Controlled transport of single biomolecules through nanopores

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Abstract:
Different types of nanopores are nowadays used for the detection of single molecules in solution. This detection is possible due to the changes in the ionic current established between two electrodes when a molecule is partially blocking the entrance of the nanopore. In this work, we have performed translocation experiments of single DNA molecules using glass nanocapillaries as molecular sensors. The obtained results show that using glass nanocapillaries we are able to determine the cross-section of the DNA double helix passing through the nanochannel and distinguish different molecular configurations. Analyzing the kinetics of the translocation process, we are able to describe it as a voltage-induced thermally activated process that follows an Arrhenius behaviour.

I. INTRODUCTION

Novel nanopore sensing techniques are recently being developed in order to detect single molecules and investigate their properties. These techniques are based on the idea of the Coulter counter [1]: the study of the current established between two electrodes placed at different reservoirs filled with a salt solution, only connected by a small hole. First Coulter counter experiments were done with micro sized pores to detect single cells. Nowadays, reaching the nanometer scale allows us to detect single molecules [2].

Different types of nanopores have been used for the detection of small molecules: biological nanopores [3], solid-state nanopores [4] and glass nanocapillaries [5]. Biological nanopores with diameters of about 1-2nm are currently used to sequence biomolecules, such as single-stranded DNA [6]. Solid-state nanopores appeared as a complementary technique with holes ranging from two nanometers to tens of nanometers. The main advantage with respect to the biological ones is the fact that they are much more stable due to its robust geometry [7]. Nevertheless, these nanopores are expensive because of their fabrication costs. A cheap alternative to solid-state nanopores are the glass nanocapillaries. They can be easily produced with pore sizes between tens and hundreds of nanometers [8].

In this work, we have studied the translocation of a long linear double-stranded DNA (dsDNA) through glass nanocapillaries ranging from 50 to 80nm, when an external electrical potential is applied. We have demonstrated that the translocation takes place when one end of the molecule enters to the nanocapillary due to the electrophoretic force. Moreover, we have determined the effective diameter of the molecule blocking the current, 2.1 ± 0.6nm, which coincides with the diameter of the double-stranded DNA (dsDNA) helix measured in crystallographic studies, proving that the molecule is stretched during the translocation process. Finally, we have studied the kinetics of dsDNA translocation through the pore and estimated the kinetic barrier and the distance to the transition state.

II. METHODS AND MATERIALS

A. Microfluidic chambers

To perform translocation experiments we have manufactured our own microfluidic chambers (see **Fig 1.a**) using two PDMS (Polydimethilsiloxanne) layers prepared at a mixing ratio of 1:10 (one part of curing agent per 10 parts of base), a glass nanocapillary and a glass microscope slide (Menzel Glaser, GmbH, Germany). Two PDMS layers of different thickness have been used: one of them of 1mm, containing two separated pools, and a second one of 0.6mm used as a cover. Nanocapillaries have been built using a laser assisted pipette puller (P-2000, Sutter Instrument) and quartz glass tubes with outer and inner diameters of 0.5and 0.3mm, respectively (Hilgenberg GmbH, Germany). Using the pipette puller protocol described in **Tab 1,** we obtained nanocapillaries with tip hole sizes between 50 and 80nm , that were used to connect the two pools (**Fig 1.b**).

Heat	Fil	Vel	Del	Pull
480	0	30	170	200

TABLE 1: Pulling protocol to obtain nanocapillaries with holes between 50 and 80nm using the glass tubes (Hilgenberg GmbH, Germany). The values from the table have not specific units, but refer to the Heat applied to the glass capillaries (Heat), the pulling velocity (Vel), the delay between the heating and the pulling (Del) and the pulling force (Pull).

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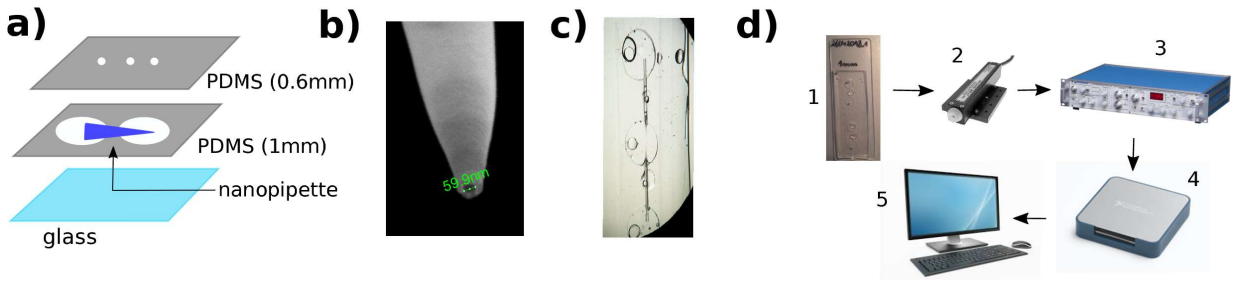

FIGURE 1: **a)** Scheme of the microfluidic chambers used for the experiments. **b)** SEM image of the tip of a nanopipette. To prevent charging effects, the pipette was coated with a 5nm gold layer. **c)** Photograph of a microfluidic chamber with two different channels. **d)** Scheme of the electrical setup where 1 is a microfluidic chamber, 2 the preamplifier of the Axopatch instrument, 3 the Axopatch 200B instrument, 4 the National Instruments digidata and 5 the computer used for the data recording. The microfluidic chamber (1) and the preamplifier (2) were placed inside a Faraday Box.

In order to assemble the whole microfluidics chamber we have used oxygen plasma techniques (Expanded plasma cleaner PDC-001, Harrick Plasma) to attach both the PDMS layers and the glass, and UV glue (NOA61, THORLABS) to fix the nanocapillary and make sure that the only connection between the pools was through the nanochannel. A picture of a microfluidics chamber is shown in **Fig 1.c**. Finally, two holes have been drilled in the PDMS cover slide to fill the chamber with the salt solution and place the silver/silver chloride (Ag/AgCl) electrodes.

B. Electrical setup

Electric measurements have been done using an Axopatch 200B instrument (Axon Instruments, USA), which is an ultra low noise amplifier, currently used for ionic current measurements. The Ag/AgCl electrodes placed inside the pools of the microfluidic chamber were directly connected to the preamplifier of the Axopatch instrument.

The current-time signals were obtained at a sampling frequency of 250kHz and filtered by the Axopatch with an intern Bessel filter at 10kHz. To minimize the electrical noise that could affect our measurements, we have placed the microfluidic chambers in Faraday box. The digitalization of the signal has been done using a National Instruments digidata. A LabView program was used to monitor and record the intensity traces. A simple scheme of the experimental setup is presented in **Fig 1.d**.

C. Buffer and samples preparation

The chambers used for the experiments were filled with a 1M KCl, 10mM TRIS and 1mM EDTA solution. TRIS helps maintaining the pH of the solution, while EDTA prevents the degradation of molecular samples. In this work, we have used a 1.6nM solution of complete λ-DNA phage, 48.5kbp, supplied by Sigma-Aldrich.

III. ANALYSIS AND RESULTS

Three different chambers with nanopore sizes of 51, 62 and 73 ± 5nm (determined from SEM images) filled with 1M KCl buffer solution have been used for the λ DNA translocation experiments. In order to effectively fill the nanopipettes, we have established an oxygen plasma cleaning treatment protocol consisting in applying plasma for 3 minutes to the chamber completely assembled before filling it. This kind of treatment makes the PDMS and the glass surfaces hydrophilic and facilitates the nanopipettes and microfluidics chamber filling.
In order to check the validity of the filled microfluidics chamber we performed two tests: (i) we studied the Ohmic response of the chamber at different applied voltages, that should follow a linear relation (see **Fig 2.a**), and the resistance estimated from the slope should be around 10MΩ for capillaries of around 50nm of diameter [9] and around 6MΩ for capillaries of around 70nm (ii) we measured the stability and noise level of the current signals. The measured resistances for the nanopores tested here ranged between 6 and 10MΩ.

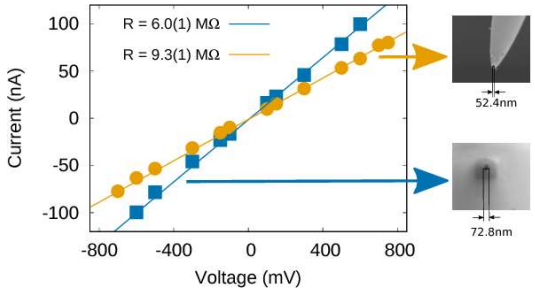

FIGURE 2: Current – Voltage curves for two different nanopores with resistances of 6.0 and 9.3MΩ in blue and yellow respectively. In the SEM images these resistances correspond to nanopore diameters of 72.8 and 52.4nm, respectively.

Considering the Johnson-Nyquist noise equation the expected thermal noise for those resistances was between

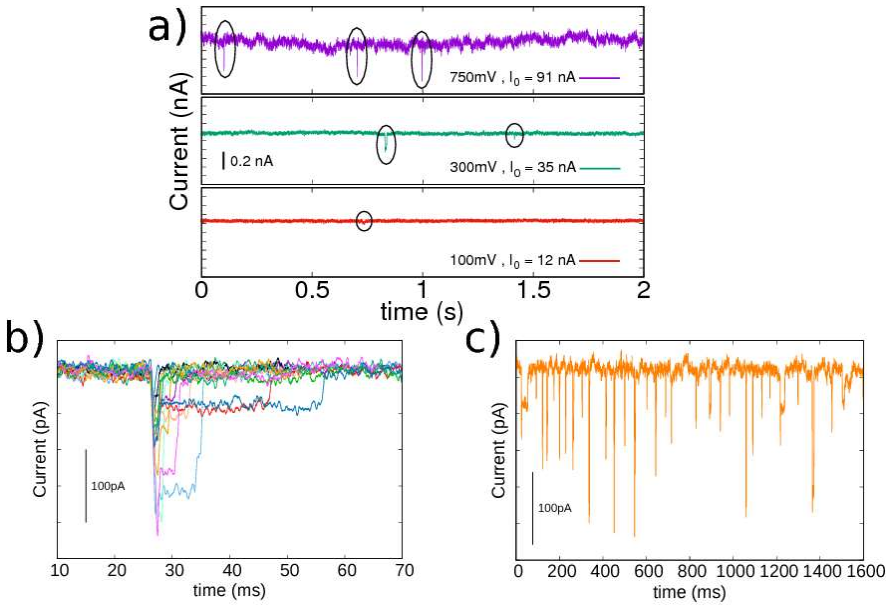

Figure 3: a) Current – time traces at different applied voltages, 150 (bottom), 300 (center) and 750mV (top). Dark circles indicate where the different translocation events took place. **b)** Superposition of different translocation events at 300mV. **c)** Translocation events at 300mV concatenated in time.

4 – 5pA [10]. In our case, we measured noise levels around 10pA, which might indicate that either the pore cannot be modelled as a simple resistance, or that some sources of external noise were not properly screened by the Faraday cage. Nevertheless, in our experimental conditions translocation events have been detected with such noise level.

We next refilled the chamber with the buffer containing the λ-DNA sample and recorded the current-time signal at a constant voltage. Sudden and transient drops in the ionic current appeared, corresponding to dsDNA translocation events through the nanocapillary. In order to further investigate this phenomenon we performed experiments at different applied voltages, between 150 and 750mV (see traces in **Fig 3.a**).

A. Size analysis

When a molecule passes through the tip of the glass nanocapillary a drop appears in the current signal (see **Fig 3**). In this case, we can distinguish different current levels, I_0 and I_b. I_0 is the current baseline measured when there is not any molecule passing through the pore, and I_b is the blockage current measured when a molecule is partially blocking the nanocapillary entrance. Going in deep, in **Fig 3.a** it can be observed that the blocking event is proportional to the applied voltage. Highlighted with black circles we can see different translocations in real current – time traces.

In **Fig3.b-c** different measured translocations at 300mV with a resistance of around 8MΩ are presented in two different ways: (**b**) few translocation events are superposed in order to see the variability in the duration time and in the blockage current level. (**c**) Several translocations can be seen arranged in time to see the different current levels presented in another way.

At a given voltage we recorded at least 300 translocation events for each microfluidic chamber. In **Fig 4.a** a zoom – in of a single translocation event is presented. For each translocation event we define three different parameters: I_0, I_b and t_{trans}. These parameters are the ones that will be studied in this work.

We have studied the variability in the blocking current events, I_0-I_b, and we have found a wide distribution that is presented in **Fig 4.b**. This distribution has been fitted to the sum of different Gaussians with peaks at ~80, ~130, ~230 and ~370 ± 10pA for the smaller pore we had (corresponding to the bigger resistance, 9.3MΩ) at 300mV. These different peaks have been related to different molecular conformations [11]. In our case, we have been able to classify mostly all our traces in three groups corresponding to different molecular conformations: single unfolded molecules, folded molecules (or groupings of two molecules translocating at the same time) and hooked molecules, with two different blockage current levels (**Fig 4.c**). Note that the main peak is the one at the lower current level, which corresponds to the stretched DNA conformation.

As a first approximation, we can assume the blockage current to be proportional to the cross-section of the entrance of the nanochannel. As a consequence, when a molecule passes through the entrance of the nanochannel,

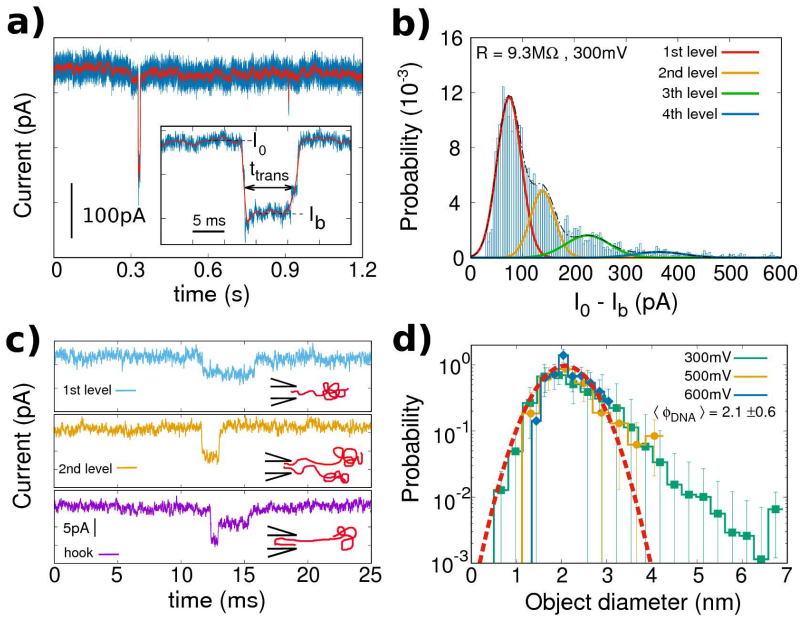

FIGURE 4: **a)** Example of a current time trace recorded where jumps in the ionic current can be clearly seen when a molecule is passing through the nanocapillary. **b)** Drop level distribution of the different translocation events recorded at an applied voltage of 300 mV with the smaller nanocapillary which corresponds to the bigger resistance. **c)** Different types of events that correspond to 3 different molecular configurations (single molecule, multiple molecule and hook). **d)** Distribution of the molecular thickness obtained for applied voltages of 300, 500 and 600mV.

That means that big molecules invade larger sections and produce larger drops in the ionic current. Following this idea, one can relate the magnitude of the blockade current levels to the diameter of the pore and the thickness of the molecule [12]:

$$\frac{I_b}{I_o} = 1 - (\frac{a}{d})^2 \qquad \textbf{(1)}$$

Where I_0 and I_b are the previously defined current levels, a is the effective diameter of the molecule and d is the diameter of the pore. Using this equation and the corresponding values for the different pores, we have obtained a molecular diameter of 2.1 ± 0.6nm (see **Fig 4.d**). This value is in agreement with the known value for the B-form of dsDNA.

B. Kinetics analysis

Single molecule translocation process can be understood as a voltage-induced thermally activated process. Activated processes exhibit in general an Arrhenius behavior: an exponential dependence between the kinetic rate and the activation energy (**eq. 2**). The kinetic rate is defined as the inverse of the mean translocation time, which is the time the molecule is partially blocking the entrance of the nanopore (see **Fig 4.a**).

$$k(V) = k_0 e^{\beta Q V} \qquad \textbf{(2)}$$

Equation 2 relates the measured kinetic rate (k) from the current-time traces with the applied voltage (V). k_0 is the kinetic attempt at zero voltage, Q is the distance to the transition state and β is the inverse of the product between the Boltzmann constant and the temperature.

From the experimental current traces we have measured the translocation time, t_{trans}, for several translocation events, about 300 events per nanopore and voltage. As expected for a first order kinetics, the distribution of t_{trans} followed an exponential behavior. Fitting an exponential function of the type $(\frac{1}{k(V)})e^{-k(V)\cdot t_{trans}}$ we found directly the value of the kinetic rate at a given voltage (see **Fig 5.a**).

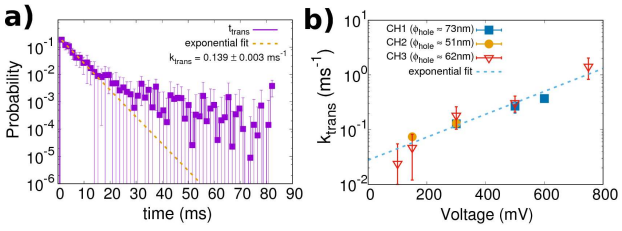

Figure 5: a) Translocation time distribution at 300mV for a pore of 50nm of diameter. **b)** Kinetic rate as a function of the applied voltage for the three different studied pore sizes.

The results for the kinetic rate as a function of voltage for the different capillaries are shown in **Fig 5.b**. As expected, we observed that the translocation process follows an Arrhenius behavior for all the different

nanopores. Moreover, it is important to point out that the observed kinetic rates are independent on the nanopore size, or at least, they do not vary for changes about 20nm on the nanohole diameter. The kinetic barrier measured for those pore sizes is $19 \pm 1 s^{-1}$ and Q is 0.15 ± 0.02 e^-. The attempt rate measured for those pore sizes is 19 ± 1 s^{-1} whereas the charge to the transition state turns out to be very small and close to Q is 0.15 ± 0.02 e^-.

IV. CONCLUSIONS

We demonstrate that using nanocapillaries with hole sizes 50 – 80nm linear dsDNA, 48.5kbp, tends to enter to the nanohole completely stretched by one of its extremities. It has been proved using the existing relation between the measured blockage current and the cross – section of the translocating molecule. Studying in detail the shape of the different current blockage events we distinguished four different molecular translocating configurations: individual stretched configuration, multiple molecule configuration, hook configuration and knot configuration. Furthermore, we have characterized the kinetic behavior of the translocation process, finding that it can be described as a voltage-induced thermally activated process that follows an Arrhenius behavior which is independent on the pore size for our range of nanohole diameters. One of the most intriguing results of this study is the reported small value of the charge to the transition state Q that is about one tenth of the electron charge. What is the physical significance of such a small charge? One might think that once a small segment of the DNA approaches the hole then the whole polymer is pulled away across the nanohole. The strong electrostatic repulsion between the glass nanohole at the tip of the nanopipette and the negative charge of the DNA backbone is such that the proximity of just a tiny fraction of the DNA phosphate charge to the nanohole is sufficient to pull the whole polymer through the nanopipette. In this regard it might be interesting to further investigate this effect by measuring Q as function of the entrance geometry of the DNA to the nanopore (**Fig 3.c**). This and other questions will be the subject of future work.

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