# DNA translocation through nanopipettes in crowded media

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**Abstract:** DNA translocation through nanopores constitutes a powerful method to study biomolecular behavior at the single molecule level. The objective of this work has been to study the effect of macromolecular crowding using PEG 8k on the translocation of double-stranded DNA through nanopipettes. These results show that crowded conditions and the direction of translocation are factors that strongly influence the dynamics of DNA translocation in nanopore systems.
**Keywords:** DNA, translocation, nanopipette, crowded media, biophysics, microfluidic chamber
**SDGs:** This work is related to SDGs 3, 4, 9 (see page 6)

## I. INTRODUCTION

During the last decades, the study of the transport of biomolecules through nanopores has proven to be a challenge. These nanopores are holes of a few nanometers in diameter (less than 100 nm) that enable the detection of single molecules, like DNA molecules. There are biological nanopores, solid-state nanopores and nanopipettes, and they can be used to study DNA translocation events. This is the process by which the DNA molecule is transported through a nanopore due to an electric field. Studying how DNA translocates allows for progress in the study of important phenomena such as DNA sequencing, thus allowing a better understanding of the genetic code. DNA translocation is also studied for bacterial and viral infections for the diagnosis of genetic diseases. Furthermore, it can help to improve the understanding of biological processes such as DNA replication and reparation.

In the late 1990s, it was first thought of nanopores as DNA sensors for sequencing and genomics applications [1]. Later in 1996 Kasianowicz and co-workers reported the first experimental results of DNA sequencing using a biological nanopore [1]. Since then, the study of DNA through nanopores has remained a subject of high scientific interest, although with many unresolved questions. For example, the behavior of these translocations has not been extensively studied in crowded environments. Therefore, it is of the utmost importance to mimic these media as we want to simulate the cell cytoplasm as best as possible. This complex intracellular environment is composed of macromolecules such as proteins, nucleic acids, lipids, carbohydrates and small solutes, resulting in an occupied volume of 30% to 40% [2]. These highly concentrated environments make it harder to study the transport of biomolecules through nanopores and their dynamics and kinetics.

To address this challenge, recent studies [3, 4] mimic the intracellular environment using polyethylene glycols (PEGs) of different molecular weights. It was concluded that when increasing the concentration of PEG 4k in the saline solution, from 10% to 40% (w/v), both the frequency of translocation events and the dwell time, which is the time a molecule spends inside the nanopore as it translocates through it, tend to increase [3]. To corroborate these results, I have conducted translocation experiments in crowded media too using PEG 8k. PEG is a usual crowding agent, water soluble and chemically inert polymer that occupies volume of the solution.

The objective of this work was to study whether the presence or absence of macromolecular crowding in the medium affects DNA translocation through nanopores. In this work, we have used nanopipettes inside a microfluidic chamber as a cell mimicking environment. We used quartz capillary nanopipettes, which function as solid-state nanopores and offer advantages over biological nanopores (such as protein-based pores) because they are cheaper, mechanically stable, and easier to fabricate and integrate into microfluidic systems [5]. To simulate crowded cytoplasmic conditions, we introduced 10%, 25% and 50% (w/v) of PEG 8k into salt solutions containing 0.5 M and 2 M NaCl.

To detect the DNA translocations, we located the microfluidic chamber inside a Faraday cage and applied a potential difference. Then, an electric field was created at the tip of the nanopipette, and the DNA was enhanced to enter due to its negative charged character. In this process, the nanopore is partially blocked, resulting in a reduction of the ion current. Moreover, the drag force and the electrophoretic force cancel each other out [6]. Recording these results, DNA translocation properties such as dwell time, interevent frequency, or the current blockade amplitude can be determined in different media conditions.

Therefore, the aim of this work is to determine how DNA translocation events are affected by the environment they take place, whether it is a crowded or non-crowded media, using the microfluidic chamber set up with nanopipettes.

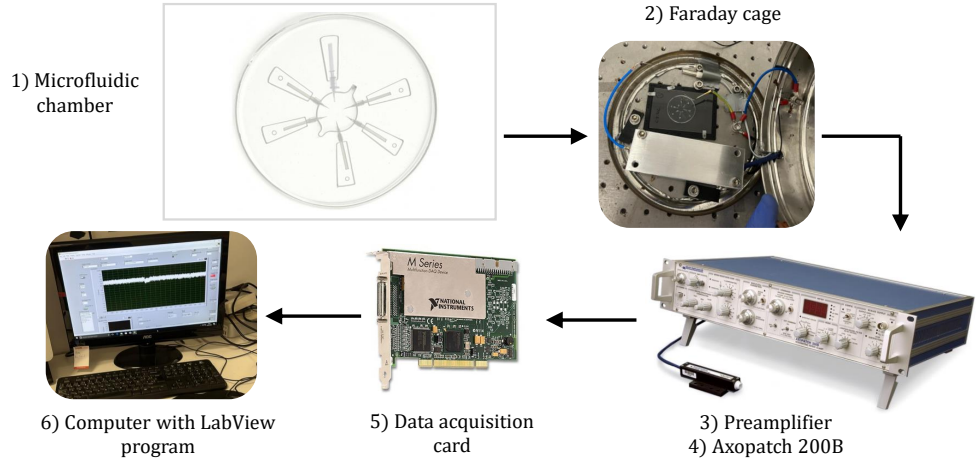


FIG. 1: Schematic representation of the microfluidic chamber and the electrical setup used for the translocation experiments.

## II. EXPERIMENTAL SETUP

### A. Nanopipettes and microfluidic chamber preparation

To fabricate the microfluidic chamber, we took a PDMS (polydimethylsiloxane) chip that had been fabricated by curating the PDMS in a mold, with six lateral chambers for the nanopipettes, one central chamber and holes to connect the electrodes (see Fig. 1).

The nanopipettes were fabricated from quartz capillaries with the P-2000 laser-based pipette puller from Sutter Instruments, then cut and placed in each chamber with the tip facing the central chamber. The capillaries were 5cm long, with a 0.2 mm inner diameter and 0.5 mm outer diameter. The optimal range for the diameter of the nanopipette tip was 20-50 nm. $\lambda$-DNA molecules have a diameter of 2 nm approximately, so a nanopipette with a tip diameter that allows the entry of dsDNA and partially blocks the nanopore is needed to detect a current blockade in the signal.

To attach the PDMS chip to a glass substrate, we place both in an oxygen plasma cleaner and then bring them together, as the plasma treatment binds the PDMS to the glass. Then, the nanopipettes were fixed with liquid PDMS. Finally, the chambers were filled with the desired solution, and after at least 1 hour in the refrigerator, we could proceed with the electrical measurements.

### B. Buffer preparation

For measurements in the microfluidic chamber without crowder, we used solutions containing 2 M NaCl and 0.5 M NaCl, 10 mM TRIS, 1 mM EDTA and pH of 7.5. NaCl allows us to measure ionic current through the nanopipette, TRIS helps to mantain the pH of the solution and EDTA stabilizes the solution. For the experiments with crowder, we added concentrations of 10%, 25%, and 50% (w/v) of PEG 8k to the previous 0.5 M NaCl solution. In the side chambers, we added the solution without DNA, and in the central chamber, the desired solution with DNA in a proportion of 100 $\mu$L of solution and 1 $\mu$L of DNA. In this project, we used a sample of double-stranded (ds) $\lambda$-DNA molecules of 48.502 base pairs (bp). The sample also contained DNA fragments shorter than the $\lambda$-DNA molecules due to degradation.

### C. Electrical setup

To measure the ionic current and thus calculate the resistance of each nanopipette, we worked with the setup shown in Fig. 1. First, we introduced the microfluidic chamber, already filled with the solution we wanted to study, inside a Faraday cage. This is done because the ionic current is in the range of nA and thus external signals are avoided. Inside the cage, we have two Ag/AgCl electrodes to apply voltage between 0-1000 mV. We then connect the lateral chamber of one of the nanopipettes and the central chamber to the positive (anode) and negative (cathode) electrodes, respectively. The $Cl^-$ ions will be attracted to the anode and the $Na^+$ ions to the cathode. These electrodes are connected to a preamplifier that sends the signal to the Axopatch 200B, which amplifies the signal and minimizes noise. The data is then sent to the data acquisition card connected to the computer.

Finally, the I(t) data of each measurement is plotted in a live computer Labview app. This is how the resistance of each nanopipette is calculated (R = V/I) to determine if any are within the optimal range for observing translocation events.

If we obtain optimal resistance, between 20 and 100 M$\Omega$ for the 2 M NaCl solution and between 80 and 400 M$\Omega$ for the 0.5 M NaCl solution, indicating that we have a nanopipette with a diameter tip between 20 and 50 nm, we then fill the central chamber with the solution with

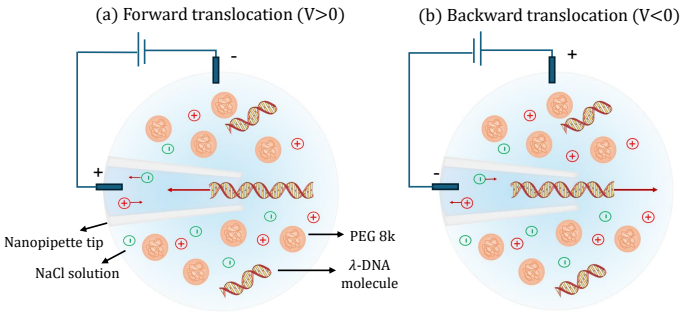


FIG. 2: Representation of the central chamber of the experimental setup. (a) Forward translocation. The DNA molecule translocates from the crowded medium to the non-crowded medium. (b) Backward translocation. The DNA molecule translocates from the non-crowded medium to the crowded medium.

λ-DNA, observe if translocation events occur and record them. As can be seen in Fig. 2, we applied a positive voltage for forward translocation events in which the DNA molecule enters the tip, from the central to the lateral chamber. Then we applied a negative voltage for backward translocation where the DNA molecule translocates from the lateral to the central chamber.

## III. RESULTS AND ANALYSIS

For each case studied, we obtained an I(t) graph, with which we could determine the base current $I_b$ that flows through the nanopipette and the blockade current $\Delta$I when the DNA molecule or single fragments block the nanopore. We also determined the resistance of the nanopipette. From these data, the characteristic parameters of each event can be obtained: the dwell time (the time the DNA blocks the nanopore), the translocation frequency and the current blockade. Later, these data were analyzed with a Matlab program that differentiated between the λ-DNA molecules and the DNA fragments, which could be in the λ-DNA sample and have much smaller dwell times.

It is important to mention beforehand that before filling the chambers with the solution and DNA, we studied the I(t) characteristic without DNA present in both crowded and non-crowded solutions to be sure that no other element besides DNA caused translocations. In both cases, we did not observe anomalous behavior. Although the PEG affected the measured electrical signal, increasing the signal noise, it did not cause current drops.

### A. Translocations in non-crowded media

As the first experimental phase, we studied the DNA forward translocations in a non-crowded media of 2 M NaCl, without PEG in the medium. The nanopipette studied had a resistance of 27.6 MΩ. We then applied 500, 750 and 1000 mV to the nanopipette and for each case a minimum of 500 events were recorded. A sample of these events can be observed in Fig. 3.

When the applied potential is increased to a nanopipette, the event mean frequency, the base current, and the current blockade amplitude increase progressively. This occurs because the negatively charged DNA molecule experiences an electrophoretic force due to the electric field induced inside the nanopipette tip. As the applied potential increases, the electric field increases, thus favoring the entry of DNA molecules into the tip of the nanopipette, and therefore, the frequency of events increases. However, the dwell time decreases when applying higher voltages. This happens because the DNA translocates faster when the voltage increases, thus having a shorter dwell time. It is also important to mention that the voltage does not change the confined configuration in which DNA translocates through a nanopipette [6].

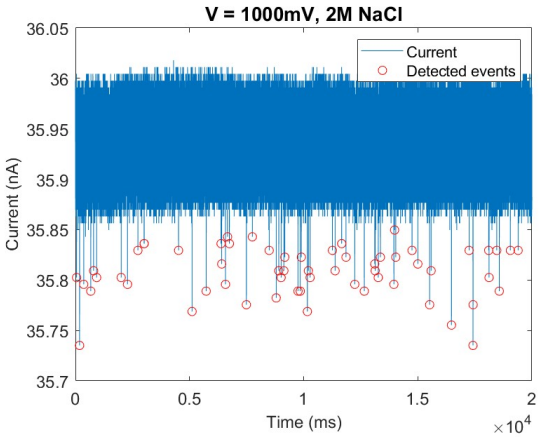


FIG. 3: Ionic current measured in 2 M NaCl solution for 1000 mV applied to the nanopipette. The downward peaks circled in red represent the dsDNA translocation events found with the Matlab algorithm.

### B. Translocations in crowded media

As the second experimental phase, we did translocation experiments with the solution of 2 M NaCl containing a concentration of 10% (w/v) of PEG 8k in the central chamber, and 2 M NaCl in the lateral chamber. However, no forward or backward translocation was observed, when a positive or negative voltage was applied, respectively (results not shown). Therefore, to better simulate the conditions of the previous study [4] in which translocations were observed in crowder media, we used 0.5 M NaCl to prepare the solution containing a 10% of PEG 8k. Since DNA is a negatively charged molecule, the positive $Na^+$ ions present in the solution accumulate around it, increasing the screening of the DNA's nega-

tive charges. This reduces the persistence length, reducing the molecule's stiffness [7]. This is why it can be better observed in 2 M NaCl than in 0.5 M NaCl under non-crowded conditions. However, when translocation events take place in a crowded environment at high ionic strength (2 M NaCl), no translocations are observed, but should be detected at low ionic strength (0.5 M NaCl). This is likely because in a crowded environment, the flexibility of the DNA molecule is counteracted. Crowding restricts molecular motion and increases the effective viscosity, which can hinder DNA dynamics and translocation, despite the high ionic strength.

It is important to note, that all the following experimental results in crowded media have been observed with the same nanopipette.

As shown in the upper panel of Fig. 4, at a positive voltage of 900 mV, clear differences can be observed in the ionic current through the nanopipette. In both cases, DNA is initially placed in the central chamber. For the experiment without crowder, using 0.5 M NaCl in both the central and lateral chambers, translocation events are clearly detectable. This current value (2.6 nA at 900 mV) indicates a high resistance of approximately 346 MΩ.

It can be interesting to compare the results shown in Fig. 4 with the ones shown in the upper-left panel of Fig. 5, as the experiments were made in the same nanopipette. We can observe a lower frequency of events at 900 mV but a similar blockade current. This occurs because inside the tip of the nanopipette, the electric field is more intense so a greater electrophoretic force attracts the DNA molecule from the lateral chamber to the tip of the nanopipette, enhancing the DNA capture efficiency. Additionally, the nanopipette geometry favors DNA backward translocations. However, once DNA does enter the nanopipette, it blocks the current in a similar way, in backward and forward translocation, which explains the comparable blockade current values.

However, when the central chamber contains DNA in a 0.5 M NaCl solution with 10% (w/v) PEG 8k, no translocation events were observed. This difference could be explained by the fact that PEG increases the viscosity of the solution, making it harder for DNA to move among the nanopore tip. Although without the presence of PEG, the DNA moves more freely and can easily enter the nanopipette, which is why translocation events are observed.

After not observing translocations at 900 mV with PEG in the central chamber, it was observed what happened when the voltage was reversed to -900 mV in the same nanopipette. In this way, the DNA that was now in the lateral chamber would translocate to the central chamber (from the non-crowded zone to the crowded zone), towards the tip of the nanopipette. And unexpectedly, DNA did translocate from a non-crowded to a crowded environment. The results were compared with the ones obtained in diluted solution at -900 mV for the same nanopipette. As shown in Fig. 5, translocation events were observed in both cases.

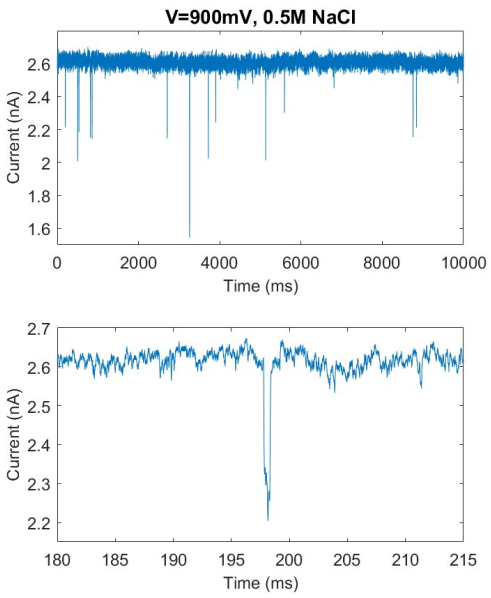


FIG. 4: Ionic current measured in 0.5 M NaCl in the central and lateral chambers, for 900 mV applied to the nanopipette. A translocation event is shown below.

The results of experiments in crowded media show the highest ionic current, with much more pronounced and frequent current drops than in the other case. Nevertheless, a higher level of noise was also observed due to the presence of PEG. Analyzing the translocation parameters, we can conclude that the mean frequency of events and the mean dwell time are higher in the crowded solution (2.4 events/s and 6.2 ms, respectively) than in the dilluted solution (1.7 events/s and 3.9 ms) in the central chamber. Strikingly, the blockade current is the parameter where the greatest difference has been observed, since the average amplitude of the current drops triples from the case under crowder (1.1 nA relative to the mean base current) to the case without crowder (0.3 nA).

One of the factors that differentiates the medium with PEG 8k from the medium without crowder is the increase in the viscosity, which results in a decrease in the translocation speed and, therefore, an increase in the dwell time of the DNA molecule through the nanopore. The volume occupied in the solution is also increased and, consequently, there is less free volume in the tip area of the nanopipette (central chamber). This can cause DNA to partially obstruct the nanopore more than in the case without a crowder, resulting in higher current blockages and longer dwell times. Additionally, it has been proved that the presence of PEG enhances the affinity of DNA for nanopipettes and helps to increase the number of DNA molecules and nanopore encounters, therefore experimenting with a greater event frequency.

Finally, we introduced the solution with PEG 8k at 25% and 50% (w/v) to the central chamber. No translocation events were observed for negative or positive ap-

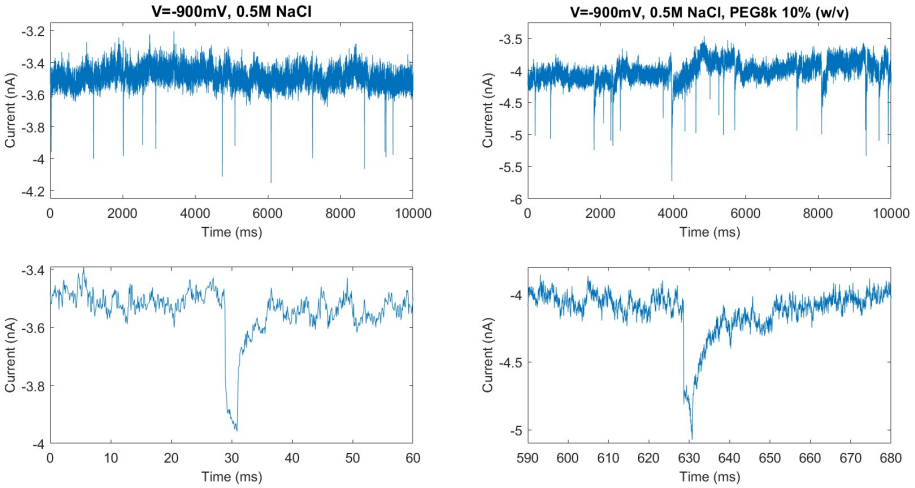


FIG. 5: Ionic current measured in 0.5 M NaCl solution with and without PEG 8k 10% (w/v) in the central chamber. A single translocation event sample for each case is shown below.

plied voltages. This effect could be due to a considerable increase in the viscosity of the medium, that would hinder DNA entering the nanopore, since the tip could be blocked by the crowding agent. Although more experimental results are needed to confirm this.

## IV. CONCLUSIONS

In this project, we have been able to observe surprising results of how the DNA molecule translocated depending on the solution these translocations took place. In the experiments in non-crowded conditions, it was observed that at higher potentials the mean event frequency and blockage current increased, while the dwell time decreased. When a 10% (w/v) of PEG 8k in the 0.5 M NaCl solution was used to simulate a crowded environment in the central chamber, the results changed significantly. With a positive voltage, no forward translocations were detected. This suggested, that the presence of PEG hinders the DNA molecule's ability to approach and enter the nanopipette. However, for backward translocations (negative voltages), translocation events began to be recorded. These were not only more frequent, but also showed longer dwell times and stronger blockade currents compared to the backward and forward translocations in non-crowded media. These findings highlight how experimental conditions, such as the composition of the medium or the applied voltage, can strongly influence the behavior of DNA during its translocation. Throughout this project, clear differences were observed, offering a clear perspective on the complexity of the process of DNA translocation when studied through nanopipettes.

### Acknowledgments

I would like to thank my advisor Isabel and Alex Colchero for the time they dedicated teaching me both inside and outside the laboratory, and for their patience and kindness over these months. I would also like to thank Félix Ritort for letting me work in the Small Biosystems Lab. Finally, I would like to thank my partner who has accompanied me throughout these academic years and has been my biggest support.

[1] Dekker, C. "Solid-state nanopores". *Nature Nanotechnology* **2**, 209–215 (2007).
[2] El-Morched, M., Barraco, C., Simionescu, R., Harroun, T., and Yan, H. "Characterization of Simulated Cytoplasmic Fluids". *Chemistry Select* **9**, (2024).
[3] Charron, M., Piguet, F., Faltova, L., and Junier, I. "Crowding-Induced DNA Translocation through a Protein Nanopore". *Anal. Chem.* **92**, 3827–3833 (2020).
[4] Chau, C. C., Radford, S. E., Hewitt, E. W., and Actis, P. "Macromolecular Crowding Enhances the Detection of DNA and Proteins by a Solid-State Nanopore". *Nano Lett.* **20**, 5553–5561 (2020).
[5] Fraccari, R. L., Ciccarella, P., Bahrami, A., Carminati, M., Ferrari, G., and Albrecht, T. "High-speed detection of DNA translocation in nanopipettes". *Nanoscale* **8**, 7604–7611 (2016).
[6] Colchero, A. "Salt-dependent DNA translocation dynamics across nanopores".(2024).
[7] Guilbaud, S., Salomé, L., Destainville, N., Manghi, M., Tardin, C. "Dependence of DNA Persistence Length on Ionic Strength and Ion Type". *Physical Review Letters* **2**, 122 (2019).

# Translocació de l'ADN a través de nanopipetes en medis crowded

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**Resum:** La translocació de l'ADN a través de nanopors constitueix un mètode potent per estudiar el comportament biomolecular a nivell de molècules individuals. L'objectiu d'aquest treball ha estat estudiar l'efecte del crowding macromolecular amb PEG 8k en la translocació de l'ADN bicatenari a través de nanopipetes. Els resultats mostren que les condicions de crowding i la direcció de la translocació són factors que influeixen fortament en la dinàmica de la translocació de l'ADN en sistemes de nanopors.
**Paraules clau:** ADN, translocació, nanopipeta, medis crowded, biofísica, cambra microfluïdica
**ODSs:** Aquest TFG està relacionat amb els Objectius de Desenvolupament Sostenible (SDGs) 3, 4, 9

**Objectius de Desenvolupament Sostenible (ODSs o SDGs)**

| | | | |
|---|---|---|---|
| 1. Fi de la es desigualtats | | 10. Reducció de les desigualtats | |
| 2. Fam zero | | 11. Ciutats i comunitats sostenibles | |
| 3. Salut i benestar | X | 12. Consum i producció responsables | |
| 4. Educació de qualitat | X | 13. Acció climàtica | |
| 5. Igualtat de gènere | | 14. Vida submarina | |
| 6. Aigua neta i sanejament | | 15. Vida terrestre | |
| 7. Energia neta i sostenible | | 16. Pau, justícia i institucions sòlides | |
| 8. Treball digne i creixement econòmic | | 17. Aliança pels objectius | |
| 9. Indústria, innovació, infraestructures | X | | |

El contingut d'aquest TFG, part d'un grau universitari de Física, es relaciona amb l'ODS 3, ja que contribueix amb l'estudi de les translocacions de l'ADN, al camp de la biomedicina i la genètica. També es pot relacionar amb l'ODS 9, fita 9.5, perquè promou la investigació científica i tecnológica, i amb l'ODS 4, fites 4.3 i 4.4, ja que contribueix a l'educació a nivell universitari.