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## COARSE-GRAINED SIMULATIONS OF HIGHLY DRIVEN DNA TRANSLOCATION FROM A CONFINING NANOTUBE

BY DAVID SEAN AND GARY W. SLATER

Consider this brain teaser: How do you pass a large knitted blanket to your cold friend waiting in a locked room where the only possible passage is a small keyhole in the door? The answer: simply find one of the two ends of the yarn and slowly thread it through the keyhole. As the blanket unravels on one side of the door it can be reconstructed on the other.

Entropy ensures that a long DNA chain stays as a random disordered mess in a liquid. Passing a long polymer like DNA from one side of a membrane to the other via a nm-scale hole can at first seem impossible. But much like the passage of the knitted blanket through the keyhole, a large DNA molecule can realize the “impossible” by simply unraveling itself. Since DNA is charged in solution, this process can be driven via an electric field.

Imagine now that salt ions are present in the solution. These charged ions will also be driven to transit through the small opening, resulting in an ionic countercurrent that can be measured. Since the dividing membrane is electrically insulating, the electric field lines converge at the small hole and the measured conductivity is extremely sensitive to what is happening near the so-called *nanopore*. The passage of the DNA molecule will impede the ionic passage for the duration of the DNA *translocation* event. In the lab, this can be observed as a sudden drop in the electrical conductivity between the two chambers. Experimental translocation data is typically extracted from the duration and amplitude of a recorded drop in the measured current readout<sup>[1]</sup>. The duration of this current blockage can provide information on the

molecule such as its total curvilinear length. In this short report, we present computational work on using DNA translocation for applications where the focus is on determining the length of a piece of DNA.

There are many applications which require finding the length of the DNA molecules present in an unknown sample. For example, forensic DNA *fingerprinting* works by breaking a long DNA molecule into smaller fragments using restriction enzymes that cleave it at sequence-specific sites. The length distribution of these smaller DNA fragments constitutes the fingerprint. Traditional *macroscopic* sizing techniques relies on the separation of these smaller pieces at a *population*-level. That is, a single DNA molecule may need to be amplified multiple times in order to create a population of DNAs which will later be fragmented and size-separated.

Nanopore translocation techniques, as described above, can be integrated into portable lab-on-a-chip devices. These can be much faster, cheaper and easier to operate than traditional methods—and could potentially only require a single DNA molecule!

Oddly, with a sample solution where all the DNA molecules are identical in size, experimental distributions of translocation times are found to be surprisingly wide. The reason for this is an example of *molecular individualism*, an expression coined by Pierre-Gilles de Gennes<sup>[2]</sup>.

Figure 1a depicts a handful of possible DNA shapes—or *conformations*—at the moment when the translocation process begins (the first monomer is inside the pore). As is obvious from the figure, we are using here a rather coarse-grained representation of DNA—a simple chain of beads and springs. The generic polymer model of  $N = 100$  beads is described in more details in ref [3]. The focusing of the electric field inside the pore completely dwarves its effect outside the pore. This permits us to simplify the problem further: the effect of



### SUMMARY

**We study the effect of pre-confining DNA in a small tube prior to driven translocation. Computer simulations are presented together with a theoretical Tension-Propagation model.**

David Sean,  
<david.sean@gmail.com> ,

and

Gary W. Slater,  
<gary.slater@uottawa.ca> ,  
University of Ottawa,  
150 Louis-Pasteur,  
Ottawa,  
ON K1N 6N5

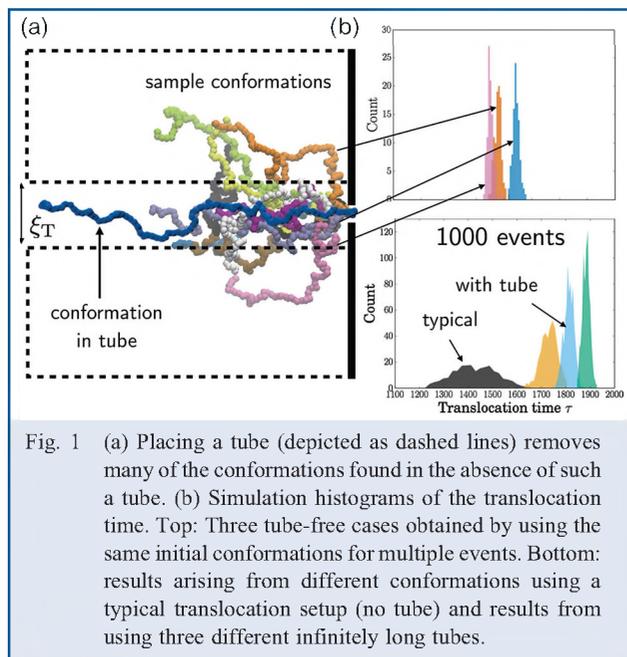


Fig. 1 (a) Placing a tube (depicted as dashed lines) removes many of the conformations found in the absence of such a tube. (b) Simulation histograms of the translocation time. Top: Three tube-free cases obtained by using the same initial conformations for multiple events. Bottom: results arising from different conformations using a typical translocation setup (no tube) and results from using three different infinitely long tubes.

the driving field is modeled as a force only applied to the beads present inside the nanopore.

We show in Fig. 1b the corresponding histograms of translocation times. The top panel is generated by repeating the simulation with the same conformation, but with different Brownian trajectories. A remarkable feature is how the different distributions are centred about their own mean translocation times. Since the translocation process occurs much faster than the time it takes for the polymer to relax (typically by two orders of magnitude), the exact positions of all the beads at the onset of translocation is a determining factor in the resulting transit time. In the lab, one cannot explicitly control for the shape of the DNA molecule once the first bead enters the pore. The resulting histograms contain a large mix of possible shapes yielding a rather wide distribution of translocation times for what is in essence a group of chemically-identical molecules! Indeed, the bottom panel of Fig. 1b shows how wide the distributions can become. Thus one way to tighten them would be to somehow limit the range of initial DNA conformations. In Fig. 1a there is a schematic of a confining tube which we use as a way to limit the possible initial conformations. We will assume that this hypothetical tube is made of a porous material such that it does not significantly affect the flow of ions or distort the field lines.

We report here the use of long (longer than the polymer) confining tube characterized only by its diameter  $\xi_T$ . By construction, the tube will limit the range of possible conformations, which by virtue of our preceding argument, should reduce the width of the distribution of translocation times.

We first generate a DNA conformation that has its first bead inside the nanopore. The presence of the tube imposes a hard cutoff  $\rho = \xi_T/2$  in the radial positions of the remaining beads. For entropic reasons, a polymer chain does not like to be compressed. When we impose radial restrictions, it reacts by swelling outwards in the axial direction. As a side-effect, a thin tube will tend to squeeze out the DNA away in the axial direction. This means that the remaining monomers will on average be positioned further from the pore and will need to travel longer to translocate to the other side, as shown in the insets of Fig. 2a.

Although it may seem that this “distancing” effect alone should yield longer translocation times, there is another—perhaps subtler—contributing factor relating to the translocation rate. Let us investigate this according to what is known as the Tension-Propagation Theory<sup>[4]</sup>.

In these out-of-equilibrium dynamics of translocation, the driving force causes a *tension* that propagates down the polymer (see red beads in Fig. 2b). If we neglect the crowding of monomers on the *trans*-side and friction in the nanopore—which contribute to minor corrections—a force-balance argument can be made to show that the instantaneous translocation rate is proportional to the number of monomers that feel this tension.

Consider the forces acting on the bead inside the pore in Fig. 2b. In the overdamped limit, the driving force  $F_d$  applied to this bead is balanced by the drag force of all moving beads under tension (coloured in red). Every bead contributes to a viscous force  $-\zeta v$ , with  $\zeta$  the bead’s friction coefficient and  $v$  the velocity. If there are  $k$  beads moving together as shown in Fig. 2b (red beads), then the total friction resisting the applied force is  $-k\zeta v$  which enables us to find the translocation rate (or terminal velocity) of  $v = F_d/k\zeta$ . Since the driving force  $F_d$  and the friction coefficient  $\zeta$  are known quantities, the resulting rate can be determined for every step of the process if we can find how many beads are affected by the applied force, i.e., how tension propagates.

Note that the beads outside the tension front do not (yet) contribute to this resistance. In fact, these beads do not even know that translocation has started. Since we consider the limit where the process is highly driven, a geometrical argument can be made to estimate the number of monomers under tension once the tension front reaches a specific bead. Figure 2b shows how the initial position of the  $i^{\text{th}}$  bead can be used to estimate the number of beads under tension once the tension front reaches it. Since the monomers under tension form a taught segment, the distance  $R_i$  between the  $i^{\text{th}}$  bead and the pore can be used to find the number of moving monomers (and the translocation rate) once the tension front reaches it.

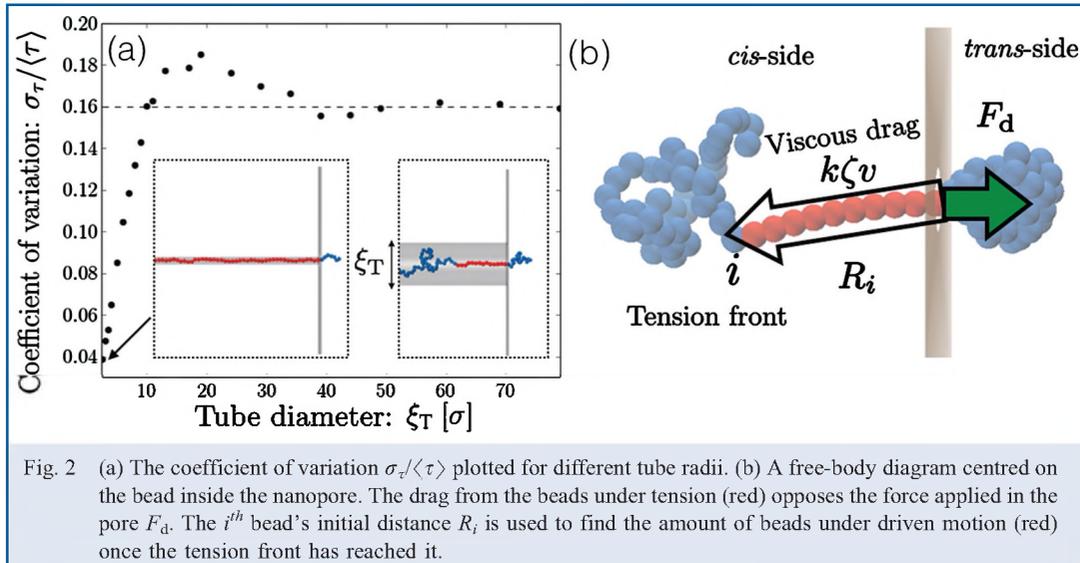


Fig. 2 (a) The coefficient of variation  $\sigma_\tau / \langle \tau \rangle$  plotted for different tube radii. (b) A free-body diagram centred on the bead inside the nanopore. The drag from the beads under tension (red) opposes the force applied in the pore  $F_d$ . The  $i^{\text{th}}$  bead's initial distance  $R_i$  is used to find the amount of beads under driven motion (red) once the tension front has reached it.

This picture highlights why the initial conformations matter so much in determining the total translocation time: the initial positions essentially determine what values of viscous damping is felt by the polymer during translocation. Thin tubes will yield longer translocation events because it also causes the system to sample what is effectively a higher amount of viscous damping. Imagine pulling a heavy 1 km long chain lined-up straight on a road, versus pulling a similar chain but which is instead neatly coiled in a pile. Displacing the lined-up chain would require moving *all* the links in unison, whereas in the coiled version, you would be able to move the chain tip for a fair amount before the accumulated drag would become overwhelming.

The total translocation time  $\tau$  can be determined by integrating the rate. This is a two-step process: there is the time needed to propagate to the last bead and a time for the final retraction<sup>[3]</sup>.

Combining them gives a total translocation time which can be written as

$$\tau = \frac{\zeta}{F_d} \int_0^N R_i di,$$

where the information in the initial conformation is completely captured by the list of initial monomer distances  $R_i$  (see Fig. 2b). Averaging the above, we obtain the mean translocation time  $\langle \tau \rangle = \zeta \langle N \rangle / F_d$ . Note that small tube diameters not only reduce the standard-deviation  $\sigma_\tau$  because they limit conformations, but also *increase* the mean translocation time  $\langle \tau \rangle$  by pushing the beads away from the pore and increasing the friction. These two effects work together in reducing the coefficient of variation  $\sigma_\tau / \langle \tau \rangle$  four-fold, as shown in Fig. 2a. When using a given mixture of multiple DNA fragments, decreasing the coefficient of variation  $\sigma_\tau / \langle \tau \rangle$  means that DNA sizing can be obtained a higher resolution.

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