

Translocation-Induced Shape Transitions in Vesicles using a Neural Network-Based Solver for the Helfrich Model

by

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Author's Declaration

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

I understand that my thesis may be made electronically available to the public.

Abstract

This thesis discusses our efforts to model the translocation of an enclosed lipid bilayer membrane (vesicle) through a circular pore. First, we will discuss the study of lipid bilayers, introduce the standard model for representing the energy of a membrane, and provide background on the many theoretical and experimental efforts in the field of membrane modeling. We then review the relevant theoretical and practical considerations regarding the simulation of vesicles and translocation, and implement a neural network-based solver for a scalar phase field. We will proceed to detail our efforts to characterize each constraint imposed on the vesicle throughout the translocation and model them within the context of the solver. Following this, we provide a variety of visual snapshots of the translocation process showing different classes of translocation and the resulting behavior of each. Equally important is the quantitative analysis of the energy landscape traversed by the vesicle, where we chart the induced bending energy imposed upon it by the narrow pore. Additionally, we introduce two types of external effects that modify the energy landscape and illustrate their impact on the total vesicle energy throughout its passage. We then map the results out onto the relevant parameter space to give a picture of where the thresholds between qualitatively different behaviors lie. As a final demonstration of our model's capabilities, we estimate the time of passage of the vesicle by modeling it diffusively using the energy landscape to calculate the effect of narrower pores on the time to translocate. This model successfully demonstrates explicit phase transitions between stable vesicle states and maps out the energy landscape throughout the unstable regime under the effects of translocation.

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Thank you to my loving partner Ruchi, my friends, and my family. Your support is why I'm able to do this and ensures that it will always be worth it.

I'd like to thank my examining committee, Dr. Robert Wickham and Dr. Mohamed Hibat-Allah. Your feedback and questions throughout our committee meetings have provided so many ideas and options that it never felt like I could get through them all.

Finally, thank you to anyone else reading this work. I hope you find it insightful and interesting, and I hope that others fascinated by this problem find this work as a useful stepping stone to build upon.

Dedication

This thesis is dedicated to all those who have supported me to bring me to where I am today. Thank you.

This work is also dedicated to all who have a passion for learning.

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Chapter 1

Introduction and Background

This chapter will provide a comprehensive review of the physical topics studied throughout this thesis, elaborating on their physical and practical significance. The discussion will begin by introducing the idea of a lipid bilayer membrane, its physical properties, and its importance in biological processes. The focus will then shift to introducing enclosed membrane surfaces as vesicles and discussing what their physical structure reveals about the geometrical restrictions they impose. Continuing, the energy of a vesicle will be classified using the standard Helfrich bending energy model and will be validated in the context of experimental and theoretical works. Following this will be details and descriptions of many of the historically significant works studying vesicles, their shapes, and their behavior under many different conditions. Finally, the translocation problem, the primary focus of this work, will be introduced, as well as the role of this thesis in attempting to shed some light on it.

1.1 Membranes and Vesicles

The field of soft matter encompasses a wide range of materials that are highly responsive to external influences, and the field plays a crucial role in both biological processes and various industrial applications. Among the diverse structures within soft matter, vesicles, which are compartments enclosed by biological membranes [1, 2], have garnered significant attention due to their role in essential biological functions such as cellular transport, membrane fusion, and drug delivery [3]. Spanning across the nanometer, nm, and micrometer, μm , scales within biophysics, their ability to dynamically reshape, fuse, and adapt to external

conditions highlights their importance not only as biological building blocks, but also as versatile platforms for biomedical innovations.

These biological membranes are self-assembled bilayers of lipids that provide structural integrity and compartmentalization for living cells. The lipids that compose them, compounds with hydrophilic (water-soluble) heads and hydrophobic (water-insoluble) tails, naturally organize themselves within aqueous environments into bilayers, forming the fundamental architecture of cellular membranes [4]. This amphiphilic organization ensures the stability of membranes and creates distinct internal and external environments, which are critical for sustaining cells, and by proxy, life. Embedded within these bilayers are all of the proteins, sterols, and carbohydrates that contribute to dynamic processes of cellular signaling, ionic transport, and adhesion. On top of that, the lateral fluidity of the bilayer itself allows membranes to rapidly reorganize, adapt to mechanical stresses, and mediate transient interactions, thereby enabling a wide range of biological responses. A visual representation of lipid bilayers, taken from the book *Permeability of Biological Membranes* by Gaspar Banfalvi [5], can be seen in Figure 1.1.

Vesicles are closed, spherical bilayer structures that encapsulate an aqueous interior. They can be formed artificially, as is the case with liposomes, or naturally, as with exosomes and endosomes. Because of their tunable size, composition, and deformability, vesicles serve as simplified, but highly effective models for studying membranes, curvature-driven processes, and self-assembly [6]. In living systems, vesicles are essential for trafficking molecules, mediating endocytosis/exocytosis, and maintaining cellular homeostasis [4]. Artificial vesicles have also been extensively explored in biotechnology, particularly on the small scale, in the delivery of therapeutic molecules, such as nucleic acids and proteins, to the interior of cells [7]. This further goes to emphasize the versatility of vesicle structures, especially in the context of transporting them into, around, or through other biological surfaces.

The core importance of membranes and vesicles for life lies in their functional versatility. Membranes define the boundary between the cell and its environment, maintain electrochemical gradients crucial for the energetics of the cell, and enable selective transport of ions and molecules [4]. Vesicles, in this case, act as transport intermediates, facilitating communication between organelles and regulating secretion pathways [3]. Beyond physiology, vesicles also play roles in immune signaling, viral infection cycles, and drug delivery applications [6, 8]. Their multifunctional nature makes them a unifying concept across cell biology, soft matter physics, and biomedical engineering. In layman’s terms, cellular biology would not function without the properties of lipid bilayers and vesicles, and so their importance to life cannot be understated.

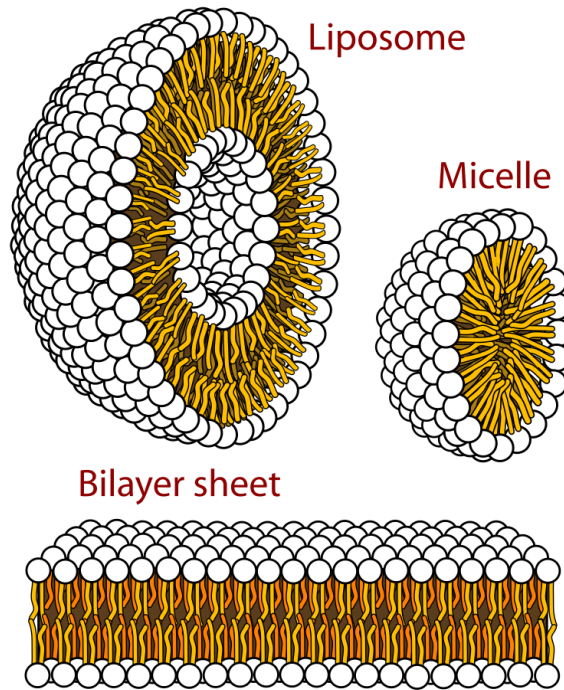


Figure 1.1: A visual representation of the structure of lipid bilayers and the different configurations they take on. The white circles represent the hydrophilic heads, while the attached orange and yellow lines represent the hydrophobic tails. The liposome is an example of an enclosed bilayer sheet, with an aqueous interior and exterior, and is a prime example of a vesicle within this analysis. The micelle is an example of another composition of lipids, in which they organize together in a spherical aggregate with no aqueous interior. This micelle will not be of interest to us, but is here to demonstrate another valid composition of lipids. The bottom visual is a depiction closer to the surface of the bilayer, showing the tightly packed lipids and their polarities. This image is extracted from the book, *Permeability of Biological Membranes*, by Gaspar Banfalvi [5].

Biophysically, studying these systems provides insights into shape transformations and the role of curvature elasticity in cellular processes. By examining how membranes bend, stretch, and remodel, research has been able to connect molecular-scale interactions with many emergent cellular phenomena [9]. Their simplicity and universality make membranes and vesicles one of the most studied systems in soft matter physics and quantitative biology, serving as baseline models for exploring cellular relaxation, non-equilibrium dynamics,

and the coupling between geometry and mechanics. Moreover, because the same physical principles govern both artificial vesicles and biological membranes, these systems provide a powerful bridge between theoretical predictions, experimental observations, and biomedical applications.

1.2 Vesicle Characteristics

At the micron length scale of the cell, which itself is much larger than the nanometer scale of the lipids making up its membrane, a fluid lipid bilayer behaves almost as an inextensible, two-dimensional fluid sheet. Its long-wavelength mechanics are captured by a curvature energy defined along its relatively smooth surface. The result is that a vesicle is often described with rigid geometric constraints in its surface area and enclosed volume. Additionally, microscopic chemical detail enters only through constant elastic moduli. The result, from a biophysical perspective, is that membranes are soft, flexible surfaces governed by bending rigidity and surface tension, with harsh constraints on surface area and enclosed volume. The remainder of this section will focus on why a lipid bilayer satisfies these conditions and how they can be incorporated into a mathematical model.

Experimentally, it has been observed that lipid bilayers are highly area-incompressible under physiological forces. This inflexibility comes as a byproduct of two main phenomena. First, following the construction of the membrane, the total number of lipids making up a particular patch is conserved. This means that the stretching of the surface primarily results in the lipids altering their spacing to one another and changing their surface density. Following this, the stretching or compressing of the surface of a membrane in this way costs an area-compressibility modulus, on the order of about ~ 0.2 N/m for biological exosomes and endosomes [10, 11]. The result is that tiny areal strains produce huge tensions on the nanometer scale of the membrane itself. As a consequence, if the membrane cannot change in area by altering its lipid count or by adjusting the space between lipids, it follows that the area of the bilayer remains almost perfectly constant following its creation. In practice, it is therefore standard to impose a global area constraint on the body, insisting that its area remains unchanged even across long timescales, despite its flexibility to deform and curve.

Additionally, the enclosed volume of a vesicle also varies very minimally following the creation of the membrane. Water and fluid exchange across the bilayer does occur, but very rarely as a result of the opening of a hole in the surface. In resulting processes, the small fluctuations in volume take place on a much slower timescale than the relaxation of the membrane shape. Given that the enclosed and surrounding fluids are incompressible

themselves, the vesicle’s enclosed volume of the membrane is another effectively conserved property of its state, in the same way as area [12, 13]. This follows as a direct consequence of the amphiphilic structure of the lipids themselves, ensuring they pack together incredibly tightly to minimize and restrict the seeping of unwanted aqueous solutions to the interior of the bilayer. This motivates a fixed volume constraint, mirroring the fixed area one, insisting that the volume of incompressible fluid enclosed by the membrane be fixed under time scales shorter than the membrane’s relaxation time.

According to the classical Helfrich model [14], the energy associated with the shape of a membrane can be modeled elastically, where the curvature of the surface plays a key role in quantifying the relaxed state of the bilayer. The model posits that the driving factor in determining the configuration of a lipid bilayer is how sharply the membrane curves about itself, in a way that is geometrically well defined. This is justified experimentally through works of fluctuation spectroscopy [15], which show vesicle undulations as being well described using two elastic bending moduli: the bending modulus, κ , and the Gaussian modulus, $\bar{\kappa}$. This model is discussed in more detail in Sections 1.4 and 1.3.

Figure 1.2 displays a single lipid layer under three possible curvatures and justifies why membrane curvature is indeed the main factor towards a vesicle’s derived energy state. Specifically, for membranes with curvature away from a natural balance, the heads or tails of the lipids are forced toward or away from each other, incurring an energetic cost in either direction. An important comment regarding the natural balancing curvature is that different bilayer structures can energetically favor a nonzero natural curvature. If one side of the bilayer is less dense with lipids than the other, the surface would naturally bend towards the sparser side to fill in gaps. This results in the layer with more lipids bending convexly, and the layer with less bending concavely to stay intact. The result is a bilayer whose natural energetic minimum is actually a surface with definite bending in one way over the other, with the resultant value of this curvature being called the membrane’s spontaneous curvature.

This elastic model also satisfies many demanded symmetries of the membrane surfaces that are necessary for their internal structure. The purely geometric metrics proposed by this model are both rotationally invariant and locally contained [14, 16, 17]. Thus, the energy of a patch of the surface can be ascertained without any additional knowledge of the membrane’s structure. In fact, the global configuration is not a requirement for this calculation, and so whether the membrane encloses a volume or not has no effect on the local curvature-based energy of the Helfrich model.

The result is arriving at lipid bilayer vesicles that are governed by a curvature-based bending energy, and strict constraints on their surface area and enclosed volume.

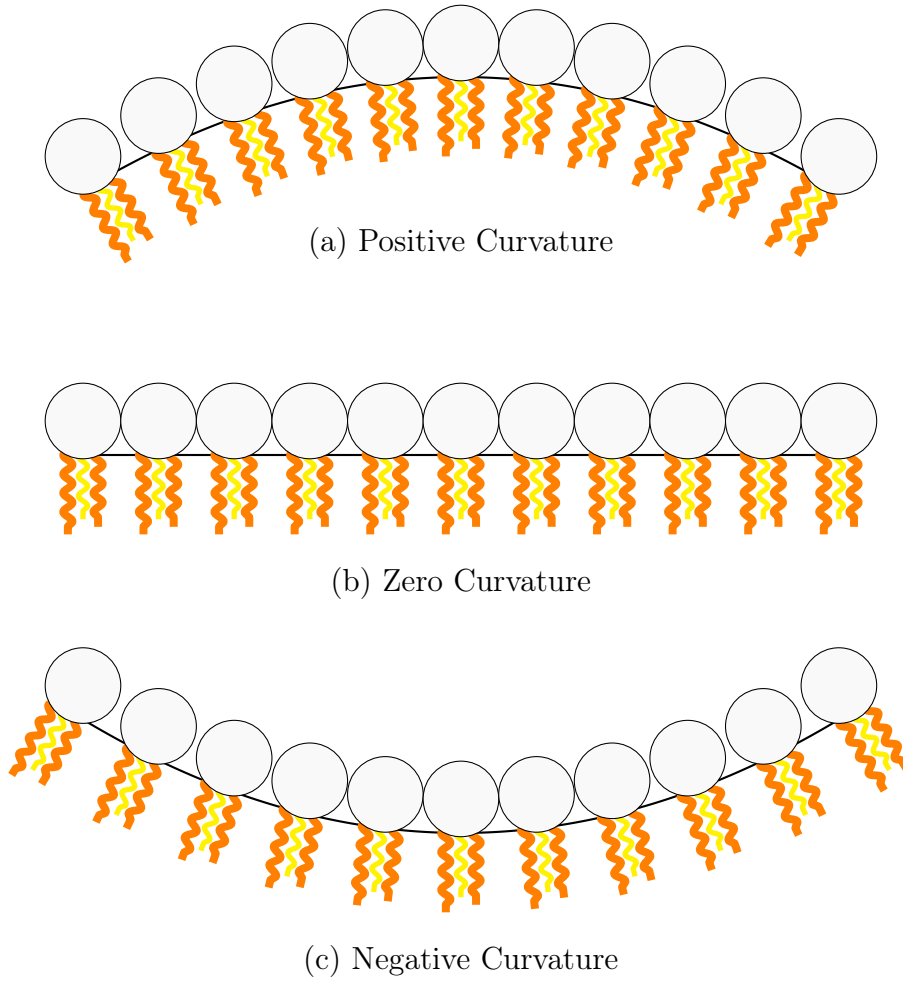


Figure 1.2: A rough schematic showing a conceptual understanding of the effects of curvature on the natural energy of the vesicle. Three different lipid monolayer patches are shown, composed of ten lipids in arrangements with differing curvature. The top patch in Figure (a) shows lipids arranged in a layer with positive curvature, where the hydrophobic tails are squeezed together on the interior more densely than the heads on the outside. The middle patch in Figure (b) shows a zero curvature surface in which the density of heads and tails remains consistent throughout. The negative curvature shown in Figure (c) shows the now hydrophilic tails being pulled apart further than normal due to the surface. This imbalance in the density of hydrophobic and hydrophilic regions in either direction can cause a higher energy interaction between the component lipids and affect the total elastic energy state of the vesicle as a whole.

Importantly, the results here are purely mathematical, with no qualitative dependence on the specific lipids used or their properties. These conditions alone still allow for much flexibility in the configuration of the membrane, leading to a variety of equilibrium shapes. Among their stable configurations, spherical vesicles are symmetric or roughly symmetric about all of their axes, prolate vesicles are elongated along one axis as a tube with lobes, while oblate vesicles are flattened like a disc [18]. The exact shape of these and others will be explained when vesicle solutions are discussed in Section 1.4. There have been many other discovered morphologies [19], of both axisymmetric and non-axisymmetric varieties, with the stability of these shapes depending on the discussed geometric factors. The formulation of these shapes and their discovery hinges on the specific elastic nature of the Helfrich model and the tools constructed to mathematically tackle it.

1.3 The Helfrich Bending Energy Model

The Helfrich bending energy has become the standard for vesicle modeling, with its advantages in both theoretical simplicity and experimental accuracy. The model treats the membrane as a thin, flexible, two-dimensional surface embedded in three-dimensional space, with its bending energy described as the square of the local curvature in the manifold. The primary idea behind this model is that values of membrane curvature far from a preferred shape incur an energetic cost [13, 20], which can be quantified using a curvature-based free energy. Additionally, the bending energy is dependent on the material of the lipid bilayers themselves through the value κ , the bending modulus, given in units of energy, J.

The mean curvature, H , at a point on the membrane surface is a purely mathematical construct composed as the arithmetic mean of the two principal curvatures, k_1 and k_2 , as can be seen in Equation 1.1. A principal curvature along a parallel axis to the surface is defined as the reciprocal of the radius of the circle that best matches the curving of the surface at that point, with a convex (outward bulging) shape defined to have positive curvature, as in Figure 1.2. As the surface is two-dimensional, there are two perpendicular curves to follow on the surface, giving two principal curvatures.

$$H = \frac{k_1 + k_2}{2} \tag{1.1}$$

According to the Helfrich model [12, 14], shown in Equation 1.2 in its general form, the elastic bending energy, E_b , of a deformable lipid bilayer is expressed as a function of both the mean and Gaussian curvatures.

$$E_b = 2\kappa \int_S [H - H_0]^2 d^2S + \bar{\kappa} \int_S K d^2S \quad (1.2)$$

Here, S is the membrane surface and d^2S is a surface element. Note that this equation is the Seifert [12] variation of the bending energy containing an extra factor of 2 at the front, as opposed to the original 1973 Helfrich definition, which does not. The Gaussian curvature, K , is the product $K = k_1 k_2$ of the principal curvatures, and is mainly a function for calculating the global topology of the membrane. For a similar reason, the Gaussian modulus, $\bar{\kappa}$, will also not be relevant in our methodology. One final parameter to introduce is the spontaneous curvature H_0 , discussed in detail in Section 1.2. The spontaneous curvature accounts for differences in bilayer density across the vesicle, which, in essence, is a measure of the ground state curvature of the surface. As mentioned in Section 1.4, this is often presented as a function of the area difference between the two lipid layers forming the sheet.

The bending modulus, κ , sets the fundamental energy scale for deformations in the Helfrich model. It quantifies the cost of bending the bilayer away from its relaxed state and is typically expressed relative to the thermal energy $k_B T$. Here, k_B denotes the Boltzmann constant, $1.381 \times 10^{-23} \text{ m}^2 \text{ kg s}^{-2} \text{ K}^{-1}$, and T is absolute temperature of the vesicle system. Experimental measurements, particularly from fluctuation spectroscopy and micropipette aspiration, have consistently reported values in the range of $10 - 40 k_B T$, with $\kappa \approx 20 k_B T$ often used as a benchmark for red blood cells and other naturally formed vesicles [6, 10, 15]. At room temperature, this corresponds to a vesicle's total elastic energy on the order of $2 \times 10^{-18} \text{ J}$. At this scale, even seemingly small curvatures or modest applied stresses can become competitive with the $k_B T$ scale of thermal noise, underscoring why vesicle curvature is so sensitive to elastic perturbations.

This form for the bending energy abides by all the necessary conditions demanded by the physical descriptors of vesicles in the previous sections. Looking at the mean curvature term specifically, as a result of bilayer symmetry, the model should only favor one side over the other through the factor of H_0 , the spontaneous curvature. Additionally, due to similar energetic restrictions in both positive and negative bending, the model must be a function of $(H - H_0)^2$. Finally, since the energy arises from lipid scale interactions, higher order terms in $(H - H_0)^2$ or their derivatives pick up additional factors of membrane width. Vesicles and membranes are typically formed on micrometer scales, while lipids are on the nanometer scale, making these higher-order terms negligible in comparison [21]. Absorbing the constant in front of this 2nd-order expansion term into κ gives the mean energy term.

This model can also be simplified based on the conditions imposed on the vesicle. For

various applications of the Helfrich model, H_0 can take on many different values [12, 14, 21], but the standard is to enforce a constant bilayer density throughout the entire membrane by setting $H_0 = 0$ for all points on the surface. Continuing to simplify Equation 1.2, the next step is to take a look at the second Gaussian term. Even though the Gaussian curvature, K , varies across the surface of a non-spherical vesicle, the integral of the Gaussian curvature is only dependent on the Euler characteristic of the body. As a result, the local quadratic nature of the Helfrich model is irrelevant since the second integral is a purely global evaluation. If the vesicle's topology changes, either by splitting into multiple shapes or through a perforated hole, there can be dynamical effects. However, in most cases, the vesicles are always topologically homeomorphic to spheres, and the second integral results in the value $4\pi\bar{\kappa}$, which is just a constant [22]. Since the focus of our analysis is to monitor the change in E_b as the surface deforms, this constant second term is ignored, and the variations in the mean curvature term are primarily studied. These assumptions are not particularly strict in most applications of the model, and so the Helfrich bending energy can be reduced down to the pleasant Equation 1.3 below.

$$E_b = 2\kappa \int_S H^2 d^2S \quad (1.3)$$

Before discussing historical efforts in tackling the Helfrich model, it should be clarified how exactly the model can be used to predict vesicle shapes. Modeling boils down to the process of trying to minimize the total bending energy of the vesicle, subject to the fixed geometric constraints on its area and volume. And so deformations in an enclosed lipid bilayer are driven by an energetic incentive to strain the surface's curvature as little as possible, while the incompressible volume and inflexible surface remain unchanged. Given values for surface area and volume, a vesicle morphology, or shape, can be classified as a corresponding stable minimum of the Helfrich energy function.

1.4 Historical Efforts to Solve the Helfrich Model

The theoretical study of vesicle shapes began with Canham in 1970, who proposed that the biconcave shape of red blood cells could be explained by minimizing a bending energy proportional to the square of the mean curvature, subject to fixed area and volume constraints [19]. Shortly thereafter, in 1973, Helfrich generalized this idea for lipid bilayers, formulating the now standard curvature elasticity energy, shown in Equation 1.2 [14]. These early works established the problem as a variational calculus problem on a curved surface, laying the groundwork for later shape analysis.

One of the first explicit solutions was developed by Deuling and Helfrich in 1976, who restricted vesicle shapes to be axisymmetric, in order to collapse the problem down into two dimensions [23]. By solving the shape equations derived from the Helfrich functional under area and volume constraints, they were able to classify the primary stable equilibrium shapes, including spheres, prolates, oblates, and stomatocytes (cup-like shapes with invaginated necks). The shape of these can be found in Figure 1.3, from the work by Seifert et al. that will be elaborated on further in a few paragraphs. This work also introduced the wide-reaching concept of a vesicle’s reduced volume, which is a measure of how much volume is on the interior relative to a sphere of the same area. This work then evolved to produce the first reduced volume phase diagram, which remains a cornerstone of vesicle shape theory.

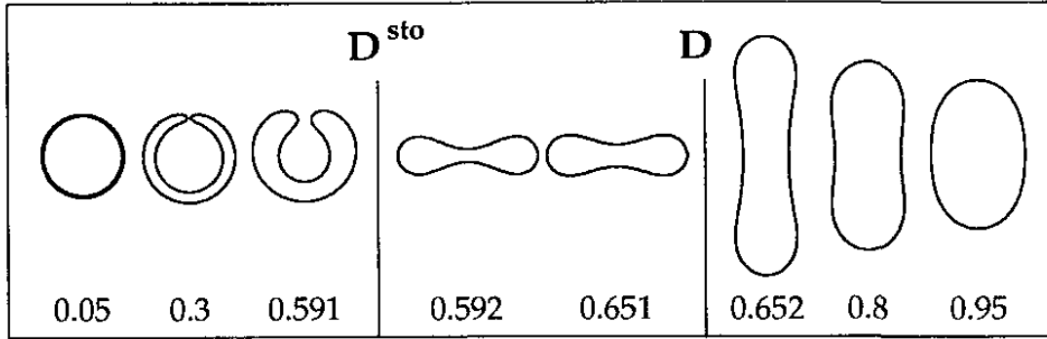


Figure 1.3: These are the three classes of primary axisymmetric vesicle shapes. In particular, due to their symmetry, they are only modeled in two dimensions and are fully determined by the nature of these cutout images. The physical three-dimensional shapes can be extracted by revolving each of these cutouts around the vertical y -axis in the figure. The numbers at the bottom correspond to the value of reduced volume where the corresponding shape is a solution to the Helfrich model. On the left are curves for stomatocytes, in the middle are curves for oblates, and on the right are curves for prolates. The D and D^{sto} labels at the top of the figure are part of the vesicle labeling system explained in the analysis of the corresponding work. This image is taken directly from the paper, *Shape transformations of vesicles: Phase diagram for spontaneous-curvature and bilayer-coupling models*, [24] by Seifert, Brendl, and Lipowsky.

Further refinements to the model incorporated asymmetry between the two lipid layers. The bilayer-couple hypothesis, introduced by Sheetz and Singer, proposed that the two layers may locally contain mismatched numbers of lipids, properly inducing spontaneous

curvatures through specific lipid mechanics [25]. This concept was extended by Svetina et al. in 1989 into the area-difference elasticity model, which adds an elastic penalty for layer area differences [18]. This model successfully explained vesicle budding and pear-shaped morphologies beyond the original Helfrich description.

Looking at Figure 1.3, the two-dimensional analogues of the three free space axisymmetric shapes can be seen. The shapes are cross-sections from stomatocytes, which are invaginated cup-like shapes that exhibit a near spherical curvature across the majority of the surface, before doubling back. This allows them to be quite energetically favorable at low reduced volumes, where the vesicle’s volume is low compared to the area of its surface. In the middle, the shapes are oblates, which, when revolved, form disc-like shapes with a central divot in the center. In many common illustrations, red blood cells take on the morphology of an oblate, as a disc with most of its volume concentrated around this central ring, far from the vesicle’s geometric center. Finally, on the right are prolates, forming long tube-like shapes, characterized by a narrow neck and two pseudo-spherical lobes on either end. Prolates are the most stable of these morphologies at the higher end of reduced volumes and are the most sphere-like in their structure. Figure 1.4 shows three-dimensional versions of the prolate and oblate using the developed method of modeling from this thesis.

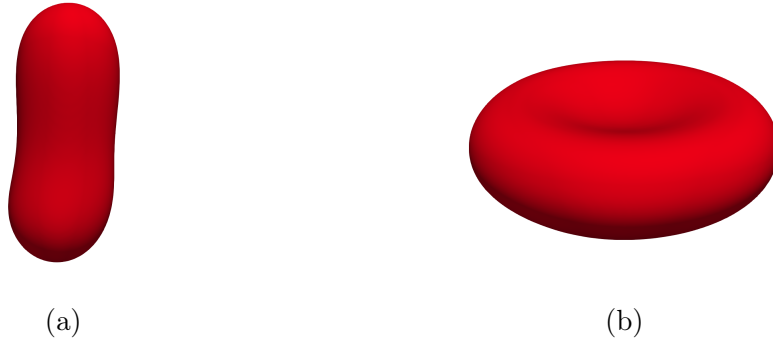


Figure 1.4: Figure (a) is an example of a prolate vesicle aligned vertically. Figure (b) is an example of an oblate vesicle aligned nearly vertically. These images are pulled directly from the simulations in this thesis, via the method described in Sections 3.2 and 3.3.

Beyond axisymmetric shapes, theoretical studies have also explored non-spherical topologies. Seifert, Berndt, and Lipowsky (1991) analyzed vesicles of toroidal topology, demonstrating that the Helfrich model allows for more complex morphologies [24]. In 1997, Seifert et al. provided a comprehensive review of some general stable non-axisymmetric

vesicle shapes and outlined the main challenges of solving the full shape equations analytically [12]. Figure 1.5a shows a diagram produced in their work that details the energies of various configurations and the stable states under specific geometric constraints.

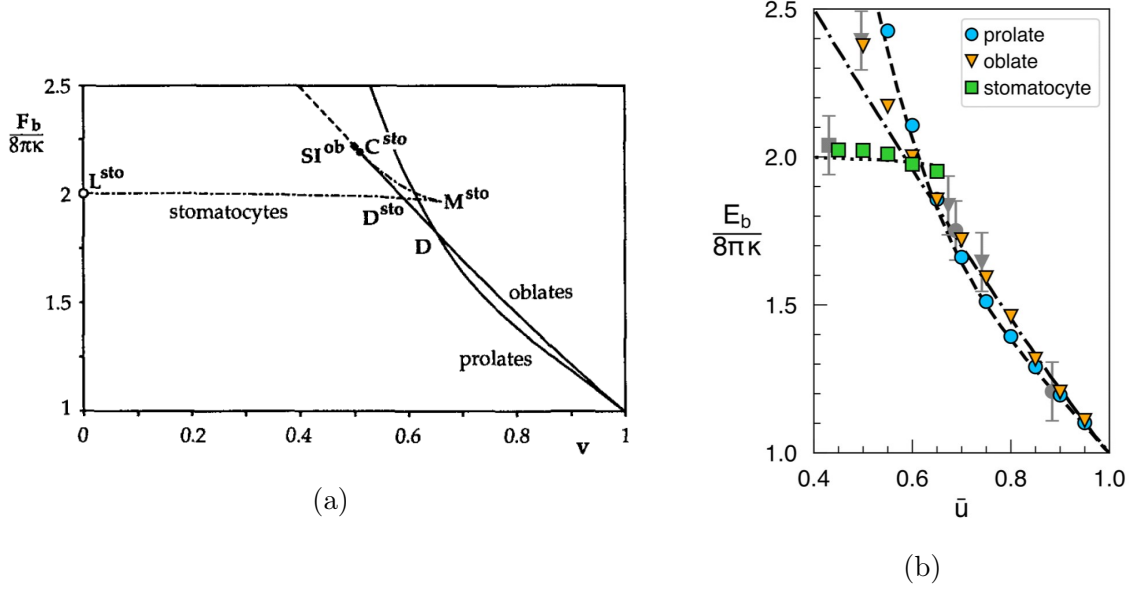


Figure 1.5: The quantified energies of the three stationary axisymmetric vesicle shapes. The $v/\bar{u}$ axes along the bottom quantify the reduced volume of the vesicle, which is a measure of the volume of the vesicle relative to its surface area. The $\frac{F_b}{8\pi\kappa} / \frac{E_b}{8\pi\kappa}$ axes on the left are measures of the energy of each vesicle configuration relative to that of a sphere of reduced volume, which itself corresponds to a reduced volume of 1. The three curves in the diagram represent the energy of that specific vesicle branch for varying values of the reduced volume. The result is that this figure shows the lowest energy ground state shape for each value of reduced volume. These diagrams agree in content, but were arrived at from entirely different methods. Figure (a) comes from the axisymmetric Seifert et al., where the energy stability of these three forms was first studied. Diagram (a) is taken directly from the paper, *Shape transformations of vesicles: Phase diagram for spontaneous-curvature and bilayer-coupling models*, [24] by Seifert, Brendl, and Lipowsky. Figure (b) is a more modern version by Rohanizadegan et al. in 2024, where a neural network-based solver of the free space Helfrich model produced the same set of curves without the necessary axisymmetric assumptions. Diagram (b) is taken directly from the paper, *Neural-network-based Solver for Vesicle Shapes Predicted by the Helfrich Model* [26] by Rohanizadegan, Li, and Chen.

This and other diagrams provided the first systematic mappings of vesicle shapes at various reduced volumes and took a major step toward understanding the stability of different shapes. This laid the foundation for quantifying just how important the geometric constraints were in determining vesicle shapes, as well as compiling the efforts from previous works in area-difference, spontaneous curvature, and non-spherical topologies. The result of this was a mass categorization of shapes, but via a method that hinged on the vesicle expressing some sort of axial symmetry, as seen in the examples in Figure 1.3. While the analytical parameterizations involved could handle some non-axisymmetric morphologies, they struggled to systematically categorize them. These developments would highlight the necessity of numerical methods for vesicle simulation in the future, as the analytical methods had become ill-equipped to manage them in full detail.

At this point, studies began to emerge that attempted to solve for vesicle shapes outside of free space. In this context, a vesicle in free space is one whose configuration is purely determined by the Helfrich model as described in Section 1.3. The vesicle in this case only has a curvature-based, elastic energy term and is not confined, restricted, or impacted by external effects. In free space, the only conditions are the purely geometric ones as a result. Following the systematic work of Seifert et al., the introduction of vesicles to non-trivial boundary conditions became a focal point of great interest to probe further into these more abstract morphologies. The translocation problem that is the focus of this thesis is one of these developments, and its history will be explored in more detail in Section 3.1.

The statistical mechanics of vesicle fluctuations was also explored to connect theory with experimental measurements. Back in 1987, Milner and Safran derived fluctuation spectra using a mean-field approximation of the Helfrich model [27], while following experimental studies by Rawicz et al. and Faucon et al. confirmed that bending elasticity accurately predicts vesicle undulation amplitudes [10, 15]. These results also enabled precise determination of the bending rigidity of lipid bilayers, validating the Helfrich model as a physically accurate and predictive framework for vesicle mechanics.

Moving forward, it is important to highlight the difficulty in the unsimplified 3D problem of vesicle modeling that is being collapsed down in many of these works. Modeling vesicles in three dimensions has always been a struggle because the Helfrich energy depends on the square of the full curvature tensor of a deformable surface, making the governing shape equations nonlinear, high-order, and highly coupled across the vesicle’s geometry. In three dimensions, vesicle surfaces can, in principle, explore a vast configuration space with non-axisymmetric fluctuations, budding events, and local instabilities that are mathematically and computationally demanding to resolve. This reduction captured the essential physics of vesicle elasticity and volume-to-area constraints, while removing the complexity of arbitrary three-dimensional deformations.

At this point, much of the stationary state mechanics of vesicles is well understood, being justified through much consistency in both theory and experiment. However, many of the nonstationary states still elude much of the analysis. In particular, the vesicle dynamics of shape transitions between stable states, and the unstable non-axisymmetric intermediary shapes that are passed through on the way. Here, many of the numerical methods for vesicle simulation have been developed, including triangulated surface models by Gompper et al., phase-field simulations by Du et al., and finite-element approaches by Elliot et al. [28, 29, 30]. These numerical methods are essential to the work on translocation, and will be discussed in detail in Section 2.2.

These computational advances allow researchers to capture behavior, such as vesicle confinement by a geometric surface, by influencing the vesicle with an irreducible 3D external factor. Modern machine learning-based solvers by Rohanizadegan et al. have found ways to exert effects from these newly accessed directions without any need for dimensional reduction. Nonetheless, one of the most striking results from these simulations is that the lowest-energy vesicle shapes under reduced volume constraints often relax to axisymmetric configurations, effectively justifying the 2D reduction in many cases. This can be seen clearly in Figure 1.5, where the unrestricted 3D simulations produce an almost identical phase diagram to the 2D analytical cross-section approach. Thus, while three-dimensional modeling is indispensable for understanding fluctuations, instabilities, and realistic dynamics in symmetry-deficient environments, the classical axisymmetric models remain central because they still represent the natural attractors of the vesicle energy landscape.

1.5 The Translocation Problem and the Contribution of this Thesis

Vesicle translocation, which refers to the movement of vesicles through pores or short channels, is a phenomenon that spans multiple disciplines in soft matter, including biophysics, polymer physics, and materials science. Specifically, given a pore of a specific shape within a barrier, the translocation problem asks if a given vesicle can successfully deform to pass through, and what the fundamental limits are in doing so. This process is especially significant in the context of cellular operations, where vesicle trafficking is crucial for functions such as endocytosis, exocytosis, and nutrient uptake [4, 31]. From a physical perspective, vesicle translocation presents a highly nontrivial interplay between geometrical restrictions, membrane mechanics, and dynamic forces, making it a central test case for the application of continuum theories of membranes. Recent studies, such as the work of

Shojaei et al. [32, 33] and Han et al. [34] on vesicle translocation through nanopores, have revealed some of the intricacies by using an analytic 0th-order approximation of the free energy landscape. These contributions have emphasized how both pore size and membrane bending rigidity govern whether a vesicle smoothly passes, deforms, or becomes trapped, thereby offering insights into the critical thresholds for successful translocation.

Many factors, including hydrodynamic drag, membrane elasticity, and surface adhesion, all couple to determine how the vesicle interacts with the pore and the barrier [35]. Although substantial experimental and computational advances have been made, many aspects of this process remain inadequately understood. The method by which a vesicle can undergo shape transitions organically and hop to a different stable state during translocation is a topic of ongoing analysis, as such transformations directly alter the effective free energy profile.

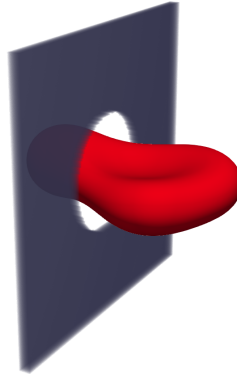


Figure 1.6: A vesicle being deformed into a non-axisymmetric shape as a result of its interaction with a pore. Here, the vesicle’s morphology exhibits that of a sort of elongated three-pronged star as it passes through. The configuration is not rotationally symmetric about any axis, and so it can only be modeled using a truly three-dimensional algorithm.

Translocation is a fundamentally 3D dynamical problem that cannot be tackled using the techniques shown throughout this chapter. Looking at Figure 1.6, a vesicle is seen transitioning between shapes as a result of its interaction with the pore. There has been no good method that could solve this problem, and so it has historically been given strict assumptions that eliminate transitional shapes like this from analysis. The method described and applied in this thesis shows that analysis of these shapes is possible,

repeatable, and fruitful. This method was originally put together by Rohanizadegan, Li, and Chen to solve free space and confined vesicles [26], and with some modifications, can properly tackle the translocation problem as well.

By combining theoretical approaches with computational techniques, this study seeks to bridge the gap between continuum membrane models and the qualitatively 3D behavior of translocation through nanopores. This study is performed by optimizing a neural network to attempt to minimize the free energy directly, given a fixed position of the vesicle relative to the pore. This approach leverages machine learning optimization to efficiently sample a high-dimensional configuration space that would be computationally prohibitive with conventional variational methods. By iterating through various positions of the barrier and then interpreting the snapshots and energies that arise as a result, a picture is formed of the sequential deformations that accompany translocation. The calculations are restricted to a set of possible vesicles to paint a picture of the energy landscape of vesicle translocation, focusing on oblate and prolate characteristic shapes. The focus will then shift to implementing external forces in the form of a linear potential and a pressure differential to see the effect on the energy information, thereby connecting the analysis to experimentally relevant conditions such as osmotic gradients and applied electric fields. Finally, through the lens of Fokker-Planck formalism [36], the mean first passage time of the translocation is computed with respect to the hole radius and external forces, offering a quantitative framework for predicting translocation timescales under varying mechanical and energetic constraints.

Chapter 2

Model and Methods

In this chapter, the tools available for the modeling and simulation of vesicles and translocation will be explored. The first step will be to parameterize the problem into a set of dimensionless values according to the fixed surface area of the vesicle, and understand the effects of these parameters on the process of translocation as a whole. Next, a more detailed discussion will be held about the various methods implemented to solve vesicle simulation, and why the phase field approach was chosen for this project. Afterwards, the phase field model will be introduced concretely to better quantify how the vesicle will be simulated and what the corresponding parameters translate to mathematically. Then, we introduce the constrained energy equation that is to be optimized and the neural network-based algorithm employed to do so. This is followed by a breakdown of the specific constraints of translocation and an incorporation of all of the discussed parameters into a master equation, to be the main tool in our analysis of the problem for the rest of the thesis. Finally, we will acknowledge certain limitations of this modeling scheme and aspects of it that may compromise the accuracy of the obtained results.

In Section 1.3, the Helfrich model is described mathematically through the lens of the mean and Gaussian curvatures. For the interests of the translocation problem, topological changes to the vesicle's structure will not be studied, and the vesicle's spontaneous curvature will be set to $H_0 = 0$. The result is that the Helfrich bending energy takes on the simplified form derived at the end of that section in Equation 1.3. It is the evaluation and optimization of this energy under the constraints of the translocation problem that becomes the focal point of this thesis. The remainder of this section outlines the parameters, techniques, and methods employed to address this issue with translocation in mind.

2.1 Dimensional Analysis and Parameters

2.1.1 Parameters of Vesicles

The problem is initialized by assuming the vesicle is an incompressible and inextensible bilayer with fixed enclosed volume U and fixed surface area A . With the exceptions of bending energy and translocation time, the quantitative analysis of vesicles will be done in a reduced system of units relative to the geometric constraints. In particular, the surface area of the membrane will be fixed to a value of $A = 4\pi \mu\text{m}^2$ with all other lengths measured relative to that specific size. It should be noted that this results in a unit length scale equal to the radius of a sphere with the same area, thus our reduced units can be turned dimensionful through a $1 \mu\text{m}$ standard length scale. The physical size of vesicles is the central factor in defining meaningful dimensionless parameters, and so will be tackled with some experimental justification.

In biological contexts, studies of many different types of vesicles have been performed. Between them, vesicle diameters often vary widely, with cell messengers (exosomes/endosomes) typically on the order of $\sim 100 \text{ nm}$, medicinal liposomes designed in the $100 - 200 \text{ nm}$ range, and red blood cells reaching radii of $5 - 10 \mu\text{m}$ [4, 7, 37, 38]. To capture this broad diversity within a unified framework, many theoretical studies adopt a vesicle radius of $1 \mu\text{m}$ as a representative benchmark [12, 39]. This choice simplifies dimensional analysis by providing a natural reference scale for reduced quantities such as surface area and reduced volume. At the same time, it preserves experimental relevance, lying squarely between the nanoscale vesicles commonly used in biomedicine and the micron-scale organelles and red blood cells that dominate physiological contexts.

As the problem is to be transitioned into a continuous phase field model in Section 2.3 with purely reduced units, the absolute size of the vesicle is not a contributing factor to the analysis, which is why this reduction can be performed at all. It is only the relative, reduced system that provides information as to the mechanics of the problem. If the individual bilayers were chosen to be simulated instead, the scale of the vesicle relative to the bilayers would change the resulting behavior.

Given a vesicle with a defined volume U and surface area A , a value for the reduced volume, $\bar{U}$, can be produced. For clarity, it is worth mentioning that in the works of Seifert and Lipowsky, this parameter is often denoted by $\mathbf{v}$ instead [12, 24]. This reduced volume, $\bar{U}$, is the primary parameter in determining the configuration of the vesicle. In essence, it quantifies the amount of volume on the interior of the vesicle relative to a sphere of the same surface area. The formula for calculating $\bar{U}$, in terms of U and A , is shown below in Equation 2.1.

$$\bar{U} = \frac{U}{\frac{4\pi}{3} \left(\frac{A}{4\pi}\right)^{\frac{3}{2}}} \quad (2.1)$$

Note that a sphere has $\bar{U} = 1$ (with $U = \frac{4\pi}{3}\mu\text{m}^3$ and $A = 4\pi\mu\text{m}^2$) with smaller values of $\bar{U}$ indexed for more geometrically restricted shapes.

In the case of a vesicle floating in free space, the membrane deforms into several stable and metastable conformations based on the value of $\bar{U}$ [24, 40]. As mentioned in Chapter 1, the two main branches of stationary states to be analyzed in this thesis are the prolate branch and oblate branch. A prolate is elongated from a sphere along a single axis, producing two lobes on either end akin to a dumbbell, as shown in Figure 1.4a from Section 1.4. An oblate, on the other hand, is flattened inward along one axis and extended along the other two to look more like a disc, as in Figure 1.4b. Both of these stable states contain a single rotational axis of symmetry, namely the vertical y -axis in the figure.

These vesicle morphologies are only stable for specific values of $\bar{U}$. As categorized by Seifert et al., many of these states are global/local energy minima for certain sets of geometric constraints, with different shapes taking precedence at different scales [12]. The stable and metastable reduced volumes for these branches are shown explicitly in Figure 1.5 from Section 1.4.

Of relevance to us, the prolate branch is metastable for values of $\bar{U} \gtrsim 0.58$ and the oblate branch is metastable for values where $0.5 \lesssim \bar{U} \lesssim 0.81$ [24, 40, 41]. Note that by definition, $\bar{U}$ cannot be higher than 1, imposing an upper limit on the range for prolate vesicles as well. Despite both being metastable on the interval of $\bar{U}$ between 0.58 and 0.81, the global energy minimum of the vesicle lies in the prolate branch for $\bar{U}$ between 0.65 and 0.81, while the oblate becomes the true minimum for the range between 0.58 and 0.65 [42, 43]. These results can be summarized in the simplified Table 2.1.

Table 2.1: Stability of prolate and oblate vesicle shapes as a function of reduced volume $\bar{U}$.

Reduced Volume $\bar{U}$	Prolate Stability	Oblate Stability
$\bar{U} \gtrsim 0.81$	Globally Stable	Unstable
$0.65 \lesssim \bar{U} \lesssim 0.81$	Globally Stable	Locally Stable (Metastable)
$0.58 \lesssim \bar{U} \lesssim 0.65$	Locally Stable (Metastable)	Globally Stable
$0.5 \lesssim \bar{U} \lesssim 0.58$	Unstable	Globally Stable

2.1.2 Parameters of Translocation

In this subsection, the parameters relevant for modeling and simulating translocation are introduced. Figure 2.1 depicts a 2D snapshot of a vesicle passing through a pore in a barrier. In general, throughout the remainder of this discussion, the axis of translocation will be the x -axis, and the vesicle will travel from right to left through the pore in the negative x -direction. The reduced pore radius $\bar{R}$, the reduced barrier width $\bar{D}$, and the reduced barrier position $\bar{h}$ are all defined relative to the fixed vesicle surface area as in Equations 2.2, 2.3, and 2.4. From this point forward, our discussion will be primarily about these reduced parameters with no particular length scale in mind. Generic points in 3D space, denoted by $\mathbf{r} = (x, y, z)$, will also be reduced to the same dimensionless scale.

$$\bar{R} = \frac{R}{\left(\frac{A}{4\pi}\right)^{\frac{1}{2}}} \quad (2.2)$$

$$\bar{D} = \frac{D}{\left(\frac{A}{4\pi}\right)^{\frac{1}{2}}} \quad (2.3)$$

$$\bar{h} = \frac{h}{\left(\frac{A}{4\pi}\right)^{\frac{1}{2}}} \quad (2.4)$$

$\bar{R}$ defines the radius of the circular pore the vesicle is to be passed through, and is the second primary parameter in our analysis of translocation, along with $\bar{U}$. For large values of $\bar{R}$, much larger than the size of the vesicle, one can imagine that the vesicle will pass straight through the opening without interacting with the barrier or being constrained any more than is normal for its $\bar{U}$. On the other hand, smaller values of $\bar{R}$ force the body to deform around the rim of the pore, causing there to be more curvature than otherwise intended. This should accordingly boost the bending energy of the vesicle as it traverses the pore, with narrower openings causing more and more extreme variations in the shape.

As the size of the opening is shrunk down, it is expected that the vesicle would go from seeing minor temporary deviations of the shape, to large-scale changes, to eventually being unable to pass through at all. As a result, our main analysis focuses on analyzing pairs of $(\bar{U}, \bar{R})$ points in parameter space and charting out a rough map for when translocations are possible and what the resulting behavior looks like.

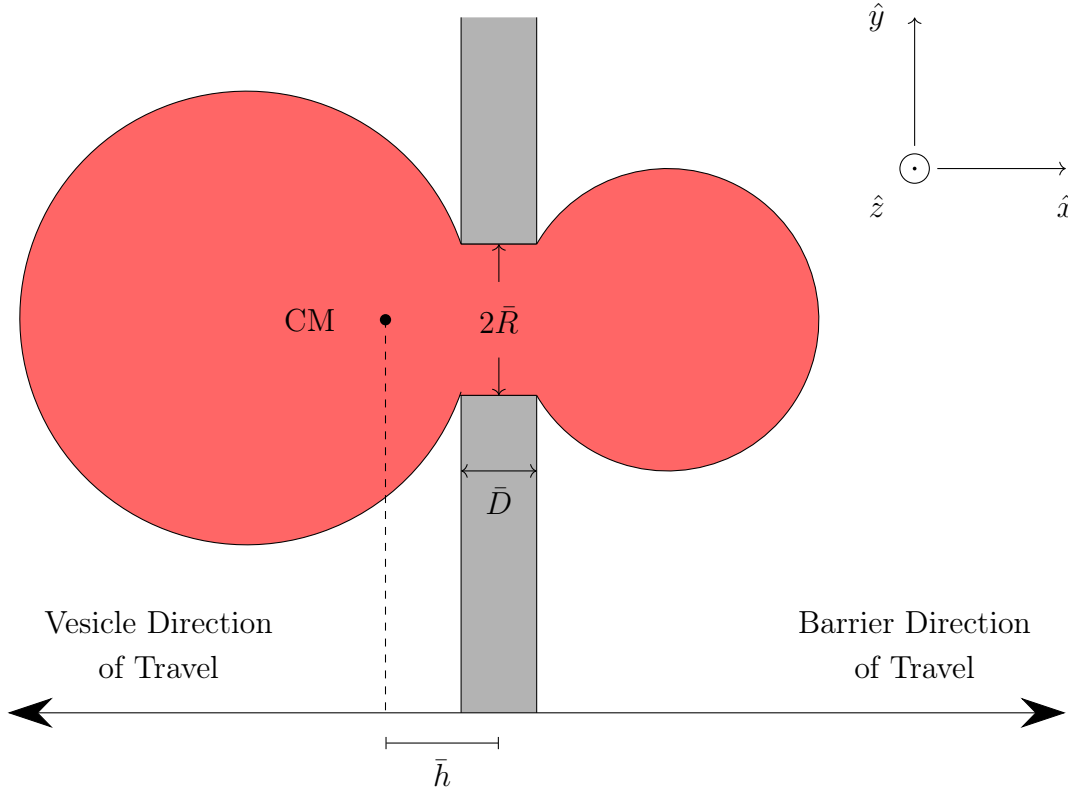


Figure 2.1: A sample 2D diagram showing a vesicle partway through translocation, as well as all the relevant parameters for the analysis. In this illustration, the gray region is the barrier, the red region is the interior of the vesicle, and the white region is free space. All lengths are dimensionless, and are reduced with respect to the radius of a sphere with surface area $4\pi\mu\text{m}^2$. $\bar{R}$ denotes the size of the pore radius, which has been modeled as described in Section 2.4.3. $\bar{D}$ represents the width of the barrier reduced in the same way as $\bar{R}$. $\bar{h}$ is the position of the barrier relative to the center of mass (CM) of the vesicle.

$\bar{D}$ defines the width of the barrier along the axis of translocation and is a value chosen to be kept fixed throughout the discussion. Larger values of $\bar{D}$ cause the barrier to be more like a tunnel for the vesicle to pass through, causing our problem to behave more like a confinement problem rather than a translocation one [44, 45, 46]. Smaller values of $\bar{D}$, on the other hand, can lead to errors in numerical precision when the mathematical processing of the problem is underway, as described in Section 2.4.2. In this work, the value of the reduced barrier width is set to $\bar{D} = 0.16$, and the remaining results are derived with an unchanged width. However, $\bar{D}$ is an excellent third parameter to tackle more

quantitatively in further studies on this problem.

$\bar{h}$ is the main translocation coordinate that dictates how far along the process is. It represents the position of the pore’s center relative to the center of mass (CM) of the vesicle. As will be elaborated upon in Sections 2.4.3 and 3.3, the vesicle will be fixed in space with the barrier moving towards and around the body. Large negative values of $\bar{h}$ indicate that the vesicle is at the start of translocation and has not yet interacted with the barrier. $\bar{h}$ near 0 indicates that the center of mass is close to the center of the pore, and thus the vesicle is in the process of translocating and deforming about the entrance. Large positive values of $\bar{h}$ indicate the barrier has passed over the vesicle and thus the translocation is complete.

In physical processes, the barrier is static and fixed relative to the incoming vesicle. Under the coordinates discussed, it would then seem that defining $\bar{h}$ as the position of the CM relative to a fixed barrier would paint a clearer picture. However, for vesicle modeling, it makes more sense to pretend that the barrier is moving and focus specifically on the vesicle’s behavior. Alternatively, this approach could be imagined as having the model be in the vesicle’s rest frame and follow its motion, making the barrier move. This is purely a numerical trick that makes the vesicle easier to quantify and iterate over multiple different values of $\bar{h}$. The barrier traveling from left to right across the vesicle in this case is indistinguishable from the vesicle moving from right to left through the pore. These specific choices will act as the standard directions of translocation, with the barrier being moved from left to right in the positive x direction.

2.2 Summary of Computational Methods

As discussed briefly in Section 1.2, the Helfrich model has been the foundation for computational studies of membrane mechanics for decades. In this section, a variety of implemented computational methods towards solving the Helfrich model will be elaborated on, as well as why the phase field model is chosen in this work. One of the earliest and most widely used strategies is direct minimization of curvature energies on discretized surfaces. In these methods, the vesicle is represented as a triangulated mesh, and the mean curvature is calculated at each vertex on the surface. Early implementations by Seifert, Gompper, and coworkers applied constrained optimization to minimize the Helfrich energy under fixed volume and area [12, 28]. The strength of these approaches lies in their direct correspondence with the continuum theory and their ability to reproduce equilibrium morphologies. However, they are computationally expensive, sensitive to mesh quality, and poorly suited for capturing dynamics or external interactions.

Another class of approaches involves spectral methods, in which the vesicle shape is expanded in spherical harmonics or related basis functions [12]. This is the specific focus of the work of Milner et al., where they introduced the idea of expressing the mesh as a combination of polyhedral groups that could each independently be relaxed quickly [27]. By truncating the expansion at a given mode number, the Helfrich energy can be expressed in terms of the expansion coefficients, allowing a heavy reduction of parameters and a relatively efficient minimization. Spectral approaches provide excellent accuracy for nearly spherical vesicles and allow analytical tractability for small deformations [47]. Their main limitation is their inflexibility: they struggle to represent highly deformed or highly non-spherical morphologies, and truncation artifacts can bias the final results. As a result, spectral methods are often most effective for perturbative studies rather than general vesicle dynamics.

The finite element method (FEM) for discrete data modeling has also been adapted to suit vesicle mechanics. In these formulations, the bilayer is treated as a thin elastic shell with bending rigidity, and the energy functional is discretized into a system of local interacting elements [48]. In this method, the system of points is treated as a set of non-linear differential equations, with the local interactions between elements quantified by the Helfrich model. This allows high flexibility in representing arbitrary geometries and applying boundary conditions, making FEM particularly useful for studying vesicles under flow, adhesion, or confinement [49, 50]. The downside of this method is its complexity—implementing bending energy terms requiring 4th-order derivatives of the surface, which demand specialized elements or stabilization techniques. Moreover, finite-element simulations are relatively costly when applied to evolving three-dimensional shapes with constraints such as volume and area conservation. The result is a loss of stability when attempting to solve for non-axisymmetric states; however, the method is still quite capable of handling external interactions.

Monte Carlo and molecular simulation approaches represent another avenue. Coarse-grained membrane models and dynamically triangulated surface methods simulate vesicles under thermal fluctuations while incorporating Helfrich-like bending energies. These simulations provide statistical information on shape fluctuations, budding, and shape transitions, going beyond mean-field energetics, as famously demonstrated by Noguchi and Gompper [51]. Their strength lies in accessing fluctuation phenomena inaccessible to other methods. This inherently non-deterministic system allows for repeated application to produce confident reaffirmations of the solution, while also providing a tool for quantifying stability under random forces. On the downside, Monte Carlo methods are limited in time resolution and may equilibrate slowly, while coarse-grained molecular dynamics, though physically rich, are still computationally intensive and often obscure the direct link to the

continuum Helfrich model.

An alternative approach that has gained popularity is the phase-field method formalized for lipid bilayers by Du, Liu, and Wang in 2004 [52]. Here, the vesicle interface is represented implicitly by a smooth, scalar phase field, and the Helfrich energy is reformulated in terms of gradients of this field. Phase field models naturally accommodate drastic physical changes, such as vesicle fission or fusion, without explicit mesh handling. They are also very well suited for coupling with hydrodynamics and other physical fields. A final advantage in the phase field model is that of consistency. The need for a fixed simulation grid can be a major disadvantage in that it requires diffuse interfaces of finite thickness, which introduces additional parameters and numerical stiffness. Accurately recovering sharp-interface Helfrich behavior often demands fine discretization, which, in the limit, can once again make computations expensive.

Finally, more recent work has focused on hybrid and numerical optimization schemes, such as boundary element methods, surface evolver techniques, and machine learning-assisted solvers. Tools, like Ken Brakke’s surface evolver, have been designed and applied specifically to minimize curvature-based energies efficiently with constraints, providing versatile shape optimization [53]. Meanwhile, newer algorithms integrate optimization with neural networks or employ reduced-order models to accelerate convergence [54]. These hybrid methods combine the flexibility of mesh-based techniques with the efficiency of Monte Carlo modeling. Still, they often inherit the limitations of their parent methods, either being tied to mesh quality or requiring careful constraint enforcement [55]. On top of this, there may also be issues in interpretability when the problem is translated into the neural network, in a way that the solutions may not be accurate representations of the Helfrich model’s dynamics. The choice of hyperparameters and the network’s internal structure are often quite hard to determine optimally for a given task, requiring special care to produce high-quality results.

The approach taken in this work is a hybrid between the scalar phase field model and a machine learning based solver originally investigated and designed by Rohanizadegan, Li, and Chen [26]. The key aspect of this work that makes it appealing for solving the translocation problem is that it allows for qualitatively three-dimensional behavior to arise with no assumptions of symmetry. Many complex boundary conditions cannot be collapsed down into anything less than 3D without forcing major assumptions of symmetry. Nonetheless, this algorithm has produced accurate and reproducible results in vesicle simulations under free space conditions and geometric confinement in an unrestricted 3D landscape. This work by Rohanizadegan et al. is the primary inspiration for this study, and many of the methods employed throughout this thesis hinge on applying aspects of their work in the context of translocation passage specifically.

The reason the phase field is chosen in this model is that it is flexible in coupling the interaction of the vesicle to other fields, namely the field describing the barrier/pore, in the process of translocation. In particular, since the method implements the bending energy, area, and volume as functions of the phase field and its derivatives, any other constraint can be implemented in much the same way by adding constraints directly onto the set. The breaking of axial symmetry that comes with this decision arises naturally as a consequence of the various interactions and the asymmetry in the chosen fields. As discussed, the main drawback of the phase field model is the necessity of a finite thickness in the membrane, which itself can only be reduced by increasing the number of parameters in the model.

Unlike other numerical solvers, a machine learning approach has excellent optimization time relative to the size of the simulation. Most other solvers scale quadratically or worse with input size, but an iteratively solved neural network remains linear. This allows the density of points to comfortably increase in such a way that the effects of the phase field’s finite thickness are manageable and predictable. A second advantage of networks is that of implementation. In the same way that the phase field model is more than capable of taking on additional constraints, neural networks can also easily be modified by adding additional terms into their cost function as necessary and monitoring the solution finding. While the large-scale time complexity of machine learning approaches is quite good, they suffer due to needing excessive amounts of input data to find the desired solution [56]. The contents of Sections 2.3 and 2.4 contain the details of the phase field model and machine learning approach used in this project.

2.3 The Phase Field Model

In this study, one method will be focused on, namely the phase field approach developed by Du et al. [52] and Blowey et al. [57]. In this, rather than model the vesicle directly, it is chosen to be represented by a particular scalar field that permeates all of space, from which all the geometric properties of the vesicle can be ascertained.

It is important to ask why a scalar phase field is being chosen to model the vesicle in the first place. In the classical Helfrich model discussed in Section 1.3, the bending energy is often defined along a 2D surface embedded into 3D space, requiring explicit knowledge of the surface’s geometry. As with many of the methods of Section 2.2, encoding it mathematically would require us to construct a function from $\mathbb{S}^2 \mapsto \mathbb{R}^3$ with $\mathbb{S}^2$ being a parameterization or triangulation of a 2-sphere [28, 58]. Thus, the problem with this method is twofold. The requirement of mapping a pure 2D parameterized surface into 3D is computationally taxing, while also being mathematically difficult.

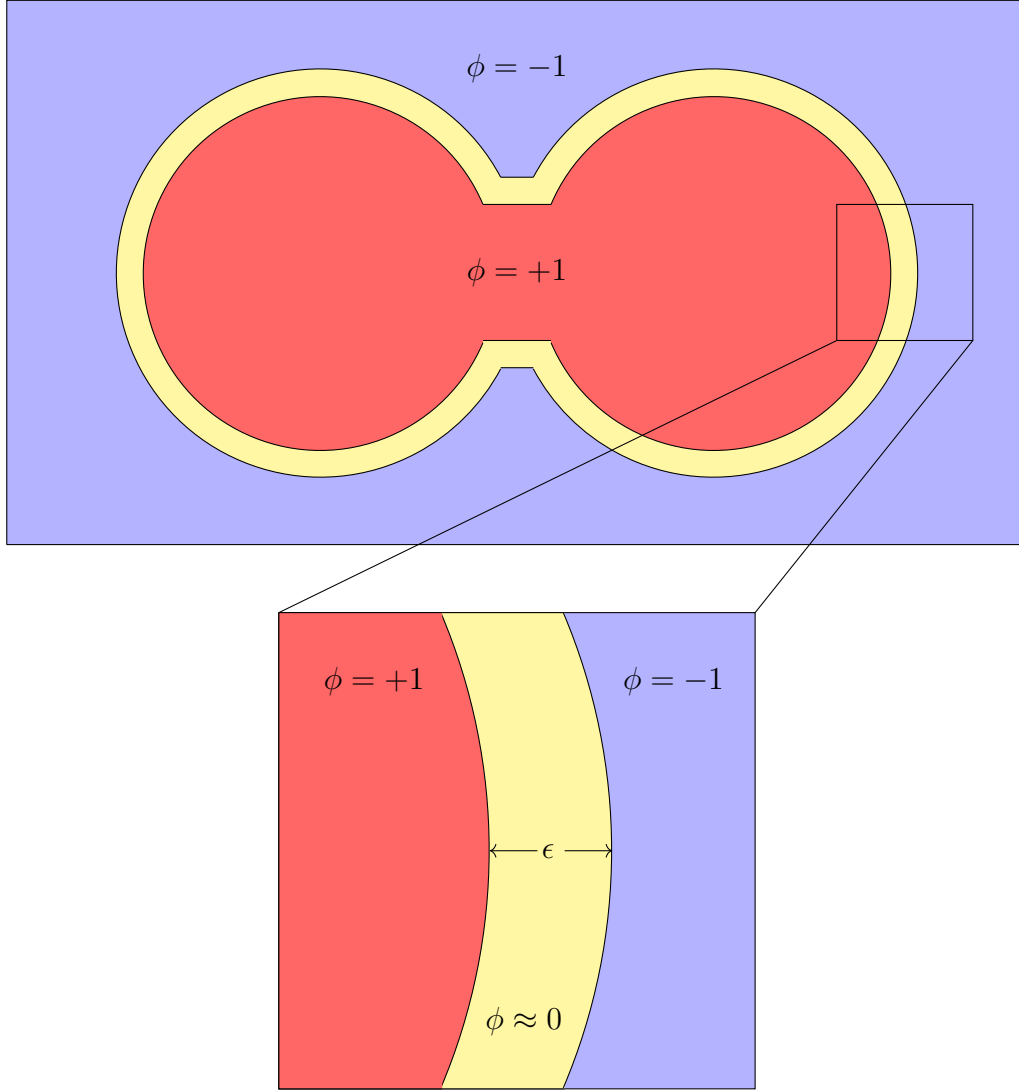


Figure 2.2: A mock heat map of the values of the phase field for a prolate-esque vesicle in 3D space. Points colored in red are the vesicle interior where $\phi(\mathbf{r})$ is very close to $+1$, while points colored in blue are the vesicle exterior where $\phi(\mathbf{r})$ is very close to -1 . The yellow region represents the interface/membrane surface, where $\phi(\mathbf{r})$ takes on intermediary values near 0. The width of this interface (in other words, the sharpness of the phase field close to the membrane) is governed by the value ϵ . This plot is idealized by three colors to provide a clear visual depiction, but the phase field is fundamentally a continuous function. The yellow region then sweeps across a wide range of ϕ between $+1$ and -1 , despite all being the same color.

Modeling our vesicle using a continuous scalar phase field approach solves both of these problems. To do this, a scalar function $\phi(\mathbf{r})$ from $\mathbb{R}^3 \mapsto \mathbb{R}$ is defined, taking in a point in physical 3D space, $\mathbf{r}$, and outputting the value of the field, ϕ . This field ranges from -1 to $+1$, with -1 being defined as the exterior of the vesicle and $+1$ being defined as the interior, as can be seen in Figure 2.2.

An important parameter to this phase field approach is the value ϵ , which will be called the interface sharpness. In producing the dynamical equations for the bending energy, area, and volume of the vesicle in terms of $\phi(\mathbf{r})$, it must be noted that a true vesicle membrane would be a surface with zero thickness. This would mean that $\phi(\mathbf{r})$ is discontinuous at the edge of the vesicle, jumping from -1 to $+1$. These discontinuities would result in issues with solving the resulting differential equations from a mathematical perspective, as well as issues in sampling data points from a computational perspective. ϵ lets us model the phase field continuously, by introducing a finite interface thickness, with smaller values of ϵ providing us with sharper, more narrowly defined membranes. In the limit as $\epsilon \rightarrow 0$, the phase field model produces a 2D surface embedded in 3D space, mathematically equivalent to models that explicitly track the evolution of the surface.

The result is that the phase field model provides both flexibility and ease of implementation, which gives it a particularly attractive quality with regard to numerical simulation. With classical surface mesh models, like in the work of Gompper et al. [28] and the original Helfrich and Seifert-Lipowsky works [14, 24], the energy, area, and volume constraints are all tackled using different fundamental methods. This results in the model needing to be reformulated to accommodate shape transitions or non-axisymmetric states, which are of primary importance in tackling translocation. Additionally, the field can be modeled on a fixed 3D grid, with the energy and constraints being functionals of the field ϕ itself. Finally, the introduction of external effects, like interaction with the barrier in the translocation problem, can be incorporated straightforwardly by coupling ϕ with additional fields and functionals.

One can then derive Equations 2.5, 2.6, and 2.7 for $E_b[\phi]$, $A[\phi]$, and $U[\phi]$, respectively. As mentioned in Sections 1.4 and 1.3, the Seifert-Lipowsky definition for E_b is used, where the bending energy of a sphere is $8\pi\kappa$ [13, 22]. The more in-depth derivation of these three functionals can be found in Appendix A.

$$E_b[\phi] = \frac{3\kappa}{4\sqrt{2}\epsilon} \int d\mathbf{r} \left[\epsilon \nabla^2 \phi(\mathbf{r}) + \frac{1}{\epsilon} \phi(\mathbf{r}) (1 - \phi(\mathbf{r})^2) \right]^2 \quad (2.5)$$

$$A[\phi] = \frac{3}{4\sqrt{2}} \int d\mathbf{r} \left[\epsilon |\nabla \phi(\mathbf{r})|^2 + \frac{1}{2\epsilon} (1 - \phi(\mathbf{r})^2)^2 \right] \quad (2.6)$$

$$U[\phi] = \frac{1}{2} \int d\mathbf{r} [1 + \phi(\mathbf{r})] \quad (2.7)$$

2.4 Optimization and Neural Network Implementation

2.4.1 The Free Space Constrained Energy

Given a free energy functional like Equation 2.5 above, our goal becomes to find the field $\phi(\mathbf{r})$ that best models the vesicle. Assuming that the relaxation of the vesicle follows an energy-minimizing gradient flow, the following dynamical evolution of the vesicle is described using Equation 2.8.

$$\frac{\partial \phi}{\partial t} = - \frac{\delta E_{\text{total}}[\phi]}{\delta \phi} \quad (2.8)$$

Equation 2.8 states that the phase field ϕ changes to decrease the total energy of the vesicle, and that if ϕ is stationary, it is because a minimum of $E_{\text{total}}[\phi]$ has been found where the right hand side is equal to 0. This total energy would account for the elastic bending energy, but also the energy associated with the lipid-scale interactions, area inflexibility, environmental forces, and many other effects. To isolate this in terms of the bending energy E_b , the decision made throughout this study is to solve the constrained energy equation under the area and volume constraints motivated in Chapter 1. Therefore, the total energy minimum is not the goal, but rather the bending energy minimum under the geometric constraints of the vesicle itself. A stable vesicle state is one satisfying the volume and area constraints while simultaneously minimizing this Helfrich free energy. To this, the use of Lagrange multipliers is employed to ensure constraints on surface area and volume. This provides us with the total constrained optimization given in Equation 2.9.

$$C_F[\phi] = E_b[\phi] + \lambda_A(A[\phi] - A_0) + \lambda_U(U[\phi] - U_0) \quad (2.9)$$

$C_F[\phi]$ denotes this as a cost function, which will be the notation used for expressions that will be minimized through the neural network. With a specific value of $\bar{U}$ in mind, $A_0 = 4\pi$ and $U_0 = \frac{4\pi}{3}\bar{U}$ are fixed to determine the geometric restrictions on the vesicle body. λ_A and λ_U are the Lagrange multipliers for the area and volume terms, respectively.

Finding a field ϕ that minimizes this function along with the associated Lagrange multipliers, corresponds directly to a solution for a free space vesicle. The method selected to deal with and manage these multipliers is discussed in Section 2.4.2.

2.4.2 Implementation of Neural Networks

At this point, many computational methods have been employed to attempt to solve Equation 2.9 from the phase field model, including the work of those who originally produced the phase field model [52, 59]. Techniques like finite difference methods, spherical harmonics, and coarse-grained modeling have been used to tackle phase field functionals of this form. However, the main struggle for these algorithms has been the sheer size and complexity of the problem. The parameter ϵ , described in Section 2.3, must be quite small to produce an accurate interface. Large values of ϵ result in the phase field method approximating the bending energy curve worse and worse, and so the interface width must be kept quite small. The same concern can be applied to narrow barriers with small values of $\bar{D}$, where points spread out too wide trivialize the structure of the barrier too much. This comes with the drawback, however, of the simulation requiring more densely packed sampling points to actually capture the behavior of ϕ at the interface. With too sparse a sampling, the solver struggles to find non-zero derivatives of the functional and fails to make progress towards a solution. Therefore, a highly dense sampling grid comes at the cost of these solvers attempting to process huge systems of nonlinear equations, often resulting in shaky final results.

This is why it has been chosen to attempt to solve the problem by using an unsupervised neural network (NN) [60, 61]. The phase field $\phi(\mathbf{r})$ is modeled as a feedforward NN, taking in 3D points and producing a value for the field at that point. Then, $\phi(\mathbf{r})$ is evaluated over a sampling grid of points, and Equation 2.9 is calculated across the grid. Note that any integrals are computed as standard numerical integrals by summing over the outputs and dividing by the density of points. The optimization equation above is then treated as the cost of the network. As a result, the problem of attempting to find a function ϕ that minimizes Equation 2.9 has turned into the problem of finding the set of network parameters that minimizes the associated cost function. The result should then be that the network calculates the phase field for a given point in 3D space. An introduction to neural networks, as well as the details of our NN code, optimization, and data, are given in Appendix C.

This method navigates around the issues of sampling methods by avoiding trying to solve a system of equations involving the points of the grid, which can often have a count over 1,000,000. Instead, our parameter space for $\phi(\mathbf{r})$ is much smaller, only involving the parameters of the network, which, depending on the network size itself, are typically in the hundreds or thousands. Additionally, the NN is more than capable of sampling data points not originally in the set used to optimize it, and so interpolating between sampled points is as straightforward as evaluating the network on the point in question. Numerical

methods resulting in a 3D array of values are much more difficult to interpolate between, often requiring re-solving the problem from the ground up.

Next is the issue of actually quantifying the Lagrange multipliers. If the whole problem were just to minimize Equation 2.9, then it becomes quite obvious that λ_A and λ_U could just be huge magnitude values that make the cost arbitrarily negative. For the constraints to actually be enforced, the Lagrange multipliers would ideally work against the minimization and ensure they are satisfied. With this in mind, the approach towards the Lagrange multiplier problem is to gradually optimize the Lagrange multipliers in the same way that the neural network optimizes its network parameters. In particular, while the network weights and biases follow a steepest descent optimization, the Lagrange multipliers are iterated to follow an optimization of steepest ascent instead. To be specific, after each batch of data points, the network parameters are adjusted to minimize the value of the bending energy/cost function as much as possible. At the same time, the multipliers in front of the constraints are set to adjust to increase the cost as much as possible instead. While this sounds to be against the goal of the problem, all it does is strengthen the effect of the constraints more and more in Equation 2.9. In order to keep the cost down, the network must put more effort into ensuring the vesicle keeps the constraints satisfied, as the multipliers try to keep them relevant. This means that to bring the cost down, the network is forced to find a phase field $\phi(\mathbf{r})$ that minimizes the bending energy while ensuring the constraints are satisfied throughout the solving.

To summarize, the NN method excels at approximating the complex, high-dimensional models induced by the phase field approach to the Helfrich model. As a result of their nonlinear internal activation functions, they can identify patterns and symmetry in the data, allowing them to quickly map vesicle shapes to their corresponding energies. Another perk of the NN method that is not seen in the others is that of real-time analysis. By monitoring the vesicle as it solves, and tuning the network’s hyperparameters, the cost can be modified in real time to push the network into a different solution branch. As discussed in Appendix C, this is implemented to form the initial vesicle fields for the networks by changing the cost throughout the optimization.

Neural networks, however, are incredibly data hungry and may often require extended solving periods to produce consistent results. Neural networks typically require a large amount of input data to learn accurate mappings between vesicle geometries, parameters (e.g. $\bar{U}$), and energies. In the Helfrich setting, generating initial states for this input data often means running simpler traditional solvers first, or attempting to construct a new solution out of a known previous one, which can cancel out the advantage of using a neural network in the early stages. Moreover, the network’s accuracy is bounded by the quality and coverage of the inputs: if the parameter space is not sampled densely enough, the

network may interpolate poorly.

On top of this, while constraints like fixed area and volume can be encoded into loss functions, neural networks only enforce them approximately, as they are not hard-coded into the network’s architecture. In practice, this means predicted vesicle shapes may slightly violate conservation laws, unlike variational solvers that guarantee exact or near-exact constraint satisfaction. These small violations can accumulate and make results unphysical, especially in dynamical studies like translocation. A final nitpick with the NN approach is that it is non-deterministic, similar to Monte Carlo simulations. Due to the highly sensitive nature of the gradient descent algorithm, repeated solving can potentially give qualitatively different results. This means that their final results can be unreliable and require consistent observation before they can be trusted as valid solutions to the problem. Even so, NNs can never answer a problem with 100% confidence, a point always worth keeping in mind as their results are interpreted.

2.4.3 Translocation Constraints

For this project, the translocation problem will be approached quasi-statically. In particular, the conditions and parameters of the system will be fixed to specific values, and then there will be an attempt to solve for the phase field at that point in parameter space. As a result, our modeling of vesicles during translocation is very similar to our method of modeling the vesicles in free space as described above. The next step is to then add additional constraints and associated Lagrange multipliers to the network’s cost function to produce vesicles experiencing the restrictions of translocation.

Our approach produces two additional constraints. First, $B[\phi]$ is the functional responsible for the interaction between the vesicle and the barrier itself. This is modeled as a direct excluded volume interaction between the bodies given by Equation 2.10 below.

$$B[\phi] = \frac{1}{2} \int d\mathbf{r} [(1 + \phi(\mathbf{r}))b(\mathbf{r})] \quad (2.10)$$

$$b(\mathbf{r}) = \sigma \left(\beta \left(\left(\frac{\bar{D}}{2} \right)^2 - (x - \bar{h})^2 \right) \right) \times \sigma (\gamma(y^2 + z^2 - \bar{R}^2))$$

Here, $b(\mathbf{r})$ is the barrier field that takes values near 1 inside the barrier, and values near 0 outside, with $\sigma(t) = \frac{1}{1+e^{-t}}$ being the sigmoid function. This sigmoid takes values near 0 when its input is very negative and takes values near +1 when its input is very positive.

β and γ are the numerical parameters that determine the sharpness of the barrier along the parallel and perpendicular axes. To explain the function, the first term only takes on values near +1 when $x \approx \bar{h}$ and the second term only takes on values near +1 when $y^2 + z^2 > \bar{R}^2$. As the sigmoid function ranges from 0 to 1, the barrier is positive only in the intersection of these two conditions, and is 0 everywhere else in space, as desired. And so the barrier has value +1 in regions of space where x is close to or at the value of $\bar{h}$ within a threshold of size $\frac{\bar{D}}{2}$, and outside the circle of radius $\bar{R}$ in the yz plane. Our analysis sets $\beta = 10^4$ and $\gamma = 20$ to ensure the barrier was highly localized along the x -axis and fairly sharp around the mouth of the pore.

Additionally, to enforce the constraint, this integral is set to 0, and thus $B_0 = 0$ for this constraint. As the integrand in Equation 2.10 is strictly nonnegative, setting it equal to 0 simply asks for there to be little to no vesicle/barrier overlap as possible. Finally, it is precisely this function $b(\mathbf{r})$ where the barrier parameters $\bar{R}$, $\bar{D}$, and $\bar{h}$ are located in our modeling.

The fourth and final condition to be enforced on the vesicle forces it to be in a particular location in 3D space. The first three constraints are all involved in determining the shape and state of the vesicle at a given position. However, there is nothing ensuring that the vesicle does not wander off and get pushed by the barrier to a location outside of the simulation box. As an aside, the term simulation box here refers to the grid of data points used as input data for the neural network. The constraint chosen to be implemented for this is a center-of-mass constraint on the vesicle, denoted by $R_{CM}^2[\phi]$, that serves the purpose of keeping the vesicle's CM at the origin of the grid at all times. To construct this constraint, one can produce the vesicle's center of mass along each of the three main axes as a weighted volume density. Equation 2.11 shows the expression for the x -component of the vesicle's CM, denoted by $R_{CM,x}[\phi]$.

$$R_{CM,x}[\phi] = \frac{1}{2} \int d\mathbf{r} [(1 + \phi(\mathbf{r}))x] \quad (2.11)$$

This is the same as the volume functional, Equation 2.7, but with each element weighted by its x -value. Repeating this process for the y and z components gives us the center of mass of the vesicle, $\mathbf{R}_{CM}[\phi]$, as a point in physical space. To ensure the vesicle stays at a particular location, it is demanded that $\mathbf{R}_{CM}[\phi] = \mathbf{R}_0$, or in other words that $R_{CM,x}[\phi] = R_{0,x}$, $R_{CM,y}[\phi] = R_{0,y}$, and $R_{CM,z}[\phi] = R_{0,z}$. Rather than introducing a separate constraint for each, however, they can be combined to produce Equation 2.12, which calculates the square of the distance of $\mathbf{R}_{CM}$ to $\mathbf{R}_0$.

$$(\mathbf{R}_{CM}[\phi] - \mathbf{R}_0)^2 = (R_{CM,x}[\phi] - R_{0,x})^2 + (R_{CM,y}[\phi] - R_{0,y})^2 + (R_{CM,z}[\phi] - R_{0,z})^2 \quad (2.12)$$

By fixing $\mathbf{R}_0 = \mathbf{0}$ at the origin of space, the functional above calculates the square of the distance of the CM of the vesicle to the origin. As such, by enforcing that this single constraint equals 0, the vesicle is penalized for moving away from the origin in any direction, causing it to remain in the same relative position in the grid throughout the modeling process. And so $R_{CM}^2[\phi]$ is defined as,

$$R_{CM}^2[\phi] = R_{CM,x}^2[\phi] + R_{CM,y}^2[\phi] + R_{CM,z}^2[\phi], \quad (2.13)$$

with $R_{CM,r_i}[\phi]$ matching the form of Equation 2.11 for each coordinate of $\mathbf{r}$.

In total, there are four constraints on the vesicle in our modeling of translocation. The area and volume constraints, $A[\phi]$ and $U[\phi]$, govern the geometric structure and size of the vesicle. The barrier constraint, $B[\phi]$, ensures the vesicle deforms around the opening of the pore. The CM constraint $R_{CM}^2[\phi]$ keeps the vesicle in a fixed location relative to the barrier and simulation box. Combining all of this produces the master equation used for the network's cost function, shown as C_T in Equation 2.14. Once again, for details regarding the initial parameters, learning rates, and optimization periods, see Appendix C.

$$C_T = E_b[\phi] + \lambda_A(A[\phi] - 4\pi) + \lambda_U(U[\phi] - \frac{4\pi}{3}\bar{U}) + \lambda_B B[\phi] + \lambda_{CM} R_{CM}^2[\phi] \quad (2.14)$$

2.5 Additional Notes and Limitations

As alluded to earlier, the passage of the vesicle is modeled quasi-statically, by iterating through a set of predetermined barrier positions relative to the vesicle's center of mass. As mentioned, the center of mass of the vesicle is fixed in space, and the barrier is moved across and around it. Physically, this is not typically what happens, as it is often the barrier being fixed in space as a vesicle is moved towards it, attempting to pass through to the receptacle on the other side. However, under this static approach, barrier movement and vesicle movement are fundamentally symmetrical processes that do not incur any issues in modeling.

On the other hand, in choosing to approach the problem from a static perspective, some critical vesicle behavior is neglected that would affect the analysis of the problem. Primarily, the vesicle is given endless time to stabilize and relax at each value, $\bar{h}$, of the barrier position. This results in a neglect of temporary deformations in the body as it stabilizes. In a sense, this means the vesicle does not really remember the previous snapshot that it came from. Our method solves each snapshot on its own using the solution of the previous snapshot as a starting point. But as a result, any information about the vesicle’s linear/angular momentum or kinetic energy is completely ignored by our analysis.

Another limitation of this system, briefly discussed in Section 2.4, is the fact that this method attempts to enforce four simultaneous constraints on the vesicle. While there has been an effort to define each of them so that they are continually satisfied throughout the optimization, the network may go down a route of parameters that optimizes some at the cost of others. As an example, it’s theoretically feasible that the network solver abandons the center-of-mass constraint if it feels that holding the vesicle in the center of the frame is too costly energetically. Despite the steepest ascent approach of the multipliers, having been designed to make the constraints stricter if they are being disobeyed, it is still possible that the vesicle finds an alternative minimum away from the supposed nearest one as a result of the tugging of these various conditions. So, even if the final stationary state satisfies the conditions, there is no guarantee that the state it arrived at was not affected by specific abuses of the cost function involved.

A final note is that of sampling bias. Given that the networks are being solved with a uniform grid of points, they are susceptible to not detecting any behavior on scales smaller than the grid spacing. Additionally, since the iteration is over a discrete number of barrier positions, critical in-between behavior can be missed, which can only be accessed by adding more snapshots into the trial. Overall, this model is fundamentally limited by discontinuities in its sampling space, which could contribute to different resulting behavior throughout solving.

Chapter 3

Simulations of Translocation

In this chapter, we will implement the method from Chapter 2 to produce 3D visualizations of vesicles undergoing translocation. First, it is essential to acknowledge the numerous previous attempts to address the translocation problem and the degree of success achieved by each. Next, the translocation problem will be defined in the context of the phase field model and the NN-based solver introduced in Section 2.3 and 2.4. Next, the $(\bar{U}, \bar{R})$ parameter space will be made clear, what region of it this thesis will explore, and the exact quantitative data by which the simulated images were produced. Afterwards, solutions of the networks, image strips of vesicles undergoing fundamentally different behavior during passage, will be shown. Additionally, there will be an attempt to justify the connection between these results and the choice of parameters. Finally, all the different classes of translocation will be compiled into the charted area of parameter space and shown explicitly.

3.1 Previous Efforts on the Translocation Problem

Before continuing on to our analysis of translocation, it is worth discussing the other recent works that helped shape the analysis in this thesis. In 2016, Shojaei and Muthukumar derived closed-form expressions for the size and shape of the free energy barriers by enforcing an approximate, but analytical form for the vesicle during passage, shown in Figure 3.1 [33]. This method included the bending/stretching of the membrane, as well as how increased external forces lowered the height of the energy barrier. As a final triumph of their model, they produced the first estimates of translocation time across the pore, quantifying the effects of the energy barrier against the vesicle by treating the passage

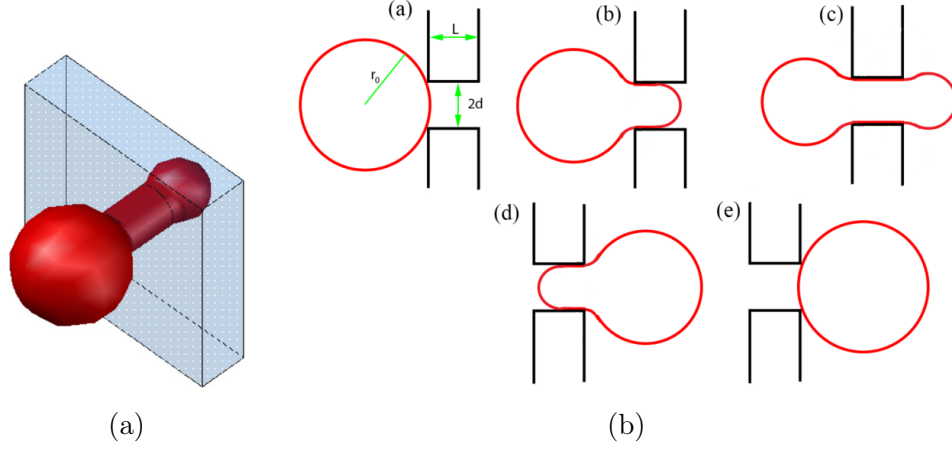


Figure 3.1: (a) A 3D diagram of translocation from the work of Muthukumar and Shojaei in their analytical analysis of the problem. The red volume is the vesicle’s interior, whereas the translucent cyan region is the barrier. (b) A 2D illustration from the work of Muthukumar and Shojaei detailing the different phases of translocation, in which they discuss the modeling technique used for each setup. These diagrams are taken directly from the paper, *Translocation of an Incompressible Vesicle through a Pore* [33], by Shojaei and Muthukumar.

through a diffusion-based, Fokker-Planck scaling. This specific Fokker-Planck derivation will be the technique employed by us to analyze our translocations in Chapter 4.

Building on this work, in 2019, Doi et al. employed Onsager’s variational principle to couple driving pressures with membrane elasticity [62]. They constructed a Rayleighian, which, when minimized with respect to shape evolution, yielded the kinetic pathway followed by the vesicle. The Rayleighian consisted of a dissipation term, representing the frictional resistance during shape changes and motion, together with the time derivative of the free energy, representing the thermodynamic driving force. This method actually softens the geometric volume/area constraints in some instances and allows the geometry of the vesicle to vary, subject to energetic penalties. Again, under the assumption of axial symmetry, this framework enabled more accurate predictions of how translocation time depends on the applied pressure and vesicle size, while also incorporating frictional resistance from the surrounding environment.

Complementing such analytic treatments, the more recent 2025 study by Baruah et al. introduced a triangulated-surface minimization approach to investigate adhesion-assisted translocation through membrane-covered pores [63]. In this framework, both the pore and

the vesicle were modeled as deformable membranes, with vesicle passage depending on the mutual deformation of the two surfaces. The pore-spanning membrane could reshape to permit vesicle entry at prescribed pore radii, while the driving force for translocation arose from the favorable adhesion energy gained upon contact between the membranes, which pulled the vesicle deeper into the pore. Using this method, the authors classified the translocation behavior of spherical and prolate vesicles into distinct regimes determined by adhesion strength, membrane rigidity, and pore size. The outcomes were categorized according to the final state of the system: complete translocation, partial translocation, or failure to translocate.

In all of these works, translocation was cast under the assumption of axial symmetry about the pore, in addition to other assumptions of structure. In all cases, though, the incoming vesicle retained a constant axis of symmetry throughout, retained its shape, and matched its axis with that of the pore. Therefore, the question of vesicles interacting with the pore at different angles, or moving dynamically from one shape to another, could not be analyzed, given the assumptions in the models. Certain features of a translocation event, like axial realignment and shape transitions, were thus omitted from analysis, as the tools being used were ill-equipped to handle them.

From an experimental perspective, high-throughput handling of giant vesicles matured in the early 2010s through the work of Arriaga et al., enabling the controlled trapping and imaging of single vesicles near constrictions or pores in microfluidic chips [64]. This development became critical for testing translocation models and is still widely used today to verify certain geometrical limits assumed by theoretical frameworks. In the 2017 studies by Krishnan, Ziegler, and Arnaut, DNA-architected membrane pores provided tunable, cell-sized conduits embedded in the membranes of large-scale vesicles [65]. These systems allowed controlled and consistent single-channel readouts, facilitating monitoring of membrane characteristics during passage and providing vesicle-compatible platforms for studying pore mechanics.

More recently, Bayley et al. demonstrated “leakless” end-to-end transport through engineered μm -scale DNA channels, achieving precise control of vesicle flux across barriers [66]. They introduced DNA nanopores as a novel strategy for controlling vesicle transport with high precision. Unlike conventional translocation studies that rely on pressure, adhesion, or mechanical deformation, this approach uses specifically engineered channels with preselected geometry and permeability. Manually placed mechanical blockers further enable temporal regulation of molecular flux, allowing controlled passage of small molecules, folded proteins, and nm-scale vesicles. By providing a programmable and highly specific mechanism for vesicle transport, this work represents a significant advance in both experimental control and the study of membrane dynamics. The ability to regulate

transport in a time-sensitive manner also moves the field closer to establishing clean benchmarks for coupling translocation mechanics with detailed experimental readouts.

3.2 The Modeling of Translocation

As originally discussed in Section 1.5, the translocation of vesicles through narrow pores or channels represents a fundamental problem in membrane biophysics, with strong relevance to cellular processes such as endocytosis, exocytosis, and viral infection. At its core, vesicle translocation refers to the passage of a closed lipid bilayer compartment from one side of a constriction to the other, driven by external forces, pressure gradients, or interactions with the pore walls. As described in the previous chapters, vesicles can be modeled as having a fixed surface area and volume over time scales shorter than their relaxation time. The restrictive geometry of the pore further forces them to squeeze into and around the opening, introducing an additional constraint that strongly influences vesicle shape. As a result, the interaction with the barrier can cause the vesicles to deform into new configurations while still obeying their original geometric constraints. These deformations may involve elongation or even shape transitions, making the process a valuable testbed for studying membrane mechanics under strong physical restrictions [12].

A vesicle may initially be considered in free space, subject only to its standard geometric constraints on A and U , where it adopts a known stationary state. Upon encountering a pore, however, its shape is altered by interactions with the barrier. This departure from the stationary state, driven specifically by contact with the pore, is what is defined as a translocation-induced deformation. During passage through the barrier, the vesicle continues to conserve A and U , but it must also satisfy the excluded volume condition imposed by the barrier. Consequently, translocation forces the vesicle into shapes that would not be stationary in free space and introduces new structural configurations to its overall morphology.

From a biophysical standpoint, translocation is governed by the competition between the Helfrich elastic forces, arising from membrane bending rigidity, and external constraining forces, which can include hydrodynamic flow or mechanical pressure across the pore. As discussed, the Helfrich model provides the baseline description of the elastic contribution, with an energy functional that penalizes deviations of the mean curvature from a preferred value [14]. As the vesicle is forced into a narrow channel, bending energy increases sharply, as interactions with the pore wall add further energetic penalties [6]. Thus, successful translocation requires overcoming energy barriers that depend on pore geometry, vesicle size, and material parameters such as the bending modulus, with an

energetic preference given to vesicles of particular configuration that line up well with the pore itself.

In such cases, the vesicle’s evolution is governed by a balance of induced bending stresses from both the surrounding fluid and the geometry of the pore, leading to a set of coupled nontrivial interactions between the fields. Consequentially, the parameter space for modeling the process becomes incredibly vast, with parameters like the reduced volume, $\bar{U}$, and spontaneous curvature, H_0 , being matched up with pore radius, $\bar{R}$, the pore width, $\bar{D}$, and the vesicle’s orientation in space. As discussed in Section 2.4, constraints on area, volume, barrier interaction, and center-of-mass enter as Lagrange multipliers, attempting to ensure the fidelity of the vesicle’s structure is maintained throughout the process.

Finally, the translocation problem can also be cast as an energetic problem of vesicle integrity, where the primary question is whether a given vesicle, under specified forces and pore geometry, can successfully deform to pass through. This involves studying the free energy landscape, identifying accessible stable vesicle configurations, and determining transition pathways between them. Under this formulation, translocation corresponds to a saddle-point problem, in which the vesicle must cross an energy barrier, through intermediary transitional states, to move from one equilibrium shape to another [13]. This highly restrictive interaction with the barrier may cause the resulting vesicle to conform to a different stable branch after passage due to its deformations, or to become trapped within the pore and lose its structural integrity, failing to pass through to the other side.

Given the tools developed in Section 2.1, the translocation problem can be quantified in the framework of our phase field and neural network approach. Given a stationary vesicle state described by a neural network formulation of the phase field $\phi(\mathbf{r})$, and a pore described by $b(\mathbf{r})$ in Equation 2.10, the process of translocation will be simulated through several static snapshots. Particularly, the barrier is fixed at a starting position, $\bar{h}$, in front of the vesicle, and the network is optimized to minimize the energy at that moment in the process under the Lagrange multiplier constraints. After a satisfactory solving period, the network is saved for that position, and the barrier is moved one step closer to the vesicle to the next value of $\bar{h}$. The network is then re-optimized and solved under the new cost using the final result of the previous snapshot as its initial state. Repeating this, the barrier is eventually moved over and across the vesicle to the other side, where the vesicle can reenter free space. As each previous snapshot is implemented into the next, the goal is to monitor the bending energy of the vesicle at each step, identifying how they transform into the final state, and what bending energies the vesicle passes through on the way.

3.3 Cases and Evaluation Methods

Each translocation event analyzed in this thesis lies in the $(\bar{U}, \bar{R})$ 2D parameter space. To be specific, an event is defined as the simulated passage of a vesicle from the free space on one side of the pore to the free space on the other, with specific monitoring of the morphology and energy of the intermediary points throughout. While other parameters, such as the initial axis of orientation and barrier width, are interesting, the reduced volume and reduced hole radius have been selected as the primary parameters in this study. In doing so, the other parameters remain fixed across all simulations to remain consistent between trials. As mentioned in Section 2.1.2, the reduced width of the barrier will remain at the value of $\bar{D} = 0.16$ across all events. As shown explicitly in Figure 1.5, for the same value of $\bar{U}$, there may be more than one stable state, which will be of note when shape transitions are discussed. This is specified more in Table 2.1.

The data collected spans two main cases. Our first case is a $\bar{U} = 0.6$ oblate vesicle passing through holes of radius $\bar{R} = 0.7$ up to $\bar{R} = 1.0$. Our second case is a more constrained one with a larger $\bar{U} = 0.8$ oblate passing through the same set of holes. For each of these data sets, the energy landscape is determined to see what elastic energies the vesicle experiences as it passes through, as well as if and when shape transitions happen throughout. At each of these reduced volumes, both oblates and prolates are stable, and so it can be observed if and when state transitions occur by contrasting the two. Note that looking at Table 2.1, oblates are globally stable down at $\bar{U} = 0.6$ while prolates are globally stable at $\bar{U} = 0.8$. This range of hole sizes is chosen to bring the vesicles near the geometrical limit of translocation so that more extreme behavior is observed. For a discussion regarding this geometric limit on $(\bar{U}, \bar{R})$ parameter space, see Appendix B.

For each of the two cases, the network is initialized to a stationary free space oblate disc with an axis of symmetry along the positive y -axis for each of the seven hole radii ($\bar{R} = 0.7$ up to $\bar{R} = 1.0$ with spacing 0.05). The barrier is placed at position $\bar{h} = -1.2$, and the first network is optimized towards an energy-minimizing solution. This specific position is chosen due to satisfying the conditions described in Sections 2.4.3 and 3.2. In particular, the oblate discs in question are far enough from the barrier to be considered in free space at this distance. Therefore, this snapshot occurs at a moment definitively before the event has begun, with no interaction between the vesicle and barrier. Mirroring this, $\bar{h} = +1.2$ is claimed to represent the moment where the event is complete and the vesicle reenters free space on the other side of the constriction. Taking the resultant network parameters, the barrier is moved a bit closer to $\bar{h} = -1.1$. Repeating this process (carrying over the network parameters and Lagrange multipliers from the previous snapshot onto the next), 25 barriers are iterated through, from -1.2 up through $+1.2$.

For each choice of $(\bar{U}, \bar{R})$, the corresponding translocation event was simulated from scratch three separate times. Only one of the sets of images is shown for each case, but any numerical data extracted is taken as an average of the three runs for each. The details and results of this repeated testing are explained further in Chapter 4. There was also an additional decision made to accelerate the solving process of our vesicles by dividing it into two distinct phases. Namely, the series phase and the parallel phase.

First, in the series phase, a stable free vesicle is started in a given configuration far from the barrier with a fixed center of mass. Then, after solving the network for some time, it is saved, and the barrier is inched one step further along in the process. This is the exact process described earlier, with the important note that the networks are not being solved for an extensive period of time in this phase. As a result, a lot of the late-stage fine-tuning of the vesicle’s shape has not yet happened. This is mainly done to produce a rough vesicle shape for each value of $\bar{h}$, which can be more thoroughly optimized in the parallel stage.

The second stage of solving is the parallel phase, where the output from each snapshot is taken from the series step and solved in isolation for a much higher number of epochs. Here, an epoch refers to a full batch of input data being filtered through the network, so one epoch in this case is every data point in the grid being fed through the network once. Since all the roughly determined snapshots have been calculated, all of them can be done simultaneously, resulting in a lot of time being saved in the solving process. The Lagrange multipliers are also initialized to be those obtained from the series step at the final moment of the corresponding stage. In this phase, each network is typically optimized for about 10-20 times longer than the corresponding series phase. For all of the solved networks shown in this project, the series phase lasted 30,000 epochs per snapshot, whereas the parallel phase lasted 500,000 epochs per snapshot. The computational cost and total solving duration can be found in Appendix C.

To produce visual plots of the events, there is some additional manipulation that occurs to ensure clean, artifact-free diagrams. These renderings are generated by sampling the solved vesicle network inside a box of size $4.8 \times 4.8 \times 4.8$ centered at the origin with 160^3 points. A step function was then applied to the phase field output of each data point to give all points a static color depending on whether they qualify as vesicle interior or exterior. Any point where $\phi > 0$ gets set to $\phi = +1$ and any $\phi \leq 0$ gets set to $\phi = 0$. Additionally, the visual barrier rendering is produced in the same way as a grid of 1’s inside and 0’s outside, by rounding $b(\mathbf{r})$ to the nearest integer for each point. Plotting these cleaned-up data sets using the 3D rendering engine, ParaView, gives us all the image strips (Figures 3.2, 3.3, 3.4, 3.5, and 3.6), in the following sections. Finally, it is worth noting that not every snapshot in the process is shown below, only the ones relevant to the discussion at hand.

3.4 $\bar{U} = 0.6$ Visual Analysis

The first case to analyze is the $\bar{U} = 0.6$ oblate and its behavior at each respective hole size. As mentioned, not all of the 25 snapshots are shown in a strip below since there is little variation between adjacent barrier positions. The general trend across the snapshots is the focus of this section. Note that at $\bar{U} = 0.6$, oblates and prolates are both stable. However, the pore being a narrow circular opening should theoretically result in prolates finding it easier to settle within the confines of the hole. This is the primary reason oblates were chosen to be the default initial state. They are worse at aligning with a narrow circular pore than a prolate, and so are more likely to have significant interactions with the barrier, causing them to undergo a shape transition into a prolate. In the $\bar{R} = 0.8$ case, shown in Figure 3.2, a transition from oblate to prolate can be seen, whereas in the $\bar{R} = 1.0$ case, shown in Figure 3.3, the oblate deforms but not quite enough to jump to the prolate branch, stabilizing back at a standard oblate shape.

In the $\bar{R} = 0.8$ tighter hole, one can see that as the disc passes through, it begins to deform and shift its volume into the other half, forming an intermediary three-pronged disc. As the neck approaches the midpoint of the passage, the vesicle rebalances its volume across the two posterior lobes and pushes them towards each other, eventually combining them. Therefore, the original vesicle's state deviated continuously from oblate to prolate, passing through non-axisymmetric states throughout. By the end of the process, the axial symmetry has reemerged in the form of a prolate. Additionally, the axis of symmetry of the original vesicle is lost in this interaction as the symmetry of the final state matches that of the barrier, not the original vesicle. For example, an oblate aligned out of the page along the z -axis would theoretically converge to the same state shown below. This suggests that, at least in the way the events are being simulated, the original axis of symmetry of the oblate is lost during the passage. Similar behavior is seen with holes $\bar{R} = 0.7$ and $\bar{R} = 0.75$, with the vesicle coming to a stable prolate aligned with the hole of the barrier once it arrives at $\bar{h} = +1.2$.

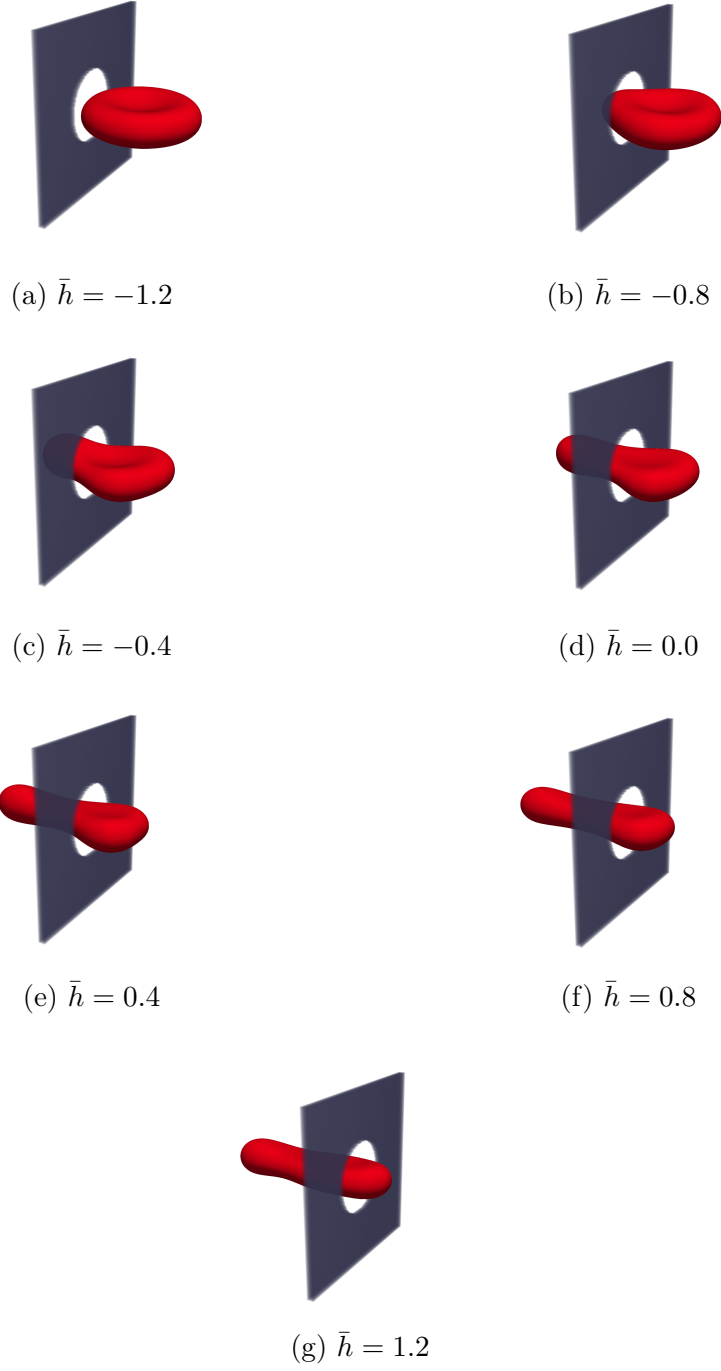


Figure 3.2: $\bar{U} = 0.6$ oblate to prolate translocation with $\bar{R} = 0.8$ for different values of the barrier position $\bar{h}$.

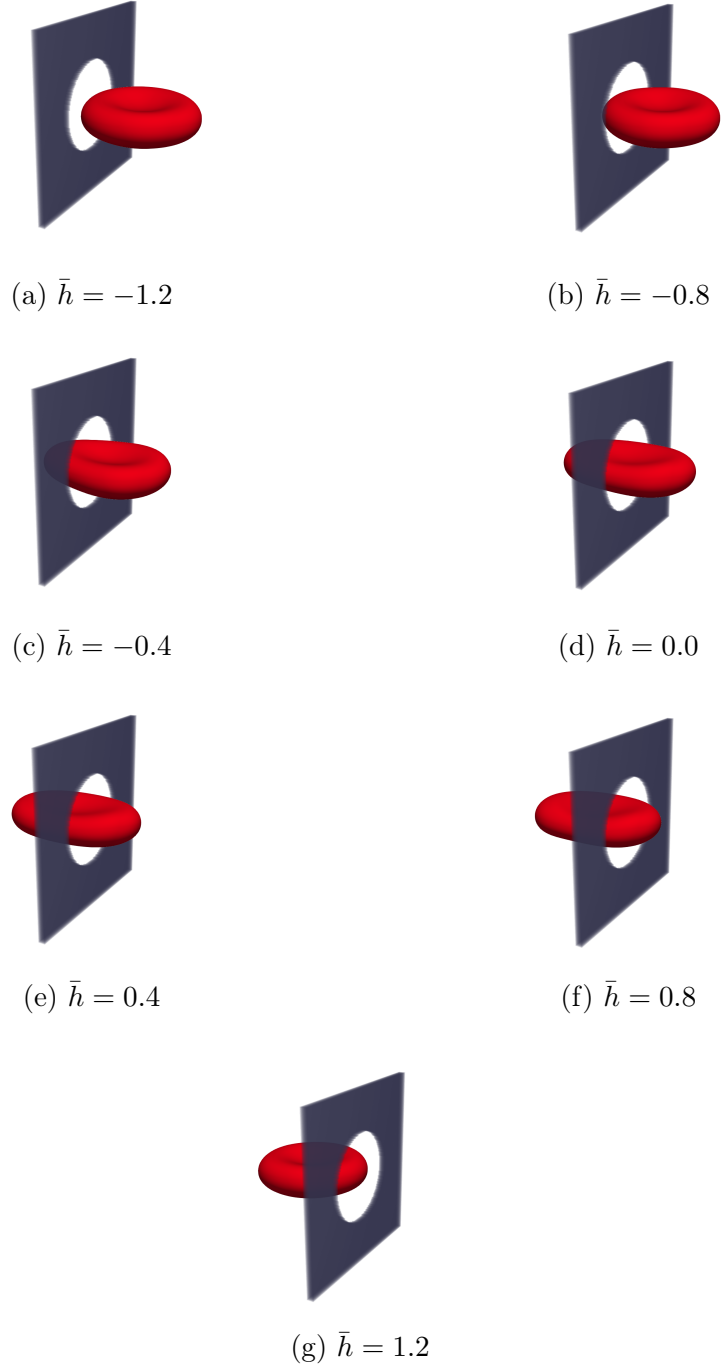


Figure 3.3: $\bar{U} = 0.6$ oblate to oblate translocation with $\bar{R} = 1.0$ for different values of the barrier position $\bar{h}$.

In the larger $\bar{R} = 1.0$ case, it is seen that as the vesicle passes through, it deforms in the same way as before, but to a lesser extent due to the less restrictive pore configuration. The result is that the vesicle elongates parallel to the translocation direction but doesn't deviate enough from an oblate to actually transition between states. In particular, the vesicle retains its disc-like form throughout the passage. The disc takes on an elliptical shape as it is still wider than the pore, but does not contract inwards to a prolate. Once the vesicle is free of the barrier, it widens back out, eventually returning to the original oblate form from before. In this case, the same atransitional behavior is seen at the other large hole sizes, $\bar{R} = 0.85 - 0.95$, with all maintaining their initial axis of symmetry and staying as an oblate after passing through.

One technical issue that presents itself is the stability of the solving process involved at smaller hole sizes. In these more and more extreme cases, the vesicle's behavior becomes wildly unpredictable and much more sensitive to the random walk optimization of the network. The data points analyzed here are specifically the ones where the qualitative results from all three trials for a specific $(\bar{U}, \bar{R})$ pair are very similar to each other. This gives us more confidence in their accuracy as a representation of the genuine behavior of the vesicle. This results in much more chaotic behavior and is a strict limit on the static approach implemented to produce this data. On the other hand, this is also where it is much more likely to see drastic changes to the state of the vesicle and see a shape transition. The numerical analysis of the energy landscape and first passage time for this case are in Sections 4.2.1 and 4.4.1.

3.5 $\bar{U} = 0.8$ Visual Analysis

Moving to the second case, the network is initialized to a $\bar{U} = 0.8$ oblate and pushed through the same set of seven holes as before. $\bar{U} = 0.8$ is chosen since it still allows for both prolate and oblate stable states, while being bulkier and less stable under the more extreme constrictions of the pore. In short, the possibility of some hole radii failing translocation is accepted with this large a vesicle.

In this case, it is worth looking in detail at three different hole sizes, rather than just two, as there is fundamentally different behavior in each. For the largest hole $\bar{R} = 1.0$, shown in Figure 3.4, the vesicle deforms slightly and passes through intermediary non-axisymmetric states before finding its original symmetry again and relaxing back to an oblate. The behavior here is very comparable to that of the $(\bar{U} = 0.6, \bar{R} = 1.0)$ vesicle from Section 3.4. Hole sizes $\bar{R} = 0.9$ and 0.95 also stay within the oblate branch, but this time $\bar{R} = 0.85$ does not. At $\bar{R} = 0.8$, shown in Figure 3.5, the vesicle overcomes the

state transitional energy and relaxes as a prolate on the other side, again similar to the ($\bar{U} = 0.6, \bar{R} = 0.8$) case from Section 3.4. However, here the vesicle clearly holds a different shape than its $\bar{U} = 0.6$ counterpart from Figure 3.2, mainly because the free space prolate it defaults to is much less eccentric. $\bar{R} = 0.85$ and $\bar{R} = 0.75$ both undergo shape transitions at $\bar{U} = 0.8$ in a similar way.

Finally, at $\bar{R} = 0.7$, the vesicle failed to pass through the pore on all three trials, resulting in the empty snapshots shown in Figure 3.6. The vesicle initially aligns itself perpendicular to the pore as it fails to find a stable conformation while being partially translocated. This results in a complete restructuring of the vesicle until the interaction becomes too costly for the network to simulate. In the final Figure 3.6g, the vesicle model has ‘blown up’, and only residual artifacts of the phase field can be seen within the simulation box. At this point, the vesicle structure has collapsed, and the event is deemed to have failed. There are two possible interpretations for this result. First, this can be the product of a genuine physical limit beyond which the local binding energy of the vesicle becomes comparable to the elastic stretching caused by the pore. In this case, this location in parameter space corresponds to a sort of energy discontinuity and is physically unrealizable.

On the other hand, it is also possible that this extreme deformation is a trade-off limit of the cost function, as mentioned in Section 2.5. Specifically, as energy costs increase, the network is more likely to sacrifice other constraints to reduce energy. Looking again at Figure 3.6, the vesicle bunches up around the pore without entering it and gets spread out along the wall until the CM is expected to pass through to the other side. At that point, the network picks up a very high cost from the CM constraint, resulting in the other parameters getting muddled and neglected. In a sense, if one of the constraints has a cost orders of magnitude higher than the others, it can be expected that the network parameters would do whatever they can to bring that down, to the detriment of the other conditions. As a result, since there is no mathematical guarantee that the vesicle can hold its form, the structural integrity of the body can just give way, and the vesicle can ‘blow up’ throughout the simulation box, as can be seen.

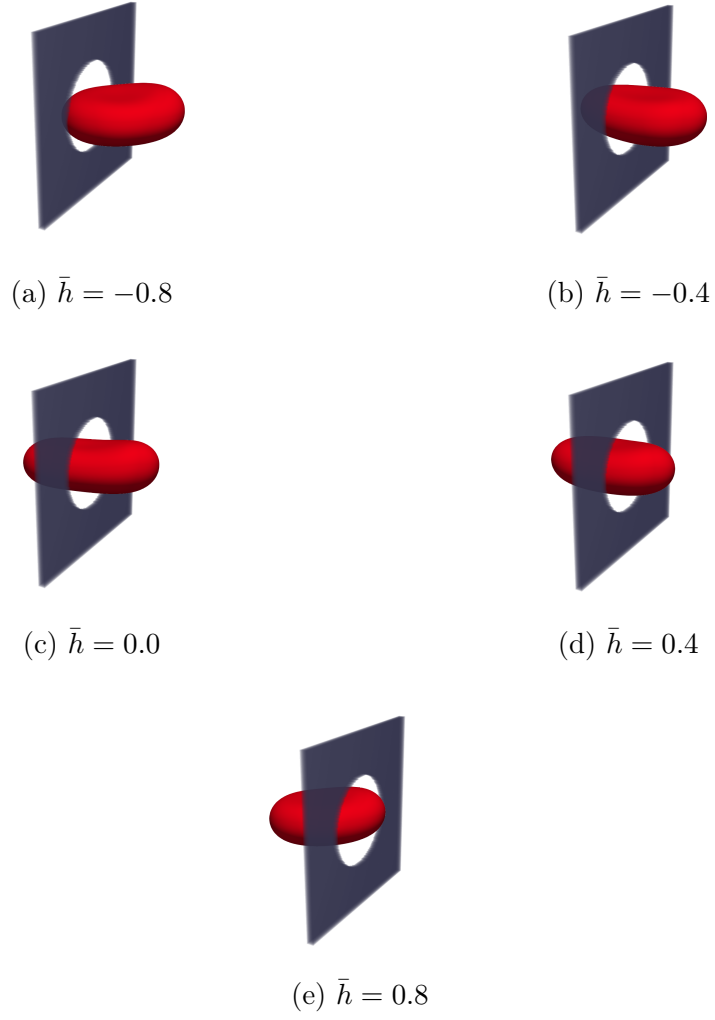


Figure 3.4: $\bar{U} = 0.8$ oblate to oblate translocation with $\bar{R} = 1.0$ for different values of the barrier position $\bar{h}$.

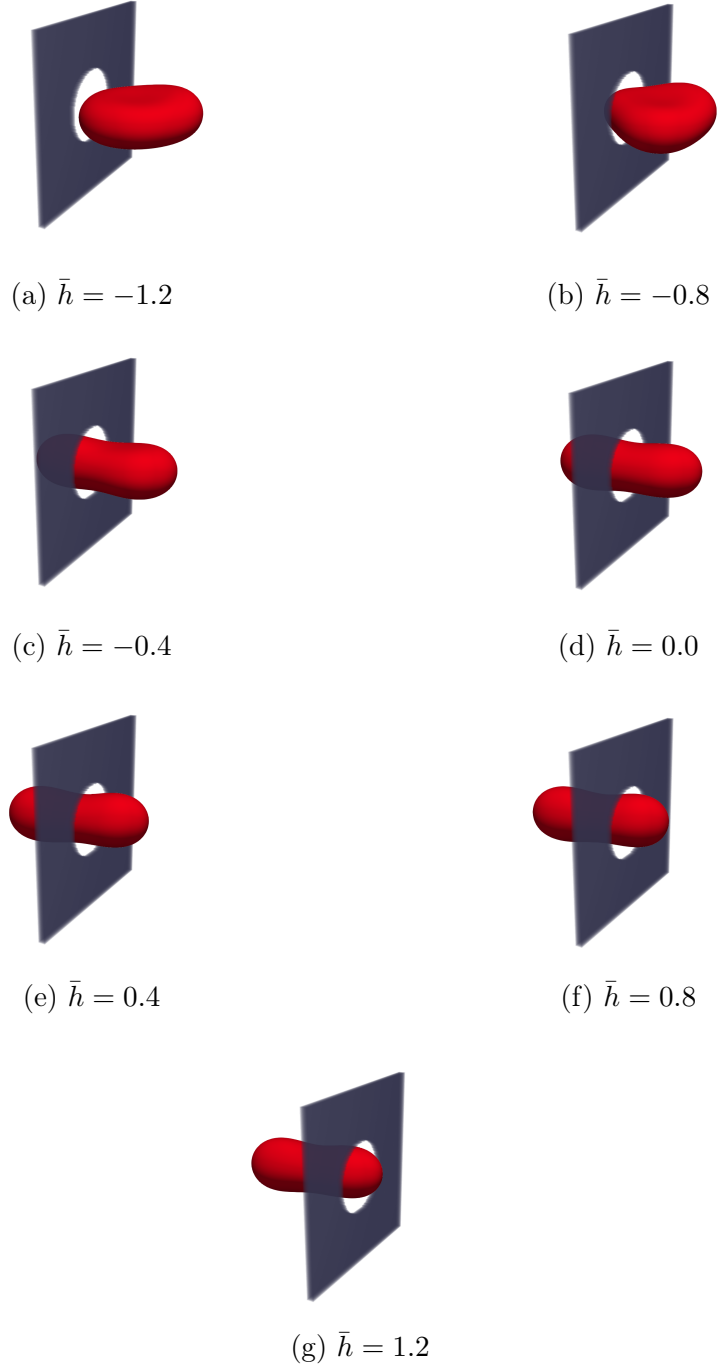


Figure 3.5: $\bar{U} = 0.8$ oblate to prolate translocation with $\bar{R} = 0.8$ for different values of the barrier position $\bar{h}$.

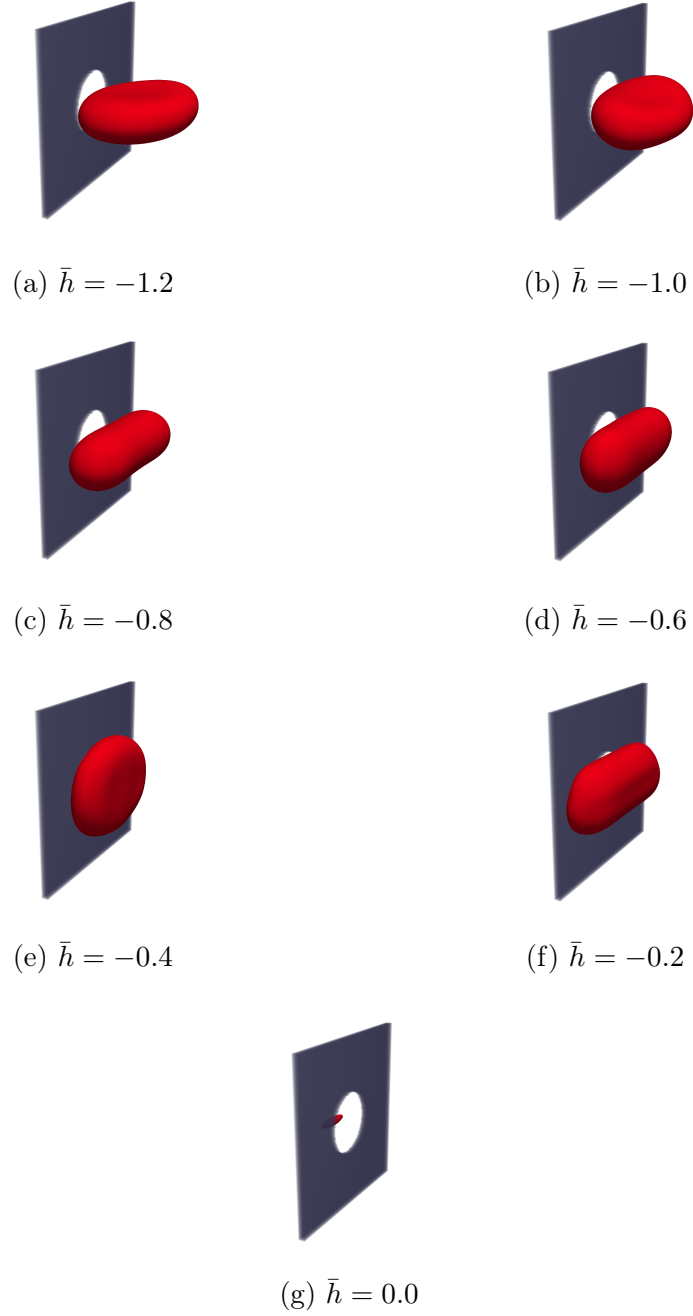


Figure 3.6: $\bar{U} = 0.8$ failed oblate translocation with $\bar{R} = 0.7$ for different values of the barrier position $\bar{h}$. In the final figure, the simulated vesicle has failed to translocate, by virtue of the model exploding the vesicle and leaving disconnected residual phase field artifacts behind.

The most important note with these $\bar{R} = 0.7$ cases is that of the axial alignment of the vesicles. In the previous cases, the final alignment of the vesicle was either the same as its initial axis (vertical along the y -axis), or parallel to the axis of the pore (horizontal along the x -axis). Here, however, the body is spread across the barrier in the z -direction instead. It should also be noted that all three trials for this produced very similar qualitative results in the vesicle extending along z before reaching a critical point. However, the breaking point of the simulations did not match up, with the final stable value of $\bar{h}$ ranging from -0.1 to $+0.2$. The numerical analyses of the energy and estimated passage time for this case are in Sections 4.2.2 and 4.4.2.

Finally, the final resting states of each case can be plotted into $(\bar{U}, \bar{R})$ parameter space to properly produce a coloring of parameter space shown in Figure 3.7. Looking at the limited set of data within this plot, there are already some meaningful conclusions worth investigating in more detail. Intuitively, it is expected that events further to the left, with lower values of $\bar{R}$, will undergo more extreme behavior. First, undergoing shape transitions to prolates, and then failing to pass through altogether. This is seen in both the $\bar{U} = 0.6$ and $\bar{U} = 0.8$ line. Varying $\bar{U}$ is less clear as only two different values were selected for the analysis, but across the board, it appears that the larger $\bar{U} = 0.8$ shapes have a tougher time undergoing a smooth passage. Particularly, at $\bar{R} = 0.7$, the $\bar{U} = 0.6$ successfully passes through, while the $\bar{U} = 0.8$ fails to. Additionally, at $\bar{R} = 0.85$, the $\bar{U} = 0.6$ undergoes no state transition as it passes, whereas the $\bar{U} = 0.8$ actually makes it over to the prolate branch.

In theory, this classification of the data could be used to divide the $(\bar{U}, \bar{R})$ parameter space into three distinct regions. One in black where vesicles fail to pass through, one in blue where vesicles pass through but undergo a shape transition to a prolate, and one in magenta where vesicles retain their oblate state while passing. However, a confident mapping would likely require a more densely packed sampling of the space and much more variation in the chosen parameters to provide any sort of value. This is a great next step to take with regard to making progress on the translocation problem in the future.

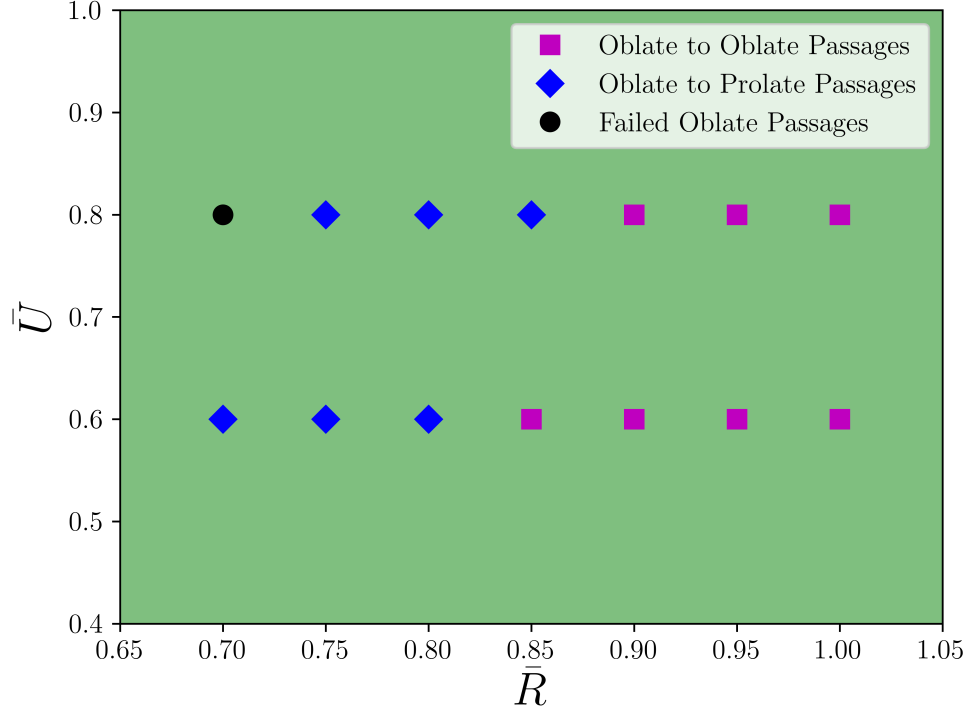


Figure 3.7: The charted map of the $(\bar{U}, \bar{R})$ space compiling the 14 cases discussed throughout this chapter. The black circle is the single failed event corresponding to $(\bar{U} = 0.8, \bar{R} = 0.7)$. The blue diamonds are the oblate to prolate transitional passages, while the magenta squares are the oblate to oblate atransitional passages. The overall trend in this diagram is that as $\bar{R}$ decreases, the events become more restrictive and energetically unstable. Additionally, for select values of $\bar{R}$, increasing $\bar{U}$ to grow the vesicle's volume can also cause the passage to be more restrictive.

Chapter 4

Energies and Times

In this chapter, we will quantitatively examine the cases presented in Chapter 3 by analyzing the associated bending energy and translocation time for each case. This will be done by first explaining the method used to extract the bending energy of each snapshot and fitting a specially chosen curve through the points. Next, an explanation will be provided on how the translocating vesicle can be influenced by external forces and pressures, as well as how these influences can be represented in the free energy landscape. Following this will be an introduction to the vesicle's mean first passage time, based on the mathematics of the Fokker-Planck Formalism using the extracted energy landscape. Throughout, we will unleash these mathematical tools on the acquired energy data to extract numerical details about all of the discussed cases. Finally, there will be an attempt to quantify a relationship between the translocation time and the size of the pore, with shape transitions in mind.

4.1 Energy Mathematics

4.1.1 Energy Curve Fitting

Following the optimization of each network, the total bending energy $E_b(\bar{h})$ can be evaluated for each barrier position to plot and see the free energy landscape navigated over the course of the event. Extracting the total bending energy of a vesicle is as simple as allowing the network to evaluate the energy density Expression 2.5 across the grid and numerically integrate the result. After evaluating the energy using this method, it can be plotted versus the barrier's position to map out the free energy landscape of the event. In

doing so, it is expected to see the vesicle at a stable energy before and after contact with the barrier, with an energy hump in the middle as it deforms due to the barrier interaction. As mentioned in Section 2.3, our phase field model assigns a bending energy of $8\pi\kappa$ to a sphere. Like most other parameters, the energies can be reduced by dividing by $8\pi\kappa$, down to the dimensionless $\bar{E}_b(\bar{h})$, so that a sphere instead has a bending energy of 1. Our energy plots will be scaled with this normalization in mind.

There has been much work put into quantifying the free energy landscape using this method. Other models used to chart out the free energy landscape of vesicle states can be found in the works of Seifert [24], Shojaei [32], Dimova [39], and Noguchi [67], in addition to the method implemented here.

Given an effective energy dataset, it can be interpolated with a curve to make the data clearer and continuous. Given 25 energy data points for each translocation event, the form chosen for the approximation curve is in Equation 4.1. Our fitted curve consists of a constant term, a sigmoid, and two Gaussians.

$$\bar{E}_b(\bar{h}) = A' + \frac{B'}{1 + e^{-C'\bar{h}}} + D'e^{-E'(\bar{h}-F')^2} + G'e^{-H'(\bar{h}-I')^2} \quad (4.1)$$

The main justification for this choice is that all of these terms flatten out far away from the barrier, which the energy is expected to do once the vesicle enters free space. Additionally, the sigmoid gives our curve the ability to flatten to different values before and after the passage, allowing for a change in energy/state. Finally, the two Gaussians are to represent the actual energy bump(s) and ideally map out the transition as well as possible. This curve picks up 9 total fit parameters (A' through I'), which can be decreased with certain assumptions of symmetry. To be clear, A' through I' in this context are pure mathematical fit parameters for the shown models, and are completely independent of the physics involved.

In the cases of more symmetrical energy landscapes (oblate to oblate passages in our case), terms asymmetric in $\bar{h}$ were removed from the expression above to reduce the parameter count. Thus, for this set of curves, the simpler fit shown below will be resorted to.

$$\bar{E}_b(\bar{h}) = A' + D'e^{-E'(\bar{h}-F')^2} + D'e^{-E'(\bar{h}+F')^2} \quad (4.2)$$

This fit removes the sigmoid term entirely and equates parameters from the Gaussians to force symmetry. In these cases, the parameter count is brought down to four, ideally ensuring the curve does not end up overfitting the data.

Overall, thirteen different events (seven at $\bar{U} = 0.6$ and six at $\bar{U} = 0.8$) were fitted to these energy curves. The collected parameter set is shown in Tables 4.1 and 4.2.

A few comments regarding the fit parameters. A' gives an estimate for the rest energy of the vesicle in either the initial or final state. For example, for the ($\bar{U} = 0.6$, $\bar{R} = 0.7$) case, 1.911 is the rest energy of the oblate before translocation, as can be seen in the table. In the cases where a shape transition occurs, the magnitude of the energy shift is captured by the B' parameter in the table. C' is a rough indication as to how quickly the state shift occurred, where a large value of C' indicates that the baseline energy level increased quite sharply. Finally, D' and G' give estimates on the size of the free energy cost induced by the barrier interaction.

Additionally, the G' , H' , and I' terms for the ($\bar{U} = 0.8$, $\bar{R} = 0.8$) case has been thrown out since I' was very large and the Gaussian was centered far from the data points. Thus, it does not contribute to the intention of the fit and has been omitted.

Table 4.1: The fitted parameter set of the energy landscape for the $\bar{U} = 0.6$ transition discussed in Section 4.2.1. The oblate to prolate transitional passages use Equation 4.1, while the oblate to oblate atranslational passages use Equation 4.2.

$\bar{U} = 0.6$							
	Ob to Pr			Ob to Ob			
$\bar{R}$	0.7	0.75	0.8	0.85	0.9	0.95	1.0
A'	1.911	1.928	1.933	1.923	1.929	1.933	1.939
B'	0.1108	0.1106	0.1161	-	-	-	-
C'	3.573	3.750	3.825	-	-	-	-
D'	0.3970	0.2411	0.1614	0.1254	0.0833	0.0530	0.0315
E'	3.247	2.976	3.156	3.090	2.874	2.386	2.975
F'	0.2472	0.1615	0.1005	0.3028	0.2669	0.1526	0.0000
G'	0.3167	0.1611	0.1052	-	-	-	-
H'	5.170	6.776	8.402	-	-	-	-
I'	-0.3896	-0.4134	-0.4165	-	-	-	-

Table 4.2: The fitted parameter set of the energy landscape for the $\bar{U} = 0.8$ transition discussed in Section 4.2.2. Due to the $\bar{R} = 0.7$ case not translocating, the fit cannot be applied to the data set in a meaningful way. The oblate to prolate transitional passages use Equation 4.1, while the oblate to oblate atransitional passages use Equation 4.2. Note that the $\bar{R} = 0.8$ case omits the G' , H' , and I' terms. That specific fit produced a dataset with $I' > 1000$, which is very clearly outside the range of the data that spans the range $-1.2 \leq \bar{h} \leq 1.2$. Therefore, the second Gaussian has been thrown out as its parameters don't actually contribute to the goal of the fit, and the single Gaussian does the job well.

$\bar{U} = 0.8$						
	Ob to Pr			Ob to Ob		
$\bar{R}$	0.75	0.8	0.85	0.9	0.95	1.0
A'	1.479	1.478	1.479	1.521	1.524	1.529
B'	0.0498	0.0509	0.0504	-	-	-
C'	-4.717	-3.842	-12.98	-	-	-
D'	0.2725	0.2767	0.2013	0.0945	0.0679	0.0385
E'	4.323	2.594	3.865	3.510	3.531	4.210
F'	0.2532	0.0154	0.0996	0.2947	0.2711	0.2208
G'	0.2217	-	0.0354	-	-	-
H'	4.593	-	10.76	-	-	-
I'	-0.2965	-	-0.4995	-	-	-

4.1.2 External Driving Forces

Throughout the analysis so far, it has been assumed that the members of the lipid bilayer only feel the potential caused by the elastic curvature of the surface. Physically, however, the only effects that would drive the vesicle to pass through the barrier, reaching the visual snapshots from Chapter 3, would be random Brownian forces from the environment. As a result, specific external forces can be induced onto the vesicle to drive it through the pore by modifying the total energy at each given value of $\bar{h}$. From Section 4.1.1, there now exists a bending energy value associated with each position of the barrier, interpolated to cover nearly the entire region from $\bar{h} = -1.2$ to $+1.2$. Combining this elastic potential barrier with additional potentials can provide insight as to how different external forces would have an effect on the passage.

These forces can be applied experimentally in several ways. In practice, two types of external drivers can be imposed on vesicles: a linear pulling potential, experimentally realizable through optical tweezers, and a pressure differential, which can be generated

by controlling flow on either side of the barrier. The most straightforward approach is to trap a bead tethered to a large vesicle and pull it through a pore using optical tweezers. In this setup, the continuous pulling force acts as the driving potential, with the added advantage of precise control over its magnitude. Another method to capture the effects of external drivers is to separate the two regions on either side of the pore with a thin film and use syringe pumps or pressure controllers to establish a pressure differential. This setup allows the role of pressure in translocation to be isolated and systematically varied. The remainder of this section will break down how these two types of external drivers can be modeled within the context of the simulated events from Chapter 3.

Micropipette aspiration and optical tweezers experiments demonstrate that forces as small as 10^{-13} N are sufficient to significantly deform a $1\text{ }\mu\text{m}$ vesicle or to drive it through a constriction [11, 68]. This magnitude corresponds to an energy change comparable to the bending rigidity itself when applied over micrometer length scales, and so is a good target energy scale for a simulated analysis. Similarly, modest pressure differences across a bilayer, on the order of 10^{-2} N/m², have been shown to substantially alter translocation pathways and energy landscapes [32, 34]. These quantitative scales highlight the delicate balance between bending energy and external forcing, providing experimental anchors for the theoretical energy curves analyzed throughout this chapter. The remainder of this section will discuss the method used to apply these effects to the vesicles of Chapter 3, and the way they will be utilized to study vesicle dynamics.

To model these forces and estimate their magnitudes, the first step is to calculate the scale of the effects and express them as convenient dimensionless quantities for numerical analysis in comparison to the elastic energy scale of the vesicle. As was discussed in Section 1.3, the typical lipid bending modulus, κ , of a $1\text{ }\mu\text{m}$ spherical vesicle is approximately $20 k_B T$, a function of the thermal energy of the body [10]. As noted in Section 4.1.1, energy values are normalized by a factor of $8\pi\kappa$, corresponding to the bending energy of a spherical vesicle. Combining this normalization with the $20 k_B T$ modulus, and assuming room temperature, yields an elastic bending energy of approximately $E_b \approx 2.1 \times 10^{-18}$ J for a spherical vesicle, mimicking the stated value from Chapter 1.

The first type of external effect to impose on the vesicles is a linear ‘gravitational’ potential. To do this, one can imagine the vesicle being moved towards the pore under the force of gravity or some other constant gradient potential. For a charged or submerged vesicle, a uniform electric field or hydrostatic pressure field would produce similar results. In this case, a linear potential is enforced that adds to the vesicle’s energy before passing through the pore, and subtracts from it afterwards. For this, the bending energy data can be utilized and combined with a term of the form $-f \times h$, which provides the vesicle with an additional potential energy as a linear function of the barrier position/center

of mass. f denotes the magnitude of a constant gradient force and is given in units of Newtons, N. For negative values of h , this expression is positive and adds energy to the vesicle state that slowly decreases as the vesicle moves towards and through the hole. Similarly, this expression is negative for positive h , dropping the energy associated with the vesicle near the end of the passage. Given that the energy has been reduced to dimensionless parameters, this force can also be simplified in terms of the translocation parameters. Namely, h can be reduced to $\bar{h}$ through Equation 2.4, and f can be reduced to the dimensionless f' , the primary force parameter in the model.

Next, it must be determined what values f' can take in a reasonable setting by seeing what value it takes on under a gravitational field. Assuming the biological vesicles are filled with a water-like solution (having density $\sim 1000 \text{ kg/m}^3$) and are on the order of a $1 \mu\text{m}$ sphere, the gravitational force on the vesicle due to the Earth comes out to about $4.1 \times 10^{-14} \text{ N}$. Across the standard $1 \mu\text{m}$ length scale, this would only result in an energy change of about $4.1 \times 10^{-20} \text{ J}$, not nearly strong enough to noticeably impact the structure of the energy landscape with elastic energy on the order of $2.1 \times 10^{-18} \text{ J}$. Following this line of logic, the dimensionless f' should represent how impactful the energy induced by the force is relative to the bending energy of the vesicle itself. Using $\bar{h} = 1$ as a distance to equate the energy scales over, the corresponding value of f' would just be the quotient of the external induced potential and the standard elastic energy. For the force of gravity discussed, $f' = \frac{4.1 \times 10^{-20} \text{ J}}{2.1 \times 10^{-18} \text{ J}} \approx 0.02$ meaning that the strength of gravity operates at about 2% the strength of the bending energy.

Given this, the values of f' chosen to be analyzed in this work are 0.08 and 0.16, corresponding to forces of size $1.7 \times 10^{-13} \text{ N}$ and $3.4 \times 10^{-13} \text{ N}$, respectively. The exact scale factor between f' and f is shown in the derivation of Equation 4.4. As will be seen when the energy plots are discussed, values of f' around 0.1 give noticeably altered, but still elastically dominant energy landscapes, which is why these values of f' were chosen. As discussed at the beginning of this section, this scale of linear force has also been probed experimentally, further justifying the choices for f' . Namely, in the work of Faizi et al. [68], they manipulate large-scale vesicles using a micropipette and induce linear forces on the $10^{-13} \text{ N} - 10^{-12} \text{ N}$ scale. Additionally, Liu et al. [69] use the method of threading optical tweezers on single cells and perform confidently at forces around 10^{-13} N .

Under the assumption that the vesicle moves slowly through the pore, relative to its relaxation time, this linear $-f' \times \bar{h}$ term can simply be added to each bending energy pulled from the network. The result is that the total energy can be expressed as the dimensionless $\bar{E}_{total}(\bar{h}) = \bar{E}_b(\bar{h}) - f' \times \bar{h}$. A notable side effect of this induced linear force, however, is that it comes with the drawback of having a non-constant energy landscape in the free space on either side of the barrier. This can produce artifacts in the energy data that are

difficult to omit, as will be seen in Section 4.2.

The second type of external effect to add is a term based on the pressure difference on either side of the hole. Treating the environment around the vesicle as a constant temperature would involve an energy term of the form $-P \times V$, where P represents the pressure of the surrounding environment and V represents the volume of the vesicle displacing fluid. This energy term represents the mechanical work required to maintain volume integrity against the environment. To implement this, the vesicle is initially placed behind the barrier to be immersed in a high-pressure fluid, and it feels a large amount of strain while pushing outward against the environment. Once part of the vesicle passes into a lower-pressure fluid on the other side of the pore, the structural integrity of the membrane there is under less stress and is pushed around less by its environment. The vesicle would then translocate by simply being pushed by the high-pressure particles in the initial box more than from the final one. Specifically, a pressure of P_1 can be associated with the environment before the barrier and a pressure of P_2 after it, with the barrier separating the two distinct regions into two constant-pressure chambers. The result, assuming that $P_2 < P_1$, is that the vesicle will be energetically favored to travel from the higher pressure container into the lower one.

In addition, a value can be constructed called the translocated volume, V , that just calculates the volume of the vesicle that has passed into the second container. This is easy to calculate using the phase field model by just integrating the volume field over the region of space beyond the barrier. V always ranges from 0 to U , where $V = 0$ refers to a vesicle completely in front of the barrier in the initial environment, and $V = U$ being one completely contained in the final container. This quantity can also be reduced relative to the total volume to get a measure for the fraction of the vesicle that has passed the barrier. The reduced translocated volume, $\bar{V}$, is defined as follows in Equation 4.3.

$$\bar{V} = \frac{V}{U} \quad (4.3)$$

$\bar{V}$ is a secondary translocation coordinate, alongside $\bar{h}$, and ranges from 0 to 1. As the vesicle passes through, an energy term of the form $-(P_2 - P_1) \times V = -\Delta P \times V$ develops based on the amount of volume that has passed through the pore. Here, ΔP is a constant, as it is being assumed that the two containers are each at a constant pressure. Once again, this term can be added to each energy data point based on the associated V to that $\bar{h}$. To put it in a similar form as the gravitational force, a factor of U can be absorbed into the ΔP term to write the effect as a function of $\bar{V}$. The reduced pressure-differential effect is then equal to $-\Delta P' \times \bar{V}$, where $\bar{V}$ is the reduced translocated volume and the

dimensionless $\Delta P'$ measures the difference of pressure between the boxes. This time, $\Delta P'$ gives the relative energy scale between the natural elastic term and the statistical pressure term, just like f' .

Again, it must be determined what values of reduced pressure difference $\Delta P'$ are relevant to the data at hand. As $\bar{h}$ and $\bar{V}$ are both roughly on the order of 1 across translocation, it applies to state that $\Delta P' = 0.08$ and 0.16 will also correspond to noticeable but not dominant pressure differentials. Following the same calculation, shown in the derivation of Equation 4.5, the values of pressure differential across the pore are $\Delta P = 4.0 \times 10^{-2} \text{ N/m}^2$ and $\Delta P = 8.0 \times 10^{-2} \text{ N/m}^2$, respectively. Additionally, in the 1999 work by Seifert and Berndl [70], they investigated the deformation and motion of vesicles under pressure flow, where the applied pressure was on the order of 10^{-2} N/m^2 . The work of Brochard et al. studies a small vesicle exiting out of a larger one through a pore, driven by pressure differences between the inside and outside of the membrane at similar scales to this work as well [71].

The result is the equation for total reduced energy including a pressure differential $\bar{E}_{total}(\bar{h}) = \bar{E}_b(\bar{h}) - \Delta P' \times \bar{V}$, and so the induced pressure model now nicely parallels the linear force model and implementing them both can be done with minimal effort.

Equations 4.4 and 4.5 show the mentioned derivation of the corresponding dimensionful values of force and pressure in terms of the dimensionless f' and $\Delta P'$. The corresponding dimensionful values for the four reduced driving effects are shown in Table 4.3. From this point forward, the 0.08 values will be referred to as the low force and low pressure driving factors, whereas the 0.16 values will be referred to as the high force and high pressure driving factors, respectively.

$$\begin{aligned}
|f'| &= \frac{|f| \times h}{E_b} \\
|f| &= \frac{|f'| \times E_b}{h} \\
|f| &= \frac{2.1 \times 10^{-18} \text{ J}}{1 \mu\text{m}} \times |f'| \\
|f| &= 2.1 \times 10^{-12} \text{ N} \times |f'|
\end{aligned} \tag{4.4}$$

$$\begin{aligned}
|\Delta P'| &= \frac{|\Delta P| \times V}{E_b} \\
|\Delta P| &= \frac{|\Delta P'| \times E_b}{V} \\
|\Delta P| &= \frac{2.1 \times 10^{-18} \text{ J}}{\frac{4\pi}{3} \times 1 \mu\text{m}^3} \times |\Delta P'| \\
|\Delta P| &= 0.50 \times 10^{-1} \text{ N/m}^2 \times |\Delta P'|
\end{aligned} \tag{4.5}$$

Table 4.3: The conversion table between the dimensionless f' and $\Delta P'$ used in the analysis of this thesis, and the dimensionful f and ΔP following the calculations showed in Equations 4.4 and 4.5. As discussed, these dimensionful values are easily attainable in experiments and have been the subject of a number of past experiments.

Driving Factor	Reduced Value	Dimensionful Value
Low Force	$f' = 0.08$	$f = 1.7 \times 10^{-13} \text{ N}$
High Force	$f' = 0.16$	$f = 3.4 \times 10^{-13} \text{ N}$
Low Pressure	$\Delta P' = 0.08$	$\Delta P = 0.40 \times 10^{-2} \text{ N/m}^2$
High Pressure	$\Delta P' = 0.16$	$\Delta P = 0.80 \times 10^{-2} \text{ N/m}^2$

4.2 Energy Analysis

4.2.1 $\bar{U} = 0.6$ Energy Data

Starting with the $\bar{U} = 0.6$ vesicle, plotting the bending energy for each hole radius, along with the fits from Section 4.1, produces Figure 4.1. As seen in Chapter 3, for the larger holes, there is no shape transition and hence the energy landscape is roughly symmetric about the barrier. Additionally, there is a roughly even spacing between the energy maxima occurring at $\bar{h} = 0.0$. Given the size of the vesicle, they seem to stop interacting with the barrier at a distance of about $\bar{h} = \pm 1.0$ as that is roughly where the curve flattens out.

To produce the error bars for each data point in the shown energy landscapes, the standard deviation for the resultant energy of all three trials is used as a benchmark. The value of the energy in the plot is the mean of the trials, and the size of the error bars is the standard deviation calculated. Calculating the errors like this is very rough and fails to take into account any sampling biases of the grid or network itself, as described in Section 2.5.

The narrower pores cause greater instability in the passage as the simulation becomes much more sensitive to the random fluctuations under the harsher constraints. Primarily, however, the vesicles converge to the higher energy of the prolate branch. The start of the event looks to be similar for all hole sizes, up to some numerical artifacts of the fit itself. In particular, the vesicles all stabilize to the same energy at $\bar{h} = -1.2$ and -1.1 . The transitional passages also appear to flatten out a bit more near the midpoint of the passage. This is somewhat expected as it is around $\bar{h} = -0.4$ where the vesicles go from taking pseudo-oblate forms to taking pseudo-prolate ones, as seen in Figure 3.2. The hole still deforms the vesicle at this point since the pore size is smaller than the free vesicle neck size, but the deviations are small relative to when it was an oblate.

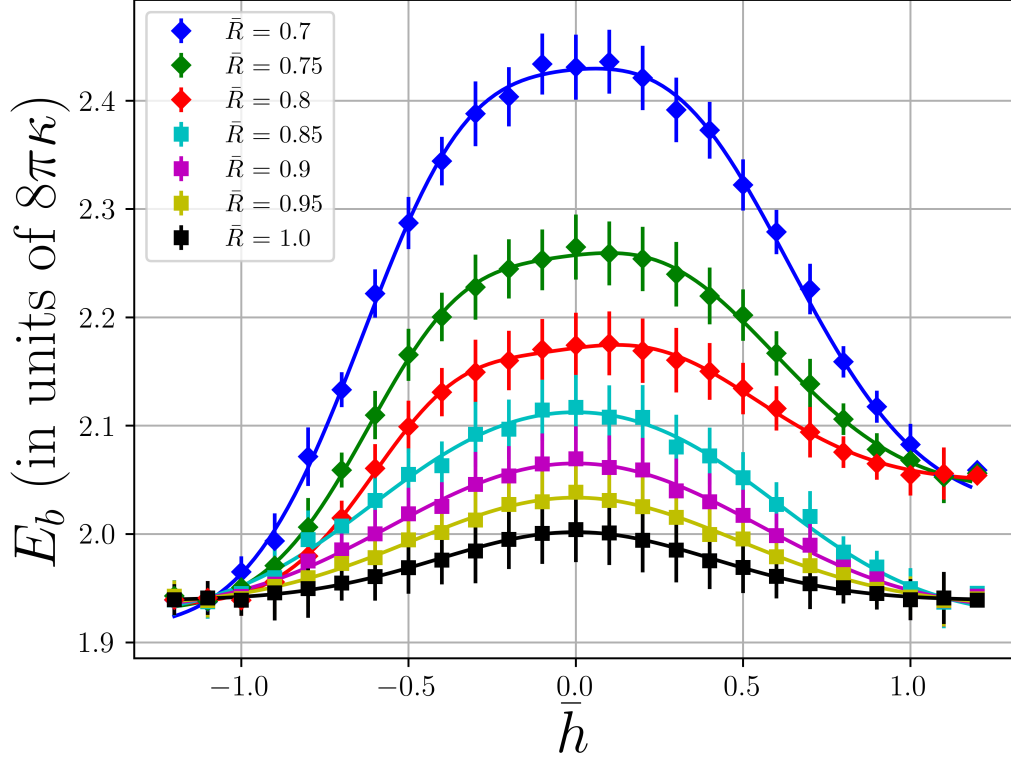


Figure 4.1: $\bar{U} = 0.6$ oblate to oblate and oblate to prolate transitional energies with no induced force or pressure. This is a plot of purely the bending energy as a function of $\bar{h}$. Each node is an extracted value of energy from the corresponding network, whereas the curves are using the fitting models described in Section 4.1.1. As earlier, diamond nodes represent shape transitional passages, while square nodes represent atransitional passages. The error bars are calculated using the standard deviation of the three trials. Two branches of events can be seen in this plot. The three narrower pores stabilize to the higher bending energy of the prolate, while the four wider pores are not strained enough and return to the original oblate once the event is complete.

Another important detail is the relative energy peak of the $\bar{R} = 0.85$ curve, and the final resting energy of the transitional curves. Specifically, the $\bar{R} = 0.85$ vesicle is pushed to a higher peak energy than should otherwise be needed to cross over to the prolate branch. Despite this, the vesicle stays as an oblate and returns to one as the other atransitional vesicles do. One explanation for this is that since the oblate and prolate are stable vesicles, they are local minima of the bending energy. Traversing the landscape to get from one

to the other takes the vesicle through certain non-axisymmetric configurations, which are much more energetically taxing than the simple gap between the two minima. The energy hill between the two valleys is significantly higher than both valleys themselves, and so significantly more deviation is required to get the vesicle over into the other's minimum. Following this logic, it can be stated that, assuming this event pushes the oblate in the direction of the prolate minimum in the energy landscape, the height of the energy hill between them is somewhere between the peaks of the $\bar{R} = 0.85$ and $\bar{R} = 0.8$ curves.

Pulling explicit numbers from the networks, the peak of the $\bar{R} = 0.85$ curve has a reduced energy value of about $E_b/8\pi\kappa = 2.117 \pm 0.031$, whereas the peak of $\bar{R} = 0.8$ curve is at about $E_b/8\pi\kappa = 2.176 \pm 0.027$. As a result, the estimated height of the potential barrier between the oblate and prolate stationary states along this type of deformation lies somewhere between these two values.

Next, the external forces can be added to the energy profile to see how the vesicle's landscape is affected under each. Note again that the value of forces/pressures is dimensionless in this study and is only valid relative to the energy level of the vesicle and each other.

The four plots in Figure 4.2 show the same energy data as above, but with low and high values of force and pressure, respectively. Without any external effects, it is not expected that the vesicle would naturally traverse the barrier, as there really is no energetic incentive to do so. Looking at the plots with induced linear forces, a similar behavior is seen to the free space case from earlier. It is worth noting the angled skew of the plots far from the barrier, where the force causes an artificial energy minimum to appear, where the vesicle should sit snugly partway into the pore. As an example, looking at plot 4.2a and $\bar{R} = 0.7$, with the weaker force, the vesicle should theoretically come to a stop around $\bar{h} = -1.1$. In plot 4.2b, however, the more powerful force allows the vesicle to get closer to the barrier before reaching the artificial minimum at $\bar{h} = -1.0$.

Another worthwhile mention is the energy curves that always slant downward as a result of the force. In these cases, the vesicle should never need to rely on stochastic movement and slide through the pore with minimal resistance. The push exerted by the force is enough to overpower any costs inflicted by the increased bending energy. None of the curves in plot 4.2a is skewed enough to have a downward slope at all points. However, plot 4.2b has two curves ($\bar{R} = 0.95, 1.0$) that should never have time to relax at a specific position, given the potential gradient enforced on them. In a few paragraphs, it will be discussed how to calculate the specific f' required for each curve to exhibit this purely downward trend.

Adding in pressure, as in plots 4.2c and 4.2d, on the other hand, the energies still

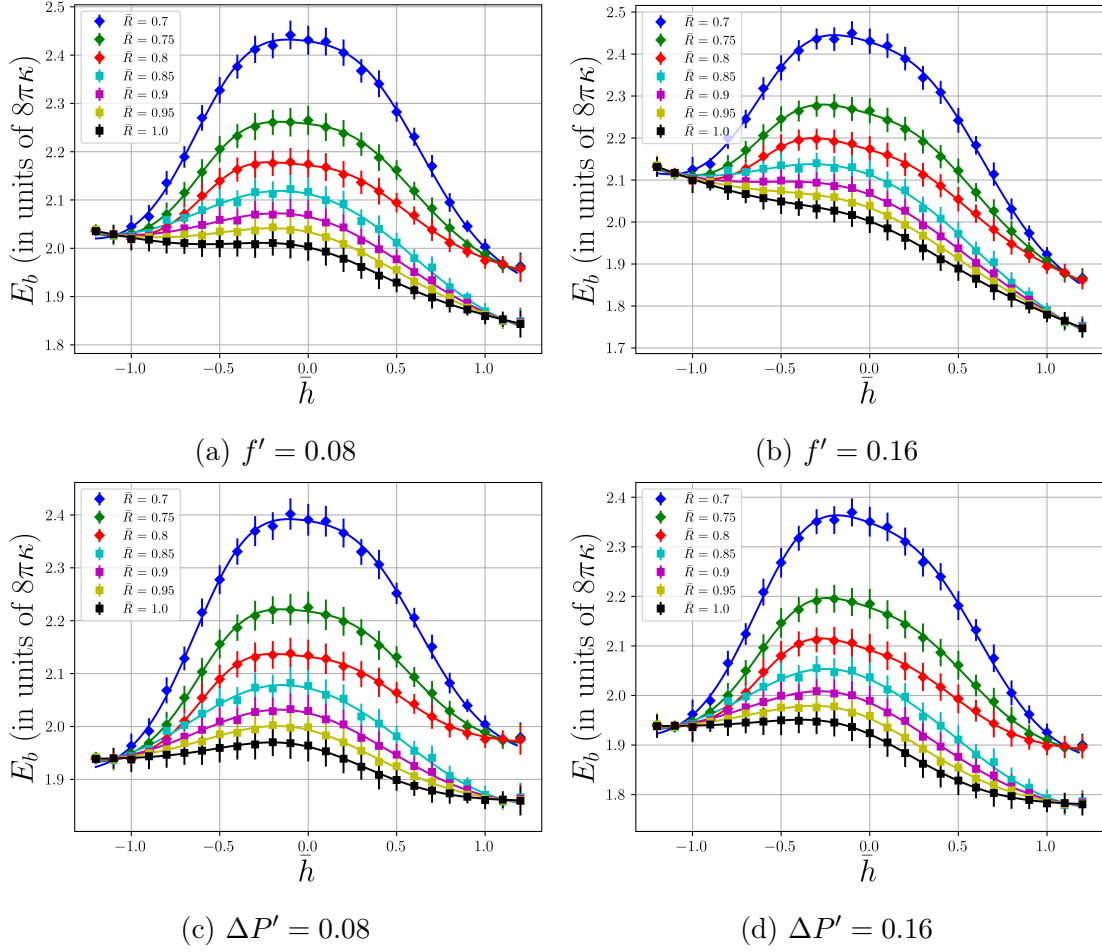


Figure 4.2: $\bar{U} = 0.6$ oblate translocation energies with various values of induced force or pressure.

stabilize out far from the barrier as they enter free space. As discussed in Section 4.1.2, the pressure model calculates the fraction of the vesicle's volume that has passed the pore $\bar{V}$, and reduces the cost proportional to that fraction. This results in no changes to the energy landscape when the vesicle is fully contained in one of the halves, and so the energy shift only varies during the central portions of the passage.

Analyzing the curves in more detail, the weaker pressure in Figure 4.2c exhibits a lot of the same behavior as the unmodified case in Figure 4.1. The most notable point to make is near the peak of the event around $\bar{h} = 0.0$. Mainly for the shape transitional

curves, there is a steadily declining plateau in the energy curve between $\bar{h} = -0.2$ and $\bar{h} = +0.2$, before dropping quite sharply. Looking back at the visualizations in Figure 3.2, it is around $\bar{h} = 0.0 - 0.4$ that the vesicle properly begins to bulge outward around its neck and transitions from oblate to prolate. Adding in the pressure shift shows a bit more clearly where the vesicle is likely undergoing its shape transition and crossing the peak of the potential barrier between the two vesicle states.

Given the form of the energy fit given in Equations 4.1 and 4.2, the derivatives of $\bar{E}_b(\bar{h})$ can be calculated to see when the curve peaks most steeply. This point of maximum slope, denoted by $\bar{h}_s$, represents the point of most resistance for the vesicle as it attempts to pass through the pore. To find this, the second derivative of the energy landscape, $\bar{E}_b''(\bar{h})$, can be calculated analytically using the fit parameters and set equal to 0. $\bar{h}_s$ will be a solution to this and represents a local maximum of the first derivative. Additionally, the first derivative, $\bar{E}_b'(\bar{h})$, can be computed at $\bar{h}_s$ and used to quantify how strong an external force field must be to overcome the wall without hitting any local minima. These results are summarized in Table 4.4.

Note that in this table, the value of force f' needed to completely override the potential barrier is actually just equal to $E_b'(\bar{h}_s)$. This table is confirmation of the claims from earlier in the section, where the hole radii with $\bar{E}_b'(\bar{h}_s) \leq 0.16$ can not withstand the higher force of $f' = 0.16$ and the vesicle smoothly passes through.

Table 4.4: The points of steepest ascent on the calculated energy curves from Section 4.2.1 for the $\bar{U} = 0.6$ case. $\bar{h}_s$ is the barrier position at which the energy climbs most sharply in each curve, and $E_b'(\bar{h}_s)$ is the value of the slope at that point.

$\bar{U} = 0.6$							
	Ob to Pr			Ob to Ob			
$\bar{R}$	0.7	0.75	0.8	0.85	0.9	0.95	1.0
$\bar{h}_s$	-0.633	-0.617	-0.601	-0.608	-0.554	-0.487	-0.410
$\bar{E}_b'(\bar{h}_s)$	0.804	0.554	0.433	0.232	0.165	0.126	0.093

4.2.2 $\bar{U} = 0.8$ Energy Data

Moving to our second case, the energy plot shown in Figure 4.3 shows some of the same tendencies as was the case for $\bar{U} = 0.6$. It is important to note that the overall bending energies are significantly lower in this case as the free space stationary states are much closer to the normalized spheres at $E_b/8\pi\kappa = 1$. The large hole cases produce symmetric

curves as the vesicle simply deviates around its natural minimum and bounces back into place as it passes the barrier. At $\bar{R} = 0.85$ and below, the vesicle pops into the prolate branch and stabilizes into that corresponding energy valley afterwards.

The first point of discussion regarding this plot is that of the stable energy values on either side. As discussed in Section 2.1.1, larger values of $\bar{U}$ favor the prolate over the oblate despite the oblate being metastable. As a result, the stable ground state prolate is at a lower energy, which is shown in the plot with the transitional curves dropping lower than the atransitional ones. This makes the state change into a prolate much more apparent. For example, comparing the $\bar{R} = 0.85$ (cyan) and $\bar{R} = 0.9$ (magenta) curves, the start of the passage is similar for both as their energy increases in tandem. Around $\bar{h} = -0.1$, the cyan curve peaks and begins declining, whereas the magenta curve is still steadily increasing. Then, despite being in the more constrained pore, the now prolate $\bar{R} = 0.85$ vesicle drops to a lower energy than the still oblate $\bar{R} = 0.9$ one around $\bar{h} = +0.4$. From there, the oblate stabilizes back at an energy of about $E_b/8\pi\kappa \approx 1.53$, while the prolate continues to drop until $E_b/8\pi\kappa \approx 1.48$.

An additional qualitative note about this data is that there is much less of a plateau near the midpoint of the passage as opposed to the $\bar{U} = 0.6$ vesicle. In particular, in Figure 4.1, near the energy peak of each curve, the value of energy was relatively constant between barrier positions. Namely, from $\bar{h} = -0.3$ to $\bar{h} = +0.3$, the peak energy attained is held for a much longer set of barrier positions. For the same case in Figure 4.3, there is instead a much narrower peak with the energy changing more rapidly near the midpoint of the event, especially for the transitional passages. Looking at the vesicles in Figure 3.5, a potential reason for this is the specific shape of prolates at $\bar{U} = 0.6$ versus $\bar{U} = 0.8$. $\bar{U} = 0.6$ prolates are more elongated and more eccentric as opposed to at $\bar{U} = 0.8$. As a result, once the transition happens, the $\bar{U} = 0.6$ prolates are better adjusted to the narrow pore and do not feel as much restriction from the barrier itself. In the $\bar{U} = 0.8$ case with the bigger prolate, the pinching of the vesicle continues all the way until the midpoint of the passage, causing them to peak more sharply at $\bar{h} = 0.0$. However, there may be other reasons for this mechanism that could be of interest in future studies.

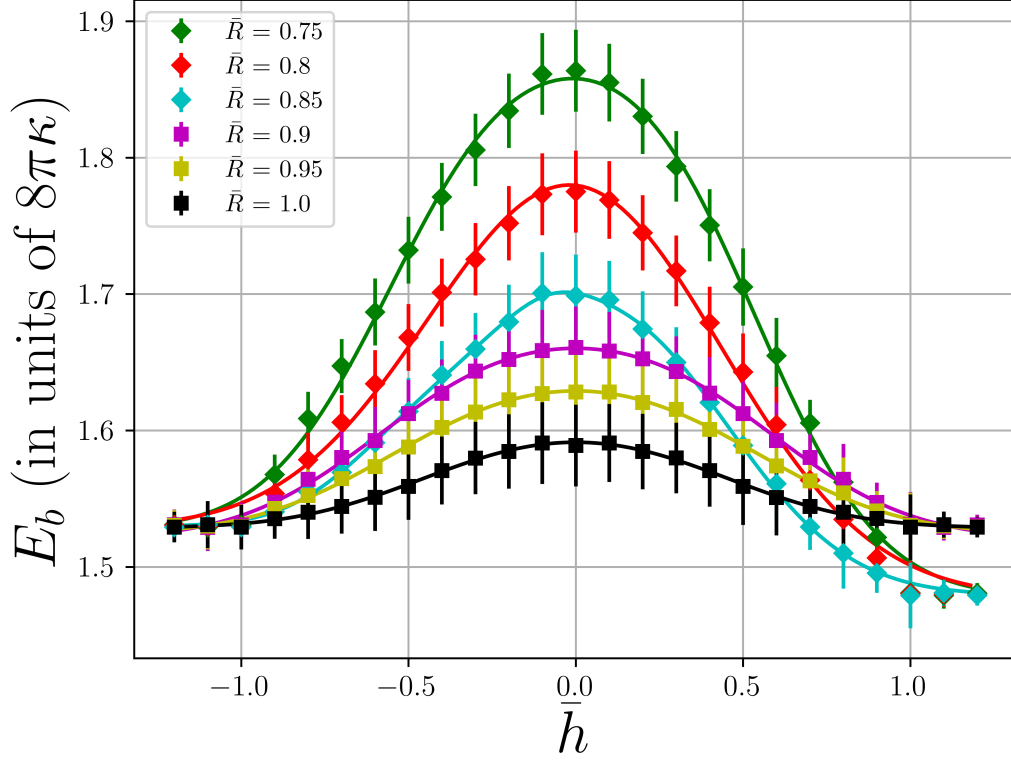


Figure 4.3: $\bar{U} = 0.8$ oblate to oblate and oblate to prolate transitional energies with no induced force or pressure. This is a plot of purely the bending energy as a function of $\bar{h}$. As earlier, diamond nodes represent shape transitional passages, while square nodes represent atransitional passages. The error bars are calculated using the standard deviation of the three trials. The energies are normalized so that a sphere has energy 1, and thus the energies are scaled down by a factor of $8\pi\kappa$. Unlike the $\bar{U} = 0.6$ case, there is a failed event occurring at $\bar{R} = 0.7$. As a result, data was only collected for the initial stages of the event before the model fell apart, and thus is not shown in the figure.

The height of the potential wall between the oblate and prolate branches for this case is also worth briefly mentioning, as was done in Section 4.2.1. Pulling from the networks, the peak of the $\bar{R} = 0.9$ curve has a reduced energy value of about 1.661 ± 0.024 , whereas the peak of the $\bar{R} = 0.85$ curve is at about 1.701 ± 0.026 . As a result, the estimated height of the potential barrier along this type of deformation lies somewhere between these two values at $\bar{U} = 0.8$.

As shown in Figure 4.4, applying force and pressure once again, the same overall

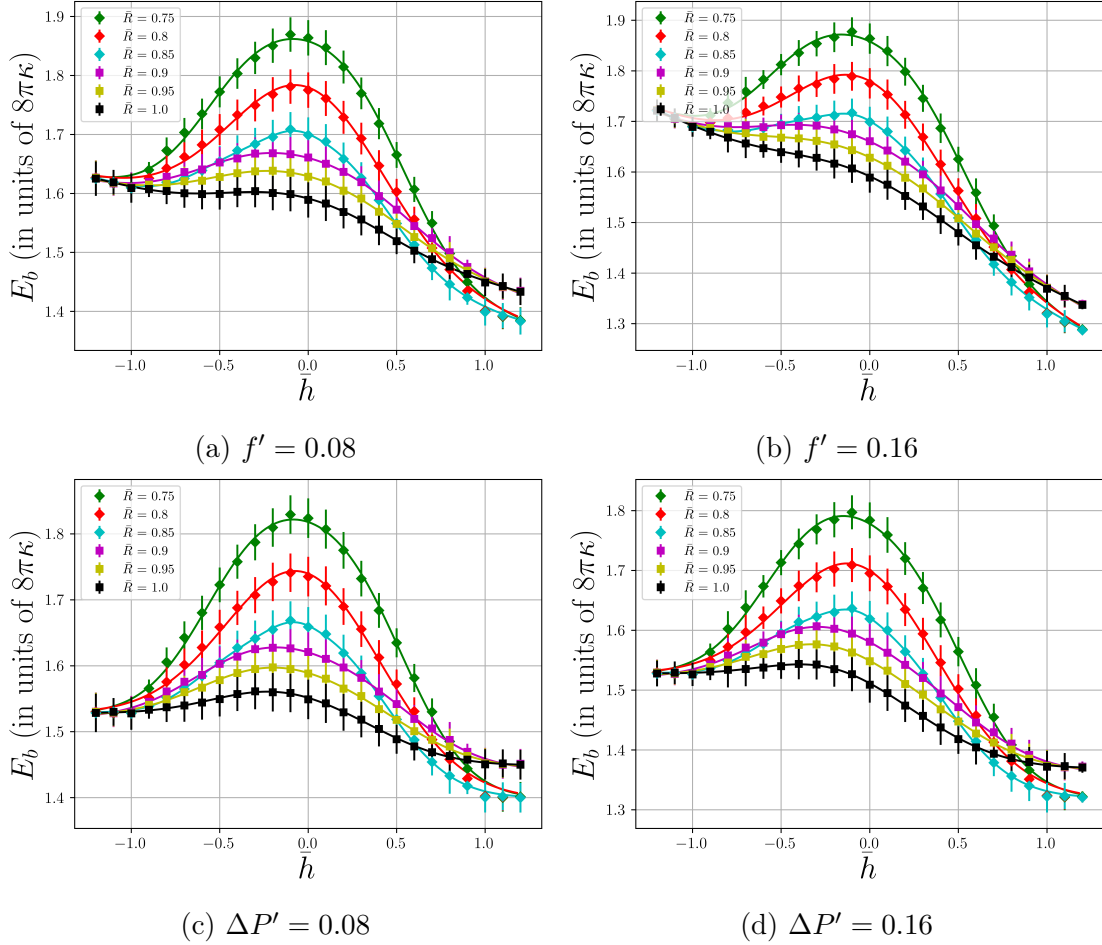


Figure 4.4: $\bar{U} = 0.8$ oblate event energies with various values of induced force or pressure.

behavior is found as in the $\bar{U} = 0.6$ analysis. Plots 4.4a and 4.4b show the translocation with low and high values of force, while plots 4.4c and 4.4d show high values of pressure difference instead. Looking once again for artificial energy minima within the induced force plots, they lie comfortably around the $\bar{h} = -1.1$ to $\bar{h} = -0.9$ region, as was the case at $\bar{U} = 0.6$ as well.

Interestingly, there are the same number of hole radii with strictly decreasing energy landscapes (resulting in a smooth passage). Once again, no hole radii for the weaker force in plot 4.4a, but even in plot 4.4b with the stronger force, only the $\bar{R} = 0.95$ and $\bar{R} = 1.0$ cases avoid an energy barrier entirely. Additionally, given the fit parameters of each curve,

the values of f' that would give it a smooth passage can be found by simply finding the maximum value of the derivative of $E_b(\bar{h})$, as done in the previous section. These values are calculated and shown in Table 4.5.

Table 4.5: The points of steepest ascent on the calculated energy curves from Section 4.2.2 for the $\bar{U} = 0.8$ case. $\bar{h}_s$ is the barrier position at which the energy climbs most sharply in each curve, and $E'_b(\bar{h}_s)$ is the value of the slope at that point.

$\bar{U} = 0.8$						
	Ob to Pr			Ob to Ob		
$\bar{R}$	0.75	0.8	0.85	0.9	0.95	1.0
$\bar{h}_s$	-0.532	-0.406	-0.073	-0.589	-0.548	-0.458
$E'_b(\bar{h}_s)$	0.517	0.410	0.325	0.182	0.138	0.092

4.3 Time Formalism

The translocation time is defined to be the characteristic time scale across which the vesicle goes from one side of the barrier to the other. Intuitively, it is expected that the narrower pores would inhibit the progress of the vesicle to a greater extent and should thus be associated with longer translocation times. The translocation time, τ , will be modeled by treating the vesicle's movement as a diffusion under the influence of random forces from the encompassing fluid, as in Brownian motion [72, 73]. The primary tool to model this follows the Fokker-Planck (FP) method of diffusion, letting the vesicle act as a point particle occupying a 1D free energy landscape, where the interaction with the pore can be modeled as a potential barrier within the landscape [36]. In doing this, the vesicle is assumed to be diffusing through the energy landscape stochastically with a probabilistic incentive to travel towards energy minima.

Before deriving the FP formula for the mean first passage time, it is important to mention other theoretical efforts in estimating the translocation time. One approach by Sung and Park to estimating translocation times of polymers through a pore relies on adapting Kramer's escape theory to soft matter systems [74]. In this framework, the vesicle's free energy landscape is mapped, and the translocation time is identified with the mean first passage time across an effective barrier. Kramers formula connects the curvature of the potential well, barrier curvature, and free energy barrier height to produce a statistical estimate for the mean first passage time. This approach has been applied in polymer and vesicle systems to capture scaling laws for translocation rates and timescales.

For instance, Sung and Park first adapted Kramers’ method to polymers, which then inspired similar treatments in vesicle dynamics as shown in the work of Linke et al. [75]. Kramers’ escape theory specifically measures the time taken for a system to cross from one metastable state to another under thermal fluctuations, in the same way as Fokker-Planck. The key difference is that the estimated time was dependent on the curvatures and features of the potential barrier, like the mentioned curvatures above, which assumed symmetries existing within its shape. While this method would work for the atransitional oblate to oblate passages, it would not be realizable for the transitional ones.

In addition to these detailed stochastic approaches, scaling theory provides simplified predictions for translocation times by relating driving forces, vesicle size, and pore geometry. These models often also draw analogies with polymer scaling theories and use dimensional analysis to predict translocation time scaling exponents. For vesicles, the balance of bending energy and entropic penalties leads to scaling predictions that can be tested numerically or experimentally. Work by Gompper and Kroll exemplifies this strategy, providing coarse-grained models for vesicle dynamics under confinement and flow that are adaptable to translocation problems [28]. A primary issue with this approach, with regard to shape transitional vesicle translocation, is assuming symmetry in the potential barrier. The dimensional analysis provided does not easily accommodate asymmetries in the data, meaning the corresponding results fail to apply.

As a result of needing to tackle the asymmetry in the barrier, the approach taken in this thesis must be more general and independent of the landscape’s structure. Following in the work of Shojaei and Muthukumar [33], the mean first passage time of this diffusion is directly obtained from the 1D Backward FP equation. This equation is a corollary of the widely encompassing FP theory for stochastic processes. The purpose of this formalism is to probabilistically model the evolution of a particle under the effects of random forces within the context of an energy landscape. In particular, Sections 3.6 and 5.2.7c in the textbook *Handbook of Stochastic Methods for Physics, Chemistry and the Natural Sciences* are the source of the following motivation for the mean first passage time [76]. This textbook also provides additional background on the fundamentals behind the original FP equation and the backward implementation used in our study.

For our purposes, the Forward Fokker-Planck equation will be taken as a starting point, and its limits on the translocation problem will be discussed later in this section. To derive the mean first passage time, it is first assumed that a particle is at an initial position, x_i , at initial time, t_i , in the free energy landscape. $P(x, t)$ is then the probability density of the particle’s current location, x , at current time t . Note that x in this context is different from the x in the translocation problem, and refers purely to the particle’s location within the FP formalism. This x will be translated into a translocation coordinate following the

derivation of the translocation time formula. The Forward Fokker-Planck equation states that $P(x, t)$ evolves as shown in Equation 4.6.

$$\frac{\partial}{\partial t}P(x, t) = \frac{\partial}{\partial x}[E'(x)P(x, t)] + D\frac{\partial^2}{\partial x^2}P(x, t) \quad (4.6)$$

$E'(x)$ is the x -derivative of the traversed energy landscape, and D is the diffusion constant given in units m^2/s . This parameter D gives the effective diffusion rate of the particle's coordinate x and is a function of both the scale and specifics of the problem. Note that despite similar notation, this value is not related to the barrier width, D , in translocation.

Through a derivation of the Backward FP equation that can be found in Section 5.2.7a of the mentioned textbook, the mean first passage time of the particle to reach position $x > x_i$ from x_i while subject to energy landscape $E(x)$ will be defined as $\tau(x)$, satisfying Equation 4.7.

$$1 = D \frac{1}{P_s(x)} \frac{d}{dx} \left[P_s(x) \frac{d\tau}{dx} \right] \quad (4.7)$$

Here, $P_s(x)$ is a solution to the stationary time-independent form of Equation 4.6. Given that D is a constant in this diffusive process, Equation 4.6 can be directly integrated under the assumption that $P(x, t) = P_s(x)$ is stationary. The result is that $P_s(x) = Ne^{\frac{-E(x)}{D}}$ for a normalizing parameter N . Substituting this expression into Equation 4.7 and rearranging provides a formula for $\tau(x)$, shown in the derivation of 4.8.

Let x_f be the ending location to which the mean first passage time is calculated from starting position x_i , with $\tau(x_f) \equiv \tau$. Equation 4.7 calculates the probability that the particle has remained on the interval from $-\infty$ up to x_f , from the starting position at x_i , by a given time τ under the diffusion mechanism from Equation 4.6. The probability that it has remained in the interval $(-\infty, x_f)$ by time τ , then becomes the probability that it has not yet reached x_f by time τ . As τ increases, the probability that the vesicle ever reaches x_f steadily increases until it reaches the mean value of $\frac{1}{2}$. The τ in which this happens is what is calculated in Equation 4.8, and is considered the mean first passage time of the process.

$$\begin{aligned}
DN e^{\frac{E(x)}{D}} \frac{d}{dx} \left[\frac{1}{N} e^{\frac{-E(x)}{D}} \frac{d\tau}{dx} \right] &= 1 \\
\frac{d}{dx} \left[e^{\frac{-E(x)}{D}} \frac{d\tau}{dx} \right] &= \frac{1}{D} e^{\frac{-E(x)}{D}} \\
e^{\frac{-E(x)}{D}} \frac{d\tau}{dx} &= \frac{1}{D} \int_{x_i}^x dx_1 e^{\frac{-E(x_1)}{D}} \\
\frac{d\tau}{dx} &= \frac{1}{D} e^{\frac{E(x)}{D}} \int_{x_i}^x dx_1 e^{\frac{-E(x_1)}{D}} \\
\tau(x) &= \frac{1}{D} \int_{x_i}^x dx_2 e^{\frac{E(x_2)}{D}} \int_{x_0}^{x_2} dx_1 e^{\frac{-E(x_1)}{D}} \\
\tau(x) &= \frac{1}{D} \int_{x_i}^x dx_2 \int_{x_i}^{x_2} dx_1 e^{\frac{E(x_2)-E(x_1)}{D}} \tag{4.8}
\end{aligned}$$

Equation 4.8 at the end here is the mean first passage time equation employed on our vesicle's fitted energy landscape. This formula has three main assumptions built into it within the context of vesicle translocation. First, this derivation hinges on the object at x being a point particle traveling in 1D. The vesicles produced by our network-based approach in Sections 3.4 and 3.5 are very clearly not point particles, bringing the evaluation into question. However, given that our model mainly monitors the vesicle's CM relative to the barrier position, $\bar{h}$, it is reasonable to assert that the CM behaves as a particle interacting with an energy barrier spawned by the vesicle's interaction with the pore. This assumption indirectly neglects the dynamics of the body, but since the vesicle's deformations are captured within the elastic energy landscape, the assumption should hold.

Second, the vesicle is locked along a straight line through the pore. As a result, the energy landscape is collapsed down into one dimension, simulated in our model, and discards any paths where the center of mass is not aligned with the axis of the hole. This forces the vesicle to move along one dimension and along a certain trajectory in that direction. This is once again necessary as a consequence of the center of mass constraint used in our model, since our simulated vesicles will only have CMs that lie on the axis of translocation. However, it could be imagined that the vesicle may find a faster path through the energy landscape that moves the CM off the translocation axis. As the problem is fundamentally 3-dimensional, this locking to the axis is indeed a non-trivial decision to consider.

The third assumption is that the vesicle translocation time occurs on a much longer timescale than the vesicle's relaxation time. This is an assumption in our initial quasi-

static modeling of the problem from Section 3.2 as well, but is especially relevant with the diffusive formalism employed here. To clarify, it is being assumed that the vesicle has enough time to reach its stable state at each snapshot before progressing to the next one. This assumption is not anything new, given the way we’ve generated the snapshots, maximally relaxing the vesicle at a certain moment in the translocation. As mentioned in Section 1.2, the Helfrich model’s volume constraint assumes that the vesicle’s relaxation occurs on a much shorter time scale than the passage of fluid in and out of the membrane. On the longer time scales of stochastic FP processes, this constraint necessarily becomes less strict. This is the primary concern with the time scales modeled by Equation 4.8, as the vesicle’s volume can undergo small fluctuations on long enough timescales. Nonetheless, Equation 4.8 can take in the acquired energy data and translocation coordinates to get a measure for the mean first passage time across the barrier.

$$\tau = \tau_0 \int_{\bar{h}_i}^{\bar{h}_f} d\bar{h}_2 \int_{\bar{h}_i}^{\bar{h}_2} d\bar{h}_1 e^{\frac{E(\bar{h}_2) - E(\bar{h}_1)}{k_B T}} \quad (4.9)$$

Equation 4.9 is the form of Equation 4.8 that will be implemented to produce the mean first passage time of each event. Here, $\bar{h}_i$ and $\bar{h}_f$ are the initial and final positions of the barrier. $E(\bar{h})$ is the effective fitted energy curve over which the translocation time is processed. $k_B T$ is the typical statistical energy scale factor, which takes the place of the diffusion coefficient D in the exponents, and will be tackled in the next paragraph. As before, τ is a measure of the mean first passage time between the points in space. Additionally, due to the choice of translocation coordinate being the dimensionless $\bar{h}$ and reduced according to the vesicle’s area, the factors of length have been absorbed into the $\frac{1}{D}$ coefficient out in front to produce the value τ_0 . τ_0 sets the fundamental time scale for the stochastic process of wrapping and is determined by nonuniversal features of the membrane, such as the relaxation of the small lipids and other microscopic details of the wrapping, and itself is proportional to $\frac{1}{D}$. Quantifying an actual value for τ_0 is beyond the scope of this project, as the phase field model is ill-equipped to describe the lipid-scale interactions that produce a value for this number. For this analysis, the translocation time will be given in units of τ_0^{-1} and the results treated purely relative to each other.

From the classic Arrhenius formula of chemical reaction theory [77], which holds for governing interactions on the small μm and nm scales of lipid bilayer membranes, it is stated that $D \approx k_B T$. Here, k_B is the Boltzmann constant and T is the absolute temperature of the interaction. This value for the diffusion constant D is the standard for membrane mechanics and is effectively a reformulation of the Einstein relation for Brownian motion $D = \frac{k_B T}{\zeta}$ applied to lipid interactions [78]. There is an additional

factor, ζ , in this expression regarding the effective friction coefficient, given in units of kg/s, between the membrane and surrounding medium, but for vesicles, it is often absorbed into lipid interactions of D leaving us with the purely statistical $k_B T$ [20]. The integrand of the double integral in Equation 4.9 then becomes $e^{\frac{\Delta E}{k_B T}}$, which predicts that the stochastic interaction time scale has a very characteristic dependence on temperature. This Boltzmann factor represents the probability that the energy will exceed that of the barrier when the system is in thermal equilibrium. Thus, it is only when that probability is satisfied that the vesicle can step forward towards the barrier and progress towards the other side of the pore. In all of the following time calculations, the fairly standard value of $\kappa = 20 k_B T$ has been selected, in agreement with the chosen values from previous Sections.

In short, Equation 4.9 looks at ordered pairs of points $(\bar{h}_1, \bar{h}_2)$ between the starting and ending locations $(\bar{h}_i, \bar{h}_f)$, on the energy landscape and quantifies the associated diffusion cost for that pair using the Boltzmann factor. Integrating over these pairs attempts to calculate the average time for the vesicle to travel stochastically through the pore. Given the method used to generate the energy landscape, the point $\bar{h}_i = -1.2$ and $\bar{h}_f = +1.2$ act as the initial and final positions of the vesicle, respectively. Under the stated assumptions, Equation 4.9 calculates the mean translocation time under the effect of random forces on the vesicle.

4.4 Time Analysis

4.4.1 $\bar{U} = 0.6$ Time Data

Figure 4.5 shows the derived first passage times versus hole radius for the unmodified landscape following the method from Section 4.3. Each data point corresponds to a single translocation event, and is calculated using evenly spaced points on the fitted energy curve between $\bar{h} = -1.2$ and 1.2 . The fitted curve was sampled 240 times, with a numerical spacing of $d\bar{h} = 0.01$ between successive samples. The error bars are once again calculated as a standard deviation of the time obtained from the three different trials. Finally, $\kappa = 20k_B T$ is the bending rigidity used in all shown time plots in this thesis.

The decaying exponential trend is expected, given the stochastic nature of the motion. However, the scale of the various passage times is quite far removed from each other due to the sensitivity of the Boltzmann factor in Equation 4.9. To get a better grasp of the behavior, the data will be made singly and doubly logarithmic to develop some sort of relation between $\bar{R}$ and τ .

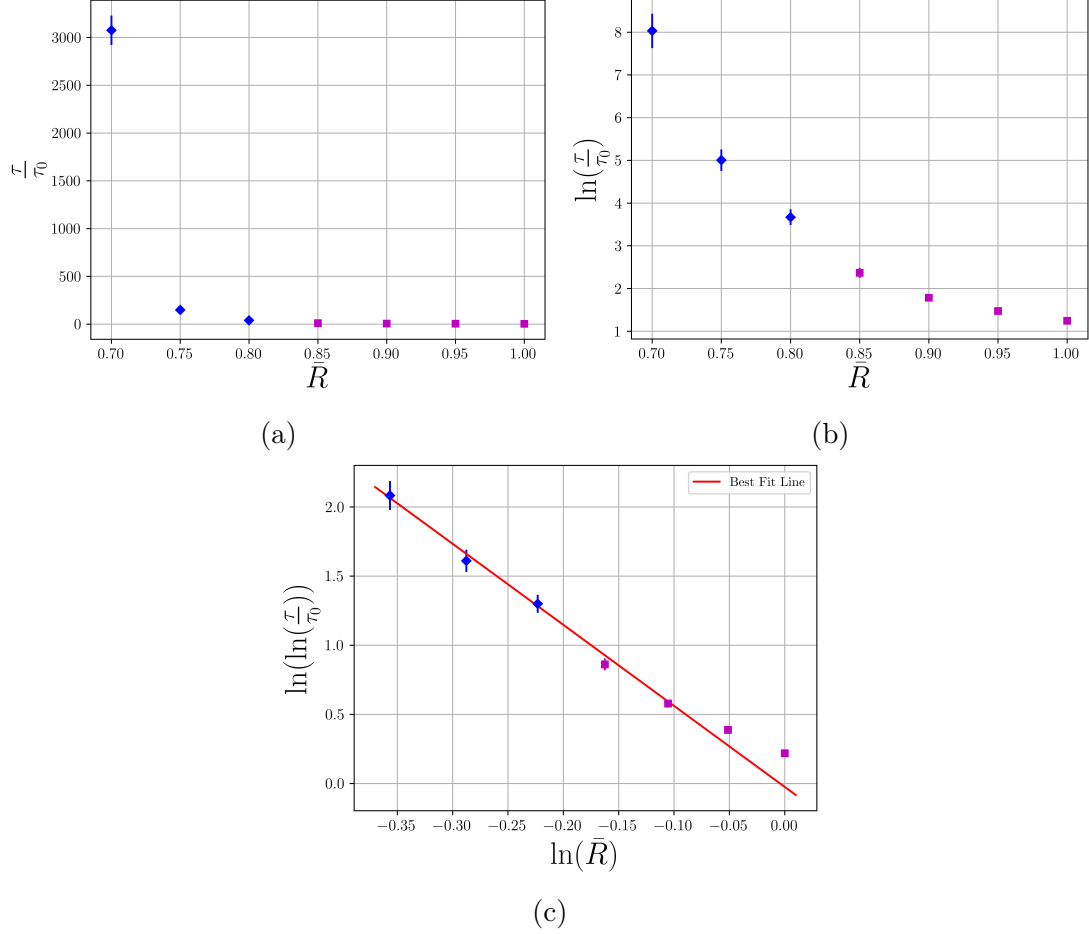


Figure 4.5: $\bar{U} = 0.6$ oblate Fokker-Planck first passage times calculated based on the energy curves from Figure 4.1 with no driving forces/pressures. Each time is given in terms of the scaling factor τ_0 in front of Expression 4.9. Error bars are calculated as an average of the three trial runs as in Section 4.2. Plot (a) shows the raw values for the time calculation plotting τ/τ_0 versus $\bar{R}$, while plot (b) is a semi-log plot graphing $\ln(\tau/\tau_0)$ versus $\bar{R}$. Plot (c) shows $\ln(\ln(\tau/\tau_0))$ versus $\ln(\bar{R})$ instead, as that plot gives us a roughly straight line to extract a fit from. Again, as before, magenta square data points are the atransitional oblate to oblate passages, while blue diamonds are transitional oblate to prolate passages. The red line in plot (c) is the best fit line according to the relation in Equation 4.10.

Looking at the plots in Figure 4.5, the range of times spans about three orders of magnitude between the narrowest and widest pores. Additionally, plot 4.5b shows that $\tau(\bar{R})$ as a function drops even more rapidly than an exponential, so the relationship between the two is incredibly sensitive. Finally, looking at plot 4.5c, a straight line is seen forming between the two variables. Note that given the extreme scaling of the axes, a straight line here refers to a relationship of the form in Equation 4.10. In fact, in the work by Muthukumar et al., their analytical approach to the problem found time dependence to also be of this form with $C_2 \approx -2$ [33]. The red line in this plot is the best-fit line with this relation in mind.

$$\tau = e^{C_1 \bar{R}^{C_2}} \quad (4.10)$$

Attempting a linear fit with the data in Figure 4.5c gives us the rough values $C_1 = 0.97$ and $C_2 = -5.86$. However, this depth of analysis is very ambitious given the number of assumptions present to arrive at this point. As a result, this quantitative analysis is likely unreliable and is more to demonstrate the incredibly sensitive relationship between translocation time and pore radius. Despite this, our model would suggest that for the pure bending energy, the relationship between τ and $\bar{R}$ takes on the form shown in Equation 4.10 for unknown values C_1 and C_2 .

Moving on, the effects of induced forces/pressures on the mean translocation times are also important to consider. Figure 4.6 shows the singly log-scaled time plots with the external forces and pressures applied.

From the perspective of translocation time, the skew caused by a linear force makes the time calculation more sensitive to the chosen start and endpoints. Choosing an endpoint immediately before/after the pore versus 0.5 units further out can cause a huge shift in the time scale of the process. This is mainly because the time scale of passage is being modeled diffusively, heavily penalizing the vesicle for needing to travel to a further point along its random walk. The pressure model, on the other hand, has the energy curve flatten at each end, due to the total energy remaining unchanged while $\bar{V}$ is constant. This likely makes the pressure calculation a better representation of the physical time scale of the passage.

All these time plots look quite similar to each other in terms of shape, so the largest point of analysis between them is that of scale. Across the board, the 4.6a and 4.6b plots (which are the induced force plots) show lower times in general. Now, it should be noted that the force value $f' = 0.08$ and the pressure value $\Delta P' = 0.08$ have no physical reason to really be comparable to each other. The only point that can be mentioned when comparing them is at specific moments, using the fact that the different translocation

coordinates agree predictably at certain snapshots. For example, at $\bar{h} = -1.0$, the reduced translocated volume has value $\bar{V} \approx 0$, and at $\bar{h} = +1.0$, $\bar{V} \approx 1$. These chosen points are roughly before and after the main events of translocation, and so the effective magnitudes of the force and pressure models are quite similar in this instance, up to a factor of 2.

Comparing the effects of no force to low force to high force, there is a drop in time by a factor of about 5 going from no force to low force, and another drop by about 4.5 going from low force to high force. Note that the axes are base- e logarithmic, so this factor is calculated by taking e to the power of the vertical difference between the data points on the log plot. Additionally, there is something else of note in these plots. In Figure 4.6b, the log-scaled time actually goes negative. This means that the first passage time integral in Equation 4.9 is actually less than τ_0 , the time scale factor in front. Thus, for those values of $\bar{R}$, the effect of the force is enough to push the vesicle through the barrier faster than it would travel through free space. This is actually the same finding as when the fit parameters were analyzed with respect to $\bar{E}(\bar{h}_s)$, where it is found that the high force case never hits an energy maximum during the passage for $\bar{R} = 0.95, 1.0$. This time plot shows an alternative perspective on the same phenomenon since the vesicle does not feel the presence of the energy barrier in a way that properly slows down the passage.

Finally, the same discussion can be performed with respect to pressure. In this case, a similar dependence is seen on the value of the pressure difference $\Delta P'$. A drop of a factor of about 2 is seen going from no pressure to low pressure. From low $\Delta P'$, in Figure 4.6c, to high $\Delta P'$, in Figure 4.6d, there is another drop in the time by a factor of about 1.8. Another important point is that the vesicle always hits a potential barrier at the pore in this case, unlike when analyzing force. Since the pressure only starts discounting the energy once it starts passing through the pore, the vesicle has to interact with the barrier before $\bar{V}$ can change simply due to the size of the vesicle. As a result, no data points go below 0 on the pressure-time plots, as the threshold for complete negation of the barrier wall is much higher.

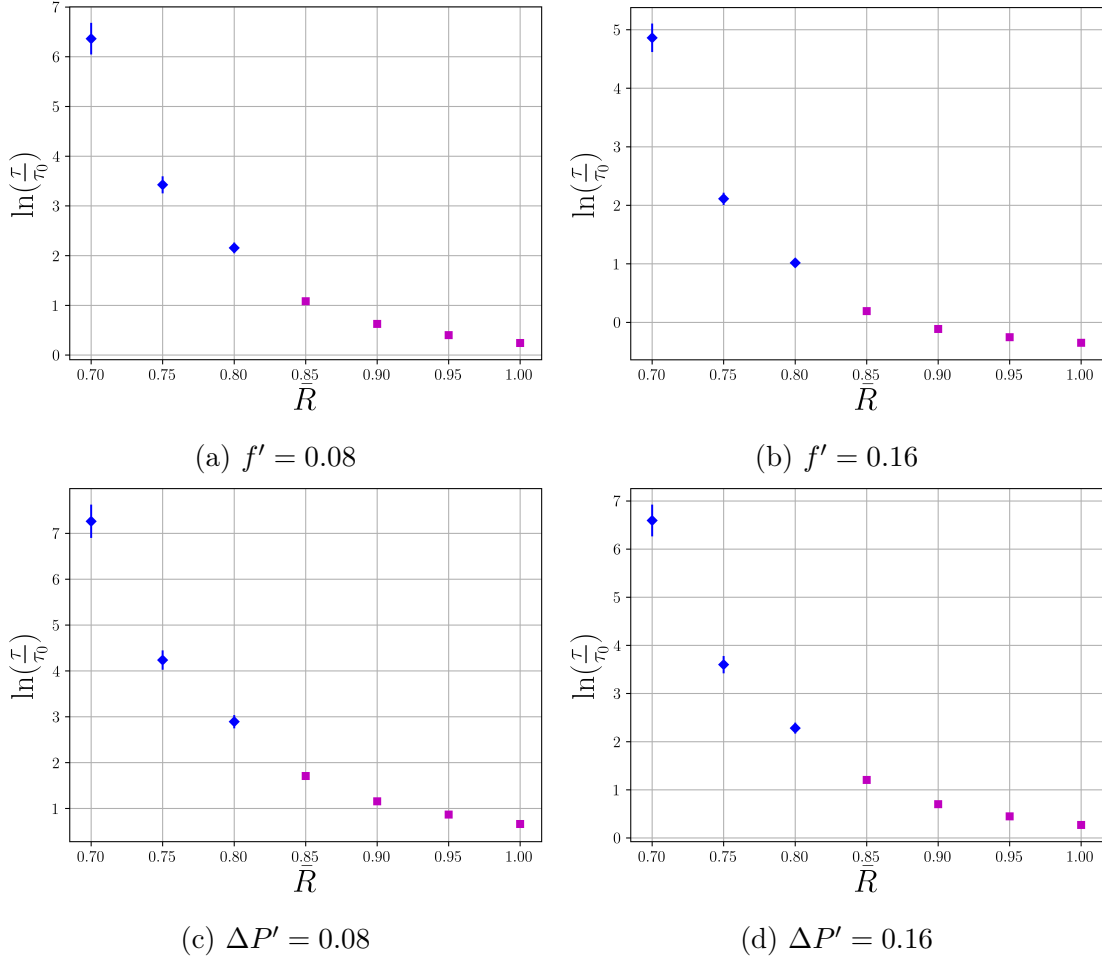


Figure 4.6: $\bar{U} = 0.6$ Fokker-Planck first passage times with various values of induced force or pressure. All plots are singly log scaled and display $\ln(\frac{\tau}{\tau_0})$ versus $\bar{R}$.

4.4.2 $\bar{U} = 0.8$ Time Data

Figure 4.7 shows the derived first passage times versus hole radius for the unmodified landscape. Once again, the data will be made singly and doubly logarithmic to develop a relation between $\bar{R}$ and τ .

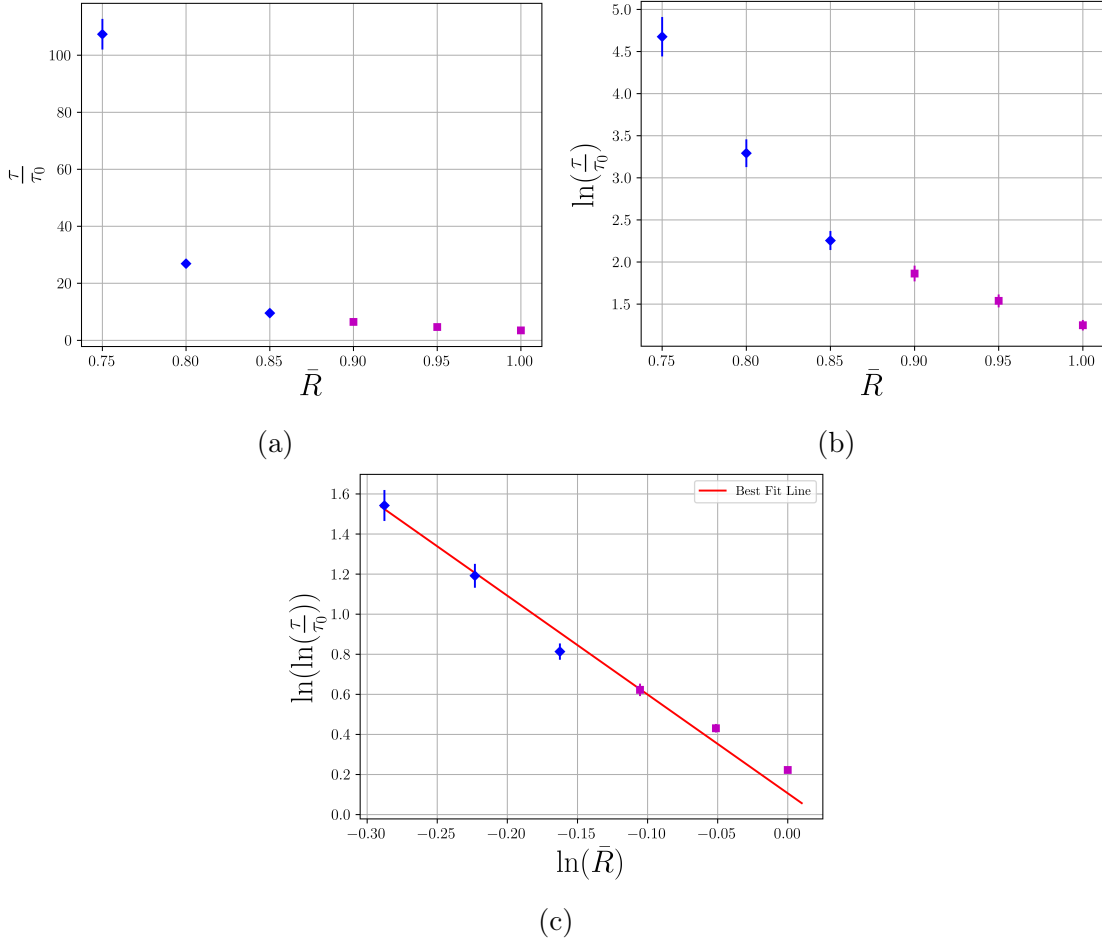


Figure 4.7: $\bar{U} = 0.8$ oblate Fokker-Planck first passage times calculated based on the energy curves from Figure 4.3 with no driving forces/pressures. Each time is given in terms of the scaling factor τ_0 in front of Equation 4.9. Plot (a) shows the raw values for the time calculation plotting τ/τ_0 versus $\bar{R}$, while plot (b) is a semi-log plot graphing $\ln(\tau/\tau_0)$ versus $\bar{R}$. Plot (c) shows $\ln(\ln(\tau/\tau_0))$ versus $\ln(\bar{R})$ instead, as that plot gives us a roughly straight line to extract a fit from. As before, magenta square data points are the atransitional oblate to oblate passages, while blue diamonds are transitional oblate to prolate passages. The red line in plot (c) is the best fit line according to the relation in Equation 4.10.

Looking at the plots in Figure 4.7, the range of times spans about two orders of magnitude between the narrowest and widest pores. Note that the smaller range is

primarily because $\bar{R} = 0.7$ was omitted this time around due to the passage failing at that step. Commenting on plot 4.7c, despite looking quite linear as was the case in Section 4.4.1, the blue and magenta points do not perfectly line up with each other. As a result, it seems that the dependence of τ on $\bar{R}$ is subtly dependent on whether the passage is transitional or not.

Attempting a linear fit with all six data points in Figure 4.7c gives us the rough values $C_1 = 1.11$ and $C_2 = -4.93$. If it were chosen to isolate the three blue oblate to prolate points in the fit, the resulting parameters would be $C_1 = 0.904$ and $C_2 = -5.72$. Finally, for the magenta oblate to oblate times in isolation, it is found that $C_1 = 1.258$ and $C_2 = -3.76$. The high unreliability of this analysis should be noted, as this fit is operating with only three data points, with data that is already a product of the many assumptions built into the Fokker-Planck diffusion mechanism. Table 4.6 shows all of the sets of these $\tau(\bar{R})$ fit parameters for both the $\bar{U} = 0.6$ and $\bar{U} = 0.8$ cases.

Table 4.6: Fit parameters for $\tau(\bar{R})$ using the first passage times obtained from Equation 4.9 for the passages with no induced forces/pressures. Fit is done separately for each size of vesicle and for each class of translocation. Ob refers to oblate vesicles, and Pr refers to prolate vesicles.

$\bar{U} = 0.6$ Fit Parameters for $\tau(\bar{R})$			
Parameters	All Hole Radii	Only Ob to Pr	Only Ob to Ob
C_1	0.976	0.891	1.203
C_2	-5.86	-6.14	-4.08
$\bar{U} = 0.8$ Fit Parameters for $\tau(\bar{R})$			
Parameters	All Hole Radii	Only Ob to Pr	Only Ob to Ob
C_1	1.112	0.904	1.258
C_2	-4.93	-5.72	-3.76

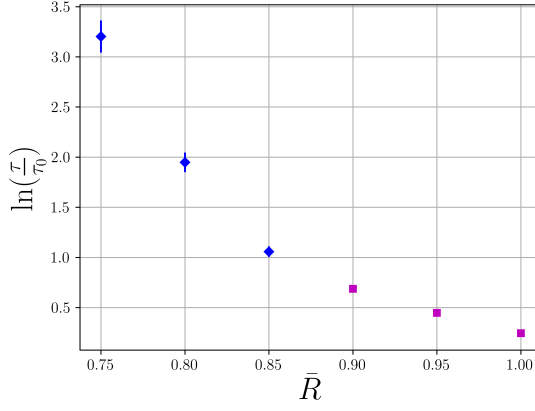
Looking at these fit parameters in Table 4.6, there are some interesting trends to note. First, all C_1 parameters are roughly 1 and so the dependence on $\bar{R}$ primarily comes from C_2 , which is the power of $\bar{R}$ in the exponent. This is likely a consequence of the normalization by τ_0 , as the ultra-wide pore behavior should look indistinguishable from free space movement, giving a pseudo-boundary condition on the translocation time values. For the atransitional oblate to oblate passages, a weaker dependence on $\bar{R}$ is seen as $C_2 \approx -4$ for both sizes of vesicle. Additionally, the transitional oblate to prolate passages are more sensitive with $C_2 \approx -6$ instead. It is also important to note that at $\bar{U} = 0.6$, the transitional energies increased throughout the passage since a prolate is a higher energy state than an oblate at that size. On the other hand, prolates are more stable than oblates at $\bar{U} = 0.8$. Despite

this, both sets of transitional passage times exhibit roughly the same behavior reflected in the value of $C_2 \approx -6$. This suggests that the size and shape of the bending energy barrier between oblates and prolates is quite similar even at different values of $\bar{U}$.

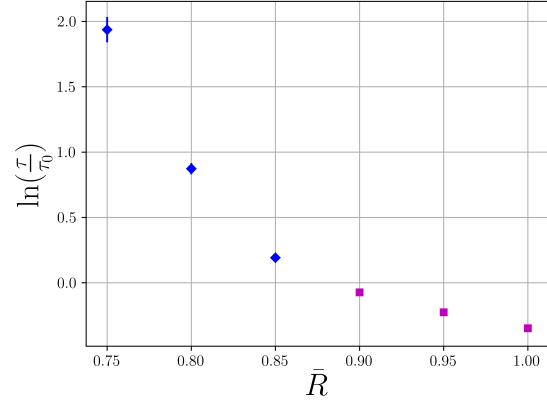
The effects of induced forces/pressures on the mean first passage time for the $\bar{U} = 0.8$ cases can be seen in Table 4.8. Overall, the results are similar to those of the $\bar{U} = 0.6$ cases, with the exception that the magnitudes are once again lower due to the highly strenuous $\bar{R} = 0.7$ case being omitted. Going from $f' = 0$ to $f' = 0.08$ to $f' = 0.16$, the time scales drop across the board by a factor of about 4.5, and then again by about 3.7 using the same method as with $\bar{U} = 0.6$. On the pressure side, the numbers are even more similar, with drops of 2 and 1.9, respectively.

Overall, it can be claimed that the fundamental structure of the mean first passage time plots is not dependent on the induced effects. One explanation for this comes directly from the FP expression itself (Equation 4.9). By far the largest contributions to the integral come from the pairs of points with the highest energy discrepancy. Since the energy difference is exponentiated, the points that maximize $\bar{E}(\bar{h}_2) - \bar{E}(\bar{h}_1)$ completely dominate the overall value of the integral. The induced effects implemented throughout this section are quite consistent in their behavior near the endpoints, and so it makes sense qualitatively that the result of their implementation would be this predictable.

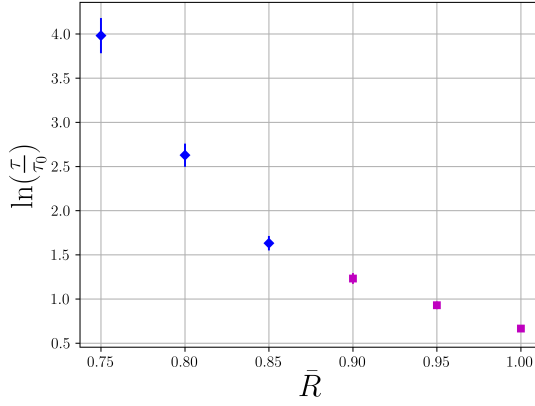
Finally, in Figure 4.8b, there are three events corresponding to logarithmically negative times, with hole radii $\bar{R} = 0.9 - 1.0$ being faster events than in completely free space. This mimics the result from the analysis of the energy curves in Table 4.5 with the maximum slope calculations. The main exception is that in that analysis, $\bar{R} = 0.9$ was not an event that showed a constant downward trend, whereas here it is still one that results in a faster overall passage time than movement through free space. Therefore, the existing energy maximum present in the landscape does not contribute enough to the time estimate to make up for the general downward slope, making up the rest of the event. That is as much as can be confidently ascertained from the data, due to the number of assumptions contributing to this depth of analysis.



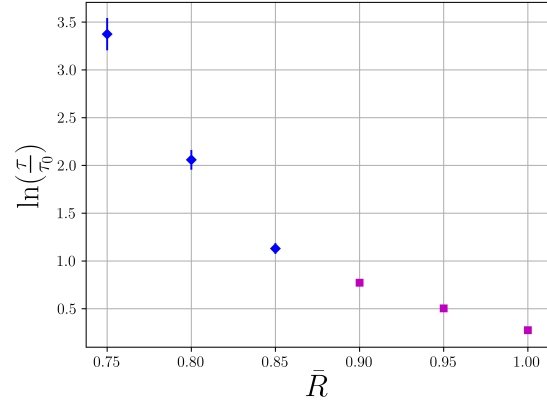
(a) $f' = 0.08$



(b) $f' = 0.16$



(c) $\Delta P' = 0.08$



(d) $\Delta P' = 0.16$

Figure 4.8: $\bar{U} = 0.8$ Fokker-Planck first passage times with various values of induced force or pressure. All plots are singly log scaled and display $\ln(\frac{\tau}{\tau_0})$ versus $\bar{R}$.

Chapter 5

Conclusion

Throughout this project, we have developed a novel algorithm designed to calculate the energy landscape and translocation time of an incompressible lipid bilayer vesicle as it quasi-statically moves through a narrow pore under the influence of externally applied forces and pressure gradients. At the core of this algorithm lies a neural network, optimized specifically to solve the constrained Helfrich bending energy functional as the vesicle progresses through the pore. The vesicle is modeled with four key constraints in mind. The enclosed volume and surface area are preserved as geometric constraints to maintain physical fidelity, while the vesicle's center of mass and its interaction with the barrier are constrained to ensure realistic deformation around the obstacle located at a predetermined position within the pore. To generate a robust representation of the system's thermodynamic behavior, the free energy landscape is computed by evaluating the bending energy across three independent trials in order to properly see the interactions of the vesicle and barrier throughout the journey. Using this energy landscape as an input, the Fokker-Planck formalism for stochastic evolution is used to calculate the average translocation time, which is identified with the mean first passage time across the pore.

In general, a significant free energy barrier arises during vesicle translocation through the pore due to the requirement of substantial elastic deformation. This energy barrier exists even in the absence of an externally applied potential, and under less restrictive conditions, it remains symmetric with respect to the two halves of the passage. The magnitude of this barrier is highly sensitive to the physical and geometrical parameters of the system, including vesicle size and pore diameter. As the system is driven to higher energy states, its imposed constraints, particularly those associated with the barrier and pore diameter, may enable the vesicle to explore alternate metastable/stable vesicle configurations. These transitions can lead to the emergence of non-axisymmetric

vesicle shapes far from equilibrium, representing transitional states not previously well-documented in the context of translocation through a confined geometry. This shape transition shows us asymmetric passages where the vesicle’s energy and interactions differ on either side of the barrier. Introducing an external driving potential, such as a gravitational or pressure gradient, serves to further break the energy landscape’s symmetry and reduce the height of the barrier. This accelerates the translocation process, and the average time for translocation decreases drastically with increasing driving potential. Furthermore, variations in pore diameter have a particularly strong effect, where sufficiently narrow pores can raise the energy barrier to a point where translocation is effectively suppressed or rendered physically impossible.

This project mainly serves as a stepping stone into NN-based modeling of the translocation problem. There are many directions in which this work may be expanded, any of which may produce genuinely new results. Firstly, continued exploration of the $(\bar{U}, \bar{R})$ parameter space with more densely chosen data points spanning a wider area. Secondly, the implementation of variation in $\bar{D}$ and stretching the pore longer and longer to connect the results from this thesis to those of vesicle confinement. Thirdly, changes in the structure of the pore, such as analyzing a narrow rectangular slit instead of a circle, and seeing if and how the vesicle transitions in that context. Finally, and most importantly, a more rigorous implementation of the numerical methods imposed throughout this project. In particular, better fine-tuning of the network parameters, energies, and times to produce more accurate and clear data that hopefully better captures the real physical behavior. There are many other avenues worth exploring, like initial vesicle orientation, but it should now be clear that the ocean of possibilities that are available to be explored in the future is vast and fruitful.

In our theoretical framework, the vesicle’s chemical composition and biophysical interactions are abstracted into a set of parameters by implementing a scalar phase field approach to the problem. These parameters are chosen to faithfully capture the universal large-scale features of vesicle behavior without committing to a specific molecular composition. This neural network-based solver aims to be broadly applicable to a variety of vesicle systems and scales across soft matter and biological physics, while remaining accessible enough to be an efficient and customizable tool for solving and optimization. We anticipate that the relationships our model uncovers, linking translocation dynamics to pore structure, vesicle state transitions, and geometric constraints, will continue to serve as a stepping stone toward future investigations. In particular, we hope that this work stimulates future investigative studies aimed at validating the role of transitional states and understanding how driving forces reshape the complex energy landscapes involved in vesicle deformation and transport through small-scale confinement.

References

- [1] Grace Brannigan, Peter F. Philips, and Frank L. H. Brown. Flexible lipid bilayers in implicit solvent. *Physical Review E*, 72(1), 2005.
- [2] Sonam Phuntsho. Impact of lipid structural variations on bilayer properties: A coarse-grained molecular dynamics study. *arXiv*, 2024.
- [3] Irene A. Chen, Robert W. Roberts, and Jack W. Szostak. The emergence of competition between model protocells. *Science*, 305(5689):1474–1476, 2004.
- [4] Bruce Alberts, Alexander Johnson, Julian Lewis, David Morgan, Martin Raff, Keith Roberts, and Peter Walter. *Molecular Biology of the Cell*. Garland Science, 6 edition, 2014.
- [5] Gaspar Banfalvi. *Permeability of Biological Membranes*. Springer, 2016.
- [6] Rumiana Dimova. Giant vesicles: a biomimetic tool for membrane characterization. *Advances in Colloid and Interface Science*, 208:225–234, 2014.
- [7] Dennis E. Discher and Adi Eisenberg. Polymersomes: tough vesicles made from diblock copolymers. *Science*, 297(5583):967–973, 2002.
- [8] Kai Simons and Dieter Toomre. Lipid rafts and signal transduction. *Nature Reviews Molecular Cell Biology*, 1(1):31–39, 2000.
- [9] Michael M. Kozlov and Leonid V. Chernomordik. Membrane curvature and deformation by proteins and lipids. *Current Opinion in Structural Biology*, 20(4):548–557, 2010.
- [10] W. Rawicz, K. C. Olbrich, T. McIntosh, D. Needham, and E. Evans. Effect of chain length and unsaturation on elasticity of lipid bilayers. *Biophysical Journal*, 79(1):328–339, 2000.

- [11] E. Evans and W. Rawicz. Entropy-driven tension and bending elasticity in condensed-fluid membranes. *Physical Review Letters*, 64(17):2094–2097, 1990.
- [12] Udo Seifert. Configurations of fluid membranes and vesicles. *Advances in Physics*, 46(1):13–137, 1997.
- [13] Reinhard Lipowsky and Erich Sackmann, editors. *Structure and Dynamics of Membranes*, volume 1 of *Handbook of Biological Physics*. Elsevier, 1995.
- [14] Wolfgang Helfrich. Elastic properties of lipid bilayers: theory and possible experiments. *Zeitschrift für Naturforschung C*, 28(11-12):693–703, 1973.
- [15] J.-F. Faucon, M. D. Mitov, P. Méléard, I. Bivas, and P. Bothorel. Bending elasticity of phosphatidylcholine bilayers: from small vesicles to giant vesicles. *Journal de Physique*, 50(17):2389–2414, 1989.
- [16] S. A. Safran. *Statistical Thermodynamics of Surfaces, Interfaces, and Membranes*. Westview Press, 1994.
- [17] Markus Deserno. Fluid lipid membranes: From differential geometry to curvature stresses. *Chemistry and Physics of Lipids*, 185:11–45, 2015.
- [18] Saša Svetina and Boštjan Žekš. Membrane bending energy and shape determination of phospholipid vesicles and red blood cells. *European Biophysics Journal*, 17(2):101–111, 1989.
- [19] P. B. Canham. The minimum energy of bending as a possible explanation of the biconcave shape of the human red blood cell. *Journal of Theoretical Biology*, 26(1):61–81, 1970.
- [20] Stephen Mirigian and Murugappan Muthukumar. Kinetics of particle wrapping by a vesicle. *The Journal of Chemical Physics*, 139(4):044908, 2013.
- [21] Derek Marsh. Elastic curvature constants of lipid monolayers and bilayers. *Chemistry and Physics of Lipids*, 144(2):146–159, 2006.
- [22] Reinhard Lipowsky. Remodeling of membrane shape and topology by curvature elasticity and membrane tension. *Advanced Biology*, 6:2101020, 2022.
- [23] Hans J. Deuling and Wolfgang Helfrich. Red blood cell shapes as explained on the basis of curvature elasticity. *Biophysical Journal*, 16(8):861–868, 1976.

- [24] Udo Seifert, Karin Berndl, and Reinhard Lipowsky. Shape transformations of vesicles: Phase diagram for spontaneous-curvature and bilayer-coupling models. *Physical Review A*, 44(2):1182–1202, 1991.
- [25] Michael P. Sheetz and S. Jonathan Singer. A microtubule-dependent bilayer couple hypothesis for microvillus formation and maintenance. *Journal of Cell Biology*, 62(1):1–11, 1974.
- [26] Yousef Rohanizadegan, Hong Li, and Jeff Z. Y. Chen. Neural-network-based solver for vesicle shapes predicted by the Helfrich model. *Soft Matter*, 20(27):5359–5366, 2024.
- [27] S. T. Milner and S. A. Safran. The shape and fluctuations of a red blood cell: a mean field approximation. *Journal de Physique*, 48(11):1665–1672, 1987.
- [28] G. Gompper and D. M. Kroll. Network models of fluid, hexatic and polymerized membranes. *Journal of Physics: Condensed Matter*, 9(42):8795, 1997.
- [29] Qiang Du, Chun Liu, and Xiaoqiang Wang. Phase field modeling of the spontaneous curvature effects in cell membranes. *Journal of Computational Physics*, 212(2):757–777, 2006.
- [30] Charles M. Elliott and Björn Stinner. Modeling and computation of two phase geometric biomembranes using surface finite elements. *Journal of Computational Physics*, 229(18):6585–6612, 2010.
- [31] Vladimir P. Torchilin. Recent advances with liposomes as pharmaceutical carriers. *Nature Reviews Drug Discovery*, 4(2):145–160, 2005.
- [32] Shojaei H.R., Khaleghi Ardabili A., and Muthukumar M. Free energy minimization for vesicle translocation through a narrow pore. *Methods in Molecular Biology*, 2186:171–183, 2021.
- [33] Hamid R. Shojaei and Murugappan Muthukumar. Translocation of an incompressible vesicle through a pore. *The Journal of Physical Chemistry B*, 120(26):6102–6109, 2016.
- [34] Yunlong Han, Hao Lin, Mingming Ding, Rui Li, and Tongfei Shi. Flow-induced translocation of vesicles through a narrow pore. *Soft Matter*, 15:3307–3314, 2019.
- [35] Gerhard Gompper and Dietrich M. Kroll. Shape transformations of vesicles: Phase diagram for spontaneous-curvature and bilayer-coupling models. *Physical Review E*, 51(5):5149–5172, 1995.

- [36] Shu Liu, Wuchen Li, Hongyuan Zha, and Haomin Zhou. Neural parametric fokker–planck equation. *SIAM Journal on Numerical Analysis*, 60(3):1385–1449, June 2022.
- [37] Narla Mohandas and Patrick G. Gallagher. Red blood cell deformability, membrane material properties and shape: regulation by transmembrane, skeletal and cytosolic proteins and lipids. *Seminars in Hematology*, 45(3):171–192, 2008.
- [38] Theresa M. Allen and Pieter R. Cullis. Liposome drug delivery systems: From concept to clinical applications. *Advanced Drug Delivery Reviews*, 65(1):36–48, 2013.
- [39] Rumiana Dimova. Giant vesicles and their use in assays for assessing membrane phase state, curvature, mechanics, and electrical properties. *Annual Review of Biophysics*, 48:93–119, 2019.
- [40] Michael Mutz, David Bensimon, and Michel J. Brienne. Phase diagram of vesicle shapes. *Physical Review Letters*, 67(7):923–926, 1991.
- [41] Jacob N. Israelachvili. *Intermolecular and Surface Forces*. Academic Press, 3 edition, 2011.
- [42] H.-G. Döbereiner, J. Käs, D. Noppl, I. Sprenger, and E. Sackmann. Mapping vesicle shapes into the phase diagram: A comparison of experiment and theory. *Biophysical Journal*, 73(1):431–442, 1997.
- [43] L. Miao, U. Seifert, M. Wortis, and H.-G. Döbereiner. Budding transitions of fluid-bilayer vesicles: The effect of area-difference elasticity. *Physical Review E*, 49(6):5389–5407, 1994.
- [44] Ashley Gannon, Bryan Quaife, and Y. N. Young. Hydrodynamics of a multicomponent vesicle under strong confinement. *Soft Matter*, 20:599–608, 2024.
- [45] Borzoo Kaoui, Tobias Krüger, and Jens Harting. How does confinement affect the dynamics of viscous vesicles and red blood cells. *Soft Matter*, 8(35):9246–9252, 2012.
- [46] Matthew S. Peterson, Aparna Baskaran, and Michael F. Hagan. Vesicle shape transformations driven by confined active filaments. *arXiv preprint arXiv:2102.02733*, 2021.
- [47] S. J. Singer and G. L. Nicolson. The fluid mosaic model of the structure of cell membranes. *Science*, 175(4023):720–731, 1972.

- [48] John W Barrett, Harald Garcke, and Robert Nürnberg. A parametric finite element method for fourth order geometric evolution equations. *Journal of Computational Physics*, 222(1):441–467, 2007.
- [49] Xiaobing Feng and Andreas Prohl. Finite element approximations of the willmore flow and related problems. *SIAM Journal on Numerical Analysis*, 44(3):1049–1072, 2006.
- [50] Pablo Vázquez-Montejo, Bojan Božič, and Jemal Guven. Equatorial deformation of homogeneous spherical fluid vesicles by a rigid ring. *Phys. Rev. E*, 111:035411, Mar 2025.
- [51] Hiroshi Noguchi and Gerhard Gompper. Fluid vesicle shape transformations by monte carlo simulations. *Physical Review E*, 64(4):041913, 2001.
- [52] Qiang Du, Chun Liu, and Xiaoqiang Wang. A phase field approach in the numerical study of the elastic bending energy for vesicle membranes. *Journal of Computational Physics*, 198(2):450–468, 2004.
- [53] Kenneth A Brakke. The surface evolver. *Experimental Mathematics*, 1(2):141–165, 1992.
- [54] R. Wang and W. Sun. A machine learning approach for solving variational problems in computational mechanics. *Computer Methods in Applied Mechanics and Engineering*, 361:112732, 2020.
- [55] Pablo A. Rueda-Contreras, Ole G. Mouritsen, and Pedro Campillo-Aleman. Activation energy and force fields during topological transitions of lipid bilayer membranes. *Communications Physics*, 5:285, 2022.
- [56] Zeyuan Allen-Zhu, Yuanzhi Li, and Zhao Song. Convergence of stochastic gradient descent for deep neural networks. *Proceedings of the 36th International Conference on Machine Learning (ICML)*, pages 242–252, 2019.
- [57] James F. Blowey and Charles M. Elliott. The cahn-hilliard gradient theory for phase separation with non-smooth free energy part i: Mathematical analysis. *European Journal of Applied Mathematics*, 2(3):233–280, 1991.
- [58] M. I. Angelova and D. S. Dimitrov. Liposome electroformation. *Faraday Discussions of the Chemical Society*, 81:303–311, 1986.

- [59] Felix Campelo and Aurora Hernández-Machado. Modeling the shape of lipid membranes: A phase-field model. *The European Physical Journal E*, 20(1):37–45, 2006.
- [60] Kyle Boniface, Samuel Bohn, and Markus Deserno. Data-driven prediction of vesicle morphologies in confined geometries using machine learning. *Biophysical Journal*, 120(19):4030–4041, 2021.
- [61] Bastiaan Olsthoorn, Petros Koumoutsakos, Larissa Rocha, et al. Inverse design of soft matter via machine learning: Progress, opportunities, and challenges. *Soft Matter*, 18(33):6388–6403, 2022.
- [62] Petch Khunpetch, Xingkun Man, Toshihiro Kawakatsu, and Masao Doi. Translocation of a vesicle through a narrow hole across a membrane. *Physical Review E*, 97(5):052406, 2018.
- [63] Nishant Baruah, Jiarul Midya, Gerhard Gompper, Anil Kumar Dasanna, and Thorsten Auth. Adhesion-driven vesicle translocation through membrane-covered pores. *Biophysical Journal*, 124(5):740–752, 2025.
- [64] L. R. Arriaga, J. Huang, T. Yang, and et al. High-throughput trapping of giant unilamellar vesicles for controlled deformation and analysis. *Lab on a Chip*, 10(5):587–594, 2010.
- [65] S. Krishnan, D. Ziegler, V. Arnaut, and et al. Programmable dna-based membrane channels for controlled molecular transport. *Nature Communications*, 7:12787, 2016.
- [66] H. Bayley, J. R. Burns, D. Stoddart, and et al. Leakless end-to-end transport of small molecules through dna nanopores. *Nature Nanotechnology*, 15:1203–1210, 2020.
- [67] Hiroshi Noguchi and Gerhard Gompper. Shape transitions of fluid vesicles and red blood cells in capillary flows. *Proceedings of the National Academy of Sciences*, 102(40):14159–14164, 2005.
- [68] Hammad A. Faizi, Cody J. Reeves, Vasil N. Georgiev, Petia M. Vlahovska, and Rumiana Dimova. Fluctuation spectroscopy of giant unilamellar vesicles using confocal and phase contrast microscopy. *Soft Matter*, 16(20):4937–4946, 2020.
- [69] Kuo-Kang Liu and Hu Zhang. Optical tweezers for single cells. *Philosophical Trans. Royal Society B*, 2008.

- [70] Udo Seifert and Karin Berndl. Shape transition and hydrodynamics of vesicles in tube flow. *Physical Review Fluids*, 5(4):043602, 2019.
- [71] François Brochard-Wyart et al. Cascades of transient pores in giant vesicles: Line tension and transport. *Biophysical Journal*, 85:X–Y, 2003.
- [72] Aljaž Godec et al. First-passage times in complex energy landscapes via fokker–planck coarse-graining. *Case Study: Nonmuscle Myosin II Assembly*, 2022.
- [73] Hannes Risken et al. Analytical theory of mean first passage time in gradient systems. *Handbook of Stochastic Methods*, 1989.
- [74] W. Sung and P. J. Park. Polymer translocation through a pore in a membrane. *Phys. Rev. Lett.*, 77(4):783–786, 1996.
- [75] Linke, G. T., Lipowsky, R., and Gruhn, T. Osmotically induced passage of vesicles through narrow pores. *Europhys. Lett.*, 74(5):916–922, 2006.
- [76] C. W. Gardiner. *Handbook of Stochastic Methods for Physics, Chemistry and the Natural Sciences*. Springer-Verlag, Berlin, 2nd edition, 1985.
- [77] Svante Arrhenius. Über die reaktionsgeschwindigkeit bei der inversion von rohrzucker durch säuren. *Zeitschrift für Physikalische Chemie*, 4(1):226–248, 1889.
- [78] Albert Einstein. Über die von der molekularkinetischen theorie der wärme geforderte bewegung von in ruhenden flüssigkeiten suspendierten teilchen. *Annalen der Physik*, 322(8):549–560, 1905.
- [79] Selim Esedoglu, Andreas Rätz, and Matthias Röger. A nonlocal surface tension model for the evolution of bilayer membranes. *Interfaces and Free Boundaries*, 16(3):375–400, 2014.
- [80] Jean Gottos and Laura De Lorenzis. Physics-informed neural networks for solving the cahn-hilliard equation with applications to phase separation and coarsening in multicomponent alloys. *Computer Methods in Applied Mechanics and Engineering*, 384:113979, 2021.
- [81] Michael Spivak. *A Comprehensive Introduction to Differential Geometry, Volume III*. Publish or Perish, Inc., Houston, TX, 3 edition, 1999. Contains “Elements of the Theory of Surfaces in $\mathbb{R}^3$, including Euler’s theorem, in Chapter 2.

- [82] Michael A. Nielsen. *Neural Networks and Deep Learning*. Determination Press (self-published), 2015. Available online; Creative Commons license.
- [83] Diederik P. Kingma and Jimmy Ba. Adam: A method for stochastic optimization. *International Conference on Learning Representations*, 2015.
- [84] Xiaoqiang Wang and Qiang Du. A phase field formulation of the willmore problem. *Nonlinearity*, 21(2):365–381, 2008.
- [85] Claire Cadart, Krassimira Venkova, Pierre Recho, et al. Cell volume fluctuations in mdck monolayers. *Biophysical Journal*, 109:170–178, 2015.

APPENDICES

Appendix A

Phase Field Formulation

In this Appendix, the goal is to construct the formulas for Equations 2.5, 2.6, and 2.7 for the bending energy, surface area, and volume of the surface, respectively. To do this, we will be following the methodology of the Ginzburg-Landau form discussed in the works of Blowey et al. and Du et al. [52, 57, 79]. It should be noted that there is a lot of freedom in how exactly ϕ should be defined. The underlying methodology used to construct the phase field is the main component governing how the energy, volume, and area are extracted from it. Therefore, this derivation has a lot of flexibility in defining $\phi(\mathbf{r})$, allowing us to make decisions that simplify the necessary calculations. The equations to be derived from Section 2.3 are repeated below.

$$E_b[\phi] = \frac{3\kappa}{4\sqrt{2}\epsilon} \int d\mathbf{r} \left[\epsilon \nabla^2 \phi(\mathbf{r}) + \frac{1}{\epsilon} \phi(\mathbf{r}) (1 - \phi(\mathbf{r})^2) \right]^2 \quad (\text{A.1})$$

$$A[\phi] = \frac{3}{4\sqrt{2}} \int d\mathbf{r} \left[\epsilon |\nabla \phi(\mathbf{r})|^2 + \frac{1}{2\epsilon} (1 - \phi(\mathbf{r})^2)^2 \right] \quad (\text{A.2})$$

$$U[\phi] = \frac{1}{2} \int d\mathbf{r} [1 + \phi(\mathbf{r})] \quad (\text{A.3})$$

A.1 Derivation of $E_b[\phi]$

The first functional to derive is $E_b[\phi]$. Given our phase field $\phi(\mathbf{r})$, our first step is to express it in terms of the signed distance $d(\mathbf{r})$. $d(\mathbf{r})$ is a measure of the signed distance between

the point $\mathbf{r}$ in space and the surface of the vesicle, S , with points on the interior of the vesicle, given the positive values of d . We will then define $\phi(\mathbf{r})$ as below in equation A.4.

$$\phi(\mathbf{r}) = \tanh\left(\frac{d(\mathbf{r})}{\sqrt{2}\epsilon}\right) \quad (\text{A.4})$$

This expression for $\phi(\mathbf{r})$ matches the boundary conditions set on the phase field as described in Section 2.3. Namely, the field ranges from -1 to $+1$, takes on values near $+1$ on the interior of the vesicle, and values near -1 on the exterior. Additionally, the sharpness between these regimes is dictated directly by the parameter ϵ . Collapsing the phase field using this ansatz allows us to work purely in terms of the effectively 1D function $d(\mathbf{r})$ while still maintaining a strong correlation to the original ϕ . The factor of $\sqrt{2}$ in the denominator also assists in cleaning up the derivation in this way.

Additionally, from our definition of mean curvature in Section 1.3, the quantity H measures the mean of the two principal curvatures k_1 and k_2 , which are the radii of the circles best matching the curvature along the two axes parallel to the surface. On the other hand, from differential geometry, Euler's theorem states that the mean curvature of a surface can also be expressed as the divergence of the normal vector to that surface. Namely, $2H = -\nabla \cdot \mathbf{n}$ where H is the mean curvature and $\mathbf{n}$ is the normal vector to the surface [17]. This theorem is explained in detail in Michael Spivak's Differential Geometry Volume III [81]. It should be noted that this theorem occasionally comes with a factor of 2 depending on the specific definition of mean curvature.

Near the surface of the vesicle (assuming the surface is smooth), $d(\mathbf{r})$ changes linearly with $\mathbf{r}$, with ∇d being very well approximated by $\mathbf{n}$. As a result, $2H(\mathbf{r}) = -\nabla^2 d(\mathbf{r})$ holds up to the first order in d . As a result, if the value $-\nabla^2 d(\mathbf{r})$ can be calculated in terms of ϕ , it can be used to quantify the elastic energy functional.

First, let us calculate $\nabla\phi(\mathbf{r})$ and $\nabla^2\phi(\mathbf{r})$, the derivatives of $\phi(\mathbf{r})$ near the vesicle's surface as expressed in Equation A.4. The $\mathbf{r}$ dependence of ϕ and d will not be explicitly stated to clean up the derivation below.

$$\begin{aligned}
\nabla\phi &= \nabla \tanh\left(\frac{d}{\sqrt{2}\epsilon}\right) \\
&= \frac{1}{\sqrt{2}\epsilon} (1 - \tanh^2(\frac{d}{\sqrt{2}\epsilon})) \nabla d \\
\nabla\phi &= \frac{1}{\sqrt{2}\epsilon} (1 - \phi^2) \nabla d
\end{aligned} \tag{A.5}$$

$$\begin{aligned}
\nabla^2\phi &= \nabla \cdot \nabla\phi \\
&= \nabla \cdot \left(\frac{1}{\sqrt{2}\epsilon} (1 - \phi^2) \nabla d \right) \\
&= \frac{1}{\sqrt{2}\epsilon} (1 - \phi^2) \nabla^2 d + \nabla d \cdot \nabla \left(\frac{1}{\sqrt{2}\epsilon} (1 - \phi^2) \right) \\
&= \frac{1}{\sqrt{2}\epsilon} (1 - \phi^2) \nabla^2 d - \frac{\sqrt{2}}{\epsilon} \phi \nabla d \cdot \nabla\phi \\
&= \frac{1}{\sqrt{2}\epsilon} (1 - \phi^2) \nabla^2 d - \frac{1}{\epsilon^2} \phi (1 - \phi^2) |\nabla d|^2 \\
\nabla^2\phi &= \frac{1}{\sqrt{2}\epsilon} (1 - \phi^2) \nabla^2 d - \frac{1}{\epsilon^2} \phi (1 - \phi^2)
\end{aligned} \tag{A.6}$$

In the final line above, note that $|\nabla d|^2 \approx 1$ simply due to being very well approximated by $\mathbf{n} \cdot \mathbf{n}$. Near the interface, $\nabla^2 d$ can be replaced with $-2H$ in Equation A.6 and rearrange to find Equation A.7.

$$-\sqrt{2}(1 - \phi^2)H = \epsilon \nabla^2\phi + \frac{1}{\epsilon} \phi(1 - \phi^2) \tag{A.7}$$

Squaring both sides and integrating over all of 3D space gives,

$$2 \int d\mathbf{r} (1 - \phi^2)^2 H^2 = \int d\mathbf{r} \left[\epsilon \nabla^2\phi + \frac{1}{\epsilon} \phi(1 - \phi^2) \right]^2. \tag{A.8}$$

Looking at the left side of Equation A.8, note that the $(1 - \phi^2)^2$ term is a function of $d(\mathbf{r})$ and thus only depends on the coordinate perpendicular to the surface. Similarly, H^2 only has dependence on the surface element in question and so, is a function of the parallel coordinates. Consequently, a change of coordinates can be implemented on the separable left integral to break it up. Namely, $d\mathbf{r} = dx dy dz = \epsilon d\rho d^2S$ where d^2S is the 2D surface

element of the vesicle and $d\rho$ is the normal perpendicular axis. The factor of ϵ comes out as a scalar by just rescaling $\rho = \frac{d(\mathbf{r})}{\epsilon}$ so that the perpendicular integral has no ϵ inside. As a result, this integral, shown in Equation A.9, can be computed directly as

$$2\epsilon \int d\rho (1 - \phi(\rho)^2)^2 \int H^2 d^2S = \int d\mathbf{r} \left[\epsilon \nabla^2 \phi(\mathbf{r}) + \frac{1}{\epsilon} \phi(\mathbf{r})(1 - \phi(\mathbf{r})^2) \right]^2, \quad (\text{A.9})$$

with $\phi(\rho) = \tanh(\frac{\rho}{\sqrt{2}})$.

This integral can be calculated analytically as ρ ranges from $-\infty$ to ∞ , and gives us the constant factor $\frac{4\sqrt{2}}{3}$. Finally, reincorporating the factor of 2κ from Equation 1.3, one can derive the phase field bending energy shown in Equation A.10,

$$\begin{aligned} 2\epsilon \frac{4\sqrt{2}}{3} \left(2\kappa \int H^2 d^2S \right) &= 2\kappa \int d\mathbf{r} \left[\epsilon \nabla^2 \phi + \frac{1}{\epsilon} \phi(1 - \phi^2) \right]^2 \\ E_b[\phi] &= \frac{3\kappa}{4\sqrt{2}\epsilon} \int d\mathbf{r} \left[\epsilon \nabla^2 \phi + \frac{1}{\epsilon} \phi(1 - \phi^2) \right]^2, \end{aligned} \quad (\text{A.10})$$

matching the form of E_b used in the analysis.

A.2 Derivation of $A[\phi]$ and $U[\phi]$

Moving on to the derivation of $A[\phi]$, much the same principles can be employed as in the derivation for E_b . This time, the integral to isolate is the same surface integral from Equation A.9, but without the factor of H^2 inside. There are many ways to approach this value from a phase field approach, but the most common one (used by Du [52]) employs a condition known as equipartition. Using Equation A.5, a value for $|\nabla\phi|^2$ can be derived, in addition to Equation A.12.

$$\begin{aligned}
\nabla\phi &= \frac{1}{\sqrt{2}\epsilon}(1-\phi^2)\nabla d \\
|\nabla\phi|^2 &= \frac{1}{2\epsilon^2}(1-\phi^2)^2|\nabla d|^2 \\
|\nabla\phi|^2 &= \frac{1}{2\epsilon^2}(1-\phi^2)^2 \tag{A.11} \\
|\nabla\phi|^2 + \frac{1}{2\epsilon^2}(1-\phi^2)^2 &= \frac{1}{\epsilon^2}(1-\phi^2)^2 \\
\epsilon|\nabla\phi|^2 + \frac{1}{2\epsilon}(1-\phi^2)^2 &= \frac{1}{\epsilon}(1-\phi^2)^2 \tag{A.12}
\end{aligned}$$

Note that in the fourth line, the right-hand side of the expression is added to both sides to get rid of the factor of 2 in the denominator. This specific choice will be justified at the end of this appendix. Looking at the right side of this expression, it appears to be a very similar term to the left-hand side of Equation A.8. This expression only depends on ρ as discussed earlier, and so both sides can be integrated over all of space.

$$\frac{1}{\epsilon} \int d\mathbf{r} (1-\phi^2)^2 = \int d\mathbf{r} \left[\epsilon|\nabla\phi|^2 + \frac{1}{2\epsilon}(1-\phi^2)^2 \right] \tag{A.13}$$

Then, expanding the left integral into the $d\rho$ and d^2S terms as was done for the bending energy, the surface area functional can be found via Equation A.14 below.

$$\begin{aligned}
\int d\rho(1-\phi^2)^2 \int d^2S &= \int d\mathbf{r} \left[\epsilon|\nabla\phi|^2 + \frac{1}{2\epsilon}(1-\phi^2)^2 \right] \\
\frac{4\sqrt{2}}{3} \left(\int d^2S \right) &= \int d\mathbf{r} \left[\epsilon|\nabla\phi|^2 + \frac{1}{2\epsilon}(1-\phi^2)^2 \right] \\
A[\phi] &= \frac{3}{4\sqrt{2}} \int d\mathbf{r} \left[\epsilon|\nabla\phi|^2 + \frac{1}{2\epsilon}(1-\phi^2)^2 \right] \tag{A.14}
\end{aligned}$$

Finally, the vesicle volume, $U[\phi]$, requires no extra preliminary computation. Looking at our approximation of $\phi(\mathbf{r})$ in terms of $d(\mathbf{r})$ in Equation A.4, this expression takes on the values of -1 outside of the vesicle, and values of $+1$ inside, with the vesicle interface taking values of 0. Following the assumption that $|\nabla d| \approx 1$, this distribution also exhibits odd symmetry about the interface, $\phi(-d(\mathbf{r})) = -\phi(d(\mathbf{r}))$. Thus $\frac{1}{2}(1+\phi(\mathbf{r}))$ symmetrically

takes the value 0 outside and 1 inside, and is a good representation of the volume density of the vesicle. Therefore, after being integrated over all of space, this expression produces $U[\phi]$ in Equation A.15.

$$U[\phi] = \frac{1}{2} \int d\mathbf{r} [1 + \phi(\mathbf{r})] \quad (\text{A.15})$$

A.3 Important Comments on Phase Field Formalism

There are some notes to make regarding this derivation. First, the initial formulation of $\phi(\mathbf{r}) = \tanh(\frac{d(\mathbf{r})}{\sqrt{2}\epsilon})$. At the end of the day, this choice of ϕ is the defining assumption made in our analysis of the phase field approach. Using this as a baseline is what allows us to construct the various identities above using ϕ and its derivatives. Additionally present in this derivation is the condition that ϕ is only a function of d , the distance to the surface of the vesicle. Physically, this is a justified claim, matching the boundary conditions and intuition of the problem. However, this is by no means an enforced reason. This form is one that gives rise to somewhat neat expressions of our three formulas as space integrals of ϕ and its derivatives, while being applicable enough to be mathematically maneuverable. Thus, the hyperbolic tangent itself does not contain a physical connection to the structure of the phase field itself, as only its global features were utilized in this derivation. The field $\phi(\mathbf{r})$ could have been presented through an unnamed 1D heteroclinic function $q(\rho)$, solving $q(\pm\infty) = \pm 1$, $q(0) = 0$ and satisfying $q' = \frac{1}{\sqrt{2}\epsilon}(1 - q^2)$ with no mentioned explicit form. It turns out that $\tanh$ uniquely solves this boundary value problem, which is the only reason an explicit equation was mentioned here.

The second note regarding this method is that of equipartition in the area functional. As another consequence of our decision to model using the $\tanh$ function, at that step, the identity in Equation A.11 can be used to rewrite $(1 - \phi^2)^2$ in a different way using $|\nabla\phi|^2$. In essence, any choice of weights for the two expressions could be chosen to combine them and produce the same result mathematically. Physically, for an actual vesicle, these expressions are not guaranteed to be equal, however, and it may be possible that the phase field of certain vesicle configurations could cause the values to be mismatched despite being indistinguishable under the $\tanh$ formulation. As a result, since it is not known which expression better captures the behavior of the vesicle, both are implemented into the model for $A[\phi]$ by taking a simple arithmetic mean of the two. This is the equipartition mentioned earlier, as our function is made up of both values in equal parts. Notably, the better an assumption our signed distance $\tanh$ is at modeling the phase field dynamics, the more closely these two terms line up, matching the vesicle behavior.

Appendix B

Geometric Limits

In this Appendix, the goal is to provide a geometric argument for which regions of parameter space are definitively impossible for translocation and what to expect near that geometric limit. In the discussion in Chapter 3, the limits of translocation and when different classes of transitions occur are discussed with specific values of $(\bar{U}, \bar{R})$. Using a geometric analysis shown in Figure B.1, it can be determined mathematically what pairs of $\bar{U}$ and $\bar{R}$ are mathematically impossible. This method works as follows. First, values are selected for $\bar{R}$ and $\bar{D}$. As $\bar{D} = 0.16$ in our analysis of translocation, that will also be its value for this analysis, and the focus will be on testing $(\bar{U}, \bar{R})$ pairs at this chosen barrier width. Given $\bar{R}$, a vesicle of $\bar{U} = 1$ is placed in front of the barrier, as shown in Figure B.1a. Then, the vesicle is slowly moved towards the barrier while analytically deforming it. In particular, we use the assumption that the configuration of the vesicle is a pseudo-prolate formed by spherical caps on either side of the barrier and a filled cylinder within the confines, as visible in Figure B.1b. As the constraint of the barrier becomes more restrictive, our vesicle cannot hold the entirety of its $\bar{U} = 1$ volume, and so the maximum volume the vesicle can take at each step is calculated analytically.

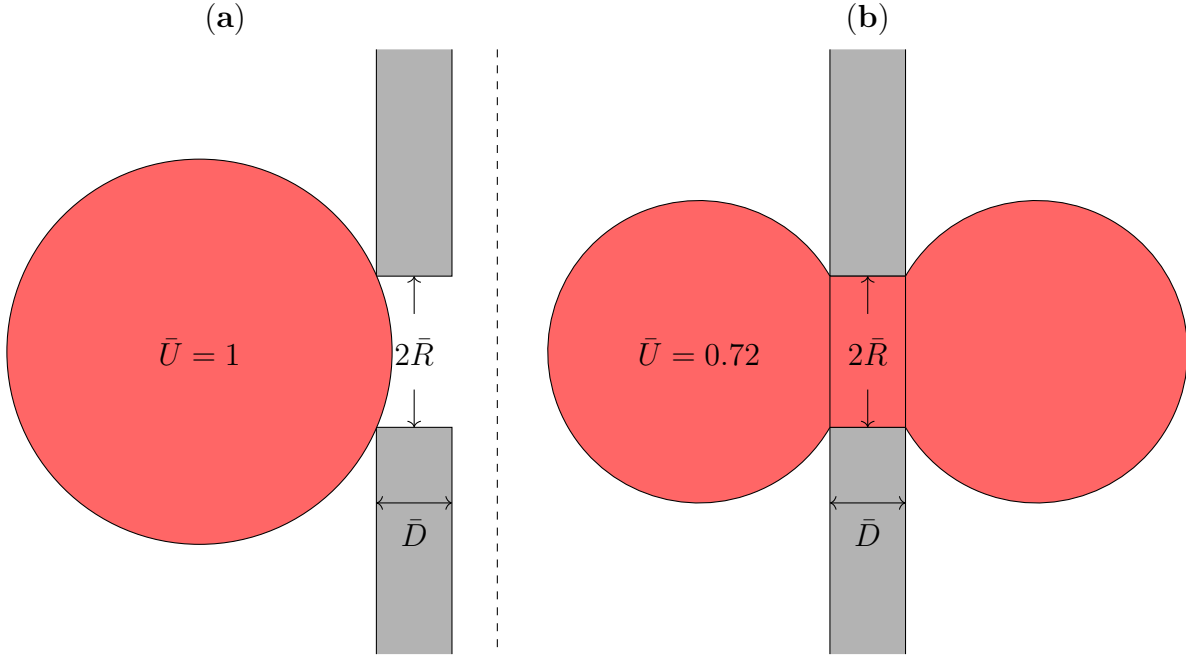


Figure B.1: An analytical geometric extreme used to qualify certain regions of parameter space as unphysical. In subfigure (a), a $\bar{U} = 1$ sphere is shown nestling into the pore as far as it can go. In particular, for the radius shown above, the vesicle in (a) cannot geometrically navigate any further, as a vesicle of that size has no flexibility to deform. As a result, this $(\bar{U}, \bar{R})$ pair is unphysical. No matter what configuration the vesicle takes, it cannot maintain its area and volume while simultaneously passing through the barrier. In (b), on the other hand, a vesicle is seen with the same surface area as the first, but less volume on the interior. Under this formulation, the vesicle's structure is that of two perfectly spherical lobes on either side of the barrier, and a filled cylinder on the inside. As a result, this vesicle has a lower $\bar{U}$ of about 0.72. Iterating through other positions of the barrier relative to the center of mass, the vesicle can always fit all of its volume within the surface, and so this $(\bar{U}, \bar{R})$ pair is considered geometrically possible. Visually, this diagram uses $\bar{R} \approx 0.4$ and $\bar{D} \approx 0.4$, but this argument can be extended to any barrier size.

Therefore, it is possible to iterate through these barrier positions and calculate the maximum allowed value of $\bar{U}$ at each one. As a result, for that given $\bar{R}$, the minimum of these $\bar{U}$ s attained across the entire translocation is the maximum value of $\bar{U}$ that can pass through the pore. In other words, $\bar{U}$ is capped from above by the most restrictive position of the barrier, and so any vesicle larger than that cap will be unable to get through

that moment of the passage. Iterating through hole radii produces a curve in the $(\bar{U}, \bar{R})$ parameter space that divides the space into geometrically possible and impossible regions.

We will do an example calculation for clarity using a theoretical $\bar{R} = 0$ pore. This pore cannot be navigated energetically as the induced bending in the vesicle’s surface would blow up to infinity, but geometrically, a nontrivial finite result can be produced. In a sense, the vesicle can be thought of as being pushed through a thin, point-sized straw through the barrier under the question of how high its volume can go while still maintaining its area. Thus, the volume of the vesicle will be split among two perfect spheres on each side, as can be seen in Figure B.2. As more volume is fed through, the spheres’ total volume decreases relative to their area until they reach a minimum value of $\bar{U} = \frac{1}{\sqrt{2}} \approx 0.707$ at the halfway point. The second half of the translocation is the same as the first but in reverse, and so this $\bar{U} = 0.707$ is the minimal value necessary for this hole radius. Thus, for $\bar{R} = 0$, only vesicles of size $\bar{U} = 0.707$ or smaller can fit through. This logic can be repeated for each hole size, using spherical caps and the filled cylinder to calculate the corresponding value for each $\bar{R}$.

In Figure B.3, the geometric limit from this argument is mapped alongside the simulated translocations from the contents of Chapter 3. However, this geometric limit is still quite far from the points in question. This constraining of parameter space has not accounted for network convergence or energetic stability of the vesicle, which are likely to fail under much less extreme conditions. Thus, it is expected that translocations will fail due to other reasons before reaching this critical curve. In theory, this mathematical model could be reformulated to ease out the kinks and symmetries that cause the distinction between the geometric and energetic limits. However, this is still an ongoing analysis. Despite this, it still gives a hard upper bound on what parameters are worth investigating. Too far from the curve will only see small deformations of the vesicle, whereas too close will cause divergence and blow up the simulation. The examined points in the main analysis of this paper were chosen under the assumption that large deformations and shape transitions would occur at the right distance from this curve.

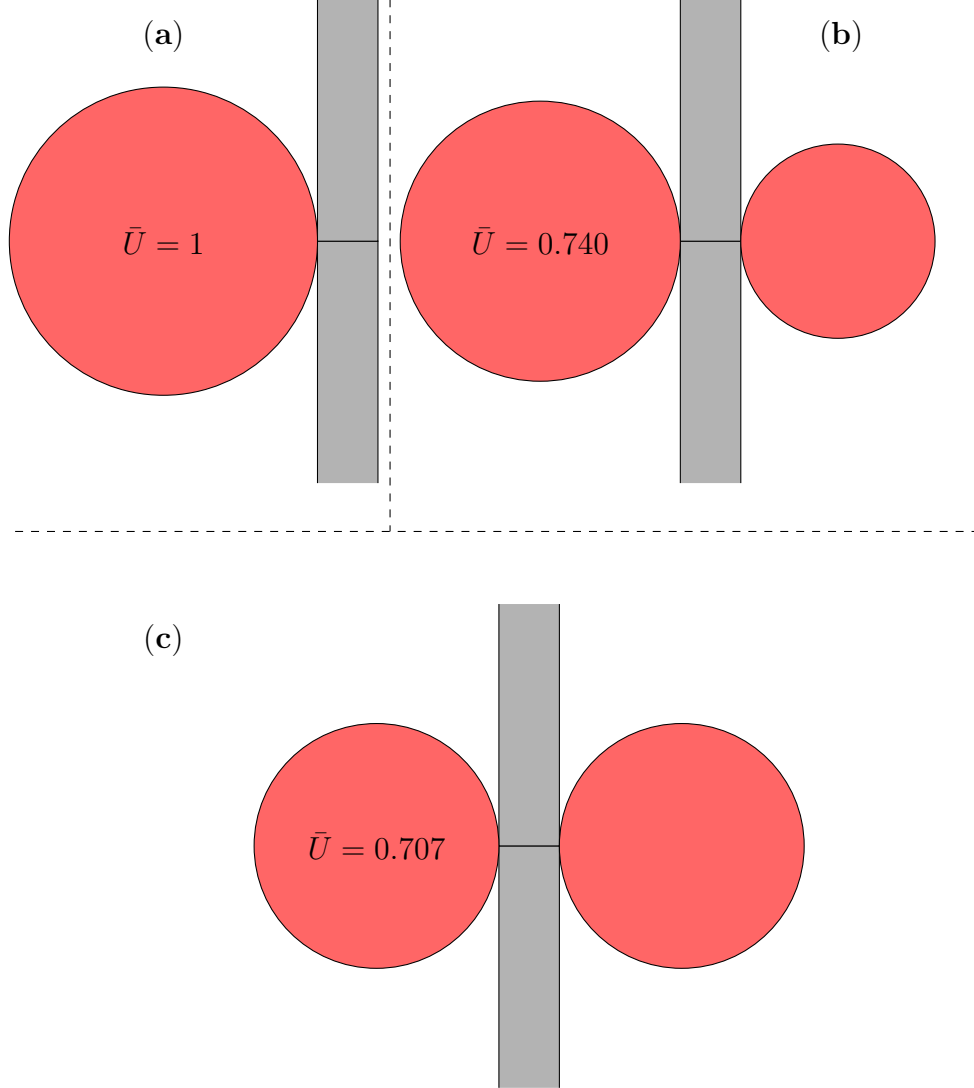
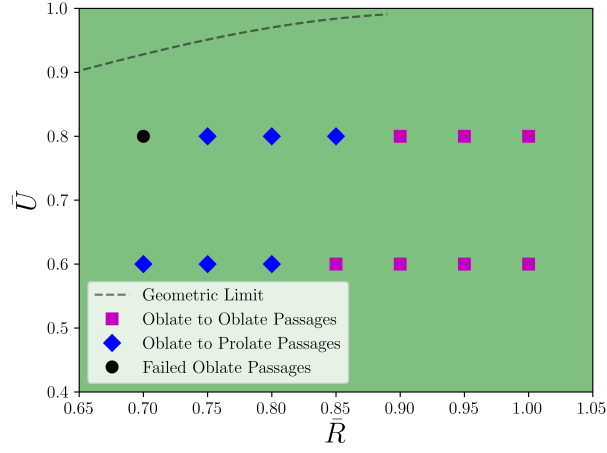
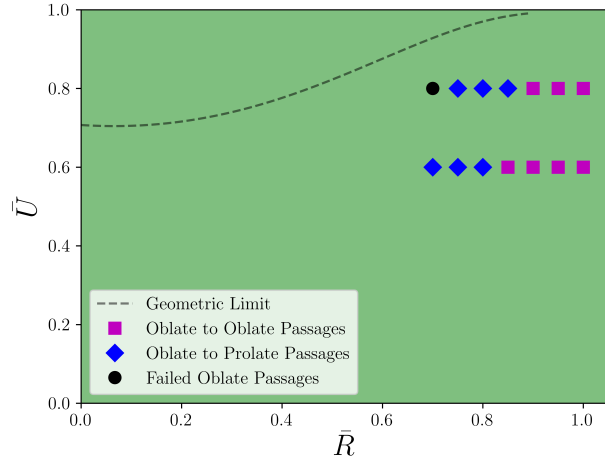


Figure B.2: Three snapshots of the algorithm described to find the unphysical regime in the $(\bar{U}, \bar{R})$ parameter space. Figure (a) shows the initial state with a $\bar{U} = 1$ vesicle preparing to pass. Figure (b) shows a moment when a quarter of the volume passes through, and the vesicle is forced to shrink down to $\bar{U} = 0.740$ to fit within its surface. Finally, figure (c) is the global minimum of $\bar{U} = 0.707$ at the halfway point, representing the largest vesicle size that can complete the entire passage through this hole.



(a)



(b)

Figure B.3: A map of the $(\bar{U}, \bar{R})$ space with the geometric limit curve. The dashed line is the calculated geometric limit for each value of $\bar{R}$ using the method described in this Appendix. The black, blue, and magenta points are the data sets analyzed within this paper. Figure (a) is zoomed in to the same scale used in the other versions of this plot. Figure (b) is zoomed out to see the entirety of the curve. Near $\bar{R} = 1$, the numerical solver used to produce the curve ran into some stability issues, likely because the pore is wide enough that enforcing the filled cylinder condition became infeasible. This is why the geometric limit stops around $\bar{R} \approx 0.88$ as the analytical model breaks down.

Appendix C

Neural Networks and Computational Efforts

C.1 Neural Network Details

A neural network (NN) is a computational model inspired by the structure of the brain, designed to recognize patterns and approximate complex relationships in data. It is built from layers of interconnected ‘neurons’, each of which computes a weighted sum of its inputs from the previous layer, applies a nonlinear activation function, and passes the result to neurons in the next layer. The first layer receives the raw input data, hidden layers transform these inputs into increasingly abstract representations, and the final layer produces one or more outputs. As a result, a network is a function with a number of tunable levers that can be adjusted to any desired output. The network’s goal is to fine-tune these parameters to better and better match the desired function, in a process called learning. This learning occurs through an algorithm called backpropagation, where the network’s initial predictions are compared to the desired outputs, a cost is calculated, and the weights are adjusted to reduce this cost. This adjustment is typically performed using gradient descent, an optimization method that gradually moves the network parameters toward values that minimize the cost.

Neural networks excel at tasks like image recognition, language processing, and constrained optimization, largely because they can learn useful features directly from data rather than relying on hand-crafted rules. However, they require large datasets, substantial computational resources, and careful tuning of hyperparameters (learning rates, layer sizes, activation functions, and so on) to achieve strong performance. Despite these challenges,

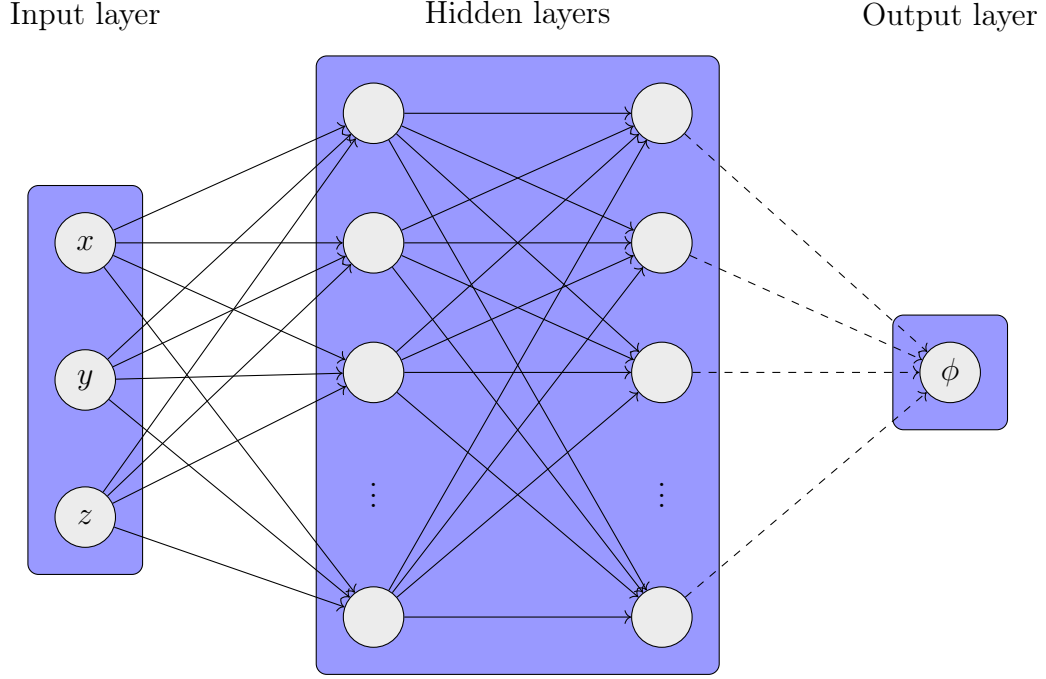


Figure C.1: Schematic of the NN representation of the phase field. The layers connected by solid arrows indicate the application of the sigmoid function to the linearly combined input at the receiving node, and the dashed arrows signify the application of the hyperbolic tangent function to the linearly combined input of the last node.

NNs form the backbone of modern deep learning and form the foundational method used in this study on vesicle translocation. For more details on NNs, see Michael Nielsen’s deep learning textbook [82].

We use a four-layer feed-forward small-scale NN to produce our vesicle. The network, shown roughly in Figure C.1, is fully connected between each layer with layer sizes of 3, 20, 10, and 1. A sigmoid activation function is used for layers 1 and 2, while a hyperbolic tangent function is used in layer 3. The specific sigmoid activation function is shown explicitly below in Equation C.1. The inputs to the network are the three coordinates of space $\mathbf{r} = (x, y, z)$ while the output is the scalar field $\phi(\mathbf{r})$. Note that $\tanh$ has a range of -1 to $+1$, meaning that the range of the network’s outputs matches that of the phase field ϕ .

$$\sigma(s) = \frac{1}{1 + e^{-s}} \quad (\text{C.1})$$

C.2 Network Initialization

As mentioned in Section 3.3, for each event, the initial network was one modeling a free space oblate aligned along the y -axis. To create this, we started by randomly selecting all of the network's weights and biases according to a normal distribution with standard deviation 1, centered at 0. The next step was to optimize the network to generate a $\bar{U} = 1$ sphere using an alternate cost function that demanded $\phi = +1$ inside a sphere of radius 1, and $\phi = -1$ outside of it. This cost function, C_{sphere} , is given explicitly in Equation C.2. This sphere is constructed by associating points within a radius of 1 of the origin with a negative cost, and those outside the radius with a positive one. This is the role of the hyperbolic tangent in Equation C.2. When multiplied by the volume density, this incentivizes the network only to take on positive values within a range of 1, creating a near-perfect sphere. The parameter ϵ can also be introduced here to quantify the interface sharpness before the next step.

$$C_{sphere} = \frac{1}{2} \int (1 + \phi(\mathbf{r})) \tanh\left(\frac{\mathbf{r}^2 - 1}{\epsilon^2}\right) d\mathbf{r} \quad (\text{C.2})$$

$$\begin{aligned} C_{Pr \rightarrow Ob} = & E_b[\phi] + \lambda_A(A[\phi] - A_0) \\ & + \lambda_U(U[\phi] - U_0) + \lambda_{CM} R_{CM}^2[\phi] + 100 \times \frac{1}{2} \int (1 + \phi(\mathbf{r})) y^2 d\mathbf{r} \end{aligned} \quad (\text{C.3})$$

Next, Equation 2.9 was implemented as the model for a free space vesicle, with $\bar{U} = 0.8$ and the center of mass constraint in place to avoid drift. Note that the Lagrange multipliers are also initialized in the same way as the network parameters. This converged the vesicle down to a prolate, due to it being a more stable configuration at this value of $\bar{U}$. To turn it into an oblate, a temporary term was added onto the cost that penalized the vesicle for being far from the xz plane. The cost used in this step is shown as $C_{Pr \rightarrow Ob}$ in Equation C.3. This cost penalizes the prolate's lobes for bulging outwards and presses the vesicle down to be as flat as possible along the xz plane. By monitoring the state of the vesicle as it solved, this flattening term could be removed once the vesicle became close enough to the oblate metastable point that it converged naturally from there. This vesicle was then optimized for an extended period of time to produce a high-quality stable oblate for the $\bar{U} = 0.8$ cases. Finally, taking this oblate, the $\bar{U}$ value was adjusted once again to 0.6 for the second dataset. Finally, this $\bar{U} = 0.6$ oblate was iterated with the same high epoch optimization as the first to prepare it for translocation.

Each epoch of solving involves evaluating the network on each of the sampling points in the grid to calculate $\phi(x, y, z)$. With this map of ϕ , the energy and constraints according to the expressions given in Sections 2.3 and 2.4, as well as Equations C.2 and C.3 from earlier can all be computed. Given the constrained energy as a cost function (with the Lagrange multipliers), the total calculated effective energy is integrated across our grid. Note that the values for each term in Equation 2.14 or C.3 are calculated separately, and so the energy, volume, area, or anything else can be extracted quite easily. Due to the even spacing of our grid points, this integration becomes akin to just summing over the values of energy density and dividing by the point density. The derivatives can also be calculated by calculating a finite difference field for each point with respect to those around it.

For all of our networks, the simulation box is of size $7.2 \times 7.2 \times 7.2$ represented by a uniformly distributed grid of 160^3 points. These points are the input data for the network, and remain unchanged as solution finding progresses. The density of points, as a result, is $(\frac{160}{7.2})^3 \approx 11,000$ points per $1 \times 1 \times 1$ unit box. The summation over the corresponding densities then gets divided by this number to calculate the integral and thus, the total value of each functional.

We take advantage of the Adam optimizer to adjust the network parameters at the end of each epoch in order to reduce the bending energy [83]. Specifically, the steepest descent Adam optimizer is run on Equation 2.9 for free vesicles, or on Equation 2.14 for translocating ones. The core of the Adam algorithm is gradient descent, which updates the network parameters by calculating the gradient of the cost with respect to each parameter and shifting them in order to reduce the cost as much as possible. Our network contains 2,641 weights and biases (network parameters), so the optimization of the network exists as a steepest descent in this parameter space. In Figure C.1, each arrow has a scalar weight attached, and each node outside of the first layer has an associated bias, which is where this total comes from. Even with 4,000,000 points per epoch, no mini-batching of the data occurs, which means the parameters are only updated once the entire epoch has been evaluated and the total cost has been computed and averaged between the data points.

Following the parameter update, the Lagrange multipliers are also updated at the end of each epoch. The Lagrange multipliers are updated using the same Adam optimizer, but now using a steepest ascent approach in order to enforce the constraints as tightly as possible. Thus, after each epoch, the Lagrange multipliers are updated to increase the cost as much as possible. This results in the network putting extra effort into ensuring that the constraints are met as the network approaches a stable solution. Finally, the learning rate is the scale factor by which the size of parameter updates is determined. When the gradient with respect to each parameter is calculated, the applied shift is multiplied by this factor, called the learning rate, and then added to the corresponding parameter. The learning

rates for the network and Lagrange parameters were tuned separately, with $\eta = 1 \times 10^{-2}$ for the network rate and $\zeta = 2 \times 10^{-4}$ for the Lagrange rate.

C.3 Convergence and Computational Weight

We did not enforce a hard convergence criterion on the networks to decide when to stop solving them. In the series phase, the networks were often seen to be far from stable convergence by the time solving was cut, especially for the tighter hole radii. Despite this, a hard limit of 30,000 epochs was set so as not to drag the phase on too long. During the parallel phases, nearly every network saw convincing convergence. Beyond about 300,000 epochs, the fluctuations in the evaluated bending energy of nearly every network dropped to less than $\sim 1\%$, and continued to fluctuate around the mean for the rest of the optimization period. On the scale of the bending energy plots in Chapter 4, this is an energy scale of about 0.02, which is slightly smaller than the standard deviation error bar approach implemented in the chapter. Seeing this, the networks were allowed to continue to optimize for about double their epoch count to 500,000 before ending the phase and finalizing the results. The only networks that failed to converge in this way were the $\bar{U} = 0.8$, $\bar{R} = 0.7$ vesicles that would often explode across the simulation box at some point during the optimization. The time for this to happen was highly unpredictable, which is why the results were not analyzed quantitatively during the analysis.

The next important point to discuss is the amount of computational resources spent on our analysis and the amount of computation performed. Using the Digital Alliance Beluga server as our benchmark, on a single node (1 GPU and 3 CPUs), our code solved the network at a rate of about 12,000 epochs/hour. Each snapshot’s network was optimized for 530,000 epochs across both the series phase and parallel phase. Given 25 snapshots per passage, 14 passages studied, and 3 trials per passage, the total number of epochs processed is roughly 550,000,000. Given our optimization rate, this converts to about 5.3 GPU years and about 16 CPU years (given 3 CPUs used per node). This is obviously an extreme amount of computational resources, but there are likely many optimizations that can be implemented to cut them down in future studies.

Assuming the network solving jobs are submitted immediately as they are needed to, simulating a single passage from scratch requires 750,000 epochs on 1 node for the series phase, and 500,000 epochs on 25 nodes for the parallel phase. Again, at the 12,000 epochs/hour rate mentioned above, the development of a single passage takes 104 hours, or a bit under 4.5 days to produce. The series phase here takes about 63 hours, and the parallel phase takes the remaining 41. Given that each passage can be modeled independently

without prerequisite from the other, this theoretically means that all the data could be reproduced over this same 4.5-day period. However, the parallel phase of this step would require an enormous number of GPU nodes and would likely need to be spread across multiple servers. Thus, by doing each passage one at a time to limit the rate of uploaded jobs to the Digital Alliance servers, the entire computational workload can be completed in 189 days, or about 6 months. All of the solving was done on the Digital Alliance Graham, Nibi, and Beluga servers. With our level of optimization, our solving rate was around 35,000 epochs/hour on the faster GPUs at Nibi and about 12,000 epochs/hour on the standard GPUs at Beluga and Graham.

The code used for this project was not developed solely by myself. A member of my group, Yousef Rohanizadegan, developed the first version of this code to model vesicles and test confinement. Using this, I restructured many aspects of the code to suit the process of translocation and tuned the various parameters and results to produce the results of this paper. For other work using the same NN-based solver of the Helfrich model, see the recent results by Rohanizadegan, Li, and Chen from 2024 [26].