

Polymer translocation through a nanopore: Controlling capture conformations using an electrical force

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Abstract

Solid-state nanopore sensors remain a promising solution to the rising global demand for genome sequencing¹. These single-molecule sensing technologies require single-file translocation for high resolution and accurate detection². This study uses molecular dynamics-lattice Boltzmann simulations of the capture of a single polymer chain under pressure-driven hydrodynamic flow to investigate a method of increasing the single-file capture and translocation rate. By using a model force of two oppositely electrically charged rings, single-file capture in hydrodynamic flow can be amplified from about 45% to 51.5%. This paper found that the optimal values of force location, force strength, and system pressure/flow velocity are neither too high nor too low and are roughly parabolic in shape near the apex. Thus, implementing an electrical force and optimizing these variables can result in a higher probability of threading a polymer through a nanopore in a single-file fashion.

1 Introduction

Polymer translocation through nanopores remain a relevant topic due to its occurrences in many biological and biotechnological systems¹. Nanopore-based single-molecule analysis has also shown increasing success in characterizing proteins, polymers, DNAs, RNAs, post-translational/epigenetic base modifications, protein-protein interactions (PPIs), protein-nucleic acid interactions (PNIs), biomarkers, and chemical reactions³. Perhaps, the greatest motivator for this field of research is the goal of using nanopore sensors as a cheaper and faster alternative to the conventional Sanger sequencing method for DNA sequencing⁴. The technologies used for sequencing and single-molecule detection rely on voltage differences across nanopores which result in ion currents through these pores⁵. The translocation of charged molecules disturb these ion currents with characteristic signatures which can be detected and used to sequence these molecules⁵. As the societal drive to sequence an overwhelming number of genomes has increased over recent years, so has the interest in developing nanopore sensors as the solution to this demand¹.

A limitation to using fast sequencing techniques with nanopore sensors is that folded threading of a polymer through the pore results in challenging data analysis and difficulty distinguishing different effects². This is due to the secondary current drops within the pore when translocation occurs in a hairpin conformation². Small diameter pores can alleviate this issue by forcing single-file translocation, however, it results in lower capture rates and often non-specific interactions with the pore wall⁵. Another method of increasing single-file translocation was shown by Ermann et al. by adjusting electrolyte concentration⁵. Their method of decreasing ionic strength, however, may result in a drop in sensor resolution². Recognizing this limitation, this study hopes to find a means to control the capture conformation of polymers translocating across a nanopore and increase single-file translocation.

This study investigates polymer translocation in a system driven

by hydrodynamic flow through the nanopore. The capture of a polymer by a nanopore is typically divided into two stages². Throughout drift and diffusion, the movement of the polymer is primarily dictated by drift-diffuse dynamics, however, the drag force causes the polymer to slowly drift towards the pore². Here the fluid shear is not strong enough to deform the polymer chain and the polymer can explore various conformations². As the increasing flow velocity begins to dominate the motion of the polymer, the capture stage begins². The shear becomes strong enough to deform the polymer as monomers closer to the pore experience greater drag². The capture process continues until a monomer enters the pore and translocation begins.

The flow itself in hydrodynamic driven translocation promotes single-file capture². One of the mechanisms by which this occurs is the pulley effect^{2,6}. The pulley effect occurs when a hairpin divides the polymer chain into two strands, possibly of varying length². The velocity of flow within the system increases while approaching the pore due to conservation of mass and momentum, resulting in a shear². The drag from the converging flow induces a slightly greater force on the leading monomer (the monomer closest to the pore) which propagates down each strand². As this tension propagates down each strand, it counteracts the drag of the flow². If this tension reaches the end of one strand (the shorter strand) and is not counteracted by an equal and opposite force on the longer strand, then the shorter strand begins to move faster than the longer strand². This results in polymer unraveling and extension². This mechanism favors single-file polymer capture because an extended polymer is more likely to have one of its ends captured first². It is also important to note that polymers with hairpin length of 0.4 to 0.5 of the polymer length are also slightly favored by the pulley effect².

The goal of this study is to exert an electrical force on an electrically charged polymer and magnify the impact of the pulley effect. The proposed mechanism is that by suspending the polymer in the flow before translocation, the polymer will have greater time for the pulley effect to take place and unravel. Ideally, the force would be placed within the flow where the shear is strong enough to cause tension propagation down the strands of the polymer, allowing for the pulley effect to take place. Details regarding the flow in the system and the electrical force are discussed in the Methods section. The results and effectiveness of the proposed mechanism are discussed in section 3 and the concluding statements are contained within section 4.

2 Methods

This study simulates the dynamics of a general 64-mer coarse-grained polymer molecule immersed in a Lattice Boltzmann (LB) fluid. Specifically, polymer translocation through a solid state pore that is smaller than the radius of gyration. The simulations were conducted using the open source Molecular Dynamics Software, LAMMPS⁷. The polymer model uses Finitely Extensible Nonlinear Elastic (FENE) bonds⁸. The potential of these bonds is given by

$$U = -\frac{1}{2}kR_0^2 \ln\left(1 - \left(\frac{r}{R_0}\right)^2\right) + 4\varepsilon\left(\left(\frac{\sigma}{r}\right)^{12} - \left(\frac{\sigma}{r}\right)^6 + \frac{1}{4}\right)H\left(2\frac{1}{6} - \frac{r}{\sigma}\right) \quad (1)$$

where r is the distance between adjacent monomers. The constant $k = 3_0\varepsilon\sigma^{-2}$ where $\varepsilon = k_B T$, k_B is the Boltzmann constant, and $T = 300$ K is temperature. The maximum bond length $R_0 = 1.5\sigma$, where σ is the Lennard-Jones (LJ) length scale.

Once of the means by which we measure the deformation of the polymer throughout translocation is using the radius of gyration. The radius of gyration gives a measure of the polymer shape is defined by equation 2,

$$R_g = \sqrt{\frac{1}{M} \sum_i m_i (r_i - r_{com})^2} \quad (2)$$

where M is the total polymer mass, r_i is the position vector of the monomer, and r_{com} is the center of mass position vector. The Flory radius (R_F) is defined as the radius of gyration in equilibrium. For the 64-mer polymer used in this system, $R_F = 8.6nm$

As stated above, the polymer in the system is implemented in a Lattice Boltzmann (LB) fluid. This is done using the LB algorithm implemented into LAMMPS by Denniston et al.^{9,10}. This algorithm provides both hydrodynamic forces and random thermal agitation into the system⁹. The algorithm works by solving an approximation of equation 3².

$$(\partial_t + e_{i\alpha} \partial_\alpha) f_i = -\frac{1}{\tau} (f_i - f_i^{eq}) + W_i \quad (3)$$

In the above equation, e_i are the velocities of the material as they move to neighbouring mesh points, τ is the relaxation time, f_i is the partial distribution function along $e_i = (e_{ix}, e_{iy}, e_{iz})$ and f_i^{eq} is its equilibrium value. W_i is a term that incorporates thermal fluctuations and solvent-particle interactions into the system. The lattice directions from a site on a cubic mesh are indicated with subscript i with Δx spacing^{9,11}. In our system, $\Delta x = 1$ nm and $\Delta t = 50$ fs.

The simulation box is 80nm x 52nm x 52nm with periodic boundaries in each direction. Placed at $x=48nm$ is an atomistic wall with dimensions 10nm x 52nm x 52nm. At the center of this wall is a pore with dimensions 10nm x 4nm x 4 nm which is wide enough to allow for translocation in a single-file fashion or with one fold. Both the fluid and the polymer interact with the wall. The polymer interacts with purely repulsive Leonard-Jones forces and the velocity of the fluid at the wall is zero (no-slip condition). To drive translocation of the polymer, a pressure jump is implemented at the boundaries in the x -direction(perpendicular to the pore). This creates a non-uniform flow that converges as it enters the nanochannel and diverges as it exits. The flow of the system is depicted graphically in fig. 1 and the velocity of the flow is depicted in fig. 2.

Multiple runs were conducted for each electrical force trialed in this study. Each run within a force's trial used a different initial polymer configuration and seed, however, the variables of each run are kept the same between force trials. Despite the varying initial polymer conformations, the center of mass of the polymer chain is initially placed at 4 times the Flory radius from the wall and in the central area of the box ($16 \text{ nm} < y < 36 \text{ nm}$, $16 \text{ nm} < z < 36 \text{ nm}$). An example of an initial configuration for the system is shown in fig. 3.

This study investigates implementing an electrical force as a potential method to increase the single-file translocation rate of a polymer in a hydrodynamic-driven system. A simplified model of

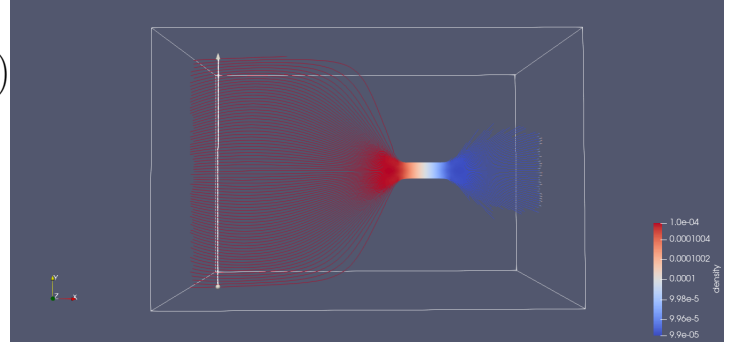


Figure 1 The flow within the system, visualized using Paraview. As depicted, the flow converges and grows stronger when approaching the pore. The flow diverges and weakens upon exiting and moving away from the pore.

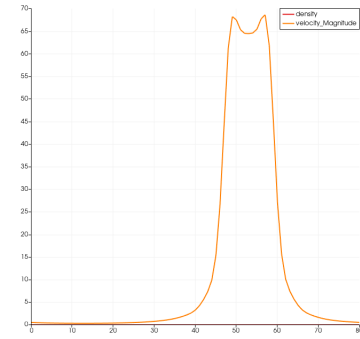


Figure 2 The x-axis represents the distance along the x-axis in the system and the y-axis represents the velocity of the fluid at the center of the box in m/s. This clearly shows the increasing flow velocity as the x-position approaches the pore and decreasing flow velocity as the x-position continues beyond the pore.

two oppositely electrically charged rings is implemented into the system. The equation for the force in the direction perpendicular to and at the center of one ring is given by equation 4.

$$E = \frac{k\lambda 2\pi R x}{(x^2 + R^2)^{\frac{3}{2}}} \quad (4)$$

By creating two oppositely charged loops at a slight distance from each other, a force is created with a profile resembling fig???. This force is implemented uniformly in the x -direction, which is perpendicular to the solid-state pore, and acts only on the polymer chain. Different variations of the force were tested using the same initial polymer conformations and seeds. These different forces and their effectiveness are discussed in the results section.

3 Results

This section will discuss the results of the various different electrical forces trialed throughout this study. A summary of the results is included in Table 1. The primary goal of these trials was to increase the single-file translocation rate from a baseline rate in pressure driven translocation. In a previous study, Afrasiabian and Denniston, using the same system, found the pressure driven single-file translocation rate to be about 45%². Thus, any improvement to this translocation rate is considered successful. Another marker of success is a more "hollow" histogram of which monomer translocates first. If more polymers translocate in a single-file fashion or with hairpins of length greater than 0.4 of the polymer length, then

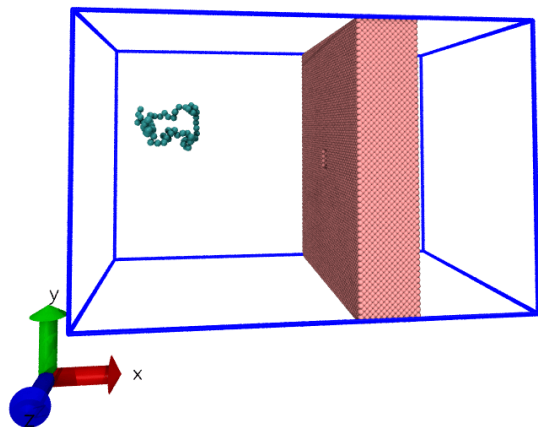


Figure 3 A graphical representation of an initial configuration of the system constructed using vmd. The blue box represents the boundaries of the system. The polymer (green) is shown on the left. The atomistic wall and solid-state pore (pink) are depicted on the right.

this would suggest that the pulley effect is having a greater effect on the polymer. This is because the pulley effect favors single-file translocation and slightly favors hairpins of 0.4 to 0.5 of the polymer length². Finally, the last marker of success we use is the extension of the polymers. These are visualized plotting the radius of gyration/Flory radius versus the distance to the pore/Flory radius. Afrasiabian and Denniston reported polymer extensions that peaked around 1.7-1.8 of the Flory radius². Extension above 1.8 of the Flory radius shows an improvement in the extension of the polymer.

3.1 Force Trials

The force-11 runs include the force depicted in fig.4 , in the x direction. This force will be used as the reference for describing the other forces. Force-11 is centered at 26.5 nm into the system. The zeros of the graph are located at $x=12.89$ and $x=40.12$. Across 33 trials, 17 runs resulted in single file translocation (fig.5). This is the most successful single-file probability tested within this study. The average translocation time was 74.87 ns, with a standard deviation of about 28.87 ns. The increase in single-file translocation rate is small, however, the histogram is subjectively "hollowed". The polymer often formed hairpins close to the ends of the polymer in these runs. This suggests that the single-file rate could be increased if translocation could be slowed before capture into the pore, allowing more time for the polymer to extend. Although these results are positive, the difference between these results and those found by Afrasiabian and Denniston² are minimal, and may be due to chance and a small sample size. These runs did show an improvement in polymer extension. Figure 6 shows that the polymer began extending at about $2 R_F$ away from the pore and extended to about $1.9 R_F$.

The force-10.2 narrow force was a narrower force that was

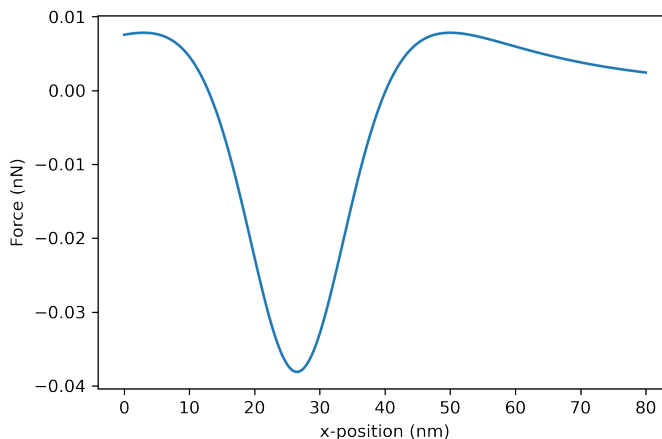


Figure 4 Force-11 is centered at 26.5, min value of 0.038, zeros at $x=12.89, 40.12$

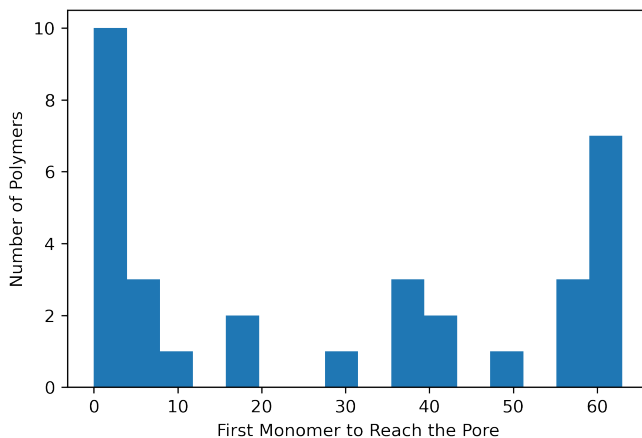


Figure 5 Force-11: histogram shows 17/33 runs, or 51.52% single-file capture rate. The histogram is notably "hollowed" where there are fewer hairpins forming in near the middle of the polymer.

moved closer to the pore as depicted in fig 7. This force also has a stronger force magnitude to account for the stronger flow near the pore. The force is centered at 39.5 nm with zeros at $x=32.11$ and $x=46.93$.

Across 10 runs, two polymers translocated in a single-file fashion. This suggests that the closer force to the pore is detrimental to single-file translocation. A possible mechanism for this result is that the stronger narrow force stalls the extending polymer too much. This causes the polymer to bunch up in the negative region of the force. This may diminish the pulley effect by counteracting the polymer extension caused by the flow. In addition to this, fig. 8 shows that the polymer extends to less than 1.75 times the Flory radius, which is less than the extension reported in a system with flow alone². It is worth noting that the polymers in these runs translocated relatively quickly, averaging about 49.58 ns between the start of the simulation and the first monomer entering the pore. This quick translocation may contribute to hairpin formation as there is less time for the polymer to unravel and extend. The faster translocation time is likely due to the positive portion of the force moving the polymer through the otherwise diffusive portion of the system (where the flow is slower) and into the portion of the system with a much stronger flow.

Table 1 This table summarizes the results for the various force trials.

Force Implemented:	Single-file Capture Rate	Average Capture Time	Standard Deviation of Capture Time
force-11	51.5	74.8	28.9
force-11x2	33.3	73.4	30.1
force-11moved	38.5	61.1	23.1
force-11movedback	38.5	65.8	23.2
force-11wide	40.0	47.6	60.5
force-11x2movednarrow	38.5	60.5	16.4
force-10.2narrow	20.0	49.6	23.4
2100forcetests	25.0	51.2	11.9
1700forcetests	30.8	81.0	27.4
1450forcetests	44.4	86.0	31.0
1100forcetests	33.3	100.8	25.3

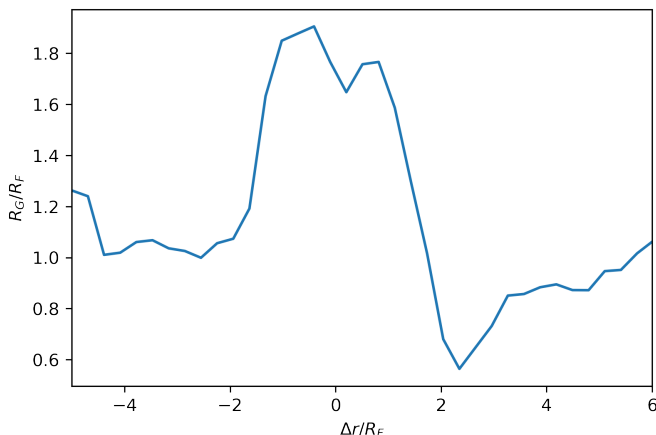


Figure 6 Force-11: graph shows the average $R_G=R_F$ vs. distance from the pore in units of $R_F=8.6$ nm. The polymer extension peaks at about 1.9 times the Flory radius, showing improved extension in the polymer.

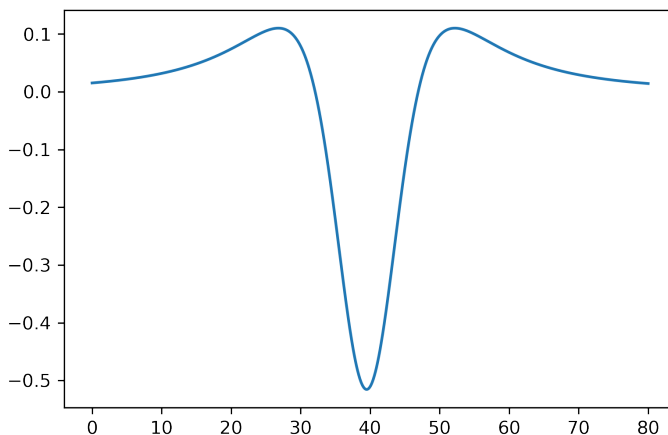


Figure 7 Force-10.2narrow: centered at 39.5, min value of 0.515, zeros at $x=32.11, 46.93$

The force-11moved runs involve a force that is the same as force-11, except it is centered at $x=35$. This force showed promising results. Although only 5/13 runs translocated in a single-file fashion, almost all the hairpins formed near the ends (within 12 monomers), or between monomer 32-40. The ends and the middle of the polymer are the areas most favored by the pulley effect. This is clearly shown in fig. 9. These results might indicate that the pulley effect had a greater influence during these runs. In addition to this, the extension of the polymer, shown in fig. 10, appears to be around the same extension as force-11. This force may be worth investigating further in the future.

The force-11wide runs include a force that is wider than force-11 and has no positive section right before the pore. Across 5 runs, two runs translocated single-file. The data appeared to show that the polymer extended as it approached the pore, however this extension was to be to a lesser extent than the Force-11 reference. This could be due to the absence of the force in the positive direction before the pore. This would mean there is less shear experienced by the polymer to exaggerate the pulley effect. Since there are too few runs, no conclusions can be confidently drawn from these runs.

The force-11x2 runs include a force that is twice as strong as the force-11 force. Only 3 of 9 runs translocated in a single file fashion, which is notably lower than the force-11 runs. The data and video analysis for the force-11x2 runs appear to show that the increased force, compared to Force-11, hinders polymer extension. It appears that the tail ends of the polymer cannot extend far from

the bulk of the polymer due to the stronger force aiding the tension along the backbone of the polymer in pulling the ends towards the bulk. This results in a more bunched up polymer which promotes capture in a hairpin conformation. This is similar to the proposed mechanism for the decreased single-file translocation rate in the force-10.2narrow runs. This mechanism is supported by fig. 11 which shows that the peak extension of the polymer is less than that of the force-11 runs (fig. 6).

This force-11moved back runs include a force that is the same as force-11 but it is centered at $x=21.5$ nm instead of $x=26.5$ nm. Out of 13 runs, 5 runs, or about 38.5%, translocated in a single-file fashion. It is possible that this force is too far back. If the force is too far back, the flow does not have enough shear for the pulley effect to take place to a noticeable extent. Thus, the force fails to amplify the pulley effect. This is a possible explanation for why the single-file translocation rate is lower than that of the force-11 runs. The lower extension can also be seen in fig. 12 where the first peak shows less extension than that of force-11 (fig. 6).

The force-11x2movednarrow runs include a force that is centered at $x=35$, narrower than Force-11, and twice as strong (graphically depicted in fig. 13). The zeros of the force are located at $x=27.6$ and 42.4 which is considerably narrower than the force-11 force. Out of 13 runs, 5 translocated in a single-file fashion. The polymer extension is also considerably lower, as shown in fig. 14, peaking at less than 1.6 times the Flory radius. Due to multiple variable changes, it is difficult to propose any mechanisms influencing this result.

The trial named 2100forcetests includes a force that is the exact

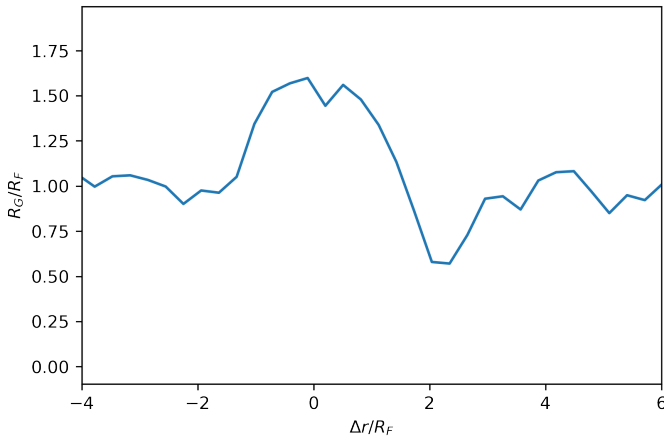


Figure 8 Force-10.2narrow: graph shows the average $R_G=R_F$ vs. distance from the pore in units of $R_F=8.6$ nm. The extension shown in this graph is less than that shown by Afrasiabian and Denniston in a system without an electric force implemented².

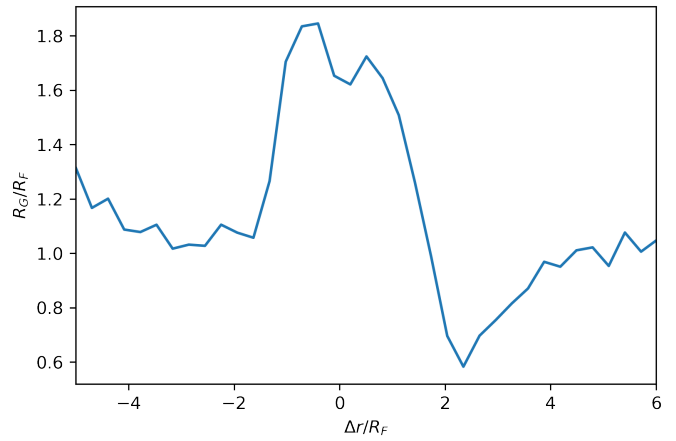


Figure 10 Force-11moved: graph shows the average $R_G=R_F$ vs. distance from the pore in units of $R_F=8.6$ nm

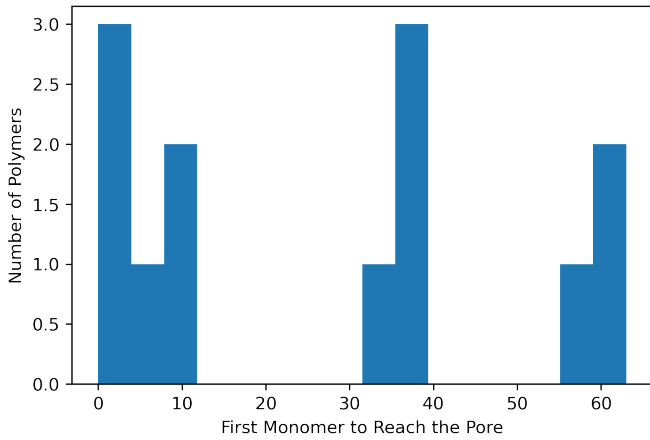


Figure 9 Force-11moved: histogram shows that 5 out of 13 or about 38.46 percent runs translocated single-file. The peaks in this graph coincide with the regions favored by the pulley effect to be captured first.

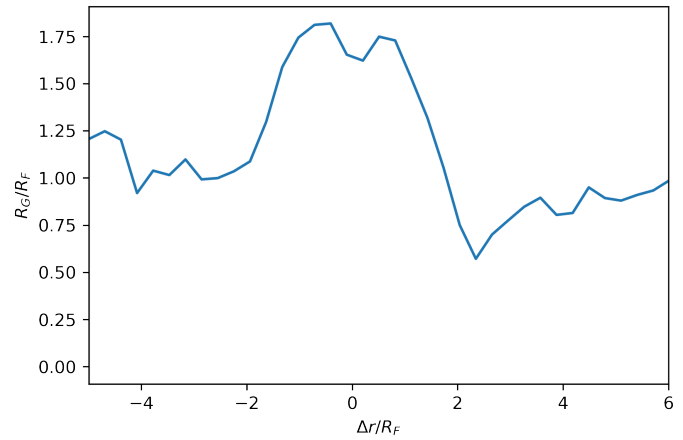


Figure 11 Force-11x2: graph shows the average $R_G=R_F$ vs. distance from the pore in units of $R_F=8.6$ nm

same as the force in the force-11 runs, but the pressure of the system is increased from 1850 to 2100. This means the pressure at the x-boundary is greater and the velocity of the flow throughout the system is faster. This would create more shear but also cause the polymer to translocate faster, which may result in the polymer not having enough time to fully unravel. Figure 15 shows that the polymer extension is similar to that of the force-11 runs (fig. 6) which makes sense due to the greater shear causing increased deformation. The low translocation time, averaging at 51.2 ns, may explain the low single-file translocation rate despite this extension as the polymer has less time to unravel. These factors may have contributed to only 2 out of 8 runs translocating single-file.

The runs within 1700forcetests also use exact same force as the force-11 runs but the pressure of the system is decreased from 1850 to 1700. This means that pressure at the x-boundary is reduced and the velocity of the flow is slower. This should result in less drag forcing the polymer towards the pore and also less shear from the converging flow entering the pore. Due to this, the translocation time of these runs were relatively longer than the translocation times of force-11 runs, averaging about 81.0 ns. Out

of 13 runs, 4 translocated in a single-file fashion. The polymer extension shown in fig. 16 is comparable to the force-11 runs (fig. 6), which is to be expected because the pressure change between these different trials is small.

This 1450forcetests runs are for the runs with the exact same force as the force-11 runs, however, the pressure of the system was decreased from 1850 to 1450. The translocation time of these runs were longer than the translocation times of force-11 and 1700forcetests runs, averaging about 86.01 ns. The lower shear in the lower pressure/flow system may be the cause for the reduced polymer extension, seen in fig. 17, compared to force-11 (fig. 6). This may also explain the lower single-file translocation rate of 4 out of 9 runs, or about 44 percent.

Based off the the previous results, we would expect similar findings in a with even lower pressure. The trials in 1100forcetests include runs with the exact same force as the force-11 runs, however, the pressure of the system was decreased from 1850 to 1100. Once again, the translocation time has increased, averaging at about 100.8 ns. In addition to this, the single-file translocation has also decreased from the 1450forcetests, with only about 2 out of 6 tests translocating single-file. Although this is a very small sample size, there does appear to be a downward trend in single-file capture as the pressure is decreased. This declining single-file capture rate is

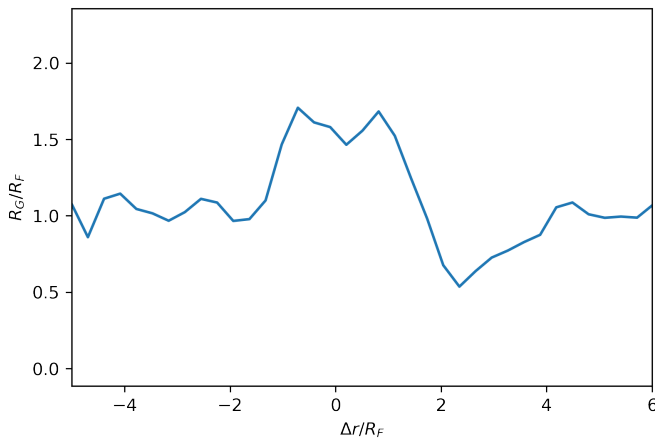


Figure 12 Force-11movedback: graph shows the average $R_G=R_F$ vs. distance from the pore in units of $R_F=8.6$ nm

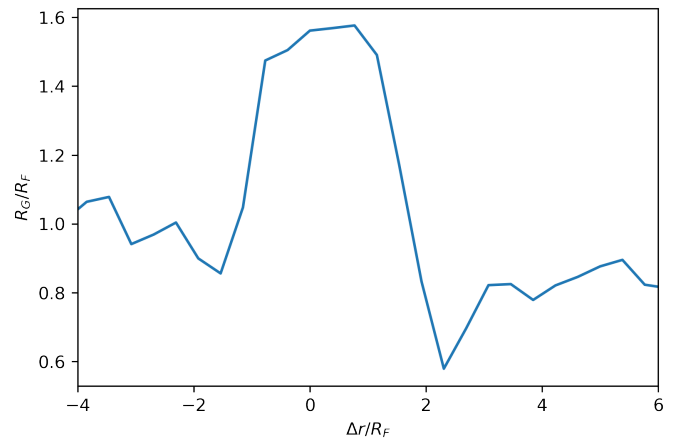


Figure 14 Force-11x2movednarrow: graph shows the average $R_G=R_F$ vs. distance from the pore in units of $R_F=8.6$ nm

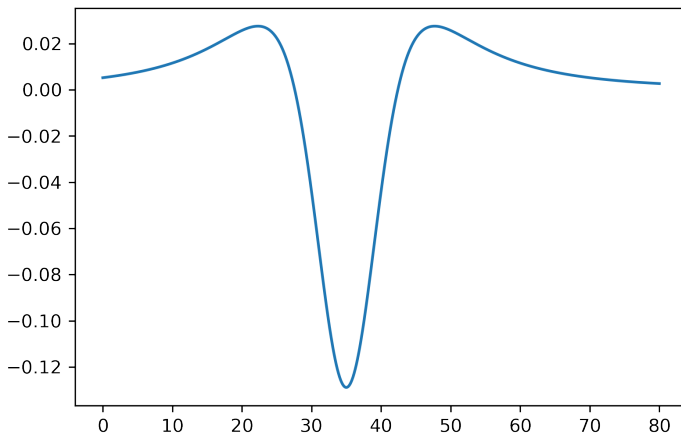


Figure 13 Force-11x2movednarrow: centered at 35, zeros at $x=27.63, 42.44$. This force is also stronger than force-11.

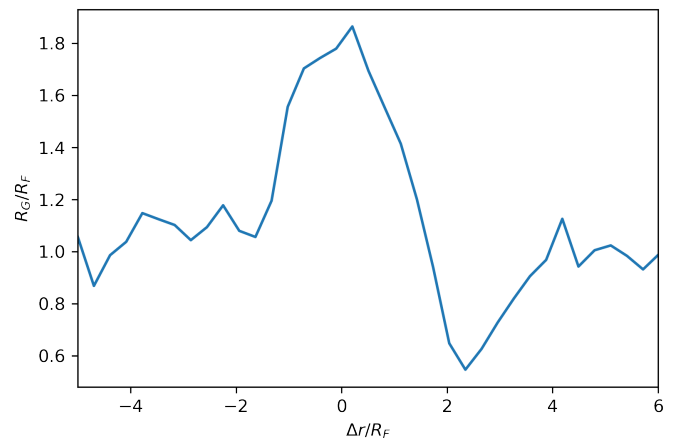


Figure 15 2100forcetest: graph shows the average $R_G=R_F$ vs. distance from the pore in units of $R_F=8.6$ nm

possibly due to the decreasing shear as the pressure in the system decreases. Contrary to predictions, however, is fig. 18, showing polymer extension to a greater extent than in the 1450forcetest (fig. 17)

3.2 Optimization

The various different forces trialed throughout this study modified various variables in attempts to determine the optimal results. The results appear to indicate a "Goldilocks effect" where there are values that are just right for the position of the force, the strength of the force, and the pressure driving the flow in the system.

Firstly, the position of the force cannot be too close or too far from the pore. If the force is too close, the polymer may not have enough time to extend and if the force is too far then there may not be enough shear in the flow for the pulley effect to noticeably take place. This is exemplified by the three trials force-11movedback, force-11 and force-11moved, and their single-file translocation rates of 0.385, 0.515 and 0.385 respectively. These numbers suggest a roughly parabolic relationship between force location and single-file translocation rate near the apex.

Next, the strength of the force must also avoid being too strong or too weak. If the force is too weak, then the effect will be too small to suspend the polymer and amplify the pulley effect. If

the force is too strong, then it may hinder the extension of the polymer as seen in some of the trials in this study. Two runs that exemplify this are force-11 and force-11x2 with single-file translocation rates of 0.515 and 0.333 respectively. This also suggests a roughly parabolic relationship between force strength and single-file translocation rate near the optimum strength value.

Finally, the pressure of the system also has an optimum value. A low pressure results in slower flow, less shear, but also slower translocation time. Slower flow and shear would decrease the influence of the pulley effect, while a slower translocation time means the polymer has more time to unravel. A high pressure results in faster flow and more shear, but faster translocation time. Faster translocation time may result in hairpins if the polymer does not have time to unravel and extend. A pressure that is not too high or low is optimal. This is exemplified by the trials, 1100forcetest, 1450forcetest, 1700forcetest, force-11(which is at 1850 pressure), and 2100forcetest. As the pressure decreases from 1850, the single-file translocation rate also decreases in the order 51.5%, 30.8%, 44.4%, 25.0%. Also, increasing the pressure from 1850 to 2100 decreased the single-file translocation to 25%. Once again, this suggests a roughly parabolic relationship between the pressure of the system (flow velocity), and the single-file translocation rate near the apex. For the system used in this study, 1850 is roughly at

