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Noncontact-AFM imaging of molecular surfaces using single-wall carbon nanotube technology

J S Bunch[1], **T N Rhodin**[2] **and P L McEuen**[1]

[1] Department of Physics, Cornell University, Ithaca, NY 14853, USA
[2] School of Applied and Engineering Physics, Cornell University, Ithaca, NY 14853, USA

E-mail: tnr1@cornell.edu (T N Rhodin)

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Abstract
Single-molecule imaging at nanometre resolution using noncontact AFM has been applied to image a protein complex on mica using single-wall carbon nanotube (CNT) technology. Hydrated RNA polymerase (RNAP)–DNA complex was imaged using constant-amplitude tapping mode AFM. CNT tips (1–2 nm diameter) prepared by chemical vapour phase deposition were mounted onto standard Pt/Ir coated AFM tips. The effectiveness of the CNT tip scanning was tested by imaging the profile of an RNAP–DNA complex. The results demonstrate the merits of high-resolution carbon nanotube nc-AFM scanning and suggest that CNT tips may provide a general tip–surface reference standard for defining tip–surface interactions on molecular-bonded organic surfaces.

(Some figures in this article are in colour only in the electronic version)

1. Introduction

Development of tip technologies to produce reproducible and understandable nc-AFM imaging of insulator, semiconductor, metal and organic surfaces, respectively, is needed to advance the theoretical understanding of high-resolution nc-AFM imaging. It has been proposed [1] that quantum mechanical modelling should predict the same nc-AFM image patterns for similar materials such as the alkaline earth fluorides (e.g. CaF_2 and BaF_2 together with CeO_2 and MgO). Other surfaces, such as semiconductors, metals and molecular-bonded materials, should show corresponding similarities. It is proposed that the mechanisms of image formation depend on the tip structure for a given class of material and for tips with identical properties images of similar surfaces should also look qualitatively similar. Hence, it is reasonable to expect reproducible imaging of similar surfaces at resolutions of 1 nm using scanning tips with radii of curvature in the nanometre range. The applicability of single-wall carbon nanotubes (CNTs) with these dimensions has been demonstrated for imaging of biological molecules [2]. The high-aspect-ratio CNTs are favoured for probing biomolecules having deep nanometre-scaled cracks and crevices. In addition, CNT scanning provides other attractive features, e.g., low tip–sample adhesion, ability to elastically resist large forces, capability of being chemically functionalized and the potential to achieve high lateral imaging resolution. However, design details of shaping and mounting CNT tips have presented practical problems, that have limited their widespread applicability [3, 4]. It is noteworthy that CNT tips are susceptible to high background thermal noise that can be minimized depending on their geometry. In addition, they can be difficult to mount onto AFM scanning probe devices. Nanometre probes fabricated of CNTs [2] have been successfully demonstrated for the fluidic scanning of biological molecules and the general applicability of CNTs to AFM imaging has been documented [5]. Problems associated with mounting and orienting CNT tips onto supporting cantilevers are being overcome [3] and new chemical routes to carbon nanotube architecture, physics and devices are being developed for nc-AFM scanning. This article specifically addresses the application of CNT nc-AFM scanning to molecular materials such as modified proteins and suggests an approach to advancing the theoretical understanding of nc-AFM tip–surface interactions.

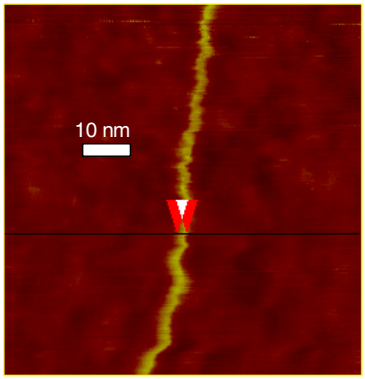

Figure 1. Nanotube imaged with a nanotube AFM tip showing high resolution and mechanical noise from the AFM instrument. The AFM instrument used in this image was the Digital Instruments Dimensions AFM mounted on a floating optical table. The height of the tube is 1.3 nm and the full width at half maximum is 2.75 nm so the effective AFM tip radius assuming a two-sphere model is 1.29 nm. All images were processed with a flattening filter.

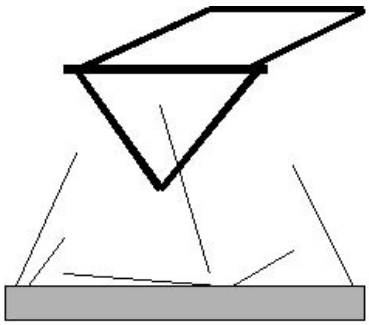

Figure 2. Schematic diagram of pick-and-stick procedure for mounting CNT tip onto cantilever.

2. Experimental method

Tapping mode AFM images were taken in air on a Multimode AFM except for figure 1 which was taken using a Dimensions AFM (Digital Instruments, Santa Barbara, CA). Standard imaging was done with Pt/Ir coated Si cantilevers with resonant frequencies of 60–100 kHz and force constants of 1.2–5.5 N m^{-1}. The best images were taken with the free amplitude typically at 1.8–2 V and the amplitude set point used was usually 1.4–1.6 V. High-resolution imaging was performed using single-walled carbon nanotube (SWNT) tips mounted on Pt/Ir cantilevers using a 'pick-up' technique whereby SWNTs are grown on a silicon substrate and during AFM imaging of the SWNT covered Si substrate a tube is picked up off the surface and then used as an imaging tip [6] (figure 2). The tips are then characterized by force–distance curves to determine whether there is a tube on the tip. Once a tube is picked up onto the Si tip, the CNT is shortened and stabilized through mechanical manipulation such as pushing it into the surface and shaking it at high velocities. The quality of the tip is usually determined by how well it can image a nanotube lying on our surface. Using a two-sphere model, our effective tip radius can be deduced from the full width at half maximum (FWHM) of the nanotube imaged. Again, similar amplitude set points were used as described above.

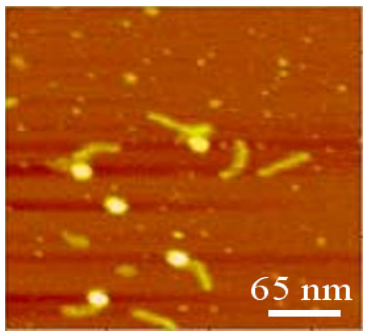

Figure 3. An nc-AFM image of RNAP–DNA complexes scanned with a silicon tip of approximately 10–20 nm diameter. It shows tip–surface interaction effects such as the polymerase complex depicted as featureless white spheres and the DNA strands as rodlike fragments. The indicated dimensions of the polymerase and the DNA strands have also been enlarged by the tip–surface interaction.

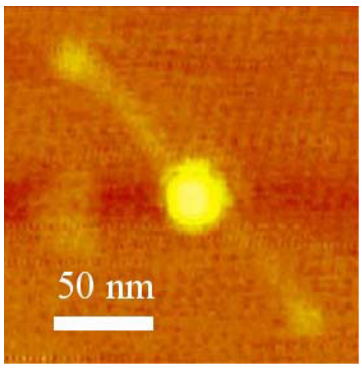

Figure 4. nc-mode AFM image using standard Pt/Ir coated tip. The DNA shown had an apparent FWHM of 11 nm.

2.1. Preparation and imaging of the RNAP–DNA elongation complex [10]

A solution of the DNA–polymerase complex was prepared by incubating a mixture of the DNA and protein solutions at 37 °C, for 10 min shortly prior to the nc-AFM measurement. One microlitre of one nanomolar RNAP–DNA complex was deposited onto a freshly cleaved mica surface and washed with ammonium acetate buffer solution. Excess liquid was removed using nitrogen flushing and the sample was inserted into the microscope. (Note: nc-AFM studies of this kind provides information on configurational features related to the dynamic modelling of proposed mechanisms for complex elongation.)

3. Results

A series of images obtained using conventional Si tips, (figure 3) and standard Pt/Ir tips (figure 4) were compared to similar images obtained by CNT-tip scanning (figure 5). A detailed analysis of the images as proposed by San Paolo and Garcia [7] for different nc-AFM modes is underway. However, some significant preliminary results are presented here. It should be noted that conventional Si tip scans gave similar images to the Pt/Ir tips. Immobilization of the molecule on the DNA appeared to stabilize the image. The magnified image of figures 5 is more sharply defined than that of figure 4 and the DNA diameter is closer to its true value. The measured diameter of the DNA in figure 5 is 3.5 nm FWHM, while in

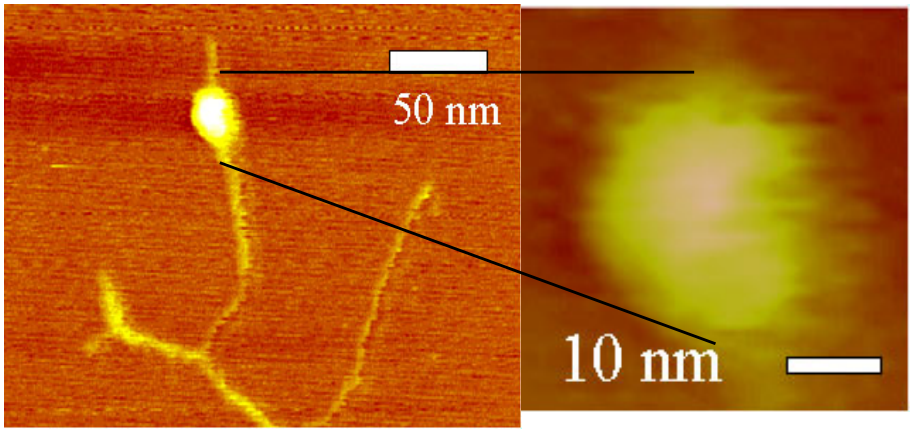

Figure 5. nc-AFM image of a single RNAP–DNA complex immobilized on a double strand of DNA with an FWHM of 3.5 nm. A blow-up of the two-dimensional profile of the RNAP–DNA complex (shown on the right) appears to reflect the intrinsic configurational profile of the polymerase complex as it is modified by formation of the elongation complex.

figure 4 it is 11 nm. Difficulties from particle dragging and distortion were absent upon repeated CNT-tip scanning. It appears that the configurational features are also intrinsically reflective of the single molecule and appear to be free of typical tip–molecule convolution effects. This conclusion is reinforced by analysis of a series of images corresponding to comparable tip-scanning procedures and sample preparation protocols (to be published). For example, it is significant that structural features for different polymerase–DNA profiles (not shown) corresponded to some of the two-dimensional features predicted from electron density plots derived from electron diffraction and x-ray diffraction measurements [8, 9].

It is significant that the polymerase molecular complex remains immobilized on the DNA substrate and is not perturbed upon multiple nc-AFM scanning. Configurational features of the complex were also found to be remarkably free of tip–molecule interaction artifacts. This was evidenced by a more detailed analysis of a gallery of nc-AFM images representative of repeated nc-AFM scans (not shown). The configurational two-dimensional features of the CNT tip scanning images suggested similarities to those predicted from x-ray and electron diffraction measurements on crystalline polymerase [9, 11].

In summary, although image resolution needs refinement, heretofore unobservable but significant features of the protein elongation complex appear to be indicated using CNT-tip scanning. These preliminary imaging results can be improved with more extensive instrumentation. It is significant that dimensional amplification associated with tip convolution is also minimized.

4. Conclusions

Single-wall carbon nanotube (CNT) tip scanning using nc-AFM imaging has been demonstrated to provide single-molecule profiles of a hydrated protein with enhanced resolution and reproducibility.

The unique features of CNT-tip scanning suggest that their special properties recommend them for developing reference scanning procedures in the imaging of molecularly bonded polymers and modified proteins on the nanometre scale.

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