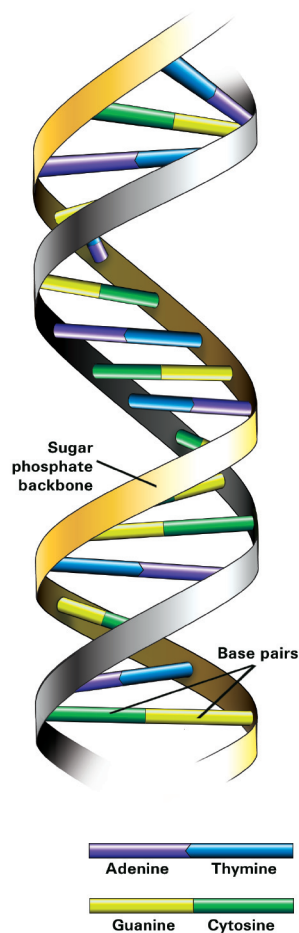




Imaging DNA in Solution with the AFM

Application Note

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The DNA double helix is composed of A-T and C-G base pairs which are attached to a negatively charged sugar-phosphate backbone.

Introduction

DNA (deoxyribonucleic acid) is a biological polymer that stores hereditary information in almost all living organisms. Each strand of DNA is a pattern for duplicating new strands of DNA, so, when cells divide, each new cell gets an exact copy of the DNA from the previous cell. DNA is composed of two strands of repeating units called nucleotides which are entwined in the shape of a double helix. Each DNA strand is 2.2 to 2.6 nanometres wide. Individual DNA molecules can contain hundreds of millions of nucleotides and can be several mm long. Each nucleotide is composed of a nucleobase, a sugar residue and a phosphate group. The nucleobases, adenine (A), cytosine (C), guanine (G) or thymine (T), interact (base pair) with a particular nucleobase in an opposing strand of the double helix by hydrogen bonding interactions; A pairs with T and C pairs with G. The sugar residues are joined together by negatively charged phosphate groups; which form phosphodiester bonds between the 5' and 3' hydroxyl groups of each sugar residue. The DNA double helix is stabilized primarily by electronic interactions between the nucleobases and Watson-Crick base pairs (hydrogen bonds) between the nucleobases in the opposing strands.

Imaging DNA with the AFM

Atomic Force Microscopy (AFM) can be used to obtain highly accurate images of DNA and other nucleic acids (such as RNA) under physiological conditions with nanometer scale resolution. This makes AFM a powerful analytical tool to study DNA [Lyubchenko 1993, Han

1997]. The technique has been used to study the structure of nucleic acids such as supercoiled, kinked and looped DNA and DNA protein complexes. In order to be resolved by AFM, samples must be immobilized onto flat, smooth, stationary substrates, so, in many cases, some form of surface modification is required to immobilize biological materials for AFM studies. In the case of DNA, its negatively charged backbone can be utilized to immobilize the biological polymer onto flat, smooth, charged substrates; predominantly via electrostatic interactions.

Immobilizing DNA onto Substrates for AFM Imaging in Liquid

When imaging under liquids, the samples must be firmly affixed to very smooth and flat surfaces in order to overcome the forces of the AFM probe and to permit resolution of the sample. Freshly cleaved muscovite mica, the surface of which is covered with siloxy groups, is often used for this purpose. The siloxy groups have many negative charges in aqueous solutions near neutral pH. The negatively charged siloxy groups can be directly exploited for the noncovalent, electrostatic immobilization of positively charged proteins (for example, ferritin), which have a large number of positively charged lysine residues near the surface. But, since DNA has a backbone that is composed of negatively charged phosphodiester groups, the siloxy groups must be treated with various chemistries to infer upon a positive charge upon them. Divalent metal cations, which can mediate the electrostatic interactions



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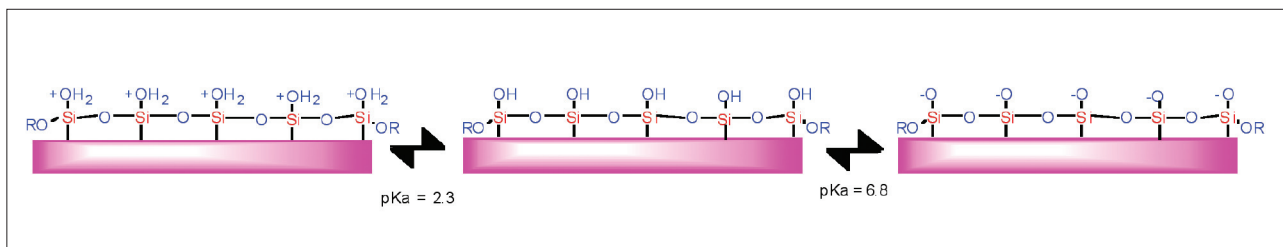


Figure 1. Siloxy groups on the surface of freshly cleaved mica.

between the negatively charged phosphate groups of the DNA backbone and the mica substrate, can be used to immobilize DNA so that it can be imaged in aqueous buffers. Other noncovalent approaches to attach DNA to mica for AFM imaging applications include inducing a positive charge to the surface of mica using amine polymers, such as polylysine, which has a net positive charge at neutral pH that can bridge the negative charges on mica and on DNA. Mica can also be covalently modified with various other reagents, such as aminosilanes or ethanolamine, which also carry positively charged amine groups, to render mica adhesive to the negatively charged DNA backbone.

Immobilizing DNA onto APTES or Ethanolamine Treated Mica

As mentioned above, the surface of muscovite mica contains numerous, reactive siloxy groups, which have a pK_a of 6.8. The siloxy groups can be chemically modified and then utilized to introduce positive charges onto the surface of mica to bind the DNA backbone via electrostatic interactions. One method that has been successfully applied for

this purpose is to modify mica with a uniform, very smooth and flat layer of positively charged amine groups using ethanolamine. When the reaction is performed in dry DMSO in the presence of molecular sieves, water is removed from the reaction, which drives the reaction in the direction of esterification resulting in a smooth layer of amine groups on the surface of the mica [Hinterdorfer 1996].

Alkoxy aminosilanes can also be used to covalently amine mica substrates [Lyubchenko 1993]. Solvents such as toluene, ethanol or acetone are often utilized in reactions involving alkoxy aminosilanes [Schumakovitch 2002, Vinckier 1998], but unless alkoxy silane reactions are performed under completely anhydrous conditions, they can hydrolyze and form partial condensation products. This adds unnecessary roughness to the otherwise atomically flat and smooth mica substrates; making it impractical for high resolution AFM imaging applications. Alkoxy aminosilane reactions that take place in the vapor phase can circumvent the negative issues associated with alkoxy silane reactions in solution, and these

reactions will generally yield a uniform layer of amine groups on the surface of mica [Lohr 2007]. Aminosilane-mica substrates that are derived from vapor phase reactions possess the necessary abundant positive charges at neutral pH for tightly immobilizing DNA, and they remain nearly atomically flat, so they can be used to immobilize, and consequently image, DNA in environments that mimic physiological conditions. The method utilizes freshly distilled aminopropyltriethoxysilane (APTES) under an inert argon atmosphere. Freshly cleaved mica strips are hung from the top of a glass or plastic dessicator jar and, after a small amount of freshly distilled APTES and *N,N*-diisopropylethylamine are placed in the bottom of the dessicator jar, the jar is purged with argon to remove all air and moisture and the jar is sealed. After 30 minutes to 2 hours, the APTES treated mica substrates are removed and used immediately or stored under argon and used within a week. A buffered solution containing DNA can then be spotted directly onto the APTES-mica substrate and the DNA imaged in aqueous buffer using MAC Mode AFM.

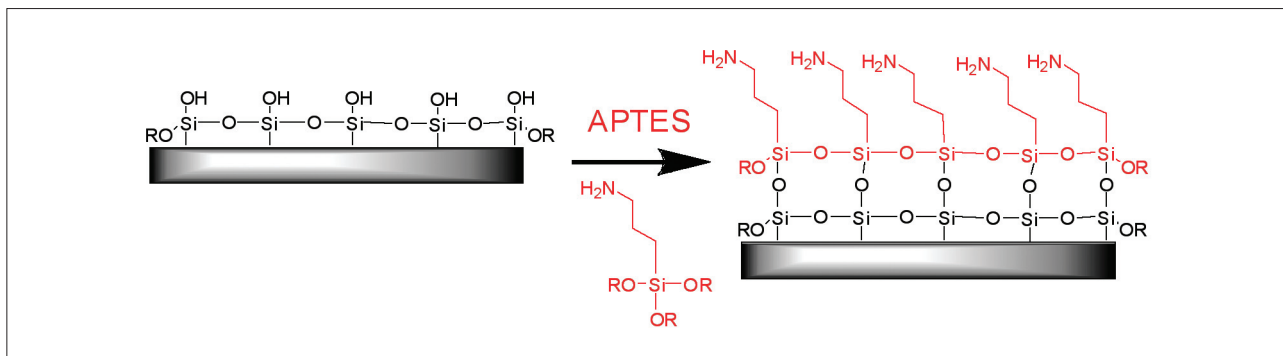


Figure 2. APTES treated mica substrate.

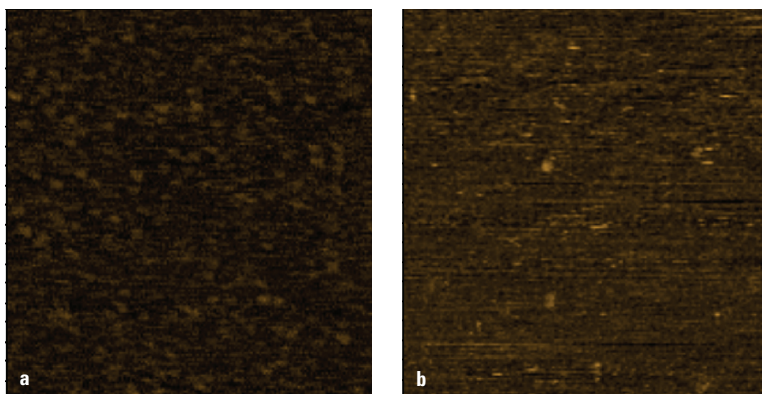


Figure 3. a) AFM images of freshly cleaved mica (rms = 0.16 nm) and b) APTES-mica (rms = 0.18 nm) that was functionalized in the vapor phase. The samples were imaged under aqueous conditions using MAC Mode. Scan size: 1 μ m.

Immobilizing DNA onto Mica with Polylysine

Polylysine coated carbon surfaces are frequently used to absorb DNA for imaging by electron microscopy. DNA can also be bound to polylysine coated mica to study DNA structure in liquid using AFM [Bussiek 2003]. In a typical procedure, polylysine is diluted to 0.01% in water and spotted onto a freshly cleaved mica substrate. After rinsing the substrate, a solution of DNA in the appropriate buffer is applied, allowed to stand for a

short period of time, washed and imaged in the imaging buffer (for example, HEPES pH 7.4) using MAC Mode AFM.

Immobilizing DNA onto Mica with Divalent Cations

Divalent cations can also be used as a bridge to immobilize negatively charged DNA molecules onto negatively charged mica surfaces, so that the DNA can be imaged in aqueous buffer [Cheng 2006]. Consequently, the pretreatment of mica with divalent cations and/or the addition

of divalent cations to the imaging solution can be a relatively straightforward and successful technique for high resolution imaging of DNA in aqueous buffers. The cations that are used to immobilize the DNA onto mica should be soluble in water and bind tightly to both the backbone of DNA and to mica with high affinity in order to withstand the forces exerted by the AFM probe during imaging. The divalent cations nickel (Ni^{2+}) and/or magnesium (Mg^{2+}) are often utilized for this purpose because they meet these criteria. Zinc (Zn^{2+}) has also been used for this purpose, but zinc is known to induce kinks in the DNA duplex. One particular technique for immobilizing DNA on mica via divalent cations involves spotting a dilute solution (for example, 2.1 $\mu\text{g}/\mu\text{L}$) of lambda phage DNA, along with small amounts of magnesium chloride and nickel chloride, in HEPES buffer (pH 7.4) onto a freshly cleaved mica substrate. After the DNA and the metal ions have been permitted to interact with the mica substrate for several minutes, the mica is washed and promptly imaged in the same buffer using MAC Mode AFM. Since the tip of an AFM probe and mica have similar surface chemistries, the cations, and the DNA can stick to AFM probes in a similar manner as they adhere to the mica. Consequently, it is important to wash the DNA- M^{++} -mica substrate carefully before imaging.

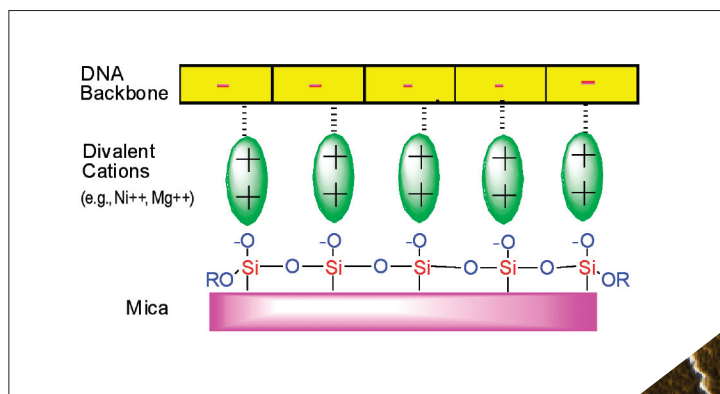


Figure 4. Immobilizing DNA on mica with divalent metal ions (M^{++}).

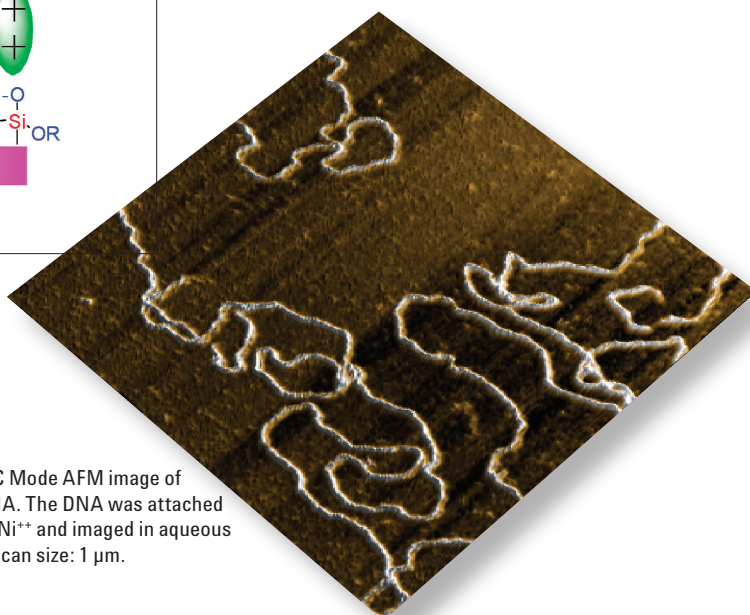


Figure 5. 3D MAC Mode AFM image of lambda phage DNA. The DNA was attached to mica by $\text{Mg}^{2+}/\text{Ni}^{2+}$ and imaged in aqueous (HEPES) buffer. Scan size: 1 μ m.

Conclusion

A combination of inorganic chemistry, bioorganic chemistry and surface chemistry can enhance the power and utility of AFM. AFM offers unprecedented resolution and some unique advantages over many other techniques for the study of biological molecules under physiological conditions. Consequently, it has become an important tool for nanometer scale imaging of biological molecules, including DNA, under physiological conditions.

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