DNA Sequencing and Translocation Studies using Electrically Addressable Nanopore Arrays

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Overview and Objectives:
Determination of the genetic code embedded in DNA molecules is fundamental to molecular biology and medicine. The most successful technique used for DNA sequencing has been the dideoxy termination mechanism developed by Sanger. Some of the main limitations of the traditional Sanger method are its relatively high cost (it heavily relies on multiple costly polymerase chain reactions), slow speed (the technique relies on gel separations), and only relatively short DNA strands can be effectively sequenced in each gel (600-800 bases). Alternative, faster and lower-cost methods for DNA sequencing are thus in high demand. There is a worldwide race towards a DNA sequencing technology that will cost no more than $1k per human genome. Such a personal genome technology promises to revolutionize medicine. Among the various proposed alternative DNA sequencing technologies, nanopore sequencing, especially with solid-state nanopore devices, is an exciting example. The objective of this NIRT program is to develop the necessary science and technology to bring nanopore DNA sequencing to reality.

Our research program [1] brings together broad expertise in nanobioscience, both experimental and theoretical, encompassing solid-state physics to biochemistry, to attack a major problem of broad scientific interest and with potentially immense societal impact. The science goals of this project include: (1) to develop low-cost approaches for fabricating addressable nanopore arrays [2] using silicon or other low-cost solid-state materials; (2) to study and to elucidate the translocation processes of biomolecules in nanopores; (3) to exploit the phenomena of electric-field driven transport of DNA molecules through nanopores for DNA sequencing [3] and rapid characterization of biomolecules.

This research program offers valuable educational experiences to students at Brown and Harvard in the emerging area of nanobioscience, empowering them with useful skills in nanofabrication, molecular biophysics, biochemistry, and statistical physics. These important skills will prepare our students well for the emerging economy of nanotechnology.

Research Findings:
In the grant period of 2004, we have engaged in a multi-front effort in attacking the nanopore sequencing problem. Since the future of nanopore DNA sequencing is largely resting on the availability of low-cost nanopore devices, we have spent a significant amount of effort in developing the necessary technology for device fabrication. This effort paid off handsomely. We now have developed a simple low-cost approach for fabricating nanopores in silicon chips, using electrochemical feedback etching. The same technique is also applicable for preparing devices with addressable nanopores.

Figure 1 is a TEM picture of one of such nanopore devices. In our approach, due to a slight imperfection in the photo mask, the final outcome is often a bi-nanopore device. The presence of

bi-nanopores, double holes, is a great advantage since it provides twice the possibility for a DNA to get through the pores without sacrificing the ionic current sensitivity.

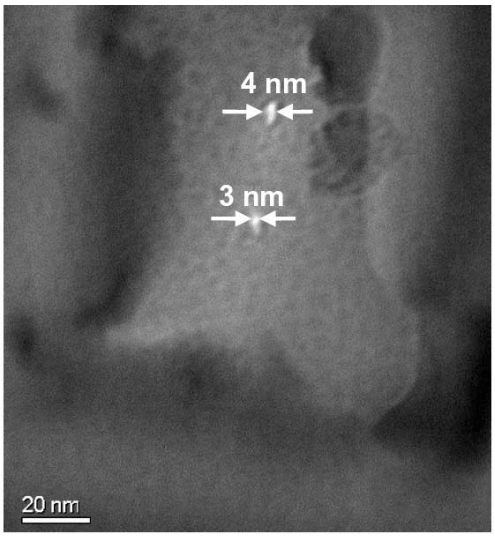

Figure 1: Transmission electron micrograph of a bi-nanopore device in a silicon chip (after oxidation). The light gray area is a thin layer of SiO_2.[4]

In the same effort, we also explored the use of laser heating to form nanopores in plastic materials with high melting temperatures. This work is of great interest for integrating biological pores such as alpha-hemolysin (α-HL) since the surface of plastics is naturally hydrophobic and can be used as host for lipid bilayers. The Ling and Meller groups are working together to put α-HL in such solid-state pores, with the purpose of reducing the electrical noise due to the membrane capacitance.

To elucidate the physics of DNA translocation dynamics in biological pore α-HL, we have carried out a systematic study [5] of the orientation dependence of ssDNA translocation and the unzipping kinetics of DNA hairpin molecules.

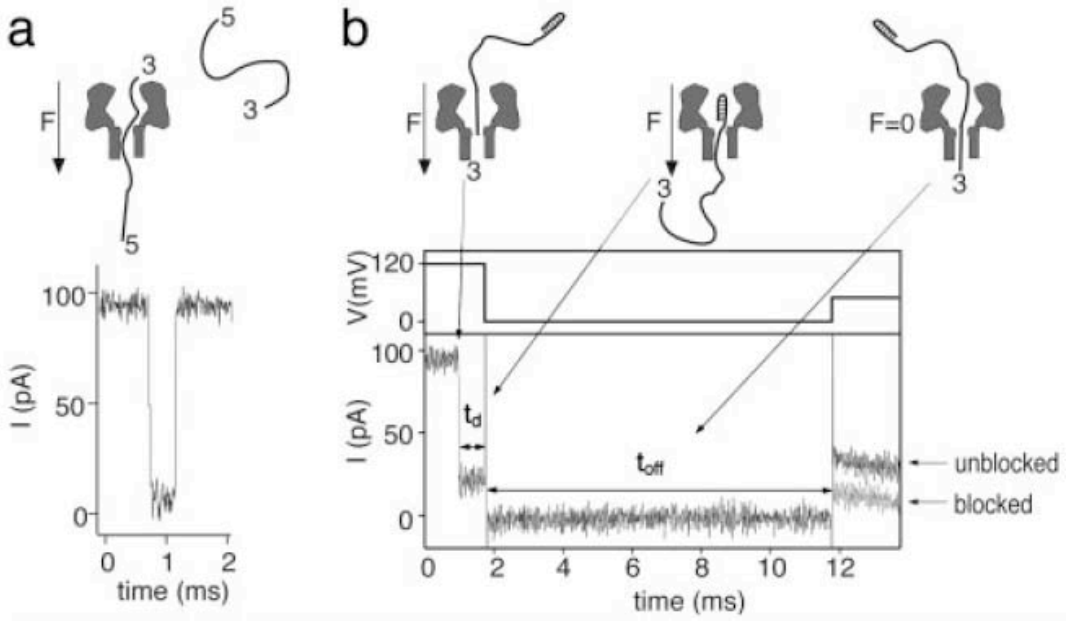

Figure 2: DNA hairpins can be used to facilitate translocation and diffusion measurements as a function of their orientation. (*a*) With ssDNA, 3" and 5" entries to the nanopore are mixed. (*Inset*) A typical translocation event measured with $V = 120$ mV. (*b*) The escape probability of DNA hairpins, with long single-stranded overhang, can be measured as a function of t_{off} by using dynamic voltage control. (*Inset*) The applied voltage and the corresponding current traces for two events. Note that at the end of the t_{off} period only one of two options can exist: an empty pore or a blocked pore, either of which are readily detected by applying a small probing voltage.[5]

On the theory front, we provided theoretical support for experiments that revealed orientation discrimination of single-stranded DNA (ssDNA) threading an alpha-hemolysin pore. Hairpins at the 3' or 5' ends were used to block complete translocation. The retraction under Browinian motion after setting the threading electric field to zero can be described by one-dimensional diffusion, but with a hard reflecting wall to account for the blocking effect of the hairpin. Fits to the probability of ssDNA escape as a function of time allowed the first measurement of the two distinct diffusion constants that control the threading of the 3' and 5' ends through the asymmetric pore. We have begun to look at the effect of an electric field E that *assists* with retraction. Fits to a "diffusion with drift" model should allow determination of the drift velocity v(E) and diffusion constant D(E) for the two orientations. These functions can be predicted theoretically, once the microscopic pore potential is known from, e.g., an ab initio computer simulation.

Presently, experiments are underway to explore the method of HANS (hybridization-assisted nanopore sequencing, Fig.4) for rapid DNA sequencing and bi-analysis.

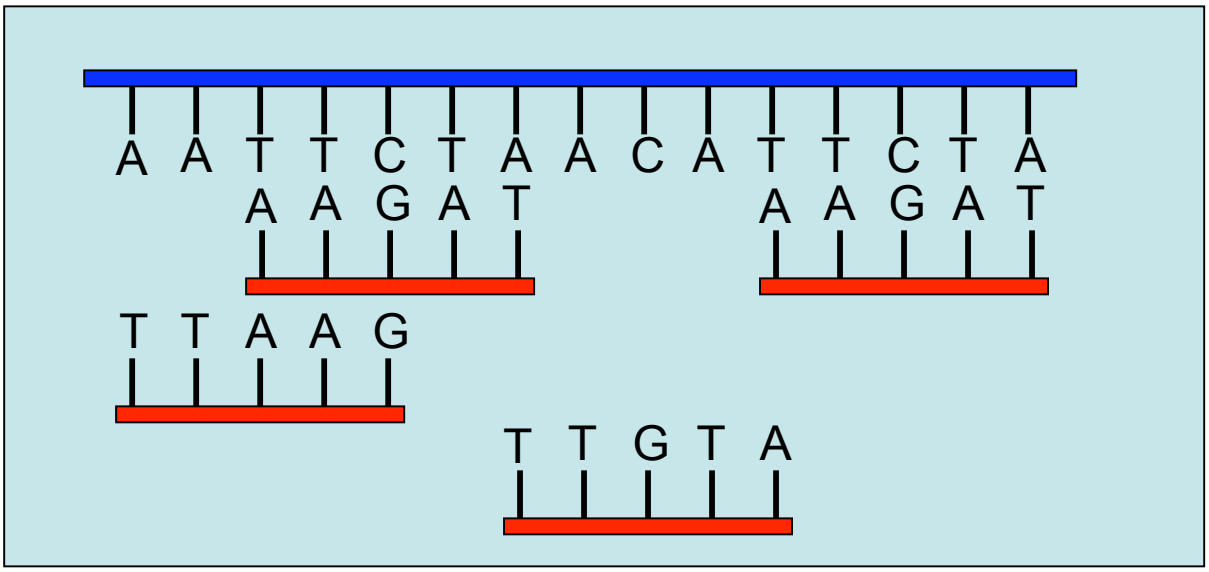

Figure 4: Hybridization-assisted nanopore sequencing of DNA. In this method, the position of each hybridizing DNA probe (in red), a short oligonucleotide, is determined from the ionic current trace using the standard patch-clamp electronics. The DNA sequence of the target DNA (in blue) can be constructed from the sequences of matching DNA probes and their positions.[3]

References

[1] For further information about this project email **xsling@brown.edu**.
[2] X. S. Ling, "Addressable nanopores and micropores including methods for making and using same", USPTO Application No. 20050127035.
[3] X.S. Ling and A. Pertsinidis, "Hybridization-assisted nanopore sequencing of DNA" (USPTO, patent disclosure, 2005); B. Bready and X.S. Ling, "DNA sequencing using positional information of probes" (USPTO, patent disclosure, 2005).
[4] S.R. Park, H. Peng, and X.S. Ling, "Formation of bi-nanopores in silicon chips", submitted to Applied Physics Letters (2005).
[5] J. Mathe, A. Aksimentiev, D.R. Nelson, K. Schulten and A. Meller, "Orientation discrimination of single stranded DNA inside the a Hemolysin membrane channel", *Proc. Natl. Acad. Sci.*, **102**, 12377-12382 (2005).