A deformable microfluidic device to control the conformation of DNA molecules before nanopore translocation

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Introduction

A solid-state nanopore is a hole of nanoscale dimensions embedded in a thin, insulating membrane separating two liquid electrolytes. DNA, a negatively-charged biomolecule, can be driven through a nanopore by applying a voltage across the membrane; as the molecule passes through the nanopore, the ionic current is modulated. The properties of the electrical signal, such as its depth and duration, can provide information on the length, size, charge, and shape of the molecule of interest. A newly-developed custom instrument, capable of simultaneously providing electrical and optical measurements of molecules translocating through a solid-state nanopore, is used to gain an understanding of the dynamics of DNA molecules in nanoconfined geometries.

The ultimate goal is to test the following hypothesis: the distribution of passage times of identical DNA molecules is influenced by the conformation of the DNA molecule at the instant of capture. To reach this goal, polydimethylsiloxane, or PDMS, microfluidic flow cells are developed and designed to modulate the confinement of DNA polymers. DNA molecules of identical lengths can coil into random conformations; the deformable PDMS allows the adjustment of the confined volume, which impacts the conformation of individual DNA molecules.[1] Fluorescence microscopy will demonstrate the relationship between the confinement volume and the conformation of the DNA molecule. Different conformations of DNA molecules are accountable for the variations in passage times through a nanopore due to varying drag forces. Controlling DNA behavior during its passage through a solid-state nanopore is necessary for a large number of applications, including low-error-rate DNA sequencing.

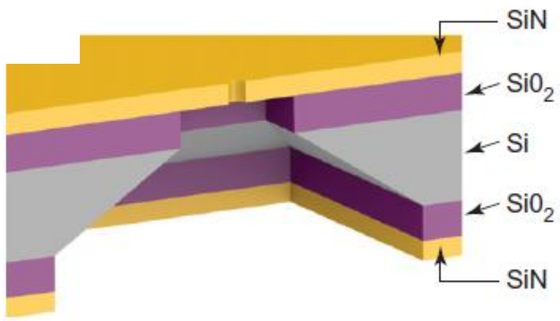

Figure 1. Solid-state nanopore

Methodology

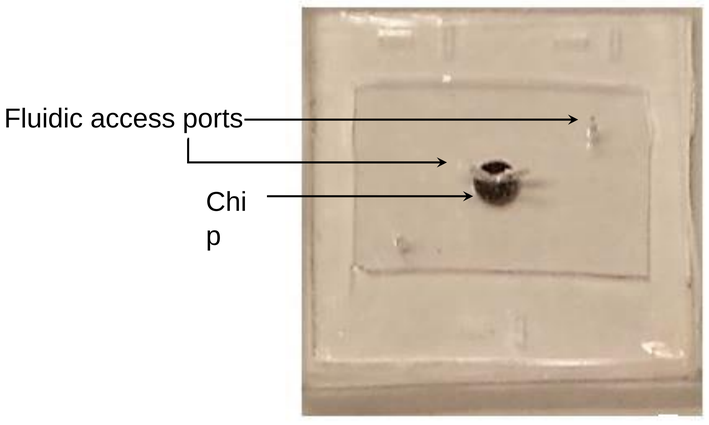

Figure 2. The top view of a microfluidic device

Microfluidic flow cells were developed and designed to modulate the confinement of DNA polymers. The devices were fabricated using polydimethylsiloxane (PDMS), a glass coverslip and a chip containing a 5-nm solid-state nanopore in a 30-nm thick silicon nitride membrane. Double-stranded λ-DNA molecules, with a length of 48.5 kbp and radius of gyration of ~700nm), are confined in a small volume that exists between an upper layer of 3-mm thick PDMS, on which the nanopore chip is bounded, and a glass coverslip. A smaller piece of PDMS (1mm in height) is shaped to create the deformable confinement volume.

The device fabrication process utilized liquid PDMS mixed with a cross-linking agent, which was consequently poured into moulds, and heated to obtain cross-linked PDMS, which is a flexible and viscoelastic polymer. Channels with a diameter of 0.75mm were punched into the PDMS, through which fluid (100mM KCl) containing YOYO stained DNA molecules would flow through using syringe pump.

Fabrication

The assembly of the device required PDMS to covalently bind to a nanopore and to a glass coverslip, which was achieved by activating the surfaces using the Oxygen Plasma System. Plasma oxidation changes the surface chemistry and produces silanol terminations (SiOH), which functionalises the surface of PDMS and allows the assembly of PDMS microdevices. The confined volume is adjusted by deforming the PDMS, which impacts the conformation of individual DNA molecules.

Figure 3. Double-stranded λ-DNA confined in a schematic of the side view of a microfluidic flow cell.

Analysis of DNA molecules

The application of a small amount of pressure to the upper portion with PDMS allows for the confinement volume (where the DNA is found) to decrease controllably. Special instruments in the T.-Cossa lab will allow for the simultaneously electrical and optical measurements of DNA molecules translocating through a solid-state nanopore. Fluorescence microscopy will demonstrate the relationship between the confinement volume and the conformation of the DNA molecule.

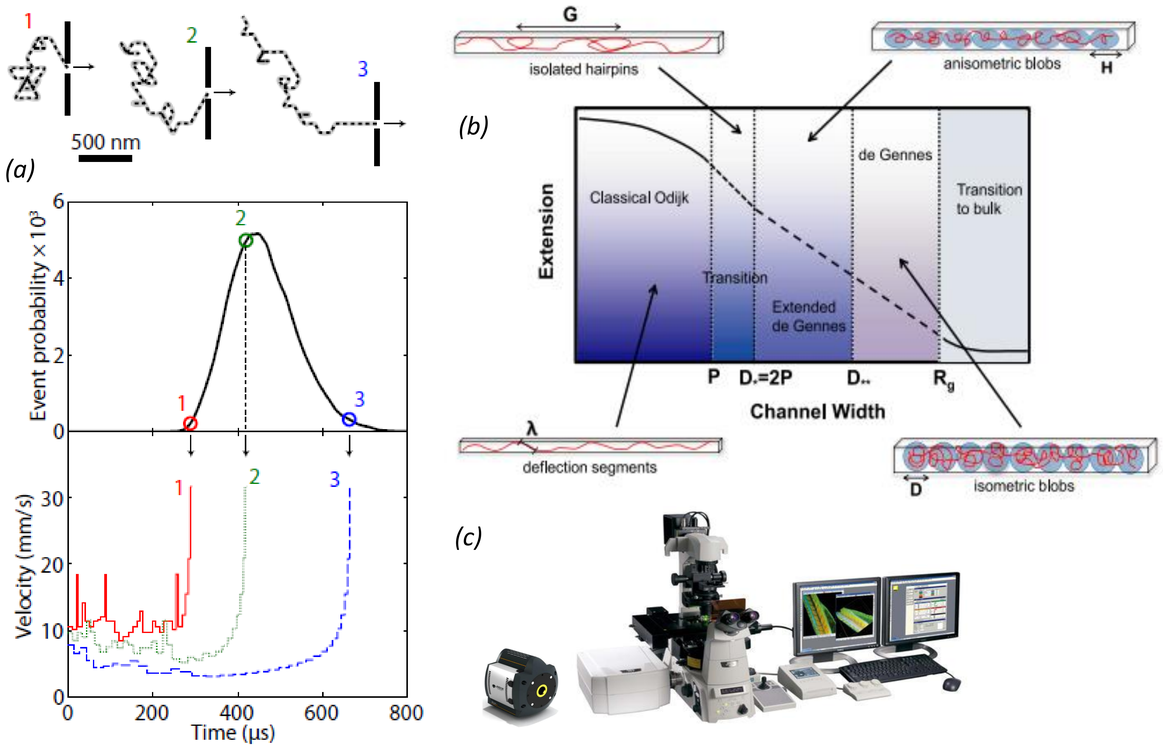

Figure 4. *(a)* Examples of velocity and translocation time fluctuations from modeling results of 10-kb DNA molecules.[1] *(b)* The various shapes of confined polymers based on their confinement.[2] *(c)*Instruments used for fluorescence microscopy; Nikon Ti-E inverted microscope, equipped with an emCCD camera.

References and Acknowledgements

[1] Lu, Bo, Fernando Albertorio, David P. Hoogerheide, and Jene A. Golovchenko. "Origins and Consequences of Velocity Fluctuations during DNA Passage through a Nanopore." *Biophysical Journal* 101.1 (2011): 70-79. Print.

[2] Reisner, Walter, Jonas N. Penderson, and Robert H Austin. "DNA confinement in nanochannels: physics and biological applications." *Reports on Progress in Physics* 75.10 (2012): 2-31. Print.

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