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Controlled translocation of DNA through nanopores in carbon nano-, silicon-nitride- and lipid-coated membranes

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We investigated experimentally and theoretically the translocation forces when a charged polymer is threaded through a solid-state nanopore and found distinct dependencies on the nanopore diameter as well as on the nano membrane material chemistry. For this purpose we utilized dedicated optical tweezers force mechanics capable of probing the insertion of negatively charged double-stranded DNA inside a helium-ion drilled nanopore. We found that both the diameter of the nanopore and the membrane material itself have significant influences on the electroosmotic flow through the nanopore and thus on the threading force. Compared to a bare silicon-nitride membrane, the threading of DNA through only 3 nm thin carbon nano membranes as well as lipid bilayer-coated nanopores increased the threading force by 15% or 85%, respectively. This finding was quantitatively described by our recently developed theoretical model that also incorporates hydrodynamic slip effects on the translocating DNA molecule and the force dependence on the membrane thickness. The additional measurements presented in this paper further support our model.

Introduction

Both electrophoretic and electroosmotic (EOF) forces play major roles when charged molecules translocate through biological or artificial nanopores (NPs).[1–7] The magnitude of the EOF depends not only on the charge of the polymer, but also on the diameter of the NP[7,9–11] and the surface charge of the membrane material containing the pore.[7,8,12] First optical tweezers measurements of DNA threading forces also confirmed the theoretical prediction that the EOF screened and reduced the bare electrostatic force acting on a charged molecule inside a NP.[13] In recent works different molecules like RNA[11] or DNA protein complexes[14] were investigated and novel techniques like optical tweezers combined with microcapillaries were introduced.[15]

In order to investigate the role of the membrane materials we utilized three different membrane systems: Silicon-nitride membranes with various thicknesses are the reliable standard system for translocation measurements. Lipid bilayer-coated silicon-nitride membranes[7] are used as a model system for biological membranes containing NPs. As a new class of membrane material, we introduce artificial carbon nano membranes (CNMs)[16,17] which were fabricated by cross-linking self-assembled aromatic monolayers.

Particularly, we scrutinized the dependence of the EOF on the diameter of the NP as well as on the electrical surface charge and thickness of these membranes, expanding our previous data base. We developed and extended a theoretical model that is based on the coupled Poisson, Nernst–Planck and Stokes equations and incorporates hydrodynamic slip effects on the translocating DNA molecule, describing all the observed experimental phenomena reasonably well.

Results and discussion

As artificial membrane material we utilized 10, 20, and 50 nm thin silicon-nitride membranes where various NPs with diameters between 6 and 80 nm were drilled into with helium-ion-microscopy.[18] In Fig. 1A we show two typical optical tweezers

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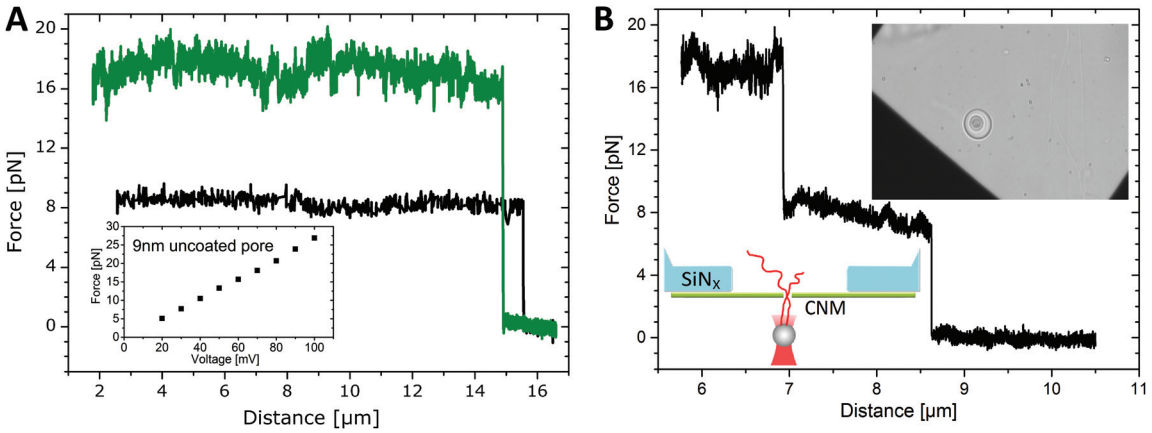

Fig. 1 (A) Optical tweezers force mechanics measurement on dsDNA inside a 41 nm NP in an uncoated silicon-nitride membrane (black line) and inside a 31 nm NP that has been coated with a lipid bilayer (green line) when a transmembrane voltage of 50 mV was applied. Upon continuously increasing the distance between bead and membrane, the force remains constant until it drops to zero when the dsDNA is completely pulled out of the NP. (Inset) The dependence of the threading force on the applied voltage was found to be linear (9 nm pore as an example). (B) Threading force of two individual dsDNA molecules inside a 60 nm NP that was drilled into a 3 nm thin freestanding carbon nano membrane (CNM).

force measurements, where a single dsDNA molecule (λ-phage DNA; contour length of 16.4 μm) is threaded out of a NP at a transmembrane voltage of 50 mV. The black curve is a step function with a constant threading force of 8 pN as we increase the vertical distance (see Fig. 3A) until the DNA-strand is completely pulled out of the pore and the force drops to zero when approaching the contour length. The threading force depended linearly on voltage over a range from 20 to 100 mV (see inset of Fig. 1A).

We found that various threading experiments through pores with different diameters in silicon-nitride membranes clearly exhibit a gradually increasing threading force with decreasing NP diameter (Fig. 2). In contrast to this finding, the electrostatic component of the threading force was independent of the NP diameter and can be estimated as to be 50 pN for a voltage of 50 mV.[13] This discrepancy is solved by taking the hydrodynamic coupling of the EOF to the threaded molecule and the NP wall into account. The EOF itself points in the opposite direction of the electrostatic force and therefore reduces the measured threading force.[9] Finally, the interplay between the drag forces both on the molecule and the NP wall leads to a gradually increasing EOF[10,11] with increasing NP diameter.

Additionally, the pore surface charge affects the magnitude of the EOF and therefore the threading force.[7] We examined this by coating a silicon-nitride membrane with an electrically neutral POPC lipid bilayer[7,12] and executed similar dsDNA threading experiments. In various measurements, individual DNA-molecules were threaded in and out of each individual coated pore, while we recorded the threading forces for voltages in a range between 20 and 80 mV.

The green graph in Fig. 1A shows the dsDNA threading force for an applied voltage of 50 mV in a lipid-coated pore with 31 nm diameter (the uncoated diameter was 41 nm). The

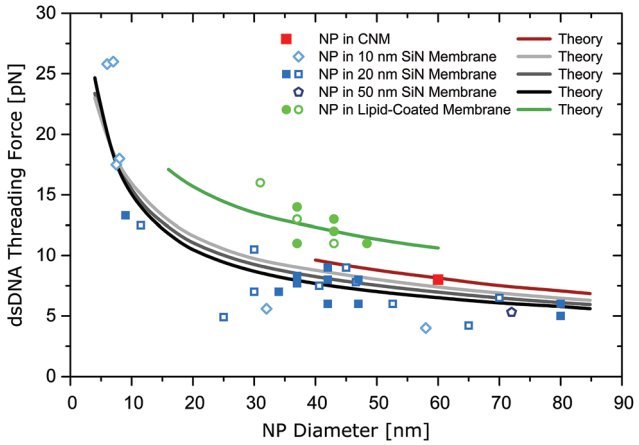

Fig. 2 The dependence of the dsDNA threading force (applied voltage of 50 mV) on the respective NP diameter for various membrane thicknesses and membrane materials. Closed symbols indicate measurements first presented in this paper. All experiments were conducted in buffer containing 20 mM KCl and 2 mM Tris/HCl at pH 8.0. Our theoretical model for silicon-nitride membranes (grey lines), a lipid-coated membrane (green line) and the CNM (red line) fits best when we adopt a surface charge density of −60, 0 and −60 mC m^{-2}, respectively, in combination with a hydrodynamic slip effect on the dsDNA surface with a slip-length of 0.5 nm. Although silicon-nitride and CNMs were found to have comparable surface charges, the CNM thickness of only 3 nm slightly reduced the magnitude of the EOF and therefore increased the threading force by about 15%.

threading force of about 16 pN is twice as large as it would be for an uncoated pore of the same diameter.

This increase can be only partially attributed to the reduction of the pore diameter due to the coating, which is 9.6 nm for POPC.[12] The change in NP size of 9.6 nm due to lipid bilayer coating[12] only accounts for a small increase in the measured force. Consequently, the strong increase of the

threading force is mainly attributed to a significant reduction in the EOF inside the NP. Because of the now electrically neutral NP wall, the EOF that was induced on the negatively charged silicon-nitride NP wall is now significantly suppressed, which in turn raises the measured threading force by about 85%, as confirmed by eight individual threading experiments.

To achieve the best fit of our theoretical model (for details see Experimental setup and methods) to the experimental findings we abandoned the usual no-slip boundary condition on the surface of the DNA strand. Instead we introduced a finite slip length of l_{slip} = 0.5 nm, whereas the no-slip condition on the pore wall was retained for all membranes (CNM, silicon-nitride and lipid-coated) with the result of a very good agreement with all experimental findings.[7] The presence of a hydrodynamic slip effect at the DNA surface is based on structural properties of the actual dsDNA: the presence of alternating hydrophilic (DNA backbone) and hydrophobic domains (nucleobases inside the grooves of the DNA) give rise to significant hydrodynamic slip effects.[19] Our best fit of l_{slip} = 0.5 nm agrees very well with the slip length in Fig. 2 of Kesselheim *et al.*[20]

As a promising new membrane material, we found the freestanding CNM to be a robust membrane with hydrophilic surface properties where large areas of several mm^2 can be fabricated and easily transferred. In contrast to freestanding graphene membranes, where a microbead inside the optical trap was instantly thermally heated and attached DNA molecules were denaturated,[21] the trapping laser had no thermal influence on the bead-DNA construct even in close proximity to the freestanding CNM. Furthermore, the CNM was mechanically stable and we observed no energy absorption or dissipation. Another advantage of CNM is the tunable surface chemistry depending on the choice of the organic molecules that were use to form a self assembled monolayer during the first step of CNM fabrication. Additionally, we found out that no sticking of DNA molecules was observed when one or more DNA strands were threaded into a NP inside a CNM.

Fig. 1B shows the threading forces on two individual dsDNA inside a 60 nm diameter NP that has been drilled into a 3 nm thin CNM (see lower left inset of Fig. 1B). When increasing the distance between bead and NP, both DNA strands were consecutively threaded out of the NP. During the experiment the applied transmembrane voltage was 50 mV, which resulted in a relatively constant threading force of 8 pN on each DNA strand.

In addition, the surface charges of the respective membranes as parameters of our theoretical model were found to be $\sigma_m = -60$ mC m^{-2} for silicon-nitride and $\sigma_m = 0$ mC m^{-2} for a lipid-coated NP membrane. For CNM, the single data point indicates a theoretically predicted surface charge comparable to that of silicon-nitride.

Although both the silicon-nitride and the CNM were modelled with identical surface charges, the threading forces through a CNM were found to be about 15% larger than for silicon-nitride membranes. We explain this by the fact that the only 3 nm thin CNM slightly reduces the magnitude of the EOF and therefore increases the measured threading force.

Furthermore, we found that realistic modifications of the DNA and membrane permittivities and of the pore shape only have negligible effects on the results. The effects of the membrane thickness are plotted in Fig. 2.

Experimental setup and methods

Single NPs with a diameter between 6 and 80 nm were fabricated, cleaned and mounted into our sample chamber as described recently.[7] The diameters of all NPs in a CNM, in silicon-nitride membranes and the diameters of NPs after lipid-coating were calculated by measuring the ionic current through the pore, according to the work of Yusko *et al.*[12] and our recent work.[7] DNA threading experiments and force measurements (see also Fig. 3A) with our video-based OT

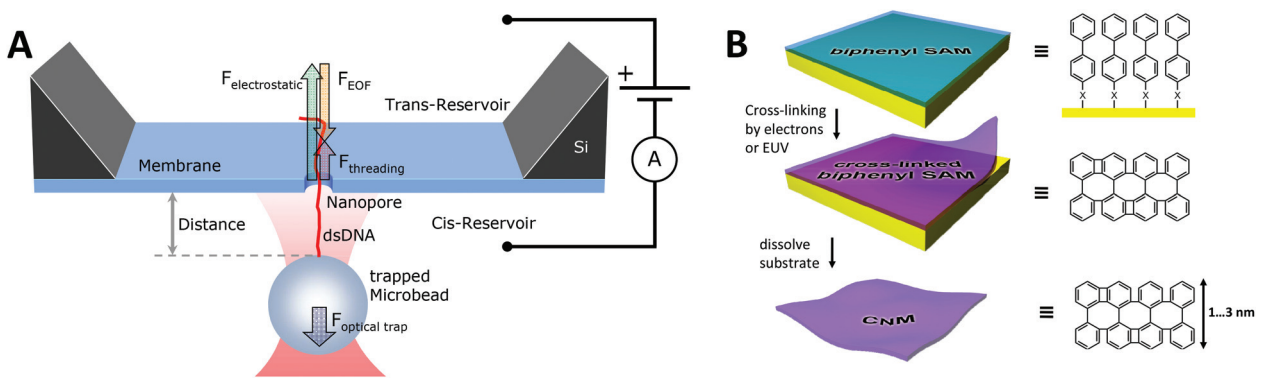

Fig. 3 (A) Experimental setup. A TEM chip contains a silicon-nitride, a lipid-coated or a CNM with a nanopore through which a single dsDNA molecule is threaded. The DNA is immobilized on the microbead and experiences a threading force, which is balanced by the optical trapping force holding the microbead. The threading force itself is composed of a strong electrostatic force acting on the charged molecule inside the pore that is opposed by a force induced by the electroosmotic flow (EOF) through the pore. (B) A CNM is fabricated by a self-assembled monolayer (SAM) on a gold surface that has been cross-linked by electron radiation and subsequent dissolving of the gold substrate.

setup[22] were performed in a buffer solution containing 20 mM KCl and 2 mM Tris/HCl at pH 8.0.

We coated the silicon-nitride membrane with a lipid bilayer by preparing small unilamellar vesicles (SUV) composed of 99.2 mol% POPC (1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine) and 0.8 mol% DOPE, introduced them into the sample chamber and let them burst on the hydrophilic silicon-nitride membrane where they merged into a complete lipid bilayer.[7,12] Subsequently, we confirmed successful bilayer coating of both the membrane and the NP wall by fluorescence recovery after photo bleaching by adding 0.8 mol% DOPE labelled with Rhodamine B as fluorescence marker to the lipid before SUV formation. As additional evidence, we monitored a reduced NP conductivity compared to the uncoated pore, which is caused by NP diameter reduction due to the lipid-coated pore walls. Finally, dsDNA molecules were threaded through the coated NP under identical salt and pH conditions that were used for uncoated NPs.

CNMs are novel, artificial, organic, polymerized membranes that were fabricated by self-assembling aromatic molecules on inorganic surfaces and subsequent electron irradiation[16,17] (see Fig. 3B). Here, we used a 3 nm thin cross-linked biphenyl-thiol CNM that was transferred onto a silicon-chip with 500 nm thick silicon-nitride membrane which contained a single 7 μm micropore (see upper right inset of Fig. 1B). This freestanding CNM covering the hole was mounted into the sample chamber, rinsed with buffer solution and confirmed as to be a gigaohm seal. Afterwards, we drilled a 60 nm NP into the freestanding CNM by helium-ion microscopy as described before[14] and performed DNA threading experiments under the conditions as described above. Due to the small thickness and the reduced overall membrane stability of this novel nano membrane it was technologically difficult to perform such an experiment. Therefore, only one data point for CNM is reported.

Theoretical methods

We developed an approach which is based on the coupled Poisson, Nernst–Planck and Stokes equations.[7,23,24–26] Concerning the dsDNA surface, we did not impose no-slip boundary conditions *a priori*. Rather, only the introduction of a finite slip length l_{slip} at the DNA surface led to a good quantitative agreement with all experimental results. For a comprehensive description, see Galla *et al.*[7]

Conclusion

We successfully demonstrated DNA threading through NPs imbedded in three different membrane materials, namely silicon-nitride, lipid bilayer-coated membrane, and CNMs as a new class of membrane material, thus substantially expanding our previously published data base. In general, a strong increase of the measured threading force could be observed upon decreasing the NP diameter, which we attribute to the strong reduction of the EOF. Interestingly the measured forces of the CNM NP indicate a theoretically predicted surface charge comparable to that of silicon-nitride. However, due to the CNM thickness of only 3 nm, the EOF is reduced, which in turn increases the threading force by 15%. Moreover, the electrical neutral lipid bilayer-coated membrane strongly suppresses the EOF, leading to an 85% increased threading force. This finding was quantitatively described by our model that includes a hydrodynamic slip effect on the translocating charged molecule (dsDNA). These results suggest that the choice of membrane material or coating is fundamental for the further improvements of NP sensor applications.

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