DNA Sequencing via Electron Tunneling

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***Abstract*—Fast and low-cost DNA sequencing methods would revolutionize medicine: a person could have his/her full genome sequenced so that drugs could be tailored to his/her specific illnesses; doctors could know in advance patients' likelihood to develop a given ailment; cures to major diseases could be found faster [1]. However, this goal of "personalized medicine" is hampered today by the high cost and slow speed of DNA sequencing methods. We will discuss the sequencing protocol we suggest which requires the measurement of the distributions of transverse currents during the translocation of single-stranded DNA into nanopores [2-6]. We will support our conclusions with a combination of molecular dynamics simulations coupled to quantum mechanical calculations of electrical current in experimentally realizable systems [2-6]. We will also discuss recent experiments that support these theoretical predictions. In addition, we will show how this relatively unexplored area of research at the interface between solids, liquids, and biomolecules at the nanometer length scale is a fertile ground to study quantum phenomena that have a classical counterpart, such as ionic quasi-particles and ionic "quantized" conductance [7,8].**

I. Introduction

Alternative methods for DNA sequencing have been increasingly of interest over the last decade. One of the primary targets is the "$1000" genome – the capability to sequence an individual's full genome for very low cost (and in a short time). Nanopore-based approaches have emerged as a promising method [1]. These are single-molecule approaches that directly detect physical differences of individual nucleotides in a strand of DNA, thereby "reading" off the sequence linearly as the DNA translocates through a pore.

A number of potential read-out mechanisms – to be coupled to the nanopore – have been proposed, from electronic transport, to ionic transport, to optical detection [1]. Of critical importance is to have a fast and reliable *physical* signature of the nucleotide present at a given location in a sequence. However, these techniques probe the DNA at the single base level and, thus, are subject to considerable fluctuations that occur at the nanoscale.

Our work has focused on theoretical investigations of nanoscale electronic transport as a method to discriminate between the DNA bases, how to limit the structural motion of the DNA, and how the surrounding aqueous, ionic environment affects the distinguishability of the bases. Experiments are starting to make progress in examining electronic transport as a means to sequence, with a number of outstanding recent results confirming our predictions [9-12].

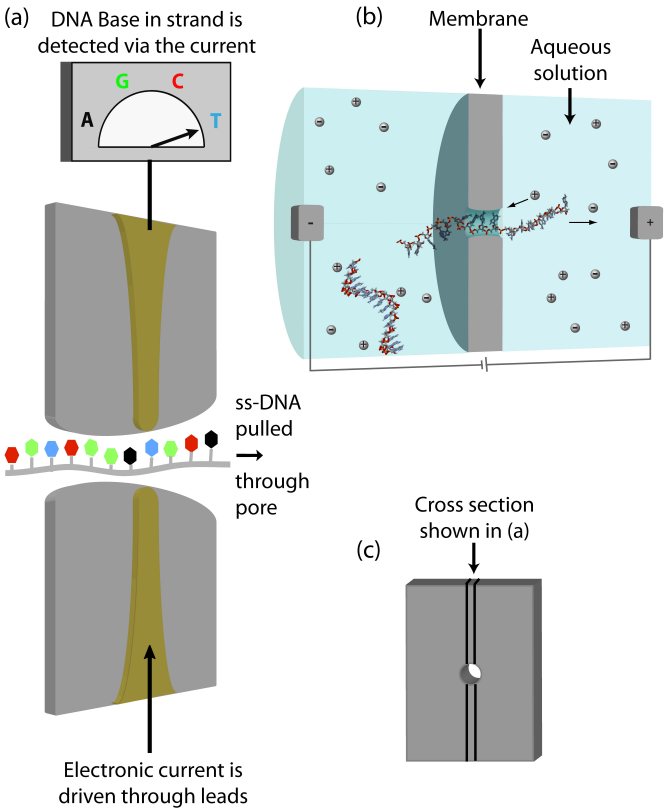

Figure 1. Schematic of sequencing via electronic transport. (a) A cross-sectional slice of a membrane (gray) with a nanopore through it is used to localize a DNA molecule (which can be pulled into the pore via an electronic bias applied across the membrane). Transverse electrodes are embedded within the nanopore that drive a current in a direction perpendicular to the DNA motion. As each DNA nucleotide in the strand passes between the electrode gap, an electronic current distribution is acquired and used to identify the base in the sequence. (b) The membrane partitions an aqueous, ionic solution which contains single-stranded DNA. (c) A frontal view of the membrane showing the cross-sectional slice taken for (a).

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II. TUNNELING CURRENTS FOR SEQUENCING

Electrons can tunnel at the nanoscale. This means that they can penetrate into classically forbidden regions and generate an electronic current. Such is the case when a nanoscale gap is made in a metallic wire. Current carrying electrons make their way across the gap, regardless whether its contents are the vacuum, molecules, fluid, etc., so long as the gap is suitably small. The contents, however, will modulate the ability of electrons to tunnel. In particular, when molecules are present they provide energy levels (i.e., molecular states) in the junction that increase the current. Depending on how far these energy states are from the Fermi level of the metallic leads, and also how well these states couple to the leads, the current will be modulated by different amounts.

This is the backdrop for using tunneling currents as a detection mechanism for reading out the sequence of DNA in nanopores [2,3]. The different DNA bases have distinct molecular structure and energy levels that will couple in differing ways to the electronic leads, thus giving each base their own electronic current signature that can be used to distinguish them, see Fig. 1.

We have theoretically investigated the electronic currents across the different DNA nucleotides ideally situated between two electrodes – where they span the gap between the two leads – and found that they indeed have different values [2]. However, the differences rely to a large extent on the couplings of the molecular states to the electrodes. Therefore, structural fluctuations of the nucleotides, e.g., due to the driving of the DNA strand through a nanopore or just due to structural motion of the DNA within the aqueous environment, will have an effect on the measured currents and the ability to detect which nucleotide is present between the electrodes [2].

Indeed, structural fluctuations turn out to have a profound effect. To address this issue we have coupled molecular dynamics simulations of DNA fluctuating within the pore with calculations of the electronic transport properties [3-6]. The molecular dynamics simulations provide the structure of single-stranded DNA, water, and ions in the junction region as the DNA translocates through the pore. We then take a Landauer approach to calculate the electronic current flowing through the atomic constituents of the junction. The current is given by

$$I = \frac{2e}{h}\int_{-\infty}^{\infty} dE\, T(E)\left[f_t(E) - f_b(E)\right],$$

where e and h are the magnitude of the electron charge and Planck's constant, respective. The integral over energy, E, contains the transmission coefficient $T(E)$ and the Fermi-Dirac distributions, $f(E)$, for the top (t) and bottom (b) electrodes.

To obtain the transmission coefficient one first computes the retarded Green's function

$$G_{DNA}(E) = \frac{1}{ES_{DNA} - H_{DNA} - \Sigma_t - \Sigma_b - \Sigma_n},$$

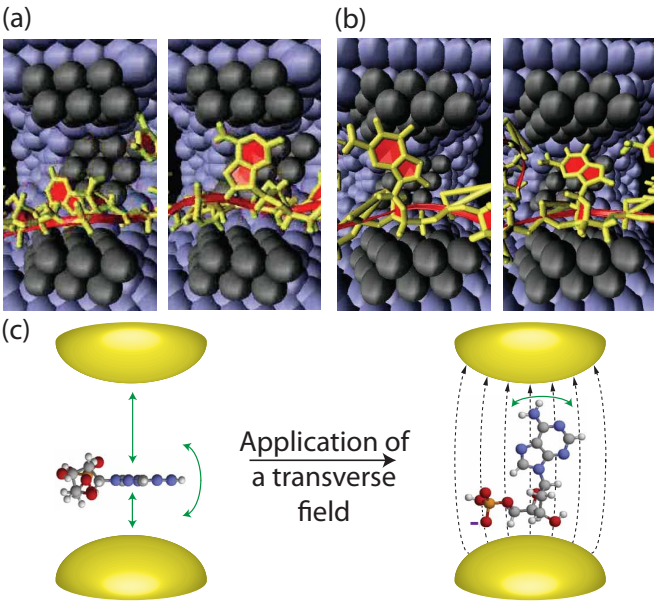

Figure 2. Snapshots from molecular dynamics simulations and the structure of DNA between the electrodes. (a) Translocation of single-stranded DNA through the nanopore without any stabilizing field. The DNA has large structural fluctuations in the pore, which results in many different orientations of the nucleotides as they pass through the junction region (here, dark spheres are gold atoms that represent the surface of the electrodes within the silicon nitride pore, shown as purple spheres). (b) DNA in the presence of a stabilizing field greatly reduces the structural fluctuations of DNA, giving the nucleotides well-defined current distributions that act as an electronic signature of the nucleotide. Water and ions are present in the simulations but have been removed to clearly see the DNA. (c) Application of a transverse field (either due to the bias driving the electronic current or an additional field across the whole device) pulls the charge on the backbone of each nucleotide to one side of the pore. Before the field is turned on there are lots of fluctuations (indicated by green arrows). Afterward the field holds the nucleotide close to configurations that are more ideal for transport (restricting motion to smaller fluctuations).

where S_{DNA} and H_{DNA} are the overlap and Hamiltonian matrices, respectively, of the contents of the junction. The remaining terms are the self-energies of the top electrode, bottom electrode, and external probes (i.e., potential sources of noise). These self-energies represent the influence of the electrodes and external noise on the DNA and other contents of the junction. In particular, when modeling noise, we include two external probes that represent scattering of the electrons in the complex fluid environment. Details can be found elsewhere [6].

The resulting transmission coefficient is

$$T(E) = \mathrm{Tr}\left[\Gamma_t G_{DNA} \Gamma_b G_{DNA}^{\dagger}\right]$$

where

$$\Gamma_{t(b)} = i\left(\Sigma_{t(b)} - \Sigma_{t(b)}^{\dagger}\right).$$

With external noise described by a two-probe model [6], the total transmission coefficient will just be this transmission coefficient between the top and bottom electrodes.

We found that, without control of the DNA motion, the tunneling currents fluctuate over orders of magnitude due to

the exponential dependence of the electronic coupling with the nucleotide-electrode distance. We therefore proposed that one should use a transverse field to control the motion of the DNA. For instance, a capacitor placed across the nanopore system, or the bias driving the current itself, can give a transverse field that will pull the (charged) backbone of DNA toward one side of the nanopore, both slowing its motion and reducing fluctuations [3]. Several snapshots from these simulations, with and without the stabilizing field, are shown in Fig. 2. While there are still fluctuations of the nucleotides between the electrodes, the DNA nucleotides acquire well-defined electronic current distributions that can be used as a signature of the base present in the electronic gap.

These current distributions are approximately log-normal under many circumstances and only have a partial overlap, making them distinguishable with only a small number of independent measurements of the current. Their main feature – that the current has Gaussian fluctuations on a log scale – is due to the exponential dependence of the electronic coupling on the distance between the molecule and electrodes. As well, transport across the junction can be modeled as due to a single electronic state on the DNA. Taking this perspective, we can obtain an approximate expression for the current

$$I(\eta) \approx \frac{2e^2V}{h}\frac{\gamma^2}{E_0^2+\eta^2},$$

where we have assumed linear response, which gives the current linearly proportional to the bias V. This expression is for a single electronic level coupled equally to both electrodes (with a strength γ) and in the presence of noise (of strength η).

As just mentioned, however, the main feature of the current is due to fluctuations of the coupling of the DNA to the electrodes. The expression above for the current, then, is just for one instant of time. As the DNA and other contents of the junction fluctuate, the coupling constant will be described by its probability distribution

$$p(\ln\gamma/\gamma_m) = \frac{1}{\sqrt{2\pi\sigma_\gamma^2}}\exp\left\{-\frac{(\ln\gamma/\gamma_m)^2}{2\sigma_\gamma^2}\right\}$$

with standard deviation σ_γ and maximum likelihood value γ_m. We emphasize that only when the DNA motion is controlled, e.g., by a transverse field pulling its backbone to one side of the pore, will the coupling constant take on (approximately) a log-normal distribution. These fits to the data from Ref. [6], in terms of the current distributions, are shown in Fig. 3(a).

This plot demonstrates an important characteristic of nanoscale electronic sequencing of DNA (and, indeed, sequencing with single-molecule approaches in general): Discriminating between the bases is not possible with just a single measurement. Only when taking several measurements and/or measuring an average current over time – while a single nucleotide is present in the junction – can one obtain a signal that allows for the different bases to be distinguished. These are issues explored in more detail in Refs. [3-5].

Further, we have also been investigating the effect of the aqueous, ionic environment on the ability to distinguish the DNA bases with electronic transport [5-6]. Water, for instance, has very little effect on the current across the junction when a nucleotide is present. Two-probe dephasing, which is present in the approximate equation, $I(\eta)$, through the noise strength η, also has little effect for moderate noise strengths. This is plotted in Fig. 3(b) for the nucleotide with Adenine. Only for noise strengths greater than the gap between the molecular energy levels and the Fermi level of the electrodes will there start to be an effect. Thus, due to the tunneling being through molecules, the signals for the different bases are protected from white noise of this type.

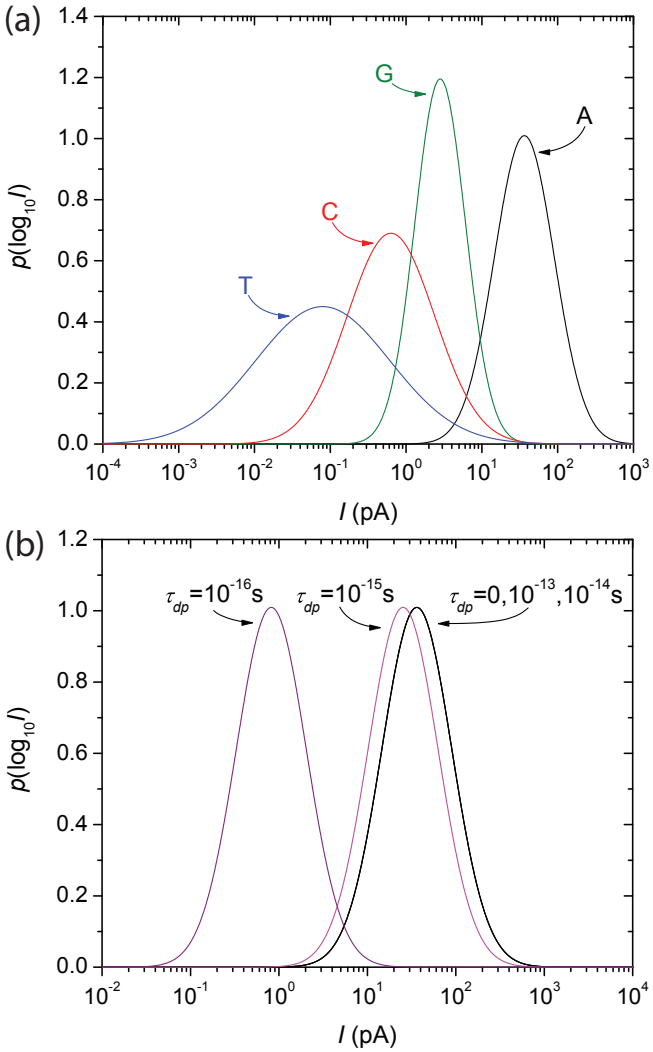

Figure 3. Current distributions for the different nucleotides between electrodes of spacing 1.4 nm and held at a bias of 1 V. These are log-normal distributions fitted to the full transport and molecular dynamic calculations of Ref. [6]. (a) All four DNA nucleotides, with bases Adenine (A), Guanine (G), Cytosine (C), and Thymine (T) are shown with zero noise (η=0). (b) The DNA nucleotide with Adenine shown for different dephasing times ($\eta = \hbar/\tau_{dp}$). Only the zero noise case is fitted to the full calculations, the other curves are from the approximate expression for the current, $I(\eta)$.

Many other issues arise in single-molecule sequencing techniques. In the case of transport, for instance, electrons tunnel through a nanopore that is embedded in an aqueous, ionic environment. Thus, there are background currents due to the presence of ions. In actual experiments, the tunneling signals indicating the presence of a base come on top of this constant background signal (see, e.g., Ref. [9]).

Experimental progress has been rapid over the past couple of years, starting from the construction of nanoscale electrodes housed in nanopores [13-18] to the measurement of tunneling currents across nucleotides/nucleosides [9-11] to devices that can simultaneously measure transverse and longitudinal currents [12]. For instance, the experiments of Refs. [9] and [10] measured tunneling currents across individual DNA nucleotides/nucleosides. They demonstrated that the bases do, in fact, have distinguishable electronic current signatures of the type shown in Fig. 3(a). While these are not working sequencing devices, they experimentally show the proof-of-principle behind sequencing via electronic transport. Refs. [11] and [12] have further shown that identification of individual bases in short DNA oligomers is achievable and that simultaneous measurement of transverse and longitudinal currents in nanopore devices is feasible, respectively. Another recent experiment explored a different electronic readout method that simultaneously measured the conductance change of a field-effect transistor and the longitudinal ionic current [19].

III. Conclusions

In addition to giving a potential route to rapid and low cost sequencing, transverse electronic transport and related nanopore setups offer the possibility to investigate a number of scientific challenges at the interface between solids, liquids, and biomolecules. For instance, we have also been investigating ionic transport phenomena in nanopores. We have shown that ions translocating through nanopores have to shed tightly bound water molecules, a process which requires a large energetic penalty to be paid. As the radius of a nanopore is decreased, more water layers have to be removed, resulting in a nonlinear stepwise energy barrier. This should be observable experimentally as quantized ionic conductance [7,8]. We believe that nanopore systems will give a number of fascinating results from understanding electronic transport in strongly fluctuating environments to understanding the motion of biomolecules. They thus promise to impact science and technology dramatically in the 21st century.

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References

[1] M. Zwolak and M. Di Ventra, "Colloquium: Physical approaches to DNA sequencing and detection," Rev. Mod. Phys., vol. 80, pp. 141-165, January 2008.

[2] M. Zwolak and M. Di Ventra, "Electronic Signature of DNA Nucleotides via Transverse Transport," Nano Lett., vol. 5, pp. 421-424, February 2005.

[3] J. Lagerqvist, M. Zwolak, and M. Di Ventra, "Fast DNA Sequencing via Transverse Electronic Transport," Nano Lett., vol. 6, pp. 779-782, March 2006.

[4] J. Lagerqvist, M. Zwolak, and M. Di Ventra, "Comment on Characterization of the tunneling conductance across DNA bases," Phys. Rev. E, vol. 76, pp. 013901, July 2007.

[5] J. Lagerqvist, M. Zwolak, and M. Di Ventra, "Influence of the Environment and Probes on Rapid DNA Sequencing via Transverse Electronic Transport," Biophys. J., vol. 93, pp. 2384-2390, October 2007.

[6] M. Krems, M. Zwolak, Y.V. Pershin, and M. Di Ventra, "Effect of Noise on DNA Sequencing via Transverse Electronic Transport," Biophys. J., vol. 97, pp. 1990-1996, October 2009.

[7] M. Zwolak, J. Lagerqvist, and M. Di Ventra, "Quantized Ionic Conductance in Nanopores," Phys. Rev.Lett., vol. 103, pp. 128102, September 2009.

[8] M. Zwolak, J. Wilson, and M. Di Ventra, "Dehydration and ionic conductance quantization in nanopores," J. Phys. Cond. Matt., vol. 22, pp. 454126, October 2010.

[9] M. Tsutsui, M. Taniguchi, K. Yokota, and T. Kawai, "Identifying single nucleotides by tunnelling current," Nature Nanotech., vol. 5, pp. 286-290, March 2010.

[10] S. Chang et al., "Electronic Signatures of all Four DNA Nucleosides in a Tunneling Gap," Nano Lett., vol. 10, pp. 1070-1075, February 2010.

[11] S. Huang et al., "Identifying single bases in a DNA oligomer with electron tunnelling," Nature Nanotech., vol. 5, pp. 868-873, November 2010.

[12] A. P. Ivano et al., "DNA Tunneling Detector Embedded in a Nanopore," Nano Lett., vol. 11, pp. 279-285, January 2010.

[13] M. D. Fischbein and M. Drndić, "Sub-10 nm Device Fabrication in a Transmission Electron Microscope," Nano Lett., vol. 7, pp. 1329-1337, May 2007.

[14] X. Liang and S. Y. Chou, "Nanogap Detector Inside Nanofluidic Channel for Fast Real-Time Label-Free DNA Analysis," Nano Lett., vol. 8, pp. 1472-1476, May 2008.

[15] B. C. Gierhart et al., "Nanopore with transverse nanoelectrodes for electrical characterization and sequencing of DNA," Sens. Actuators B, vol. 132, pp. 593-600, June 2008.

[16] M. Tsutsui, M. Taniguchi, and T. Kawai, "Transverse Field Effects on DNA-Sized Particle Dynamics," Nano Lett., vol. 9, pp. 1659-1662, April 2009.

[17] M. Taniguchi, M. Tsutsui, K. Yokota, and T. Kawai, "Fabrication of the gating nanopore device," Appl. Phys. Lett., vol. 95, pp. 123701, September 2009.

[18] T. Maleki, S. Mohammadi, and B. Ziaie, "A nanofluidic channel with embedded transverse nanoelectrodes," Nanotech., vol. 20, pp. 105302, February 2009.

[19] P. Xie, Q. Xiong, Y. Fang, Q. Qing, and C. M. Lieber, "Local electrical potential detection of DNA by nanowire-nanopore sensors," Nature Nanotech., vol. 7, pp. 119-125, February 2012.