New and Notable

Reading Nanopore Clocks in Single-Molecule Electrophoresis Experiments

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In this issue of the *Biophysical Journal*, Carson et al. (1) arrive at the provocative conclusion that the diffusion constant and the velocity of dsDNA in single molecule electrophoresis are proportional to each other at all voltages. Such a claim, if valid, would destroy the distinction between Newton's law of projectiles and Einstein's law of diffusion. Naturally, the authors' claim cannot be valid. How did the authors come to their erroneous conclusion? The origin of this mishap lies in their data analysis, which uses a model inappropriate for their experiments. While the data are for long dsDNA molecules threading through a narrow-and-thin pore in a single-file manner, their model is for a colloidal particle with a uniform velocity undergoing diffusion in an infinitely long pore. The use of a single-file polymer model, appropriate to their experiments, is shown below to capture the essentials of their data, without transgressing the fundamental laws of physics. Additionally, the polymer model predicts that the average time for single-file translocations through the pore is proportional to the polymer length for very long polymers. This prediction is in agreement with the experimental data (1), but contradicts the authors' claim that the average translocation time is universally proportional to 1.37-power of DNA length.

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Single molecule electrophoresis, as deployed in the experiments by Carson et al. (1), has emerged as an important technique for characterizing biopolymers and synthetic polymers (2). In this technique, single molecules are driven through a protein pore or a narrow-and-thin, solid-state nanopore under an electric field, and temporary blockades in the ionic current passing through the pore are monitored. In ideal situations, the levels of blocked ionic current should uniquely represent the chemical identity of the constituents of the polymer (such as DNA and proteins), ultimately enabling their sequencing. Additionally, the duration of current blockade should enable the narrow pore to act as a clock to measure the time taken by a single molecule to thread through the pore in a single-file. This would in turn reveal the contour length (proportional to molar mass) of the molecule. In real situations, the extent and durations of current blockades are broadly distributed, even when identical molecules pass by each time (3–7). Combinations of diffusion and electrophoretic drift of the molecule, entropic barriers for threading into the pore, pore-molecule interactions, hydrodynamic forces, electroosmotic flow, etc., contribute to such behavior (8). One of the continuing challenges is to experimentally reduce the broad distributions in the readout and thereby enable understanding of polymer translocation in a simplified experimental system.

Carson et al. (1) demonstrate the best time-resolution in solid-state nanopore experiments reported so far in the literature. The authors have accomplished this by increased high bandwidth measurements and by using narrow-and-thin silicon nitride nanopores that just snuggle the passing DNA. Their data show significant reduction in the distributions of the extent and duration of current blockades, and better time-resolution. How do we learn about the molecular details of translocation from such experimental accomplishments? The authors' analysis of the data led them to argue that the average velocity v and the diffusion constant D of DNA undergoing single-file translocation follow the relation

$$\frac{v}{D} = 0.43 \pm 0.13 \ \text{nm}^{-1}, \qquad (1)$$

which is independent of voltage V. This result is startling in view of its violation of the well-established Einstein-Smoluchowski law,

$$\frac{v}{D} = \frac{QE}{k_B T} \sim V, \qquad (2)$$

where Q is the net charge of the polymer, E is the electric field proportional to the voltage V, and $k_B T$ is the Boltzmann constant times the absolute temperature. Also, Carson et al. (1) argue for a universal power law for the dependence of the average translocation time $\langle \tau \rangle$ on DNA length (N),

$$\langle \tau \rangle \sim N^{1.37} \qquad (3)$$

(N = 35–20,000 bp). The authors use quantitative agreement between their MD simulations and their deduced values of D from their experiments in support of Eq. 1, and previous literature (9) in support of Eq. 3.

The striking discrepancy of Eq. 1 with the Einstein-Smoluchowski law can be traced to the particle-virtual-pore model (Fig. 1 *a*) used by the authors to interpret their data. It is imagined that the whole dsDNA molecule is a single structureless particle with a uniform velocity v and diffusion constant D in a one-dimensional virtual pore extending from $-\infty$ to $b + L$ (where b ~ 6 nm is the pore thickness and L ~ (35–20,000) × 0.34 nm is the DNA contour length). Let the particle be placed initially at the entrance of the actual physical pore. Using the absorption boundary condition at $-\infty$ and $b + L$ in this semiinfinite pore for the particle, and allowing the particle to have uniform velocity everywhere inside the virtual pore

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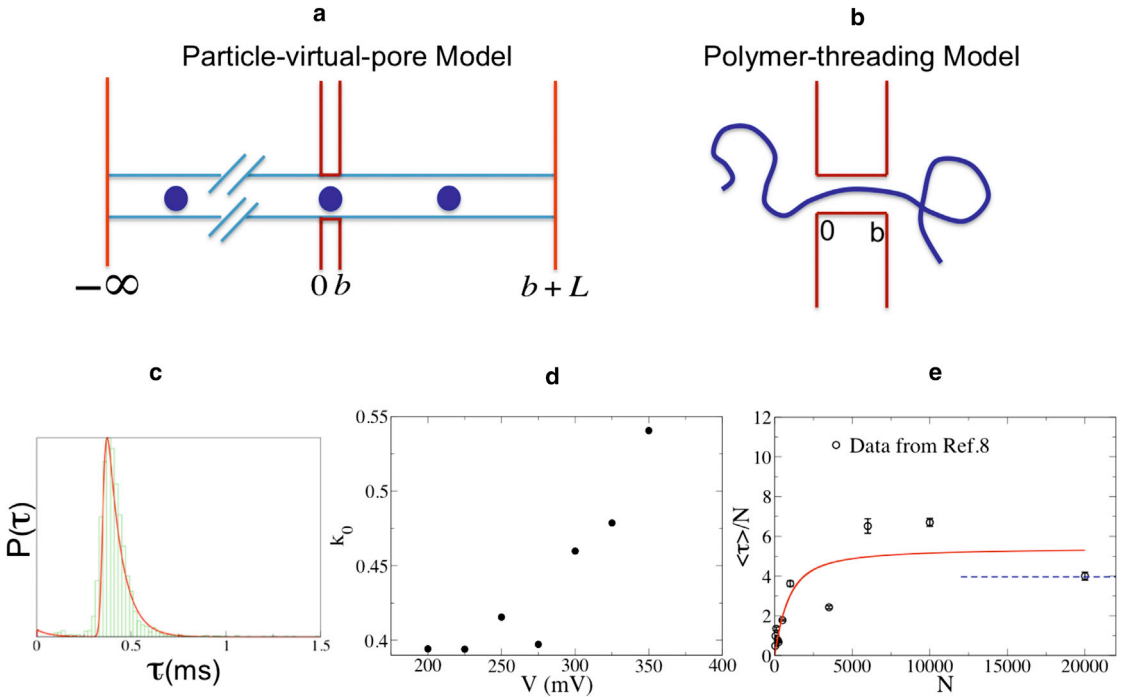

FIGURE 1 Theoretical models for polymer translocation. (*a*) Particle-virtual-pore model: a structureless particle (representing the translocation of DNA molecule) moves with uniform velocity v and diffusion constant D everywhere between $-\infty$ and $b + L$, while the actual pore is between 0 and b, and L is the contour length of DNA. D and v are parameters in this model. (*b*) Polymer-threading model: The voltage gradient acts only across the physical pore and entering monomers accelerate inside the pore against friction due to pore-polymer interactions. The effective monomer diffusion constant k_0 is a parameter in this model. (*c*) Fit of the calculated histogram (*red curve*), $P(\tau)$, from the polymer-threading model with data (*green*) of Fig. 2 *a* of Carson et al. (1) for $N = 500$ and $V = 300$ mV. For very small values of τ, a discrepancy between theory and experimental data occurs due to choices of the binning size in the numerical calculations. (*d*) The parameter k_0, from fits as in panel *c*, increases with voltage ($N = 500$). (*e*) Plot of $\langle\tau\rangle/N$ versus N using the same data (see Table S1 in Carson et al. (1)) used in Fig. 6 *c* of Carson et al. (1) for $V = 200$ mV. (*Smooth curve*) Fit ($A\{1 + (N^*/N)[\exp(-(N/N^*)) - 1]\}$, with $A = 5.44$, and $N^* = 518.2$) to the data exhibiting a cross-over behavior to the asymptotic limit of $\langle\tau\rangle \sim N$. For smaller values of N compared to N^*, the contributions from the polymer diffusion and pore-polymer interactions dominate over the drift contribution. For values of DNA length much higher than N^*, such that $VN >> 1$, the drift dominates over all other contributions (8). (*Dashed line*) Asymptotic value from the polymer-threading model for large N at $V = 200$ mV.

(even outside the physical pore), the theoretical histogram of the translocation time is described by a simple Gaussian-like formula (1) in terms of D and v. By fitting the experimental histograms with this formula, the authors obtained their values of D and v, and Eq. 1. The violation of the Einstein-Smoluchowski law by Eq. 1 has its roots in the inapplicability of the particle-virtual-pore model to the single-file experiment. Further, in their MD simulations, Eq. 2 is in fact used to get D, which is claimed to be in quantitative agreement with their experimental values—thus internally contradicting Eq. 1.

Hence, the paradoxical analysis of Carson et al. in fact shows that single-file translocations cannot be mapped into the particle-virtual-pore model. A polymer-threading model (10) sketched in Fig. 1 *b* is better suited to capture the essentials of the experimental data for this problem. Let b and L be the pore length and DNA contour length, respectively. These are equivalently written in terms of the DNA repeat distance as M and N, respectively ($b \equiv M \times 0.34$ nm and $L \equiv N \times 0.34$ nm). As DNA is translocated through a pore, the repeat units (monomers) that are present within the pore accelerate due to the strong electric field across the pore. This acceleration is opposed by frictional resistance from the pore walls and hydrodynamics inside the pore. The rest of the monomers of DNA residing outside the pore are subjected to relatively weak electric fields. In addition, as new monomers enter the pore they lose conformational freedom, which is released once they exit the pore. Using the theory of Muthukumar (10), the probability $P(m,t)$ that m monomers have translocated out of the pore in time t is

$$\frac{\partial P(m,t)}{\partial t} = k_0 \frac{\partial}{\partial m}\left[\frac{\partial F(m)}{\partial m} P(m,t) + \frac{\partial P(m,t)}{\partial m}\right], \quad (4)$$

where the free energy landscape $F(m)$ is given by Eqs. 26–28 of Muthukumar (10). Here, k_0 is a parameter denoting the effective diffusion constant of one monomer determined by the

local pore-polymer interaction. The histogram $g(\tau)$ of the translocation time τ is related to $P(m,\tau)$ given by Eq. 4 as (8)

$$g(\tau) = -\frac{d}{d\tau}\int_0^{N+M} dmP(m,\tau). \quad (5)$$

Taking the charge of a monomer q as $-0.3e$, the pore-monomer interaction energy ϵ as ln2, and $b = 6$ nm, and ignoring the weak logarithmic terms in Eqs. 26–28 of Muthukumar (10), histograms of τ are numerically computed from Eq. 5 with k_0 as the only free parameter for each voltage used in Carson et al. (1). The value k_0 is related to ϵ and depends on V. A typical good fit with experimental data of Carson et al. (1) is given in Fig. 1 *c*, as an example ($V = 300$ mV, $N = 500$). In general, theoretical histograms are narrower than the experimental data of Carson et al. (1), especially for higher voltages. Given the complexity of the problem and the simplicity of the polymer-threading model of Muthukumar (10), the fitting is reasonable. It offers a measure of k_0, which is found to increase slightly with V (Fig. 1 *d*), as expected from the reduction of the frictional barrier at higher voltages.

One of the main consequences of the polymer-threading model of Fig. 1 *b* is that the dependence of the mean translocation time $\langle\tau\rangle$ on DNA length N is not a universal law, $\langle\tau\rangle \sim N^{\alpha}$, with α as a single universal value for all values of N. The relation between τ and N should exhibit a crossover behavior depending on N, the voltage V, and the pore-polymer interactions. However, in the asymptotic limit of very large values of N such that $NV >> 1$, the mean translocation time is proportional to DNA length as the universal law,

$$\langle\tau\rangle \sim N, \quad (6)$$

and the single nonlinear dependence of Eq. 3 for all values of N cannot be valid. The raw data of Fig. 6 *c* and Table S1 in Carson et al. (1) for $V =$ 200 mV are plotted as $\langle\tau\rangle/N$ versus N in Fig. 1 *e*. This figure clearly shows that the authors' claim of Eq. 3 is not supported, although the same data are used in the plot. In Fig. 1 *e*, there appears to be a crossover from $\langle\tau\rangle \sim N^2$ behavior at small N to $\langle\tau\rangle \sim N$ behavior at large N. A similar crossover behavior was observed in Storm et al. (9), although with a different conclusion in the large N limit. The asymptotic value from the polymer-threading model (Fig. 1 *b* and Eq. 6) is indicated by the horizontal dashed line in Fig.1 *e* for this voltage. The data and theory are in qualitative agreement in the asymptotic limit of large enough N, consistent with Eq. 6. There are several possible avenues to improve the existing polymer-threading models, but even this elementary model describes the essentials of the experimental data without violating any fundamental theorems in physical sciences.

The use of nanopores as clocks to time the passage of macromolecules, and as sensors to infer the chemical signatures of analytes, requires implementation of relevant models and correct theoretical ideas in interpreting hard-earned experimental data. This necessity is as important as the high standards required to generate excellent data, as clearly displayed by Carson et al. (1), in order to ensure the correct inference of molecular details in single-molecule electrophoresis experiments.

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