Towards Entropic Trapping of DNA in Solid-State Nanopores

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Abstract

INTRODUCTION

Better nanofluidic control over DNA will yield exciting leaps in technology, such as biochemical labs-on-a-chip and so-called DNA hard drives. We intend to get a few steps closer by making an isolation chamber to hold single DNA molecule in place. Such an isolation chamber would allow for the outcome of biochemical experiments to be observed on a per-molecule basis. It could also act as a storage medium for data encoded in DNA which can hold the equivalent of one million CDs in a single gram for 10,000 years[1]. We believe we can create such an isolation chamber based on entropic trapping, and combine it with a molecular detector called a nanopore.

In order to isolate a single DNA molecule in the chamber, we need a way to deliver it and to know when it enters. Nanopores are the ideal device for such purposes. A nanopore is a small hole in a membrane, be it biological or synthetic. Solid-state nanopores consist of a hole approximately 10 nanometers across in some larger solid-state membrane. For contrast, double-helical DNA is approximately 2.5 nanometers across. When placed between two reservoirs of ionic solution, solid-state nanopores can detect the passage of a single DNA molecule. This passage event is also known as a translocation. When a voltage bias is placed across the two reservoirs, current forms from ions flowing through the pore. DNA, driven by electrophoretic forces on its slight negative charge, travels toward the pore, eventually getting sucked through. Its passage displaces some flowing ions, causing an observable drop in the ionic current through the pore.

In order to trap DNA in its isolation chamber, we intend to take advantage of the DNA's configurational entropy. At equilibrium, DNA forms a random coil whose spherical shape has a size characterized by the radius of gyration (Rg). If we could get DNA in a cavity that is roughly the size of the radius of gyration and that has holes smaller than the radius of gyration on either side, we predict that the molecule will have a near-zero probability of diffusing out (Del Bonis-O'Donnel); it would have to squeeze too far out of equilibrium to exit this entropic trap.

The device we envisioned to trap individual DNA molecules is shown in Fig. 1. (Note that the molecule pictured is not trapped.) Capturing a DNA molecule in the cavity can be thought of as a competition between two timescales: the time required to push the center of mass of the DNA through the structure and the time it takes the DNA molecule to

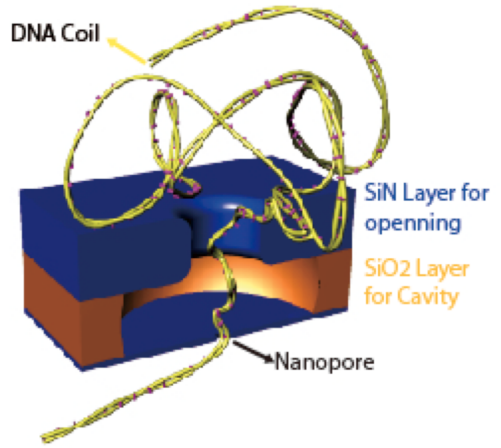

FIG. 1: A schematic of a silicon structure containing a nanopore and adjacent chamber during a DNA translocation event. The structure has 3 main layers. From bottom to top they are the nanopore, the cavity, and a large hole with diameter approximately 500 nm on average.

equilibrate in the cavity so it cannot traverse the large hole. A translocation starts when the leading tip of DNA - or someplace nearby on the polymer - enters the nanopore. A drop in current is observed. The molecule is driven through the cavity by local electric fields. As it is driven, Brownian motion causes the molecule to start to equilibrate and expand to fill the chamber. However, translocation happens much faster than equilibration, so the molecule remains "skinny" when compared to the large hole; continuing to push it will cause it to exit the structure. Thus, the probability of exit is dependent on the cavity length and hole diameter. When the molecule finishes translocating the current will return to its original baseline value. Turning off the driving voltage at the right moment (too ambiguous? i feel like we could turn off voltage a little before translocation ends if we could calculate it on the fly) will allow the molecule to equilibrate inside the cavity, trapping it.

Placing such a structure between two reservoirs of ionic solution yields the translocation dynamics described above with a twist: the structure's asymmetry means translocations from either side of the pore are not equivalent. Although the effects of the asymmetric structure on translocation dynamics will be touched on, Karri DiPetrillo's thesis takes a deeper look at those phenomena. The focus of this thesis will be trapping DNA molecules approaching the exposed nanopore (the bottom layer in Fig. 1). Our objectives are thus twofold: to develop the hardware (pores-plus-chambers) and the software (control electronics and data analysis) to make our vision a reality. My contributions were software for analy-

sis and recommendations for programming control electronics informed by my preliminary experiments and theoretical calculation. In addition to creating more intricate labs-on-a-chip and possibly storing DNA hard drives, creating such entropic traps would yield deeper understanding of DNA as a polymer more generally.

THEORY

Quantifying DNA as a Polymer

In order to supply numbers to our currently hand-waving model of a trapped molecule, we need to explore both the statistical and dynamical properties of DNA.

The simplest statistical model of a polymer in equilibrium has two parameters: total length L and persistence length $p \ll L$. These two numbers describe a freely-jointed chain of length L in which rigid lengths of polymer of size p are connected by joints that can take any bond angle. The resulting shape of such an object is a sphere. Mathematically the chain can be described by a three-dimensional random walk. As such, one would expect the radius of the sphere, also known as the radius of gyration, to scale with the square root of the length of the chain: $R_g \propto L^{1/2}$. The flaw in such reasoning is that real polymers are self-avoiding, meaning that links in the chain cannot occupy the same space. The Flory model modifies the random walk by using a mean field approach (where segments encounter one another with equal probability) to define a new parameter, the Flory exponent ν_F, where $R_g \propto L^{\nu_F}$. The Flory model predicts that, for DNA, $\nu_F = \frac{3}{5}$. Experiments yield an exponent of approximately .588. To make the Flory model even more realistic, we must take into account Brownian motion. The Rouse model describes a polymer as a collection of beads connected by springs in which beads feel the effects of thermal forces and drags. It does not, however, take into account self-avoidance and as such is as useless as the Flory model.

Timescales are provided by the Zimm model. Zimm combined the Flory and Rouse models by including the hydrodynamic interactions between different parts of the chain as mediated by the solvent. The Zimm model's predictions most exactly agree with experiment. The Zimm model predicts that a polymer enters its equilibrium state from any other state within a characteristic relaxation time τ_z. That is, if one stretched a DNA molecule to be

completely straight, it would reach a spherical conformation in τ_z seconds, maximum. The process of reaching such a sphere is known as relaxation. For λ-DNA $\tau_z \approx 100$ms. Our translocations last $\approx$ 2ms, so molecules do not have time to equilibrate until long after they translate. Thus our translocations are called "fast translocations." To paint a physical picture of what that means, we imagine a randomly coiled rope being pulled off a table. As it is pulled, individual folds are sequentially straightened and pulled off the table. Only a small length of rope (the most recently straightened fold) is involved in the motion. The rest of the rope is stationary. The same things happens with DNA, but on a much smaller length scale (put in how many orders of magnitude here, for kicks). Folds of the molecule are sequentially straightened as they are sucked through the pore while the rest of the molecule is effectively frozen.

Trapping in More Detail

Trapping can be thought of as a competition between the movement of DNA through the pore-plus-cavity structure and the equilibration of the molecule in the cavity. The major force pushing the DNA molecule though the structure is electrophoretic. From charge conservation we know that, to a good approximation, the current traveling through the pore (our measured quantity) is equal to the current passing through the area of hemisphere enclosing the pore:

$$I = 2\pi R^2 J(R) \tag{1}$$

where I is current, R is distance from pore, and $J(R)$ is the current density. Solving Eq. 1 for $J(R)$ we see

$$J(R) = \frac{I}{2\pi R^2}.$$

By Ohm's Law we know

$$\overrightarrow{J}(R) \equiv \sigma \overrightarrow{E}(R),$$

where σ is two-dimensional charge density. Combining these equations and solving for E yields:

$$E(R) = \frac{I}{\sigma 2\pi R^2} \tag{2}$$

We know the velocity of the DNA molecule a distance R from the pore:

$$v_{\mathrm{DNA}} = \mu_{\mathrm{DNA}} E(R). \tag{3}$$

Plugging in Eq. 2 we see that

$$v_{\mathrm{DNA}} = \frac{\mathrm{d}R}{\mathrm{d}t} = \mu_{\mathrm{DNA}} \frac{I}{\sigma 2\pi R^2},$$

where μ_{DNA} is the electrophoretic mobility of DNA. Integrating yields the tip's distance from the pore and the elapsed time:

$$\int_{R=0}^{R(\Delta t)} \frac{\sigma 2\pi R^2}{\mu_{\mathrm{DNA}} I} \mathrm{d}R = \int_{t=0}^{\Delta t} \mathrm{d}t$$

yields an equation

$$\frac{1}{3} \frac{\sigma 2\pi R^3}{\mu_{DNA} I} = \Delta t,$$

which can be solved for $R(\Delta t)$:

$$R(\Delta t) = \sqrt[3]{\frac{3\mu_{DNA} I}{\sigma 2\pi} \Delta t}. \tag{4}$$

Eq. 4 quantifies the location of the tip as a function of time. If $R(\Delta t)$ is less than the height of the cavity, the molecule should be trapped. Even if the tip could travel outside of the chamber (that is, $R(\Delta t)$ greater than cavity height), we believe the molecule will still be trapped with high probability if the center of mass is within the cavity. The center of mass, the point from which R_g is measured, should be at approximately $R(\Delta t)/2$. The major assumption in this calculation is that current is conserved across a hemispherical geometry within the cavity. That may be true to a first approximation, but in reality all current must flow through the hole at the other end of the structure and thus the fields are "squeezed" into that hole; the molecule will travel slightly farther than expected as it reaches the far side.

Now we will find an actual value for cavity size. Plugging in numbers from our experiments, $\mu_{DNA} = 3.75 \times 10^{-3}\mathrm{cm}^2/\mathrm{V{\cdot}s}$, $= 3.75 \times 10^{-7}\mathrm{m}^2/\mathrm{V{\cdot}s}$ [2] $I = 20\mathrm{nA} = 2 \times 10^{-8}\mathrm{A}$, $\Delta t = 2\mathrm{ms} = \mathrm{ms} = 2 \times 10^{-3}\mathrm{s}$ and $\sigma = 100\mathrm{mS/m} = 10\ \mathrm{S/m}$[3], we see that the molecule travels

$$R(\Delta t) = \sqrt[3]{\frac{3\mu_{DNA} I}{\sigma 2\pi} \Delta t} = \sqrt[3]{\frac{3 \cdot 3.75 \times 10^{-7} \cdot 2 \times 10^{-8}}{10 \cdot 2 \cdot \pi} 2 \times 10^{-3}}$$
$$\approx 8.95 \times 10^{-7}\mathrm{m} = 895\mathrm{nm}$$

in the time it takes to translocate. Half of this (that is, 450nm) gives us a lower bound for cavity length. Because our cavities were roughly 400nm long and we did not kill the voltage immediately after translocation, we captured no DNA molecules in our preliminary trials. We now understand why and are seeking to fabricate devices with longer chambers and build and program better control electronics to improve responsiveness.

EXPERIMENT

Synthesis of Nanopores

DNA Preparation and Apparatus Setup

Trapping Procedure

Data Analysis with MatLab

After collecting current vs. time data at a rate of 250kHz we ran it through our MatLab data analysis pipeline. Each data file contains one minute of data. Current is measured in nanoamps.

Monodirectional Translocations

The data analysis pipeline for monodirectional translocations starts with data sanitation. We run once through a given file, recording where events start and end. Our heuristic for detecting translocations in monodirectional experiments is deviation from a baseline value defined by the moving average of the previous 1ms of data (250 data points). [figure here] We also average the next 7 points, called the "lookahead." If the lookahead is far enough away from the baseline (far enough being dependent on driving voltage and specified by the user, typically a quarter of a nanoamp at 100mV driving voltage), the "new event" variable is set to true. While true, [need to talk to Xu about the event end heuristic... don't quite get it]

"Ping-Pong" Translocations

The data analysis pipeline for "Ping-Pong" experiments proceeds largely as above, but with more complicated event start and end detection. The added complication is due to the nastiness of the data [show a picture with whacked out baseline here]. We tried multiple ways to fit the exponential-seeming decay of the data and subtract it to allow us to use the exact event detection method above but failed. We resorted to using a moving average of the derivative of sorts (explain in more detail) to detect events as well.

RESULTS AND DISCUSSION

CONCLUSION

[1] *Double helix serves double duty* (2013), URL `http://www.nytimes.com/2013/01/29/science/using-dna-to-store-digital-information.html`.

[2] R. P. Stellwagen NC, Gelfi C, Biopo **42**, 687 (1997).

[3] W. M. Haynes, ed., *CRC Handbook of Chemistry and Physics* (CRC Press, 2012-2013), 93rd ed., URL `www.hbcpnetbase.com`.