Imaging Biological Samples with Atomic Force Microscopy

Pedro J. de Pablo and Mariano Carrión-Vázquez

Atomic force microscopy (AFM) is an invaluable tool both for obtaining high-resolution topographical images and for determining the values of mechanical and structural properties of specimens adsorbed onto a surface. AFM is useful in an array of fields and applications, from materials science to biology. It is an extremely versatile technique that can be applied to almost any surface-mounted sample and can be operated in ambient air, ultrahigh vacuum, and, most importantly for biology, liquids. AFM can be used to explore samples ranging in size from atoms to molecules, molecular aggregates, and cells. Individual biomolecules can be viewed and manipulated at the nanoscale, providing fundamental biological information. In particular, the study of the mechanical properties of biomolecular aggregates at the nanoscale constitutes an important source of data to elaborate mechanochemical structural/function models of single-particle biomachines, expanding and complementing the information obtained from bulk experiments.

BASICS OF ATOMIC FORCE MICROSCOPY

The first thing that comes to mind on hearing the word "microscope" is an optical device that manipulates light to obtain a magnified image of a sample. As a consequence, the first question that is usually posed when seeing an atomic force microscope for the first time is the following: Where do I have to look to see the specimen? A microscope is generally considered a machine in which a source emits particles such as photons or electrons that are used as probes directed onto or emitted from the specimen. These particles are registered by a detector and subsequently analyzed, yielding information about the sample. There are two kinds of microscopes that fit readily into this source–specimen–detector–analyzer scheme. The first is the optical microscope, where the photons emitted from an incandescent lamp are manipulated by a system of lenses and mirrors located both before and after interacting with the specimen, and arriving at the eyepiece, where the detector (i.e., the eyes) collects the information. A typical optical microscope can reach a resolution of $\lambda/2 \approx 200$ nm ($\lambda$ being the wavelength of the light). Antonie van Leeuwenhoek (1632–1723) is credited with bringing the optical microscope to the attention of biologists, even although simple magnifying lenses were already being produced in the 1500s.

The electron microscope (EM) is a little more complicated. In this case, the particles that act as probes are not photons but electrons produced by thermionic emission from an incandescent wire. Here, electromagnetic lenses are used to manipulate and focus the electron beam to provoke the right interaction with the specimen. The electrons are then collected by a screen, which is conveniently monitored. The first prototype electron microscope was built in 1931 by the German engineers Ernst Ruska and Max Knoll. Two years later, Ruska constructed an electron microscope that exceeded the resolution possible with an optical microscope, reaching $\sim 1$ nm.

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In scanning probe microscopy, a sharp tip a few nanometers in diameter, which can be considered a probe, approaches the surface of the sample. The first member of this family of microscopes was the scanning tunneling microscope (STM) invented by Binnig and Rohrer (1982), who received the Nobel Prize for Physics with Ruska in 1986. This system is based on a quantum effect (tunneling) that occurs when a sharp metallic tip is brought to a distance (z) of <1 nm from a conductive surface. This effect involves the flow of an electronic current (I) between the surface and the tip according to the formula

\[ I \propto \exp\left(-\sqrt{4\xi}\right), \]

where \( \phi \) is the work function of the metallic surface (Chen 1993). The strong dependence of the current on the tip-to-surface distance can be used to obtain topographic and electronic maps of the sample by moving (i.e., scanning) the tip on the surface, while keeping the tip–sample distance constant through a feedback algorithm. Although this tool provides true atomic resolution in ultrahigh vacuum (UHV) conditions, a prerequisite is that both the tip and sample should be conductive. Therefore, it follows that STM is not suitable for biological samples because these are mainly insulators, which would need to be covered with a metallic layer (Baró et al. 1985).

In 1986, Binnig et al. (1986) invented the atomic force microscope, combining the principles of both the STM and the so-called stylus profilometer (Schmalz 1929). In an atomic force microscope, a sharp stylus (approximately tenths of a nanometer in diameter) attached to the end of a cantilever is brought close to the surface. As a consequence of this interaction, a force appears between the tip and surface that can be attractive or repulsive (see below), causing the cantilever to bend. When this bending is controlled with a feedback algorithm, it is possible to obtain a topographic map by scanning the surface in a plane perpendicular to the tip. In the original paper (Binnig et al. 1986), the topographic profiles of a ceramic sample (an insulator) were shown. This is one of the main advantages of AFM: Both tip and sample may be insulators. This property greatly expands the range of possibilities for scanning probe microscopy, making it possible to study biological samples (e.g., proteins, membranes, and whole cells).

**AFM IMPLEMENTATION: TECHNICAL ISSUES**

Although there are a variety of ways to control the deflection of the cantilever in AFM, here we will focus on the beam deflection method (Meyer and Amer 1988), because it is commonly used when working with biological samples. The beam deflection system involves focusing a laser beam on the end of the cantilever and collecting the reflected light with a photodiode. As a consequence, any bending of the cantilever will affect the position of the reflected laser spot on the photodiode. A normal bending generates a normal force \( F_N \) on the photodiode sectors, whereas a lateral torsion will result in a lateral force \( F_L \). The core of an atomic force microscope is the head (Fig. 1A) in which the beam deflection system is integrated along with the piezoelectric tube that moves the sample in three directions (x, y, and z). In Figure 1A, an atomic force microscope head configuration is shown in which the tip is fixed and a piezo tube moves the sample. In another configuration, known as “stand alone,” the sample remains stationary while the tip scans the sample surface. The electronic components receive the signals coming from the photodiode, mainly \( F_N \) and \( F_L \), and provide high voltages (≈240 V) to move the piezo tube to which the sample is attached. A computer manages the data and calculates all of the parameters required to move the piezo tube.

Integrated tip and cantilever assemblies can be fabricated from silicon or silicon nitride using photolithographic techniques. More than 1000 tip and cantilever assemblies can be produced on a single silicon wafer. The cantilevers can be rectangular (see Fig. 1B) or V-shaped and they typically range from 60 to 200 μm in length, 10 to 40 μm in width, and 0.3 to 2 μm in thickness. The typical tip radius is ≈30 nm, although sometimes smaller diameters can be obtained (Fig. 1C). The cantilever
spring constant \( k \) ranges between 0.02 and 40 N/m, and it strongly depends on the cantilever’s dimensions. For example, for a rectangular cantilever

\[
k = \frac{E W (T/T)}{L}
\]

where \( E \) is the Young modulus of the cantilever, and \( W, T, \) and \( L \) are the cantilever’s width, thickness, and length, respectively (Fig. 1B). Whereas \( W \) and \( L \) can be fairly precisely known, \( T \) is always difficult to measure. As a consequence, manufacturers normally provide the cantilever spring constant with an error of 10%–30% and users should calibrate each cantilever (Sader et al. 1999). The spring constant \( k \) is used to calculate the Hookean force applied on the cantilever as a function of bending \( \Delta z \), that is \( F = k \times \Delta z \) (Fig. 1D).

The vertical resolution of the cantilever, \( \Delta z \), strongly depends on the noise of the photodiode, because it defines the minimum significant displacement, \( \Delta z \), on the detector (see Fig. 1D). A typical cantilever of 100 \( \mu \)m length has \( \sim 0.1 \) \( \AA \) resolution, assuming a signal-to-noise ratio of \( \sim 1 \) (Meyer and Amer 1988).

Another parameter that can influence the vertical resolution is thermal noise (Butt and Jaszke 1995). The cantilever oscillates at its resonance frequency with an amplitude

\[
\Delta z = \sqrt{\frac{k_B T}{k}}
\]

where \( k_B \) is the Boltzmann constant, \( T \) is the absolute temperature, and \( k \) is the cantilever spring constant. For example, at room temperature, there is a noise of \( \sim 5 \) \( \AA \) for a cantilever of spring constant 0.02 N/m.
Interaction Between the Atomic Force Microscope Tip and the Sample Surface

To understand the interaction between the tip and sample, we shall refer to potentials rather than forces. Physicists prefer this because potentials are scalar, and therefore easier to deal with, than vectors such as forces. For this purpose, let us graphically depict the Lennard–Jones potential (see dotted graph in Fig. 2A), \( U(r) = -A/r^6 + B/r^{12} \), where \( r \) is the tip–sample distance, \( A = 10^{-7} \text{ Jm}^6 \), and \( B = 10^{-134} \text{ Jm}^{12} \).

This describes the interaction of all the atoms in a particular solid (Israelachvili 2002). A rough approximation to the atomic force microscope tip–sample interaction is to consider the approach of two such atoms where, as depicted in the inset in Figure 2A, the lower part is a solid surface and above it is the apex of a sharp tip that moves downward. We can find an interaction force such that \( F(r) = -dU/dr \), and it can be seen that this force is attractive \( (F < 0) \) when \( r > r_0 \) or repulsive \( (F > 0) \) when \( r < r_0 \) (solid line graph in Fig. 2A). These regions define the attractive and the repulsive regimes.

**FIGURE 2.** Force curves, feedback, and lateral resolution. (A) The potential of the tip–sample interaction is shown. The dotted line represents a Lennard–Jones potential as a function of the distance between atoms, \( r \), mimicking the tip–surface interaction (represented in the inset). The solid line is the interaction force obtained from the potential. The numbers inside the circles indicate the cantilever instabilities (see text). (B) A force vs. z-piezo displacement curve in air. (C) A force vs. z-piezo displacement curve in liquid. (D) Contact mode showing the variation of \( F_{
abla} \) (green), and z-piezo tube voltage (topography, pink) as a function of a step (blue). (E) The geometric features of the tip–surface contact.
of operation, respectively. Now let us consider the role of these regimes in a force versus distance (F–z) AFM experiment that involves the tip approaching the surface (Fig. 2B). The experiment starts with the tip situated far from the surface in the attractive regime. As the tip is approaching and as soon as the gradient (i.e., the slope) of the force equals the cantilever spring constant, the tip jumps to the surface from black point 1 to black point 2 (both connected by the slope). This is seen in the F–z of Figure 2B at point A like a sudden jump of the cantilever deflection (vertical scale). Thus the tip establishes mechanical contact with the surface and it rapidly enters the repulsive regime (F_N > 0). The z-piezo (horizontal scale) is elongated until a given F_N or deflection value is reached and then stops (point B of Fig. 2B). The external loading force F_ext can be calculated as the difference between the zero deflection position (i.e., before the jump to contact in A) and the deflection at point B. Because the vertical scale is ~10 nm per division and the cantilever spring constant k = 0.1 N/m, then F_ext ~0.5 div × 10 nm/div × 0.1 nN/nm = 0.5 nN. Subsequently, the z-piezo retrace cycle starts, and the tip is released from the surface at C (i.e., once more where the derivative of the tip–surface force equals the cantilever spring constant), jumping from the red point 1 to 2 following the red arrow. The cantilever deflection jumps off to zero with a damped oscillation. This jump-off is known as the adhesion force F_adh (in this case F_adh ~ 2 div × 10 nm/div × 0.1 nN/nm = 2 nN). The total force at point B is the sum of F_ext and F_adh (i.e., 2.5 nN). It is interesting to note that no matter how small F_ext is, the total force applied to the surface will always be at least F_adh.

Contact Mode
Contact mode is the simplest AFM operational method and it was the first to be developed (Binnig et al. 1986). Here the tip is brought into contact with the surface until a given deflection in the cantilever (F_N) is reached and the tip then scans a square area of the surface to obtain a topographic map. By elongating or retracting the z-piezo, the feedback algorithm tries to maintain a constant cantilever deflection by comparing the F_N signal with a set-point reference value established by the user. The topographic data are obtained by recording the z-piezo voltage that the feedback algorithm is applying to correct the cantilever deflection at each position on the surface. Because the z-piezo is calibrated, the voltages are transformed into heights and a topographic map is obtained. Let us consider a simple example in which the tip is scanning a step (blue line in Fig. 2D) with F_N = k × Δz. When the cantilever moves to the upper part of the step, it undergoes a deflection greater than Δz. Therefore the feedback algorithm retracts the z-piezo to achieve the same deflection Δz as when the tip was in the lower part of the step. As a consequence, a topographic profile of the step is obtained (pink line in Fig. 2D). On the other hand, F_N varies at the step that is corrected by the feedback, which can be observed as a peak in the deflection signal (green line in Fig. 2D). The latter is known as the constant deflection mode or the constant height mode, because the z-piezo is not modified and a map of the changes in F_N is obtained. The reader is encouraged to reproduce Figure 2D when the tip goes down the step.

Let us now consider the lateral resolution that can be achieved in contact mode. We can estimate this parameter by applying the Hertz theory (Johnson 1985), which accounts for the deformation of solids in contact. Once the tip is in contact with the sample, the radius r of the tip–surface contact area is given by (Fig. 2E)

$$r = \left( \frac{3 \times F \times R}{4E^*} \right)^{1/3},$$

where F is the applied force F_N and E^* is the effective Young modulus expressed by

$$\frac{1}{E^*} = \frac{1 - \nu_t^2}{E_t} + \frac{1 - \nu_s^2}{E_s},$$

with (E_t, \nu_t) and (E_s, \nu_s) being the Young modulus and Poisson ratio for the tip and sample, respectively. R is the effective radius, expressed as a combination of the tip radius R_t and sample radius R_s.
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\[
\frac{1}{R} = \frac{1}{R_{dp}} + \frac{1}{R_{sample}}
\]

In the case of metals, \( E = 100 \text{ GPa}, \nu \approx 0.5, \) and the mechanical thermal noise of the cantilever is 10 pN. With \( R_c \approx 20 \text{ nm}, \) the radius of contact \( \rho \) is \( \sim 0.15 \text{ nm}, \) which implies atomic resolution. However, the adhesion force in air (see below) is \( \sim 5 \text{ nN}, \) increasing \( \rho \) to \( \sim 1 \text{ nm}. \) Atomic resolution can be achieved by either working in liquids (Ohnesorge and Binnig 1993) or in UHV conditions (Giessibl 1995), where even individual atoms can be chemically identified (Sugimoto et al. 2007).

Geometrical Dilation

When the surface asperities are comparable to the tip radius (which is common in AFM experiments), the size of the tip plays an important role. The tip distorts the image owing to the dilation of certain features of the image by the finite tip size (Villarrubia 1997). Such dilation effects occur in all AFM operational modes. An example can be seen in Figure 3, A and B, in which single-walled carbon nanotubes have been imaged. These are graphene in the form of a cylinder a few nanometers in diameter. In Figure 3C two profiles of similar carbon nanotubes are compared, showing that the nanotubes of Figure 3B are wider than those in Figure 3A. The geometric dilation effect can be seen in Figure 3C, in which scanning of the carbon nanotube by the tip results in the red profile, because the

![Figure 3. Geometrical dilation and sample preparation. (A,B) Atomic force microscope images of the same samples of carbon nanotubes adsorbed on silicon dioxide. (C) The topographic profiles obtained along the green lines in A and B show different widths due to dilation. (D) The geometric parameters in the dilation process. The red line depicts the dilated section of the carbon nanotube section (gray circle). (E) Illustration of sample preparation for an experiment to study a DNA-protein complex. (F) The DNA-protein complexes imaged by AFM in air (see text). (Adapted from Dame et al. 2002.)](https://www.cshprotocols.org/3/3/fig3)

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tip cannot get closer to the tube than the tip radius $r_t$. Therefore, based on geometrical considerations in Figure 3D, the tip radius $r_t$ can be calculated as $r_t = b^2/2h$. The topographies of Figure 3, A and B, have been obtained using tips with a radius of 15 nm and 70 nm, respectively. It is evident that the sharper the tip, the better it is for imaging.

A very popular and impressively experiment used to teach AFM is to image graphite (in particular, highly oriented pyrolytic graphite) in air conditions (Martí et al. 1988), where the elastic deformation of the tip–sample contact plus the dilation effects result in atomic corrugation. Although the image seems to provide atomic resolution, atomic defects are not visualized.

**Dynamic Modes**

Dynamic modes (DMs) (Martín et al. 1987) are those in which the cantilever is made to oscillate near or at its resonance frequency ($\omega_0 \propto \sqrt{E} T/l^3$ for a rectangular cantilever). As the tip approaches the sample, the oscillating amplitude decreases until it establishes contact with the sample, following a similar cycle as that in Figure 2B, but now with oscillation. Therefore, the feedback loop involves the amplitude rather than the $F_n$, and by keeping the oscillation amplitude constant, a topographical map can be obtained. The amplitude is reduced because the resonance frequency ($\Delta \omega$) changes with the tip–sample distance $z$ and with the tip–sample interaction force $F_n$ according to

$$ \frac{\Delta \omega}{\omega_0} \propto \left( \frac{1}{2k} \right) \frac{dF_n}{dr}, $$

thereby decreasing with attractive forces. The new resonance frequency $\omega$ is positioned to the left of $\omega_0$ and the cantilever is still oscillating at $\omega_0$, the cantilever amplitude decreases.

The very high lateral forces that are applied to the surface in the contact mode (Carpick et al. 1997) can damage the sample. This is especially problematic for single biomolecules adsorbed onto a surface, because these are delicate samples from which to obtain images. However, when operated in non-contact mode (Garcia and Perez 2002), DM does not apply large dragging forces and so it is commonly used to image molecules weakly attached to surfaces in air. Maps other than topographical maps can also be obtained in DM, such as a phase map (the time difference between the excitation and the response of the cantilever), which in air carries information on the composition of the sample (see specific details in Garcia and Perez 2002).

When DM is used in liquid, the landscape completely changes, as the viscosity of water reduces the resonance frequency approximately fourfold and the quality factor (a measure of the cantilever damping) of the oscillation is reduced from ~100 to 10. When oscillating the cantilever in liquid, a mechanical contact between tip and sample is established, resulting in the application of lateral and normal forces that may damage the specimen (Legleiter et al. 2006).

**Jumping or Pulse Force Mode**

Jumping or pulse force mode (JM) (Miyatani et al. 1997; de Pablo et al. 1998) is a contact mode in which lateral tip displacement occurs when the tip and sample are not in mechanical contact, thereby avoiding shear forces and the corresponding damage to the tip–sample system. An $F$–$z$ curve (Fig. 2B) is obtained at every point of the image, moving the tip to the next point at the end of each cycle when the tip and sample lose contact. Feedback is engaged at point B of Figure 2B, moving the z-piezo so that a constant deflection or loading force $F_{ext}$ is maintained. Adhesion force maps can provide compositional or geometrical information about the surface (de Pablo et al. 1999). The adhesion force between the tip and the surface can be described as $F_{adh} = 4\pi R y_L \cos \theta + 1.5 \gamma$ $\bar{y}\delta R$, where $y_L$ is the water surface tension and $\theta$ is the angle of the water meniscus present between the tip and surface, $R$ is the effective radius (described above), and $\gamma$ $\bar{y}\delta$ is the tip–surface energy difference. Although the effective radius $R$ is present in both terms, the first one provides information mainly about the hydrophobicity of the sample and as such a rough estimate in air at room temperature results in ~7 nN for an $R_t$ of ~20 nm. In air, the first term is the main contribution to $F_{adh}$ whereas the second
one depends mainly on the tip–surface geometry. The importance of the first term can be appreciated by comparing the $F$–$z$ curves taken from glass in both air (Fig. 2B) and liquid (Fig. 2C). In liquid the adhesion force is almost absent, because there is no water meniscus between the tip and sample, although some hysteresis appears in the $F$–$z$ owing to the dragging of water on the cantilever.

**USING AFM TO IMAGE BIOLOGICAL SAMPLES**

**Imaging Biological Samples in Air**

AFM is used in dynamic mode for imaging DNA on mica. As DNA and mica are both negatively charged, MgCl$_2$ is added to the DNA solution. The Mg$^{2+}$ ions become sandwiched between DNA and mica, allowing the DNA molecules to adsorb onto the surface. The sample is then dried and NM-AFM is used to image the DNA molecules on the surface. DNA itself has been the focus of much research using AFM (Hansma et al. 1992), and more recently, AFM has been used to investigate the binding of proteins to DNA (Lyubchenko et al. 1995; Dame et al. 2003; Janicijevic et al. 2003). In these kinds of single-molecule experiments, the researcher is not interested in the average result of the bulk reaction but rather in the action of individual proteins on DNA. Hence, once the protein is pipetted into the DNA solution (Fig. 3E) the reaction starts. The DNA–protein complex is then adsorbed onto the mica at the desired time points where it is air dried (Fig. 3E). AFM thus provides a snapshot of the process that is taking place between the proteins and DNA, and the topographic map that is generated provides single-molecule information about the protein–DNA complex. For example, this technique has been used to study how the *Escherichia coli* H-NS enzyme interferes with DNA polymerase activity (Dame et al. 2002). AFM images revealed that the DNA polymerase (the big round blob on the DNA filament) becomes trapped between two pieces of DNA that are bridged by the HNS enzyme (Fig. 3F). This entrapment appears to be sufficient to stop transcription initiation.

**Imaging Biological Samples in Aqueous Media**

When imaging samples in buffer or other aqueous media, proper attachment of the biomolecule to the supporting surface is required to achieve good resolution during the imaging process. Although biomolecules can be covalently linked to a chemically modified surface (Wagner et al. 1995), covalent modifications could potentially damage the biomolecules. Fortunately, physisorption is usually sufficient and thus, specimens in a physiological buffer can be directly adsorbed to the desired surface. The physisorption process is driven by van der Waals forces, the electrostatic double-layer force (EDL force), and the hydrophobic effect (Muller et al. 1997). The EDL force depends strongly on the concentration and valence of charged solutes, as well as the surface charge density of both surface and specimen. The EDL force between two equally charged surfaces is repulsive and hence opposite to the van der Waals attraction (Muller et al. 2002). Contact mode AFM has been used extensively to image two-dimensional protein crystals in liquid, such as membranes. Biological macromolecules become attached to the surface (e.g., mica, silicon, gold, or glass) when there is a net attractive force between the macromolecules and the surface. The Derjaguin–Landau–Verwey–Overbeek force ($F_{DLVO}$) can be estimated (Israelachvili 2002) as the sum of the electrostatic force between surface and molecule $F_{el}$, and the van der Waals interaction $F_{vdW}$,

$$F_{DLVO} = F_{el}(z) + F_{vdW}(x) = \frac{2 \sigma_{surf} \sigma_{sample}}{e_r e_0} e^{-z/\lambda_D} - \frac{H_a}{6 \pi z^3},$$

where $z$ is the distance between the surface and specimen; $\sigma_{surf}$ and $\sigma_{sample}$ are the charge densities of surface and specimen, respectively; $\epsilon_r$ and $\epsilon_0$ are the dielectric constants of the electrolyte and the vacuum, respectively; $\lambda_D$ is the Debye length, which depends on the electrolyte valence (Muller et al. 1997); and $H_a$ is the Hamaker constant.
The adsorption of a sample onto freshly cleaved mica (atomically flat) can be manipulated by adjusting both the ion content and the pH of the buffer solution. An estimate of the $F_{\text{DLVO}}$ between a purple membrane (a two-dimensional crystal lattice formed by bacteriorhodopsin) and mica is shown in Figure 4A, highlighting the strong influence of the electrolyte concentration (Muller et al. 1997). Figure 4B shows a region of purple membrane adsorbed onto mica (Muller et al. 1995). Interestingly, in this type of setup, the cantilever can be used to apply forces that trigger conformational changes in single proteins, such as GroEL (Viani et al. 2000).

AFM can also be used to visualize single proteins at work. To do so, AFM is used in dynamic mode and in liquid (Moreno-Herrero et al. 2004). Maximum peak forces of a few nanoNewtons are applied, relative to the stiffness of the sample, for very short periods of time (Legleiter et al. 2006; Xu et al. 2008), to avoid damaging the sample. For example, the force application period could be 10% of an oscillating period (i.e., for a cantilever with a resonance frequency of 10 kHz in liquid, the forces are applied for 10 μs every 100 μs). Using this method, it has been possible to visualize the activity of RNA polymerase on DNA (Kasas et al. 1997), and the conformational changes in a DNA-repair complex on binding DNA (Moreno-Herrero et al. 2005).

**Imaging Viruses Using AFM**

Structural and chemophysical characterization has been critical for our understanding of the biology of viruses. X-ray crystallography and EM (cryo-EM/IR) techniques have traditionally been used. Although they provide direct three-dimensional structural information and allow the interior and the surface of the virus to be visualized, these are averaging ("bulk") techniques, and thus they present an average time and space model of the entire population of particles found in the crystal or on the EM grid. These techniques provide limited information about the characteristics of individuals within a population of viruses that distinguishes them from the average. For this reason,

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**FIGURE 4.** Imaging the purple membrane and single viruses. (A) Establishing the buffer conditions for imaging. The interaction force between the purple membrane and the mica surface is depicted as a function of the distance and the electrolyte concentrations. (B) A typical atomic force microscope image of a purple membrane in liquid with bacteriorhodopsin, a light-absorbing membrane protein. (Reprinted, with permission, from Muller et al. 1997 and Muller et al. 1995, © Elsevier.) (C–E) Three MVM particles showing threefold, twofold, and fivefold symmetry, respectively. (Adapted from Carrasco et al. 2006.)

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the beautifully symmetrical models of larger viruses derived from these techniques may be somewhat deceptive and not fully representative of every individual virus particle within a population (Plomp et al. 2002).

Because viruses are individual particles that adsorb weakly to typical surfaces, they are prone to destruction by lateral forces when imaged in AFM contact mode. It is preferable to use the tapping mode because loading forces can be accurately controlled to avoid the application of lateral forces. Figure 4, C–E, shows single viral particles of the minute virus of mice (MVM) adsorbed in threefold, twofold, and fivefold symmetry, respectively. By making nanoindentations with F–z curves on single viruses, it has been shown that single-stranded DNA within the MVM virus contributes to the overall mechanical stiffness of the virus particles, which could be important for viral stability during the extracellular cycle (Carrasco et al. 2006). Moreover, it is possible to selectively disrupt these DNA–protein interactions and thereby engineer a virus with altered mechanical properties (Carrasco et al. 2008).

SUMMARY AND FUTURE DIRECTIONS

A typical AFM image takes minutes to acquire, making it difficult to view dynamic biological processes. Work is underway to increase the rate of AFM image capture, so that biological processes can be visualized in real time. For example, conformational changes of single myosin V proteins on mica have been observed by using high video rates (80 m/sec/frame) and very soft cantilevers with a high frequency of resonance in liquids (Ando et al. 2001). This was achieved by decreasing the cantilever thickness and reducing the cantilever width and length proportionally. In addition to obtaining topographical images to understand the structure and dynamics of a system, AFM can also extract information about mechanical properties (e.g., stiffness) by using the phase-in dynamic modes in liquid (Melcher et al. 2009).

Finally, noninvasive imaging techniques are being developed that minimize sample destruction. For example, frequency modulation AFM (Hoogenboom et al. 2006) is a dynamic technique in which forces on the order of tens of picoNewtons can be applied to the surface. This promising technique is based on the use of three simultaneous feedback systems. A phase lock loop ensures that the cantilever is always in resonance, whereas a second feedback process (working over the phase lock loop) changes the tip–sample gap to keep a set point frequency, such that its output gives the topography. Finally, a third feedback component is used to maintain the oscillation amplitude constant by changing the amplitude of the cantilever driving signal, which results in more stable operation.

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