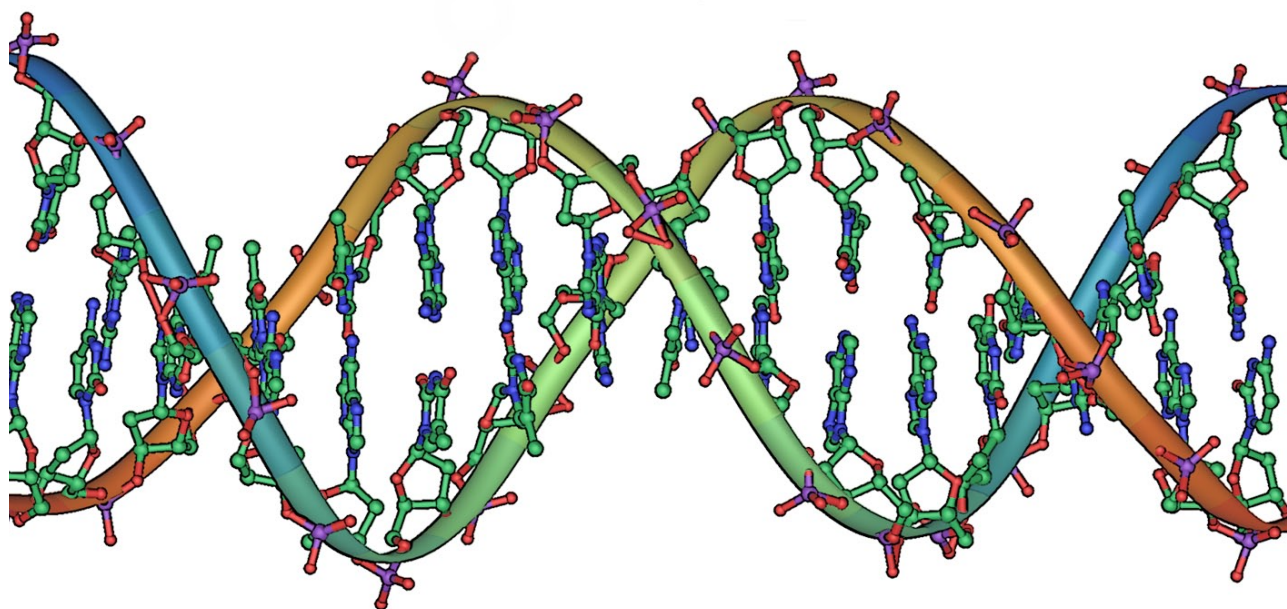


Imaging DNA with the AFM



Introduction

Long before the discovery of deoxyribonucleic acid (DNA), mankind observed that certain human characteristics are passed on from parents to their offspring. For a long time this concept was used for breeding animals or cultivating plants with distinctive characteristics. The science behind hereditary processes would, however, remain unknown for quite some time.

In 1865, Gregor Mendel discovered that inherited traits follow certain rules. Mendel's work was largely ignored until it was rediscovered in the early 20th century by Hugo de Vries and Carl Correns. This rediscovery of Mendel's work laid the foundation for modern genetics and the concept of genes as building blocks of hereditary information.

DNA was found to be the material that the genetic code was written in. Although its composition was analyzed early on, the exact organization and 3-dimensional structure of DNA was a highly debated topic. It wasn't until 1953, when James Watson and Francis Crick formulated their double-helix model, that the real structure of DNA and the mystery of the genetic code was solved. DNA is a polymer chain that stores hereditary information, and is composed of two

strands of repeating elements (nucleotides) that are folded in a double helix. The genetic code is embedded in the sequence of small groups of three nucleotides. Depending on the organism, DNA molecules contain up to hundreds of millions of nucleotides, and can therefore be anywhere between several micrometers (bacteria) and meters (mammals) in length.

X-ray diffraction data of Rosalind Franklin has played an important part in solving the structure of DNA, and this technique remains important even today, where X-ray crystallography and electron microscopy are the main tools used to image DNA at high resolution. Recently, however, scanning probe techniques have gained importance due to their high signal to noise ratio, the absence of a necessity to crystallize the DNA prior to imaging, and the possibility to study protein–DNA interactions and processes as they occur.

Imaging DNA

Atomic Force Microscopy (AFM) is used to image DNA with high accuracy and under physiological conditions^[1,2]. In order to be imaged by AFM, samples must be immobilized onto a flat surface. The negatively charged backbone of the DNA can be utilized for immobilization onto charged substrates by means of electrostatic interactions. Freshly cleaved

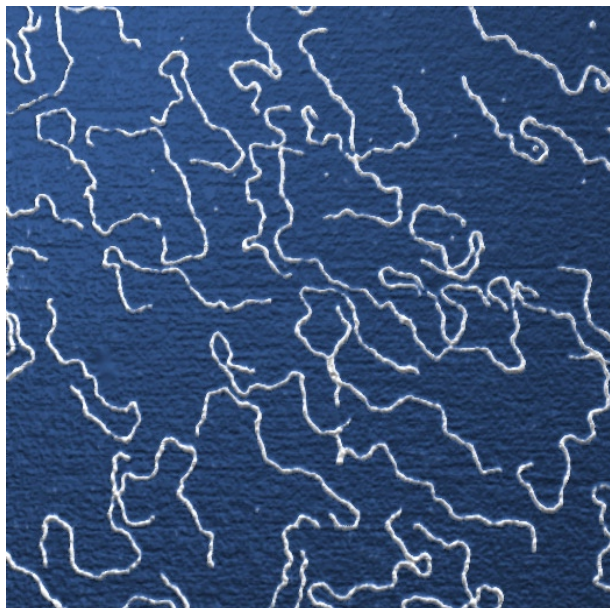


Figure 1: Linearized Plasmid DNA (pGem7zf+ from Promega) adsorbed onto muscovite mica. Image scan area corresponds to $2\ \mu\text{m} \times 2\ \mu\text{m}$.

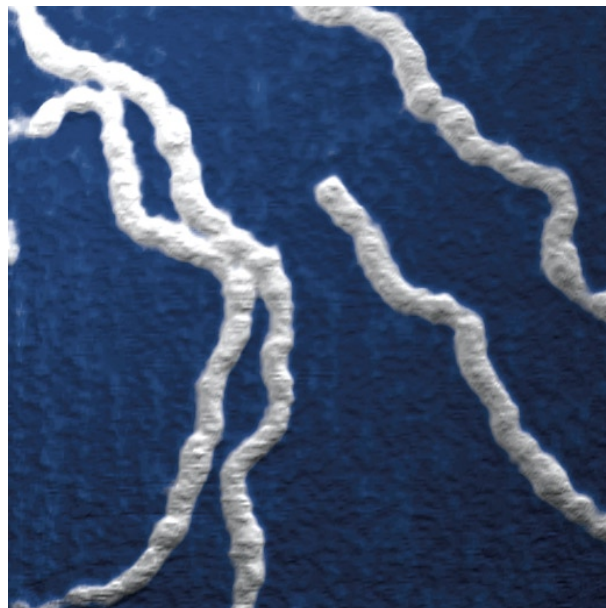


Figure 2: Close-up of the same DNA on mica preparation. Image scan area corresponds to $250\ \text{nm} \times 250\ \text{nm}$.

muscovite mica is often used for this purpose. Divalent cations can be used as a bridge to immobilize charged DNA molecules onto mica surfaces^[3]. The cations used should be soluble in water and bind tightly to both the backbone of the DNA and to the mica. Nickel (Ni^{2+}) and magnesium (Mg^{2+}) have these characteristics and are therefore often used. After the DNA and the cations have adsorbed onto the mica, the surface should be washed, air-dried, and promptly imaged.

References

1. Lyubchenko Y, Shlyakhtenko L, Harrington R, Oden P, Lindsay S. (1993). "Atomic force microscopy of long DNA: imaging in air and

under water". *Proc Natl Acad Sci USA* **90**, 2137-2140.

2. Han W, Dlakic M, Zhu YJ, Lindsay SM, Harrington RE (1997). "Strained DNA is kinked by low concentrations of Zn^{2+} ". *Proc Natl Acad Sci USA* **94**, 10565-10570.
3. Cheng H, Zhang K, Libera JA, Olvera de la Cruz M, Bedzyk MJ (2006). "Polynucleotide adsorption to negatively charged surfaces in divalent salt solutions". *Biophys. Journal* **90**, 1164-1174.

Instruments used

All measurements were performed with a high-resolution Nanosurf easyScan 2 FlexAFM ($10\ \mu\text{m}$ scan range) operated in Dynamic mode in air.

Nanosurf AG
Grammetstrasse 14
CH-4410 Liestal
Switzerland

Phone: +41-61-927 56 46
Fax: +41-61-927 56 47

Nanosurf is a leading provider of easy-to-use atomic force and scanning tunneling microscopes. Nanosurf products and services are trusted by professionals worldwide to help them measure, analyze, and present 3D surface information.

Nanosurf Inc
2125 Center Avenue, Suite 507
Fort Lee, NJ 07024
USA

Phone: 201-720-2829
Fax: 201-302-6062