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Review

Atomic force microscopy of virus shells

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ABSTRACT

Microscopes are used to characterize small specimens with the help of probes, such as photons and electrons in optical and electron microscopies, respectively. In atomic force microscopy (AFM) the probe is a nanometric tip located at the end of a microcantilever which palpates the specimen under study as a blind person manages a white cane to explore the surrounding. In this way, AFM allows obtaining nanometric resolution images of individual protein shells, such as viruses, in liquid milieu. Beyond imaging, AFM also enables the manipulation of single protein cages, and the characterization of every physico-chemical property able of inducing any measurable mechanical perturbation to the microcantilever that holds the tip. Here we describe several AFM approaches to study individual protein cages, including imaging and spectroscopic methodologies for extracting mechanical and electrostatic properties. In addition, AFM allows discovering and testing the self-healing capabilities of protein cages because occasionally they may recover fractures induced by the AFM tip. Beyond the protein shells, AFM also is able of exploring the genome inside, obtaining, for instance, the condensation state of dsDNA and measuring its diffusion when the protein cage breaks.

Contents

1. Introduction

A protein cage can be roughly stated as any closed structure built out of protein subunits that defines a closed cavity at the nanometer scale. Although viruses illustrate at most the definition of protein cages, non-viral structures, such as bacterial microcompartments (BMCs) [1], vault particles [2] and artificial virus-like structures [3–5] can also be included in this description. The basic architecture of a virus consists of the capsid, a shell made up of repeating protein subunits (capsomers), packing within the viral genome [6]. Viruses are highly dynamic nucleoprotein complexes that transport and deliver their genome from host to host. Viral particles are endorsed

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with specific physicochemical properties which confer to their structures certain meta-stability whose modulation permits fulfilling each task of the viral cycle at the right time [7]. These natural designed capabilities have impelled using viral capsids as protein containers of artificial cargoes (drugs, polymers, enzymes, minerals) [8] with applications in biomedical and materials sciences. Both natural and artificial protein cages have to protect their cargo against a variety of physicochemical aggressive environments, including molecular impacts in highly crowded media [9], thermal and chemical stresses [10], and osmotic shocks [11]. Thus, it is important to use methodologies that supply information about protein cages stability under different environments and its evolution upon structural changes. In this vein, structural biology techniques such as electron microscopy (EM) and X-ray are used to unveil the structure–function interplay, revealing high resolution impressive structures of protein cages [12]. However, these methodologies require a heavy average of millions of particles present in the crystal (X-ray) or thousands of structures for the model reconstruction (cryo-EM). Thus, they provide limited information on possible structural differences between individual particles in the population that distinguish them from the average structure. In addition, these approaches require conditions (vacuum, ice, etc.) far away of those where protein shells are functional (liquid). Thus, these techniques preclude the characterization of protein shells dynamics and properties in real time. Indeed, the advent of single molecule technologies has demonstrated that mechanical properties of biological molecular aggregates are essential to their function [13]. It is evident that the exploration of these properties would complete the structural biology methodologies (EM and X-ray) to find the structure–function–property interplay of protein cages. Atomic force microscopy (AFM) characterizes the structure of individual protein particles in liquid milieu and measure physicochemical properties of each particle. In addition, the nano-dissection abilities of AFM allow the local manipulation of protein shells to learn about their assembly/disassembly. In this review, I pretend to give a general overview of how to apply AFM methods to protein shells. It starts with a basic review describing the most successful modes for imaging protein shells with AFM. Subsequently it describes the nanoindentation methodology, which probes the stiffness, breaking force, brittleness, etc., of individual protein shells. I briefly refer here to the self-healing abilities of protein shells. Afterwards I focus in the effects of mechanical fatigue on individual particles, and the combination of AFM with fluorescence microscopy. In the last part, I illustrate the methodologies to measure the electrostatics of individual protein particles.

2. Imaging viruses with AFM

As it happens with every specimen to be examined with AFM, immobilization of protein shells to a flat solid substrate is a *sine qua non*-requisite. Viral shells are typically attached to substrates by using physical interactions of viral shells with substrate, including polar, non-polar, and van der Waals interactions [14]. Physisorption traps protein cages on the surface without creating chemical bonds that might alter their structure. Each type of protein shell has individualized features such as hydrophobic patches or local charge densities [15] that can be used for adsorption, via hydrophobic and/or electrostatic interactions, on different substrates, such as glass, mica and HOPG (highly oriented pyrolytic graphite) (see details in [16]).

Fig. 1 shows T7 bacteriophage, human adenovirus (HAdV) and herpes simplex particles adsorbed on HOPG, mica and silanized glass, respectively [17–19]. These are typical cases of non-enveloped viruses with icosahedral shape. Thus, after adsorption on the surface they can present 5-fold, 3-fold and 2-fold symmetry axes (inset, Fig. 1A–C). Nevertheless, AFM can also visualize viruses with other shapes and morphologies, including isolated appendages such as viral fibers. For instance Fig. 1D shows the cylindrical structure of tobacco mosaic virus (TMV) [20]. In spite of their undefined morphology conferred by the external lipid membrane, AFM can also image enveloped particles, such as influenza [21] (Fig. 1E) and HIV [22] (Fig. 1F). Fig. 1G presents the AFM topography of T4 phage isolated fibers [23]. One could say that each type of virus has a preferred surface, since each structure exposes different residues in the external layer, thus requiring a particular adsorption methodology. From a practical point of view predictions on proteins shells adsorption are difficult to make, and one uses the "trial and error" methodology to find the best conditions [16].

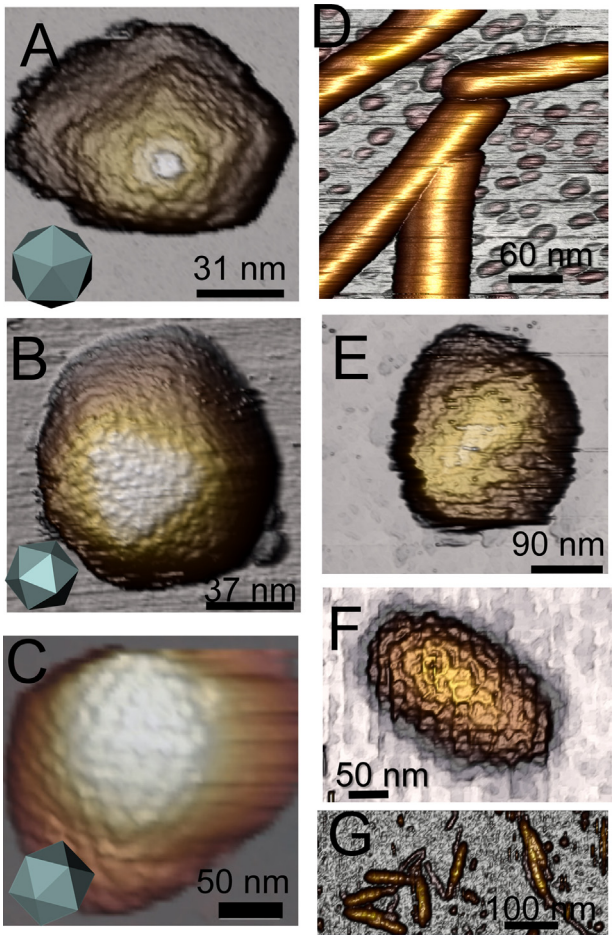

Fig. 1. *AFM images of single viruses.* (A) T7 bacteriophage mature capsid adsorbed at 5-fold symmetry orientation [17]. (B) HAdV particle adsorbed on a triangular facet [18]. (C) Herpes simplex virus particle showing a 2-fold orientation [19]. (D) TMV viruses [20] imaged in dynamic AFM. (E) Enveloped influenza virus [21]. (F) Enveloped HIV virus [22]. (G) Viral fibers of T4 bacteriophage [23].

Typically, in AFM the tip scans the sample in x, y and z directions by using piezo actuators. While x and y scanners move the sample over a square region, the cantilever bends following the surface topography. The cantilever deflects perpendicularly to the surface applying a normal force (F_n) (Fig. 2A), and also bends laterally by torsion exerting a dragging force parallel to the surface (F_l) (Fig. 2B). Both F_n and F_l are monitored by focusing a laser beam at the end of the cantilever, whose reflection is registered in a four quadrant photodiode. Thus, each pixel of the image located at a particular position of planar coordinates (x,y) will be associated with certain bending values of the cantilever F_n and F_l. If the virus particle is not strongly attached or if it is too soft, it can be swept or modified under large bending forces. To avoid this effect as much