Single Polymer Dynamics in Confined Geometries: Capsid Ejection and Sedimentation

Joonas Piili
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A doctoral dissertation completed for the degree of Doctor of Science (Technology) to be defended, with the permission of the Aalto University School of Science, at a public examination held at the lecture hall F239a of the school on 8 December 2017 at 12.

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Abstract

Polymer physics has inspired scientists partly because by using fairly general statistical physics models, one can gain understanding on the most fundamental aspects of life. The polymer models, some of which are even analytically solvable, can be used to describe characteristics of DNA, RNA, and proteins to a good precision. In this thesis, we use coarse-grained simulations for studying the general dynamics of sedimentation of knotted polymers and capsid ejection. Sedimentation and electrophoresis are standard tools used in DNA research. Capsid ejection is vital in understanding how viruses of certain type function.

In sedimentation the polymer is moved through a fluid by gravitational force. We study how the knot topology of such polymers reflect the sedimentation velocity. Using a direct model that makes no assumptions of the polymer conformation or its interaction with the fluid we find that there is a linear relationship between the sedimentation velocity and the average crossing number of the knot topology to a good precision. The reason is that the radius of gyration of the knotted polymer is inversely proportional to its average crossing number. When sedimentation is modeled in a slit strongly restricting the polymer's conformation, the linear relation between sedimentation velocity and average crossing number is sustained, albeit it is not as precise as in free solvent.

In capsid ejection a polymer, initially densely packed inside a spherical capsid, escapes through a narrow pore. In this thesis, no external forces are applied, but the ejection is driven only by the polymer's internal pressure and entropy difference. We study different aspects and dynamic regimes relevant for the ejection dynamics. We find that the semidilute assumption often used to theoretically describe the ejection dynamics of flexible polymer chains is not valid when starting the ejection from strong confinement. More precisely, in strong confinement the monomer-monomer interactions become dominant. This shows in the force at the pore increasing exponentially with the number of monomers inside the capsid. This is reflected on the ejection dynamics. The cumulative waiting times, i.e., the time it takes for each monomer to eject the capsid, increases exponentially with the number of ejected beads.

Hydrodynamic interactions are always present in real-world capsids as the natural polymers reside in aqueous solutions. We characterize the effects of hydrodynamics in capsid ejection. We find that while the inclusion of hydrodynamics speeds up the ejection, the dynamic characteristics are not changed.

When bending rigidity is included in the polymer model, the capsid ejection enters a completely different dynamic regime. This shows as the cumulative waiting times scaling with the number of ejected beads when the persistence length of the polymer is sufficiently large compared to the capsid diameter.

Keywords polymers, molecular dynamics, capsid ejection, virus, sedimentation, knots
**Tekijä**
Joonas Piiili

**Väittöskirjan nimi**
Yksittäisen polymeerin dynamiikka altaissa geometrioisissa: kapsidiejktio ja sedimentaatio

**Julkaisija**
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**Käsittelemä**

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**Tiivistelmä**


Nestedyynamiset vuorovaikutukset ovat aina läsnä luonnossa esiintyvissä kapsideissa. Kuvaamme kuinka nestedyynamikka vaikuttaa ejektoon dynamiikkaan. Näätämme, että nestedyynamikka nopeuttaa ejektia, mutta ei oleellisesti muuta ejektoon dynamiikkaa piirteitä.

Taivutuspotentialina lisääminen kapsidiejektion polymeerimalliin muuttaa täysin ejektoon dynamiisit piirteet. Kun polymeerin taivutusjäykkyys on riittävän suuri verrattuna kapsin halkeaituun, kertymäodotusjat skaalavat ejektoituneiden monomeereiden lukumäärän funktion.

**Avainsanat**
polymeer, molekyylidynamiikka, kapsidiejktio, virus, sedimentaatio, solmu


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Preface

The work of this thesis was carried out in the Computational Biophysics group at the Department of Computer Science in Aalto University during 2013-2017. Until 2015 the group was a part of The Department of Biomedical Engineering and Computational Science (BECS).

I wish to sincerely thank my advisor and the group leader Dr. Riku Linna for his encouraging support, constant guidance, and substantial dedication to this work throughout the process. He has taught me a great deal about doing research and has always been generating and sharing new ideas with the whole group. I would like to thank my supervisor Professor Jouko Lampinen for his support to this thesis and appreciation for the work of our research group. I would also like to thank Professor Kimmo Kaski for supervision during the beginning of working on this thesis when the group was still a part of BECS.

I wish to thank Pauli Suhonen for being a great coworker and a friend. I wish to also thank Davide Marenduzzo for collaboration in Publication I and Jaakko Moisio for collaboration in software development in the beginning of this work.

I would also like thank to Professor Kai Nordlund and Professor Mikko Karttunen for their careful and punctual preliminary examination of this thesis.

I express my gratitude to my loving parents Mikko and Marjut who have always given me unconditional support throughout my life. Thank you Sini for being by my side and always encouraging me. I am also grateful to all of my friends with whom it has been great to do and think about everything else but this thesis.

The scholarships awarded by The Emil Aaltonen Foundation (2 years) and The Finnish Foundation for Technology Promotion (1 year) have al-
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Helsinki, November 3, 2017,

Joonas Piili
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List of Publications

This thesis consists of an overview and of the following publications which are referred to in the text by their Roman numerals.


Author’s Contribution

Publication I: “Sedimentation of knotted polymers”

The author performed all the computational work and wrote the first draft of the manuscript.

Publication II: “Polymer ejection from strong spherical confinement”

The author performed all the computational work and wrote the first draft of the manuscript.

Publication III: “Uniform description of polymer ejection dynamics from capsid with and without hydrodynamics”

The author performed all the computational work concerning stochastic rotation dynamics simulations and wrote the first draft of the manuscript.

Publication IV: “Rigidity-induced scale invariance in polymer ejection from capsid”

The author performed all the computational work concerning stochastic rotation dynamics simulations and wrote the first draft of the manuscript.

During the PhD studies, the author also contributed in publishing additional articles [1,2] and the manuscript [3], not included in this thesis.
Author's Contribution
# List of Abbreviations

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<th>Abbreviation</th>
<th>Full Form</th>
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<tbody>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic Acid</td>
</tr>
<tr>
<td>ssDNA</td>
<td>Single stranded DNA</td>
</tr>
<tr>
<td>dsDNA</td>
<td>Double stranded DNA</td>
</tr>
<tr>
<td>RNA</td>
<td>Ribonucleic Acid</td>
</tr>
<tr>
<td>FJC</td>
<td>Freely Jointed Chain</td>
</tr>
<tr>
<td>WLC</td>
<td>Worm-like Chain</td>
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<tr>
<td>SRD</td>
<td>Stochastic rotation dynamics</td>
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<tr>
<td>MD</td>
<td>Molecular Dynamics</td>
</tr>
<tr>
<td>LD</td>
<td>Langevin Dynamics</td>
</tr>
<tr>
<td>MPC</td>
<td>Multi-Particle collision dynamics (alias for SRD)</td>
</tr>
<tr>
<td>CSG</td>
<td>Constructive Solid Geometry</td>
</tr>
<tr>
<td>LJ</td>
<td>Lennard-Jones (potential)</td>
</tr>
<tr>
<td>FENE</td>
<td>Finitely Extensible Nonlinear Elastic (potential)</td>
</tr>
<tr>
<td>cis</td>
<td>The side from where the polymer translocates (inside capsid)</td>
</tr>
<tr>
<td>trans</td>
<td>The side to which the polymer translocates (outside capsid)</td>
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List of Symbols

\(N_0\) number of monomers in a polymer
\(v_{\text{cm}}\) center-of-mass velocity
\(i\) index of a polymer bead or a solvent particle
\(r_i\) position of the particle \(i\)
\(v_i\) velocity of the particle \(i\)
\(\Omega\) Rotation matrix
\(R_g\) radius of gyration
\(b\) bond length
\(\lambda_p\) persistence length
\(m_b\) mass of a polymer bead
\(m_s\) mass of a solvent particle
\(\nu\) Flory exponent

\(\tilde{s}\) sedimentation constant
\(f_d\) gravitational force in sedimentation
\(n_{\text{min}}\) minimal crossing number of a knot
\(n_c\) average crossing number of a knot
\(R_g,\perp\) component of radius of gyration perpendicular to \(f_d\)
\(R_g,\parallel\) component of radius of gyration parallel to \(f_d\)
\(\rho_s\) radius of a sedimenting sphere
List of Symbols

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
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<tr>
<td>$s$</td>
<td>translocation coordinate</td>
</tr>
<tr>
<td>$s/N_0$</td>
<td>normalized translocation coordinate</td>
</tr>
<tr>
<td>$t(s)$</td>
<td>cumulative waiting time profile</td>
</tr>
<tr>
<td>$\hat{t}(s/N_0)$</td>
<td>cumulative waiting time profile as a function of normalized translocation coordinate</td>
</tr>
<tr>
<td>$t_f(s)$</td>
<td>first-passage time</td>
</tr>
<tr>
<td>$t_w(s)$</td>
<td>waiting time profile</td>
</tr>
<tr>
<td>$\hat{t}_w(s/N_0)$</td>
<td>waiting time profile as a function of normalized translocation coordinate</td>
</tr>
<tr>
<td>$\tau$</td>
<td>(total) ejection time</td>
</tr>
<tr>
<td>$f$</td>
<td>pore force in capsid ejection</td>
</tr>
<tr>
<td>$f_{ent}$</td>
<td>force measured at the capsid pore entrance</td>
</tr>
<tr>
<td>$R_{g,\text{trans}}$</td>
<td>radius of gyration on the trans side (outside capsid)</td>
</tr>
<tr>
<td>$R_0$</td>
<td>capsid inner radius</td>
</tr>
<tr>
<td>$\rho_0$</td>
<td>initial monomer density inside the capsid</td>
</tr>
<tr>
<td>$\rho$</td>
<td>monomer density inside the capsid</td>
</tr>
<tr>
<td>$\rho_e$</td>
<td>effective monomer density inside the capsid</td>
</tr>
<tr>
<td>$N$</td>
<td>number of monomers inside the capsid</td>
</tr>
<tr>
<td>$V$</td>
<td>inner volume of the capsid</td>
</tr>
<tr>
<td>$V_e$</td>
<td>effective inner volume of the capsid</td>
</tr>
<tr>
<td>$\zeta$</td>
<td>capsid volume/density correction parameter</td>
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<tr>
<td>$\kappa$</td>
<td>bending potential parameter</td>
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Functioning of living cells depends heavily on movement, transportation, and manipulation of biopolymers such as DNA, RNA, or proteins. These processes are essential for cells to replicate, pass genetic information, or in short, to function at all. A polymer is a long molecule consisting of multiple repeated units of smaller molecules, monomers, that are connected by chemical bonds. For example, DNA consists of repeated nucleotides. At the cell level, the biopolymers are immersed in water and move in narrow channels and between cell compartments. Viruses are the ultimate example of extreme confinement and transportation of DNA (or RNA). Reproduction of the virus depends entirely on the transportation of the genetic information of the virus to a host cell it infects. In order to transfer the genetic information, the virus needs to transport its DNA past the host cell’s defense mechanisms. The importance of the biopolymer transport processes for human health has inspired intense research on biological macromolecule systems.

The objective of this thesis is to gain understanding on the fundamental principles governing the dynamics of polymer transport in confined geometries. The main focus is on capsid ejection, while also sedimentation of knotted polymers is studied. To understand the basic principles, we use simulation models that are generic and sufficiently simple to allow for systems that are large enough to be statistically and thermodynamically relevant. We avoid a great deal of details of realistic biopolymer systems, such as the exact molecular structures and interaction potentials, as they would only perturb the generic properties, functional forms, and relevant regimes of the studied systems. The focus is in understanding how generic features such as polymer length, strength of confinement, hydrodynamic interactions, and bending rigidity affect the dynamics of polymers. The obtained principles in this thesis can be used to understand results from
real experiments or realistic molecular dynamics simulations. We use only a few of the tools of non-equilibrium statistical physics, but hope that this study promotes interesting new views also in that field.

The main computational method is coarse-grained molecular dynamics coupled with stochastic rotation dynamics (SRD) to model hydrodynamic interactions. Occasionally, when hydrodynamics is not needed, the Langevin dynamics is used due to it being theoretically more established and computationally more efficient. Coarse-graining and computational efficiency are important factors for the models, since the probabilistic nature and the large number of degrees of freedom involved in the studied systems generally require large statistics, i.e., a great number of independent simulations.

In Publication I we study how the sedimentation velocity of a circular polymer depends on its knot topology. Sedimentation and electrophoresis have been standard tools in the experimental research of biopolymers for decades. This study shows the linear dependence between knot complexity and sedimentation velocity using a model that takes directly into account hydrodynamic interactions and makes no assumptions about the polymer conformations during sedimentation. We study sedimentation in a narrow channel and compare the results to the well understood sedimentation in free, non-confining solvent. This system serves as the perfect test bench to verify that the used SRD method can be used to accurately model hydrodynamic interactions in confinement. Therefore, this study lays the foundation for understanding hydrodynamic interactions of the more complex capsid ejection, where confinement is the key property of the process.

In studying sedimentation, the identification of knots was necessary. While knots have a role in the dynamics of capsid ejection [4–6], the accurate identification of knots during capsid ejection proved to be computationally overwhelming. Therefore we decided not to explicitly analyze the effect of knots in capsid ejection.

In Publication II the fundamentals and basic principles of capsid ejection are studied. The focus is in comparing the obtained numerical results with the existing theoretical framework. Details, such as hydrodynamic interactions and the bending rigidity, are excluded to simplify the analysis. We use waiting time and force measurements, generally omitted in the analysis of capsid ejection, to understand the dynamic nature of capsid ejection. This study lays the foundation for understanding the dynamic
features in capsid ejection.

In Publication III we extend the investigation of capsid ejection of flexible chains by including hydrodynamic modes in the simulations. Their effect on the ejection is studied by comparing the measured results with those obtained without hydrodynamics. In real biological systems hydrodynamic interactions are, of course, always present. The forces involved in ejection are also studied more carefully than in Publication II by using the computationally more effective Langevin dynamics. SRD is compared with Langevin dynamics in detail. The more detailed force measurements in this publication lead to more accurate description of the ejection dynamics. The results of Publications II and III are relevant for understanding dynamics of single stranded (ss)DNA and proteins that are very flexible.

Publication IV is the final study of capsid ejection in this thesis. The complexity of the ejection process is increased by introducing bending rigidity to the polymers making them semiflexible chains. The effect of bending rigidity and its magnitude to the dynamics of capsid ejection is studied. The bending rigidity is essential in modeling double stranded (ds)DNA in viral capsids, since in such systems the capsid size is comparable to the dsDNA's persistence length. The article shows that the dynamics of ejection is completely different for flexible and semiflexible polymer chains. In fact, there is a crossover from one dynamic regime to another.

This thesis is organized as follows. In Chapter 2 we present the relevant concepts needed to analyze the results of this thesis. We also introduce the most relevant previous research. The computational models and methods are presented in Chapter 3. Chapter 4 presents the main results of the publications included in this thesis. We summarize the results and suggest future research topics in Chapter 5.
2. Theory and background

2.1 Basic properties of polymers

In this section we present two fundamental properties of polymers, radius of gyration and persistence length. They are generic and theoretically well defined quantities that we use as tools for analysis throughout this thesis. Note that in this thesis we use the words monomer and bead interchangeably.

2.1.1 Radius of gyration

In polymer physics, we need a measure of the polymer size. The most straightforward measure is the contour length of the polymer \( b(N_0 - 1) \), where \( b \) is the bond length and \( N_0 \) is the number of beads in the polymer. However, the contour length does not describe how large a volume the polymer conformation takes. A measure very often used to describe the size of a polymer conformation is the radius of gyration \( R_g \), defined as

\[
R_g^2 = \frac{1}{N_0} \sum_{i=1}^{N_0} (r_i - r_{cm})^2,
\]

where \( r_i \) is the position of the bead with index \( i \), \( r_{cm} \) is the center-of-mass position of the polymer, and \( N_0 \) is the number of beads in the polymer. \( R_g \) is larger for polymers that have expanded to cover a larger volume. For self-avoiding polymer chains in equilibrium in free solvent the radius of gyration can be shown to follow the power law

\[
R_g \sim b N_0^{\nu},
\]

where \( \nu \) is the Flory exponent. Using simple free energy arguments, Flory derived that for self-avoiding chains \( \nu = 3/5 \) [7, 8]. A more precise derivation from renormalization group theory yields \( \nu = 0.588 \) [9–13]. Remark-
ably, this scaling law has been observed experimentally for many real polymers of different kinds [14–17]. In other words, it is not dependent on the structure of the molecule studied.

In studying sedimentation we find it useful to determine the components of \( R_g \) that are parallel and perpendicular to the applied sedimentation force

\[
R^2_{g\parallel} = \frac{1}{N_0} \sum_{i=1}^{N_0} (r_{i,z} - r_{cm,z})^2,
\]

\[
R^2_{g\perp} = \frac{1}{N_0} \sum_{i=1}^{N_0} \left[ (r_{i,x} - r_{cm,x})^2 + (r_{i,y} - r_{cm,y})^2 \right].
\]

In capsid ejection we also measure \( R_g \) of the ejected part of the polymer, in which case the number of beads \( N_0 \) and the limits in the summation in Eq. (2.1) are changed to contain only the part of the polymer studied. Comparing \( R_g \) of a segment during ejection to \( R_g \) of a corresponding polymer of equal length in equilibrium allows us to determine if the polymer segment is close to equilibrium.

### 2.1.2 Persistence length

An integral part of some polymers is their resistance to bending, i.e., bending rigidity. For instance, real double stranded (ds)DNA molecules resist the bending of its backbone, i.e., persist orientation. Persistence length \( \lambda_p \) is a measure that describes how strong the bending rigidity of a polymer is.

In the following, we introduce persistence length to the model polymer as presented in Ref. [18]. Let us define the bond vector \( b_i \) of bond \( i \) as \( b_i = r_{i+1} - r_i \). Hence, we can write the dot product of two bond vectors as

\[
b_i \cdot b_j = |b_i||b_j| \cos \theta_{ij}
\]

\[
= b^2 \cos \theta_{ij},
\]

if we assume a fixed bond length \( b \). In the simulations the bond length fluctuates. The expectation value of the dot product, which gives a measure for the orientational correlation of bonds \( i \) and \( i + s \), is

\[
\langle b_i \cdot b_{i+s} \rangle = b^2 \langle \cos \theta_{i,i+s} \rangle \\
= b^2 \langle \cos \theta \rangle^s \\
= b^2 \exp \left( s \ln \langle \cos \theta \rangle \right) \\
= b^2 \exp \left( -\frac{bs}{\lambda_p} \right),
\]

6
where on the second row we have assumed that the angles $\theta$ are independent of each other, i.e., a worm-like-chain with no excluded volume interactions. This is not exactly true for simulations where excluded volume interactions are present as we discuss in Sec. 3.6. The final form defines the persistence length

$$\lambda_p = \frac{b}{-\ln \langle \cos \theta \rangle},$$

(2.8)

which describes how far in the chain the orientational correlations persist. The greater the persistence length, the more rigid is the polymer.

### 2.2 Knots and ideal knots

Knots have been studied as mathematical objects for more than two decades [19,20]. Mathematically, knot is a one-dimensional circle embedded in three dimensional space. The circle has neither a starting nor an ending point but it is a continuous loop. Different knot topologies are such that a knot of a certain topology cannot be transformed to a knot of a different topology without cutting and pasting the loop. Knot topologies are often named by using the Alexander-Briggs notation in the form $X_Y$, where $X$ is the minimal crossing number and $Y$ is an arbitrarily chosen index to separate different knots having the same crossing number [21].

The minimal crossing number is defined as the number of crossings in the knot’s minimal projection that is a two dimensional projection of the knot with minimum number of crossings. In the upper row of Fig. 2.1 we show knots $3_1$, $6_1$, and $10_1$ in their minimal projection. Finding the knot topology of an arbitrary knot in an arbitrary conformation is still a difficult mathematical problem [22].

Minimal crossing number can be regarded as a measure of the knot complexity. However, it does not contain information detailed enough for quantifying differences in, say, knots with the same minimal crossing number. Therefore, we introduce a more complicated measure of knot complexity, named *average crossing number* [23]. The average crossing number is a property of a given topology. More specifically, it is defined as the average crossing number of infinitely many two-dimensional projections of a corresponding ideal knot equisampling the sphere from all directions. An ideal knot is a conformation of a knotted tube that has the highest possible volume to surface ratio [23, 24]. In other words, given a constant radius of a knotted tube, the ideal knot has the shortest pos-
Theory and background

Figure 2.1. Knots (A) 3₁, (B) 6₁, and (C) 10₁ in their initial conformations (top row) and during sedimentation (bottom row). The figure is reprinted from Publication 1 with permission.

sible tube that can close the knot. The average crossing number is then computed for the inner core of this tube.

It has been known for long that DNA forms knots under various conditions [4, 25–31]. It is believed that enzymes have to be actively working to unknot the DNA, since knotting is detrimental for correct genome function [32]. Especially in bacteriophage capsids, knotting of DNA is typically inevitable [33, 34].

2.3 Sedimentation of knotted polymers

In biological research, there is a great demand for classification of knots in DNA [35, 36]. Knots can be classified by using electron microscopy, but this is extremely tedious. Dean et al. observed that polymers of the same minimal crossing number comigrated in gels [37]. Inspired by this Stasiak et al. published their seminal paper reporting that electrophoretic mobility depends linearly (affinely) on the average crossing number of the knot [25]. To justify this result they showed that the average inverse distance, which is a measure of polymer compactness, is linearly proportional to the average crossing number. Also the average crossing number of ideal knots is linearly proportional to the average crossing number of knots undergoing thermal fluctuations in equilibrium [24]. Rybenkov et
al. [38] developed a method to estimate the sedimentation coefficient of a polymer from a given exact conformation. Based on this Vologodskii et al. [26] estimated the sedimentation coefficients for polymer sets simulated in thermal equilibrium.

Our primary objective is to computationally verify the origin of the linear dependence between sedimentation coefficient and the average crossing number by taking hydrodynamic interactions fully and directly into account without making any assumptions on the polymer conformations. This way we do not have to assume that the polymer conformations are in equilibrium but allow hydrodynamic interactions and thermal fluctuations to alter the conformations freely.

Our second objective is to examine the effect the confinement has on the sedimentation process. In addition to simulations in free solvent (periodic boundary conditions, no walls) we simulate sedimentation between two parallel walls that are so close to each other that they flatten the polymer conformations. We do this not only to better understand the effect of confinement, but also because this kind of setup may prove useful for manipulating polymers on lab-on-a-chip type of experimental setups. These simulations also serve as a test bench for verifying that the stochastic rotation dynamics (SRD) method [39, 40] computes hydrodynamic interactions correctly in a confined space.

Here, we consider sedimentation as a process where a polymer migrates in a solvent due to a constant (gravitational) force. This differs from the much studied gel electrophoresis in that there a charged polymer is driven by an electric field. Sedimentation better sets the foundation for our research on capsid ejection, since there is no force field due to electrical charge but only hydrodynamics and Brownian motion affect the conformation of the polymer. Figure 2.1 shows snapshots of knots in their initial conformation and conformations during sedimentation in our simulations.

The sedimentation coefficient $\tilde{s}$ is defined as

$$\tilde{s} = \frac{v_t}{a},$$

(2.9)

where $v_t$ is the terminal average velocity of the polymer and $a = f_d/M$ is the acceleration applied to the polymer [41]. $f_d$ and $M$ are the applied sedimentation force and mass of the polymer, respectively. In the case of the gravitational force, $a$ would be equal to the gravitational acceleration, but in real experiments centrifuges are often used. In this case the parameter is controlled by rotational speed of the centrifuge.
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The reason for higher sedimentation velocity of knots of higher complexity is that they are smaller and therefore experience less frictional drag by the solvent. For a sphere sedimenting in a Stokesian fluid the frictional drag would be equal to 

\[ f_{\text{drag}} = 6\pi \eta r, \]

where \( \eta \) is the viscosity of the fluid. Thus the sedimentation coefficient of such a sphere at terminal velocity would equal

\[ \tilde{s} = \frac{M}{6\pi \eta r}, \]  

(2.10)

where \( M \) is the mass of the sphere. We use this simple sphere approximation to compare the sedimentation coefficients of polymers by relating the radius \( r_s \) to the radius of gyration of a polymer. It is noteworthy that in order to study sedimentation, modeling hydrodynamics is essential, since without hydrodynamic interactions the friction would be determined solely by the number of beads without any dependence on the polymer conformation [42].

2.4 Capsid ejection

In this thesis, capsid ejection means the process where a polymer chain initially confined inside a spherical capsid escapes outside without a driving force. Snapshots showing the ejection at different times are shown in Fig. 2.2. During ejection the polymer is not subject to any external force, but the ejection is driven only by the free energy difference inside and outside the capsid. In the following sections we introduce the most fundamental concepts used in studying the ejection process. We also present

![Figure 2.2](image-url)
the computations outlined in Ref. [43] which presents a fairly complete theoretical description of the ejection dynamics.

2.4.1 Biological relevance of capsid ejection

While the fundamental motivation for studying capsid ejection is to understand its complex dynamics and non-equilibrium statistical physics, in general the process is also highly relevant due to its role in biology [44,45]. A key part of the life cycle of viruses is the infection of the host cell. This they do by injecting their DNA (or RNA) into the cell in order to use it to produce more viruses [46]. There exists a multitude of viruses that contain their DNA inside a more or less spherical shell from which they are ejected to the host cell through a small pore or a syringe. Some examples of such viruses are the T2, T4, T7, and $\lambda$ phage [46]. They are viral bacteriophages that infect bacteria. Understanding the ejection process is a key factor in understanding how viruses of this type function. Actually T2 phage played an important role in determining that it is specifically DNA that transmits genetic information [47].

The DNA of bacteriophages is packed to almost crystalline densities [48]. Ref. [46] reports diameters of different bacteriophages ranging from 26 nm to 125 nm. For instance, the diameter of bacteriophage $\lambda$ is 64 nm, while the dsDNA inside of it has 48502 base pairs [49] that translates to the length of 16500 nm (using 0.34 nm/bp). This means that the length of the dsDNA is huge compared to the size of the capsid. More specifically, the length of $\lambda$-DNA is 260 times the capsid diameter. In addition, the persistence length of dsDNA is 50 nm [50], i.e., of the same order as the capsid size.

By considering that the diameter of dsDNA is of the order 2 nm [41] and that the inner radius of the $\lambda$ phage is about 27.5 nm we can estimate that the dsDNA occupies 60% of the capsid inner volume (using the outer diameter we obtain that the DNA occupies 42% of $\lambda$ phage volume as in [51]). In experiments it has been verified that the extremely high packaging densities also lead to extremely large pressures (several tens of atmospheres) that persist throughout the ejection [44].
2.4.2 Ejection time, waiting time, cumulative waiting time, and first-passage time

Capsid ejection is generally characterized by the ejection time $\tau$, which is the time it takes for the polymer to completely eject the capsid. The common assumption is that when starting the ejection from constant monomer density $\rho_0$, the (average) ejection time depends on polymer length $N_0$ in a scaling law fashion

$$\tau \sim N_0^\beta,$$  \hspace{1cm} (2.11)

where $\beta$ is the scaling exponent.

The ejection time is also the first-passage time of the last bead of the polymer chain. While ejection time can arguably be considered as the most fundamental measure of ejection dynamics, relying on studying only ejection times easily leads to wrong conclusions about ejection dynamics [52].

It is useful to define the first-passage time $t_f$ for each bead with index $s$. This is the first time the bead $s$ ejects the capsid. Note that the first-passage time of bead $N_0$ is equal to the ejection time, i.e., $\tau = t_f(N_0)$, since we consider the ejection complete when the last bead ejects the capsid the first time.

Since capsid ejection is a stochastic process, it is often the case that the first-passage time is not the last time when bead $s$ ejects the capsid. This time is defined as the cumulative waiting time $t$ of bead $s$. Note that the first-passage time and the cumulative waiting time of the last bead $N_0$ are the same since the ejection is considered complete when the last bead has exited the capsid, i.e., $t(N_0) = t_f(N_0) = \tau$. In this thesis we mainly consider cumulative waiting times, since they are more commonly studied in the context of forced translocation. However, occasionally we study the first-passage times due to them being theoretically more simple, as discussed in Section 2.4.7.

The waiting time $t_w$ is defined as the time it takes for bead $s$ to eject the capsid the last time after bead $s-1$ has ejected the capsid the last time [53]. This can be written using the cumulative waiting times as

$$t_w(s) = t(s) - t(s - 1) \approx \frac{d}{ds} t(s).$$  \hspace{1cm} (2.12)

Accordingly, the cumulative waiting time can be obtained from the wait-
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Waiting times as a sum

\[ t(s) = \sum_{s' = 1}^{s} t_w(s') \approx \int_{0}^{s} t_w(s') \, ds', \]  

(2.13)

where we have used the integral approximation to emphasize the relation between the waiting time and the cumulative waiting time. The usefulness of studying \( t_w \) lies in that it directly relates to the instantaneous rate of ejection. The ejection speed is inversely proportional to the waiting time.

We use the term *waiting time profile*, denoted by \( t(s) \), to describe the measured waiting times given for each \( s \). Similarly, *cumulative waiting time profile* means \( t(s) \) for each \( s \). We occasionally plot \( t_w \) and \( t \) as a function of the normalized translocation coordinate \( s/N_0 \). We denote these profiles as \( \hat{t}(s/N_0) \) and \( \hat{t}_w(s/N_0) \) to emphasize that \( t \) and \( t_w \) are plotted as a function of the normalized translocation coordinate.

### 2.4.3 Theoretical background of capsid ejection

In this section, we briefly outline the history of studying the dynamics of capsid ejection. The theoretical study of capsid ejection can be considered to have started by Sung and Park [54]. They studied the ejection of ideal chains omitting excluded volume interactions. Also, polymers were assumed to be in equilibrium at all times. Muthukumar carried out theoretical analysis for his Monte Carlo simulations [52] of polymers with excluded volume interactions. He assumed that the free energy of confinement is

\[ F \sim kTN/R_0^{1/\nu}, \]

from which the waiting time is derived to scale as

\[ \tau = N_0 R_0^{1/\nu} = N_0 \left( \frac{N_0}{\rho_0} \right)^{1/3\nu}. \]  

(2.14)

This result seemed to be supported by Monte Carlo simulations. This analysis did not take into account the more complex form of confinement energy in spherical confinement [55,56], as pointed out in Ref. [57] by Cacciuto and Luijten. They studied ejection dynamics using the free energy derived by Grosberg and Khokhlov [55]

\[ \Delta \frac{F}{kT} \sim \left( \frac{R_2}{R_0} \right)^{3/(3\nu-1)} \sim N_0 \phi^{1/(3\nu-1)}, \]  

(2.15)

that was shown to be correct for polymers in semidilute conditions [58]. This was verified in Ref. [56] for stronger confinement also. Scaling arguments of Cacciuto and Luijten lead to ejection times scaling as

\[ \tau \sim N_0^{1+\nu} \phi^{1/(1-3\nu)}. \]  

(2.16)
This result was also verified by Monte Carlo simulations (in the same article). However, also this analysis omits the details of ejection and does not consider the decaying number of monomers inside the capsid. This was addressed by Sakaue and Yoshinaga in Ref. [43], where the dynamic nature of confinement energy is taken into account. We present the relevant computations in detail in the following section. We believe that the seemingly good correspondence between different theories and their corresponding simulations is due to short ranges and different regimes. In the preceding work only the final ejection times were considered and the measurement of, for instance, the cumulative waiting times changes the view as we will shown in Section 4.2.

2.4.4 Dynamics of polymer ejection from semidilute conditions

In this section, we present the computations outlined by Sakaue and Yoshinaga in Ref. [43]. This article presents the most complete theoretical analysis of capsid ejection of flexible polymers assuming initial semidilute condition. Our motivation is to compare the computational results of our simulations to the theoretical treatment of their article.

In the following, we show the derivation of the dynamics of capsid ejection during the driven part of the ejection. As a starting point, we take the free energy difference as presented in Ref. [55]

\[
\frac{\Delta F}{kT} \approx \left( \frac{a}{R_0} \right)^{3/(3\nu-1)} N(t)^{3\nu/(3\nu-1)},
\]

where \( N(t) \) is the number of monomers inside the capsid, \( R_0 \) is the capsid radius, and \( a \) is the monomer size. The force is obtained by taking the derivative of \( \Delta F \) with respect to the translocation coordinate \( s \)

\[
f = \frac{\Delta F}{kT} \approx \frac{3\nu}{3\nu-1} \left( \frac{4}{3} \pi a^3 \rho(t) \right)^{1/(3\nu-1)}.
\]

The dissipation is given by

\[
T \dot{S}(t) \approx \eta \left[ \frac{\dot{N}(t)a}{\xi(t)} \right]^2 \xi(t)^3,
\]

where \( \eta \) is the solvent viscosity and \( \xi(t) = a \phi^{\nu/(1-3\nu)} = a \left( a^3 N(t)/R_0^3 \right)^{\nu/(1-3\nu)} \) is the blob size. The time-evolution can be evaluated as

\[
\Delta \dot{F}(t) = -T \dot{S}(s),
\]

which gives the differential equation

\[
\frac{3\nu kT}{3\nu - 1} \left( \frac{a}{R_0} \right)^{3/(3\nu-1)} \dot{N}(t) N(t)^{1/(3\nu-1)} = -\eta \left[ \frac{\dot{N}(t)a}{\xi(t)} \right]^2 \xi(t)^3.
\]
Simplifying the above and substituting the blob size yields

\[
\dot{N}(t) = -\frac{3\nu}{3\nu - 1} \frac{kT}{\eta a^3} \left( \frac{a}{R_0} \right)^{(3\nu+3)/(3\nu-1)} N(t)^{(\nu+1)/(3\nu-1)}.
\] (2.22)

Note that a differential equation of this form simply states that the rate of change is directly proportional to the force and inversely proportional to the friction. This differential equation has the solution

\[
N(t) = N_0 \left( 1 + \frac{t}{\tau_1} \right)^{\frac{1-3\nu}{2(1-\nu)}},
\] (2.23)

where we have used the initial condition \( N(t=0) = N_0 \). The value for the constant is \( \tau_1 = \eta a^3/(kT)\phi_0^{(1+\nu)/(1-3\nu)} N_0 \), where \( \phi_0 = a^3 N_0/R_0^3 \) is the initial volume fraction. We compare the computational results with Eq. (2.23) by adjusting the constant \( \tau_1 \) for the best fit. We can also solve the time as a function of beads inside the capsid as

\[
t = \tau_1 \left[ \left( \frac{N(t)}{N_0} \right)^{\frac{2(1-\nu)}{1-3\nu}} - 1 \right].
\] (2.24)

This roughly corresponds to the cumulative waiting time that we frequently use in analyzing our results. We can also derive the differential of time with respect to translocation coordinate \( s \) (note that \( N = N_0 - s \)), which roughly corresponds to the waiting time

\[
t_w \approx \frac{d}{ds} t = \frac{2(1-\nu)}{3\nu - 1} \frac{\tau_1}{N_0} \left( \frac{N(t)}{N_0} \right)^{\frac{1+\nu}{1-3\nu}}.
\] (2.25)

This formula predicts that the differential waiting times fall on top of each other for different \( N_0 \) as \( \tau_1 \sim N_0 \) in the driven part of ejection. Sakaue and Yoshinaga also predict that the driven part ends when the radius of gyration of the polymer segment inside the capsid equals the capsid radius, i.e., \( R_0 = R_g = aN^\nu \), which gives \( N = (R_0/a)^{1/\nu} = (N_0/\phi_0)^{1/(3\nu)} \equiv g_0 \), where we have defined \( g_0 \) as the number of beads inside the capsid for which the pressure inside the capsid drops to zero. The ejection is in the pressure-driven regime when \( N > g_0 \) and in the diffusion driven regime when \( N < g_0 \). Sakaue and Yoshinaga predict that after \( N \) has decayed to \( g_0 \), the diffusive part of the ejection takes the time \( \tau_D = R_0^2 / D N_0 \), where \( D N_0 \) is the diffusion constant of a polymer of length \( N_0 \).

In Section 4.2.2 we compare our results with these computations.

### 2.4.5 Applicability of the semidilute regime

In Ref. [56] a simple estimate for approximating the average number of monomers in a blob was derived. Assume that a single blob of a polymer
has $N_b$ monomers in it. Thus the average radius of a single blob is approximately $r_b = A_0 N_b^{\nu}$, where $A_0 \approx 0.6$ for self-avoiding chains used in Ref. [56]. The monomer density within a blob is $\rho_b = \frac{N_b}{4/3\pi r_b^3}$. Setting the global density inside the capsid equal to the density within a blob $\rho_b = \rho$ we get the average number of monomers per blob as

$$N_b = \left(\frac{4}{3}\pi A_0^3 \rho \right)^{1/(1-3\nu)}.$$  \hspace{1cm} (2.26)

For example, $\rho = 0.5$ gives $N_b = 2.7$ and $\rho = 1.0$ gives $N_b = 1.1$. Consequently, it is expected that the blob picture is not valid for densities this high, since there is a very small amount of beads per blob.

### 2.4.6 Ejection dynamics with exponential force

From the simulations in Publication II, we observed that the pore force grows approximately exponentially with the number of monomers inside the capsid $N$. This finding is in contrast with the form of free energy in Eq. (2.17). In Publication II we derive the cumulative waiting times for the exponential force dependence by replacing the free energy of Eq. (2.17) in the above computation with the form derived from pore force $f$. Assuming that $f$ is of the form

$$f = C \exp \left( A \frac{N}{N_0} \right),$$  \hspace{1cm} (2.27)

where $C$ and $A$ are constants, we can derive the free energy as the total energy needed to package $N$ polymer beads inside the capsid as

$$\Delta F \approx \sum_{i=1}^{N} f_i \Delta l_i \approx \int f(n) dn = \int C \exp \left( A \frac{n}{N_0} \right) dn$$  \hspace{1cm} (2.28)

$$= \frac{CN_0}{A} \left[ \exp \left( A \frac{N}{N_0} \right) - D \right],$$  \hspace{1cm} (2.29)

where $f_i$ is the force required to move bead $i$ inside the pore and $\Delta l_i$ is the distance the bead needs to be moved, in this case the bond length. By combining Eqs. (2.28) and (2.20) we obtain the differential equation

$$\dot{N}(t) = -\frac{C}{\eta a^2 \xi(t)} \exp \left( A N(t) \frac{N_0}{N_0} \right).$$  \hspace{1cm} (2.30)

If we approximate the correlation length, or blob size, to be constant $\xi(t) = \xi$, we can solve for

$$N(t) = \frac{N_0}{A} \ln \left( \frac{AC}{N_0 \eta a^2 \xi} + e^{-A} \right).$$  \hspace{1cm} (2.31)

where we have used the initial condition $N(t = 0) = N_0$. The constant correlation length approximation can be justified by noting that when polymers eject from high monomer densities, the correlation is not expected
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to change appreciably during the ejection. This is, of course, not exactly true, but allows us to derive the solution for \( N(t) \). Note also that Eq. (2.30) essentially states that the rate of change of \( N(t) \) is directly proportional to the force at the pore. In the calculations of Ref. [43] the force is of different form and \( \xi(t) \) is not assumed constant.

From Eq. (2.31) we can solve \( t \) as a function of \( N \) as

\[
\frac{N_0 \eta a^2 \xi}{AC} \left[ \exp \left( - \frac{A}{N_0} N \right) - \exp (-A) \right],
\]

Equation (2.32)

The waiting time is obtained as a derivative of \( t \) with respect to \( s \),

\[
t_w = \frac{\eta a^2 \xi}{C} \exp \left( - \frac{A}{N_0} N \right)
\]

Equation (2.33)

\[
= \frac{\eta a^2 \xi}{C} \exp (-A) \exp \left( \frac{A}{N_0} s \right),
\]

Equation (2.34)

where we have substituted \( N = N_0 - s \) to emphasize that this computation predicts strictly exponential waiting times also as a function of \( s \).

2.4.7 First-passage time of a random walk in a potential field

In this section we show yet another way to derive the dynamic behavior of polymer ejection, as shown in Publication III. Although this approach can be considered even simpler than the ones presented above, it takes thermal fluctuations into account more explicitly. Let us assume that the translocation coordinate \( s \) of the ejecting polymer behaves similarly to a single particle diffusing in a force field. We can use the one-dimensional Langevin equation as a starting point

\[
ma = -\xi mv + f(s) + \eta(t),
\]

Equation (2.35)

where \( m \) is the mass, \( a \) the acceleration, \( v \) the velocity, and \( f(s) \) the force field of the particle. \( \eta(t) \) is zero-mean Gaussian white noise that satisfies

\[
\langle \eta(t) \eta(t') \rangle = 2\xi mkT \delta(t - t'),
\]

Equation (2.36)

where \( kT \) is the temperature of the system. For simplicity, we assume that the inertial terms are so minimal that they can be neglected and set \( ma = 0 \). In other words, we are in the dissipative regime where inertial effects play no role, which is generally true for polymer systems [55] (also known as the overdamped limit). This approximation gives us the stochastic differential equation of the form

\[
\frac{ds}{dt} = \frac{1}{\xi m} F(s) + \frac{1}{\xi m} \eta(t),
\]

Equation (2.37)
which can be be written more formally in the Itô form [59]

\[ ds = \frac{1}{\xi_m} F(s) dt + \frac{1}{\xi_m} d\eta_i(t). \]  

(2.38)

The first-passage time for such a system can be shown to equal [59]

\[ t_f(s) = \frac{kT}{\xi_m} \int_0^s \exp \left( -\frac{U(y)}{kT} \right) \int_y^s \exp \left( \frac{U(z)}{kT} \right) dz \, dy, \]  

(2.39)

where \( U(s) = -\int_0^s f(s) ds \) is the potential of the force field \( f(s) \). Often \( t_f(s) \) is not analytically solvable for the relevant \( f(s) \). Such is the case, for instance, for the force of the form

\[ f(s) = C \left[ \exp \left( B \frac{N_0 - s}{N_0} \right) - 1 \right], \]  

(2.40)

which leads to the potential

\[ U(s) = \frac{C}{B} \left[ N_0 \exp \left( B \frac{N_0 - s}{N_0} \right) + Bs \right]. \]  

(2.41)

In Section 4.2.5 we compare numerical solutions of \( t_f(s) \) with this potential to first-passage times measured from the capsid ejection simulations.
3. Computational models and methods

3.1 Polymer model

In all publications included in this thesis, the polymer is modeled as a chain of point-like beads connected via simple potentials. Between all subsequent bead pairs we have the finitely extensible nonlinear elastic (FENE) potential \[ U_F = -\frac{K}{2} r_{\text{max}}^2 \ln \left( 1 - \left( \frac{r}{r_{\text{max}}} \right)^2 \right), \quad r < r_{\text{max}}, \] (3.1)

where \( r \) is the distance between the bead pair. \( K \) and \( r_{\text{max}} \) are parameters that define the form and the strength of the potential. FENE potential is attractive and defines the maximum bond length \( r_{\text{max}} \) between subsequent beads. Excluded volume effects are modeled with the truncated and shifted Lennard-Jones (LJ) potential (also known as Weeks-Chandler-Andersen potential \[ U_{\text{LJ}} = \begin{cases} 4.8 \epsilon \left[ \left( \frac{\sigma}{r_{ij}} \right)^{12} - \left( \frac{\sigma}{r_{ij}} \right)^{6} \right] + 1.2 \epsilon, & r_{ij} \leq \frac{\sqrt{2}}{\sigma} \\ 0, & r_{ij} > \frac{\sqrt{2}}{\sigma} \end{cases}. \] (3.2)

Here, \( \epsilon \) and \( \sigma \) are the potential parameters, \( i \) and \( j \) denote bead indices, and \( r_{ij} = |r_i - r_j| \) is their mutual distance. The potential is repulsive for distances smaller than \( \sqrt{2} \) and zero elsewhere. As it has no attractive part, it models a good solvent. In all publications we use the potential parameters \( \epsilon = 1, \sigma = 1, K = 30/\sigma^2 \), and \( r_{\text{max}} = 1.5\sigma \) in reduced units (reduced units explained in Ref. [62]).

In Publication IV we use also the bending potential given as

\[ U_{\text{bend}} = -\kappa (\vec{r}_{i+1} - \vec{r}_i) \cdot (\vec{r}_i - \vec{r}_{i-1}), \] (3.3)
3.2 Stochastic rotation dynamics

To model the solvent, we mainly use stochastic rotation dynamics (SRD), also known as Multi-Particle Collision Dynamics [39, 40]. It is a particle based method that models both hydrodynamics and thermal fluctuations. It has the benefit of being computationally relatively effective while also allowing for switching hydrodynamic modes on and off. We use SRD completely without hydrodynamics in Publication II.

The SRD solvent constitutes of point-like massive particles having positions and velocities. The update rules for the solvent particles are divided into two steps, namely the *streaming step* and the *collision step*. In the streaming step the positions of the particles are updated as

\[ \mathbf{r}_i(t + \Delta t) = \mathbf{r}_i(t) + \mathbf{v}_i(t) \Delta t, \quad (3.4) \]

where \( \mathbf{r}_i \) and \( \mathbf{v}_i \) are the position and velocity of particle \( i \), respectively, and \( \Delta t \) is the SRD time step. If in this step, a solvent particle hits a wall, it is bounced back to the direction of incidence and its velocity is flipped, \( \mathbf{v}_i(t) = -\mathbf{v}_i(t) \). This is the no-slip boundary condition.

For the collision step, the simulation space is divided into a grid of cubic cells of edge length 1.0. In each cell the velocities of particles are updated...
as
\[ v_i(t + \Delta t) = \Omega [v_i(t) - v_{cm}(t)] + v_{cm}(t), \]  

(3.5)

where \( v_{cm} \) is the center-of-mass velocity of the cell and \( \Omega \) is a random rotation matrix. The rotation axis of \( \Omega \) is chosen randomly for each cell at each time step. The fixed rotation angle was chosen to be \( 3\pi/4 \) as this value gives relatively high value for the solvent viscosity [63, 64]. The value has also been verified to work out well in previous studies [65–68]. In order to maintain Galilean invariance in the simulations the grid is shifted randomly at each collision step [69]. Modeling collisions in this way rotates the random parts of the velocities while preserving the total momentum of the particles in each cell. Collision steps also conserve energy in each cell.

When hydrodynamics is disabled, we randomly shuffle the particle velocities and positions. This way the momentum correlations are lost and hydrodynamic effects vanish.

While the SRD steps conserve energy and momentum in each cell, we ensure that the cumulation of numerical errors does not change the solvent temperature by using a thermostat. The thermostat sets the solvent temperature at \( kT = 1.0 \) by rescaling the random part of particle velocities such that equipartition theorem holds at each time step [70].

### 3.3 Velocity-Verlet algorithm and polymer coupling to solvent

The polymer dynamics is propagated in time using the velocity-Verlet algorithm [70, 71]. In the method the positions \( r_i \) of the polymer beads are updated as
\[ r_i(t + \delta t) = r_i(t) + v_i(t)\delta t + \frac{f_i(t)}{2m_b}\delta t^2, \]  

(3.6)

where \( \delta t \) is the velocity-Verlet time step, \( f_i \) is the sum of all forces exerted on bead \( i \) at time \( t \), and \( m_b \) is the bead mass. The velocities \( v_i \) are updated as
\[ v_i(t + \delta t) = v_i(t) + \frac{f_i(t + \delta t) + f_i(t)}{2m_b}\delta t. \]  

(3.7)

The velocity-Verlet method is energy conserving to a good precision. It has the accuracy of order \( \delta t^3 \) and is equivalent to the standard Verlet algorithm [70].

The velocity form of Verlet algorithm allows coupling of the polymer dynamics to the SRD solvent. When the SRD collision step is performed
the polymer particles are taken into account by treating them identically to the solvent particles in the update rule of Eq. (3.5). More precisely, the polymer particles' velocities are included in the computation of the center-of-mass velocity $v_{cm}$ and the velocities are rotated using the rotation matrix $\Omega$ of the cell the particles reside in. This way the polymer exchanges momentum with the solvent. The velocity-Verlet and SRD methods take turns such that after $\Delta t/\delta t$ velocity-Verlet steps a single SRD step is performed.

3.4 Langevin dynamics

In addition to SRD, we also use Langevin dynamics (LD) in Publications III and IV. LD is theoretically more thoroughly understood than SRD and therefore serves as the perfect model for comparison. LD is computationally much more efficient than SRD and is therefore used in force measurements and polymer packaging in some parts of this thesis. On the downside, LD does not model hydrodynamic modes.

In contrast to SRD, the solvent is not modeled explicitly in LD. Instead, the polymer beads follow the Langevin equation\[72\]

$$m_b \frac{dv_i(t)}{dt} = -\xi m_b v_i(t) + \eta_i(t) + f_i(t), \quad (3.8)$$

where $i$ is the index of the polymer bead, $\xi$ is the friction constant, $\eta_i(t)$ is a random force exerted on bead $i$, and $f_i$ is the sum of all other forces exerted on bead $i$. $\eta_i(t)$ is a zero-mean, $\delta$-correlated Gaussian process that follows

$$\langle \eta_i(t) \cdot \eta_j(t') \rangle = 2\xi kT m_b \delta(t - t') \delta_{ij}, \quad (3.9)$$

and models the heat bath the polymer is immersed in. In other words, $\eta$ in the Langevin equation models a solvent that is homogenous everywhere and does not include hydrodynamic modes but exerts random kicks on the polymer particles. We use Ermak's implementation for integrating the Langevin equation in time [62, 73].

3.5 Constructive solid geometry in molecular dynamics

The capsid ejection simulations in Publications II,III, and IV rely heavily on the simulation geometry and its accurate implementation. Hence, a considerable part of the work in this thesis comprises implementing
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and testing the constructive solid geometry (CSG) technique [74] used to model the capsid geometry. We also use this implementation in Ref. [3] to model toroidal pore in forced translocation. The idea in CSG is to use simple geometric objects like spheres and cylinders to construct more complex ones, such as the capsid in the present case. In this section we introduce the main concepts behind the implementation.

CSG method is widely used in different applications such as computer-assisted design (CAD) and computer graphics [74–76]. The implementation used here has its basis in ray tracing [77, 78], a technique used to create images with a computer by tracking trajectories of individual “light rays”. In Figs. 3.2 we show some ray-traced images of objects that have been created with our implementation of CSG method. More precisely, the intersections with light rays and the normals of the objects in the figure have been computed in the same way as the particle collisions in SRD and LD simulations.

3.5.1 Particle-wall interactions

The objective of the method is to track where a particle hits a wall and then bounce it from the wall applying either a slip or a no-slip boundary condition as illustrated in Figs. 3.3 (a) and (b). This section shows how the collisions between the particles and the walls are modeled assuming that the collision point is known.

The intersections of the particle trajectories and the object walls are tracked for every position update of the particles. More precisely, in a particle position update step we normally attempt to update a particle from the initial position $r_i(t) \equiv r_0$ to the trial position $\tilde{r}_i(t + \delta t) \equiv \tilde{r}_1$. If there is no wall between $r_0$ and $\tilde{r}_1$, the trial position is accepted as the new position $r_i(t + \delta t) \equiv r_1$. Similarly, the velocity $v_i(t) \equiv v_0$ is updated to $\tilde{v}_i(t + \delta t) \equiv \tilde{v}_1$, where $\tilde{v}_1$ is obtained from the update rule of the method in question (velocity-Verlet, SRD, or Langevin dynamics). On the other hand, if there is a wall between $r_0$ and $\tilde{r}_1$, the trial position and velocity are rejected and the update is restarted, usually with a smaller time step.

![Figure 3.2. Ray traced images of objects created with the CSG method implemented for this thesis. The rightmost image is a close-up of the pore of a capsid.](image-url)
Figure 3.3. Boundary conditions of a particle hitting a (curved, red) wall. (a) No-slip boundary condition (b) Slip boundary condition. If there is a wall between the initial position $r_0$ and the trial position $\tilde{r}_1$, the particle is bounced to position $r_1$ and the direction of velocity is flipped accordingly.

Figure 3.4. Vectors involved in the slip collision update rule of Eqs. (3.12) and (3.13). The horizontal line depicts the wall. (a) The velocity of the particle is flipped such that the angle of incidence is the same as angle of prevalence with respect to the surface normal. (b) The particle position is updated according to the normal and travel distance.

Hand, if there is a wall between points $r_0$ and $\tilde{r}_1$ at a distance $d$ from the point $r_0$, the particle hits a wall at point $r_{hit} = r_0 + \hat{v}d$, where $\hat{v} = \frac{\tilde{r}_1 - r_0}{|\tilde{r}_1 - r_0|}$ is the direction vector of the particle.

No-slip boundary conditions are used for SRD solvent particles. In this case the position and the velocity are updated as

$$r_1 = \tilde{r}_1 + (2d - w)\hat{v}, \quad (3.10)$$
$$v_1 = -\tilde{v}_1, \quad (3.11)$$

where $w = |\tilde{r}_1 - r_0|$ is the total distance traveled by the particle in one simulation step. Note that if $d > \frac{1}{2}w$, we actually need to search for walls also in the direction of incidence. If a wall is found also in this direction, a similar update scheme as shown above is used again. The no-slip boundary condition is used to ensure that the fluid flow in the wall surface is zero [79].

The slip boundary condition is used, for the polymer particles. In this case the normal vector $\hat{n}$ of the collision point $r_{hit}$ is needed for the update...
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Figure 3.5. Examples of different CSG operations. (a) A sphere $S$ and a rectangle $R$. (b) Union $S \cup R$, (c) difference $S \setminus R$, and (d) intersection $S \cap R$ of the sphere and the rectangle.

rule. The position and the velocity are updated as

$$v_{\text{tmp}} = v - 2(v \cdot \hat{n})\hat{n}, \quad (3.12)$$

$$r_1 = r_0 + d\tilde{v} + (w - d)\frac{v_{\text{tmp}}}{|v_{\text{tmp}}|}, \quad (3.13)$$

$$v_1 = \tilde{v}_1 - 2(\tilde{v}_1 \cdot \hat{n})\hat{n}, \quad (3.14)$$

where $v = (\tilde{r}_1 - r_0)/\delta t$ is the velocity required to go from $r_0$ to $\tilde{r}_1$ through a straight line during one time step. The update rules and vectors involved are illustrated in Figs. 3.4 (a) and (b). Since the polymer is integrated using the velocity-Verlet update scheme, the Verlet velocity needs to be updated separately using Eq. (3.14) (in velocity-Verlet the particles move through second order curves instead of lines).

In principle, it is possible that a particle would hit two walls in one simulation step. However, since we use a small time step in our simulations, this never happens. Therefore such cases need not be taken into account. If the time step needs to be increased, a possible workaround is to use no-slip boundary conditions in cases where $r_1$ is inside a wall. Implementing a recursive scheme is also possible, but this requires non-trivial bookkeeping to avoid a particle hitting the same wall at the same point $r_{\text{hit}}$ again due to finite machine precision.

3.5.2 Finding the wall-particle intersection for CSG objects

In this section, we sketch how the collision points $r_{\text{hit}}$ needed for the update rules are found. The idea in using CSG is to combine simple objects, called primitives, to create a more complex CSG object. A CSG object comprises two other objects object combined using CSG operations difference “\,” union “$\cup$”, or intersection “$\cap$” as illustrated in Fig. 3.5. For instance, the capsid geometry can be created by combining spheres, infinite cylinders, and semi-infinite boxes. The construction is a binary tree of objects, as shown in Fig 3.6.

In the algorithm, each primitive can return the distances $d$ on the line
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Figure 3.6. A spherical capsid with cylindrical pore can be constructed from primitives sphere $S_1$, sphere $S_2$, infinite cylinder $C$, and semi-infinite box $B$ by using CSG operations forming a binary tree. Capsid = $(S_1 \setminus S_2) \setminus (C \cap B)$.

Figure 3.7. Intersections of a line and a CSG object. Depending on the chosen CSG operation the line-primitive intersections are classified differently. Union $S \cup R$: 1 and 4 are real, 2 and 3 are phantom [Fig. 3.5 (b)]. Difference $S \setminus R$, all intersections are real [see Fig. 3.5 (c)]. Intersection $S \cap R$, 2 and 3 are real, 1 and 4 are phantom [see Fig. 3.5 (d)].

$r_0 + dv$ that intersects it. For example, the intersection of the line and a sphere, whose center is at $c_0$ and radius is $R_0$, is obtained by solving $d$ from the second order equation

$$[(r_0 + dv) - c_0]^2 = R_0^2.$$  \hspace{1cm} (3.15)

Similar simple equations can be derived for all primitives. The primitive can also return its normal vector in this point if slip boundary conditions are used.

A CSG object consists of two objects (that can also be CSG objects). A CSG object tracks all the intersections of these objects and the line $r_0 + dv$. We classify these intersections as real and phantom, as illustrated in Fig. 3.7. A real intersection lies in the shell of the final CSG object. A phantom intersection is between the line and an object surface that is excluded after the CSG operation, i.e., not a real intersection.

We do not explicitly go through the algorithm used to deduce which hits are real and which are phantom as these can be found in many textbooks concerning ray tracers [77]. Instead, let us consider an example of a union of the sphere and the rectangle of Fig. 3.7. We assume that initially, in
minus infinity ($d = -\infty$), we are outside both objects. Therefore the first intersection 1 must be a real intersection, since we are entering either of the two shapes. The second intersection is real if and only if it involves the same object as before. The intersection 2 is a phantom intersection, because it involves a different shape than we entered. After intersection 2 we are inside both objects. Therefore the next intersection must be a phantom intersection because we can exit only one object at a time. Thus, the intersection 3 is a phantom intersection. Finally, the intersection 4 occurs to a different object than the previous intersection 3 and must therefore be a real intersection where we exit the whole CSG object. By similar deduction and bookkeeping we can derive all the intersections for CSG objects of other types also. When tracking particle-wall intersections only the closest real intersection, i.e., smallest $d > 0$, is important. However, all the intersections need to be tracked from $d = -\infty$ to classify real and phantom intersections.

A useful property of CSG objects is that we can treat CSG objects similarly to any other objects. Hence, complicated shapes can be created using a binary tree structure as is done for the case of a capsid in Fig. 3.6. In Publication IV we use a torus pore instead of the cylinder pore in the illustration. The binary tree of the capsid with the torus pore is a bit more complicated.

### 3.6 Persistence length measured from simulations

In this section we illustrate how the value of the bending potential parameter $\kappa$ affects the persistence length of polymers in free solvent [see Eq. (3.3)]. Recalling Section 2.1.2, the orientational correlation of bonds is expected to decrease exponentially. Figure 3.8 shows the correlations between all bond pairs. The values are averages over 5 runs where each run is averaged over 1000 samples during 500 000 time units. We see
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Figure 3.9. Correlation of bonds as a function of distance between bonds along the chain $|i-j|$. The lines are exponentials whose parameters are shown in Table 3.1.

Table 3.1. Bond length $b$ and persistence length $\lambda_p$ defined for a polymer of length $N_0$ in free solvent for different values of $\kappa$.

<table>
<thead>
<tr>
<th>$\kappa$</th>
<th>$b$</th>
<th>$\lambda_p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.980</td>
<td>0.889</td>
</tr>
<tr>
<td>5</td>
<td>0.988</td>
<td>4.35</td>
</tr>
<tr>
<td>10</td>
<td>1.00</td>
<td>9.41</td>
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<tr>
<td>15</td>
<td>1.01</td>
<td>15.3</td>
</tr>
<tr>
<td>20</td>
<td>1.03</td>
<td>21.9</td>
</tr>
</tbody>
</table>

that for small $\kappa$, the correlations vanish quickly, i.e., correlation is large only very close to the diagonal. As $\kappa$ is increased the correlations persist further in the chain. The matrix is symmetric by definition.

To determine the persistence length $\lambda_p$ [18], we need to plot correlations as a function of distance $|i-j|$ along the chain. This is shown in Fig. 3.9 along with exponential curves of the form $b^2 \exp \left( -\frac{b|i-j|}{\lambda_p} \right)$. The values of $\lambda_p$ and the bond length $b$ corresponding to the curves in Fig. 3.9 are shown in Table 3.1. We observe that $\kappa$ is close $\lambda_p$ in most cases. Figure 3.9 shows that for sufficiently large $\kappa$, correlations decrease exponentially, while for smaller $\kappa$ the orientational correlations decay slower than exponentially. This comes from the exponential decay being accurate only for chains without excluded volume interactions. The excluded volume interactions increase the orientational correlations for bonds.

3.7 Simulation parameters and their relevance to experiments

As the body of the thesis concerns simulations, it is beneficial to have some insight on how the length scales in simulations relate to the real world. The mapping between simulation units and the real world can be done in several ways. Here, we show one possible way, which cannot be regarded
Table 3.2. Simulation parameters used in different publications. Reduced units.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Publication I</th>
<th>Publications II-IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length scale $\sigma$</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Energy scale $\epsilon$</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Bead mass $m_b$</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>LJ parameter $K$</td>
<td>$30/\sigma^2$</td>
<td>$30/\sigma^2$</td>
</tr>
<tr>
<td>LJ parameter $r_{\max}$</td>
<td>$1.5\sigma$</td>
<td>$1.5\sigma$</td>
</tr>
<tr>
<td>Verlet time step $\delta t$</td>
<td>0.002</td>
<td>0.0002</td>
</tr>
<tr>
<td>SRD cell edge length</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>SRD time step $\Delta t$</td>
<td>1.0</td>
<td>0.5</td>
</tr>
<tr>
<td>SRD solvent density</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>SRD particle mass $m_s$</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Rotation angle of $\Omega$</td>
<td>$3\pi/4$</td>
<td>$3\pi/4$</td>
</tr>
<tr>
<td>SRD temperature $kT$</td>
<td>1.0</td>
<td>1.0</td>
</tr>
</tbody>
</table>

as the only one. This means that the simulations do not produce accurate numeric values corresponding to real world measurements. Obtaining such a mapping would require performing real experiments and carefully comparing those with the simulation results.

The used forms of polymer potentials define the length scales of the simulations. The simulation parameters are shown in Table 3.2. All the parameters are in reduced units [62]. Since we set $\sigma = 1.0$ as our simulation length scale, we can in principle take one simulation unit to correspond to an arbitrary length in nature. As the most relevant counterpart for the simulated polymers is DNA, it is reasonable to map the simulation length scales according to properties of DNA. A fundamental property of DNA is its persistence length. However, the persistence length of dsDNA and ssDNA are of different orders of magnitude (50 nm and 4 nm, respectively [50, 80]). Therefore, we propose a mapping for both.

Publications I-III involve only flexible polymer chains ($\kappa = 0$). Hence, here the more flexible ssDNA is the relevant counterpart in nature. The persistence length of ssDNA is approximately 4 nm [80]. As indicated in Table 3.1, the persistence length of flexible chains in our simulations is approximately 0.9 simulation units. Therefore, we can conclude that one simulation unit corresponds to roughly 4 nm\(^1\). Since the length of

\(^1\)In Publication III we assumed $\lambda_p \approx \frac{1}{2} b$, which is valid for freely-jointed-chains. Therefore one simulation unit is taken to correspond to roughly 8 nm in the article, which leads to a different mapping.
one polymer segment is approximately one simulation unit, a polymer of
length $N_0$ corresponds to a ssDNA of length $4N_0$ nm having $12N_0$ bases
(using 0.37 nm/base [81]). Hence, in our simulations the lengths of studied polymers can be taken to range from $111$ nm / $300$ bases ($N_0 = 25$) to $1800$ nm / $4800$ bases ($N_0 = 400$). The polymer length $N_0 = 215$ used in Publication I corresponds to $960$ nm / $2600$ bases long ssDNA. The interaction distance of the repulsive Lennard-Jones potential implies a width of about one simulation unit for the simulated polymers. As the width of ssDNA is approximately 1 nm there can be considered to be a factor of 4 mismatch between simulations and ssDNA mapping.

The initial monomer density $\rho_0 = 1.0$ receives the most attention in Publications II and III. For this initial density, the inner diameter of capsids range from 3.6 to 9.1 simulation units, which corresponds to 16 nm and 41 nm, respectively. Recalling Section 2.4.1, this falls within the same range of radii as many real viral capsids whose outer diameter can range from 26 nm to 125 nm [46]. The results in Publications II and III can be thus considered applicable for understanding capsid ejection of ssDNA (or RNA) that, although less studied, exist in nature (e.g., leviviridae [82,83]).

In Publication IV we introduce the bending potential which, opens the possibility to model also dsDNA more credibly. The persistence length and width of dsDNA is 50 nm and 2 nm, respectively. Hence, the polymer for which $\kappa = 20$ has the persistence length of 44 nm and the width 2 nm, if we take one simulation unit to correspond to 2 nm, which would best correspond to the properties of dsDNA. To thoroughly understand the role of bending rigidity in capsid ejection, we use multiple values of $\kappa$ in Publication IV. The capsid diameters can be considered to range from 7 nm to 14 nm for $N_0 = 25$ (50 nm) and $N_0 = 200$ (400 nm) for $\rho_0 = 0.75$, respectively. Hence, we are in the correct length scale range. However, in nature the length of dsDNA is typically much longer compared to the capsid size than in our simulations [84]. For example, for $\lambda$ phage the $\lambda$-DNA is 260 times the capsid diameter [46,49].
4. Results and discussion

4.1 Sedimentation of knotted polymers

We simulated sedimentation of knotted polymers by exerting a constant force \( f_d = 0.1 \) on each polymer bead. The length of the simulated polymers is \( N_0 = 215 \). The knotted polymer conformations were created with the Knotplot computer software [85]. In order to create independent starting conformations with the same topology, we let the polymers equilibrate for a sufficiently long time before the sedimentation simulation is begun.

In Fig. 4.1 (a) we show the sedimentation coefficient \( \tilde{s} \) as a function of the average crossing number \( n_c \) of the corresponding ideal knot (see Section 2.2) in bulk, i.e., free solvent with periodic boundary conditions. The solid line is a linear (affine) function fitted to the data. It seems to describe the data fairly well, especially for knots with \( n_c < 13 \). This is in agreement with the findings in Ref. [25].

In order to study the origin of the linear dependence we show the inverse of radius of gyration \( R_g^{-1} \) during sedimentation as a function of average crossing number \( n_c \) in Fig. 4.1 (b). The figure shows that also \( R_g^{-1} \) during

![Figure 4.1](image-url)

Figure 4.1. In bulk. (a) Sedimentation coefficient \( \tilde{s} \) as a function of average crossing number \( n_c \). (b) Inverse of radius of gyration \( R_g^{-1} \) during sedimentation as a function of average crossing number. (c) Sedimentation coefficient \( \tilde{s} \) as a function of inverse of radius of gyration \( R_g^{-1} \) during sedimentation. The dashed (red) line shows Eq. (2.10), where \( \rho_s = 1.33R_g \). Values of \( n_c \) obtained from [23].

The figures are reprinted from Publication I with permission.
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Figure 4.2. (a) Different appropriately normalized components of radius of gyration during sedimentation as a function of average crossing number. $\hat{R}_g = R_g / \sqrt{3}$, $R_{g\perp} = R_{g\perp} / \sqrt{2}$, see Eq. (2.3)
(b) Different components of radius of gyration during sedimentation between parallel walls. Force is applied in the $z$-direction.
(c) Sedimentation coefficient $\hat{s}$ as a function of average crossing number $n_c$ between parallel walls.

The figures are reprinted from Publication I with permission.

Sedimentation depends linearly on the knot topology. Deviations from linearity are similar in $n_c$ and $R_g^{-1}$, which suggests that $\hat{s}$ can, indeed, be explained by $R_g^{-1}$. We plot $\hat{s}$ versus $R_g^{-1}$ in Fig. 4.1 (c). $R_g$ can clearly be regarded as the explaining factor of sedimentation velocity when using these simulation parameters. The dashed line shows the sedimentation coefficient calculated for a sphere of radius $1.33R_g$ using Eq. (2.10). A reasonable correspondence with the data is obtained. The viscosity $\eta = 4.78$ is obtained using the formulas in Ref. [63]. This approximation is clearly not sufficient to describe sedimentation of knotted polymers but it gives a reasonable estimate of the process.

One of the motivations for this study lies in understanding the sedimentation process without making any assumptions about the polymer conformation or hydrodynamic interactions. Therefore, we measure the different components of $R_g$ during sedimentation, see Fig. 4.2 (a). The plot shows that the polymer conformations do not remain isometric as the different (normalized) components of $R_g$ do not collapse. For knots of small complexity the polymer is slightly more elongated in the direction parallel to the driving force. This, however, changes as knot complexity increases and polymers’ elongation in the direction perpendicular to the force increases. This shows that the polymer is not in equilibrium during sedimentation and a direct model without assumptions about the polymer conformation is in order.

In addition to the sedimentation in the bulk, we have studied sedimentation between two parallel infinite plates that are 5 simulation length units (bond lengths) apart. This allows us to study the effect of confinement, which might be present in the laboratory and even used to control
Figure 4.3. Between parallel walls. (a) Sedimentation coefficient $\tilde{s}$ as a function of average crossing number $n_c$. (b) Sedimentation coefficient $\tilde{s}$ as a function of inverse of radius of gyration $R_g^{-1}$. (c) Sedimentation coefficient $\tilde{s}$ as a function of inverse of radius of gyration $R_g^{-1}$.

The figures are reprinted from Publication I with permission.

sedimentation. We use no-slip boundary conditions for the solvent particles hitting the walls and an LJ potential truncated at a distance 0.1 from the wall for the polymer particles. Figure 4.2 (b) are shown the components of radius of gyration of different knots. The $x$-component of the radius of gyration $R_{gx}$ is almost completely restricted by the walls that are parallel to the $yz$ plane. However, in other directions the knot topology determines the elongation of the polymer. We find a similar trend as in the bulk case, namely that $R_{gy}$, which is perpendicular to the force, is smaller than $R_{gz}$, which is parallel to the force, for simple knots and vice versa for more complex knots. However, in the slit these differences are much smaller.

Even in this strong confinement in the $x$ direction we find a linear relation between $\tilde{s}$ and $n_c$, as shown in Fig. 4.2 (c), although not quite as precise as in the bulk. The linear dependence between $R_g^{-1}$ and $n_c$ is found to a good precision, see Fig. 4.3 (a). Although the walls clearly disturb the sedimentation process, the knot complexity still (linearly) determines the radius of gyration of the polymer.

Figure 4.3 (b) shows $\tilde{s}$ as a function of $R_g^{-1}$ measured in the slit. Based on the above observations it would be unrealistic to assume a perfect fit but still, a reasonably good linear dependence is obtained. A more precise analysis shows that the dominating factor in explaining the sedimentation coefficient is the inverse of the $y$ component of radius of gyration, as shown in Fig. 4.3 (c). This is the component that is parallel to the walls but perpendicular to the sedimenting force. Actually, this is the dependence that is also visible in Fig 4.3 (b) as the $x$ and $y$ components are dominating and closely reminiscent. The $z$ component shows only as a small constant.

In conclusion, we find that the explanation for $\tilde{s}$ to depend linearly on
Results and discussion

$n_c$ is that $R_g$ is also almost directly linearly dependent on $n_c$. Between parallel walls the linear dependence between $\tilde{s}$ and $n_c$ is weaker than in the bulk, but the inverse of the $y$ component of the radius of gyration $R_{gy}^{-1}$ and $\tilde{s}$ have a linear dependence.

4.2 Capsid ejection of flexible chains

In this section we present the main results of Publications II and III considering the ejection of flexible polymer chains. The simulations were performed by ejecting polymers from a capsid that has a shell of thickness 3. We use a cylindrical pore of radius 0.4 for the polymer and 0.8 for the solvent through which the polymer ejects, see Fig. 2.2. The initial polymer conformations were created by capsid injection. The polymers were created initially outside the capsid, after which they were packed inside the capsid via capsid injection using a force at the pore. The force was ramped up until the beads were inside the capsid. The ejection began when the bead at the pore is released for ejection. No external forces were exerted on the polymer after the polymer was released. The presented quantities are always averages over at least 45 independent simulations.

![Figure 4.4](image)

**Figure 4.4.** (Color online) Ejection time $\tau$ as a function of polymer length $N_0$ for SRD without hydrodynamics. Initial monomer density from top to bottom: $\rho_0 = 0.5, 0.75, 1.00, 1.25, 1.50$. The lines show functions $\tau \sim N_0^\beta$. The fitted values of $\beta$ are shown in Table 4.1.

The figure is reprinted from Publication III with permission.
4.2.1 Ejection times

In Publications II and III we study the ejection of flexible polymer chains. In polymer translocation processes the ejection time is commonly used to characterize the process. As discussed in Section 2.4 various studies suggest that the ejection times $\tau$ of polymers of different lengths $N_0$ follow a scaling relation of the form

$$\tau \sim N_0^\beta,$$  \hfill (4.1)

when starting the ejection from a constant initial monomer density $\rho_0 = N_0/V$, where $V$ is the inner volume of the capsid. $\beta$ is the scaling exponent. Fig. 4.4 shows $\tau$ as a function of $N_0$ for a few different $\rho_0$. The ejection times fall fairly well on a line on logarithmic scale, which implies that Eq. (4.1) holds quite well in this regime. The fitted values of $\beta$ are shown in Table 4.1. The exponent $\beta$ decreases as $\rho_0$ increases, which is in agreement with previous findings in capsid ejection [86] and with forced translocation where increasing pore force decreases the exponent in a similar scaling relation. This result seems to corroborate the proposed scaling relation between ejection time and polymer length. However, examining the ejection dynamics more carefully with the aid of waiting times reveals that relying only on ejection times leads to deficient conclusions.

Table 4.1. The exponents $\beta$ of the fitted curves $\tau \sim N_0^\beta$ for various models. For SRD without hydrodynamics (noHD) the curves are shown in Fig. 4.4. Included are $\beta$ for SRD with hydrodynamics (HD, Fig. 4.13) and Langevin Dynamics (LD).

<table>
<thead>
<tr>
<th>$\rho_0$</th>
<th>HD</th>
<th>noHD</th>
<th>LD</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.50</td>
<td>1.30</td>
<td>1.36</td>
<td>1.37</td>
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<td>0.75</td>
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<td>1.22</td>
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<td>1.29</td>
</tr>
<tr>
<td>1.50</td>
<td>1.22</td>
<td>1.29</td>
<td>1.27</td>
</tr>
</tbody>
</table>

4.2.2 Waiting times and cumulative waiting times

In Fig. 4.5 (a) the cumulative waiting time $t$ is shown as a function of translocation coordinate $s$ for polymers of different lengths on logarithmic scale. Note that $t$ does not scale as a function of $s$ even though the endpoints $t(N_0) = \tau$ for different polymer lengths scale according to (4.1). In other words, the cumulative waiting time does not follow the relation
Results and discussion

Figure 4.5. (Color online) (a) Cumulative waiting time $t(s)$ for various polymer lengths $N_0$ for initial monomer density $\rho_0 = 1.0$. (b) Cumulative waiting time as a function of normalized reaction coordinate $s/N_0$ on semi-logarithmic scale. Figure (a) is reprinted from Publication III with permission. Figure (b) is reprinted from Publication II with permission.

$t(s) \sim s^\beta$, which is in contrast to forced translocation (see, e.g., Ref. [87], Eqs. (14a) and (14b)). Plotting the cumulative waiting times on semi-logarithmic scale reveals that they increase almost exponentially after about 20% of the polymer has escaped. This is shown in Fig. 4.5 (b) where $t$ is plotted versus the normalized translocation coordinate $s/N_0$. Also, note that the cumulative waiting time profiles $\hat{t}(s/N_0)$ are closely of the same form when plotted this way. The distances in the $y$ direction between the $\hat{t}(s/N_0)$ curves with different $N_0$ are almost constant throughout the ejection. In the semi-logarithmic scale this means that the different curves differ only by approximately a constant factor.

The above observations call for a more careful investigation of momentary ejection rates. The waiting time $t_w(s) = t(s) - t(s - 1)$ proves to be a valuable tool for this. The higher the value of $t_w(s)$ is, the longer the bead $s$ has to wait for ejection after bead $s - 1$ has ejected. In Fig. 4.6 we show $t_w$ as a function of the normalized translocation coordinate $s/N_0$, i.e., $\hat{t}(s/N_0)$, for a few different $N_0$. Since $t(s)$ increase exponentially, also $t_w(s)$ follow an exponential increase fairly accurately. $\hat{t}_w(s/N_0)$ follow approximately a straight line on the semi-logarithmic scale until the final part of the ejection. An integral characteristic is also that $\hat{t}_w(s/N_0)$ fall on top of each other except for this final part. If $\hat{t}(s/N_0)$ were strictly of the same form for all $N_0$, the total ejection time would, by definition, be linearly dependent of $N_0^\beta$. Indeed, Fig. 4.6 indicates, that as $N_0$ increases, the curves do fall on top of each other better. This suggests that $\beta \to 1$, in the observed $\tau \sim N_0^{\beta}$ relation when $N_0 \to \infty$.

Ejection time for a polymer of length $N_0$ can be written as $\tau = t(N_0) = \int_0^{N_0} t_w(s)ds = N_0 \int_0^1 \hat{t}_w(x)dx$, using the normalized waiting time profile $\hat{t}_w(s/N_0)$. 

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Figure 4.6. Waiting time $t_w$ as a function of normalized translocation coordinate $s/N_0$ for a few polymer lengths. Initial monomer density $\rho_0 = 1.0$.

Let us compare $t(s)$ and $t_w(s)$ obtained from simulations to the theoretical predictions derived by Sakaue and Yoshinaga [43] (see Section 2.4.4). Figure 4.7 (a) shows $t(s)$ along with Eq. (2.24), where the parameter $\tau_1$ is fitted to $t(s)$ for polymers of $N_0 = 400$. The curves are truncated where Sakaue and Yoshinaga argue that the driven part of the translocation ends, i.e., when the number of beads inside the capsid $N$ equals $g_0$. Indeed, there is a reasonably good correspondence with the predicted functional form and the simulation data.

A similar comparison is shown for $\hat{t}_w(s)$ in Fig. 4.7 (b) for polymers of

Figure 4.7. (a) Cumulative waiting time $t$ as a function of translocation coordinate $s$ (blue, dotted line) along with the theoretical formula Eq. (2.24) by Sakaue and Yoshinaga (red, solid line). The theoretical lines are truncated where the driven regime is expected to end, i.e., $s = N_0 - g_0$. (b) Waiting time $t_w$ as a function of normalized translocation coordinate $s/N_0$ along with the theoretical prediction Eq. (2.25). The (blue) points show where the driven regime ends for polymers of different length. Data obtained from LD simulations for initial monomer density $\rho_0 = 1.0$ in both figures. The constant $\tau_1$ obtained by fitting.
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different lengths. The blue stars denote the points where the driven regime ends, i.e., $g_0 = N$ for polymers of different length (from left to right $N_0 = 25, 50, 100, 200, 400$). Indeed, the correspondence is relatively good again for the driven part. The analytical estimate, however, is more strongly increasing in both cases. In other words, the analytical formula underestimates the waiting time in the beginning of the ejection and overestimates in the end of the ejection.

In the following sections we investigate the reason for this mismatch by force measurements. We show that by taking the form of the force into account more accurately we can derive more precise estimates for the waiting times and better understand the dynamics of ejection.

4.2.3 Equilibrium force at the capsid pore

To explain the cause of the exponential increase of waiting times $t_w$ as a function of $s$ we measured the force $f$ required to hold the polymer bead at the pore at rest. The measurement is performed as follows. We start with a fully packed conformation and hold the polymer bead $s = 1$ in the pore using a harmonic force. We then let the polymer to equilibrate for a time $t_{eq}$, after which we measure average force for a time $t_{mt} = t_{eq}$. The force measured this way is regarded as the pore force for bead 1. After this we move the next bead $s = 2$ to the pore and perform the same equilibration and measurement procedure. We continue like this to obtain the pore force for each $s$. We measure the pore force at two different positions, namely in the pore entrance as show in Fig. 4.8 (a) and in the middle of the pore Fig. 4.8 (b). The measured force values are always averages over at least 100 independent Langevin dynamics simulations. We chose equilibration and measurement times long enough, so that the measured force values did not change by further increasing these times. Thus the measured force values correspond to close-to-equilibrium values of force.

![Figure 4.8](image)

**Figure 4.8.** The force exerted on the bead at the pore is measured by tracking the force required to hold the bead still.

The figures are reprinted from Publication III with permission.
This also means that they do at best reflect indirectly the force present during ejection.

Figure 4.9 (a) shows the force as a function of $s$ for polymers of length $N_0 = 200$ and for $\rho_0 = 1.0$ measured in the two different places at the pore using SRD and LD. Figure confirms that the obtained force values are identical for LD and SRD. In addition, force measured in the middle of the pore $f_{\text{mid}}$ is smaller than force measured at the pore entrance $f_{\text{ent}}$. Note that unlike $f_{\text{mid}}$, $f_{\text{ent}}$ does not decay to zero even at the end of the ejection when $s = N_0$. The reduction of degrees of freedom in the pore entrance creates an asymmetry which induces a bias towards the exit of the pore. In Publication II we used only the entrance measurement as the entrance point residing at the spherical confinement boundary seemed like the logical position to obtain $f$ that best describes the ejection dynamics. In the following we focus mainly on the measurements performed at the pore middle and therefore define $f \equiv f_{\text{mid}}$.

In the remaining part of this section, we study how the force measured here compares to the force proposed by previous theoretical predictions. We then show that the functional form of $f$ vs $\rho$ for different polymer lengths is sensitive to the exact interpretation of the capsid inner volume. In the next section we show how the force measurements can be used to infer the ejection dynamics characteristics via first-passage times.

**Functional form of force and theoretical predictions**

Figure 4.9 (b) shows the (average) force as a function of the monomer density $\rho = 3N/(4\pi R_0^3)$ inside the capsid for the single polymer length $N_0 = 400$. The dashed line shows the curve $f \sim \rho^{1/(3\nu - 1)} = \rho^{1.25}$, where $\nu = 0.6$. This $f$ corresponds to the form of free energy obtained from blob-scaling [55]. Obviously, blob-scaling form does not describe the free energy in our simulations. The reason is that blob-scaling assumptions are not valid in the high monomer densities used in this study, because there are only of the order of one monomer per blob and therefore the monomer-monomer interactions are dominating, see Section 2.4.5 and Ref. [56]. In addition, the form for the free energy $\Delta F = kTN/R_0^{1/\nu} \Rightarrow f \sim \text{constant}$ used in Ref. [52] is also incorrect.

Sakaue and Yoshinaga used the free energy corresponding to form $f \sim \rho^{1.25}$ in their derivation of ejection dynamics [43]. Despite the free energy in their computation not reflecting force in our simulations, the correspondence with our simulation results is fairly good as shown in Figs. 4.7.
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Cacciuto and Luijten measured that the free energy of spherical confinement follows $\Delta F \sim \rho^{2.97}$ [56], which corresponds to force $f \sim \rho^{1.97}$. The authors proposed that this occurs due to “screening of the excluded-volume interactions in concentrated solution [88, 89], which reduces the effective Flory exponent to its Gaussian value”. The Gaussian value of $\nu = 0.5$ implies $f \sim \rho^2$, which indeed describes the force in our simulations fairly well as shown by the solid line in 4.9 (b). However, Cacciuto and Luijten do not comment on how this form affects the ejection dynamics, but they use $\nu = 0.6$ in their later article considering capsid ejection [57]. The scaling $f \sim \rho^2$ does not hold for very high densities. This shows better in 4.9 (d) plotted on natural scale. The deviation increases even further for densities as high as $\rho = 1.5$ (not shown).

Fig. 4.9 (c) shows the same force data as in Fig. 4.9 (b) on semi-logarithmic scale. For $\rho$ higher than $\sim 0.4$ the measured force values fall very closely on a line. In other words, for higher densities $f$ increases exponentially as a function of $\rho$. This can be explained by the Flory-Huggins theory [42], as
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Figure 4.10. (a) Force $f$ measured at the pore center as a function of density $\rho$ inside the capsid for few different polymer lengths. Initial monomer density $\rho_0 = 1.0$. (b) The same force $f$ plotted against effective density $\rho_e$ inside the capsid, see Eq. (4.2). Shifted exponential and $\rho_e^2$ functions are also shown.

was pointed out by T. Sakaue in private communication during the writing of Publication II. For lower $\rho$, the dependence of $f$ on $\rho$ is not exponential.

In Publication II we based the analysis only on force measurements $f_{\text{ent}}$ at the pore entrance. This measurement gives strictly exponential dependence for $f$ vs $\rho$, even at low monomer densities. The more detailed force measurements with LD and in the middle of the pore in Publication III revealed that $f(s)$ actually decays to zero when $s = N_0$, i.e., $\rho = 0$. Hence, in Fig. 4.9 (c), we plot the curve of the form $f \sim \exp(2.9\rho) - 1$ that is exponential for higher densities but decays to exactly zero when there is exactly zero monomers inside the capsid. This functional form captures the behavior of $f$ at high monomer densities as well as the decay of force for low densities. Finally, to compare different functional forms we plot $f$ vs $\rho$ on natural scale in Fig. 4.9 (c). The shifted exponential is seen to capture the form of $f$ vs $\rho$ for all $\rho$ more precisely than $\rho_e^2$. In Section 4.2.5 we compare the relation between first-passage times and force using the integral formula shown in Section 2.4.7.

**Force and effective density**

Above, we considered force measurements only for distinct polymer lengths $N_0 = 200$ and $N_0 = 400$ for constant $\rho_0$. Since understanding how the length $N_0$ of the polymer affects the ejection is an important means of characterizing capsid ejection, we plot the measured force as a function of monomer density inside the capsid for polymers of multiple lengths in

2Flory-Huggins theory predicts that the free energy per lattice site diverges as $(1 - \phi)\ln(1 - \phi)$, when $\phi \to 1$, for hard sphere lattice polymers. Here $\phi$ is the fraction of lattice sites occupied by polymer segments. This form resembles the exponential form assuming that $\rho \sim \phi$. More details in Publication II.
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Fig. 4.10. We see that for different $N_0$, force values do not collapse but the longer polymers experience stronger force at the pore than the shorter ones. It turns out that this is caused by part of the monomers’ repulsive potential overlapping with the capsid shell. This is due to LJ potential having a spherical interaction range of $\sqrt[3]{2}$ while the interactions between the monomers and capsid walls are treated with boundary conditions that consider monomers as point-like particles.

Let us define the effective monomer density

$$\rho_e = \frac{N}{\frac{4}{3}\pi (R_0 + \zeta)^3} = \left(1 + \frac{\zeta}{R_0}\right)^{-3}\frac{N}{V}, \quad (4.2)$$

where $V = 4/3\pi R_0^3$ and $R_0$ is a function of the chosen initial condition $R_0 = \sqrt[3]{3N_0/(4\pi \rho_0)}$. In Fig. 4.10 (b) we show the force as a function of the effective monomer density when the correction parameter $\zeta = 0.3$ is chosen to give a good collapse for the force measured for polymers of different lengths. The effect of the correction should diminish as the polymer length increases, because $R_0$ increases when $N_0$ is increased. In other words, $\rho_e \to \rho$ when $N_0 \to \infty$. Within our limited range of $N_0$, the effect is clearly visible and this detail cannot be completely disregarded when interpreting the results of ejection. However, since the correction is a linear operator for constant $N_0$ and constant $\rho_0$, it does not have an effect on the exact functional form between $f$ and $N$ or $\rho$. The force curves collapse also for the different initial monomer densities studied here (not shown). It can therefore be concluded that for flexible chains, the equilibrium force is closely a function of the effective density only.

4.2.4 Relation between the force and the waiting time

In Sections 4.2.2 and 4.2.3 we showed that the waiting time $t_w$ and $f$ are of approximately exponential form. Therefore, it is reasonable to expect that there is a clear relation between $t_w$ and $f$. To check this, we plot $t_w$ and the inverse of the force in Fig. 4.11 (a) in semi-logarithmic scale for $N_0 = 400$. Note that we have scaled $1/f$ with a constant to get it in the same range as $t_w$. Clearly, there is a very good correspondence between $1/f$ and $t_w$, especially in the beginning of the ejection. Up to around $s \approx 250$ the force almost solely determines the waiting time.

In Publication II we argued that if the force is of exponential form, then the waiting times are also of this form (see Eq. (2.33)). Hence, we plot the exponential curve $\sim \exp(2.8/N_0s)$ in the same figure to show that, indeed, in the beginning of ejection $t_w(s)$ and $1/f(s)$ are close to an expo-
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Figure 4.11. (a) Waiting time $t_w$ along with inverse of force $1/f$ in equilibrium as a function of translocation coordinate for $N_0 = 400$ and $\rho_0 = 1.0$. Semi-logarithmic scale. (b) Waiting times for polymers of different lengths with double exponential curves of Eq. (4.3).

Figure (b) is reprinted from Publication III with permission.

Closer investigation of waiting times reveals that they can be very accurately described by a sum of two exponentials

$$t_w = E \left\{ \exp \left( \frac{2.8}{N_0} s \right) + \exp \left[ \frac{10.8}{N_0} (s - 0.625N_0) \right] \right\}, \quad (4.3)$$

as shown in Fig. 4.11 (b) for simulations using LD. The constant $E = 47$ was chosen to give a good correspondence with data. The formula works remarkably well also for polymers of different lengths. Although the formula is not derived from theoretical predictions, its form can be justified with simple arguments. In the beginning the momentary ejection rate is determined by the force as is evident from Fig. 4.11. As the ejection proceeds, there is a smooth transition to a regime where the force decays so much that it no longer drives the ejection. This occurs when about 63% of the polymer has ejected the capsid (for $\rho_0 = 1$). Note that since the double exponential is a function of $s/N_0$, it should lead to linear scaling of $\tau$ similarly as a single exponential function. Only the final retraction of the ejection deviates from the double exponential form. As the significance of the final retraction diminishes as $N_0$ increases, this should lead to linear scaling for extremely large $N_0$. 


4.2.5 First passage time derived from the pore force

It is useful to have one more analytical approach to the ejection process of flexible polymers. Here we show that the first-passage time formula Eq. (2.39) with the shifted exponential force of form $f \sim [\exp(B\rho) - 1]$ is capable of producing first-passage time curves that are in good agreement with the results obtained from simulations. This treatment differs from the theoretical calculations presented above in that the first-passage time formula models also diffusion. This property makes sure that the ejection finishes also with the force that decays exactly to zero at the end of the ejection. In other words, if we used force of the form $f \sim [\exp(B\rho) - 1]$, the waiting times would diverge assuming that $t_w \sim 1/f$.

In Fig. 4.12 (a) we show Eq. (2.39) solved numerically for each $s$ along with the first-passage times obtained from LD simulations. The free parameters $kT = 5.23$ and $\xi_m = 254.5$ in Eq. (2.39) were chosen to give good correspondence with the LD simulations for all points, emphasizing the endpoints. This shows that the form of the force is very good in explaining the form of the first-passage times of real simulations throughout the ejection for multiple polymer lengths.

However, it is in order to note that, for simplicity, we have not taken into account that the forces in LD simulations differ from forces in the first-passage time formula, since the first-passage time formula uses $f = f(\rho)$, while in the LD simulations the force is actually a function of the effective density $f = f(\rho_e)$.

The result obtained from the first-passage time formula is very sensitive to the exact choice of the functional form of the force, even assuming that it is only a function of $\rho$. If the force is above zero also for the endpoint
where \( s/N_0 = 1 \), the ejection time leads to linear scaling. However, if in the end there is a regime where the force is absolutely zero, \( i.e. \), pure diffusion, the formula gives scaling with exponent 2. The presented case where \( f = 0 \) for \( s/N_0 = 1 \) is a special case that, by numerical integration, seems to give linear scaling for extremely large \( N_0 \). This property also explains why we obtain such a nice superlinear scaling of ejection times for our limited range of \( N_0 \), while the waiting times seem to predict linear scaling for long polymers.

### 4.3 Hydrodynamic modes in capsid ejection

In the quest for more accurate models and description of capsid ejection it is essential to carefully study the effect of hydrodynamics. Above we considered capsid ejection with simulation models that do not include hydrodynamic effects. In this section we show how the inclusion of hydrodynamic modes changes the dynamics of capsid ejection.

In Fig. 4.13 we plot ejection time \( \tau \) as a function of polymer length \( N_0 \) for ejection simulations where hydrodynamics is modeled using stochastic rotation dynamics. The logarithmic plot shows that there seems to be a very similar scaling relation \( \tau \sim N_0^\beta \) with and without hydrodynamics, see Fig. 4.4. The exponents of the lines are shown in Table 4.1. Inclusion of hydrodynamics makes the exponents \( \beta \) smaller, similarly as for forced polymer translocation [66,90]. This occurs due to hydrodynamic effects reducing friction everywhere, but less so in the pore, where large part of
the friction is caused by the pore geometry. In translocation, local friction at the pore reduces the $\beta$ exponent towards unity, i.e., linear dependence between $\tau$ and $N_0$. A similar effect is present in capsid ejection.

As was the case without hydrodynamics, the analysis based only on ejection times is not very conclusive with hydrodynamics either. In Fig. 4.14 (a) we show waiting times as a function of translocation coordinate $s$ together with a plot of Eq. (4.3), where $E = 32$ instead of 42 as was the case without hydrodynamics. The plot shows that similar double exponential behavior is present without hydrodynamics as with hydrodynamics, only with a smaller prefactor. To compare the differences between ejection dynamics with and without hydrodynamics more closely, we plot cumulative

![Figure 4.14](image_url)

**Figure 4.14.** Hydrodynamics included. (a) Waiting time $t_w$ as a function of translocation coordinate $s$. The curves show double exponential functions of Eq. (4.3). (b) Cumulative waiting time $t$ as a function of $s$ without hydrodynamics (blue, dashed) and with hydrodynamics (red, solid) multiplied by $42/32$ in order to align them for comparison. Figure (a) is reprinted from Publication III with permission.

The friction is caused by the pore geometry. In translocation, local friction at the pore reduces the $\beta$ exponent towards unity, i.e., linear dependence between $\tau$ and $N_0$. A similar effect is present in capsid ejection.

As was the case without hydrodynamics, the analysis based only on ejection times is not very conclusive with hydrodynamics either. In Fig. 4.14 (a) we show waiting times as a function of translocation coordinate $s$ together with a plot of Eq. (4.3), where $E = 32$ instead of 42 as was the case without hydrodynamics. The plot shows that similar double exponential behavior is present without hydrodynamics as with hydrodynamics, only with a smaller prefactor. To compare the differences between ejection dynamics with and without hydrodynamics more closely, we plot cumulative

![Figure 4.15](image_url)

**Figure 4.15.** Radius of gyration $R_g$ outside the capsid, i.e., trans side, as a function of translocation coordinate $s$ during ejection with and without hydrodynamic modes for a few different polymer lengths $N_0$. Initial monomer density $\rho_0 = 1.0$. $R_{g, eq}$ shows the radius of gyration of a polymer of corresponding length in equilibrium.

The figure is reprinted from Publication III with permission.
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waiting times and scale \( t(s) \) for the case of included hydrodynamics by \( 42/32 = 1.3125 \) to allow for easier comparison of their forms in Fig. 4.14 (b). The cumulative waiting times are remarkably similar in form. The differing \( \beta \) exponents show as a slight deviation of the curves, especially at the end of ejection. In summary, the inclusion of hydrodynamic modes speeds up ejection, but has only a small effect on general characteristics of ejection dynamics.

In Fig. 4.15 we show the radius of gyration \( R_{g,\text{trans}} \) of the part of the polymer outside the capsid (\( \text{trans} \) side) as a function of \( s \) with and without hydrodynamics for a few polymer lengths. We see that the polymer segment on the \( \text{trans} \) side is smaller than the equilibrium radius of gyration \( R_{g,\text{eq}} \) of a polymer of corresponding length. This means that the ejection occurs faster than the relaxation on the \( \text{trans} \) side. Although the ejection is faster when hydrodynamics is included, \( R_{g,\text{trans}} \) is larger with hydrodynamics than without hydrodynamics. In other words, hydrodynamics speeds up the relaxation more than ejection.

4.4 Capsid ejection of semiflexible chains

In Publication IV we study the more complex capsid ejection of semiflexible polymer chains. Semiflexible polymers resist bending, \( \text{i.e.,} \) they have bending rigidity. Bending rigidity is an integral part of many biopolymers, the most relevant being the dsDNA in the context of capsids. In this section we show that the inclusion of bending rigidity dramatically changes ejection dynamics.

Here, we redefine the initial monomer density \( \rho_0 \) to take into account the overlap of the Lennard-Jones potential and the capsid walls. The motivation is to set \( \rho_0 \) such that the force for flexible chains is constant for different polymer lengths, see Fig. 4.10. We define

\[
\rho_0 = \frac{N_0}{\frac{4}{3} \pi (R_0 + \zeta)^3},
\]

where \( \zeta = 0.3 \) (in the previous sections \( \zeta = 0 \)). For instance, \( \rho_0 = 0.75 \) in this section corresponds to \( \rho_0 = 1.01 \) in the previous sections for \( N_0 = 100 \). Similarly, \( \rho_0 = 0.75 \) here corresponds to \( \rho_0 = 0.95 \) for \( N_0 = 200 \).

In this section we mainly use the torus pore instead of the cylinder pore used in previous simulations, see Fig. 4.16. This is necessary in order to improve the pore force measurements and maintain the correspondence between measured force magnitudes and those present in the simulated
ejections. The torus pore also has the advantage of lower pore friction, especially for polymers of high bending rigidity. This allows us to focus more on the dynamic characteristics of ejection. We also briefly compare the ejection times of the torus pore to those of the more frictional cylinder pore.

4.4.1 Packaging rate and ejection time

It has been shown that the initial packaged conformation of the polymer has a considerable effect on ejection rate [91]. Therefore, it is necessary to pay special attention to the exact packaging method in order to obtain results that are comparable for polymers of different lengths. In the case of semiflexible polymers we chose to package the beads one bead at a time. We start by holding bead $s = N_0$ in the pore and the rest of the polymer outside the capsid and let the polymer equilibrate for time $t_{eq}$. After this we pull the next bead $s = N_0 - 1$ to the pore and again let the polymer equilibrate for time $t_{eq}$. We continue packaging the polymer one bead at a time this way, until the whole polymer is inside the capsid (except for the small tail left inside the pore). The method is similar to that used in force measurements of flexible polymers, only here the polymer moves in the opposite direction. Since long equilibration times $t_{eq}$ are required, we use Langevin dynamics in the packaging process to save CPU time.

Figure 4.17 (a) shows normalized ejection time of polymers packaged using different values of $t_{eq}$ for two different polymer lengths. Here, also the ejection simulations were performed with Langevin dynamics. The
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Figure 4.17. (a) Ejection time $\tau$ as a function of time used to relax the polymer for each bead during packaging $t_{eq}$. Packaging and ejection simulated using Langevin dynamics. Initial monomer densities $\rho_0 = 0.743$ for $N_0 = 100$ and $\rho_0 = 0.826$ for $N_0 = 400$. (b) Ejection time $\tau$ as a function of bending potential parameter $\kappa$ for $N_0 = 200$ with and without hydrodynamics and with torus and cylinder pore.

The figures are reprinted from Publication IV with permission.

notation $\tau_{4000}$ means the average ejection time $\tau$ when packaged using $t_{eq} = 4000$. Figure shows that, indeed, the ejection time is dependent on the rate of packaging. The ejection is slower when the polymer is allowed more time for equilibration. This occurs because the increased equilibration time allows the polymers to find deeper local energy minima. Correspondingly, the time to release the polymer from the minima increases. Obviously, longer polymers require a larger $t_{eq}$ for the ejection time to converge, since they have longer relaxation times. We chose to package all the polymers using $t_{eq} = 2000$ in the simulations hereafter, since polymer conformations can be considered sufficiently equilibrated using this value. It is worth noting that the packaging takes a considerable amount of CPU time, which restricts the choice of $t_{eq}$.

4.4.2 Ejection rate vs. persistence length

Let us study how the ejection time $\tau$ depends on the bending potential parameter $\kappa$ for different models using a single polymer length $N_0 = 200$ and $\rho_0 = 0.75$. Figure 4.17 (b) shows $\tau$ as a function of $\kappa$ for the two pore models with and without hydrodynamics. All models show that $\tau$ consistently increases with increasing $\kappa$. For all values of $\kappa$ ejections with hydrodynamics are faster than ejections without hydrodynamics, as was observed for flexible chains in Section 4.3. Similarly, the smaller friction of the torus pore gives rise to consistently faster ejection compared to the cylinder pore.

Figure 4.17 (b) shows also that for simulations with hydrodynamics $\tau$
increases proportionately less as a function of $\kappa$ than for simulations without hydrodynamics. This phenomenon can be understood as follows. Both the inclusion of hydrodynamics and larger $\kappa$ increase correlation length. However, they have an opposite effect on ejection rate. A larger $\kappa$ means larger friction induced by the solvent, since when one bead moves also the neighboring beads need to move along with it. When hydrodynamics is present, the momentum transfer through the solvent reduces this friction and therefore increases ejection rate contrary to the effect of increasing $\kappa$. In what follows, we focus mainly on ejection where hydrodynamics is included, since it is more relevant for biological applications. In addition, the torus pore is mainly used, since it allows for better force measurements and shows the dynamic behavior of ejection more clearly.

In order to understand in more detail how $\kappa$ affects the ejection dynamics, we must study cumulative waiting times again. Figure 4.18 (a) shows the cumulative waiting time $t$ as a function of translocation coordinate $s$ for a few different values of $\kappa$. As already observed for $\tau$, increasing $\kappa$ slows down the ejection. In addition, $t$ begins to scale with $s$ when $\kappa = 20$. The cumulative waiting time follows $t \sim s^{1.33}$ quite accurately almost throughout the ejection. This is better seen in Fig. 4.18 (b), where the cumulative waiting times are plotted on logarithmic scale. The deviation from the scaling occurs only at the very end of the ejection, where the polymer no longer touches the capsid walls, as seen in Fig. 4.18 (a). This slows down the ejection considerably because the polymer is no longer pushed out of the capsid due to bending rigidity. Hence, the final ejection occurs by diffusion only.
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Figure 4.19. Force $f$ as a function of translocation coordinate $s$ for different values of $\kappa$ for polymers of length $N_0 = 200$. The figure is reprinted from Publication IV with permission.

4.4.3 Pore force

In the case of flexible chains, we found that the ejection dynamics is governed by the pore force $f$ and that $t_w$ is approximately inversely proportional to the inverse of $f$, especially at the beginning of the ejection. Here, we look at the pore force measured in the case of semiflexible polymers. The force measurement is performed similarly as for flexible chains. The only differences are that here we use the torus pore model instead of the cylinder pore model and that the capsid volume is defined differently due to volume correction, see Eq. (4.4). In Fig. 4.19 $f$ is plotted as a function of $s$ for polymers of length $N_0 = 200$ for different values of $\kappa$. Increasing $\kappa$ inevitably increases the free energy due to confinement. The figure shows that for smaller densities, i.e., larger values of $s$, the force is higher for higher $\kappa$. For small $s$, i.e., high density, this is not the case. Instead, the force curves are in a seemingly random order.

Note also how the relative magnitudes of the force for different $\kappa$ are close to each other at low $s$. This suggests that at high densities, the repulsive Lennard-Jones potential contributes to the force more than bending rigidity. For smaller densities, the contribution of bending rigidity becomes more dominant. For instance, at $s \approx 150$ the force for $\kappa = 20$ is three times the force for $\kappa = 0$. The general conclusion is that for the most part increasing $\kappa$ increases pore force. In spite of this, the ejection rate is consistently slower for higher $\kappa$. This confirms that as $\kappa$ increases, the friction increases more strongly than the spring-like force of bending rigidity.

Let us study how the pore force depends on the polymer length $N_0$ for two different values of $\kappa$. For $\kappa = 0$, $f$ for different $N_0$ almost collapse as shown in Fig. 4.20 (a). This is no surprise since we have defined $\rho_0$ to take
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into account the volume overlap of bead potentials and capsid walls, see Section 4.2.3. For the semiflexible polymers of $\kappa = 20$ the curves do not collapse as a function of $s/N_0$, as seen in Fig. 4.20 (b). Together with the force data, we plot functions of the form

$$f(s/N_0) \sim (1/N_0)^{0.4} \exp(-1.3s/N_0), \quad (4.5)$$

which are seen as lines on the semi-logarithmic scale. This function roughly describes the obtained force values. $f$ is larger for the smaller polymer, because due to choosing constant $\rho_0$, the capsid volume is smaller for shorter polymers. More precisely, we have $R_0 \sim N_0^{1/3}$. Hence, we can write that $f \sim (\lambda_p/R_0)^{1.2}$, where the exponent is quite close to 1. This means that there is close to inverse proportionality between $f$ and $R_0$ for a sufficiently large $\kappa$. Also, note that for $\kappa = 20$ and constant $s$, $f$ scales like $f \sim N_0^{-0.4}$ for a large part of ejection. In the section below, we study how these observations reflect to the cumulative waiting times for polymers of different lengths.

4.4.4 Dependence of dynamics on the polymer length

In this section we study how the ejection dynamics’ dependence on polymer length changes with increasing persistence length. In Fig. 4.21 we show the ejection time $\tau$ as a function of polymer length for different $\kappa$ when hydrodynamics is included. For all $\kappa$, the ejection time follows a scaling relation of the form $\tau \sim N_0^\beta$. The lines show these relations with fitted exponents, whose values are shown in Table 4.1. Equally good scaling relations are obtained for simulations without hydrodynamics and with the cylinder pore. These exponent values are also shown in Table 4.2. The larger friction of the cylinder pore shows as smaller $\beta$ values. This is

Figure 4.20. Pore force $f$ as a function of the normalized translocation coordinate $s/N_0$ for polymers of different lengths. (a) $\kappa = 0$ (b) $\kappa = 20$. The lines show $f = 12.5(1/N_0)^{0.4} \exp(-1.3s/N_0)$. The figures are reprinted from Publication IV with permission.
similar to forced translocation where higher pore friction takes the exponent closer to 1. Likewise, exponents are smaller for simulations with hydrodynamics, which is the case also for forced translocation [66, 90]. This is due to the friction of the system decreasing everywhere except in the pore, since the pore friction is mainly geometrical. However, for $\kappa = 20$, $\beta$ is smaller without hydrodynamics than with hydrodynamics. This change indicates a change in dynamics for sufficiently rigid polymers.

In Fig. 4.21 we show the radius of gyration of the trans side $R_{g,trans}$ during ejection as a function of the translocation coordinate $s$. Note that for the same $s$, there is an equal amount of beads outside the capsid for polymers of different lengths $N_0$. Naturally, $R_{g,trans}$ is larger when $\kappa$ is larger due to higher persistence length. For high $\kappa$, values of $R_{g,trans}$ are very similar for any given $s$ for different $N_0$. Therefore it is reasonable to assume that trans side friction is the same for different $N_0$. For smaller

<table>
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Results and discussion

Figure 4.22. Cumulative waiting times $t$ as a function of translocation coordinate $s$. Torus pore. From (a) to (e): $\kappa = 0, 2.5, 5, 10, 20$. Hydrodynamics included. (f) Cumulative waiting time with and without hydrodynamics. For shortest and longest studied polymer length.

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$k$ the differences in $R_{g,\text{trans}}$ are larger, but friction is smaller for smaller $\kappa$. In Publication III we showed that for $\kappa = 0$ the trans side has only a relatively small effect on the ejection rate. Based on these measurements we conclude that for constant $\kappa$ the contribution of the trans side friction is similar for different $N_0$.

Figures 4.22 (a)-(e) show cumulative waiting times $t$ as a function of translocation coordinate $s$ for polymers of different lengths. For the smallest bending rigidities, i.e., $\kappa = 0$ and $\kappa = 2.5$, $t(s)$ is larger for shorter polymers. This means that longer polymers eject $s$ beads faster than shorter polymers. This is a direct consequence of $f$ being approximately a (decreasing) function of $s/N_0$. For instance, for a polymer of length $N_0 = 200$ there is a considerable pore force for $s = 50$, while for the polymer of length $N_0 = 50$, $f \approx 0$ close to $s = 50$. As $\kappa$ increases this changes com-
Results and discussion

For $\kappa = 10$ and $\kappa = 20$, $t(s)$ for the driven part of the ejection is always larger for a longer polymer. In other words, ignoring the final diffusion part, shorter polymers eject proportionately more quickly than longer polymers. This can be explained by the pore force being larger for shorter polymers as shown in Fig. 4.20 (b) (this is explained by the smaller $R_0$ for smaller $N_0$ due to constant $\rho_0$). Another contribution emerges due to friction on the cis side being larger for larger $N_0$.

Figures 4.22 (a)-(f) show that the increase of $\kappa$ straightens the curves for each polymer length. As seen in Fig. 4.18 the cumulative waiting time for $N_0$ scales as $t \sim s^{1.33}$. Figure 4.22 (e) shows that all the studied polymers of different lengths scale with the same exponent, only the prefactor varying. Similar scaling is obtained for simulations without hydrodynamics. Figure 4.22 (f) shows the waiting times with and without hydrodynamics for polymer lengths $N_0 = 25$ and $N_0 = 200$. This figure shows the diffusion part of the ejection more clearly. The final part of the ejection is faster with than without hydrodynamics. This is due to higher diffusion constant when hydrodynamics is included. The cumulative waiting time curves straighten on logarithmic scale when $\kappa$ is increased and the different regimes of ejection become more distinct. For small $\kappa$ the diffusive and driven regimes are strongly overlapping. As persistence length is increased, the regimes separate almost completely.

The differences in the diffusion regime of ejection explain why the exponents $\beta$ in Table 4.1 are higher for $\kappa = 20$ when hydrodynamics is enabled while for smaller $\kappa$, $\beta$ values are smaller with hydrodynamics. The effect of diffusion is enhanced for short polymers. Therefore the final diffusion increases $\tau$ more for short than for long polymers in the absence of hydrodynamics. This shows clearly on the logarithmic scale in Fig. 4.13 (f).

Finally, we show that for all $\kappa$, it is possible to make an accurate representation of the data that collapses the cumulative waiting times for different polymer lengths. Recall that we found that force approximately follows $f \sim N_0^{-0.4} \exp(-1.3s/N_0)$ for $\kappa = 20$. This means that $fN_0^{0.4}$ as a function of $s/N_0$ approximately collapses for different $N_0$. Similarly, we find that for $\kappa = 20$, plotting $t/N_0^{0.55}$ as a function of $s$ the driven part of cumulative waiting time curves collapse for different $N_0$ as shown in Fig. 4.23. This means that for $\kappa = 20$ the driven part of cumulative waiting time follows

$$t(s) \sim N_0^{-0.55}s^{1.33}.$$  \hspace{1cm} (4.6)

Similar functional form was found with and without hydrodynamics, as
Results and discussion

Figure 4.23. Scaled cumulative waiting times $s/N_0^{0.55}$ as a function of translocation coordinate $s$ for $\kappa = 20$ for polymer lengths $N_0 = 25, 50, 100$, and $200$. (a) With hydrodynamics; (b) without hydrodynamics. The separate dashed line shows $s^{1.33}$ for reference.

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Figs. 4.23 (a) and (b) show.

Further investigation of the functional form of the cumulative waiting time profiles shows that, in fact, for all studied values of $\kappa$ the cumulative waiting time of the driven part of ejection can be expressed as

$$t(s) = N_0^p \Gamma \left( \frac{s}{N_0^q} \right),$$

(4.7)

where the exponents $p$ and $q$ and the function $\Gamma$ depend on the used $\kappa$. Remarkably, when plotting $t/N_0^p$ as a function of $s/N_0^q$, curves for different $N_0$ collapse for each $\kappa$, using appropriately chosen $p$ and $q$. In Fig. 4.24 we show the data collapse when assuming dependence of this type for (a) $\kappa = 0$ and (b) $\kappa = 20$. For flexible chains we have $p = 1.2$ and $q = 1$ (without the volume correction the exponent $p$ is close to 1 in this regime). For the most rigid polymer, $\kappa = 20$, we have $p = 2$ and $q = 1.1$. This finding suggests that there might be a scaling hypothesis for the confinement energy that correctly explains the ejection dynamics. Nevertheless, the Figs. 4.24 (a) and (b) highlight the change from exponential ejection dynamics to scaling ejection dynamics. In this transition of one dynamic regime to another $\kappa$ acts as a control parameter.

The data collapse for the exponent $p = 1.2$ in Fig. 4.24 (a) suggests that for flexible chains, if the volume is appropriately corrected, the observed scaling $\tau \sim N_0^\beta$ might tend to $\tau \sim N_0^{1.2}$ for extremely long polymers. This observation is different from that in Fig. 4.6 where the collapse of waiting times indicate that $\beta$ should approach one.
Figure 4.24. Appropriately scaled cumulative waiting times as a function of appropriately scaled translocation coordinate for (a) $\kappa = 0$ (semi-logarithmic scale) and (b) $\kappa = 20$ (logarithmic scale). Exponents of $N_0$ chosen such that a good data collapse is obtained.

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Results and discussion
5. Summary and conclusions

5.1 Overview

We have studied the basic principles of single polymer dynamics in two different systems, namely sedimentation of knotted polymers and capsid ejection. We used generic coarse-grained polymer simulations to gain understanding on different regimes of polymer dynamics, caused by e.g., strength of confinement, hydrodynamic effects, polymer length, and polymer rigidity. The polymer was simulated using molecular dynamics integrated in time by the velocity-Verlet algorithm. It was coupled to the solvent modeled using stochastic rotation dynamics (SRD) that supports hydrodynamic modes. In addition, we used the more established Langevin dynamics (LD) method in order to verify that SRD gives correct results. The computational efficiency of LD allowed us to obtain better statistics, when modeling hydrodynamics was not necessary. To model the capsid and its interactions with polymer and solvent particles we implemented a constructive solid geometry model for usage in molecular dynamics simulations. The method simplistically models the interactions with the capsid walls and simulation particles as slip and no-slip collisions.

5.2 Sedimentation of knotted polymers

Sedimentation and electrophoresis have been standard tools in DNA research for decades. In 1985 it was found by electrophoresis that DNA molecules of different knot topologies migrate at different speeds [37]. Our motivation in studying sedimentation of knotted polymers was to computationally test the linear dependence of sedimentation coefficient $s$ and the average crossing number $n_c$ of corresponding ideal knot with-
out making any assumptions about the polymer conformation or hydrodynamic interactions. We found that the linear dependence quite accurately holds and verified the arguments commonly used to explain the observed linearity. Furthermore, the radius of gyration $R_g$ was found to be the explaining factor for this linear dependence.

We studied sedimentation also in a confinement where the polymer sediments between two parallel walls. The walls are so close to each other that the polymers’ conformations are restricted. The walls perturb the linear dependence between the inverse of radius of gyration $R_g^{-1}$ and the average crossing number $n_c$. This also weakens the linear dependence between $s$ and $n_c$. However, there is a linear dependence between the inverse of radius of gyration perpendicular to the sedimenting force and $s$ to a good precision. With some adjustments it might therefore be possible to classify knots by sedimentation even in a setup using a narrow slit. The sound results of sedimentation in the confinement gave confidence that the simulation method can be used to model the more complex and more confined polymer system in capsid ejection.

5.3 Capsid ejection

We studied capsid ejection from strong spherical confinement in different scenarios and regimes. The problem turned out to be intricate with its multiple details and complex dependencies between different features of ejection. This inspired us to investigate on the generic properties of ejection dynamics with its various nuances. We characterized the ejection dynamics of both flexible and semiflexible chains. We also defined the role of hydrodynamics in capsid ejection for both models.

5.3.1 Flexible chains

Using accurate measurements of pore force $f$ in equilibrium, we found that the free energy obtained from a semidilute assumption that has previously been used to characterize the ejection dynamics of flexible chains is invalid in strong confinement. This directly shows in the waiting time profiles $t_w(s)$ and cumulative waiting time profiles $t(s)$ that proved to be the most useful tools in analyzing the dynamics of ejection. We found that $t_w(s)$ is approximately a sum of two exponentials for all polymer lengths $N_0$, with and without hydrodynamics. Hydrodynamics was found to speed
up the ejection but to maintain its dynamic characteristics, *i.e.*, the forms of \( t_w(s) \). The form of \( t_w(s) \) is a direct consequence of the measured pore force \( f(s) \), which was found to be of shifted exponential form. When force decays close to zero, \( t_w(s) \) begins to increase with a different exponent than in the beginning of ejection.

For constant initial monomer density \( \rho_0 \) the ejection times were found to scale in the studied range of \( N_0 \) as \( \tau \sim N_0^\beta \), where \( \beta \) is of the order \( 1.2 - 1.5 \) depending on the studied model. However, we presented multiple results that indicate that in spite of this, \( \beta \) should approach one for extremely long polymers. For instance, \( t_w \) for different polymer lengths collapse when plotted as a function of normalized translocation coordinate \( s/N_0 \) except for the final part of ejection. The collapse is more precise for large \( N_0 \). By definition, if \( t_w \) is exactly a function of \( s/N_0 \), the ejection time scales linearly with respect to \( N_0 \). First-passage time formula solved numerically for the observed shifted exponential form of the pore force gives scaling \( \tau \sim N_0^{1.08} \) for polymers whose length is of the order \( 10^5 \). It is also noteworthy that the cumulative waiting times \( t \) do not scale as a function of translocation coordinate \( s \) for flexible chains.

The pore force magnitudes as a function of \( s/N_0 \) measured for different \( N_0 \) and constant \( \rho_0 \) were found to collapse when we applied a volume correction that takes into account the finite size effect due to polymer-capsid interaction. This indicates that it could be the valid way to select the comparable initial conditions. If that is the case, the final ejection times seem to scale superlinearly as a function of \( N_0 \). On the other hand, the calculation of first-passage times for such a force gives linear scaling for extremely long polymers. To find a definite answer to this question would require simulating polymers that are multiple orders of magnitude longer than those simulated in the present studies. This is beyond the currently available computing resources.

### 5.3.2 Semiflexible chains

We found that including bending rigidity for polymers changes the dynamics of capsid ejection to a completely different regime. Remarkably, when the persistence length of polymers is of the order of the capsid diameter, the cumulative waiting time \( t \) scales as a function of translocation coordinate \( s \) for all \( N_0 \). This indicates that there is a transition from one dynamic regime to another where the bending potential parameter \( \kappa \) acts as a control parameter. This can be regarded as a dynamic phase tran-
Summary and conclusions

In addition, the relative magnitudes of cumulative waiting times change such that for the same $s$, $t(s)$ is smaller for shorter polymers than for longer polymers which is in contrast to ejection of flexible polymers. For rigid polymers there is no direct relation between the pore force and the waiting times.

For all studied polymer rigidities we find a data collapse of the form $t(s) \sim N_0^p \Gamma(s/N_0^q)$, where $p$, $q$ and function $\Gamma$ depend on the rigidity of the polymer studied.

5.4 Outlook to further research

The results of this thesis can be used to interpret the results of real experiments and realistic simulations to understand the relevant dynamic regimes. They cannot be regarded as accurate descriptions of real systems in nature as such. Instead, the findings of the thesis describe the underlying generic dynamics, whose understanding is essential when investigating real-world systems.

The logical step to continue the research of sedimentation of knotted polymers would be to study sedimentation of supercoils and the effect of electrostatic interactions.

For studying capsids, the most natural step to take would be to include electrostatic interactions, highly relevant in real viral capsids. More complex capsid geometries might show interesting features. For example, many capsids in nature are icosahedral. Some viral capsids, like the T7 phage, have an inner core [92] that affects the packaging conformation [93] and most likely also the ejection dynamics in a non-trivial way. Knots have been shown to affect capsid ejection [4–6]. It would be interesting to study if there is a direct relation between the knot complexity, say $n_c$, and the ejection time $\tau$.

The scaling function found in this thesis suggests there may exist a valid scaling hypothesis for the free energy of the spherically confined polymers that would give the correct ejection dynamics and a possible finite size scaling. A theoretical study in this direction would be most intriguing.
References


Errata

Publication II

Page 5, left column, line 7, before Eq. (4): There is a minus sign missing. It should read that $-T \dot{S}(t) \approx -\eta \left[a \dot{N}(t)/\xi(t)\right]^2 \xi(t)^3$. 
Polymer physics research has inspired scientists partly because quite general models often give valuable information on how biologically relevant polymers, such as DNA, RNA, and proteins behave. Understanding the principles of how they move in fluids provides insight into processes such as viral DNA ejection and sedimentation of DNA.

In this thesis we study the dynamics of polymers in confined geometries using computational coarse-grained polymer models. The emphasis is on understanding the most fundamental characteristics of the studied systems. We study how features such as strength of confinement, polymer length, polymer rigidity, hydrodynamics, and knots affect the movement of polymers in fluid. The generic understanding obtained in this thesis can be used for interpreting results from real experiments and realistic molecular dynamics simulations.