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Dynamics of DNA translocation in a solid-state nanopore immersed in aqueous glycerol

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Abstract
Nanopore-based technologies have attracted much attention recently for their promising use in low-cost and high-throughput genome sequencing. To achieve single-base resolution of DNA sequencing, it is critical to slow and control the translocation of DNA, which has been achieved in a protein nanopore but not yet in a solid-state nanopore. Using all-atom molecular dynamics simulations, we investigated the dynamics of a single-stranded DNA (ssDNA) molecule in an aqueous glycerol solution confined in a SiO\textsubscript{2} nanopore. The friction coefficient $\xi$ of the ssDNA molecule is found to be approximately 18 times larger in glycerol than in water, which can dramatically slow the motion of ssDNA. The electrophoretic mobility $\mu$ of ssDNA in glycerol, however, decreases by almost the same factor, yielding the effective charge ($\xi\mu$) of ssDNA being roughly the same as in water. This is counterintuitive since the ssDNA effective charge predicted from the counterion condensation theory varies with the dielectric constant of a solvent. Due to the larger friction coefficient of ssDNA in glycerol, we further show that glycerol can improve trapping of ssDNA in the DNA transistor, a nanodevice that can be used to control the motion of ssDNA in a solid-state nanopore. Simulation results of slowing ssDNA translocation were confirmed in our nanopore experiment.

Online supplementary data available from stacks.iop.org/Nano/23/455102/mmedia

(Some figures may appear in colour only in the online journal)

1. Introduction

Nanopore-based DNA sequencing technologies have advanced dramatically [1–3] since the first experimental demonstration of the feasibility of ssDNA translocation through an $\alpha$-hemolysin nanopore [4]. Both theoretical and experimental efforts on investigating the dynamics of single-stranded DNA (ssDNA) in a protein pore have inspired designs of engineered ones that provide better constriction sites for ssDNA sensing. Currently, when a ssDNA molecule is in an engineered protein nanopore, the sensitivity of an ionic current through the pore has been improved from a ssDNA homopolymer [5] to a single nucleotide in ssDNA [6–8] with gradually increasing accuracy. During ssDNA translocation, it is possible to use a DNA polymerase bound to the ssDNA to control the ssDNA position or slow down ssDNA motion [9, 10]. Motivated by the success of protein pores, a solid-state nanopore for DNA sequencing has been suggested [11] and has attracted extensive interest [1, 2, 12]. State-of-the-art fabrication techniques allow a nanopore to be drilled (using a focused ion or electron beam) through a synthetic solid membrane containing functional materials, such as doped semiconductors in the nanopore capacitor [13], metal electrodes in the DNA transistor [14], and carbon...
nanotubes [15–17] or graphene [18–20] in a nanopore sensor. Advantages of a solid-state nanopore include robustness, scalability, high-throughput, and integrability to an electronic nanodevice. Despite being promising, one challenge for sequencing ssDNA in a solid-state nanopore is to control the motion of ssDNA near a sensor.

One encouraging method for sensing DNA in a solid-state nanopore is to measure the tunneling current through a DNA base that bridges two nano-electrodes exposed on the pore surface [21–23]. Recent experimental work [23] showed that hundreds of independent measurements of the tunneling current are required to call a DNA base according to the distribution of measured values. Ideally, a DNA molecule should transit a pore slowly enough to have each base sensed accurately or at a high signal-to-noise ratio. However, the typical translocation speed of DNA in a solid-state nanopore is about 30 bases $\mu$s$^{-1}$, far beyond the capability of current sensing technologies. Previously, it has been demonstrated experimentally, that an aqueous glycerol solution (referred as ‘glycerol’ hereafter) can slow the translocation speed of DNA by a factor of 5.5 [24]. Theoretical work on the DNA transistor (a nanopore employing electric trapping fields) [25] suggested that ssDNA can be base-by-base ratcheted through a nanopore. If the duration of a trapped state of ssDNA is long enough, the base adjacent to a sensor can be accurately measured. Because the viscosity is larger in glycerol than in water, the duration of a trapped state for ssDNA in glycerol should be longer according to the reaction-rate theory. Therefore, in order to take advantage of this, it is important to understand the dynamics of ssDNA translocation through a solid-state nanopore ‘solvated’ in glycerol.

As a single molecule sensor, a nanopore has been used to interrogate physical properties (such as size and conformation) of a single biological molecule. Although nanopore-based sensing techniques are advancing quickly, they are still very challenging in experiment [2]. Computer simulations have provided valuable insights in nanoscale research of biochemistry [26, 27] and bioengineering [12, 28]. In this paper, using molecular dynamics (MD) simulations, we investigated the driven motion of ssDNA in glycerol confined in a 4 nm-diameter SiO$_2$ nanopore. We measured physical quantities, such as the friction coefficient and electrophoretic mobility, that govern the translocation of ssDNA in a nanopore. These results were compared to those for ssDNA translocation in a nanopore filled with water. We also studied how glycerol can be used to improve the performance of the DNA transistor.

2. Simulation details

Figure 1 illustrates the simulation system. A channel of 4 nm in diameter was ‘drilled’ (in silico) through amorphous SiO$_2$ that was simulated using the BKS force field [29]. The simulated SiO$_2$ solid measures $65 \times 65 \times 148$ Å$^3$. The ssDNA molecule, consisting of 20 adenine nucleotides, was confined near the symmetry axis of the channel by applying a harmonic spring (constant: 1.44 kcal Å$^{-2}$ mol$^{-1}$) force on the center of mass of all phosphorus atoms, mimicking a DNA–repellent surface (e.g. surface coated with a self-assembled monolayer with hydrophobic end groups [30]). The interaction between DNA and a pore surface could make the motion of DNA even more difficult to control. Since the ssDNA molecule is covalently linked to itself through the periodic boundary of the system, the ssDNA molecule is in a linear and stretched conformation [25] that is ideal for sequencing. Because of the periodic boundary condition, effectively, the ssDNA molecule as well as the nanopore are infinitely long, ignoring motion of the DNA fragment outside the pore. The average spacing $d$ between neighboring phosphate groups in the ssDNA molecule is 7.4 Å. When the channel pressure is approximately 1 bar, the electrolyte confined inside the channel contains 1792 water molecules, 1000 glycerol molecules, 3 Na$^+$ and 3 Cl$^-$ ions (about 0.1 M ion concentration). The volume percentage of glycerol molecules is about 69%, that is larger than the 50% used in a previous [24] and our (see below) experiment, and is less than the 90% used in our previous experiment of nanopore electrochemistry [31]. The complex solvent was mixed and equilibrated for 10 ns in simulation. A total charge of $-20e$ (e, the electron charge) was uniformly distributed to surface atoms of the channel. The net charge of the whole system is zero.

The program NAMD [32] was used to carry out all MD simulations. The Charmm force field [33] was used for ssDNA and glycerol molecules; the TIP3P model [34] for water molecules; the standard force field for ions [35] and silica force field [36] for the SiO$_2$ solid interacting with water. All simulations were performed in the NVT ensemble at 300 K. The constant temperature was maintained by applying a Langevin thermostat [37] to all SiO$_2$ atoms, which were harmonically constrained to their respective original positions.
positions. Periodic boundary conditions were applied in all three dimensions. Long-range Coulomb interactions were computed using the particle-mesh Ewald (PME) method over a $64 \times 64 \times 136$ grid. The simulation time-step was 1 fs. A smooth ($10–12$ Å) cutoff was used to calculate the van der Waals interaction between atoms.

3. Results

3.1. Friction coefficient

To obtain the friction coefficient of the ssDNA molecule in the confined viscous solvent, we used the steered molecular dynamics (SMD) method [38] to pull the ssDNA molecule along the symmetry axis of the channel. One end of the pulling spring (100 pN Å$^{-1}$) was attached to the center of mass of all phosphorus atoms in ssDNA and the other end was attached to the stage, which moved at a constant velocity $v$, varying from 0.1 to 10 nm ns$^{-1}$. While the pulling force $F$ in the spring is balanced by friction, the time-dependent pulling force in the spring saturates, as shown in figure 2. Overall, the spring force increases with the pulling velocity.

Assuming that $F = \xi v$, the friction coefficient $\xi$ of ssDNA can be obtained from the saturated spring forces in figure 2. The inset of figure 2 shows the relation between friction coefficients and pulling velocities. When the pulling velocity is below a critical value $v_{cr}$ ($\sim 1$ nm ns$^{-1}$), the friction coefficients are nearly constant, i.e., $\xi(v < v_{cr}) = 0.54$ nN ns nm$^{-1}$. Above the critical value, friction coefficients decrease with an increase of pulling velocities, indicating the shear-thinning effect that commonly occurs in a viscous solvent. Note that the critical velocity ($\sim 1$ nm ns$^{-1}$) is much larger than a typical translocation velocity of DNA measured experimentally [24]. Thus, the shear-thinning effect is negligible in experiment.

At a pulling velocity $v_0$ of 0.5 nm ns$^{-1}$, the flow profiles for water and glycerol molecules between the ssDNA molecule and the channel surface are shown in figure 3. The radius-dependent flow velocities were computed for oxygen atoms in water molecules and for the middle carbon atoms in glycerol molecules. Theoretically, the flow profile between two concentric cylinders can be derived from the Stokes equation $\nabla^2 v = 0$, assuming that the boundary conditions $v(R_{ssDNA}) = v_0$ and $v(R) = 0$. Here $R_{ssDNA}$ is the effective radius (5.5 Å) of ssDNA and $R$ is the radius of the outer cylinder. We choose $R = R_0 = 17.5$ Å, where $R_0 (= 2$ nm) is the channel radius and $D$ is the thickness of an inaccessible region near the channel surface due to the van der Waals radii of atoms. The theoretical result is that $v = v_0 \ln(r/R)/\ln(R_{ssDNA}/R)$. Figure 3 shows that the flow profiles obtained from simulations agree well with the theoretical prediction. Previously, a similar continuum theory [39, 40] of electro-hydrodynamics was successfully used to account for experimentally measured forces on a tethered DNA molecule inside a solid-state nanopore [41]. Thus, continuum theories could be deployed to describe a nanoscale solvent confined in a nanochannel.

3.2. Electrophoretic mobility

In a biasing electric field along the z-axis, negatively charged ssDNA in the channel moves to the opposite direction of the electric field, i.e. ssDNA electrophoresis in the nanochannel. The surface of the nanochannel serves as a boundary for the flow induced by the electrophoretic motion of the ssDNA molecule. Thus, the translocation velocity of the ssDNA molecule can be affected by surface properties, such as roughness [42] and hydrophobicity [30]. The surface used in our simulations is smooth (with atomic scale roughness only) and the hydrophobic surface [25] becomes effectively hydrophilic after the glycerol-treatment. Figure S1 (in supplementary data available at stacks.iop.org/Nano/23/455102/mmedia) shows that near the pore surface the number concentration of glycerol molecules is higher than that of water molecules. Due to this surface effect, the mean
concentration of glycerol in a solid-state nanopore may differ from the bulk concentration.

Figure 4 shows the electrophoretic motion of ssDNA in various biasing electric fields. When the electric driving force was balanced by the hydrodynamic friction on ssDNA, the ssDNA molecule moved nearly at a constant velocity, which is indicated by the slope of each line in the plot of time-dependent ssDNA positions (figure 4). The inset in figure 4 shows that the mean translocation velocity of ssDNA increases linearly with the biasing electric field. The slope of the linear relation yields the electrophoretic mobility $\mu$ of the ssDNA molecule, since $v = \mu E$. The calculated electrophoretic mobility of the ssDNA molecule is about 5.3 nm$^2$ ns$^{-1}$ V$^{-1}$, which is about 17 times less than the electrophoretic mobility (90.4 nm$^2$ ns$^{-1}$ V$^{-1}$) of ssDNA in water confined in the same pore [25]. It is worth noting that in the previous experiment of ssDNA translocation through an $\alpha$-hemolysin nanopore, the ssDNA translocation velocity was reduced by 20 times in a 63% glycerol/water mixture relative to an aqueous solution [44]. Note that the obtained larger viscosity of glycerol can slow the ssDNA translocation and affect the translocation dynamics of ssDNA in the DNA transistor (supplementary data available at stacks.iop.org/Nano/23/455102/mmedia).

After we finished simulations, to verify simulation results, we carried out further corresponding experiments of ssDNA translocation through a nanopore filled with 0%, 20% or 50% glycerol. The detailed experimental setup is in the supplementary data (available at stacks.iop.org/Nano/23/455102/mmedia). Given the limitation of wettability and detectable signals of DNA translocation, the nanopore in experiment is 6 nm in diameter (larger than the 4 nm pore used in simulation). However, we expect to have similar simulation results for ssDNA in a 6 nm pore. Theoretically, the ssDNA velocity $v = 2\epsilon E(\zeta_D - \zeta)/\eta$ [43], where $\epsilon$ and $\eta$ are the dielectric constant and the viscosity of glycerol respectively; $\zeta_D$ and $\zeta$ are the $\zeta$-potentials of DNA and the pore surface respectively. Since the radii of both pores are larger than the Debye length (1 nm for a 0.1 M electrolyte), $\zeta_D$ and $\zeta$ can be approximated by their corresponding bulk values. Thus, the translocation velocity is not sensitive to the radii of pores we used.

As shown in figure 5(a) (0% glycerol) and figure 5(b) (50% glycerol), each blockade of an open-pore current corresponds to an ssDNA translocation event. From the duration of a blockade (or the dwell time), the translocation velocity of ssDNA can be estimated. The dwell time of a typical ssDNA translocation event is much longer in 50% glycerol than in water. Figure 5(c) shows distinctive scatter plots for current signals of ssDNA translocation in different solutions. Compared to the case of ssDNA in water, the mean translocation (or dwell) times are about 5 and 27 times larger when ssDNA molecules are in 20% and 50% glycerol, respectively. These results agree with previous experimental ones [24, 44] and are also consistent with our simulation data. Note that in experiments ssDNA may move away from the symmetry axis of the pore and interact with the pore surface. Therefore, in 50% glycerol, the ssDNA velocity was slowed down by 27 times instead of 17 times as predicted in simulation.

With both the friction coefficient $\xi$ and the electrophoretic mobility $\mu$ of the ssDNA molecule, the effective charge $q_{\text{eff}}$ can be calculated since $q_{\text{eff}} = \xi \mu$ [42]. This relation was first proposed theoretically [45] and was later demonstrated in simulation [42]. For tethered DNA confined in a nanopore in an electric field [41], the mechanical tether force balances the electric driving force on DNA, i.e. $F = q_{\text{eff}}E$. The simultaneous action of a mechanical force and an electric force on a tethered DNA molecule can be decomposed into two independent motions: (a) mechanically driven motion when the mechanical driving force is balanced by the hydrodynamic friction, i.e. $F = \xi \nu$; (b) electrically driven motion (or electrophoresis of DNA) that is described by $v = \mu E$. Combining these two equations, one finds that $F = \xi \mu E$. Therefore, the effective charge of DNA is $\xi \mu$. It is possible to obtain $q_{\text{eff}}$ by experimentally measuring the tether force on DNA [41, 40], however that experiment is one of the most elegant yet difficult nanopore-based experiments. Alternatively, the effective charge of DNA could be obtained indirectly, by measuring the friction coefficient and the electrophoretic mobility of DNA separately. For the ssDNA molecule in glycerol used in our simulations, the effective charge is 17.9 $e$ using the measured $\xi$ and $\mu$ above. Thus, $q_{\text{eff}} = 0.89Q$, where $Q (=20e)$ is the charge of bare ssDNA. Experimentally, the effective charge of dsDNA in a nanopore was found to be only 25% of the charge of bare dsDNA. However, this measured effective charge of dsDNA was affected by an electro-osmotic flow in a nanopore and thus depends on the size of the pore [40]. Additionally, previous MD simulations also demonstrated that the effective charge of DNA depends on the surface properties of a nanopore, such as roughness [42], hydrophobicity [30, 46] and charge densities [47].

In our previous simulation of the same ssDNA molecule in water confined in the same nanochannel [25], the friction coefficient is 0.03 nN ns nm$^{-1}$ and the electrophoretic mobility of the ssDNA molecule is about 5 bases ns$^{-1}$. Theoretically, the ssDNA velocity is $v = 5 \epsilon E(\zeta_D - \zeta)/\eta$ [43].
mobility is 90.4 nm$^2$ ns$^{-1}$ V$^{-1}$, which yields the effective charge of 0.86$Q$ close to that of the ssDNA molecule in viscous glycerol. This is due to the fact that the friction coefficient is proportional to the viscosity [40] while the electrophoretic mobility is inversely proportional to the viscosity [43]. Therefore their product, which is the effective charge of the ssDNA molecule, is independent on the solvent viscosity.

This conclusion cannot be accounted for by the counterion condensation theory [48], which predicts that $q_{\text{eff}}$ changes with the dielectric constant of a solvent. Here, the dielectric constant of glycerol is less than that of water. It is also debatable whether the counterion condensation occurs for DNA in a mono- or di-valent electrolyte. The small-angle x-ray scattering (SAXS) experiment showed that mono- or di-valent ions only form a compact cloud around DNA [49] and a previous simulation showed that a typical residence time for a potassium ion on DNA is only about a few picoseconds [42].

4. Discussion and conclusions

Because of the periodic boundary condition used in simulations, the effects of ssDNA entering and exiting a nanopore are not considered. However, because of the large aspect ratio (~20) between the thickness of a solid membrane and the diameter of a pore, the conditions for the pore region that is several nanometers away from the entrance and the exit should be similar to those in the nanochannel used in simulations. A ssDNA molecule in a bulk solvent is typically in a coiled conformation because of a short persistence length (~2 nm). The ssDNA molecule confined in the nanochannel is assumed to be in a stretched and linear conformation, which is ideal for sequencing ssDNA base-by-base. The stretched conformation of the ssDNA molecule can be realized by the non-uniform biasing electric field [50] or the hydrodynamic flow [51, 42] in a nanopore. Additionally, electric fields (in opposite directions) in the DNA transistor [14] can also stretch the ssDNA molecule.

In summary, we have studied the dynamics of ssDNA transiting a solid-state nanopore immersed in glycerol. From MD simulations, we found that the shear-thinning effect (which is common in a viscous solvent) does not occur when the translocation velocity of the ssDNA molecule is less than 1 nm ns$^{-1}$. Thus, the shear-thinning effect in glycerol can be ignored in experiment. Compared to the ssDNA molecule in water, the translocation velocity of the ssDNA molecule in glycerol is substantially reduced. Namely, the friction coefficient for the ssDNA molecule is 18 times larger in glycerol than in water, consistent with stronger inter-molecular interactions (via hydrogen bonds) in glycerol. Accordingly, the electrophoretic mobility of the ssDNA molecule is 18 times larger in glycerol than in water, consistent with stronger inter-molecular interactions (via hydrogen bonds) in glycerol. Note that the electro-osmotic flow of an electrolyte between ssDNA and a pore surface can play an important role in the hydrodynamic and electric screening of ssDNA [42]. However, we found that the effective charge of ssDNA is independent of the viscosity of a solvent. Our simulation results were compared both with predictions of continuum theories in hydrodynamics and existing experimental data (including ours), and they agree with each other fairly well.

As for DNA sequencing, glycerol is not only useful for slowing the translocation velocity of ssDNA in a nanopore, but also important for functionalities of the DNA transistor. Experimentally, we have shown that
electrochemical corrosion of nano-electrodes in the DNA transistor is negligible when the DNA transistor is solvated with glycerol [31]. In simulations, we found that the duration of a trapped state of the ssDNA molecule in the DNA transistor can be much longer in glycerol than in water (see supplementary data available at stacks.iop.org/Nano/23/455102/mmedia). In a trapped state, the DNA base in front of a sensor can be measured. With current sensing technologies, such as measuring the tunneling current through a base [22, 23], each DNA base should pause in front of a sensor for a sufficiently long time to allow multiple independent measurements, in order to improve the signal-to-noise ratio. Therefore, it is desirable to have the trapping time of ssDNA being larger than the time for measurements, which is potentially possible in experiments of ssDNA trapped in the DNA transistor filled with aqueous glycerol. Besides the DNA sequencing, a nanopore can also be used as an ultra-sensitive biosensor [52] to analyze conformations of a transported molecule, such as the detection of microRNAs [53] and monitoring of an ATP-binding aptamer [54]. Increasing the solvent viscosity can limit the number of translocation events potentially possible in experiments of ssDNA trapped in the DNA transistor at 5 Å resolution [31].

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