Using Electron Tunneling for Direct Sequencing of DNA

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ABSTRACT

We report an electronic method that has the capacity to directly read bases without any chemical manipulation or labeling of DNA. Our method consists of stretching DNA on a conductive substrate which can then be analyzed using inelastic electron tunneling spectroscopy (IETS). We have developed techniques to stretch DNA molecules on conductive substrates and have experimentally validated our method using a scanning tunneling microscope (STM) in identification of monolayers of oligonucleotides on gold substrates..

Keywords: DNA, sequencing, IETS, STM, electron tunneling

1 BACKGROUND

Genomic information has assumed a central role in the way biology in general and cell function in particular are understood today. Methods that can sequence DNA rapidly, accurately, and cost-effectively are needed in order to expand our knowledge of the core biology and realize the promise of genomics in areas such as personalized medicine.

DNA sequencing methods in use today, while substantially faster and less expensive than older technology, still rely on some of the earliest developed techniques to sequence DNA.[1] In particular the chain termination method, also known as Sanger sequencing, is commonly used today and suffers from substantial limitations including an average read length of only 805 bases. Our approach potentially avoids the limitations of other sequencing methods by directly identifying the individual bases on a single strand of DNA. Because each base is identified directly, there is no need for fluorescent dies or other labeling techniques.

The basis of our sequencing approach is inelastic electron tunneling spectroscopy (IETS).[2-4] We use IETS to identify unique spectroscopic signatures of each individual DNA base. When a molecule is placed between two electrodes connected to a sufficient bias voltage, a tunneling current results. As the voltage increases, the energy of electrons tunneling through the molecules also increase until it reaches the point where the electrons have enough energy to excite a resonant mode in the molecule, allowing an increased electron transmission rate. This results in a current-voltage (I-V) curve for the electrodes that is kinked at points where new resonant modes are excited. These kinks appear as peaks in a second derivative representation of the I-V curve. The different chemical structure of each base in DNA results in a distinct resonance signature in the I-V curves allowing for direct identification of the molecules.

IETS spectra are generally obtained at temperatures below 77 K and produce superior results at temperatures below 5 K. At increased temperatures, peaks in IETS spectra broaden due to thermal energy, which makes them more difficult to visually distinguish than at reduced temperatures. We demonstrate that even at room temperature it is possible to identify differences between spectra even where no sharp peaks are visible.

For our experiments, we used an atomically flat gold substrate as one electrode, and a sharpened Pt/Ir STM tip as the other electrode for IETS measurements as illustrated in figure 1.

In order for our approach to be applied to sequencing individual strands of DNA, we must first develop techniques to stretch DNA molecules on conductive substrates. In particular, we must be able to create stretched DNA which is non-bundled and uncoiled. We have developed techniques to reliably stretch DNA on both highly ordered pyrolytic graphite (HOPG) and Au (111) substrates.

As an intermediate step towards the ultimate goal of sequencing strands of DNA, we have developed techniques to modify gold substrates with a monolayer of oligonucleotides. We have performed IETS measurements on these substrates and have developed the ability to differentiate between these oligonucleotides.

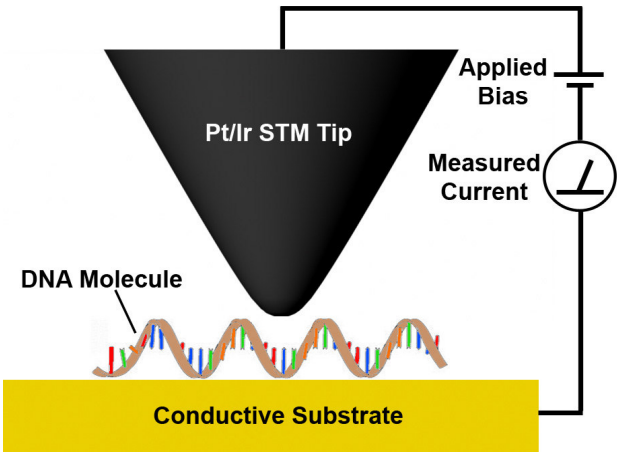

Figure 1: Experimental setup showing a DNA molecule immobilized on atomically flat gold and an STM tip close to the surface to perform IETS measurements.

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2 MATERIALS AND METHODS

2.1 DNA Stretching

Our approach to stretching DNA utilized two common types of conductive substrates used in STM imaging: HOPG and atomically flat gold. For these experiments we utilized two types of DNA: λ phage DNA with and without Hind III digest and Virion ϕX174 (New England Biolabs). The λ phage DNA was double stranded DNA (ds-DNA) and was melted to produce ss-DNA by heating at 95°C for 5 min in 10 mM Tris, 0.1 mM EDTA buffer (TE buffer) at pH 7.5. For stretching ssDNA on HOPG, we first cleaved the HOPG and soaked it in a 10 mM $MgCl_2$ solution for 10 minutes. The $MgCl_2$ binds to the freshly-cleaved HOPG and promotes adhesion to the DNA molecules. Following

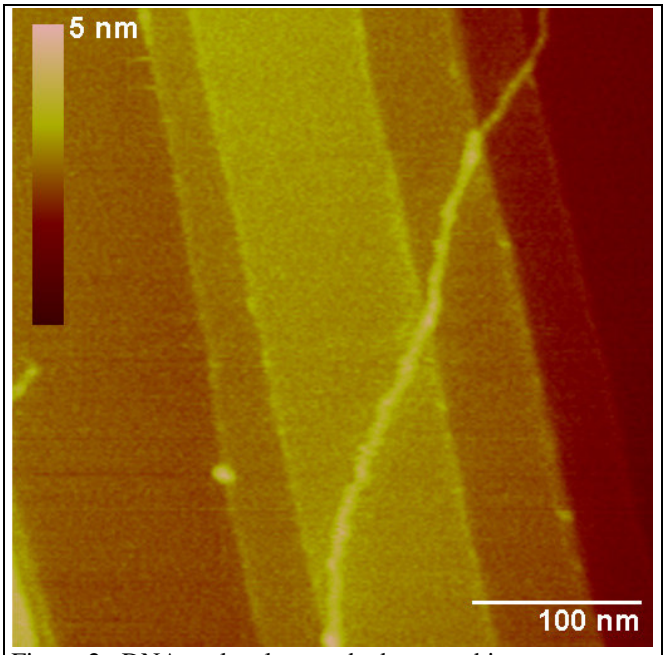

Figure 2: DNA molecule stretched on graphite.

exposure to $MgCl_2$, the graphite surface was rinsed with de-ionized water and dried with air. Five microliters of the DNA solution of interest was dispensed on one edge of the HOPG surface. We then dragged the droplet at the rate of 0.5 mm/s using a glass cover slip. We then rinsed the substrate with deionized water and dried it with air prior to imaging. An atomic force microscope (AFM) image of DNA stretched on HOPG is shown in figure 2.

We prepared mixed SAMs containing mercaptundecylamine and dodecanethiol on Au(111) substrates (Agilent) from a solution of both compounds (1 mol% amine:99 mol% dodecanethiol) in ethanol. The substrates were rinsed copiously using ethanol and dried in a vacuum dessicator. We extended non-methylated λ -phage DNA (48 kbp; New England Biolabs) using a syringe pump (New Era Syringe Pumps, NY) to draw a drop of λ -phage DNA (5 μL, 2.5 μg/mL) over the mixed SAM on Au(111); the rate of withdrawal was 1 mL/min.

We characterized the extended λ-phage DNA molecule using tapping-mode AFM. An AFM image of DNA stretched on Au (111) is shown in figure 3.

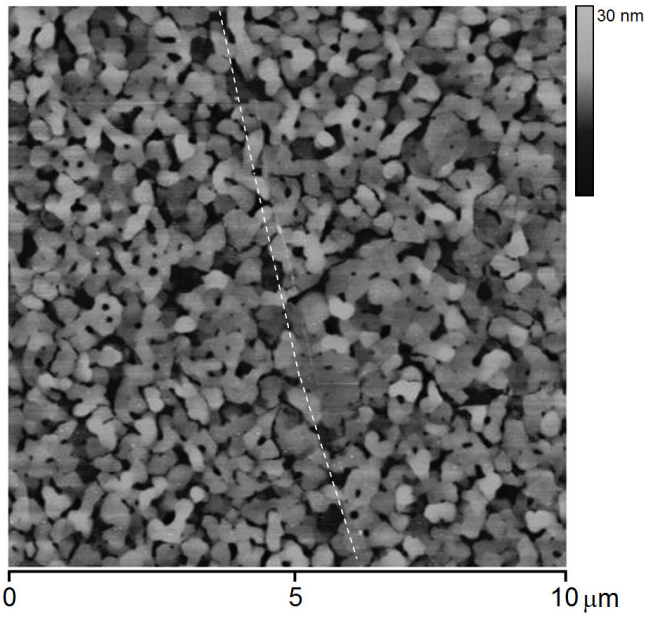

Figure 3: AFM of an extended molecule of λ-phage DNA. (a) The dashed line is used as an aid to the reader to locate the extended molecule, which appears to the right of the line. λ-phage DNA is ~15 μm long; our ability to resolve the molecule is limited to scan windows < 10 μm side.

2.2 IETS Setup

To perform IETS measurements with an STM tip, we modified a commercially available STM (Agilent 4500 AFM/SPM, model 300 STM scanner). We measured all first and second derivative signals directly using a lock-in amplifier (Stanford Research model 830) tuned to the first and second harmonic of a sinusoidal reference signal. A bias voltage signal was provided by a data acquisition card (National Instruments PCI-6281). The bias signal was combined with a reference sine wave (750 Hz, 40 mV amplitude) using OP-27 operational amplifiers. Our electronic setup was similar in design to those used in previously published research.[4]

The STM was wired normally; however, the sample bias signal was relayed in such a way that the sample bias provided by the microscope could be replaced by the aforementioned sine wave-modified signal necessary for conducting IETS measurements. The Agilent STM scanner contains an internal 10^9 transimpedance amplifier which amplifies the tunneling current and converts it to a voltage signal. We connected this signal (which is also used for STM feedback) to the input of the lock-in amplifier.

To conduct IETS measurements, we used the STM to image a sample of interest and position the STM tip at a desired location. We then turned off the STM feedback, switched to the custom sample bias, and measured the output from the lock-in amplifier using a different channel of the same data acquisition card. To ensure reliable data, the integration time on the lock-in amplifier was set to 1 second and the rolloff was set to 24 dB/oct. Because of this, the first several seconds of output from the lock-in were unreliable and had to be discarded. A typical measurement included data 19-20 seconds after initially applying the bias. After the measurement was collected, we turned the feedback on again to reset the tip to its setpoint current of 50 pA at 300 mV bias. We identified the background noise and instrumentation offset error by running an IETS measurement with no AC component. The results of this experiment indicated the background error to be negligible.

2.3 Attachment of Thiolated Bases

To verify the viability of identifying individual DNA bases on a metallic substrate we attached thiolated 5-mer molecules to gold substrates and performed IETS measurements on these samples. Each substrate was modified with only one of the four DNA bases (adenine, thymine, cytosine, and guanine).

We obtained the 5 base long thiolated bases from Integrated DNA Technologies. They are modified at 5' by the thiol modifier C6 S-S. The structure of each of these bases is inset in figure 4. We incubated the thiolated bases with 2.5 mM Tris (2-carboxyethyl) phosphine hydrochloride (TCEP) (Sigma) in the ratio of 1:30 for 2 hours at room temperature. This resulted in reduction of the disulphide (S-S) bonds to thiol (–SH) bonds which would react with the gold substrate upon incubation. Next, we soaked these thiol bases with Au (111) (Agilent) in 10 mM sample ethanolic solution (HPLC grade) for 1 hour. We then rinsed the gold substrate with ethanol and dried it with nitrogen gas. This procedure was repeated for all four bases.

3 EXPERIMENT PROCEDURE

We conducted IETS measurements on each sample. We first imaged the sample with the STM until the drift in the setup diminished to a negligible value, at which point we positioned the tip at the center of an atomically-flat gold domain. We then started an automated IETS measurement program which took 25 20 second measurements at each of 200 discrete voltage values between -300 mV and +300 mV. The collected data was then averaged over 25 measurements. Between each 20 second measurement, the STM feedback was turned on for 1 second to allow the sample-tip gap to reset. Given the time required to take each measurement, it took approximately 28 hours to collect an IETS spectra. Figure 4 shows the resulting IETS spectra for each of the 4 thiolated bases.

In addition to collecting IETS spectra for each of the 4 thiolated bases, we also collected IETS spectra for a blank gold substrate. Eventually we hope to use this system to identify bases on a single strand of DNA resting on a gold substrate, therefore, it is vital that we identify differences between all four bases with respect to the blank gold sample.

In order to compare the IETS spectra for the bases with the IETS for blank gold we normalized each set of data by dividing the data by the sum of the absolute value of each data point. We then subtracted the normalized blank gold IETS result from each of the four thiolated base spectra.

4 RESULTS

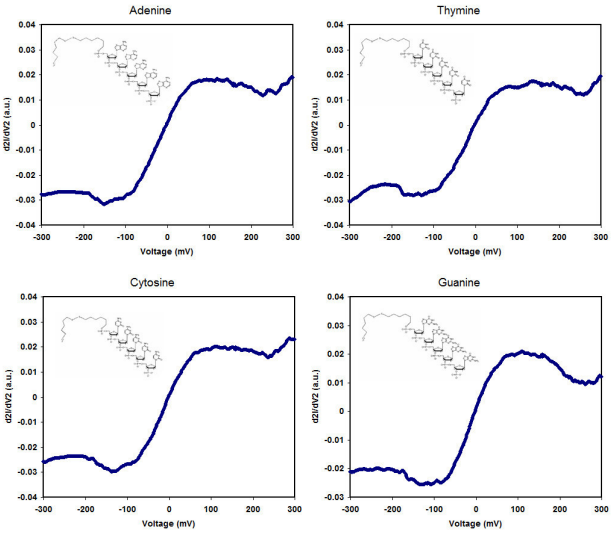

Figure 4: IETS spectra for thiolated 5-mer adenine, thymine, cytosine, and guanine.

The IETS spectra obtained for each base is shown in figure 4; however, this is insufficient to identify unique aspects of each spectrum. To see the differences between each spectrum, we looked at the difference between the thiolated base spectra and blank gold. Figure 5 shows the result of removing the blank gold spectrum from each of the thiolated base spectra. Each curve has its own unique features verifying the ability to differentiate between these thiolated bases on gold even at room temperature.

One of the best ways to verify that an IETS spectrum is accurate is to compare the second derivative data (which has peaks at resonant energy modes) with the first derivative data. Using a lock-in amplifier allowed us to measure directly the first and second derivative (eliminating noise resulting from mathematical differentiation); however, it is still critical to verify the proper operation of the amplifier. Figure 6 shows both the first and second derivative of the IETS measurement for thiolated Cytosine. This is a good verification that the measured second

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harmonic from the lock-in amplifier accurately corresponds to the second derivative of the I-V spectrum.

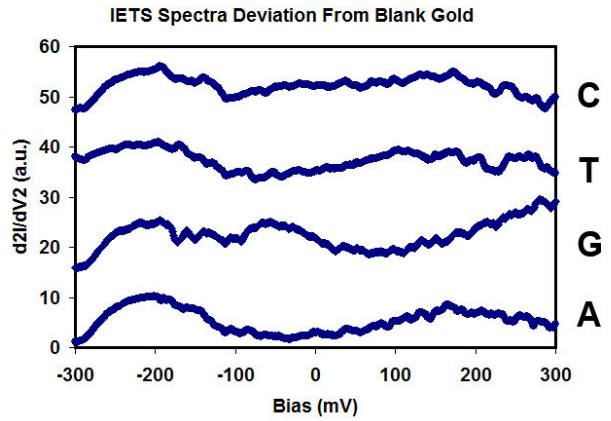

Figure 5: IETS spectrum for each base with the blank gold spectrum removed.

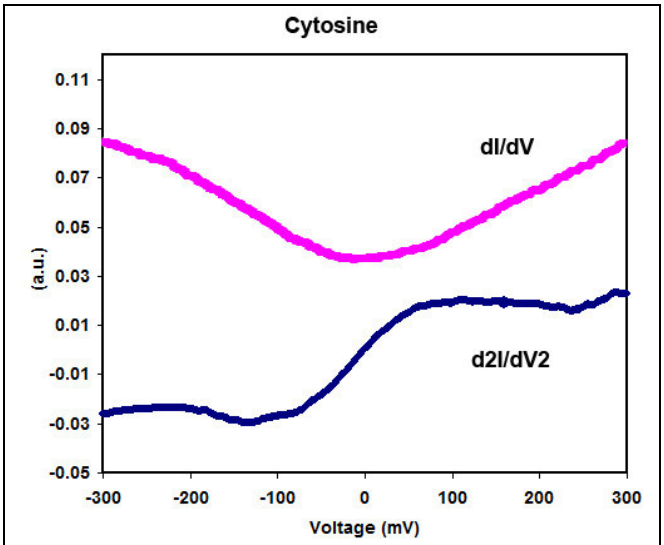

Figure 6: first and second derivative of the Cytosine spectra as measured by the first and second harmonic of the lock-in amplifier.

5 CONCLUSION

We have proposed a method for sequencing DNA using electron tunneling. As evidence that our method is viable we have demonstrated the ability to stretch single-stranded DNA on conductive substrates. To verify our proposed electron tunneling measurement approach we have developed a method to modify atomically-flat gold substrates with thiolated DNA bases and measured the room temperature IETS spectra for each of the four DNA bases. The results of these IETS measurements, when contrasted with the background IETS measurement for bare gold, indicates that it is possible to differentiate between DNA bases using room temperature IETS analysis.

The work we have presented is a starting point for a much larger body of research. We hope to apply the techniques and results described herein to eventually sequence strands of DNA on conductive substrates.

6 ACKNOWLEDGEMENTS

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