisms were exposed. The histograms show the variance of the recorded signal. As one can notice the cantilever oscillations dramatically decrease when an antibiotic is introduced into the analysis chamber.

Encouraged by these results, we recently suggested to send such a device onto Mars or any extraterrestrial body to track possible living extraterrestrial organisms. The advantage of such an instrument is its lightweight and its independence towards the chemistry of the putative extraterrestrial organisms [5].

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