FINAL REPORT
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STSM Topic:
Atomic force microscopy investigation of virus nanomechanical properties.

Introduction.

I spent one month at the Department of Condensed Matter Physics, Universidad Autonoma de Madrid (Spain), within a short- term scientific mission, under the supervision of Prof. Pedro José de Pablo.

The purpose of the stay was to improve my knowledge in:

- nanomechanical characterization of virus systems by using the Atomic Force Microscopy (AFM).
- theoretical models used to obtain quantitative information on the elasticity of viruses.

During the stay I characterized both wild-type and mutant form of the Tomato Bushy Stunt Virus (TBSV) by nanoindentation and extracted information on their elasticity.

Background and purpose.

Over the last years the approach on viruses has undergone a shift from considering them just as infective agents to considering them as very promising biomolecules for a wide range of nanotechnology-related applications ranging from novel drugs to biosensors. Functionalization of these natural nanoparticles has already been obtained successfully through modification of their external or internal surfaces. Indeed, a profound knowledge of their material and mechanical properties is essential for their optimal use as building blocks in electronics, physics, medicine and chemistry.

From this perspective plant viruses have simple structures, large potential for self assembly and they are non human pathogenic.

Tomato Bushy Stunt Virus is a spherical plant virus, belonging to the family of *Tombusviridiae*, characterized by an icosahedral symmetry. TBSV represents an efficient and versatile system for the production of viral nanoparticles (VNPs) because it is very stable and easy to purify from host plant.

To directly probe its mechanical strength and elasticity, and in order to obtain some information about the structure of the TBSV wild type (WT) and of its mutant, FLAG, I applied the technique of nanoindentation, based on AFM. By these measurements I was able to calculate the Young’s modulus, the spring constant of the virus, and determine the force response of the viral particles as a function of the indentation.

Work carried out.

During this month nanoindentation measurements have been performed by using an atomic force microscope, Nanotec, Madrid, operated in “jumping mode” in liquid. To optimally carry out this type of experiments, a key aspect is the choice of the substrate. In fact the viral particles must be well attached on the surface of the substrate to avoid any movement during the indentation. The mica treated with $NiCl_2$ proved to be the most suitable substrate for this virus.

To begin with, the capsids were imaged with low maximal force and at a low resolution to determine their position and orientation. The AFM images of both the wild-type and FLAG did not show such clear distinctions, the height of the particles was about 33-34 nm for both forms.

After individual particles were imaged, indentation experiments were performed. To investigate quantitatively the elastic response and the stability of the capsid, single force-distance (FZ) curves were recorded after positioning the cantilever tip on the center of an individual virus. After each curve, the capsid was reimaged, and its height was redetermined to see if there was evident damage.

FZ curves and topography before and after the indentation are shown in Fig. 1 and 2. The initial linear regime of the first force-indentation curves was used to deduce the effective spring constant and also the force and the indentation depth at which the shell started to respond nonlinearly from these curves.

For both viruses, the acquired FZ curves showed an initial linear behavior, which indicated that they can be regarded as thin-shelled objects. The capsid and the cantilever can be considered as two linear springs in series. The spring constant of the capsid, k_{cap}, is related to the effective spring constant, k_{eff}, and the cantilever spring constant, k_c: $k_{\mathrm{cap}} = k_{\mathrm{c}}\, k_{\mathrm{eff}}\, (k_{\mathrm{c}} - k_{\mathrm{eff}})^{-1}$. The k_{cap} values of two TBSV types were similar: 0.9 ± 0.4 N/m for the wild type and 0.8 ± 0.3 N/m for the mutant one.

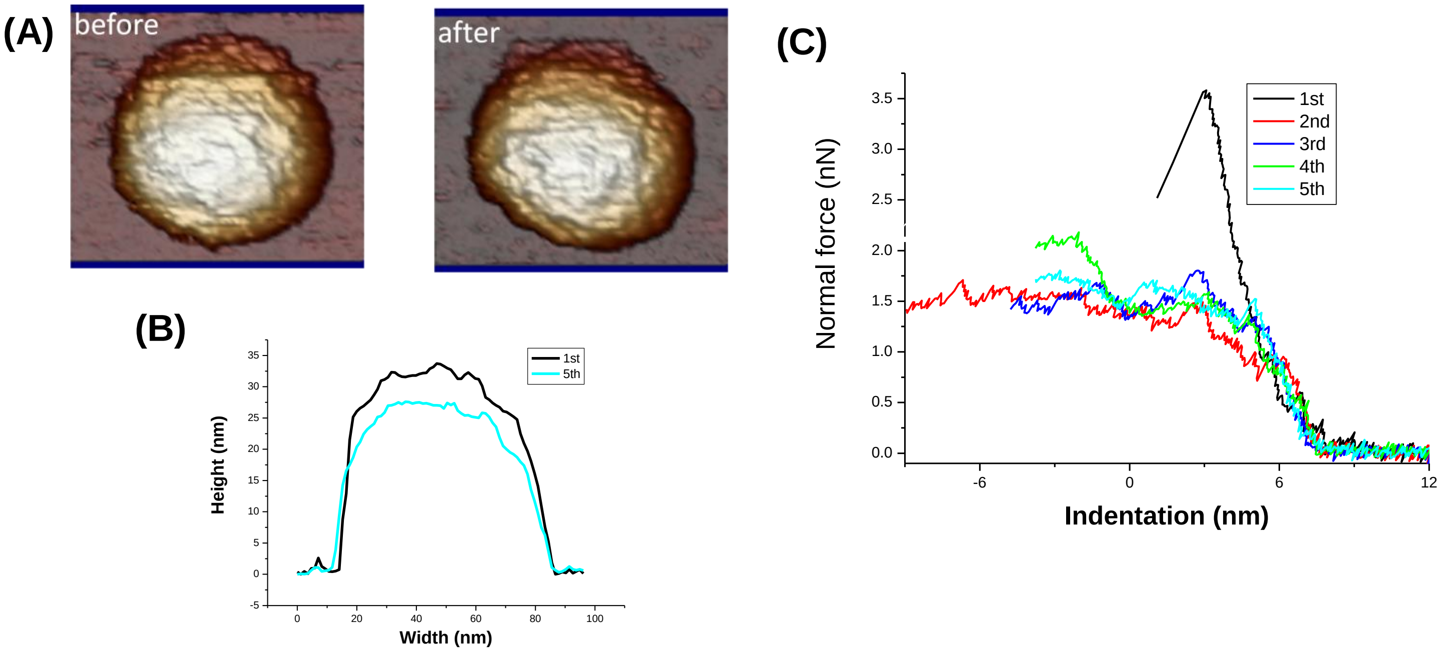

Figure 1. **(A)** Two images of a TBSV-WT particle before and after acquiring five FZ curves. **(B)** The profiles of the particle before and after the five indentations. **(C)** The five FZ curves obtained by indenting the same viral particle. From the first curve it is possible to extrapolate the values of the spring constant, the breaking force and the critical indentation of the indented viral particle.

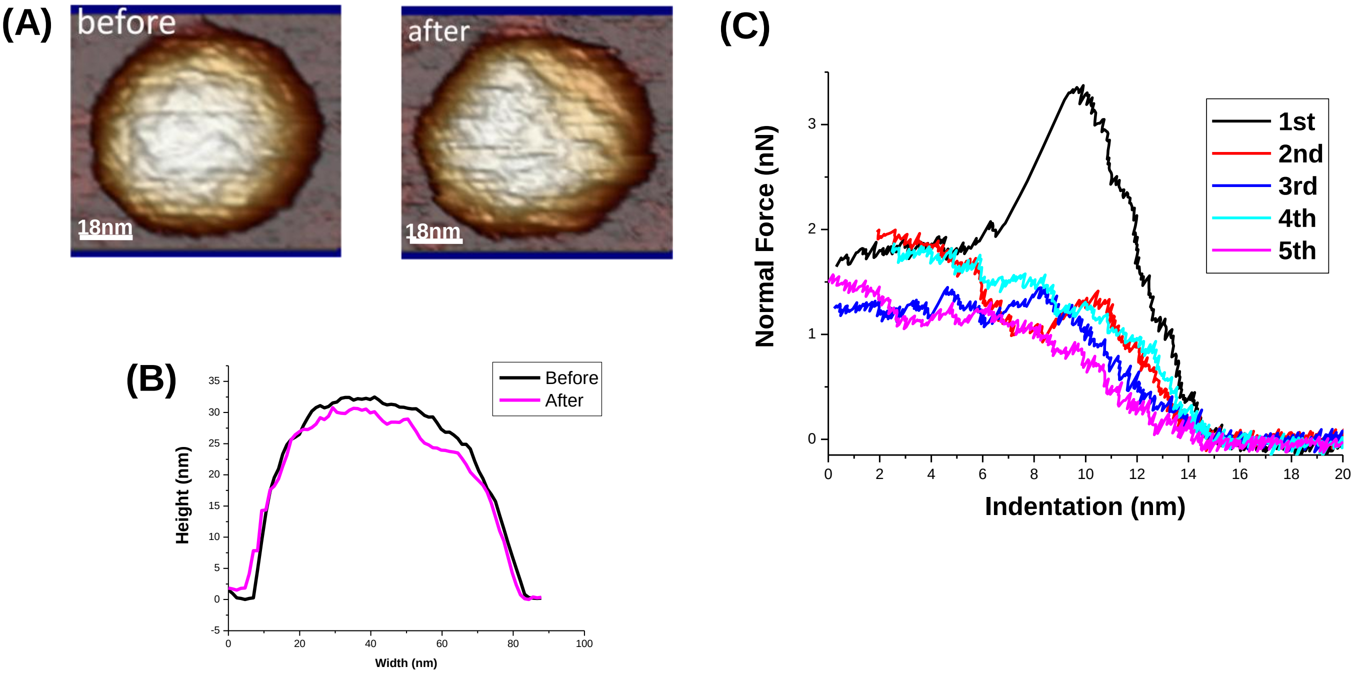

Figure 2. **(A)** Two images of the same TBSV-FLAG particle before and after the indentations (five FZ curves). **(B)** The topographic profiles of the particle before and after the indentations. **(C)** Five FZ curves acquired on this particle.

Finally, the virus Young's modulus, E, can be extracted from k_{cap} and dimensions, the outer radius, R, and the wall thickness, h. The simplest model for the elastic response is to assume the capsid as an elastic thin spherical shell undergoing small deformations: $k_{cap} = \alpha E h^2/R$ (where α is a geometry-dependent proportionality factor). For both types of TBSV the value of the Young's modulus was about 0.7 GPa.

In conclusion, this Short-Term Scientific Mission was particularly useful to improve my knowledge in this specific technique and in the field of AFM. Moreover, this experience was highly worthwhile in relation to the aspect of the new collaboration established, which will hopefully be continued in the future.