Published in final edited form as:

Nanoscale. 2010 April 8; 2(4): 468–483. doi:10.1039/b9nr00275h.

Deciphering ionic current signatures of DNA transport through a nanopore

Aleksei Aksimentiev*

Department of Physics and Beckman Institute for Advanced Science and Technology, University of Illinois at Urbana-Champaign

Abstract

Within just a decade from the pioneering work demonstrating the utility of nanopores for molecular sensing, nanopores have emerged as versatile systems for single molecule manipulation and analysis. In a typical setup, a gradient of the electrostatic potential captures charged solutes from the solution and forces them to move through a single nanopore, across otherwise impermeable membrane. The ionic current blockades resulting from the presence of a solute in a nanopore can reveal the type of the solute, for example, the nucleotide makeup of a DNA strand. Despite great successes, the microscopic mechanisms underlying the functionality of such stochastic sensors remain largely unknown, as it is not currently possible to characterize the microscopic conformations of single biomolecules directly in a nanopore and thereby unequivocally establish the causal relationship between the observables and the microscopic events. Such a relationship can be determined using molecular dynamics—a computational method that can accurately predicts the time evolution of a molecular system starting from a given microscopic state. This article describes recent applications of this method to the process of DNA transport through biological and synthetic nanopores.

1 Introduction

Imaging pulling a thread through a needle's eye, just a nanometer in diameter, a thousand times per minute, in the dark. That is precisely what so-called nanopore technology [1] enables. Figure 1 illustrates the basic principle. A nanometers-thin membrane with a single nanopore in it separates an aqueous solution into two compartments, connected through the nanopore. A transmembrane voltage bias drives charged solutes, such as biomolecules and dissociated salt ions through the nanopore, from one compartment to the other. The specific distribution of the electric field, which is high near and inside the nanopore but negligible away from the membrane, performs the hard task of navigating the thread (biomolecule) through the needle eye (nanopore), enabling automatic reloading of the pore with a new biomolecule as soon as another one exits the pore (hence is the throughput). The steric constraints of the nanopore ensure that only one molecule is loaded at a time.

What are the merits of such a remarkable technology? For a period that can be as brief as a few nanoseconds [2,3] or as long as a few seconds [4,5], a fragment of or an entire (small) biomolecule can be confined to a volume of just several cubic nanometers. Thus, the nanopore confinement enables properties of a confined molecule to be examined, tens of atoms at a time. In particular, single file transport of a linear, unfolded polymer sequentially exposes its fragments to the examination volume—a process ideal for protein or nucleic acid sequencing. Although various methods to perform the examination have been proposed and

*Corresponding author: aksiment@illinois.edu.

realized, including transverse tunneling current recordings [6,7], capacitance measurements [8,9] and fluorescent readout [10], the most useful method to date is also the one most easily realizable in practice: measurement of the ionic current co-passing through the pore along with the analyte molecule. In the absence of an analyte, the geometry and the fixed charge of a nanopore determines its ionic conductance. The presence of an analyte alters the nanopore conductance, which is registered by an electric amplifier. In a way, the *modus operandi* of a nanopore detector is similar to that of a Coulter counter—a device routinely used in biology labs to count cells by measuring ionic current blockades they produce passing through a microns-size aperture.

The first experimental realization of a molecular Coulter counter was done using the alamethicin channel [11], where the noise spectrum revealed permeation of polyethyleneglycol polymers. About the same time the idea of using nanopores to sequence DNA was articulated by Deamer, Brandon and, independently, Church [12]. In 1996, Kasianowicz and co-workers jump-started the field by demonstrating a sequence-dependence of the ionic current blockades produced by transport of RNA molecules through the α -hemolysin channel [13]. Since then, nanopores have been employed to detect nucleotide content of DNA and RNA molecules [14–20], to measure the type and the concentration of small analytes [21–24], to detect proteins [25–33], to identify the stereoisomers of a common drug [34], to determine the mass spectrum of a polymer mixture [35], to exert forces on biomolecules [4,5,36–39], and to sort proteins [40,41]. The largest technology drive beyond the explosion of publications in the nanopore field is the possible application of nanopores in DNA sequencing [42–44], with major biotech companies already eyeing nanopores as prospective generation IV sequencing platforms. Various spin-off applications such as general purpose stochastic sensors [45–47], single molecule manipulators [4,36,37,48], ion filters [49–53], nanofluidic electronics [54–57], etc. ensures that the nanopore field will be flourishing long after the DNA sequencing goals are met.

Apart from interest in using nanopores for various nano(bio)technology applications, transport of solutes through nanopores presents a rich and non-trivial physics problem. In fact, the microscopic mechanism underlying the basic nanopore functionality—ionic current blockades—remains largely unknown, as it is not currently possible to characterize the microscopic conformations of single biomolecules directly in a nanopore and thereby unequivocally establish the causal relationship between the ionic current measurements and the underlying microscopic events. Many unanswered questions pertain to the mechanism of a solute's capture, the role of hydrodynamic interactions, solvation forces, and entropic forces of confinement, the electro-osmotic effect, mechanical deformation of solutes, atomic-scale friction, interaction of inorganic surfaces with biomolecules, sorption/desorption of solutes to/from the nanopore walls, spontaneous closure of biological nanopores, noise in nanoelectrofluidic systems, etc. Nanopore translocation experiments present a unique opportunity to test physical models of diverse nanoscale phenomena at the single-molecule level.

The high throughput and the relatively simple setup of the nanopore experiments come with the price: the ionic current is often the only observable that can be directly measured. Microscopic simulations, in particular all-atom molecular dynamics (MD), can be used to interpret the results of a nanopore experiment as they can not only describe the dynamics of a polymer in a nanopore but also predict the associated ionic current values. Below we discuss the capability of the MD method in answering to the following questions:

- For a pore of fixed geometry and charge, what is the magnitude of the ionic current and how does it depend on electrolyte concentration and make-up?

- For a solute of a known structure, a pore of known geometry and an electrolyte of known concentration, what permeation pathway is the most likely and what is the permeation rate?

Once a method is available to answer these questions, it will become possible to reconstruct molecular events underlying measured ionic current blockades. This reconstruction method will enable further optimization of the nanopore structure, solvent properties and external conditions (transmembrane bias, temperature, etc.) to control the permeation mechanism and increase the sensitivity of ionic current blockades to the underlying molecular events.

2 Biological and solid-state nanopores

Nanopores are ubiquitous in nature. Equipped with voltage-driven levers, selective gates, and electrochemical motors, nanometer-size pores in lipid membranes transport biomolecules in and out of living cells and between the intracellular organelles. Viruses use nanopores to infect susceptible cells. Bacteria deploy nanopores for horizontal gene transfer. Cells divide, change their shapes and move in space in part due to the transport of biomolecules through nanometer-cross section porous filaments, i.e, microtubules and flagella, that allows the filaments to grow or contract.

Nanopores are ubiquitous in engineering. They can be deployed in a variety of applications, ranging from high-performance light-weight materials, thermal insulators, and catalytic membranes to hydrogen gas storage, protein filters and drug delivery systems. Here, we focus on a particular type of nanopores that can serve as models for biological systems and that can be deployed for various applications in bionanotechnology. Incorporated or produced in a nanometer-thick biological or synthetic membrane, such pores have diameters from one to tens of nanometers.

Until recently, bacterial toxin α -hemolysin (Fig. 2 a) was the system of choice for the nanopore translocation experiments, as this protein easily forms a water-filled pore in a lipid bilayer membrane [58,59] and retains such conformation for extended periods of time, up to several days [60]. The utility of the α -hemolysin pore as a stochastic sensor can be enhanced by placing a molecular adapter near the constriction of the pore [21]. Through genetic engineering, specific sites [61,62] or even entire proteins [63,64] can be incorporated into the nanopore's structure, further increasing the sensor's sensitivity. To be detected, the analytes do not have to permeate the pore. Indeed, a toggling-type auxiliary molecule can transduce the binding of an analyte at the periphery of the channel into a pattern of conductance states, which can be recognized using a support vector machine [27].

Another biological pore that was recently shown to have extraordinary potential for molecular sensing is MspA—a porin protein from the outer membrane of *Mycobacterium smegmatis*, Fig. 2 b. The X-ray structure of MspA [67] revealed its unusual architecture: eight beta-strand monomers form a goblet-like structure, with the inner diameter of the constriction ~ 1 nm and inner diameter of the widest part ~ 4.5 nm. This particular shape of the MspA pore makes it an ideal platform for stochastic sensing applications [47]. The main difficulty with using an MspA pore for ionic current recordings was its propensity for gating, which produces ionic current blockades similar to blockades resulting from transport of solutes. Recently an interdisciplinary team of researchers succeeded in genetically modifying the MspA pore to produce a channel pore that remains open indefinitely in bilayer recording experiments [68].

The main advantage of the α -hemolysin and MspA systems is that they always form nanopores of the same three-dimensional structure, which are known to atomic-level detail [67,69]. An obvious disadvantage is the mechanical fragility of a lipid bilayer membrane,

and a rather tedious procedure is required to incorporate a single biological pore in a lipid bilayer membrane. Experimental work [70,71] is underway to decrease the mechanical instability of the lipid bilayer, which is the main drawback of this system.

Recent advances in semiconductor nanotechnology allow pores to be fabricated with nanometer-size diameters (Fig. 2 c) using highly focused ion [72,73] and electron [66,74–76] beams (reviewed in Ref. [77]). Such pores are mechanically stable, do not change their shapes after extensive use, can operate at various temperatures and pH conditions [78,79], and can withstand high electric fields [80]. The pores can also be made of any size [74,75,81], can be arranged into arrays [76], and can be functionalized with various organic molecules [82] including oligonucleotides [83,84]. The main disadvantage of the solid-state pores is that, even when manufactured using the same procedure, all pores will have slightly different shapes and surface charges, which affect their transport properties. Coating the nanopores with an atomic layer of alumina was shown to reduce some surface effects [85].

Nanopores in metal-oxide-semiconductor membranes (Fig. 2 d) are similar to those in silicon-based insulators, but have the additional advantage of allowing direct measurement of the electrostatic field of transported solutes [8,86]. Typical metal-oxide-semiconductor membranes used in nanopore experiments have three layers: a layer of SiO₂ separating two layers of doped crystalline and polycrystalline silicon. The SiO₂ layer can be made as thin as 7 Å [87]. Single or multiple-layer structures can be manufactured using standard semiconductor fabrication procedures as well. Nanopores in such membranes can be produced using a highly focused electron beam [8,86] or through a sequence of alternating etching and atomic layer deposition procedures. The latter method can produce large nanopore arrays and is suitable for mass production.

3 Theoretical and computational models of nanopore transport

The process of polymer transport through a nanopore has attracted attention of many theoreticians who brought to the field expertise from diverse areas of physics. Consequently, the spectrum of methods used to model this process is broad, and a comprehensive review is beyond the scope of this article. Below, we briefly describe the most popular methods.

Continuum, transport and polymer physics models

The first theoretical models of polymer transport aimed to provide a theoretical account of the measurements (reviewed in Refs. [88,89]). The three most popular approaches were based on transport equations, scaling concepts of polymer physics, and continuum mechanics. In the first approach, a Fokker–Planck equation or its equivalent is used to describe polymer flux through a nanopore. The same type of models can also predict ionic current [90–92]. Concepts of semiconductor physics were employed to describe ion transport and the effective force on DNA in narrow pores [93,94]. The second approach relies on scaling laws of polymer physics [95] and is most often used to predict the dependence of the polymer transport time on the polymer length and transmembrane bias. Thus, by describing DNA as an ideal polymer chain, the free-energy barrier for the DNA translocation [96,97], the average translocation time as a function of the pore dimensions and the length of DNA [98,99], and the total polymer flux induced by the external electrical field [100] could be computed. Mathematical models describing the diffusion of polymers through pores have been used to investigate the origin of the measured non-Gaussian distributions of DNA passage times [101–103]. The third class of models treat polymer transport through nanopores at the level of continuum mechanics. Such models were instrumental in elucidating the role of hydrodynamic interactions in nanopore transport [104–106]. A clear advantage of these approaches is that they describe the translocation process in statistical terms and thus can be compared with an ensemble average determined

by experiment. A disadvantage is that these models are built *assuming* certain types of microscopic interactions between the polymer and the nanopore, solvent, and itself and cannot easily incorporate atom-scale information.

All-atom molecular dynamics

At the other side of the spectrum of approaches is MD, which is a computational method that allows the microscopic dynamics of a molecular system to be modeled at atomic resolution. In this method, a molecular system is approximated by an ensemble of virtual atoms interacting with each other according to the molecular force field [107], which has been developed and calibrated to quantitatively reproduce the physical properties of a simulated system. The trajectory of each atom is obtained by integrating the Newton's equation $\vec{a}_i(t) = -\nabla \mathcal{U}(\vec{r}_1(t), \dots, \vec{r}_N(t))/m_i$ using the Verlet algorithm [108], where $\vec{a}_i(t)$, $\vec{r}_i(t)$ and m_i are the acceleration, coordinates, and mass of atom i , respectively, and N is the total number of atoms in the system. For MD simulations of biomolecules, the molecular force field, e.g., $\mathcal{U}(\vec{r}(t))$, is derived from quantum-mechanics calculations. Parameters of the molecular force field thus obtained are further refined to reproduce the physical properties of biomolecules in water [109,110].

To model the transport of biomolecules through nanopores, an all-atom model of the experimental system is constructed using known crystallographic or other structures of the biomolecules, lipids, and inorganic materials. To model the nanopores in synthetic membranes, the force field describing the synthetic component is transcribed in the form of potential functions used by the biomolecular force fields, such as AMBER [110] and CHARMM [109]. Adapting the methodology developed for simulation of membrane proteins [65,90,115–124], a simulation of the electric field-driven translocation of biomolecules through nanopores can be accomplished [66,112]. Figure 3 illustrates the process of building atom-scale models of biological [65] and synthetic [112] nanopores (reviewed in [111,125]).

The main advantage of the MD method is its atomic resolution and the possibility of direct comparison between simulation and experiment [65,79,80,126]. The main disadvantage of the method is its time scale, which is currently limited to several microseconds.

Coarse-grained, Brownian dynamics and Monte-Carlo simulations

Occupying a niche between all-atom and continuum models, this class of approaches relies on a less detailed description of the system than all-atom MD, while preserving enough details to incorporate some structural features of a nanopore and a polymer. In coarse-grained (CG) MD [127,128], the polymer, nanopore and, sometimes, solvent are described as beads, each representing several atoms. In addition to reducing the number of degrees of freedom, a CG representation increases the time scale of an MD simulation by allowing for larger integration time steps. Additional speed-up originates from effective smoothing of the interbead interactions [129]. In Brownian dynamics or Langevin models [130–133], the solvent effects enter as stochastic forces that apply to beads according to the underlying dynamical model [107]. Monte-Carlo simulations [134–137] are often used to find a solution to the polymer physics models of nanopore transport. The time scale of these methods permits simulation of a polymer capture by the pore and complete permeation trajectories, in some cases allowing the distribution of the permeation times to be reconstructed [132]. However, the computational efficiency comes at the price of accuracy, as all atomic-level details of the nanopore and polymer structure are washed out or blurred in this approach. Accordingly, these methods are much less successful in predicting the ionic current blockades and, generally, do not account for electro-kinetic effects.

Multiscale approaches

Escaping the overidealization of a continuum approach and beating the time and length scale limits of a particle-based model, multiscale simulations typically combine a detailed description of the system in the vicinity of the nanopore with a coarser description of the system everywhere else. These types of models only recently have been applied to study polymer transport through nanopores. Thus, a CG MD model of dsDNA and a solid-state nanopore was coupled to a Lattice-Boltzmann (LB) model of an electrolyte [138,139] to study the so-called fast translocation regime [140] that is thought to be governed by hydrodynamic interactions. In another study, a continuum electrostatic model was coupled to LB hydrodynamics to predict ionic current blockades [141]. A multiscale model combining all-atom MD and a continuum electrostatic solver was used to predict electric signatures resulting from DNA transport through a metal-oxide-semiconductor capacitor [8,142]. This class of models has the ability to provide answers to almost all relevant questions of the field; however, it is also the least developed one.

4 MD simulations of nanopore experiments

The indirect nature of information furnished by the nanopore translocation experiments creates the opportunity for molecular modeling to play a major role in the research process. Successful modeling approaches must not only accurately describe the process of polymer transport through a nanopore but also predict, with a quantitative accuracy, the associated ionic current blockades—a capability required for direct comparison between simulation and experiment. The all-atom MD method is ideally suited to interpret the nanopore experiments, as it can directly relate the conformation of a molecular system to the ionic current [65,112,126]. The fundamental problem, however, is that the time scale of processes that can be investigated using this method is limited to several microseconds, whereas a typical translocation process takes milliseconds or more. Even using the newest generation of special purpose supercomputers that promise to push the simulation time into the submillisecond regime [143], a full account of the measurements cannot be obtained by the MD approach alone, as the permeation process is highly stochastic and must be characterized in statistical terms, which requires simulations of a large number of permeation events.

While coarse-grained and multiscale computational approaches that preserve the accuracy of an all-atom MD simulation at a fraction of the computational cost are not yet available, all-atom MD simulations can be used to provide valuable information about a select class of translocation phenomena. For example, a typical polymer translocation event involves capture of the polymer by the pore and subsequent translocation. The capture process is difficult to characterize by all-atom MD, as diffusion governs DNA arrival at the pore's mouth and the subsequent search for the conformation that enables translocation. Hence, in a typical MD simulation of polymer transport, the polymer is placed in a conformation that enables translocation at the very beginning of the simulation. Such an approach is justified if the study's main focus is on the translocation events, not the capture. Furthermore, in some systems (for example, α -hemolysin, Fig. 3 (i)), the translocation process is too slow for the all-atom MD even when the simulation is initiated from a conformation that is expected to occur during the translocation. In this case, a viable approach is to take advantage of the thousandfold difference in the time scales of ion and polymer transport and characterize in statistical terms the ensemble of conformations a polymer can adopt inside the nanopore and compute (using MD) the corresponding distribution of the ionic current.

Below we describe the use of all-atom MD simulations for deciphering ionic current traces produced by polymer transport through biological and solid-state nanopores. Molecules of single- and double-stranded DNA (ssDNA and dsDNA) are used as model polymers,

representing the case when the polymer's persistence length is much smaller (ssDNA) and much larger (dsDNA) than the membrane's thickness. The task of predicting the ionic current traces can be split into four parts: (i) Predict ionic current through an open pore; (ii) predicting a typical translocation trajectory of a polymer through the pore; (iii) predict ionic current blockades produced by the polymer in the nanopore; (iv) take into account the electro-osmotic effect that couples the force experiences by the polymer in a nanopore to the ionic current.

4.1 Open pore current

The task of predicting the ionic current through a nanometer-size pore can be far from trivial: computing the conductance of even the simplest biological ion channels remains a major challenge [122,144]. Luckily, nanopores used for DNA translocation experiments are considerably wider than the pores of biological ion channels. Not only predicting the conductance of a larger-diameter pore requires less computation, the simulations are more tolerable to the imperfections of the molecular force field [118].

4.1.1 Bulk conductance—A necessary but not sufficient condition for predicting the ionic conductance of a nanopore in an MD simulation is the ability of the method to properly describe bulk conductance of the electrolyte. To test the molecular force field, a small volume ($\sim 5 \times 5 \times 5 \text{ nm}^3$) of electrolyte is subject to an external electric field; the resulting displacement of ions is analyzed to obtain the currents of anionic and cationic species and the total current. Using the set of parameters that comes with the standard CHARMM force field [109], the simulated conductivity of bulk KCl is in excellent quantitative agreement (10% error of the absolute value) with experiment for the ion concentration around 1 M [65]. Although the ion conductivity of an electrolyte decreases with the ion concentration, the simulated dependence does not comply with the Kohlrausch law [65,145], in particular around 0.1 M. Considerable deviations of the simulated KCl conductance from experiment were reported for the high ($>2 \text{ M}$) concentration range [146]. The simulated dependence of the KCl conductance on temperature (at 1 M) was found to be in reasonable agreement with experiment, suggesting that the all-atom model satisfactorily accounts for the ion pairing effect [147]. Coincidentally, most of the nanopore experiments are conducted at 1 M KCl—the concentration the all-atom model performs the best. To simulate experiments carried out at a different bulk ion concentration, it is advisable to scale the absolute values of the nanopore currents with the ratio of experimentally measured and simulated bulk conductances. The inaccuracy of the force field at low and high ion concentrations may pose a problem for predicting the open pore current even at 1 M if the local ion concentration varies considerably along within pore.

In addition to the force field, other factors can influence the bulk conductivity of electrolyte in an MD simulation, for example, the type and the parameters of the thermostat [107] used to maintain the temperature of the system constant, the use of rigid or flexible bonds in the water model [147]. One should be particularly careful when determining the relative contribution of the cationic and anionic species to the total current, because the entire simulated system can randomly drift in space [65] as a result of the particle-mesh Ewalds electrostatics [148] and/or langevin thermostat.

4.1.2 Ionic current through biological and synthetic nanopores—The steps required for computing the ionic current through an open pore using the all-atom MD method are summarized in Figure 4. After assembling the all-atom model of a nanopore system, the model is simulated for a short period (typically $<10 \text{ ns}$) in the constant pressure ensemble, allowing the system's volume and the conformation of the system's constituents to attain equilibrium. Subsequently, a constant uniform electric field is applied normal to the

membrane, polarizing the dielectric material (water and membrane) and redistributing the ions. The dielectric polarization, which occurs faster than the ion redistribution, governs the behavior of the system immediately after the external electric field has been switched on [149]. Given enough time, the electrostatic potential of the bulk electrolyte at both sides of the membrane becomes uniform (electrolyte is a conductor), and the voltage drop across the membrane is given by the product of the electric field and the size of the system in the direction of the applied field: $V = -EL_E$. Note that even though the applied external electric field is uniform, the resulting effective field acting on a probe charge in the system is highly non-uniform. The distribution of the electrostatic potential can be visualized using the so-called PME potential map method [65], see Fig. 4 b for an example.

Driven by the effective electrostatic potential, ions of both types permeate through the nanopore in opposite directions. The instantaneous value of the current can be determined by summing up local displacements of individual ions between consecutive frames of an MD trajectory [65,150,151], which are usually recorded with the rate of one frame per 1 to 50 ps. The obtained instantaneous currents are very noisy. Hence, determining the value of the open pore current requires averaging over an extended period of time. In general, the standard error in determination of the current decreases as a square root of the number of ion permeation events, or the square root of the simulation time in the steady state regime. The statistical nature of the current evaluation makes predictions of the ionic current less computationally expensive for high-concentration electrolytes and at high transmembrane bias potentials.

Although the first MD simulations of an open pore current were performed using solid-state nanopores [112,150], it was the simulation of the α -hemolysin channel that demonstrated the ability of the MD method to quantitatively predict the ionic current [65]. The α -hemolysin was an ideal protein for this study because of its relatively large conductance and the known X-ray structure. The simulation of ionic current through α -hemolysin channel was carried out at different values of the transmembrane voltage, yielding the current-voltage (IV) curve, Fig 4 e. The simulations revealed features of a real channel, such a slight asymmetry of the IV dependence and moderate selectivity to potassium ions. A major advancement reported in that study was the ability of the MD method to predict the ionic current at the voltage-bias conditions accessible to experiment, enabling direct verification of the simulation results. In the case of α -hemolysin, the agreement between the simulated and measured IV curves was quantitative. Moreover, changing the protonation states of seven histidine residues near the pore constriction in an MD simulation was observed to alter the ionic current, which suggests that spontaneous protonation-deprotonation of these residues could be the source of the experimentally observed pH-dependent noise in the open pore current recordings [152,153]. In addition to α -hemolysin, the MD method was applied to characterize the ionic conductance of MscS [154], Kv1.2 [144], and OmpF [146,147].

Determining the accuracy of the MD method in the case of solid-state nanopores is more difficult, as the structure of these pores is not known to atomic detail and, therefore, direct comparison to experiment is not possible. Nevertheless, all simulations carried out to date showed a qualitative agreement of the nanopore conductance values obtained in simulation and experiment [4,112,126]. The most recent study of Si_3N_4 [126] and SiO_2 [155] nanopores demonstrated high sensitivity of the open pore currents to atomic-scale roughness of the nanopore surface. The slow ion binding kinetics in Si_3N_4 pores requires long (10–50 ns) simulation times to reach a steady-state conductance regime, in particular in the case of large-area (15–20-nm membrane thickness) pores.

To illustrate the current capabilities of the MD method, let's estimate the supercomputer and real-life time required to simulate ionic conductance of an MspA channel [67], Fig. 2(a).

The molecular dynamics code NAMD [156] can apply thousands of CPU to a single MD run. The runtime performance of the code scales linearly with the number of CPUs available, reaching the limit of scalability at about 50 to 100 atoms/per CPU, depending on the hardware architecture. A typical simulation system incorporating one copy of MspA, a lipid bilayer membrane, water and ions amounts to about 350,000 atoms. Using 1024 CPUs in one run, a 100-ns simulation of the MspA system requires about 10 days to complete. The simulation time required to compute the ionic current I to desired accuracy $\delta = \Delta I / I = 0.4(I\tau)^{-1/2}$, assuming that the standard deviation of the current is proportional to the square root of the number of elementary charges passing through the channel (which is the case for MD simulations). I and τ in the above formula have units of nA and ns, respectively. Determining the MspA open current ($I_0 = 450$ pA) at a 200 mV bias to 10% accuracy will require a 35.5-ns MD simulation, and will take about 3 days to complete. The improved communication network of a new generation of supercomputers is expected to reduce the actual time required for a 100-ns simulation of an MspA system to about 1 day by increasing the number of CPUs that can be applied in one run. In theory, using a special-purpose supercomputer [143], a 1- μ s simulation of the MspA system could be accomplished in one day.

4.2 DNA transport

Because the setup of an MD simulation can faithfully reproduce the experimental conditions, no special arrangements are required, in principle, to simulate DNA transport through a nanopore: driven by the gradient of the electrostatic potential, DNA, like any other charged solute, will find its way through a nanopore. The only limitation of this approach is the time scale of an MD simulation. Several methods have been developed to simulate DNA permeation when the experimental time scale of DNA transport greatly exceeds the range on conventional MD.

4.2.1 Electric field-driven transport—The most common approach to economize the simulation time is to place DNA at the beginning of the simulation in a conformation that enables translocation. This task can be accomplished “by hand” [112], or using a more sophisticated approach, for example a Monte Carlo simulation [126]. Once the translocation process has been initiated, its completion will require as much time in an MD simulation as in experiment. Figure 5 shows examples of the simulated DNA translocation trajectories.

In the case of relatively wide (>4 nm in diameter) solid-state nanopores, the translocation rate of dsDNA is of the order of one base pair per 30 ns at a 200 mV bias [3,85] and increases with the bias [157]. Similar translocation rates were observed in MD simulations [126]. Furthermore, as solid state membranes can withstand voltage biases of 1 V and higher, the translocation rates can be directly compared between simulation and experiment [79,158]. The translocation rate of DNA through narrow (<4 nm in diameter) pores is determined by the DNA’s interaction with the nanopore surface [101,112,157,159]. In particular, nucleobases of ssDNA were observed to bind Si_3N_4 surfaces with high affinity, often stalling the translocation in an MD simulation [112]. The phosphate groups of dsDNA were observed to bind to the surfaces of crystalline Si_3N_4 and amorphous SiO_2 solid as well (unpublished). As neither the accuracy of the force field that describes the interactions of a solid-state membrane with DNA in an MD simulation nor the structure and chemical composition of the nanopore surface used in actual experiment are known, direct quantitative comparison between simulation and experiment is, most of the time, not possible.

One noticeable exception is simulations and experiments pertaining to the so-called voltage threshold for DNA translocation [79,80], which is observed when molecules of DNA are

forced to permeate through nanopores smaller in diameter than the translocating molecules. At a low transmembrane bias, DNA transport through such nanopores is sterically forbidden. However, if the force of the electric field in a nanopore is high enough to stretch the DNA molecule (reducing its cross section), the permeation can take place. This voltage threshold should not be confused with the entropic barrier for polymer transport [97]. The voltage threshold for dsDNA translocation was first observed in MD simulations of Si_3N_4 pores and subsequently demonstrated in experiment [79,80]. It is possible to trap a dsDNA molecule in a nanopore by switching off the electric field from a value above the translocation threshold before the DNA escapes the pore [158]. The voltage threshold phenomena were also observed in experiments using DNA hairpins [126,160] and DNA-restriction enzyme assemblies [4,161].

Stretching a dsDNA molecule in a nanopore requires a high transmembrane bias. Therefore, subsequent translocation of short (up to 1000 base pair) dsDNA fragments proceeds too fast to be detected as an ionic current blockade in experiment. Hence, the fact of DNA translocation (or its absence) is established by biochemical analysis of the solution samples taken from both sides of the membrane before and after the translocation experiment [80]. In the absence of reliable experimental data on the ionic current blockades, the only quantity that can be directly compared between simulation and experiment is the value of the voltage threshold. Thus far, the simulations and experiments produced consistent values of the voltage threshold, which is quite remarkable given that the only information about experiment used in the MD simulations are the TEM image of the pore and the nucleotide sequence of DNA.

Transport of ssDNA through biological pores typically proceeds much slower than transport of dsDNA through solid-state pores, most likely because the constriction of the biological pore (α -hemolysin or MspA) is of the same size as the diameter of a single DNA strand. For example, the transport rate of DNA homopolymers through α -hemolysin is of the order of one nucleotide per μs at a 120 mV bias [15]. This is consistent with the following MD result: at a 240 mV bias, DNA maintains the same position in the pore of α -hemolysin during a 150-ns simulation (unpublished). It is also clear that the utility of a conventional MD approach for characterizing the ssDNA translocation rate is severely limited.

A typical method to accelerate the rate of ssDNA transport in an MD simulation is to increase the transmembrane bias. Often, scaling the bias by a factor of ten can yield the desired results [112,113], however the dependence of the DNA transport on the bias is often nonlinear [162]. In experiment, a solid state membrane can withstand a transmembrane bias up to 5V [79], whereas a lipid bilayer ruptures at about ± 300 mV. Electroporation of a lipid bilayer in an MD simulation is observed at voltage biases of 2V and higher, which could also depend on the overall duration of the MD run. The electroporation leads to ion leakage, which subsequently distorts the driving potential and thereby results in a simulation of an unrealistic permeation event. Grid-steered MD (G-SMD)[163] is recommended for simulations at transmembrane biases higher than the threshold for electroporation (see below).

4.2.2 Steered molecular dynamics—A popular method to simulate events whose time scale exceeds the range of conventional MD is to apply an external force to drive the system along the desired path, measuring the response of the system. In this method dubbed steered molecular dynamics (SMD) [164,165], an external force is applied by attaching a moving harmonic (spring-like) restraint to one or more atoms in the system [164–166]. Repeating the SMD simulations many times can yield the potential of mean force through Jarzinsky's identity [167,168]. Although SMD is a popular method to study permeation of small solutes through membrane channels and transporters [124,169,170], its application to the problem

of polymer transport through nanopores is troublesome [163]. Thus, pulling with a steering force one end of a polymer is likely to distort the polymer's conformation, for example overstretch dsDNA or strain the conformation of ssDNA's random coil. Because the timescale of the SMD simulation is too short to observe relaxation of the polymer conformation, it will enter the nanopore in a distorted, unrealistic conformation. Applying the SMD force to the center of mass of the solute produces similar distortions [163].

Due to these limitations, conventional SMD is seldom used to investigate polymer transport through nanopores. Nevertheless, this approach is preferred to the electric field-driven simulation when the force applied to a DNA polymer in a nanopore needs to be controlled or measured. Thus, using conventional SMD, it was discovered that a pathway (stretching or unzipping) a DNA hairpin undertakes to permeate through a nanopore that is smaller in diameter than its duplex part depends on the nanopore diameter [160]. The SMD method was employed to determine the order the bonds between the restriction enzyme and DNA break in a nanopore force spectroscopy measurement [161]. The G-SMD method (see next) could have been used to obtain these results as well.

4.2.3 Grid-steered molecular dynamics—A combination of the above two approaches is the co-called grid-steered MD (G-SMD) method [163] that in tens of nanoseconds can provide a realistic account of a permeation event that would require a millisecond simulation using conventional MD. In this method, the average distribution of the electrostatic potential in a nanopore under a transmembrane bias of interest (for example, the one shown in Fig. 4 b) is determined first from an all-atom MD simulation [65]. This electrostatic potential, scaled with an amplification factor that controls the translocation rate, is subsequently applied to a charged polymer to steer its permeation through the nanopore in another all-atom MD simulation. Doing so facilitates faster polymer transport without straining the structure of the nanopore or the membrane. Moreover, as the shape of the potential applied to the polymer exactly matches the transmembrane potential driving the polymer in experiment, the field of forces is very realistic, and because the forces are distributed over the entire polymer, the strain introduced into the polymer's conformation by the amplified forces is much smaller than in conventional steered MD [164]. G-SMD has been implemented directly in NAMD [156], and introduces virtually no performance penalty, making it a very flexible and efficient tool.

Using this method, multiple complete permeation events of ssDNA through α -hemolysin could be simulated [163]. Despite the dramatic acceleration of the permeation process, the results of the simulations were in good agreement with experiment: the ratio of permeation rates for (dA)₅₈, (dC)₅₈, and (dAdC)₂₉ strands matched experiment. The ratio of the translocation rates of a poly(dA)₅₈ strand in two possible global orientations (lead by 3'- or 5'-end) was found in quantitative agreement with experiment [113,163]. The G-SMD method can be used to simulate protein transport through α -hemolysin as well as unzipping of DNA hairpins [163]. The main deficiency of the method is that it cannot predict the absolute translocation times. Another imperfection of the approach is disproportionate scaling of the driving and stochastic forces, which may overestimate the effect of nanopore-DNA interactions on the translocation kinetics. Despite that, the method can already be applied to study slow (μ s/DNA nucleotide) permeation processes. Work is underway to enable on-the-fly recalculations of the steering potential, making the simulation scheme self-consistent, and to better describe the stochastic forces that affect the permeation process.

4.3 Ionic current blockades

Transient reductions of the ionic current are recorded in almost all polymer translocation experiments. The next two sections discuss the physical effects that determine the level of

the ionic current blockade and describe the use of the MD method to relate the microscopic conformations of a DNA polymer to the resulting departures of the nanopore current from the open pore level.

4.3.1 Factors that affect ionic current blockades—The presence of a DNA molecule in a nanopore excludes a fraction of the volume available for ionic transport and alters the local ion concentration. The competition of these two effects determines the resulting ionic current through a blocked by DNA nanopore, which usually appears as an ionic current reduction but might as well appear as an enhancement. Indeed, in the case of dsDNA translocation through 10-nm-diameter SiO₂ pores, ionic current blockades could be transformed into enhancements by lowering the concentration of the bulk electrolyte below ~0.3 M [171,172]. Considering dsDNA as a charged cylinder of diameter d_{DNA} and charge density per unit length q_{DNA}^* , the change in conductance due to the presence of dsDNA in a nanopore can be described as [172]

$$\Delta G = \frac{1}{L_{\text{pore}}} \left(-\frac{\pi}{4} d_{\text{DNA}}^2 (\mu_{\text{K}} + \mu_{\text{Cl}}) n_{\text{KCl}} e + \mu_{\text{K}}^* q_{\text{DNA}}^* \right), \quad (1)$$

where μ_{K} and μ_{Cl} are the electrophoretic mobilities of the potassium and chloride ions, respectively, μ_{K}^* is the effective mobility of the potassium ions along dsDNA, n_{KCl} is the number density of potassium or chloride ions in the empty pore, and e is the elementary charge. The first term in Eq. 1 describes the reduction of the open pore current due to the excluded volume effect, whereas the second term describes the excess current of potassium ions along the DNA surface. Although Eq. 1 was derived assuming the density of the charge carries and the driving electric field are constant inside a nanopore, it adequately describes the results of the measurements when dsDNA maintains its canonical conformation in a nanopore that is much wider than the DNA's cross section.

In addition to bulk ion concentration, MD simulations have determined that the depth and the sign of a nanopore's conductance change critically depend on the DNA's conformation and the surface properties of the nanopore. Figure 6 a–c illustrates an MD simulation of dsDNA permeation through a 3.0-nm-diameter pore in a 10-nm-thick Si₃N₄ membrane driven by a 1.3-V bias. The KCl concentration in this simulation was 0.1 M. Figure 6 d shows the simulated ionic current. As the leading edge of dsDNA approaches the pore constriction, the current is reduced by more than tenfold. About that time, the leading base pair adheres to the surface of the pore while the rest of the molecule continues to move through it. At the end of the simulation, dsDNA forms a loop in the exit compartment, piercing the nanopore constriction twice, whereas the ionic current returns to the open-pore level. This MD simulation highlights the importance of knowing the conformation of a DNA molecule in a nanopore for proper interpretation of the ionic current blockades.

To reproduce the ionic current blockades recorded during the DNA trap experiments [158], MD simulations of a long dsDNA fragment in a 2.6-nm-diameter nanopore were carried out using two models of the nanopore surface: crystalline Si₃N₄ [112] and annealed amorphous SiO₂ solid [155,173]. The two surfaces were previously found to have different affinity to the ions: the Si₃N₄ surface adsorbs both K⁺ and, to a lesser extent, Cl[−] ions [126], whereas the annealed SiO₂ surface does not adsorb ions [155]. In experiment, the presence of dsDNA in a nanopore was observed to reduce the nanopore current to 50–70% of the open pore level in a 0.1-M KCl electrolyte [158]. The simulated ionic currents blockades in the Si₃N₄ pore were ~60% of the open pore level, whereas in the annealed SiO₂ pore [155] the current was enhanced to 130%. As both nanopore materials used in the simulations had neutral surfaces,

the simulations demonstrate that atomic-scale roughness can play a major role in determining the nanopore current.

4.3.2 Detection of molecular events—The first simulations of DNA transport through Si_3N_4 pores [112] investigated the feasibility of distinguishing the ionic current blockades produced by DNA translocation from DNA bounces. The latter refers to the situation when DNA occludes the nanopore mouth in the orientation parallel to the membrane surface, blocking the current but not moving through the pore. In experiment, the two types of events are distinguished by the depth and duration of the blockades—shallow and short-lived blockades are usually associated with bounces. Also, the duration of ionic current blockades associated with bounces is assumed to be independent of the polymer length. The separation of such molecular events is possible in the case of α -hemolysin [13–15] and, although being less straightforward, in the case of wide (>4 nm diameter) solid state nanopores [157]. MD simulations [112] demonstrated that distinguishing the two types of events by the level of the ionic current blockades is difficult in the case of narrow (2–3 nm diameter) pores as in both cases similar ionic current blockades are produced. The assumption about the independence of a bounce duration of the DNA length may also be invalid, because even in the conformation parallel to the membrane surface, the DNA can experience a force sufficient to tether it to the nanopore mouth for an extended period of time while allowing for one-dimensional reptation without leaving the nanopore mouth. Using a tiny patch of a metal-oxide-semiconductor membranes, the two types of event can be distinguished, in principle, by measuring the electrostatic potential at the top and bottom layers of the membrane [8].

Hairpin DNA molecules are exceptionally attractive subjects for nanopore experiments because their double-helical portion can be trapped in a pore of the appropriate diameter while the remaining portion is threaded through the constriction, providing ample time to carry out the ionic current measurement [18,36,160,174–176]. The arrested state does not last forever because the duplex part of the hairpin can thermally denature, allowing the hairpin to complete the translocation. In the case of α -hemolysin, the kinetics of duplex disruption can be used to discriminate molecules of different sequences [175]. Contrary to α -hemolysin, in the case of solid-state nanopores, there are several plausible scenarios for how translocation of hairpin DNA could occur: a hairpin molecule could enter the pore in the coil-first orientation and complete its translocation by either mechanical unzipping or stretching of the duplex part, or a hairpin molecule could enter in the loop-first orientation and complete the translocation by stretching the duplex, as shown in Fig. 5 i–m. In the DNA hairpin experiments [126,160], well-defined and recurring levels of ionic current below and above the open pore level I_0 were observed *within one translocation event*, varying from 0.1 I_0 to 2.0 I_0 at 1M KCl. Through MD simulations, some of the experimentally observed ionic current levels could be associated with the particular conformations of the hairpin DNA–nanopore system [126]. Thus, the deepest blockades were observed when the loop of the DNA hairpin blocked the pore constriction, while the highest current levels ($> 2I_0$) developed when a large portion of the DNA hairpin accumulated in the exit chamber of the nanopore.

Figure 7 illustrates the non-trivial dependence of the ionic current on the DNA conformation. During this particular trajectory, the ionic current changed from a 35% blockade to a 210% enhancement as hairpin DNA moved through the pore constriction. The plots of the ion concentrations corresponding to different conformations of a DNA hairpin in the nanopore demonstrate the complexity of the problem. In the absence of DNA, the local concentration of K^+ and Cl^- ions are not only much lower in the nanopore than in the bulk but also asymmetric with respect to the pore constriction because of the applied bias (the nanopore itself is symmetric and electrically neutral). The presence of DNA in the nanopore

increases the local concentration of both K^+ and Cl^- ions, but only a particular combination of the excluded volume and the enhanced ion concentrations results in the ionic current enhancement. Predicting this effect based on Eq. 1 is not possible, because the ion density, the DNA conformation and the distribution of the electrostatic potential are highly non-uniform. This example supports the notion that despite being computationally expensive, MD simulation is the only practical means to deal with the complexity of the problem.

Despite extensive efforts, ionic current blockades that are specific to the nucleotide sequence of a DNA molecule have not been reported yet from experiments employing solid-state nanopores. MD simulations suggest [112] that a critical condition for obtaining such sequence-specific data from both ss- and dsDNA is the ability to restrain the DNA conformation for the duration of the ionic current measurement [158]. Moving the same fragment of DNA back and forth through the pore [9] is another possible strategy to beat the conformation noise.

MD simulations of ionic current blockades in α -hemolysin have been tempered by a rather small conductance of the channel in the blocked state (10–20 pA). A set of simulations was performed at a higher bias (1.2 V) to answer the question of an experimentalist (A. Meller, Boston U.) who challenged the author to predict how a DNA strand's global orientation affects its transport and ionic current blockades in α -hemolysin. In this blind test, no experimental data were given. The simulations of two α -hemolysin systems with DNA threaded in two opposite directions showed that DNA nucleotides in confined spaces tend to tilt toward the 5'-end, which was linked to the observed differences in the DNA translocation rate and the ionic current blockades [113]. While these simulations provided a qualitatively correct picture, the absolute values of the currents were considerably higher than what one could expect from a linear scaling of the pore conductance with the voltage. The most recent simulations of the ionic current blockades in α -hemolysin were carried out at an experimentally accessible bias of 240 mV, yielding the ionic current at 15.6 ± 8.3 pA, a value consistent with experiment [113]. From a simulation point of view, MspA is a much easier system to model, because its genetically engineered variants show stable but much higher than α -hemolysin ion conductance in both open and blocked by DNA states [68].

4.4 The electro-kinetic effect

Quantitative characterization of the force experienced by a polymer in a nanopore is critical for predicting the polymer's translocation time. The notion of an effective charge q^* can conveniently relate the force F experienced by a charged polymer in a nanopore to the applied electric field E : $F = q^*E$, where q^* is generally smaller than the nominal charge of the polymer. However, the value of the effective charge alone does not specify the physical origin of the polymer's charge reduction. Thus, the force required to stall dsDNA transport through a 10-nm-diameter Si_3N_4 nanopore was directly measured by trapping one end of the DNA molecule with optical tweezers while the other end was subject to an electric field in a nanopore [37]. The obtained dependence of the force on the transmembrane bias indicated a 75% reduction of the dsDNA charge—a value that seemed to be in perfect agreement with the Manning ion condensation theory [177]. However the applicability of this theory to describe the force experienced by a charged polymer in an electric field was under question [178].

For a problem like that, MD simulations can serve not only as a microscope revealing atom-scale processes, but also as a probe measuring the forces involved. To determine the origin and the magnitude of the DNA's effective charge, a fragment of dsDNA was threaded through a nanopore and subject to a harmonic constraint so that displacement of the DNA in an electric field revealed the effective force. Using the setup shown in Figure 8, the effective

reduction of the DNA's charge was found to be produced by the hydrodynamic drag of the electro-osmotic flow created by the ionic current [179]. A similar conclusion was reached independently by two other groups [39,180], however, only the all-atom MD study [179] could fully describe the microscopic events giving rise to the effective screening and reproduce the results of the measurements without any adjustable parameters. Furthermore, the simulations determined that the effective force obeys the following dependence [178]: $F = \xi\mu E$, where ξ and μ are the friction coefficient and the electrophoretic mobility of DNA, respectively (see Fig. 8 b). This result enables determining the effective force on DNA in a nanopore without a laborious direct force measurement, as ξ and μ can be deduced from DNA escape [113,174,181] and translocation [13–15,157] experiments.

It is now clear that the electro-osmotic flow plays an important role in determining the time scale of a polymer translocation. The feed-back loop is far from trivial, as the force experienced by a DNA fragment determines its conformation, which in turn affects the ionic current, which in turn affects the effective force. As the pattern of the flow depends on many factors, such as the surface properties of the nanopore, the local concentration of ionic species, and the electrolyte viscosity, MD simulations may be the only feasible approach to characterize the transport of flexible DNA molecules, such as ssDNA or DNA hairpins. Although the effect of the electro-osmotic flow on ssDNA transport through biological nanopores is not known, it is expected to have a considerable influence on the DNA capture rate [182].

5 Outlook

Clearly, molecular simulations can play a major role in the development of the nanopore research field. The simulations can be used both to interpret the results of the actual measurements and to carry out nanopore translocation experiments *in silico*. Particular attractive are simulations of the engineered protein nanopores, whose experimental characterization is very laborious. Due to the time scale limit of the simulation methods and the force field inaccuracy, computational experiments are unlikely to eliminate the need for real experiments in the nearest future, although as the field matures it can be expected to follow the trend observed in other branches of technology, for example, in aerospace industry, where computer simulations have already taken over experiment.

It is also clear that the MD method alone cannot address all the questions posed by experiment, even in the even the range of an MD simulation reaches a millisecond. Due to the local character of the translocation processes, the length scale problem can be tractable using a multiscale approach. Modeling the processive transport of a long (longer than the membrane thickness) polymer will require the ability to dynamically switch between the coarse and the finer representations of the same polymer fragment during the simulation. Although such a reduced description will not solve the time scale problem (the time scale will still be limited by the simulation of the highest-resolution fragment), it will dramatically improve the throughput, enabling statistical description of the translocation process.

The ability of brute-force MD to resolve ionic current blockades is limited by the time scale of the method and by the molecular force field. In the case of biological nanopores, the force-field bias can emerge as a systematic error caused by the difference between the simulated and measured bulk electrolyte conductance, systematic errors arising from the description of the interactions between the protein and the electrolyte, and yet unknown effects of the force field on microsecond dynamics of protein nanopores. Here, an important factor for future development will be the availability of experimental methods that can reliably characterize conformation dynamics of a protein at the microsecond time scale so

that the force field accuracy can be unequivocally tested and refined. Stochastic gating of the nanopore current might be used to calibrate the protein force field.

In the case of solid state nanopores, conformational fluctuations are negligible within the time scale of an MD simulation. In this case, extensive investments into development of an accurate force field to describe interactions between inorganic membranes and biomolecules can bring only modest improvement in the overall predicting power of the MD method because the atomic structure or even typical chemical makeup of a solid-state nanopore is not known and can dramatically vary from one sample to another (single wall carbon nanotubes are notable exception). Thus, the progress toward improving the accuracy of the simulations is limited by the advancements in the experimental methodology to characterize the atomic structure of the nanopores. Another possibility is to use in experiments solid-state nanopores coated with a molecular layer of a well-characterized chemical compound [82–84]. Such nanopores will not only make molecular simulations more reliable, but also provide the experimentalists with a means to manufacture nanopores that have reproducible properties.

Despite all the challenges and the short comings, microscopic simulations of the nanopore transport will remain a very active and exciting research field within the next decade, with the \$1000-genome technology coming about in 2014, which may or may not utilize nanopores for DNA sequence detection. Having the \$1000-genome goal accomplished, researchers will continue to explore nanopores for protein sequence detection, post-translational modification analysis and as a general tool to probe nanoscale interactions.

Acknowledgments

A.A. gratefully acknowledge contributions from his collaborators G. Timp, B. Luan, J. Comer and D. Wells. This work was supported by grants from the National Institutes of Health (Nos. R01-HG003713, R01-HG005115 and PHS 5 P41-RR05969), National Science Foundation (PHY0822613), and the Petroleum Research Fund (48352-G6). The authors gladly acknowledge supercomputer time provided by the Texas Advanced Computing Center, Pittsburgh Supercomputer Center and the National Center for Supercomputing Applications via Large Resources Allocation grant No. MCA05S028.

References

1. Dekker C. Solid-state nanopores. *Nature Nanotech.* 2007; 2:209–215.
2. Chen P, Gu J, Brandin E, Kim Y-R, Wang Q, Branton D. Probing single DNA molecule transpore using fabricated nanopores. *Nano Lett.* 2004; 4:2293–2298.
3. Storm AJ, Chen JH, Zandbergen HW, Dekker C. Translocation of double-strand DNA through a silicon oxide nanopore. *Phys. Rev. E.* 2005; 71:051903–051913.
4. Zhao Q, Sigalov G, Dimitrov V, Dorvel B, Mirsaidov U, Sligar S, Aksimentiev A, Timp G. Detecting SNPs using a synthetic nanopore. *Nano Lett.* 2007; 7:1680–1685. [PubMed: 17500578]
5. Hornblower B, Coombs A, Whitaker RD, Kolomeisky A, Picone SJ, Meller A, Akeson M. Single-molecule analysis of DNA-protein complexes using nanopores. *Nature Mater.* 2007; 4:315–317.
6. Lagerqvist J, Zwolak M, Ventra MD. Fast DNA Sequencing via Transverse Electronic Transport. *Nano Lett.* 2006; 6:779–782. [PubMed: 16608283]
7. Zwolak M, Ventra MD. Electronic signature of dna nucleotides via transverse transport. *Nano Lett.* 2005; 5:421–424. [PubMed: 15755087]
8. Gracheva ME, Xiong A, Leburton J-P, Aksimentiev A, Schulten K, Timp G. Simulation of the electric response of DNA translocation through a semiconductor nanopore-capacitor. *Nanotechnology.* 2006; 17:622–633.
9. Sigalov G, Comer J, Timp G, Aksimentiev A. Detection of DNA sequence using an alternating electric field in a nanopore capacitor. *Nano Lett.* 2008; 8:56–63. [PubMed: 18069865]

10. Soni GV, Meller A. Progress toward ultrafast DNA sequencing using solid-state nanopores. *Clinical Chemistry*. 2007; 53:1996–2001. [PubMed: 17890440]
11. Bezrukov SM, Vodyanoy I, Parsegian VA. Counting polymers moving through a single ion channel. *Nature*. 1994; 370:279–281. [PubMed: 7518571]
12. Church G. Genomes for all. *Sci. Am*. 2006; 294:46–54. [PubMed: 16468433]
13. Kasianowicz JJ, Brandin E, Branton D, Deamer DW. Characterization of individual polynucleotide molecules using a membrane channel. *Proc. Natl. Acad. Sci. USA*. 1996; 93:13770–13773. [PubMed: 8943010]
14. Akeson M, Branton D, Kasianowicz JJ, Brandin E, Deamer DW. Microsecond time-scale discrimination among polycytidylic acid, polyadenylic acid, and polyuridylic acid as homopolymers or as segments within single RNA molecules. *Biophys. J*. 1999; 77:3227–3233. [PubMed: 10585944]
15. Meller A, Nivon L, Brandin E, Golovchenko J, Branton D. Rapid nanopore discrimination between single polynucleotide molecules. *Proc. Natl. Acad. Sci. USA*. 2000; 97:1079–1084. [PubMed: 10655487]
16. Vercoutere W, Winters-Hilt S, Olsen H, Deamer D, Haussler D, Akeson M. Rapid discrimination among individual DNA hairpin molecules at single-nucleotide resolution using an ion channel. *Nature Biotech*. 2001; 19:248–252.
17. Vercoutere WA, Winters-Hilt S, DeGuzman VS, Deamer D, Ridino SE, Rodgers JT, Olsen HE, Marziali A, Akeson M. Discrimination among individual Watson-Crick base pairs at the termini of single DNA hairpin molecules. *Nucl. Acids Res*. 2003; 31:1311–1318. [PubMed: 12582251]
18. Ashkenasy N, Sánchez-Quesada J, Bayley H, Ghadiri MR. Recognizing a single base in an individual DNA strand: A step toward DNA sequencing in nanopores. *Angew. Chem. Int. Ed. Engl*. 2005; 44:1401–1404. [PubMed: 15666419]
19. Wang Y, Rader AJ, Bahar I, Jernigan RL. Global ribosome motions revealed with elastic network model. *J. Struct. Biol*. 2004; 147:302–314. [PubMed: 15450299]
20. Iqbal S, Akin D, Bashir R. Solid-state nanopore channels with DNA selectivity. *Nature Nanotech*. 2007; 2:243–248.
21. Gu Q, Braha O, Conlan S, Cheley S, Bayley H. Stochastic sensing of organic analytes by a pore-forming protein containing a molecular adapter. *Nature*. 1999; 398:686–690. [PubMed: 10227291]
22. Braha O, Gu LQ, Zhou L, Lu X, Cheley S, Bayley H. Simultaneous stochastic sensing of divalent metal ions. *Nature Biotech*. 2000; 18:1005–1007.
23. Bezrukov SM. Ion Channels as Molecular Coulter Counters to Probe Metabolite Transport. *J. Membr. Biol*. 2000; 174:1–13. [PubMed: 10741427]
24. Merzlyak P, Capistrano M-F, Valeva A, Kasianowicz J, Krasilnikov O. Conductance and ion selectivity of a mesoscopic protein nanopore probed with cysteine scanning mutagenesis. *Biophys. J*. 2005; 89:3059–3070. [PubMed: 16085767]
25. Movileanu L, Howorka S, Braha O, Bayley H. Detecting protein analytes that modulate transmembrane movement of a polymer chain within a single protein pore. *Nature Biotech*. 2000; 18:1091–1095.
26. Kasianowicz JJ, Henrickson SE, Weetall HH, Robertson B. Simultaneous multianalyte detection with a nanometer-scale pore. *Anal. Chem*. 2001; 73:2268–2272. [PubMed: 11393851]
27. Winters-Hilt S, Akeson M. Nanopore cheminformatics. *DNA and Cell Biology*. 2004; 23:675–683. [PubMed: 15585125]
28. Sutherland T, Long Y-T, Stefureac R-I, Bediako-Amoa I, Kraatz H-B, Lee J. Structure of peptides investigated by nanopore analysis. *Nano Lett*. 2004; 4:1273–1277.
29. L. Movileanu JS, Schmittschmitt JP, Bayley H. Interactions of peptides with a protein pore. *Biophys. J*. 2005; 89:1030–1045. [PubMed: 15923222]
30. Han A, Scharmann G, Mondin G, Bitterli R, Hegelbach N, de Rooij NF, Staufer U. Sensing protein molecules using nanofabricated pores. *Appl. Phys. Lett*. 2006; 88:093901–93903.
31. Pastoriza-Gallego M, Oukhaled G, Mathe J, Thiebot B, Betton J, Auvray L, Pelta J. Urea denaturation of α -hemolysin pore inserted in planar lipid bilayer detected by single nanopore recording: Loss of structural asymmetry. *FEBS Lett*. 2007; 581:3371–3376. [PubMed: 17601577]

32. Oukhaled G, Mathe J, Biance A, Bacri L, Betton J, Lairez D, Pelta J, Auvray L. Unfolding of proteins and long transient conformations detected by single nanopore recording. *Phys. Rev. Lett.* 2007; 98:158101–158104. [PubMed: 17501386]
33. Benner S, Chen R, Wilson N, Abu-Shumays R, Hurt N, Lieberman K, Deamer D, Dunbar W, Akeson M. Sequence-specific detection of individual DNA polymerase complexes in real time using a nanopore. *Nature Nanotech.* 2007; 2:718–724.
34. Kang XF, Cheley S, Guan XY, Bayley H. Stochastic detection of enantiomers. *J. Am. Chem. Soc.* 2006; 128:10684–10685. [PubMed: 16910655]
35. Robertson JWF, Rodrigues CG, Stanford VM, Robinson KA, Krasilnikov OV, Kasianowicz JJ. Single-molecule mass spectrometry in solution using a solitary nanopore. *Proc. Natl. Acad. Sci. USA.* 2007; 104:8207–8211. [PubMed: 17494764]
36. Tropini C, Marziali A. Multi-nanopore force Spectroscopy for DNA analysis. *Biophys. J.* 2007; 95:1632–1637. [PubMed: 17158571]
37. Keyser U, Koeleman B, Dorp S, Krapf D, Smeets R, Lemay S, Dekker N, Dekker C. Direct force measurements on DNA in a solid-state nanopore. *Nature Phys.* 2006; 2:473–477.
38. H. Trepagnier E, Radenovic A, Sivak DPPG, Liphardt J. Controlling dna capture and propagation through artificial nanopores. *Nano Lett.* 2007; 7:2824–2830. [PubMed: 17705552]
39. van Dorp SS, UF UFK, Dekker NH, Dekker C, Lemay SG. Origin of the elec-trophoretic force on dna in solid-state nanopores. *Nature Phys.* 2009; 5:347–351.
40. Striemer CC, Gaborski TR, McGrath JL, Fauchet PM. Charge- and size-based separation of macromolecules using ultrathin silicon membranes. *Nature.* 2007; 445:749–753. [PubMed: 17301789]
41. Wolfe A, Mohammad M, Cheley S, Bayley H, Movileanu L. Catalyzing the translocation of polypeptides through attractive interactions. *J. Am. Chem. Soc.* 2007; 129:14034–14041. [PubMed: 17949000]
42. Branton D, et al. The potential and challenges of nanopore sequencing. *Nature Biotech.* 2008; 26:1146–1153.
43. Bayley H. Sequencing single molecules of DNA. *Curr. Op. Chem. Biol.* 2006; 10:628–637.
44. Service RF. The race for the \$1000 genome. *Science.* 2006; 311:1544–1546. [PubMed: 16543431]
45. Bayley H, Cremer PS. Stochastic sensors inspired by biology. *Nature.* 2001; 413:226–230. [PubMed: 11557992]
46. Schmidt J. Stochastic sensors. *J. Mater. Chem.* 2005; 15:831–840.
47. Kasianowicz J, Robertson JWF, Chan ER, Reiner JE, Stanford VM. Nanoscopic porous sensors. *Ann. Rev. Anal. Chem.* 2008; 1:737–766.
48. Wilson NA, Abu-Shumays R, B BG, Wang H, Lieberman K, M MA, Dunbar W. Electronic control of dna polymerase binding and unbinding to single dna molecules. *ACS Nano.* 2009; 3:995–1003. [PubMed: 19338283]
49. Siwy ZS. Ion current rectification in nanopores and nanotubes with broken symmetry revisited. *Adv. Funct. Mater.* 2006; 16:735.
50. Gracheva M, Leburton J-P. Electrolytic charge inversion at the liquid-solid interface in a nanopore in a doped semiconductor membrane. *Nanotechnology.* 2007; 18:145704–145710.
51. Vidal J, Gracheva M, Leburton J-P. Electrically tunable solid-state silicon nanopore ion filter. *Nanoscale Res. Lett.* 2007; 2:61–68.
52. Gracheva M, Leburton J-P. Simulation of electrically tunable semiconductor nanopores for ion current/single bio-molecule manipulation. *J. Comput. Electronics.* 2008; 7:6–9.
53. Gracheva M, Melnikov D, Leburton J-P. Multilayered semiconductor membranes for nanopore ionic conductance modulation. *ACS Nano.* 2008; 2:2349–2355. [PubMed: 19206402]
54. Martin CR, Siwy ZS. Learning nature's way: Biosensing with synthetic nanopores. *Science.* 2007; 317:331. [PubMed: 17641190]
55. Vlassiouk I, Siwy ZS. Nanofluidic diode. *Nano Lett.* 2007; 7:552. [PubMed: 17311462]
56. Cheng L-J, Guo L. Rectified ion transport through concentration gradient in homogeneous silica nanochannels. *Nano Lett.* 2007; 7:3165–3171. [PubMed: 17894519]

57. Karnik R, Duan C, Castelino K, Daiguji H, Majumdar A. Rectification of ionic current in a nanofluidic diode. *Nano Lett.* 2007; 7:547–551. [PubMed: 17311461]
58. Bhakdi S, Trantum-Jensen J. Alpha-toxin of *staphylococcus aureus*. *Microbiol. Rev.* 1991; 55:733–751. [PubMed: 1779933]
59. Gouaux E. α -hemolysin from *staphylococcus aureus*: An archetype of β -barrel, channel-forming toxins. *J. Struct. Biol.* 1998; 121:110–122. [PubMed: 9615434]
60. Menestrina G. Ionic channels formed by *staphylococcus aureus* alpha-toxin: Voltage-dependent inhibition by divalent and trivalent cations. *J. Membr. Biol.* 1986; 90:177–190. [PubMed: 2425095]
61. Howorka S, Movileanu L, Lu X, Magnon M, Cheley S, Braha O, Bayley H. A protein pore with a single polymer chain tethered within the lumen. *J. Am. Chem. Soc.* 2000; 122:2411–2416.
62. Howorka S, Bayley H. Probing distance and electrical potential within a protein pore with tethered DNA. *Biophys. J.* 2002; 83:3202–3210. [PubMed: 12496089]
63. Jung Y, Cheley S, Braha O, Bayley H. The internal cavity of the staphylococcal alpha-hemolysin pore accommodates approximately 175 exogenous amino acid residues. *Biochemistry.* 2005; 44:8919–8929. [PubMed: 15966717]
64. Cheley S, Xie H, Bayley H. A genetically encoded pore for the stochastic detection of a protein kinase. *ChemBiochem.* 2006
65. Aksimentiev A, Schulten K. Imaging alpha-hemolysin with molecular dynamics: Ionic conductance, osmotic permeability and the electrostatic potential map. *Biophys. J.* 2005; 88:3745–3761. [PubMed: 15764651]
66. Heng JB, Ho C, Kim T, Timp R, Aksimentiev A, Grinkova YV, Sligar S, Schulten K, Timp G. Sizing DNA using a nanometer-diameter pore. *Biophys. J.* 2004; 87:2905–2911. [PubMed: 15326034]
67. Faller M, Niederweis M, Schultz G. The structure of a mycobacterial outer membrane channel. *Science.* 2004; 303:1189–1192. [PubMed: 14976314]
68. Butler T, Pavlenok M, Derrington I, Niederweis M, Gundlach J. Single-molecule DNA detection with an engineered MspA protein nanopore. *Proc. Natl. Acad. Sci. USA.* 2008; 105:20647. [PubMed: 19098105]
69. Song L, Hobaugh MR, Shustak C, Cheley S, Bayley H, Gouaux JE. Structure of staphylococcal α -hemolysin, a heptameric transmembrane pore. *Science.* 1996; 274:1859–1865. [PubMed: 8943190]
70. Shenoy D, Barger W, Singh A, Panchal R, Misakian M, Stanford V, Kasianowicz J. Functional reconstitution of protein ion channels into planar polymerizable phospholipid membranes. *Nano Lett.* 2005; 5:1181–1185. [PubMed: 15943465]
71. Kang, X-f; Cheley, S.; Rice-Ficht, A.; Bayley, H. A storable encapsulated bilayer chip containing a single protein nanopore. *J. Am. Chem. Soc.* 2007; 129:4701–4705. [PubMed: 17375923]
72. Li J, Stein D, McMullan C, Branton D, Aziz MJ, Golovchenko JA. Ion-beam sculpting at nanometre length scales. *Nature.* 2001; 412:166–169. [PubMed: 11449268]
73. Li J, Gershow M, Stein D, Brandin E, Golovchenko JA. DNA molecules and configurations in a solid-state nanopore microscope. *Nature Mater.* 2003; 2:611–615. [PubMed: 12942073]
74. Storm AJ, Chen JH, Ling XS, Zandbergen HW, Dekker C. Fabrication of solid-state nanopore with single-nanometre precision. *Nature Mater.* 2003; 2:537–540. [PubMed: 12858166]
75. Ho C, Qiao R, Chatterjee A, Timp RJ, Aluru NR, Timp G. Electrolytic transport through a synthetic nanometer-diameter pore. *Proc. Natl. Acad. Sci. USA.* 2005; 102:10445–14450. [PubMed: 16020525]
76. Kim D, Park J, Shin J, Kim P, Lim G, Shoji S. An extended gate FET-based biosensor integrated with a Si microfluidic channel for detection of protein complexes. *Sensor Actuat. B-Chem.* 2006; 117:488–494.
77. Rhee M, Burns MA. Nanopore sequencing technology: nanopore preparations. *Trends Biotech.* 2007; 25:174–181.
78. Fologea D, Gershow M, Ledden B, McNabb DS, Golovchenko JA, Li J. Detecting single stranded DNA with a solid state nanopore. *Nano Lett.* 2005; 5:1905–1909. [PubMed: 16218707]

79. Heng JB, Aksimentiev A, Ho C, Marks P, Grinkova YV, Sligar S, Schulten K, Timp G. The electromechanics of DNA in a synthetic nanopore. *Biophys. J.* 2006; 90:1098–1106. [PubMed: 16284270]
80. Heng JB, Aksimentiev A, Ho C, Marks P, Grinkova YV, Sligar S, Schulten K, Timp G. Stretching DNA using an electric field in a synthetic nanopore. *Nano Lett.* 2005; 5:1883–1888. [PubMed: 16218703]
81. Cai Q, Ledden B, Krueger E, Golovchenko JA, Li J. Nanopore sculpting with noble gas ions. *J. Appl. Phys.* 2006; 100 024914.
82. Wanunu M, Meller A. Chemically modified solid-state nanopores. *Nano Lett.* 2007; 7:1580–1585. [PubMed: 17503868]
83. Nilsson J, Lee JRI, Ratto TV, Letant SE. Localized functionalization of single nanopores. *Adv. Mater.* 2006; 18:427–431.
84. Iqbal SM, Akin D, Bashir R. Solid-state nanopore channels with DNA selectivity. *Nature Nanotech.* 2007; 2:243–248.
85. Chen P, Mitsui T, Farmer D, Golovchenko J, Gordon R, Branton D. Atomic layer deposition to fine-tune the surface properties and diameters of fabricated nanopores. *Nano Lett.* 2004; 4:1333–1337.
86. Heng JB, Aksimentiev A, Ho C, Dimitrov V, Sorsch T, Miner J, Mansfield W, Schulten K, Timp G. Beyond the gene chip. *Bell Labs Tech. J.* 2005; 10:5–22. [PubMed: 18815623]
87. Timp G, et al. Progress toward 10 nm CMOS devices. *Tech. Dig. - Int. Electron Devices Meet.* 1998:615–618.
88. Meller A. Dynamics of polynucleotide transport through nanometre-scale pores. *J. Phys.: Condens. Matter.* 2003; 15:R581–R607.
89. Muthukumar M. Mechanism of DNA transport through pores. *Annu. Rev. Biophys. Biomol. Struct.* 2007; 36:435–450. [PubMed: 17311526]
90. Im W, Roux B. Ions and counterions in a biological channel: a molecular dynamics study of OmpF porin from *Escherichia coli* in an explicit membrane with 1 M KCl aqueous salt solution. *J. Mol. Biol.* 2002; 319:1177–1197. [PubMed: 12079356]
91. Noskov SY, Im W, Roux B. Ion permeation through the α -hemolysin channel: Theoretical studies based on Brownian Dynamics and Poisson-Nernst-Planck electrodiffusion theory. *Biophys. J.* 2004; 87:2299–2309. [PubMed: 15454431]
92. Saraniti M, Aboud S, Eisenderg R. The simulation of ionic charge transport in biological ion channels: an introduction to numerical methods. *Rev. Comp. Chem.* 2006; 22:229–294.
93. Bonthuis DJ, Zhang J, Hornblower B, Mathe J, Shklovskii BI, Meller A. Self-energy-limited ion transport in subnanometer channels. *Phys. Rev. Lett.* 2006; 97:128104. [PubMed: 17026003]
94. Zhang J, Shklovskii BI. Effective charge and free energy of DNA inside an ion channel. *Phys. Rev. E.* 2007; 75 021906.
95. de Gennes, PG. *Scaling concepts in polymer physics.* Cornell University Press; 1979.
96. Sung S-S, Wu X-W. Molecular dynamics simulations of synthetic peptide folding. *Proteins: Struct., Func., Gen.* 1996; 25:202–214.
97. Muthukumar M. Polymer translocation through a hole. *J. Chem. Phys.* 1999; 111:10371–10374.
98. Slonkina E, Kolomeisky AB. Polymer translocation through a long nanopore. *J. Chem. Phys.* 2003; 118:7112–7118.
99. Muthukumar M. Polymer escape through a nanopore. *J. Chem. Phys.* 2003; 118:5174–5184.
100. Ambjörnsson T, Apell SP, Konkoli Z, Marzio EAD, Kasianowicz JJ. Charged polymer membrane translocation. *Biophys. J.* 2002; 117:4063–4073.
101. Lubensky DK, Nelson DR. Driven polymer translocation through a narrow pore. *Biophys. J.* 1999; 77:1824–1838. [PubMed: 10512806]
102. Matzler R, Klafter J. When translocation dynamics became anomalous. *Biophys. J.* 2003; 85:2776–2779. [PubMed: 14507739]
103. Fromenborn O, Klafter J. Translocation of a single-stranded DNA through a conformationally changing nanopore. *Biophys. J.* 2004; 86:3576–3584. [PubMed: 15189855]

104. Wong C, Muthukumar M. Polymer capture by electro-osmotic flow of oppositely charged nanopores. *J. Chem. Phys.* 2007; 126:164903. [PubMed: 17477630]
105. Ghosal S. Electrophoresis of a polyelectrolyte through a nanopore. *Phys. Rev. E.* 2006; 74:041901.
106. Ghosal S. Effect of salt concentration on the electrophoretic speed of a polyelectrolyte through a nanopore. *Phys. Rev. Lett.* 2007; 98:238104. [PubMed: 17677940]
107. Allen, MP.; Tildesley, DJ. *Computer Simulation of Liquids.* Oxford University Press; 1987.
108. Verlet L. Computer 'experiments' on classical fluids: I. Thermodynamical properties of Lennard-Jones molecules. *Physical Review.* 1967; 159:98–103.
109. MacKerell A Jr, et al. All-atom empirical potential for molecular modeling and dynamics studies of proteins. *J. Phys. Chem. B.* 1998; 102:3586–3616.
110. Cornell WD, Cieplak P, Bayly CI, Gould IR, Merz KM Jr, Ferguson DM, Spellmeyer DC, Fox T, Caldwell JW, Kollman PA. A second generation force field for the simulation of proteins, nucleic acids, and organic molecules. *J. Am. Chem. Soc.* 1995; 117:5179–5197.
111. Aksimentiev A, Brunner R, Cruz-Chu ER, Comer J, Schulten K. Modeling transport through synthetic nanopores. *IEEE Nanotech. Mag.* 2009; 3:20–28.
112. Aksimentiev A, Heng JB, Timp G, Schulten K. Microscopic kinetics of DNA translocation through synthetic nanopores. *Biophys. J.* 2004; 87:2086–2097. [PubMed: 15345583]
113. Mathé J, Aksimentiev A, Nelson DR, Schulten K, Meller A. Orientation discrimination of single stranded DNA inside the α -hemolysin membrane channel. *Proc. Natl. Acad. Sci. USA.* 2005; 102:12377–12382. [PubMed: 16113083]
114. <http://webusers.physics.illinois.edu/~alek/tutorials/>.
115. de Groot BL, Grubmüller H. Water permeation across biological membranes: Mechanism and dynamics of aquaporin-1 and GlpF. *Science.* 2001; 294:2353–2357. [PubMed: 11743202]
116. Tajkhorshid E, Nollert P, Jensen MØ, Miercke LJW, O'Connell J, Stroud RM, Schulten K. Control of the selectivity of the aquaporin water channel family by global orientational tuning. *Science.* 2002; 296:525–530. [PubMed: 11964478]
117. Ilan B, Tajkhorshid E, Schulten K, Voth GA. The mechanism of proton exclusion in aquaporin channels. *Proteins: Struct., Func., Bioinf.* 2004; 55:223–228.
118. Bernèche S, Roux B. Energetics of ion conduction through the K^+ channel. *Nature.* 2001; 414:73–77. [PubMed: 11689945]
119. Domene, C.; Bond, PJ.; Sansom, MSP. Membrane protein simulations: Ion channels and bacterial outer membrane proteins. In: Richards, FM.; Eisenberg, DS.; Kuriyan, J., editors. *APC.* Vol. vol. 66. Elsevier Academic Press; 2003. p. 159-193.
120. Allen T, Andersen O, Roux B. Energetics of ion conduction through the gramicidin channel. *Proc. Natl. Acad. Sci. USA.* 2004; 101:117–122. [PubMed: 14691245]
121. Monticelli L, Robertson K, MacCallum J, Tieleman D. Computer simulation of the KvAP voltage-gated potassium channel: steered molecular dynamics of the voltage sensor. *FEBS Lett.* 2004; 564:325–332. [PubMed: 15111117]
122. Noskov S, Berneche S, Roux B. Control of ion selectivity in potassium channels by electrostatic and dynamic properties of carbonyl ligands. *Nature.* 2004; 431:830–834. [PubMed: 15483608]
123. Robertson KM, Tieleman DP. Orientation and interactions of dipolar molecules during transport through OmpF porin. *FEBS Lett.* 2002; 528:53–57. [PubMed: 12297279]
124. Ceccarelli M, Danelon C, Laio A, Parrinello M. Microscopic mechanism of antibiotics translocation through porin. *Biophys. J.* 2004; 87:58–64. [PubMed: 15240444]
125. Aksimentiev, A., et al. *Nanostructure Design: Methods and Protocols*, vol. 474 of *Methods in Molecular Biology.* Humana Press; 2008. *Computer Modeling in Biotechnology: a Partner in Development*; p. 181-234.
126. Comer J, Dimitrov V, Zhao Q, Timp G, Aksimentiev A. Microscopic mechanics of hairpin DNA translocation through synthetic nanopores. *Biophys. J.* 2009; 96:593–608. [PubMed: 19167307]
127. Milchev A, Binder K, Bhattacharya A. Polymer translocation through a nanopore induced by adsorption: Monte Carlo simulations of a coarse-grained model. *J. Chem. Phys.* 2004; 121:6042–6051. [PubMed: 15367033]

128. Matysiak S, Montesi A, Pasquali M, Kolomeisky A, Clementi C. Dynamics of polymer translocation through nanopore: Theory meets experiment. *Phys. Rev. Lett.* 2006; 96:118103. [PubMed: 16605877]
129. Marrink SJ, de Vries AH, Mark AE. Coarse grained model for semiquantitative lipid simulations. *J. Phys. Chem. B.* 2004; 108:750–760.
130. Chauang J, Kantor Y, Kardar M. Anomalous dynamics of translocation. *Phys. Rev. E.* 2001; 65 011802.
131. Kong CY, Muthukumar M. Modeling of polynucleotide translocation through protein pores and nanotubes. *Electrophoresis.* 2002; 23:2697–2703. [PubMed: 12210174]
132. Muthukumar M, Kong CY. Simulation of polymer translocation through protein channels. *Proc. Natl. Acad. Sci. USA.* 2006; 103:5273–5278. [PubMed: 16567657]
133. Forrey C, Muthukumar M. Langevin dynamics simulations of ds-DNA translocation through synthetic nanopores. *J. Chem. Phys.* 2007; 127 015102.
134. Muthukumar M. Translocation of a confined polymer through a hole. *Phys. Rev. Lett.* 2001; 86:3188–3191. [PubMed: 11290139]
135. Chern SS, Cárdenas AE, Coalson RD. Three-dimensional dynamic Monte Carlo simulations of driven polymer transport through a hole in a wall. *J. Chem. Phys.* 2001; 115:7772–7782.
136. Chen C, Peng E. Nanopore sequencing of polynucleotides assisted by a rotating electric field. *Appl. Phys. Lett.* 2003; 82:1308–1310.
137. Loebl HC, Randel R, Goodwin SP, Matthai CC. Simulation studied of polymer translocation through a channel. *Phys. Rev. E.* 2003; 67 041913.
138. Fyta MG, Melchionna S, Kaxiras E, Succi S. Multiscale coupling of molecular dynamics and hydrodynamics: Application to DNA translocation through a nanopore. *Multiscale Model. Simul.* 2006; 5:1156–1173.
139. Fyta M, Melchionna S, Succi S, Kaxiras E. Hydrodynamic correlations in the translocation of a biopolymer through a nanopore: Theory and multiscale simulations. *Phys. Rev. E.* 2008; 78 036704.
140. Storm AJ, Storm C, Chen J, Zandbergen H, Joanny J-F, Dekker C. Fast DNA translocation through a solid-state nanopore. *Nano Lett.* 2005; 7:1193–1197. [PubMed: 16178209]
141. Reboux S, Capuani F, Gonzalez-Segredo N, Frenkel D. Lattice-Boltzmann simulations of ionic current modulation by DNA translocation. *J. Chem. Theory Comput.* 2006; 2:495–503.
142. Gracheva M, Xiong A, Aksimentiev A, Schulten K, Timp G, Leburton J-P. Simulation of the electric response of DNA translocation through a semiconductor nanopore-capacitor. *Nanotechnology.* 2006; 17:622–633.
143. Shaw, DE., et al. International Symposium on Computer Architecture. ACM; 2007. Anton, a special-purpose machine for molecular dynamics simulation; p. 1-12.
144. Khalili-Araghi F, Tajkhorshid E, Schulten K. Dynamics of K^+ ion conduction through Kv1.2. *Biophys. J.* 2006; 91:L72–L74. [PubMed: 16844753]
145. Atkins, PW. *Physical Chemistry.* Oxford University Press; 1998.
146. Pezeshki S, Chimere C, Bessonov AN, Winterhalter M, Kleinekathofer U. Understanding ion conductance on a molecular level: An all-atom modeling of the bacterial porin OmpF. *Biophys. J.* 2009; XX XXXX–XXXX.
147. Chimere C, Movileanu L, Pezeshki S, Winterhalter M, Kleinekathofer U. Transport at the nanoscale: temperature dependence of ion conductance. *Eur. Biophys. J.* 2008; 38:121–125. [PubMed: 18726094]
148. Batcho PF, Case DA, Schlick T. Optimized particle-mesh Ewald/multiple-time step integration for molecular dynamics simulations. *J. Chem. Phys.* 2001; 115:4003–4018.
149. Böckmann RA, de Groot BL, Kakorin S, Neumann E, Grubmüller H. Kinetics, statistics, and energetics of lipid membrane electroporation studied by molecular dynamics simulations. *Biophys. J.* 2008; 95:1837–1850. [PubMed: 18469089]
150. Crozier PS, Henderson D, Rowley RL, Busath DD. Model channel ion currents in NaCl-extended simple point charge water solution with applied-field molecular dynamics. *Biophys. J.* 2001; 81:3077–3089. [PubMed: 11720976]

151. Nonner W, Peyser A, Gillespie D, Eisenberg B. Relating microscopic charge movement to macroscopic currents: The ramo-shockley theorem applied to ion channels. *Biophys. J.* 2004; 87:3716–3722. [PubMed: 15465857]
152. Bezrukov SM, Kasianowicz JJ. Current noise reveals protonation kinetics and number of ionizable sites in an open protein ion channel. *Phys. Rev. Lett.* 1993; 70:2352–2355. [PubMed: 10053539]
153. Kasianowicz JJ, Bezrukov SM. Protonation dynamics of the α -toxin ion channel from spectral analysis of ph dependent current fluctuations. *Biophys. J.* 1995; 69:94–105. [PubMed: 7545444]
154. Sotomayor M, Vasquez V, Perozo E, Schulten K. Ion conduction through MscS as determined by electrophysiology and simulation. *Biophys. J.* 2007; 92:886–902. [PubMed: 17114233]
155. Cruz-Chu ER, Aksimentiev A, Schulten K. Ionic current rectification through silica nanopores. *J. Phys. Chem. C.* 2009; 113:1850–1862.
156. Phillips JC, Braun R, Wang W, Gumbart J, Tajkhorshid E, Villa E, Chipot C, Skeel RD, Kale L, Schulten K. Scalable molecular dynamics with NAMD. *J. Comp. Chem.* 2005; 26:1781–1802. [PubMed: 16222654]
157. Wanunu M, Sutin J, B BM, Chow A, Meller A. Dna translocation governed by interactions with solid-state nanopores. *Biophys. J.* 2008; 95:4716–4725. [PubMed: 18708467]
158. Mirsaidov U, Comer J, Dimitrov V, Wang D, Timp W, Aksimentiev A, Timp G. Using a nanopore to slow and trap a double-stranded DNA molecule, submitted. 2009
159. Yeh IC, Hummer G. Diffusion and electrophoretic mobility of single-stranded RNA from molecular dynamics simulations. *Biophys. J.* 2004; 86:681–689. [PubMed: 14747307]
160. Zhao Q, Comer J, Dimitrov V, Aksimentiev A, Timp G. Stretching and unzipping nucleic acid hairpins using a synthetic nanopore. *Nucl. Acids Res.* 2008; 36:1532–1541. [PubMed: 18208842]
161. Dorvel B, Sigalov G, Zhao Q, Comer J, Dimitrov V, Mirsaidov U, Aksimentiev A, Timp G. Analyzing the forces binding a restriction endonuclease to DNA using a synthetic nanopore. *Nucl. Acids Res.* 2009; 37:4170–4179. [PubMed: 19433506]
162. Meller A, Nivon L, Branton D. Voltage-driven dna translocations through a nanopore. *Phys. Rev. Lett.* 2001; 86:3435–3438. [PubMed: 11327989]
163. Wells DB, Abramkina V, Aksimentiev A. Exploring transmembrane transport through alpha-hemolysin with grid-based steered molecular dynamics. *J. Chem. Phys.* 2007; 127:125101. [PubMed: 17902937]
164. Israilewitz B, Izrailev S, Schulten K. Binding pathway of retinal to bacterio-opsin: A prediction by molecular dynamics simulations. *Biophys. J.* 1997; 73:2972–2979. [PubMed: 9414212]
165. Grubmüller H, Heymann B, Tavan P. Ligand binding and molecular mechanics calculation of the streptavidin-biotin rupture force. *Science.* 1996; 271:997–999. [PubMed: 8584939]
166. Marrink S-J, Berger O, Tieleman P, Jähnig F. Adhesion forces of lipids in a phospholipid membrane studied by molecular dynamics simulations. *Biophys. J.* 1998; 74:931–943. [PubMed: 9533704]
167. Jarzynski C. Nonequilibrium equality for free energy differences. *Phys. Rev. Lett.* 1997; 78:2690–2693.
168. Park S, Schulten K. Calculating potentials of mean force from steered molecular dynamics simulations. *J. Chem. Phys.* 2004; 120:5946–5961. [PubMed: 15267476]
169. Wang Y, Schulten K, Tajkhorshid E. What makes an aquaporin a glycerol channel: A comparative study of AqpZ and GlpF. *Structure.* 2005; 13:1107–1118. [PubMed: 16084383]
170. Gumbart J, Schulten K. Molecular dynamics studies of the archaeal translocon. *Biophys. J.* 2006; 90:2356–2367. [PubMed: 16415058]
171. Chang H, Kosari F, Andreadakis G, Alam MA, Vasmatzis G, Bashir R. DNA-mediated fluctuations in ionic current through silicon oxide nanopore channels. *Nano Lett.* 2004; 4:1551–1556.
172. Smeets RMM, Keyser UF, Krapf D, Wu MY, Dekker NH, Dekker C. Salt dependence of ion transport and DNA translocation through solid-state nanopores. *Nano Lett.* 2006; 6:89–95. [PubMed: 16402793]

173. Cruz-Chu ER, Aksimentiev A, Schulten K. Water-silica force field for simulating nanodevices. *J. Phys. Chem. B*. 2006; 110:21497–21508. [PubMed: 17064100]
174. Bates M, Burns M, Meller A. Dynamics of DNA molecules in a membrane channel probed by active control techniques. *Biophys. J*. 2003; 84:2366–2372. [PubMed: 12668445]
175. Nakane J, Wiggins M, Marziali A. A nanosensor for transmembrane capture and identification of single nucleic acid molecules. *Biophys. J*. 2004; 87:615–621. [PubMed: 15240494]
176. McNally B, Wanunu M, Meller A. Electromechanical unzipping of individual DNA molecules using synthetic sub-2 nm pores. *Nano Lett*. 2008; 8:3418–3422. [PubMed: 18759490]
177. Manning G. Limiting laws and counterion condensation in polyelectrolyte solutions. *J. Phys. Chem*. 1981; 85:1506.
178. Long D, Viovy JL, Ajdari A. Simultaneous action of electric fields and nonelectric forces on a polyelectrolyte: Motion and deformation. *Phys. Rev. Lett*. 1996; 76:3858. [PubMed: 10061127]
179. Luan B, Aksimentiev A. Electro-osmotic screening of the dna charge in a nanopore. *Phys. Rev. E*. 2008; 78 021912.
180. Ghosal S. Electrokinetic-flow-induced viscous drag on a tethered dna inside a nanopore. *Phys. Rev. E*. 2008; 76 061916.
181. Wiggins M, Tropini C, Tabard-Cossa V, Jetha N, Marziali A. Nonexponential kinetics of dna escape from alpha-hemolysin nanopores. *Biophys. J*. 2008; 95:5317–5323. [PubMed: 18775965]
182. Chou T. Enhancement of charged macromolecule capture by nanopores in a salt gradient. *J. Chem. Phys*. 2009; 131 034703.

Figure 1. Experimental setup of a single molecule nanopore experiment. Driven by the electrical field between two electrodes, a biomolecule transits the pore in a thin, synthetic membrane (*left*), inducing a transient blockade of the ionic current (*right*), measured by the amplifier.

Figure 2. Biological and solid-state nanopores. (a, b) All-atom model of the α -hemolysin (a) and MspA (b) channels suspended in a lipid bilayer membrane [65]. (c) TEM image of a nanopore in a Si_3N_4 membrane [66]. (d) TEM image of a metal-oxide-semiconductor capacitor membrane [8].

Figure 3.

Atom-scale models of solid-state (top row) and biological (bottom row) nanopores. To build a microscopic model of a solid-state nanopore, a unit cell (a) of Si_3N_4 or SiO_2 crystal is replicated in 3D to produce a solid-state membrane (b). A nanometer size pore is produced by removing atoms according to the desired shape (c). An optional step is to melt and resolidify the membrane material to obtain an amorphous surface (not shown) [111]. Next, a fragment of DNA is placed near the nanopore's entrance (d), water molecules are added to fill the volume of the simulated system (e), select water molecules are replaced with the ions of the electrolyte according to the specified concentration (f). Similar steps are required to build a microscopic model of a biological nanopore: an X-ray structure of the pore (g) is combined with the nucleic acid in the desired conformation (h) and embedded in a lipid bilayer membrane (i). The resulting system is solvated (j) and ionized (k). The procedures are described in detail in Refs. [112] and [65,113] and online tutorials [114].

Figure 4.

MD simulation of ionic current. (a) The microscopic model of a nanopore system is subject to an external electric field. Under the action of the field, water and ions rearrange, focusing the electric field to the vicinity of the membrane. (b) The resulting distribution of the electrostatic potential. A transmembrane bias of 0.6 V was imposed in this simulation. (c) Instantaneous currents sampled at 1 (black) and 10 (red) ps. The noise decreases as a square root of the sampling rate. (d) Integrated currents at different voltage biases. A linear cumulative current trace indicates a steady state conductance. (e) The simulated current–voltage curve of α -hemolysin [65].

Figure 5.

MD simulation of DNA translocation. (a–c) Electric field-driven transport of ssDNA through α -hemolysin. This proof-of-principle MD trajectory was obtained applying a very high transmembrane bias of 15 V; protein and lipids were restrained. A more realistic trajectory can be obtained using the G-SMD method [163]. (d–h) Transport of dsDNA transport Si_3N_4 pore. The DNA was observed to interact strongly with the surface of the solid-state membrane [112]. (i–m) DNA hairpin permeation through Si_3N_4 pore. The duplex part of the hairpin stretches under the influence of the electric field (panels j–l), which enables the translocation [126].

Figure 6.

The effect of dsDNA conformation on the ionic current blockades. (a–c) Snapshots from a MD simulation of dsDNA translocation through a 3.0-nm-diameter pore in Si_3N_4 . In this simulation, the Si_3N_4 membrane is 10-nm thick; the transmembrane bias is 1.3 V, the KCl concentration is 0.1 M. (d) The simulated ionic current. The ionic current returns to an open pore value while dsDNA is still in the pore.

Figure 7.

Influence of the local ion concentration on the nanopore current. The ion concentration profiles are shown for (a) an empty (no DNA) pore in, (b) an ionic current blockade, and (c) an ionic current enhancement. The pore images are faithfully aligned with the position axes of the corresponding plots. The arrows indicate the direction of the applied electric field. The bulk ion concentration in all simulations was 1 M. Other simulation conditions are described in Ref. [126].

Figure 8.

MD simulations of the effective force. (a) Setup of the simulations. A virtual spring limits displacement of the DNA fragment in an external electric field E . The effective force F is the product of the spring constant k and the DNA displacement Δx . (b) The simulated effective force F versus the product of the electrophoretic mobility μ , the friction coefficient ξ and the applied field E ; μ and ξ were determined from independent MD simulations. The symbols indicate the results obtained for three different pores and two values of the applied field [179].