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Atomic force microscopy observation and characterization of single virions and virus-like particles by nano-indentation

M Marchetti[1], GJL Wuite[1] and WH Roos[1,2]

Structure and function of viruses are intimately related, and one of the goals in virology is to elucidate the mechanisms behind this relation. A variety of research endeavours is focused on studying these mechanisms and a relatively new technique in this field is Atomic Force Microscopy (AFM). Using AFM virions and virus-like particles can be imaged and manipulated at the single particle level. Here we review recent AFM nano-indentations studies unveiling for instance the mechanics of capsid–genome interactions, morphological changes that drive viral maturation, capsid stabilizing factors and viral uncoating. We show that in an increasing amount of literature a clear link between mechanics and infectivity is observed, which not only provides us with new fundamental insights into virology, but also provides ways to improve virus-like particles for applications in nanomedicine and nanotechnology.

Addresses
[1] Natuur- en Sterrenkunde & LaserLab, Vrije Universiteit, De Boelelaan 1081, 1081 HV Amsterdam, The Netherlands
[2] Moleculaire Biofysica, Zernike instituut, Rijksuniversiteit Groningen, Nijenborgh 4, 9747 AG Groningen, The Netherlands

Corresponding author: Roos, WH (w.h.roos@rug.nl)

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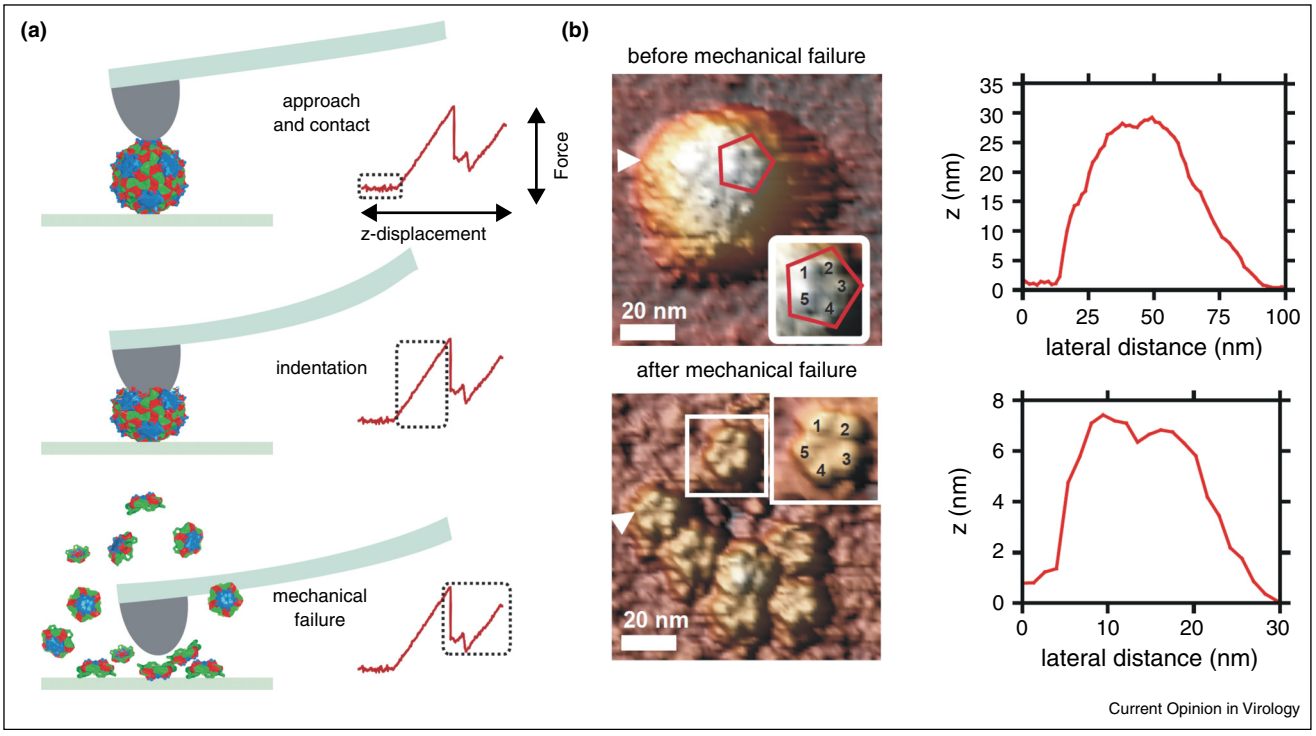
Introduction

One key aim in life sciences is to explain the correlation between the molecular structure and its biological function. For example, by investigating the structural and material properties of viruses, some viral biological functions can be correlated to capsid morphology. In particular, Structural Virology provides high-resolution molecular structures [1]: it reveals capsid protein arrangements such as inter-subunit and intra-subunit organization, as well as genome–capsid interactions that are established during or after the assembly process. Mechanical Virology, on the other hand, quantifies capsid mechanical and material properties and investigates its relation to structural changes and the virus self-assembly process [2]. These two Physical Virology branches, together with biological, biochemical and chemical approaches, create a deeper understanding of how viral proteins and capsomers are able to self-assemble in highly ordered shells, and how conformational changes inside the capsid are related to genome packaging or release [3]. These key insights not only elucidate the biology of viruses, but also allow to manipulate viruses and to create virus like-particles with numerous applications both in nanomedicine [4] and nanotechnology [5]. This review provides an overview of recent studies on the material properties of virions and virus-like particles. The main technique for these studies is Atomic Force Microscopy (AFM) imaging and manipulation.

AFM nano-indentation approach

AFM force spectroscopy allows the investigation of the nano-mechanical properties of individual molecules and particles under near-to physiological conditions [6,7]. One of the key strengths of AFM is that it allows for imaging as well as manipulation of the biological samples [8–10]. To study viral particles typically first an image is recorded by scanning the AFM cantilever over the virus covered-surface. Afterwards, the AFM cantilever is directed towards the centre of one of the viruses. Next the virus is indented and its response is analyzed as a function of the indentation (Figure 1). The recorded force–distance curve shows how the force changes during the indentation cycle. The initial part of the force–indentation curve is often approximately linear which permits the easy quantification of the virus spring constant (stiffness). However, in more elaborate analyses also non-linear effects can be taken into account [11]. To describe the viral material properties independent from particle geometry, size and shell thickness, the Young's modulus E is often used [8]. The Young's modulus can be approximated by ($k = \alpha(Eh^2/R)$), with the virus spring constant k, the radius R and shell thickness h. Although k is extracted from the force–indentation curve, an average value for R and h can be extracted from the virus PDB structure. The proportionality factor α has been shown to be roughly 1 and can therefore be omitted [12]. In above approximation the capsid is idealized as a homogenous material with constant radius and shell thickness. Of course this is not a realistic description of its actual structure. The heterogeneity of a viral shell can be taken

Figure 1



AFM nanoindentation. **(a)** Schematic of experimental approach with force–indentation curves. **(b)** Triatoma virus before and after failure. Both before and after failure the pentameric substructures of which the capsid is built up from can be observed.
Source: Modified from Ref. [36••] with permission.

into account by more elaborate approaches using computational methods such as (coarse grained) molecular dynamics simulations, normal mode analysis, elastic network models and finite element modelling [13–17]. Another parameter to characterize material properties of a virus is the maximum force it can resist before it deforms irreversibly (often called breaking force). Repeated force indentations (or alternatively repetitive imaging at low imaging force) allows quantification of the virus resistance to mechanical fatigue and sometimes show progressive dismantling of its structure (Figure 1).

Mechanical properties of viruses

In the following we will discuss recent literature on probing the mechanical properties of viruses. For a review of older literature on AFM manipulation experiments on viruses, please see Refs. [2,3,18,19].

Mechanics of maturation

Capsid maturation is the process of concerted conformational changes of the already formed viral protein shell, resulting in the formation of a virion which is ready for infection. Bacteriophage capsid maturation often involves large structural transitions [20]. Bacteriophage HK97 is a model system for maturation studies and the different steps of the maturation pathway are well described from a structural point of view [21,22]. Recently the mechanics of this process was also studied [17]. HK97 particles self-assemble into the immature Prohead I structure, which is a metastable particle in which the gp5 capsid protein still includes the Δ-domain. The N-terminal Δ-domain, which has a similar function to scaffolding proteins in other viruses, is subsequently cleaved off by the encapsidated protease. This leads to the formation of strong three-fold interactions between the hexamers. AFM nano-indentation has shown that this transition to Prohead-II leads to much more rigid particles. In particular the spring constant increases from 0.018 N/m to 0.12 N/m in this maturation step. Next, in the maturation process, expansion and covalent crosslinking occurs and the Head II particle is formed. It was shown by AFM that this results in a major strengthening and stabilization of the capsid (Figure 2a). It occurs concomitantly in three independent ways: increasing the capsid's Young's modulus from 0.3 GPa to 1 GPa, increasing the breaking force from 0.56 nN to 0.9 nN, as well increasing the resistance against material fatigue. This study showed for the first time that maturation of bacteriophages goes hand-in-hand with a mechanical stabilization of the particles, fitting with the requirements of the virus to be soft and easily adaptable during self-assembly (to correct for mistakes) and strong after maturation (to withstand the