Single molecule microscopy with the AFM

Sergio Santos, Daniel Billingsley, Jennifer Kirkham, William Bonass, Neil Thomson

Introduction

Single molecule microscopy is an extensive field of research in bio nanotechnology. Studying fundamental biological processes, interactions and phenomena at the single molecule level allows details to be elucidated that can be lost or obscured in ensemble measurements due to averaging over many, many molecules. The atomic force microscope (AFM) is a well established tool for single molecule studies. The AFM is a versatile high resolution microscope that can image in native-like environments such as ambient and liquid. It is a surface profiling microscopy that measures force between a sharp probe and sample, using a cantilever force sensor, to map out the local topography and mechanical or chemical properties. It has the advantage of not requiring surface coatings or stains to obtain contrast, enabling molecules to be imaged in native-like environments.

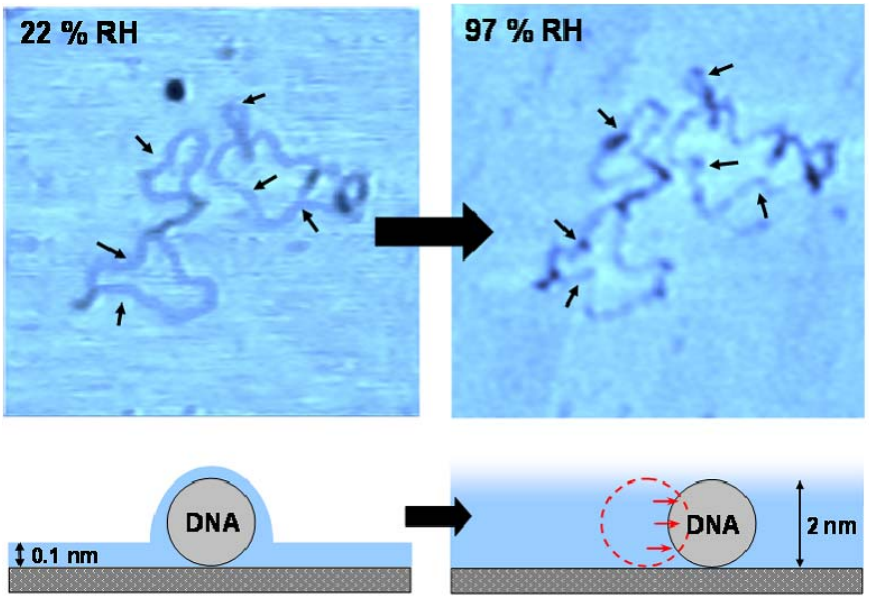

Figure 1: two supercoiled plasmid DNA molecules physisorbed onto mica surfaces imaged at 22% RH and 97% RH. The small black arrows mark regions where the local conformation of the molecules has altered due to the increase in the water layer depth, from sub-nanometre to around 2nm.

The role of hydration in ambient dynamic AFM

The AFM can be operated in the dynamic mode where the cantilever is vibrated while interacting with the surface. This mode is particularly important when imaging soft matter since frictional forces are almost eliminated. Furthermore, dynamic modes work well in ultra-high vacuum, ambient conditions and under liquid. Liquid imaging has some disadvantages to both sample preparation and instrument operation compared to ambient imaging. Molecular motion in liquid reduces stability and resolution. In ambient conditions a water layer typically forms on hydrophilic surfaces. The water layer is of the order of several angstroms to 2nm in thickness, depending on relative humidity. This layer has been commonly associated with instabilities, high adhesion and reduced resolution. We have found however, that the role of capillary interactions between probe and sample are strongly dependent on the cantilever characteristics. That is, while relatively un-sharp probes with compliant cantilevers tend to produce larger amounts of noise, stiffer cantilevers in combination with ultra sharp tips can greatly increase resolution.

Humidity-controlled AFM

The AFM can be operated under different humidity environments by enclosing the scan head in an environmental chamber. As the humidity is increased, the thickness of water layers on hydrophilic surfaces, such as mica crystals, increase to about 2nm at 90% relative humidity (RH). Recently, we have shown that this can be used to interrogate supercoiling of individual closed circular DNA molecules (Figure 1). As the thickness of the water layer approaches the diameter of the DNA, discrete and irreversible conformational changes in the molecules ensue. This is characterised by localised changes in DNA backbone, such as kinking, condensation and the rotation of loops. The interpretation is that strain energy initially taken up as DNA twist converts into local changes in the writhe of the molecule as the strain energy is released when the DNA molecules reside in a sufficient volume of water.

Apparent and true height in AFM

Since the field of Nanotechnology is defined through the dimensions of the nanoscale objects, it is clear that instruments capable of measuring true height and width with high precision are paramount for technological advances. While one of the main uses of the AFM is to measure the height of biomolecules, nanoparticles, the rugosity of the surface features, etc. The instrument, however, is notorious for always producing values which are lower the true ones. We have modeled the tip-sample interaction to show that this is due the area of interaction between the tip and the sample being finite. For example, even for the sharpest tip (i.e. an atom) the region of the sample interacting with it at relatively long distances is of the order of the size of atoms. This implies that there are certain limitations as to, for example, measure the true height of subatomic features even with a one atom tip. In terms of single molecular imaging, the finite geometry of the tip implies that there is a certain averaging between the height of the sample and the height of the supporting surface. Now that we have established the principle of height reconstruction is understood, recovering the true height is possible with reliable models of cantilever dynamics.

Publications

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