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Molding Single DNA Molecules in Metals and Sample Preparation for Electronic Sequencing

John A. Lund, Babak A. Parviz *Senior Member, IEEE*

***Abstract*— We demonstrate the molding of single DNA molecules in 8 nm thin platinum molds. The molded structures have an apparent depth of 1 nm under STM imaging, and closely follow the contours of the DNA molecules. We have confirmed the presence of the embedded molecules and have verified the ability of this technique to scale down to single molecules. Additionally, we have utilized this method to perform electron tunneling analysis on embedded DNA molecules.**

I. Introduction

DNA sequencing is among the most important techniques in any modern examination of biological organisms. The field of DNA sequencing has expanded rapidly since the initial development of the Sanger sequencing method in the early 1970s.[1, 2] Modern sequencing techniques do away with many of the traditional limitations of Sanger sequencing by eliminating the need for electrophoresis and by performing massively parallel sequencing using dense arrays.[3-5] However, even these next-generation non-electrophoretic techniques have a host of their own limitations including short read lengths (<500 bases) and the need for repeated costly reagent cycles.[6] A number of proposed future-generation sequencing techniques use methods for DNA sequencing which avoid these limitations. Among these techniques is DNA sequencing using scanning probe microscopy, in particular, the use of scanning tunneling microscopy (STM) to perform electron-tunneling analysis of DNA molecules.[7] However, the analysis of DNA with STM has proved to be a daunting task, which has led to many erroneous reports of DNA identification.[8] In many instances, the DNA molecules being examined exhibit insulating behavior, which results in contrast inversion, or a complete inability to image the molecules.[9] Even when imaging is possible, DNA molecules are easily disrupted by a scanning tunneling microscope tip,[10] making reliable and prolonged analysis of a single molecule difficult. We present a method for molding DNA molecules in a thin film of pure platinum, which we demonstrate to be useful for reliable imaging, identification, and analysis using scanning tunneling microscopy.

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B. A. Parviz is with the Electrical Engineering Department at the University of Washington Seattle, WA 98195 (206-616-4038; e-mail: babak@ee.washington.edu).

J. A. Lund is with the Electrical Engineering Department at the University of Washington Seattle, WA 98195 (e-mail: jlund@ee.washington.edu).

II. Materials and Methods

A. Molding Overview

Our molding procedure begins by stretching single or double-stranded DNA molecules on a mica surface, followed by dehydration of the sample and evaporation of a thin platinum layer on top of the molecules. We then peel away the platinum layer, leaving the DNA molecules embedded in the platinum.

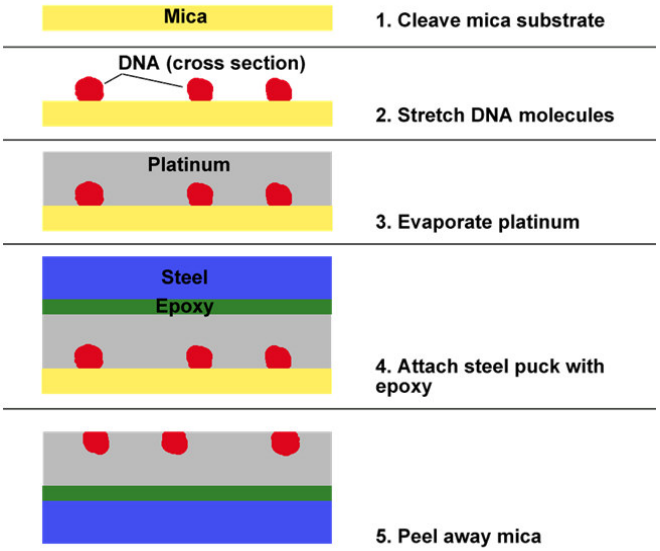

Figure 1: DNA molding procedure.

B. DNA Stretching

Prior to molding, we stretched λ-phage DNA molecules on an atomically flat freshly cleaved mica substrate. To do this, we diluted the DNA to a concentration of 40 µg/mL in a 0.45 mM $Mg(Ac)_2$ solution. The magnesium, a divalent cation, promotes adhesion between the negatively charged DNA and the negatively charged mica surface. [11] We used λ-phage DNA (Sigma-Aldrich) and a synthetic vector DNA molecule Litmus28i (New England Biolabs) which we cut with the Hind III restriction enzyme (Sigma-Aldrich). We were able to successfully stretch both the long (~16 µm) λ-phage DNA and the short (~1 µm) Litmus vector DNA. A 3 µL droplet of the DNA was blown across the surface at approximately 500 µm/s using compressed N_2. As the droplet moves along the surface, the receding meniscus of the droplet straightens and aligns the molecules. The result is an atomically-flat mica surface containing well-stretched DNA molecules as

shown in step 2 of figure 1.

Following DNA stretching, we rinsed the surface of the mica with ethanol to dehydrate the DNA molecules and prepare them for evaporation.

C. *Evaporation*

Following the DNA stretching, we evaporated an 8 nm layer of platinum on the DNA at a rate of <0.5 Å/s using an electron-beam evaporator. A slow evaporation rate is critical to the proper embedding of the DNA molecules. Evaporation at a high rate will result in platinum adatoms migrating underneath the DNA instead of on the top and sides of the molecules as illustrated in step 3 of figure 1. The thickness of the evaporated platinum layer is also critical. We found thin layers of platinum to be unstable, quickly forming discontinuities. We also found that platinum layers that were too thick tended to crack once we removed the film from the mica

D. *Removal of the platinum film*

After the evaporation procedure, we used epoxy (Devcon S-208) to glue steel wafers (Ted Pella #16218) to the platinum surface. The stainless steel chucks were thoroughly cleaned with DI water and isopropyl alcohol immediately prior to attachment to the epoxy to remove any residue from the steel surface and ensure proper adhesion. After curing, we gently peeled the steel chuck away from the mica. The platinum separates from the mica easily, resulting in a layer of platinum bonded to a steel wafer with DNA molecules embedded in the top surface, as show in step 5 of figure 1. When the mica is peeled away, the DNA molecules separate from the mica and remain embedded in the surface of the platinum. The thin platinum film is highly conductive, making an excellent substrate for STM investigation. The metal source for the film was 99.999% platinum, which offers a more pure substrate for electron tunneling spectroscopy than similar sample preparation procedures which use platinum-iridium metals and carbon stabilizing layers.[12]

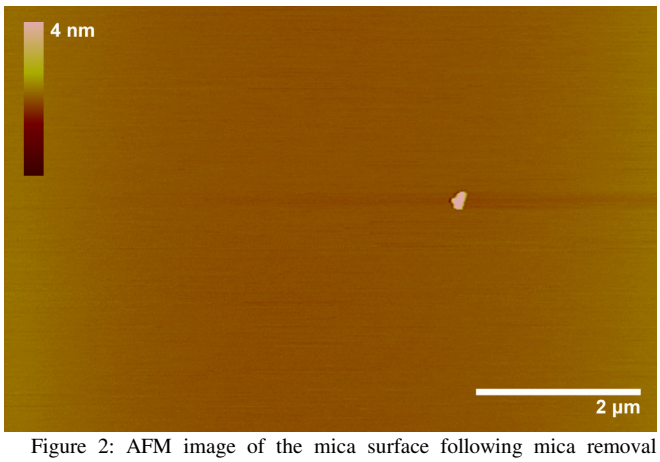

Figure 2: AFM image of the mica surface following mica removal showing the DNA molecules are removed during molding

III. Results

We confirmed the removal of the DNA molecules by imaging the mica after separation from the platinum. As illustrated in figure 2, the resulting mica surface was clean and smooth, completely free of DNA molecules indicating they remained in the platinum.

We also imaged the platinum surface with atomic force microscopy (AFM). As shown in figure 3, no DNA molecules were visible by AFM inspection, indicating the molecules are not resting on the surface of the platinum, but are embedded therein.

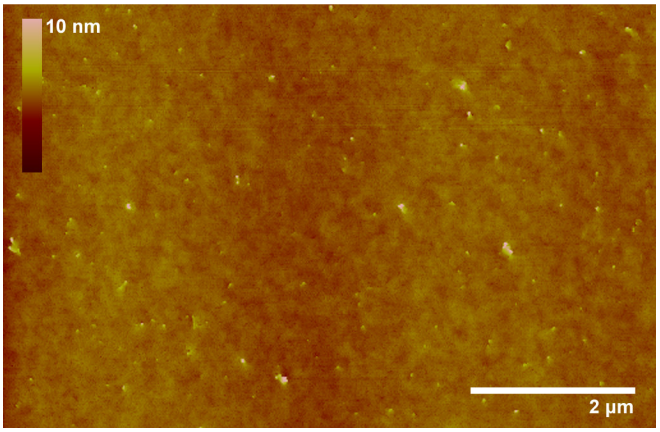

Figure 3: AFM image of the platinum surface following mica removal. The DNA molecules are not visibly raised, indicating they are embedded in the molded metal.

Scanning tunneling microscope (STM) analysis of the platinum surface showed well defined and clearly visible molecules embedded in the surface with an apparent width of approximately 3 nm and an apparent depth of 1 nm. Figure 4 shows an STM image of an embedded Litmus vector DNA molecule and an AFM image of another Litmus molecule stretched on mica for comparison.

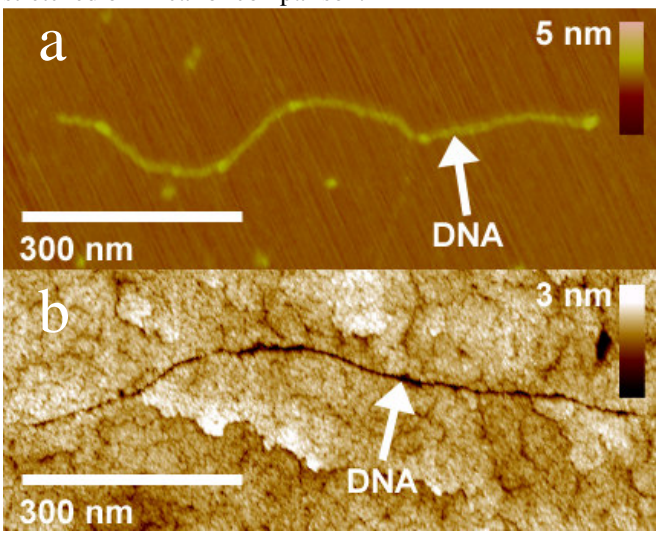

Figure 4: TM-AFM image of a Hind III restricted Litmus 28i DNA molecule on a mica substrate (a) and an STM image of a Litmus 28i DNA molecule embedded in an 8 nm platinum layer (b).

In addition to scanning probe analysis, we imaged the molded molecules using transmission electron microscopy

(TEM). We used a microtome to cut 60 nm thick cross-sections of the platinum film and imaged the depressions formed by the embedded molecules. Figure 5 shows the cross-section of embedded DNA molecules in the platinum film.

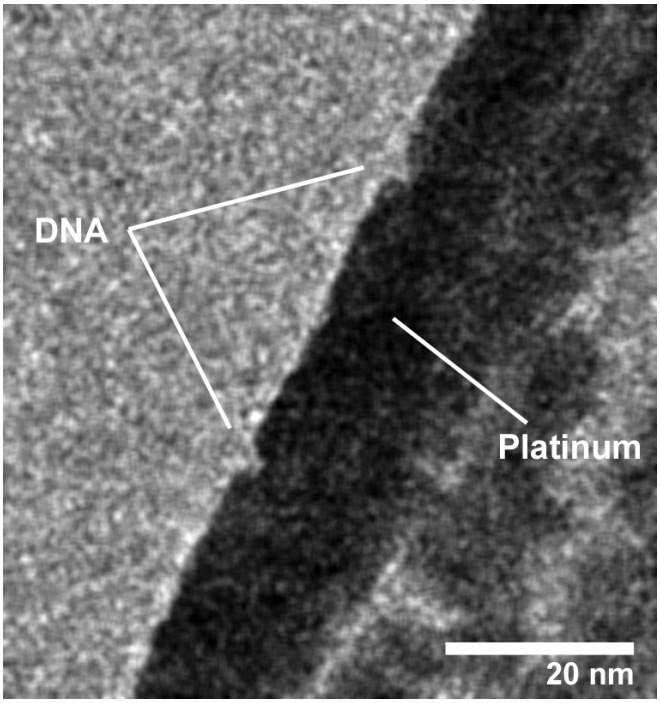

Figure 5: TEM image showing cross-sections of embedded DNA molecules. The sample was microtomed to a thickness of 60 nm and the DNA molecules are oriented perpendicular to the image plane, hence, only the very narrow cross section of the impression made in the metal by the molecule is observed.

The spacing of the impressions observed under TEM inspection corresponded to the spacing of the DNA molecules stretched on mica prior to the platinum molding, indicating they are indeed depressions made by the molded DNA molecules.

IV. Discussion

The method we have developed for molding single DNA molecules in high-purity metal films is useful for wide variety of applications, including generating nanoscale geometric features in metal films and characterizing molecular geometries. Because the molecules are embedded in the surface, they are not easily removed or displaced by an STM probe. This allows for reliable identification of the position and orientation of the molecules, and ensures the molecules remain stationary during electronic analysis.

A number of electronic analysis techniques exist for identifying molecular-level information. One form of electronic analysis in particular is orbital-mediated tunneling spectroscopy (OMTS). OMTS identifies energies at which electron tunneling events occur by measuring the first derivative of the tunneling current through a molecule. One intended use of OMTS and similar spectroscopy methods is to identify the bases in a DNA molecule to determine its sequence. This is a critical application for some future-generation DNA sequencing technologies. To this end, we have used a modified STM to perform an OMTS measurement in the range of +/-1 V. We performed this measurement with the STM tip positioned on top of an embedded DNA molecule using custom built control software that traverses an embedded DNA molecule while performing analysis on the center of the embedded molecule. For our off DNA measurements, we used the same microscope system to follow and analyze naturally occurring cracks in a thin platinum film evaporated on bare mica without DNA molecules. The preliminary results of this experiment are shown in figure 6 and provide a good indication that using our single molecule embedding technique with customized hardware and software built to analyze embedded molecules, it is possible to distinguish the molecule from the surrounding area.

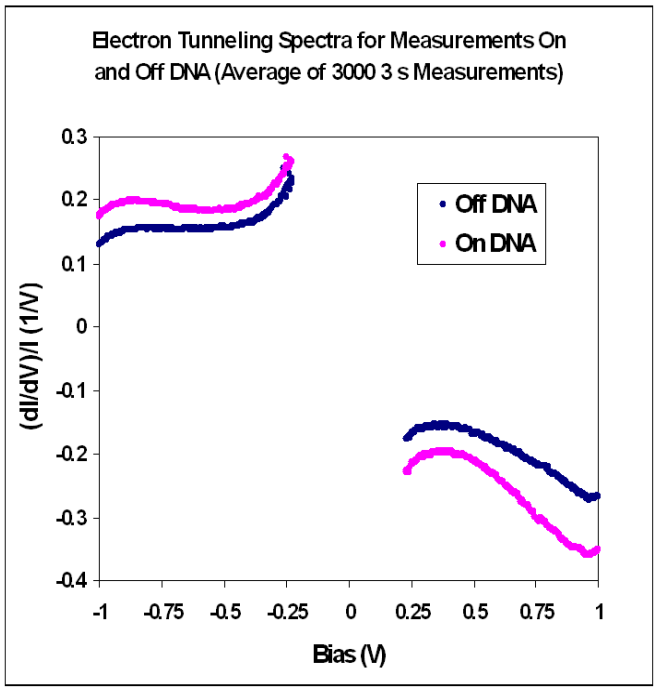

Figure 6: Electron tunneling spectra collected on and off embedded DNA molecules. Data between -23 mV and +23 mV has been excluded as it is dominated by noise resulting from division by extremely small currents.

V. Conclusion

We have developed a technique to mold single molecules in high-purity metal films. The nature of this method for embedding single molecules lends itself for applications involving STM analysis where the molecules under test may be difficult to image and analyze on an atomically flat surface. In particular, we have demonstrated the usefulness of this embedding technique for preparing and analyzing embedded DNA molecules. We have verified the location and orientation of the embedded molecules using STM, AFM, and TEM analysis. Additionally, we have performed initial spectroscopy studies on embedded DNA molecules,

demonstrating the application of this technique for single molecule analysis that we hope will be beneficial for future-generation DNA sequencing applications.

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