Molecular Dynamics Simulations of DNA Nanosystems

Annual Report for
Blue Waters Allocation

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Executive summary

The idea of sequencing nucleic acids (DNA and RNA; NAs) by measuring the ionic current blockade as a molecule translocates through the nanopore has established itself as the next frontier of genomic sequencing. Owing to the simplicity of its apparatus, this technique has the potential to provide low-cost NA sequencing. In the recent past, our group has made significant strides in this area by pioneering all-atom molecular dynamics simulation studies of DNA translocation through biological and solid-state nanopores. Currently, we are excited about our proposed double nanopore design that decouples the translocation and blockade sensing forces. The design will allow flossing a NA molecule many times through the two nanopores, providing high resolution data for accurate sequencing. The power of the Blue Waters supercomputer will be used to run multiple long-timescale all-atom molecular dynamics simulations that will provide insights into the design and demonstrate its sequencing capabilities. In this report, we describe our achievements using the JQ5 allocation for the year of 2018, which includes characterization of DNA–lipid interactions, demonstration of a novel mechanism for gating a biological membrane channel and the study of nucleosomal DNA. We request 240,000 node-hours for 2019 to investigate the design of the double nanopore system for high-accuracy NA sequencing.

Confidentiality

This report includes confidential or restricted information. Please share its contents only with the Blue Waters team and the National Science Foundation.

Description of research activities and results

Key challenges

Over the past decades, nucleic acid (NA) sequencing has undergone spectacular developments [1, 2, 3], lowering the cost of whole-genome sequencing by six order of magnitude [4]. By eliminating the need for NA amplification and by allowing arbitrarily-long read lengths, nanopores sequencing platforms are poised to bring further improvements to the sequencing technology, making it suitable for routine use in personalized health care. Nanopore sequencing works by decrypting the ionic current blockade produced by the translocation of an NA molecule through the nanopore [5]. Biological nanopore sequencing utilizes an NA processing enzymes to control the speed of the NA translocation through the pore. A double nanopore system has been recently proposed to circumvent these limitations by trapping the NA in a molecular tug-of-war between two in-plane nanopores [6, 7, 8]. Flossing a NA through such a double nanopore setup multiple times will increase the accuracy of NA sequencing, allowing detection and characterization of nucleotide modifications—critical biomarkers that are lost in present-day sequencing methods. While the double-pore sequencing idea is appealing, it raises a host of questions about the nature of molecular interactions that may affect the DNA transport and the extent to which such interactions can be controlled to improve NA sequencing accuracy. In this project, we will use all-atom molecular dynamics (MD) simulation to evaluate and improve biological and solid-state nanopores for NA sequencing.

Why it Matters

Improving the accuracy and reducing the cost of genome sequencing is a burgeoning area of interest for scientists all across the globe. The research in this area has led the human genome project to the world's largest collaborative biological project. Biological and solid-state nanopores provide a promising approach towards nanopore sequencing [9, 10]. All-atom MD simulations have been an integral part of the development of NA nanopore sequencing platforms by providing a detailed microscopic description of the translocation process. Thus, MD simulations of the ionic current blockades produced by NA translocation have been instrumental to design new class of nanopore sequencing systems and elucidate the physical mechanisms of their operation [11, 12, 13]. MD simulations can quantify aspects of the system that are not accessible to experiment, such as the forces between a NA and nanopore surfaces and the conformational fluctuations of the NA, critical information for design of the nanopore sequencing platform. Our group continues to be a pioneer in the field of NA nanopore sequencing, leading the computational exploration to develop realistic, accurate and cost effective NA sequencing systems.

Why Blue Waters

To mimic double nanopore NA sequencing in atomistic MD simulations, we have already built the all-atom models of biological and boron-nitride nanopores (see Plans for the New Year section). In order to simulate the flossing of NA sequence in the double pore, we have designed our simulation to run with the multiple-copy feature of NAMD [14]. Based on our preliminary simulations and past experience in nanopore modeling [7, 12, 13, 15], we expect that multiple, microsecond-timescale simulations will be required to sample the configurations and determine a suitable design of a double nanopore system for NA sequencing. Explicit solvent all-atom MD simulations of our systems require an amount of computational resources that can only be provided by a supercomputer of the scale of Blue Waters.

Accomplishments

For our 2018 allocation, we used Blue Waters to perform MD simulations of nanoscale systems, exploring their biophysical applications. Lipid-anchored DNA can attach functional cargo to bilayer membranes with applications in DNA nanotechnology, synthetic biology, and cell biology research. In collaboration with the Howorka group at University College London, we have combined MD simulations with experiments to determine how the membrane binding of cholesterol-modified DNA depends on factors including lipid headgroup charge, duplexed or single-stranded DNA, and the buffer composition [16]. In a collaboration with three experimental groups (Dekker, Joo and Maglia, the Netherlands), we demonstrated a new mechanism of gating and modulating a membrane channel's conductance: the inclusion of a removable gate that can be electro-mechanically stretched by an applied voltage [17]. Over the past year, we have also completed two projects that investigated the effects of DNA sequence on interactions relevant to chromatin organization. In collaboration with the Kim lab from Ulsan National Institute of Science and Technology, Korea, we investigated the effects of nucleotide sequence and chemical modification on DNA condensation [18]. In the second study, we elucidated how DNA detaches from a nucleosome, an essential process of gene transcription in eukaryotic cells [19].

A1. Dynamic interactions between lipid-tethered DNA and phospholipid membranes

A lipid molecule attached to the end of a DNA strand can anchor the strand to a lipid bilayer membrane [20]. This simple approach has been implemented for several applications in the fields of bionanotechnology [21], biosensing [22] and synthetic biology [23]. Recently, our group has shown that nanopores made from lipid-anchored DNA can insert into lipid bilayer membranes to mimic naturally occurring protein nanopores [24, 25, 26, 27]. To facilitated design of more complex lipid-anchored DNA nanostructures, we quantitatively characterized the interactions between lipid-terminated DNA and lipid bilayer membranes. We have accomplished that by combining the all-atom MD simulations of lipid membranes decorated with cholesterol-modified DNA, Fig. 1A,B, with the gel-shift assay that characterized such interaction experimentally, Fig. 1C. The main result of our study was quantification of the binding affinity of lipid-conjugated DNA to lipid bilayer membranes, something which had previously been not known.

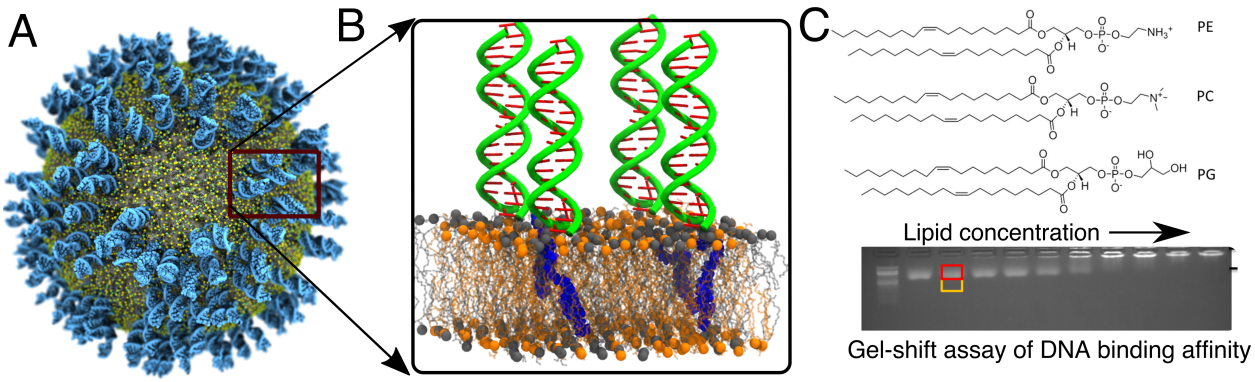

Figure 1: The interactions between lipid-tethered DNA and lipid bilayer membranes characterized by simulation and experiment [16]. (A) Atomistic representation of a lipid vesicle decorated with cholesterol-modified double-stranded DNA. The nitrogen/phosphorous atoms of the lipid head groups are shown in green and yellow, the lipid tails are shown in white and gray, and the DNA molecules are shown in blue. (B) All-atom model of a representative simulation system. The DNA backbone is shown in green, DNA bases in red, and the nitrogen/phosphorous atoms of the lipid head groups shown in gray and yellow, respectively; water and ions are not shown. (C) Top panel: chemical structure of the three phospholipids used in the study. Bottom panel: a representative result of a gel-shift assay that characterized the binding of cholesterol-modified DNA to a lipid bilayer membrane.

Complementing the gel-shift experiments carried out by the Howorka group, we built and simulated several all-atom systems that differed by the composition of the lipid membrane, the type of DNA molecules and the buffer conditions. The simulations and experiments have shown that the composition of a lipid membranes, or of the buffer electrolyte, can considerably affect the ability of a cholesterol-terminated DNA molecule to insert into a lipid membrane. We also found that, while being tethered to lipid bilayers, the DNA molecules can freely diffuse along the membrane surface. Finally, the simulations elucidated the dynamic nature of anchored DNA, showing a mushroom-like conformation of single-stranded DNA hovering over the bilayer surface that contrasts with a straight-up conformation of double-stranded DNA. The insights into the binding strength to membranes as well as the molecular accessibility of DNA for hybridization to molecular cargo is expected to facilitate the creation of biomimetic DNA versions of natural membrane nanopores and cytoskeletons for research and nanobiotechnology. A manuscript describing these results was published in *Langmuir* and was featured on the cover [16].

A2. Conductance modulation of a nanopore using a removable gate.

Ion channels form the basis of information processing in livings cells by facilitating the exchange of electrical signals across and along cellular membranes [28]. Applying the same principles to man-made systems requires development of synthetic ion channels that can alter their conductance in response to a variety of external manipulations. Generally, the gating of a membrane channel involves the opening or closing of the transmembrane pore that connects the opposite sides of a membrane with a water-filled passage that allows for ion transport across it [29]. In collaboration with the experimental groups of C. Dekker (TU Delft), C. Joo (TU Delft) and G. Maglia (U Groningen), we demonstrated a new type of a

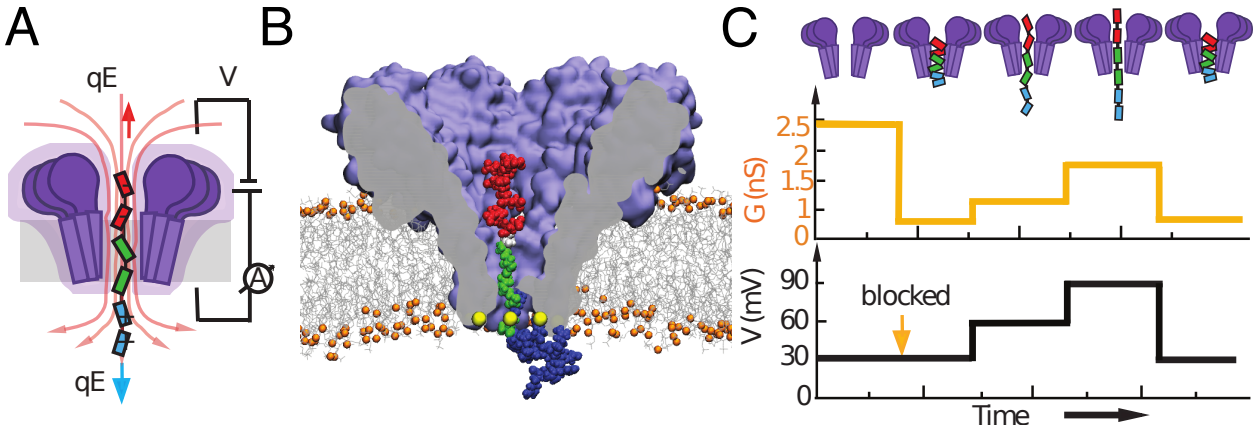

Figure 2: Electro-mechanical gating of a nanopore's conductance. (A) Schematics of a nanopore system where stretching of a bipolar peptide alters the nanopore conductance. (B) All-atom model of a typical simulation system featuring a bipolar peptide gate trapped inside a FraC nanopore that is embedded in a lipid bilayer membrane (water and ions are not shown). (C) Voltage-induced stretching of the peptide produces stepwise modulation in the nanopore conductance. Adopted from Ref. [17]

hybrid nanopore system that allows for both a stepwise change of the nanopore conductance and a nonlinear current-voltage modulation, Fig. 2. The key element of the system is a removable peptide gate that stretches when subjected to a transmembrane voltage.

Using a combination of atomistic MD simulations and single molecule experiments, we elucidated the working mechanism of our electro-mechanical gate. To characterize the effect of the electro-mechanical gate on FraC conductance, we varied the bias in the system containing the bipolar peptide trapped inside the FraC nanopore. Quantitative analysis of the MD trajectories established a relationship between the transmembrane voltage, the stretching of the peptide gate, the density of the gate's amino acids and the nanopore current. The simulation results were confirmed by experiment, where electro-mechanical modulation of the nanopore conductance was demonstrated at a single-molecule level. Based on the results of simulation and experiment, we arrived at a model where a higher transmembrane bias stretches the removable gate, thereby occupying less volume inside the FraC constriction and allowing more ions to pass through. We envision a range of applications of this removable-gate nanopore system, e.g. from an element of biological computing circuits to a test bed for probing the elasticity of intrinsically disordered proteins [30]. Our findings are described in a manuscript published in *ACS Nano* [17].

A3. The structure and dynamics of chromatin

Negatively charged DNA molecules repel one another in solution but are tightly condensed in the cell nucleus, forming arrays of nucleosomes, ultimately organizing into chromosomes. In order for DNA to become available for transcription, replication, and repair, DNA-histone assemblies must be unwrapped. Experiment has indicated that the outer stretches of nucleosomal DNA "breathe" by spontaneously detaching from and reattaching to the histone core [31, 32]. Other preliminary *in vitro* experimental and simulation studies have elucidated the process of nucleosomes unwrapping and the effect of various factors such as the external force, DNA sequence, etc. [33, 34, 35, 36]. However, the very mechanism of DNA unwrapping from a histone core had remained unclear. We used Blue Waters

to perform atomistic MD simulations of spontaneous and reversible nucleosome unraveling, elucidating the microscopic mechanism of the breathing process.

Nucleosome particles were simulated under a variety of salt conditions, revealing that higher salt concentrations lead to a greater extent of DNA unwrapping. Fig. 3A shows overlaid instantaneous snapshots of the initial fully-wound state, and a nucleosome with over two complete turns of DNA spontaneously unraveled. To examine the effect of sequence on nucleosomal breathing, we simulated four different sequences; poly-AT, 5S mRNA gene (43% CG), Widom 601L (57% CG) and poly-GC, Fig. 3B. It is evident from our simulations that DNA sequences with greater CG content may form more stable nucleosomes, in agreement with bioinformatics analysis [37, 38, 39]. By closely analyzing the simulation trajectories, we recognized a clear two-step mechanism of detachment: (1) periodically placed DNA transitions from being fully bound to a specific positively charged residue of the histone core to being only partially bound, and then (2) roughly one turn of DNA unbinds completely. We also find that histone–DNA contacts enhance the stability of the central region of nucleosomal DNA. Overall, the outcome of our simulation study suggests the possibility of a mechanism whereby AT-rich segments of DNA could form less stable nucleosomes and signal the initiation of transcription. The results of this study have been summarized in a manuscript that was recently published in the *Journal of Molecular Biology* [19].

The physical properties of DNA have been suggested to play a central role in the spatiotemporal organization of chromosomes. Previous experimental results have established correlations between the local nucleotide content of DNA, nucleosome positioning, and the frequency of inter- and intra-chromosomal contacts [40, 41]. In a study conducted in collaboration with the Kim group (UNIST, Korea), we combined fluorescence resonance energy transfer (FRET) measurements, precipitation assays, and MD simulations to characterize the effects of DNA nucleotide content, nucleotide sequence, and methylation on inter-DNA association and its correlation with DNA bending.

Using Blue Waters, we have characterized the affinity of two DNA molecules as a function of the inter-DNA distance for various combinations of the nucleotide sequence in the presence

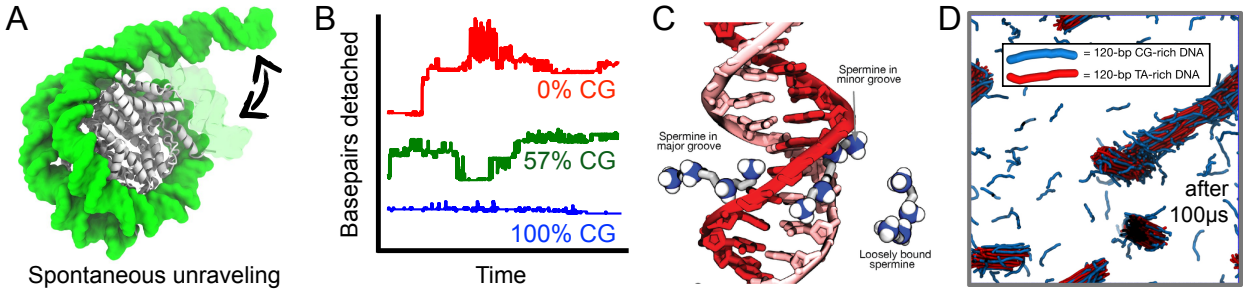

Figure 3: Sequence-dependent DNA interactions. (A) A model nucleosome system. A segment of DNA (green) was observed, in an MD simulation, to spontaneously and reversibly unravel from a histone core (white) in high salt. (B) The number of endpoint base pairs detached as a function of simulation time. Colors denote DNA sequences: poly-AT (i.e. ATAT...; red), Widom 601L (green), and poly-CG (i.e. CGCG...; blue). Greater CG content was found to correlate with less unwrapping. (C) Types of interactions between spermine^{4+} and DNA observed in all-atom MD simulations. Experiment and MD simulations were used to examine how polycations mediate DNA-DNA interactions. (D) A coarse-grained simulation of many DNA fragments (120 bp each; one-bead-per-10 bp) revealed that TA-rich DNA aggregates more readily than CG-rich DNA.

of poly-lysine peptide, spermine and spermidine polycations. These polycations, Fig. 3C, were chosen to mimic the effect of histone tails that extend from the surface of nucleosomes. In both simulation and experiment, we observed that polycations condense TA-rich DNA more strongly than DNA sequences rich in CG basepairs. Specifically, our simulations demonstrate that the presence and spatial arrangement of C5 methyl groups determines the strength of inter-DNA attraction, partially explaining why RNA resists condensation. Moreover, our results suggest the possibility that a common biophysical mechanism underlies DNA condensation and DNA looping, orchestrating DNA organization into chromosomes. Using a custom coarse-grained model, we showed that the sequence dependence of DNA condensation can produce microphase separation in dense DNA mixtures, Fig. 3D, an effect that could play a role in the structural organization of chromatin. The findings from this study were published in *Nucleic Acid Research* [18].

List of publications and presentations associated with this work

- S. Zhao, L. R. -Përez, M. Soskine, G. Maglia, C. Joo, C. Dekker and A Aksimentiev. Electro-mechanical conductance modulation of a nanopore using a removable gate *ACS Nano*, doi:10.1021/acsnano.8b09266, 2019.

- D. Winogradoff and A. Aksimentiev. Molecular mechanism of spontaneous nucleosome unraveling. *Journal of Molecular Biology*, 431(2):323335, 2019.

- J. Wilson and A. Aksimentiev. Water-compression Gating of Nanopore Transport. *Physical Review Letters*, 120(26), 268101, 2018.

- J. Yoo, A. Aksimentiev. New tricks for old dogs: improving the accuracy of biomolecular force fields by pair-specific corrections to non-bonded interactions. *Physical Chemistry - Chemical Physics,*, 20(13):8432-8449, 2018.

- P. M. Arnott, H. Joshi, A. Aksimentiev and S. Howorka. Dynamic Interactions between Lipid-Tethered DNA and Phospholipid Membranes. *Langmuir*, 34(49):15084-15092, 2018

- H. Kang, J. Yoo, B.-Y. Sohn, S.-W. Lee, H. S. Lee, W. Ma, J.-M. Kee, A. Aksimentiev, and H. Kim. Sequence-dependent DNA condensation as a driving force of DNA phase separation *Nucleic Acids Research.* 46(8):9401-9413, 2018.

- E. A. Hemmig, C. Fitzgerald, C. Maffeo, L. Hecker, S. E. Ochmann, A. Aksimentiev, P. Tinnefeld, and U. F. Keyser. Optical voltage sensing using DNA origami. *Nano Letters*, 18(3):1962-1971, 2018.

- (Invited talk) A. Aksimentiev. "DNA, a Molecule Full of Surprises". Department of Medical Biochemistry and Biophysics, Karolinska Institute, Stockholm, Sweden, March 2018.

- (Invited talk) A. Aksimentiev. "Microscopic Simulations at the Interface of Biology and Nanotechnology." CNRS-UIUC International Laboratory Kick-off Meeting, Nancy, France, Februarly, 2018.
- (Invited talk) A. Aksimentiev. "Molecular Dynamics Adventures with DNA." CECAMLorentz Joint Workshop: Multiscale-modelling of nucleosomes and their positioning on DNA, Lausanne, Switzerland, January. 2018.
- (Invited talk) A. Aksimentiev. "Surprising Physics at and near a Solid-State Nanopore." Nanofluidics in physics and biology, Lyon, France, July 2018.
- (Invited talk) A. Aksimentiev. "Surprising Physics of Nanopore Transport." Bernard L. Feringa Advanced Chemistry Lecture, East China University of Science and Technology, Shanghai, October 2018.
- (Invited talk) D. Winogradoff. "All-atom structure and ionic conductivity of the nuclear pore complex." Telluride Workshop on Nuclear Pore Complexes and Smart Polymers, Telluride, Colorado. August, 2018.

Plan for the next year

Biological nanopores such as MspA and CsgG have been successfully used to sequence DNA by monitoring the ionic current through the nanopore as DNA translocates through it [3, 9, 42, 43]. However, DNA under a transmembrane bias travels through nanopores much too rapidly for the ionic current signal to provide an accurate read of the sequence, necessitating the use of a biological motor to slow down the translocation process. In collaboration with the experimental group of C. Dekker (TU Delft), we are developing enzyme-free approaches to control DNA translocation through nanopores. In such systems, the speed and direction of NA transport is determined by the tug-of-war of electrophoretic forces applied to pull the NA molecule in opposite directions. Using Blue Waters, we will optimize the design of biological and solid-state double nanopores to advance the field of DNA and RNA sequencing.

Using the latest version of the NAMD package [44, 45], we have found that a 1 ns (NS) simulation of a 1-million-atom (MA) system requires 80 node hours (NH) on Blue Waters. We will use the 80 NH/MANS factor to estimate the requested allocation for each subproject. As detailed below, our projects P1 and P2 will require 180,000 and 60,000 node hours, respectively, resulting in the **total requested allocation of 240,000 node hours**. P1 will be performed during Q1, Q2 and Q3, and P2 will be performed during Q3 and Q4. We estimate our Blue Waters Professor allocation usage schedule throughout the year to be Q1: 15%, Q2: 35%, Q3: 35%, Q4: 15%.

P1. Nucleic acid sequencing using a double biological nanopore system.

Previously, we have demonstrated a considerable reduction of DNA translocation velocity using a system of two nanopores separated by a distance shorter than the length of the DNA

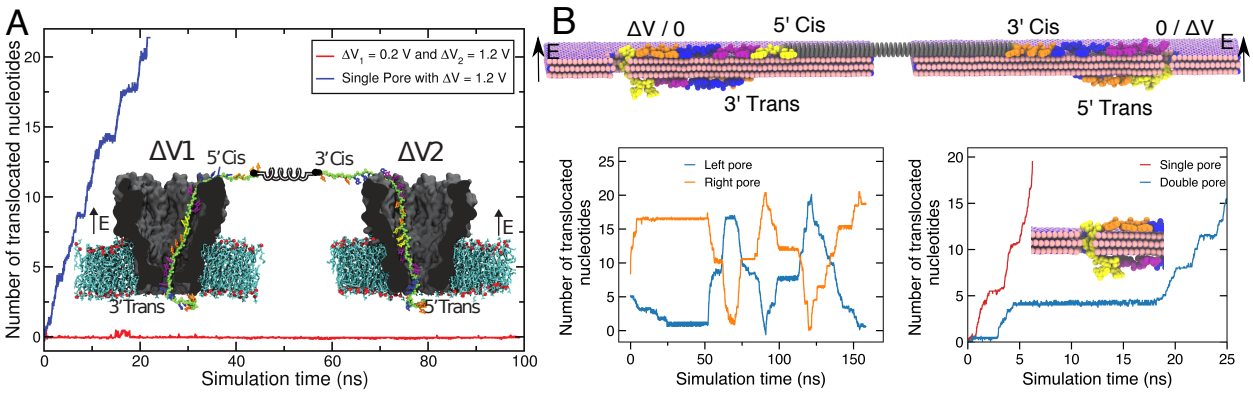

Figure 4: Controlling NA translocation across two nanopores. (A) A double biological nanopore system: two all-atom MspA systems, ssDNA in each coupled by a harmonic spring. The number nucleotides translocated across single (blue) and double (red) nanopore systems with respect to simulation time. (B) The atomistic model of NA translocation across a double boron nitride nanopore system. Bottom left: the number of translocated nucleotides with respect to simulation time across the left and right nanopores as the ssDNA flosses. Bottom right: the number of translocated nucleotides across single and double nanopore systems. The zoomed-in image shows a representative conformation of ssDNA near the pore.

molecules [7]. When a DNA molecule begins threading through one of the nanopores, the other end of the molecule can be captured by the second nanopore. Similar electrophoretic forces acting on the DNA molecule at both nanopores mechanically arrest the DNA translocation. Matching predictions of MD simulations, our experimental collaborators have succeeded in slowing down the translocation of DNA by three to four orders of magnitude in comparison to single pore translocation [7].

Although the proof-of-principle demonstration of double-pore trapping has been accomplished, it does not immediately lead to successful DNA sequencing. To sequence DNA using the double-pore approach, the force on the DNA in each pore needs to be individually controlled. More importantly, the ionic current blockades produced by DNA must carry information about the DNA sequence. A system of two individually addressable MspA pores meets all of the above requirements. By individually adjusting the transmembrane bias in each of the two pores, the DNA molecules could be transported, in principle, in any direction, allowing for repeated sequencing of the same DNA fragment and eliminating the need for biological enzymes to control the translocation. While our experimental collaborators are developing a system for the placement of MspA into individually addressable electric chambers, we propose to evaluate the performance of a dual MspA system with regard to controlling the motion of ssDNA and ssRNA, Fig. 4A. Specifically, we aim to determine if the dual MspA system reduces the "sequence flickering" noise [13] that occurs because of the longitudinal displacement of the DNA in the pore.

Instead of performing a brute force simulation of a system containing two MspA pores separated by a ∼50 nm ssDNA linker, we plan to simulate two much smaller systems in parallel, each containing a fragment of ssDNA pre-threaded through an MspA pore. The effect of the ssDNA tether will be accounted for by exchanging the forces on the terminal fragments of the ssDNA molecules via the multiple-copy feature in NAMD [14]. We have previously simulated individual MspA nanopore systems, finding a 20-nucleotide ssDNA fragment to move through the MspA constriction in one microsecond at the experimental

bias of 180 mV [11]. Using all-atom MD simulations, we will examine the translocation rate of DNA trapped between two MspA nanopores under the same bias (diffusion under tension), and under unequal bias – searching for a regime in which the translocation is best controlled. Each of the two MspA nanopore systems includes a lipid membrane, protein nanopore, electrolyte, and a strand of ssDNA and contains ∼250,000 atoms. The tension force on the terminal nucleotides of the ssDNA fragments will be computed according to the observed displacement of the nucleotides and the effective spring constant of the linker DNA fragment. For a 50 nm pore-pore distance, the spring constant of the ssDNA linker is 3.6 pN/nm [46].

To measure the effect of tension on the trapping efficiency we will perform a series of simulations where each of the pores is under a different bias, but the difference between the bias is fixed: 50 mV/230 mV, 100 mV/280 mV,—as well as one simulation of the same length with equal bias—180 mV/180 mV. In each simulation under an 180 mV bias differential, we aim to observe at least 30 nucleotides permeating through one of the nanopores, which will require about 1.5 μs of simulation time. The voltage clamp simulations (where both pores are subject to the same bias) will be run for the same duration to investigate intrinsic diffusive motion under a dual-bias condition.

This part of the project will require **(2×0.25 M atom system) × (1500 ns) × (3 bias combinations) = 2250 MANS, × (80 node hours/MANS) = 180,000 Node hours** on Blue Waters.

P2. Nucleic acid sequencing using a double boron-nitride nanopore system.

In collaboration with C. Dekker's group, we are also developing a purely solid-state approach to NA sequencing that utilizes two boron-nitride (BN) nanopores. Fig. 4B illustrates the results of our preliminary simulations of such a dual-nanopore setup containing two ∼0.125 M atom BN nanopore systems. Flossing a DNA molecule through the double nanopore system is expected to place its nucleotides in statistically similar conformations, enabling determination of their chemical identify via ionic current measurement. Fig. 4B (bottom right) shows the number of DNA nucleotides displaced through the double nanopore system in response to switching the magnitude of the transmembrane bias in our preliminary simulations.

Using Blue Waters, we will optimize the design of the solid-state double-pore system by varying the BN nanopore geometry. From our previous work on graphene nanopores [12, 47], we know that the translocation velocity and the conformation of the DNA nucleotides sensitively depends on the number of layers in the graphene membrane. For that reason, we will carry out our simulations of DNA flossing using one-, three- and six-layer BN systems. The six-layer BN nanopores will be designed to mimic the constriction region of the MspA nanopore which has shown to have DNA sequence recognition ability [42, 43]. Using an alternating bias of 0.5 V, we will floss DNA across the double nanopore systems. From our preliminary simulations, we estimate that 1000 ns will be required to floss DNA several times and thereby obtain a statistically significant number of DNA conformations to determine the nucleotide-specific ionic current signal using a steric exclusion method (SEM) developed within our lab [48]. The outcome of these simulations will be used to guide the development

of an experimental solid-state nanopore sequencing system.

Carrying out project P2 will require **(2×0.125 M atom system) × (3 systems with different pore geometries) × (1000 ns) = 750 MANS, × (80 node hours/MANS) = 60,000 Node hours** on Blue Waters.

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