On The Interaction And Motion Of Inclusions In Lipid Membranes

Xinyu Liao
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On The Interaction And Motion Of Inclusions In Lipid Membranes

Abstract
In this thesis, we analytically investigate three problems in the mechanics of lipid membranes and inclusions embedded in them. We study (a) irradiation-induced oxidation of membranes, (b) elastic and entropic interactions of inclusions on membranes due to bending and kinetics of their self-assembly, and (c) membrane thickness mediated interactions of inclusions and kinetics of self-assembly. Oxidative damage to cell lipid membranes is a ubiquitous phenomenon in biology that often leads to cell death. Oxidative damage occurs in two steps: per molecule area increase, followed by vesicle shrinkage due to formation of pores. We employ a model to study the first step which focuses on thermal fluctuations, tension-area relation and the change in per molecule area caused by irradiation. Our model makes predictions of vesicle shapes, adhesion kinetics and forces under various conditions of irradiation. These results may potentially be applied in photodynamic therapy where controlled oxidative damage is harnessed for killing diseased cells. Interactions between inclusions in lipid membranes is an important topic in biophysics as it can lead to self-assembly (of proteins) that influences a host of biological processes, including exo- and endo-cytosis. The rate of self-assembly of inclusions is of interest since interventions made at the right time could help to block the assembly of viruses. We develop a model based on theory of stochastic processes that casts self-assembly of two inclusions as a first passage time problem. A partial differential equation (PDE) is derived to compute the mean first passage time of self-assembly. The validity of the PDE is verified by running Langevin dynamic simulations to estimate the mean first passage time. Our methods provide new ways to study self-assembly which could complement existing methods based on molecular dynamics simulations. We separately study the interactions between inclusions caused by bending deformations and thickness deformations and use both the PDE and Langevin equation to compute the mean first passage time for self-assembly of variously shaped inclusions. Hydrodynamic interactions and rotational diffusion are also considered in our models. Finally, we apply our stochastic model mentioned above to compute the mean and second moment of the first passage time of MutSa protein searching for lesions through one-dimensional diffusion on DNA. The results could enhance the understanding of post-replicative mismatch repair (MMR) for fixing errors in DNA.

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ON THE INTERACTION AND MOTION OF INCLUSIONS IN LIPID MEMBRANES

Xinyu Liao

A DISSERTATION

in

Applied Mathematics and Computational Science

Presented to the Faculties of the University of Pennsylvania in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy

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ABSTRACT

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Xinyu Liao
Prashant K. Purohit

In this thesis, we analytically investigate three problems in the mechanics of lipid membranes and inclusions embedded in them. We study (a) irradiation-induced oxidation of membranes, (b) elastic and entropic interactions of inclusions on membranes due to bending and kinetics of their self-assembly, and (c) membrane thickness mediated interactions of inclusions and kinetics of self-assembly.

Oxidative damage to cell lipid membranes is a ubiquitous phenomenon in biology that often leads to cell death. Oxidative damage occurs in two steps: per molecule area increase, followed by vesicle shrinkage due to formation of pores. We employ a model to study the first step which focuses on thermal fluctuations, tension-area relation and the change in per molecule area caused by irradiation. Our model makes predictions of vesicle shapes, adhesion kinetics and forces under various conditions of irradiation. These results may potentially be applied in photodynamic therapy where controlled oxidative damage is harnessed for killing diseased cells.

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We separately study the interactions between inclusions caused by bending deformations and thickness deformations and use both the PDE and Langevin equation to compute the mean first passage time for self-assembly of variously shaped inclusions. Hydrodynamic interactions and rotational diffusion are also considered in our models.

Finally, we apply our stochastic model mentioned above to compute the mean and second moment of the first passage time of MutSα protein searching for lesions through one-dimensional diffusion on DNA. The results could enhance the understanding of post-replicative mismatch repair (MMR) for fixing errors in DNA.
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Chapter 1

Introduction

The lipid bilayer is the most common form of cell membrane in living species. It is a two dimensional fluid with polar head groups exposed to aqueous environments and hydrophobic tails made of hydrocarbon chains which group together to avoid the aqueous environment (see Fig 1.1). A lipid bilayer acts as a barrier, which controls the movements of ions, molecules, proteins across the cell, transporting nutrients into the cell and toxic materials out of the cell, and creates an ideal environment for organelles to perform biological functions and reactions inside the cell. The composition of lipids in species living in different environments are different in order to adapt to the local temperature and climate. In some unicellular species lipid bilayer even plays a role in endo- and exo-cytosis through folding and unfolding. Hence, it is important to keep the lipid membrane intact and functionally well for the survival of species. In the following we give a brief introduction to various problems
Figure 1.1: The structure of lipid bilayer. The polar headgroups are exposed to the aqueous environment, while the hydrocarbon tails are huddled together in the interior to avoid the aqueous environment. The figure is derived from https://www.chegg.com/learn/chemistry/organic-chemistry/lipid-bilayer-in-cell-membrane.

analyzed in this thesis which ultimately stem from maintaining the functionality of lipid membranes.

1.0.1 Mechanics of oxidatively damaged lipid membranes

Damage due to oxidation of lipid membrane could lead to cell death and even diseases like Alzheimer’s disease [1]. On the other hand, phototherapy is a medical treatment to cure diseases like seasonal affective disorder and hemolysis [2] by exposure to light or irradiation. For these reasons it is important to understand the chemical and mechanical response of lipid membranes to oxidation. Oxidation of
lipids consists of two steps. In the first step, there is a 15-20% increase in the area per molecule in the lipids due to the oxidized double bonds. In the second step, oxidation causes scission of lipid tails accompanied by pore formation that results in vesicle shrinkage. While a lot of research has focused on chemical changes to the lipid membranes oxidized by irradiation, the mechanical effects of these chemical changes have not been investigated. Similarly, many researchers have worked on the kinetics and mechanics of a vesicle detached from a substrate under micropipette aspiration (see Fig 1.2), few have paid attention to mechanics of lipid membranes during the irradiation-induced structural changes and micropipette aspiration. In the second chapter of this thesis, we use analytical models to investigate the mechanics of irradiated vesicles under various boundary conditions caused by adhesion, detachment and micropipette aspiration. The emphasis in this chapter is on mechanics and connections to experiment which is different from the rest of this thesis in which we delve into first passage time problems in lipid membranes from a mathematical perspective.

1.0.2 Self-assembly of proteins on lipid membranes

Numerous proteins embedded on cell membranes play important roles in cell functions (see Fig 1.3). For instance, they could exchange signals with other cells or connect them with each other. They also serve to maintain the structure and shape of the cell and provide localization information. These membrane proteins inter-
Figure 1.2: Top: A vesicle adhered to an endothelium cell (EC). Middle: The vesicle detached from the EC at the critical force $4 \pm 1$ nN. Bottom: The vesicle fully detached from the EC with a rate of loading $600 \pm 150$ pN/sec. The figure is obtained from [7].
Figure 1.3: Membrane proteins perform various functions and play important roles in regulating the cell cycle. The proteins self-assemble into various functional structures by diffusing on the cell membrane. The fact that self-assembly occurs suggests that there are attractive interactions between membrane proteins. We investigate mechanical and entropic origins of these interactions and analyze the rate of self-assembly due to these interactions. The figure is derived from https://ib.bioninja.com.au/standard-level/topic-1-cell-biology/13-membrane-structure/membrane-proteins.html.
act with each other and diffuse on lipid membranes. The interactions between inclusions could be thickness-mediated [9, 10, 11], bending deformation mediated [12, 13, 14, 15], entropic [16] and electrostatic [17]. Bending deformation has its origin in the mid-plane deflections of the lipid bilayer. The interaction energy expression for two inclusions based on bending deformations only has asymptotic forms for two circular or elliptic inclusions [12, 18] in an infinitely large membrane. Thickness deformation originates from bilayer thickness mismatch due to embedded proteins that alter the thickness of their nearby hydrophobic region (see Fig 1.4). The energy functional is given by [19, 10, 11] from which the Euler–Lagrange equation can be obtained which is a fourth-order partial differential equation. Then, the solution of thickness deformation field can be derived by solving a boundary value problem. In this thesis, we focus on membrane inclusion interactions dominated by bending and thickness deformations since they are common and can be computed efficiently using analytical and numerical methods. Under some conditions the interactions between inclusions could lead to self-assembly which is important in key life functions such as pore formation, budding of endo- and exo-cytotic vesicles.

The literature on the bilayer mediated interactions of proteins is quite big [13, 16, 10, 11, 21, 22, 23, 24, 25]. Here we give just a few examples that point to the importance of studying the type of problems we have tackled in this thesis (i.e., kinetics of self-assembly). [9] showed that under thermodynamic equilibrium protein monomers could self-assemble into different membrane protein oligomeric
Figure 1.4: Thickness deformation of lipid bilayer due to embedded proteins. The embedded proteins have hydrophobic and hydrophilic regions which align with hydrophobic and hydrophilic regions of the lipid membrane. If the thickness of the hydrophobic region of the protein is different from that of the lipid membrane then the membrane suffers thickness deformations which cost energy, states which results in different protein functions, but they do not pay attention to the time scales over which the assembly occurs. Self-assembly is also relevant to the infectivity of viruses. Using a statistical thermodynamics model, [26] found that icosahedral symmetry for viral capsids is not a direct consequence from self-assembly of protein subunits. Reversible self-assembly was discovered to spontaneously occur in several viruses including the tobacco mosaic virus [27] and sphere-like plant viruses [28], and the self-assembly of a viral protein on a vesicle is finished on a time scale of seconds [29] (see Fig 1.5). [30] used interferometric scattering microscopy to investigate the self-assembly process of individual MS2 bacteriophage capsids around their RNA. Their results showed that nucleation is a prerequisite for capsid self-assembly and the time scale of this process is a few hundred seconds. It is
Figure 1.5: This figure shows images of viral proteins self-assembling on a micron-sized vesicle. The proteins diffuse on the vesicle before they oligomerize. Notice the time scale over which self-assembly occurs – it is on the order of seconds. The figure comes from [29] which is one of the few experimental studies showing the time to assembly.

It is crucial to understand the kinetics of viral self-assembly pathways but it is difficult to measure due to the weak interactions between viral protein subunits [31] and a large span of timescales from less than one second [32] to hours [33]. There are some studies using simulations to determine the conditions of self-assembly of viruses [34, 23], but they say little about the rate of self-assembly. In this thesis we develop a partial differential equation for the mean first passage time of self-assembly of two inclusions on a lipid membrane. We can also simulate the self-assembly of multiple inclusions (of different sizes) and account for hydrodynamic interactions between them. Our PDE based method provides a fast way to compute the rate of self-assembly that is difficult to measure using experimental techniques.
1.0.3 Diffusion of proteins on DNA

The analytical machinery that we developed for solving first-passage time problems on two-dimensional lipid membranes can also be applied to one-dimensional DNA. Just as proteins diffuse on lipid membranes and allosterically interact with each other through deformations of the membranes itself, proteins diffuse on DNA and allosterically interact with each other through deformations of the DNA. An important class of such proteins are enzymes that correct errors in DNA sequence caused by mechanical or chemical damage. The post-replicative mismatch repair (MMR) apparatus is a collection of proteins that identify and correct errors in DNA. In eukaryotes MutSα and MutLα are core members that perform MMR. In the first step, MutSα finds DNA mismatch sites through 1D sliding or 3D diffusion. Then MutLα locates mismatch-bound MutSα through 1D hopping or 3D diffusion \[35\] (see Fig 1.6). Apart from MMR, CRISPR-Cas is another system of proteins that trigger immune responses in bacteria \[36, 37\]. CRISPR-Cas is classified as two types based on the type of effector proteins \[38\]. Cas12a is one of Class 2 type proteins that plays an important role in genome editing. It is found that Cas12a facilitated its target search through 1D diffusion \[36\]. These two examples show that 1D diffusion is a crucial path for enzymes to conduct biological functions in DNA. In this thesis, we focus on the kinetics of target search by MutSα through 1D-sliding. An important aspect is that we consider allostERIC interaction as well as applied tension in our model. The former diminishes after a few tens of base-pairs
while the effect of the latter can be felt over long distances. We view the DNA target search as a 1D first passage time problem and we compute mean and second moment of the first passage time assuming various diffusion coefficients and applied tensions. The results could help to understand under which conditions DNA target search process could be sped up or slowed down. Concurrently, we also show that our PDE based method to compute first passage times can also predict the second moment (or variances) of the first passage time under various conditions.

1.0.4 Organization of this thesis

This thesis is organized as follows. In Chapter 2, we study the mechanics of irradiated lipid vesicles. In Sec. 2.2 we revisit an analytical framework employed by [39] which focuses on adhered axisymmetric vesicles. Then, we extend this model to the scenario when irradiation is present. Irradiation changes the tension-area relation of the lipid membrane by increasing the area per molecule and altering the bending modulus. We compute the shape of vesicles in equilibrium under several conditions like adhesion, irradiation, tensile/compressive forces and compare them with experimental results. In Sec. 2.3, we move to the kinetics of the adhesion process of vesicles under irradiation. Following the work in [40], we can derive an ordinary differential equation for the contact radius of the adhered vesicles under irradiation. This provides an efficient way to compute the vesicles shape change when irradiation is applied. In Sec. 2.4, we analyze the detachment of the vesicle
Figure 1.6: This figure shows how MutSα searches the mismatch site or lesion, and is released from MutSα-MutLα complex after ATP hydrolysis is done to repair the mismatch site. The important point is that 1D diffusion along DNA is a mechanism for the proteins to perform their search for targets along the DNA. We investigate how allostERIC interactions due to deformation of the DNA and due to applied tension affect the time to find the target. The figure comes from [35].
subjected to irradiation. Particularly we focus on the critical force which leads to the detachment.

In Chapter 3, we develop a model based on the theory of stochastic processes to study the self-assembly of inclusions on a lipid membrane that can be viewed as a first passage time problem. In Sec. 3.2, we first compute the free energy of elastic and entropic interactions between two circular inclusions using a semi-analytic method based on Gaussian integrals. Then, we use a Langevin equation to model the self-assembly of two inclusions as a stochastic process with a stopping time at the inner boundary of the lipid membrane marking the occurrence of self-assembly. The mean first passage time which characterizes the rate of the self-assembly can be estimated using Langevin equations. We also derive an ODE for the mean first passage time for two circular inclusions, the solution of which is in excellent agreement with the result from Langevin equations. In Sec. 3.3, we focus on the self-assembly of two ellipse shaped inclusions. We first fit the interaction free energy using an analytical expression derived by [18]. Then, we derive a PDE for the mean first passage time of self-assembly between two anisotropic inclusions which can be regarded as a general version of the ODE for the isotropic case. The accuracy of the PDE has been verified once again by comparing to the result of Langevin equations. Rotational diffusion of anisotropic inclusions can also be considered in our model. In Sec. 3.4, we add hydrodynamic interactions of the inclusions. The result shows that hydrodynamic interactions could speed up self-
assembly. Finally, in Sec. 3.5 we use Langevin equations to study the self-assembly of multiple inclusions.

In Chapter 4, we extend our model developed in Chapter 3 to study interactions between inclusions caused by thickness deformations of lipid membranes. In Sec. 4.2, we develop a finite difference scheme based on equilateral triangular grids to compute the interaction energy between two inclusions. The shape of inclusions considered in our model includes hexagon-shaped, ellipse-shaped and star-shaped and the energy calculated by our finite difference scheme is in excellent agreement in most cases with the result using analytical solution derived by [10, 11]. In Sec. 4.3 and 4.4, we extend the ODE and PDE developed in Chapter 4 to account for a more physically realistic boundary condition: an absorbing wall at the inner boundary and a reflecting wall at the outer boundary. In Sec. 4.5 we discuss our simulation results in the context of other work in the field.

In Chapter 5, we apply the framework of the previous chapters to a one-dimensional scenario concerning MutSα searching DNA mismatch sites through 1D sliding. In Sec. 5.2, we fit the interaction free energy between two DNA-binding proteins as a function of distance separating them as in [41] using an analytical expression. Allosteric interactions are considered in our energy expression since it plays a role in short-range interactions (a few tens base-pairs). In Sec. 5.3 we compute the first and second moment of the first passage time for MutSα target search. We compare our results with the experimental data on the DNA target
search time by MutSα in [35]. Then, we compute the first and second moment of
the first passage time under various applied tensions and diffusion coefficients.

In Chapter 6, we briefly summarize the findings in this thesis from Chapter 2 to Chapter 5 and point to future directions.

In Appendix A to Appendix G, we attach proofs for all the theorems that appeared in this thesis as well as important calculation steps.
Chapter 2

Mechanics of irradiation induced structural changes in a lipid vesicle


2.1 Introduction

Oxidative damage to lipids in cell membranes is increasingly being implicated as a cause of cell death, diseases and ageing since at least the early 1990s [5, 43]. Controlled oxidative damage has also been used in treatment of certain diseases by a
procedure called photodynamic therapy. The treatment induces oxidative damage in diseased cells through the use of photosensitizer, light and oxygen in a proper combination [44, 5]. The target for oxidation are poly-unsaturated lipids, or lipids with double-bonds in their hydrocarbon chains [3, 4, 44]. Even though in experiments and in photodynamic therapy reactive oxygen is produced using photosensitive molecules these are also generated enzymatically and through respiration [5] during normal life processes. As such, much is known about the chemical changes to the lipid molecules due to oxidation [4, 5, 6], but little attention has been paid to the mechanics of lipid membranes subjected to irradiation induced oxidation. In particular, irradiation results in time varying mechanical properties of vesicles [45], yet there are no models that account for this variation in vesicle mechanics.

Oxidative damage to lipids is known to proceed in two major steps [3]. In the first step oxidation of double bonds causes an increase in the area per molecule of the lipid membrane by about 15-20% [4]. This is accompanied by a decrease of area expansion modulus $K_A$ and an increase in thermal undulations [45]. In the second step (whose effects are prominent if irradiation continues for long periods) scission of the hydrocarbon chain may occur and vesicle shrinkage occurs due to formation of pores [3]. The kinetics of both these oxidation steps can be described as first order reactions [6] and the rate constants appearing in them have been fitted to experimental data [3]. However, the mechanics of a vesicle under simultaneous action of forces and irradiation remains to be investigated even though it plays an
important role in cell membrane functions \[46\]. For example, membrane tension is known to regulate exo- and endo-cytosis, actin network assembly and motility in live cells \[47\] and may also contribute to cell growth \[48\]. Therefore, it is conceivable that changes in membrane tension caused by irradiation induced area increases could affect cellular processes.

In this paper we will build on previous experimental studies and develop a model to analyze vesicle shapes and mechanics under the action of forces and irradiation. Since adhesion and micropipette aspiration are routinely used in experimental measurements we will focus on vesicle shapes in these assays. We will analyze both equilibrium and kinetics of irradiation induced shape changes and take account of thermal fluctuations which contribute significantly to membrane biophysical behavior \[49\]. We will constrain our analysis to the first step of oxidation which involves structural changes that increase area and decrease elastic moduli, and to axisymmetric vesicles since these are analytically tractable \[50\], \[51\]. The mechanics in the second step of oxidation is more challenging and is not treated here since it involves lipid hydrocarbon chain scission and pore formation which are amenable to molecular simulation. Our model connects vesicle shape, mechanics, and kinetics of irradiation induced structural changes within a tractable analytical framework that can be used to interpret experiments and generate falsifiable predictions. We are not aware of any other models that accomplish this even though there are detailed analyses of the kinetics of lipid oxidation \[52\], \[53\], and a multitude of papers
that predict vesicle shape changes in response to applied forces or other mechanical constraints [53, 39, 54]. In the following we consider (a) equilibrium shapes of vesicles under the action of forces and irradiation induced structural change in section 2, and (b) the kinetics of shape evolution due to loading and irradiation induced structural change in sections 3 and 4. We give a brief conclusion in section 5.

2.2 Equilibrium shapes of adhered vesicles under irradiation

2.2.1 Review of equations to compute shapes of adhered vesicles under applied force

To see how area changes due to irradiation enter into the computation of vesicle shape and mechanics we must first review the mechanics of adhered vesicles. Consider a vesicle that is originally spherical with radius $R_0$. The vesicle is surrounded by fluid (usually water for lipid vesicles) that may contain osmolytes to control its volume [53]. When it adheres to a surface it remains axisymmetric but might not be spherical. Hence, the three-dimensional shape of the vesicle can be described by a closed curve in two dimensions with an axis of rotational symmetry [55]. Then, the pressure difference $p$ is related to the tension $\tau$ and local mean curvature through
the Young-Laplace law:

\[ p = 2\tau H = \tau(\kappa_1 + \kappa_2), \quad (2.2.1) \]

where \( \kappa_1 \) is the meridional principal curvature, \( \kappa_2 \) is the principal curvature along lines perpendicular to the meridians and \( H = \frac{\kappa_1 + \kappa_2}{2} \) is the mean curvature. In experiments the deformation of the vesicle happens in such a way as the volume remains fixed (due to osmotic constraints), so the pressure difference \( p \) must change and we will determine it using the Young-Laplace law given above.

Adhesion changes the tension in the membrane and also causes an increase in the area by \( 1 - 2\% \) \([40, 56]\). However, this area change is not taken into account in most analyses of adhering vesicles \([8, 39, 55]\). Typically, in experiments the tension is in the region where the fluctuations of the vesicle get pulled out to cause the increase in area. Let \( A \) be the area of the membrane in the absence of fluctuations and tension.

Let \( A_{\text{red}}(\tau) \) represent the amount by which the projected area is reduced below the area \( A \) by thermal fluctuations at tension \( \tau \) and temperature \( T \), then \([57, 55]\)

\[ \frac{A_{\text{red}}(0) - A_{\text{red}}(\tau)}{A} = \frac{k_B T}{8\pi K_b} \log(1 + \frac{\tau A}{\pi^2 K_b}) + \frac{\tau}{K_A}, \quad (2.2.2) \]

where \( k_B \) is the Boltzmann constant, \( K_b \) is the bending modulus of the membrane and \( K_A \) is the stretching or area expansion modulus. This relation is obtained by using equipartition theorem of statistical mechanics which says that every normal mode of the fluctuating membrane has average energy \( \frac{k_B T}{2} \) at equilibrium.

Now, let us assume that the vesicle is adhered to a substrate and a force \( F \) is exerted on it by a micropipette while it remains axisymmetric. \( F > 0 \) when the
force is pushing the vesicle against the substrate. If we make a cut perpendicular to
the axis of the vesicle where the radius is \( r(s) \) and the tangent angle to the contour
of the axisymmetric shape is \( \phi(s) \), \( s \) being a position parameter along the arc-length
of the closed curve, then equilibrium demands that

\[
2\pi r(s) \sin \phi(s) = \pi r^2(s)p - F. \tag{2.2.3}
\]

The vesicle makes contact with the substrate over a circular region of radius \( r_1 \).
The angle \( \phi_1 \) at \( r = r_1 \) depends on the adhesion energy per unit area between the
vesicle and the substrate. Springman and Bassani \[58\] shows that adhesion energy
is not uniform everywhere and depends on the \( r \). However, for simplicity we assume
the adhesion energy is concentrated at \( r = r_1 \) and use \( \Gamma \) to denote the adhesion
energy per unit area, then

\[
\Gamma = \tau (1 - \cos \phi_1), \tag{2.2.4}
\]

which is known as the Young-Dupre equilibrium \[39\]. To get the vesicle shape we
note that

\[
\frac{dr}{ds} = \cos \phi(s) = \sqrt{1 - \sin^2 \phi(s)}, \quad \frac{dz}{ds} = \sin \phi(s), \tag{2.2.5}
\]

where \( \sin \phi(s) \) is given by Eq (2.2.3) in terms of \( r(s) \) and \( F \), and \( z(s) \) is the height
of point \( s \) with \( z(0) = 0 \) at the equator of the vesicle. We can therefore integrate
the differential equation for \( r(s) \) and get

\[
r(s) = \frac{\sqrt{2\tau}}{p} \sqrt{1 + \frac{Fp}{2\pi\tau^2} + \sqrt{1 + \frac{Fp}{\pi\tau^2} \cos \frac{ps}{\tau}}}, \tag{2.2.6}
\]

20
Now, we claim that the term $\kappa_1 + \kappa_2$ in Eq (2.2.1) is a constant. As pointed out in standard texts in differential geometry \[59\] the mean curvature \( \frac{\kappa_1 + \kappa_2}{2} \) equals a constant \( c_0 \) for an axisymmetric shape if and only if

\[
\frac{d[r(dz)]}{ds} = c_0 \frac{d(r^2)}{ds}.
\] (2.2.7)

By using Eq (2.2.3) and Eq (2.2.5), it is easy to show that Eq (2.2.7) is equivalent to

\[
\frac{p}{2\tau} = c_0,
\] (2.2.8)

which implies that $\kappa_1 + \kappa_2 = \frac{p}{\tau}$. Therefore, the Young-Laplace law Eq (2.2.1) is automatically satisfied on the non-adhered part of the vesicle. Let \( s = s_1 \) be such that \( r(s_1) = r_1 \) and \( \phi(s_1) = \phi_1 \) at the bottom of the vesicle where adhesion occurs. Then the expression for \( s_1 \) becomes:

\[
\cos \frac{ps_1}{\tau} = \frac{\frac{p^2 r_1^2}{2\tau^2} - 1 - \frac{Fp}{2\pi \tau^2}}{\sqrt{1 + \frac{Fp}{\pi \tau^2}}}.
\] (2.2.9)

From Eq (2.2.9) we get

\[
s_1 = -\frac{\tau}{p} \cos^{-1} \left( \frac{\frac{p^2 r_1^2}{2\tau^2} - 1 - \frac{Fp}{2\pi \tau^2}}{\sqrt{1 + \frac{Fp}{\pi \tau^2}}} \right).
\] (2.2.10)

We must solve for $\sin \phi_1$ using the Young-Dupre equilibrium Eq (2.2.4)

\[
\sin \phi_1 = \sqrt{1 - \left( 1 - \frac{\Gamma}{\tau} \right)^2} = \frac{pr_1}{2\tau} - \frac{F}{2\pi r_1 \tau}.
\] (2.2.11)

By using Eq (2.2.11), \( r_1 \) can be solved,

\[
r_1 = \frac{\tau}{p} \left( \sin \phi_1 + \sqrt{\sin^2 \phi_1 + \frac{Fp}{\pi \tau^2}} \right).
\] (2.2.12)
At the top of the vesicle \( r = r_2 \). If \( s = s_2 \) at the top of the vesicle then it can be determined using

\[
s_2 = \frac{\tau}{p} \cos^{-1} \left( \frac{p^2 r_2^2 - 1 - \frac{F p}{2\pi \tau^2}}{\sqrt{1 + \frac{F p}{2\pi \tau^2}}} \right). \tag{2.2.13}
\]

It should be noted that \( 1 + \frac{F p}{2\pi \tau^2} > \sqrt{1 + \frac{F p}{\tau^2}} \), which implies that \( r_2 \neq 0 \) if \( F \neq 0 \).

Integrating Eq (2.2.5) for \( z(s) \) we get

\[
z(s) = \int_0^s \sin \phi(s) \, ds = \int_0^s \left( \frac{p r(s)}{2\tau} - \frac{F}{2\pi \tau r(s)} \right) \, ds, \tag{2.2.14}
\]

where \( r(s) \) is given by Eq (2.2.6) above. We substitute for \( r(s) \) to get

\[
z(s) = \frac{1}{\sqrt{2}} \int_0^s \sqrt{1 + \frac{F p}{2\pi \tau^2} + \sqrt{1 + \frac{F p}{\tau^2}}} \, ds
- \frac{F p}{2\sqrt{2\pi \tau^2}} \int_0^s \frac{ds}{\sqrt{1 + \frac{F p}{2\pi \tau^2} + \sqrt{1 + \frac{F p}{\tau^2}}} - 2\sqrt{1 + \frac{F p}{\tau^2} \sin^2 \frac{ps}{2\tau}}} \tag{2.2.15}
\]

This can be reduced to

\[
z(s) = \frac{\tau}{p} \left( 1 + \sqrt{1 + \frac{F p}{\tau^2}} \right) \int_0^{\frac{ps}{2\tau}} \sqrt{1 - m^2 \sin^2 \theta} \, d\theta
- \frac{F}{\pi \tau} \frac{1}{1 + \sqrt{1 + \frac{F p}{\tau^2}}} \int_0^{\frac{ps}{2\tau}} \frac{d\theta}{\sqrt{1 - m^2 \sin^2 \theta}}, \tag{2.2.16}
\]

where

\[
m^2 = \frac{2\sqrt{1 + \frac{F p}{\tau^2}}}{1 + \frac{F p}{2\pi \tau^2} + \sqrt{1 + \frac{F p}{\tau^2}}} \leq 1, \tag{2.2.17}
\]

Recognizing the incomplete elliptic integrals above we write

\[
z(s) = \frac{\tau}{p} \left( 1 + \sqrt{1 + \frac{F p}{\tau^2}} \right) E \left( \frac{ps}{2\tau} | m \right)
- \frac{F}{\pi \tau} \frac{1}{1 + \sqrt{1 + \frac{F p}{\tau^2}}} F \left( \frac{ps}{2\tau} | m \right), \tag{2.2.18}
\]
where $E\left(\frac{ps}{2\tau}\mid m\right)$ is the incomplete elliptic integral of the second kind with modulus $m$ and $F\left(\frac{ps}{2\tau}\mid m\right)$ is the incomplete elliptic integral of the first kind with modulus $m$. We use Eq (2.2.6) and Eq (2.2.18) to plot vesicle shapes later in the paper.

When $F = 0$, from Eq (2.2.12) and Eq (2.2.9) we see that

$$r_1 = \frac{2\tau}{p} \sqrt{\frac{2\Gamma}{\tau} - \frac{\Gamma^2}{\tau^2}};$$

$$\cos \frac{ps_1}{\tau} = 2 \left( \frac{2\Gamma}{\tau} - \frac{\Gamma^2}{\tau^2} \right)^{-1}.$$ (2.2.20)

Hence, the shape of the adhered vesicle is described by

$$r(s) = \frac{2\tau}{p} \cos \frac{ps}{2\tau},$$

$$z(s) = \frac{2\tau}{p} \sin \frac{ps}{2\tau},$$

$$s_1 = -\text{sgn} (\cos \phi_1) \frac{\tau}{p} \cos^{-1} \left( \frac{4\Gamma}{\tau} - \frac{2\Gamma^2}{\tau^2} - 1 \right),$$

$$s_2 = \frac{\tau}{p} \cos^{-1} \left( \frac{p^2 r_2}{2\tau^2} - 1 \right).$$ (2.2.24)

Now, let us consider the case of a spherical vesicle of radius $R_0$ about to adhere to a substrate. The vesicle is a spherical section of radius $R$ after adhesion and its shape is given by Eq (2.2.21) - Eq (2.2.24). Then the volume is easily computed:

$$V = \frac{1}{2} \pi \left( \sqrt{R^2 - r_2^2} + \cos \phi_1 R \right) \cdot \left( R^2 \sin^2 \phi_1 + r_2^2 + \frac{1}{3} \left( \sqrt{R^2 - r_2^2} + \cos \phi_1 R \right)^2 \right),$$ (2.2.25)

where $R = \frac{2\tau}{p}$ is derived from the Young-Laplace law Eq (2.2.1). Similarly, the surface area of the spherical section can be computed:

$$A_{sp} = 2\pi R \left( \sqrt{R^2 - r_2^2} + \cos \phi_1 R \right).$$ (2.2.26)
The area of the circular portion in contact with the substrate is \( \pi R^2 \sin^2 \phi_1 \). Let \( A_0 \) be the contour area when the vesicle is a perfect sphere. Then we have \( A_0 = A_{\text{red}}(0) + 4\pi R_0^2 \). If the changes in area are due to stretching out of thermal fluctuations then \( A_{\text{red}}(0) = A_0 \frac{k_B T}{8\pi K_b} \log \frac{A_0}{b^2} \). Hence, we can compute \( A_0 \) through
\[
4\pi R_0^2 = A_0 \left(1 - \frac{k_B T}{8\pi K_b} \log \frac{A_0}{b^2}\right), 
\]
(2.2.27)
where \( b = 1 \) nm is intermolecular spacing \[55\].

### 2.2.2 Application to an adhered vesicle

We will now use the ideas above to estimate the adhesion energy per unit area, \( \Gamma \), from experimental data. In an experiment conducted by Aoki et al. \[60\], the radius of a 1,2-dioleoyl-sn-glycero-3-phosphatidyl-choline (DOPC) giant unilamellar vesicle (GUV) after adhesion is estimated to be \( R = 1.73 \times 10^{-5} \) m (using the scale bar in figure 1c of \[60\]). We will take stretching modulus \( K_A = 230 \) mN \cdot m\(^{-1}\) and bending modulus \( K_b = 21k_B T = 21 \times 4.1 \) pN \cdot nm at \( T = 300 \) K as typical values for DOPC vesicles \[45, 55\]. Then, \( A_0 \) can be numerically solved from Eq (2.2.27).

We assume there is no thermal fluctuation in the part of the vesicle attached to the substrate. Let \( A^f \) denote the contour area of the fluctuating part of the vesicle (\( A^f \) would be the area at \( T = 0 \)), then we have \( A^f = A_0 - \pi R^2 \sin^2 \phi_1 - \pi r_2^2 \). Under tension \( \tau \), \( A_{\text{red}}(\tau) \) can be computed through
\[
A_{\text{red}}(\tau) = A^f - 2\pi R \left(\sqrt{R^2 - r_2^2} + \cos \phi_1 R\right). \quad (2.2.28)
\]
At present, $\phi_1$, $R_0$ and $\tau$ are all unknown. They will be determined by the Young-Dupre equilibrium Eq (2.2.4), tension area relation and volume constraints, respectively, through

$$
\frac{A^t k_B T}{8\pi K_b} \log \frac{A^t}{\ell^2} = A^t + 2\pi R \left( \sqrt{R^2 - r_2^2} + \cos \phi_1 R \right) = \frac{k_B T}{8\pi K_b} \log \left( 1 + \frac{\tau A^t}{\pi^2 K_b} \right) + \frac{\tau}{K_A},
$$

(2.2.29)

$$
\frac{4}{3} \pi R_0^3 = \frac{1}{2} \pi \left( \sqrt{R^2 - r_2^2} + \cos \phi_1 R \right)
\cdot \left( R^2 \sin^2 \phi_1 + r_2^2 + \frac{1}{3} \left( \sqrt{R^2 - r_2^2} + \cos \phi_1 R \right)^2 \right).
$$

(2.2.30)

Here we take $r_2 = 0$ since there is no pipette or adhering surface on the top of the vesicle. Now, Eq (2.2.29), Eq (2.2.30), Eq (2.2.4) are three equations for three unknowns $R_0$, $\tau$ and $\phi_1$ if the adhesion energy per unit area $\Gamma$ is given. However, $\Gamma$ is not immediately known from the experiments of Aoki et al. [60] who report that $\frac{\Gamma}{R_0}$ lies between 0.5 ~ 0.7 when there is no irradiation. Using this information $R_0, \tau, \phi_1$ can be numerically computed by choosing $\frac{\Gamma}{R_0}$ to be in this range and combining with Eq (2.2.29), Eq (2.2.30). Then, $\Gamma$ is estimated from Eq (2.2.4). We find that $\Gamma$ is between $10^{-10}$ N/m ~ $10^{-7}$ N/m. Other experimental and theoretical works [7, 40] estimate that $\Gamma$ mostly lies between $10^{-5}$ N/m ~ $10^{-2}$ N/m. Trial and error reveals that the estimated $\Gamma$ is quite sensitive to changes in $\frac{\Gamma}{R_0}$. To make progress, we take $\Gamma = 2.53 \times 10^{-5}$ N/m which corresponds to $\frac{\Gamma}{R_0} \approx 0.8$ for experiments in Aoki et al. [60]. The uncertainty in $\Gamma$ is probably also the reason why Sankhagowit et al. [3] measure changes in area using optical traps rather than adhesion.
2.2.3 Shapes of adhered vesicles before and after irradiation with zero force

Once the three unknowns \( R_0 \), \( \tau \) and \( \phi_1 \) have been solved we can plot the shape of an adhered vesicle using eqns. (2.2.21)-(2.2.24). Next, we must account for increases in vesicle surface area caused by irradiation. Let \( A \) be the area after irradiation which can be represented as

\[
A = A_0 \times \alpha \quad (\alpha > 1).
\]

(2.2.31)

In Aoki et al. \cite{60} the maximum surface area ratio is \( \alpha = 1.1801, 1.1878, 1.1936 \) under irradiation powers 1/4, 1/32, 1, respectively. Here, 1 represents maximum radiation intensity and we refer the reader to Aoki et al. \cite{60} for quantitative details. We take \( \alpha = 1.1878 \) in Eq (2.2.31) as an example. We are not aware of measurements of \( K_b \) as a vesicle suffers oxidation. However, \cite{55} points out that \( K_b \) is proportional to \( K_A \), or \( K_b = \zeta K_A \) where \( \zeta \) is a constant depending on the bilayer thickness. The bilayer thickness is determined by the length of the hydro-carbon chains in the lipid tails which does not change in the first step of oxidation before chain scission happens. Hence, from the known values of \( K_A \) after irradiation is completed we can estimate the \( K_b \) of oxidized vesicles. Using \( K_A = 80 \text{ mN} \cdot \text{m}^{-1} \)\cite{45} after irradiation for DOPC, the corresponding \( K_b = 7.3 k_B T \). Note that \( A_0 \) is dependent on \( K_b \) through Eq (2.2.27), hence it will change when \( K_b \) is changed. Then, with \( \Gamma \) known from the previous section we can solve for the new values of \( R, \phi_1 \) and \( \tau \) in the
adhered vesicle after irradiation. Accordingly, the adhered vesicle shapes before and after irradiation can be plotted using eqns. (2.2.21)-(2.2.24). The shapes are shown in Figure 2.1.

![Figure 2.1](image)

Figure 2.1: Computed vesicle shapes before (solid line) and after (dashed line) irradiation for parameters in Aoki et al. [60]. These shapes are in qualitative agreement with shapes in Aoki et al. [60] validating our model.

In both situations (with and without irradiation), the shapes of the vesicle adhering to the substrate under no force in the experiments of Aoki et al. [60] are qualitatively described by our model.
2.2.4 Shapes of adhered vesicles under tensile and compressive forces after irradiation

Tensile forces are exerted on adhered vesicles (with known mechanical properties) to estimate the adhesion energy density $\Gamma$ on various substrates [8], while compressive forces are exerted on cells to estimate membrane tension [17]. The changes of vesicle (or cell) shape under these circumstances can be measured and used to estimate $\Gamma$, $\tau$, etc. In a previous work Lin and Freund [39] studied the shapes of an adhered vesicle under pulling force in the absence of irradiation. Our goal here is to determine how shapes change when both pulling force and irradiation are applied. Following the assumptions of deGennes [8], we consider a vesicle bound on one side to a pipette with fixed pipette radius $r_2 = 0.2R_0$ and constant contact angle $\phi_1 = \pi/4$. The height of the cylindrical portion of the vesicle in the pipette, $h$, is adjustable [61]. For convenience, we assume the top of the vesicle is flat and that there is no thermal fluctuation in the part of the vesicle inside the pipette and the part that is adhered. When $F \neq 0$, the constraint equations are only the tension-area relation and volume constraint. Since $\Gamma$ is given and $\phi_1$ is fixed, therefore $\tau$ can easily be obtained from Eq (2.2.4). Thus, Eq (2.2.29) and Eq (2.2.30) are respectively replaced by,

$$\frac{A_t}{8\pi K_b} \frac{k_B T \log \frac{A_t}{b^2}}{A_t} - \left( A_t - 2\pi \int_{s_1}^{s_2} r(s) ds \right) = \frac{k_B T}{8\pi K_b} \log \left( 1 + \frac{\tau A_t}{\pi^2 K_b} \right) + \frac{\tau}{K_A}, \quad (2.2.32)$$

$$\frac{4}{3} \pi R_0^3 - \pi r_2^2 h = \tau^3 \frac{3}{p^3} \pi \left( 1 + \sqrt{1 + \frac{Fp}{\pi \tau^2}} \right)^3 \int_{\frac{p^2}{2\tau}}^{\frac{p^2}{p^2}} \left( \sqrt{1 - m^2 \sin^2 \theta} \right)^3 d\theta$$
\[-\frac{F_τ}{p^2} \left( 1 + \sqrt{1 + \frac{Fp}{\pi τ^2}} \right) \int_{\frac{ρ_2}{2τ}}^{\frac{ρ_2}{2τ}} \sqrt{1 - m^2 \sin^2 θ} dθ, \quad (2.2.33)\]

where the area of the fluctuating part of the vesicle $A_t$ in Eq (2.2.32) is replaced by,

$$A_t = A - (\pi r_1^2 + \pi r_2^2 + 2πr_2h). \quad (2.2.34)$$

Recall that $r_2$ is the radius of the flat region of the vesicle inside the pipette. We have two equations Eq (2.2.32) and Eq (2.2.33) for two unknowns $p$ and $h$, which can be solved numerically.
Figure 2.2: (a) Prediction of dependence of dimensionless contact radius $r_1/R_0$ on dimensionless pulling force $F/pR_0^2$ under irradiation. (b) Prediction of shapes during detachment. In (b) the height of the vesicle inside the pipette is not fully shown to focus on vesicle shapes. The heights inside the pipette at $A_1, B_1, C_1$ are, respectively, $h_{A_1}/R_0 = 3.73$, $h_{B_1}/R_0 = 3.65$, $h_{C_1}/R_0 = 3.89$. The non-monotonic dependence of $r_1$ on $F$ is similar to Lin and Freund [39].
Figure 2.3: (a) Prediction of dependence of dimensionless contact radius $r_1/R_0$ on dimensionless pushing force $F/pR_0^2$ and dependence of dimensionless pressure difference $p/p_0$ on $F/pR_0^2$ under irradiation where $p_0$ is the pressure difference in the absence of force. (b) Prediction of shapes during compression. In (b) the height of the vesicle in the pipette are not fully shown to focus on vesicle shapes. The heights inside the pipette at $A_2, B_2, C_2$ are, respectively, $h_{A_2}/R_0 = 3.73$, $h_{B_2}/R_0 = 3.49$, $h_{C_2}/R_0 = 2.96$. 
Once we solve for $p$ and $h$, we can plot the shapes of the vesicle for both $F < 0$ (tensile force causing detachment) and $F > 0$ (compressive force causing adhesion). In Fig. 2.2(b) we plot the shapes when a tensile force $F$ is applied. The corresponding contact radii are plotted in Fig. 2.2(a). Similarly, in Fig. 2.3 we plot the shapes and the corresponding contact radii when a compressive force $F$ is applied. The predictions in these figures can be tested with routine micro-pipette experiments.

For $F < 0$, there are three important points $A_1$, $B_1$ and $C_1$, where $A_1$ represents the start of the detachment process when the contact radius is maximum, $B_1$ represents the state of maximum achievable detachment force, which is $F_{cr} \approx 0.43 \ pR_0^2$ compared to $F_{cr} \approx 0.30 \ pR_0^2$ in the case without irradiation studied by Lin and Freund [39]. Since the non-dimensional initial contact radius $r_1/R_0 \approx 0.7$ in our work is slightly larger than the one $r_1/R_0 \approx 0.6$ in [39], it requires stronger force to detach the vesicle from the substrate after irradiation that increases contact area. In addition, $C_1$ represents the state when detachment is about to occur. As pointed out by Lin and Freund [39] the vesicle is in stable equilibrium from $A_1$ to $B_1$ while from $B_1$ to $C_1$ it is not. As for $F > 0$ we found the pressure difference and contact area monotonically increase as the compressive force increases, as expected.
2.3 Kinetics of attachment of vesicle under irradiation

So far, we have gained some understanding of how vesicle shapes and mechanics can be modulated by adhesion, applied forces and irradiation. Our methods can be used to estimate difficult to measure quantities (i.e., pressure difference, adhesion energy, tension) when other experimental observables (i.e., contact radius, vesicle height sucked into pipette) are measured under various applied forces and irradiation intensities. Next, we hope to garner some insights into the kinetics of processes, such as, adhesion and oxidation, that happen simultaneously in experiments.

2.3.1 Oxidation kinetics and corresponding area increase

In this section, we study a palmitoyl-oleoyl-phosphatidyl-choline (POPC) vesicle with radius \( R_0 = 0.75 \times 10^{-5} \, \text{m} \) under irradiation at constant intensity. In the experiment conducted by Weber \textit{et al.} \[45\], the top of the vesicle is sucked into a micropipette with radius \( r_2 = 0.47 \, \mu\text{m} \) and the constant membrane tension is fixed at 0.7 mN m\(^{-1}\). Since there is no adhesion, the contact radius \( r_1 = 0 \).

Sankhagowit \textit{et al.} \[3\] illustrated that oxidation has two stages where OX1 is an oxidized lipid which is the product of the first reaction and it occupies more membrane area than non-oxidized DOPC and OX2 is the product of the second reaction, which occupies less surface area than DOPC. For simplicity, we assume
only OX1 appears during the irradiation and the model built for DOPC could be applied to POPC, since both have double bonds. Also, the bending modulus of POPC is about $20.7 k_B T$ at room temperature $T = 303 \text{ K}$ [62] that is not too different from the bending modulus of DOPC. The second reaction which involves OX2 is known to be slower than that involving OX1 and its effects (decrease in area) are seen when irradiation is continued for a long time [3]. We will confine our analysis to only the early stages of irradiation when area increases are seen.

Sankhagowit et al. [3] gives the formula to compute the concentration of OX1,

$$C_{OX1}(t) = 1 - e^{-k_1 t}, \quad (2.3.1)$$

where $k_1$ scales roughly as $I^{1/2}$ where $I$ is the radiation intensity or radiation power.

Let $A_{OX1}$ be the area per lipid of OX1 relative to that of POPC (i.e. $A_{POPC} = 1, A_{OX1} > 1$). Let $S(t)$ be the the ratio of the surface area increment at time $t$ to the original membrane area. Then it can be expressed as,

$$S(t) = A_{POPC}(1 - C_{OX1}) + A_{OX1}C_{OX1} - 1 = (A_{OX1} - 1)(1 - e^{-k_1 t}). \quad (2.3.2)$$

This relation is obtained from a ‘rule of mixtures’ applied to oxidized and unoxidized lipids which have different area per headgroup. When the irradiation time is sufficiently long, the surface area will reach $\max_{t \geq 0} S(t)$ and then not change. This requires that $A_{OX1} = \max_{t \geq 0} S(t) + 1$. In order to be consistent with the experimental data in Fig. 3(A) of Weber et al. [45], we need to set $A_{OX1} = 1.1388, k_1 = 0.1979 \text{ s}^{-1}$, as shown in Fig 2.4(a) by the orange line. Note that the value of $k_1$ used here
is in the same range as those reported in Sankhagowit et al. The good fit of the orange curve to the experimental data from shows that our assumption of confining attention to the first step of oxidation is justified. Now, Eq (2.2.32) and Eq (2.2.33) can be used again to solve the two unknowns \( p, h \). In order to do so we need to (a) set \( r_1 = 0, r_2 = 4.7 \, \mu \text{m} \) in Eq (2.2.34), (b) in Eq (2.2.31) the surface area should be a function of time \( A(t) = A_0 \times (1 + S(t)) \), where \( A_0 \) can be solved numerically from Eq (2.2.27) based on vesicle initial radius \( R_0 = 0.75 \times 10^{-5} \, \text{m} \), (c) \( S(t) \) is given in Eq (2.3.2) and (d) the mechanical moduli \( K_A \) and \( K_B \) vary with time as discussed below.
Figure 2.4: (a) Prediction of dependence of dimensionless pipette height $h/R_0$ (left $y$-axis) on irradiation time $t$. The orange line is a fit of eqn. (2.3.2) to the experimental data of Weber et al. [45] for fractional change in vesicle area (right $y$-axis) as function of time. (b) Prediction of dependence of dimensionless pressure difference $p/p_0$ on irradiation time $t$. 

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Weber et al. [45] point out that the stretching modulus of vesicles, $K_A$, varies as irradiation progresses. Ordinarily, the mechanical properties of homogeneous vesicles are constants, but the appearance of an oxidized phase during irradiation decreases $K_A$. This oxidized phase appears everywhere on the vesicle since irradiation is applied globally, hence $K_A$ is taken to be independent of position. Since the fraction of the oxidized phase increases with time the modulus $K_A$ decreases with time. We use the information in Fig.5 of [45] to treat $K_A$ as a function of irradiation time $t$: $K_A = 230 \text{ mN m}^{-1} - 1.6 \text{ mN m}^{-1}\text{s}^{-1} \cdot t$, $0 \leq t \leq 50 \text{ s}$. Since $K_b$ is proportional to $K_A$ the variation of $K_b$ with time can be estimated if the initial $K_b = 20.7k_B T$ for the POPC vesicle before irradiation is known. Note that $K_b$ plays an important role at low tensions, while $K_A$ weighs in at high tension $\tau > 10^{-4} \text{ N/m}$. This can be understood from the fact that in the low tension regime the log term dominates in eqn. (2.2.2) since $\frac{\tau}{K_A} << \frac{k_B T}{8\pi K_b} \log(1 + \frac{\tau A_i}{\pi^2 K_b})$, while at high tension the thermal fluctuations are pulled out and the area increases linearly with tension as dictated by $K_A$. The result for the predicted pressure difference $p/p_0$ as irradiation progresses is shown in figure Fig 2.4(b) and the result for the predicted pipette height $h/h_0$ is shown in Fig 2.4(a). Note that the $h/R_0$ values for $\tau = 0.7 \text{ mN m}^{-1}$ are similar to the insets in Fig. 3A of [45] where $h/R_0$ is between 1 and 2 suggesting that our analysis is reasonable.
2.3.2 ODE model of contact radius increase without irradiation

Now, we study the dynamics of an initially spherical vesicle (with initial contact radius $r_{\text{init}}^1 = 10$ nm and initial radius $R_0 = 1.68 \times 10^{-5}$ m consistent with the experiment in Aoki et al. [60]) adhering to the substrate in the absence of irradiation ($\alpha = 1$). In this context, we do not consider a pipette for aspiration on the top ($h = 0, r_2 = 0, F = 0$). Note that $S(t) = 0, \forall t \geq 0$ when there is no irradiation. As there is no applied force, the vesicle remains spherical in the unadhered region.

Let $R(t)$ denote the radius of the spherical cap at time $t$. Then, $A'(0) = A_0$, and

$$A'(t) = A_0 \cdot (1 + S(t)) - \pi R^2(t) \sin^2 \phi_1(t).$$

From [40] we know that the tension $\tau$ is determined by

$$\tau = \frac{\pi^2 K_b}{a_0^2} \exp \left( -\frac{8\pi K_b}{k_B T} \epsilon \right), \quad (2.3.3)$$

where $a_0^2$ is the surface area of a lipid molecule with linear size $a_0 \approx 0.8$ nm, and

$$\epsilon = (A'(t) - 2\pi R^2(t)(1 + \cos \phi_1)) / (2\pi R^2(t)(1 + \cos \phi_1))$$

denotes the area strain of the membrane, which is the proportion of the surface area stored in the undulations.

Note that Eq (2.3.3) is very close to the inverse formula of eqn. (8.74) in Boal [55] which is valid in the low tension regime. The constraint for fixed volume Eq (2.2.32) at time $t$ can be rewritten as,

$$\frac{4}{3} \pi R_0^3 = \pi R(t)^3 \left( \frac{2}{3} + \cos \phi_1(t) - \frac{\cos^3 \phi_1(t)}{3} \right). \quad (2.3.4)$$
Thus, \( R(t) \) can be expressed as a function of contact angle \( \phi_1(t) \),

\[
R(t) = \frac{2R_0}{(4 + 6 \cos \phi_1(t) - 2 \cos^3 \phi_1(t))^{\frac{3}{2}}}. \tag{2.3.5}
\]

If we know the contact radius \( r_1(t) \) at time \( t \), then we should have \( R(t) \sin \phi_1(t) = r_1(t) \). Putting this into Eq (2.3.5), we have

\[
\frac{r_1(t)}{2R_0} = \frac{\sqrt{1 - \cos^2 \phi_1(t)}}{(4 + 6 \cos \phi_1(t) - 2 \cos^3 \phi_1(t))^{\frac{3}{2}}}. \tag{2.3.6}
\]

Thus, \( r_1(t) \) is monotonically decreasing as \( \cos \phi_1(t) \) is increasing and we can find an implicit relation as

\[
\cos \phi_1(t) = f_1(r_1(t)). \tag{2.3.7}
\]

Substituting this into Eq (2.3.7), we have

\[
R(t) = \frac{2R_0}{(4 + 6 f_1(r_1(t)) - 2 f_1^3(r_1(t)))^{\frac{3}{2}}} \triangleq f_2(r_1(t)), \tag{2.3.8}
\]

then \( A'(t) \) can be written as

\[
A'(t) = A_0 \cdot (1 + S(t)) - \pi f_2^2(r_1(t))(1 - f_1^2(r_1(t))) \triangleq f_3(r_1(t), t). \tag{2.3.9}
\]

Putting Eq (2.3.7) - Eq (2.3.9) into the tension-area relation Eq (2.3.3), we have

\[
\tau = \frac{\pi^2 K_b}{a_0^2} \exp \left( -\frac{8\pi K_b}{k_B T} \cdot \frac{f_3(r_1(t), t) - 2\pi f_2^2(r_1(t))(1 + f_1(r_1(t)))}{2\pi f_2^2(r_1(t))(1 + f_1(r_1(t))}\right) \triangleq f_4(r_1(t), t). \tag{2.3.10}
\]

Also, from (40) we know the contact radius \( r_1 \) propagates toward the Young-Dupre equilibrium Eq (2.2.4) at a rate

\[
\dot{r}_1(t) = \frac{\Gamma - (1 - \cos \phi_1(t))\tau}{\eta_0 c_s}, \tag{2.3.11}
\]

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where $\eta_0 \approx 10^{-3}$ Pa·s denotes the viscosity of the aqueous medium [63], and $c_s$ denotes surface drag coefficient ranging from hydrodynamic limit ($c_s \approx 1$), through the membrane friction dominated range ($c_s \approx 10^3$), up to surface inhomogeneity governed values ($c_s \approx 10^6$) [40]. In our work we study the membrane friction dominated range ($c_s \approx 10^3$) used in [40]. Dragging Eq (2.3.7) and Eq (2.3.10) into Eq (2.3.11), the contact radius rate $\dot{r}_1(t)$ has the form,

$$
\dot{r}_1(t) = \frac{\Gamma - (1 - f_1(r_1(t))) f_4(r_1(t), t)}{\eta_0 c_s}. 
$$

(2.3.12)

Although there is no analytic solution for Eq (2.3.12), numerical results can be obtained using a fourth order Runge-Kutta method.
Figure 2.5: (a) Prediction of time evolution of contact radius $r_1$ in the absence of irradiation. (b) Comparison between different $\Gamma$ for the time of vesicle finishing adhesion on substrate in the absence of irradiation.

In Fig 2.5(a) we plot the evolution of contact radius by solving the ODE
Eq (2.3.12) for a vesicle with initial contact radius $r_{\text{init}}^1 = 10 \text{ nm}$, initial radius $R_0 = 1.68 \times 10^{-5} \text{ m}$ and bending modulus $K_b = 21k_B T$ under the adhesion energy per unit area $\Gamma = 2.53 \times 10^{-5} \text{ N/m}$. Equilibrium is reached at time $t^* = 0.56 \text{ s}$. Our estimate for $t^*$ agrees with a recent theoretical work [40]. Next, we study the effect of $\Gamma$ on $t^*$. We choose $\Gamma = 2.53 \times 10^{-4} \text{ N/m}$, $\Gamma = 2.53 \times 10^{-5} \text{ N/m}$ and $\Gamma = 2.53 \times 10^{-6} \text{ N/m}$ corresponding to $t^*_4, t^*_5, t^*_6$ (with initial contact radius $r_{\text{init}}^1 = 10 \text{ nm}$). The results for the evolution of $r_1(t)$ are shown in figure Fig 2.5(b). We can see that $t^*_4 \sim 10^{-2} \text{ s}$, $t^*_5 \sim 10^{-1} \text{ s}$, $t^*_6 \sim 10^0 \text{ s}$. As expected, increasing $\Gamma$ increases the speed at which the vesicle finishes its adhesion on the substrate and reaches equilibrium.

2.3.3 ODE model of contact radius increase with irradiation

Figure 2.5(a) suggests that $t^*$ is about 0.56 s, which implies that the kinetics of the vesicle adhesion on the substrate is much faster (at least for large enough $\Gamma$) than the kinetics of the irradiation which is hundreds of seconds in Aoki et al [60] and tens of seconds in Sankhagowit et al. [3]. This shows that the vesicle will completely finish adhesion on the substrate and reach equilibrium as soon as the irradiation is applied. As a consequence, we can separate these two kinetics and only consider the kinetics of irradiation induced oxidation. Here we consider the three irradiation powers used in Aoki et al. [60]. Let $S_1, S_2, S_3$ be the surface area increment under irradiation powers 1, 1/4, 1/32, respectively. Then, in order to fit the model in
Eq (2.3.1) and Eq (2.3.2), we need to set $k_1 = 0.0427, 0.0066, 0.0017$ and $A_{OX1} = 1.1936, 1.1801, 1.1878$ under the irradiation power $1, 1/4, 1/32$, respectively. The model in section 3.2 can be used here except that we need to replace $S(t) = 0$ by $S_1(t), S_2(t), S_3(t)$. Then, the contact radius $r_1(t)$ at time $t$ can be determined through the Young-Dupre equilibrium Eq (2.2.4)

$$\Gamma = f_4(r_1(t), t) \cdot (1 - f_1(r_1(t))). \quad (2.3.13)$$

The results are plotted in Fig 2.6a which shows that higher irradiation power leads to quicker completion of area increase. The $k_1$ values we found above increase with increasing radiation power, as expected [3]. The curves in Fig 2.6a qualitatively capture the experimental results in Aoki et al. [60] as shown in the inset; a quantitative match may require that we account for the kinetics of OX2. As an alternative, we have used linear interpolation to construct continuous functions for $S_1, S_2, S_3$ based on discrete data extracted from Aoki et al. [60] and performed the exercise above to get better agreement with the experimental data (not shown here). In Fig 2.6b we predict that the tension $\tau$ decreases as irradiation progresses. Intuitively, the decrease in tension is expected since the vesicle area increases while its volume is held fixed. Also, experimental evidence [3] shows an increase in thermal fluctuations of the vesicles after irradiation which is consistent with a reduction in tension. Such a decrease in tension could lead to functional changes in live cells [47]. For example, it has been shown that artificially decreasing membrane tension in fibroblasts increases the rate of cell spreading and lamellipodial extension and
stimulates new lamellipodial extensions \[64\]. Although it has long been known that oxidative damage can cause cell death and diseases \[5\], changes in cell behavior due to irradiation induced changes in membrane tension have not been tested in experiments. If such experiments are performed in live cells then insights from our analysis can inform models for the evolution of cell membrane tension.
Figure 2.6: (a) Prediction of time evolution of contact radius $r_1$ in equilibrium as irradiation is progressing under three different irradiation powers in Aoki et al. [60]. The irradiation power affects the rate constant $k_1$ which increases as irradiation power increases. The inset is a comparison between fitted functions (solid line) and raw experimental data (dashed line) for surface area increment function $S(t)$ under three different irradiation powers in Aoki et al. [60] showing qualitative agreement.
2.4 Kinetics of detachment of vesicle after irradiation

Finally, it is important to study the kinetics of detachment of vesicles from substrates because it represents a stage in cellular processes such as endo- and exocytosis, immune response and cell-tissue interaction \([65]\). Here our goal is to determine how irradiation induced area changes can change the kinetics of detachment.

2.4.1 Critical force to cause detachment

We consider a detachment process in which the dissociation of bonded ligand-receptor pairs is considered after irradiation has occurred. Following the model in Lin and Freund \([39]\) and Boulbitch \([65]\), we use \(\rho_l\), which is a number per unit area, to denote the local concentration of free ligands in the substrate. Similarly, we use \(\rho_r\) to denote the concentration of free receptors in the vesicle wall. Let \(\rho_{lr}\) denote the concentration of bonded ligand-receptor pairs. Also, let \(k_+\) be the association rate constant, \(k_-\) be the dissociation rate constant. We assume that \(\rho_{lr} = \rho_{lr}^{(0)}\) is constant everywhere. Here \(\rho_{lr}^{(0)}\) is the density of bonded ligand-receptor
pairs at equilibrium. Then, the following local rate equation is given by [39],

$$\frac{d\rho_{lr}}{dt} = k_+ \rho_1 \rho_r - k_- \rho_{lr}^{(0)}, \quad (2.4.1)$$

where the dissociation rate $k_-$ is given by [66],

$$k_-(f) = k_-^{(0)} \exp \left( \frac{af}{k_B T} \right), \quad (2.4.2)$$

where $a$ is a length on the order of 0.1 nm, and $f$ is the force on a single bond. We also assume that the vesicle is in equilibrium adhesive contact with the substrate in the absence of detachment force [39]. Then the LHS of Eq (2.4.1) should vanish, and we have

$$k_-^{(0)} \rho_{lr}^{(0)} = k_+ \rho_1 \rho_r. \quad (2.4.3)$$

Putting this into Eq (2.4.1), we get,

$$k_-(f) = \frac{k_+ \rho_1 \rho_r}{\rho_{lr}^{(0)}} \exp \left( \frac{af}{k_B T} \right). \quad (2.4.4)$$

Lin and Freund [39] supposed the applied force on each stretched bond is uniform:

$$f = F(t)/2\pi r_1(t)b_0 \rho_{lr}^{(0)},$$

where $r_1(t)$ is the radius of the adhesion zone at time $t$ and $b_0 \approx 10$ nm is the width of bending zone. Then they show that,

$$\frac{dr_1}{dt} = \frac{b_0 k_+ \rho_1 \rho_r}{\rho_{lr}^{(0)}} \left[ 1 - \exp \left( \frac{F(t)a}{2\pi b_0 r_1(t) \rho_{lr}^{(0)} k_B T} \right) \right]. \quad (2.4.5)$$

We first follow the model in [39] to consider the case when the pulling force is suddenly applied and remains constant during the unbinding process, i.e. $F(t) =
F, t > 0. Evans and Ritchie [67] have pointed out that detachment will occur under any level of pulling force if it is held for sufficient time. Therefore, we are interested in the dependence of the dimensionless detachment time $\tau^*$ on the constant applied force $F$ with and without irradiation. Let $\beta_1 = \frac{Fa}{2\pi b_0 k_B T \rho_r^{(0)} R_1}$, where $R_1$ is the initial radius of the adhesion zone under irradiation. Following [39], we use dimensionless variables $\tau_1 = (b_0 k_+ \rho_1 \rho_r) t / (\rho_r^{(0)} R_1)$, $\xi_1 = r_1 / R_1$. Then, Eq (2.4.5) becomes

$$\frac{d\xi_1}{d\tau_1} = 1 - \exp \left( \frac{\beta_1}{\xi_1} \right),$$

(2.4.6)

with the initial condition $\xi_1(0) = 1$. Similarly, we can define $\beta_2, \tau_2, \xi_2$ and the ODE with respect to $R_2$ which is the initial radius of the adhesion zone in the absence of irradiation. It is not hard to see that at a given $F$, $\beta_1 = C \beta_2$ where $C$ is a constant.

In Boulbitch [65], $k_+ \rho_1$ is estimated to be $0.18 \times 10^5$/s and in Lin and Freund [39] the receptor density on the substrate surface is about $\rho_r = 1/\mu$m$^2$. In Alon et al. [68], $k_+^{(0)}$ is estimated to be $0.95$ s$^{-1}$. From Fig 2.1 in section 2.3 we know $R_1 = 2.38 \times 10^{-5}$ m and $R_2 = 1.34 \times 10^{-5}$ m. Then, $\beta_1, \beta_2$ should be in the range $4.30 \times 10^{-9} < \beta_1 < 4.30 \times 10^{-6}$ and $7.64 \times 10^{-9} < \beta_2 < 7.64 \times 10^{-6}$, respectively, which corresponds to $0.5$ pN$< F < 500$ pN used by [39]. In particular, for a given $F$, $\frac{\beta_2}{\beta_1} \approx 1.78$. We numerically integrate eqn. (2.4.5) using these numbers and plot the result in figure 2.7(a) in which $\beta = \beta_1$ is a ‘reference dimensionless variable’.

Within the force range mentioned above, the dependence of the dimensionless detachment time $\tau_1^*, \tau_2^*$ (corresponding to dimensionless detachment time with and
without irradiation) respectively on $\beta_1, \beta_2$ are

$$\tau_1^* \propto \beta_1^{-1.00}, \quad \tau_2^* \propto \beta_2^{-1.00} \quad (2.4.7)$$

Since $\beta_1, \beta_2$ are proportional to $F$, then we have,

$$\tau^* \propto F^{-1.00}, \quad (F \text{ between } 0.5 \text{ pN and } 500 \text{ pN}). \quad (2.4.8)$$

Note that Eq (2.4.8) holds for both cases (with and without irradiation). The time to detachment will be higher after irradiation because a larger area is adhered.

Now, we consider a time-dependent force at constant rate $\dot{F}$ as proposed by [39].

Let $F(t) = \dot{F}t$, then Eq (2.4.8) can be rewritten as,

$$\frac{d\xi}{d\tau} = 1 - \exp\left(\eta \frac{\tau}{\xi}\right), \quad (2.4.9)$$

where $\eta = \dot{F}a/(2\pi \rho_0^2 k_+ \rho_1 \rho_r k_B T)$. Using the values of these parameters mentioned above, we have $\eta = 2.15 \times 10^9 \dot{F}$. As pointed out by [7] the force rate $\dot{F}$ in experiments is estimated to be between 20 pN/s and 4 nN/s, which corresponds to $\eta$ between 0.043 to 8.6.
Figure 2.7:  (a) Prediction of dependence of dimensionless detachment time $\tau^*$ on $\beta_1$ and $\beta_2$ under the same constant force $F$. (b) Prediction of dependence of dimensionless detachment time $\tau^*$ on $\eta$ which implies the dependence of real detachment time $\mu^*$ on loading rate $\dot{F}$.
Eq (2.4.9) can be integrated numerically and the result is plotted in Fig 2.7(b) as symbols. The fitted line reveals that $\tau^* \approx \eta^{-0.59}$. Let the real detachment time be $\mu^*$. We want to study the dependence of the dimensionless detachment time $\tau^*$ on $\eta$ and, accordingly, the dependence of the real detachment time $\mu^*$ on the force rate $\dot{F}$ with and without irradiation. Recall that $\mu^*$ is proportional to $\tau^*$ and $\dot{F}$ is proportional to $\eta$. It follows from Fig 2.7(b) that within the range $0.043 < \eta < 8.6$, the dependence of the real detachment time $\mu^*$ on the force rate $\dot{F}$ can be approximated by a power law as $\mu^* \propto (\dot{F})^{-0.59}$. Since the detachment force $F^* = \dot{F} \mu^*$, this implies,

$$F^* \propto (\dot{F})^{0.41} \quad (2.4.10)$$

We note that Fig 2.7(b) and Eq (2.4.10) works for both cases (with and without irradiation). The only difference comes from the real detachment time $\mu^*$. If we use $\mu_1^*$ to denote the detachment time under irradiation and $\mu_2^*$ without irradiation, then we should have, as expected, $\mu_1^*/\mu_2^* = R_1/R_2 = 1.78$. 

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Chapter 3

Self-assembly on a lipid membrane viewed as a first passage time problem


3.1 Introduction

Interactions between inclusions on lipid membranes and their self-assembly leads to a host of biologically important functions such as budding of endo- and exocytotic vesicles, pore formation and assembly of viruses. These interactions could
be curvature mediated $^{13, 20}$, membrane thickness mediated $^{9}$, entropic $^{13, 16}$ and even electrostatic $^{17}$. There is a large literature spanning a few decades on such interaction problems on membranes $^{13, 20, 71, 21, 22, 23, 24, 71, 5, 25}$. There is a smaller literature exploring the dynamics of self-assembly on lipid membranes $^{12, 23, 34}$, including continuum models $^{72, 73}$. Most papers on dynamics of self-assembly assume phenomenological forms for the interactions between particles, rather than computing them from membrane deformations; this is because it is computationally expensive to do so. Many of these simulations have focused on the assembly of viruses on the surface of membranes due its relevance to disease and to the technologically important problem of drug delivery using nano-containers $^{23, 34}$. As such, the focus on these papers is on determining the conditions that lead to the assembly of full (virus) particles from the inclusions, but they say little about how the interaction forces between inclusions affect the time to assembly. However, an experimental paper by Shnyrova et al. $^{29}$ shows that self-assembly of spherical buds formed by self-assembly of a viral protein occurs on the time scale of few seconds on vesicles that are a few microns in diameter $^{29}$. Shnyrova et al. show using experiments that the interactions between these viral proteins are non-electrostatic, but the proteins induce local curvature of the membrane.

We have shown previously using a coarse-grained model that we can recover asymptotic forms for the curvature mediated elastic and entropic interactions of inclusions on membranes $^{15, 14}$. In particular, we had assumed that the inclu-
sions were rigid, and hence had not accounted for the Gaussian curvature following arguments in Kim et al. [20]. However, the $1/r^4$ asymptotic form for the entropic interactions for circular inclusions separated by distance $r$ on a lipid membrane happens to hold even if the inclusions are not rigid as shown by Lin et al. [74]. In fact, Lin et al. recover the $1/r^4$ dependence even in the presence of line tension at the inclusion-membrane interface. Another point to note is that Lin et al. [74] showed that the inclusions’ shape and mechanical properties enter the calculation of the fluctuation induced forces through a characteristic matrix. In our computations too the inclusions’ shape and mechanical properties enter through a single ‘stiffness’ matrix. The general idea behind the calculation of fluctuation induced interaction forces is exactly the same in our work and that of [74] (i.e., quadratic energies and Gaussian integrals for computing the partition function), except they do it analytically, while we compute the fluctuation determinants numerically. Thus, although we will stick with the simpler case of rigid inclusions in this work the paper of Lin et al. [74] shows that our methods can be extended to compliant inclusions as well.

An important result in [14] was that for rigid circular inclusions the competition of repulsive elastic forces and attractive entropic forces could lead to a maximum in the interaction free energy. The presence of a maximum indicates that at small separations there are attractive forces between inclusions which should lead to self-assembly. We had not demonstrated this self-assembly earlier since our coarse-grained model based on Gaussian integrals [75] was for equilibrium only. Here
we go beyond equilibrium by combining our computational method with Langevin
dynamic simulations to model the evolution of the membrane-inclusion system. Unlike most other papers on dynamic self-assembly our focus is not on particle and
membrane shape which can be easily visualized through our calculations; rather, we
are interested in the time to self-assembly for a few inclusions diffusing on a lipid
membrane. We do not assume phenomenological forms for the interactions between
inclusions; instead, we compute these interaction forces on the fly from membrane
deformations caused by the boundary conditions imposed by the inclusions. We also
take account of hydrodynamic interactions between inclusions which are known to
speed up self-assembly \[34\]. Since most inclusions may not be circular we study
the self-assembly of elliptical inclusions in this paper and show that their time to
assembly is of the same order as that for circular inclusions of similar size.

An advantage of using Langevin dynamics combined with known asymptotic
forms of the interaction energy between two inclusions is that we can invoke the
Fokker-Planck equation to understand the evolution of the system. This allows us
to make analytical progress with the problem of self-assembly instead of relying
entirely on computations. If we fix one of the two inclusions at the center of the
lipid membrane while allowing the other to diffuse under the action of the elastic
and entropic forces then the time for coalescence of the two inclusions can be cast as
a first passage time problem. For some special boundary conditions we can derive
a differential equation for the mean first passage time starting from the Fokker-
Planck equation. This allows us to verify our Langevin dynamic simulations against solutions from partial differential equations. To the best of our knowledge, such an exercise has not been attempted in the literature on self-assembly of inclusions on membranes. We show that our analytical techniques based on partial differential equations give reliable results much quicker than Langevin dynamic simulations.

In the remainder of the paper we first focus on circular inclusions in section 2. We show the correspondence between the Langevin dynamics and Fokker-Planck equation based approaches. Then, in section 3 we extend our analysis to elliptical inclusions; we compute the elastic and entropic interaction forces between them and again demonstrate the correspondence of the Langevin dynamics and Fokker-Planck equation based approaches. This section illustrates the generality of our approach since the problem is not radially symmetric when the inclusions are elliptical. In section 4 we go back to circular inclusions, but we account for hydrodynamic interactions between them. We show yet again that the Langevin dynamics and Fokker-Planck equation based approaches give identical results. In section 5 we analyze the self-assembly of multiple inclusions using Langevin dynamics and show the role of interaction forces at small separations. Finally, we conclude the paper with a brief summary of results.
3.2 Self-assembly of two circular inclusions in a lipid membrane

In this section we study the self-assembly of two circular inclusions on a square lipid membrane of finite size. We assume that one inclusion is fixed at the center of the membrane and the other is diffusing around driven by the entropic and elastic forces of interaction and due to Brownian motion. We cast the self-assembly of these two inclusions as a ‘first passage time’ problem within the theory of stochastic processes. Our expectation is that the elastic and entropic interactions between inclusions will affect this first passage time and determine the rate at which self-assembly occurs.

3.2.1 Energy landscape

It is well known that inclusions on lipid membranes interact via elastic and entropic forces. Asymptotic forms of these interactions are available for circular inclusions on an infinite membrane which deforms by bending only. It is understood that entropy (or fluctuations) is dominant at large separations between inclusions, while the elastic force plays a bigger role at small separations. This could yield a maximum in the free energy at a critical separation. Asymptotically, the entropic and elastic energy both depend on the inverse separation.
the fourth power (leading order term) using perturbation theory \cite{13, 12},

\begin{align*}
E_{el}(r) &= \frac{a_{el}}{r^4} + O \left( \frac{1}{r^6} \right) \quad (3.2.1) \\
E_{en}(r) &= \frac{a_{en}}{r^4} + O \left( \frac{1}{r^6} \right), \quad (3.2.2)
\end{align*}

where \( r \) is distance between two rigid circular inclusions on a lipid membrane. \( a_{el} \) scales linearly with the bending modulus of the membrane and quadratically with the radius of each inclusion and the contact angle \( \lambda \) between the inclusion and the membrane for small contact angles; \( a_{en} \) scales linearly with the thermal energy scale \( k_B T \) (where \( k_B \) is the Boltzmann constant and \( T \) is absolute temperature) and quadratically with the radius of each inclusion. These dependencies have been computed analytically \cite{12} and verified numerically using a method based on Gaussian integrals \cite{15, 14, 78}. 

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Figure 3.1: Fitting of the total free energy by Eq (3.2.1) and Eq (3.2.2). The panel on the top left shows two inclusions, one fixed at the center and the other located at \((r, \psi_c)\) in a polar coordinate system. The remaining panels show the variation of the total free energy (elastic + entropic) of the membrane inclusion system as a function of \(r\) for various \(\psi_c\). The symbols are obtained using our semi-analytic method based on Gaussian integrals \([14]\) and the lines are fits using Eq (3.2.1) and Eq (3.2.2). The free energy is practically independent of \(\psi_c\) and depends only on \(r\).

In Fig 3.1 we choose a small contact angle \(\lambda = 0.1\) radian, membrane bending modulus \(K_b = 20k_B T\), and \(T = 300\) K (at which \(k_B T = 4.1\) pNnm) to plot the free energy for two inclusions on a membrane of dimensions 500 nm \(\times\) 500 nm. Due
to the limitations of our finite difference grid we can only have inclusions that are hexagonal in shape, so we choose two inclusions whose ‘radii’ are $R_1 = R_2 = 2l = 5$ nm, where $l = 2.5$ nm is the length of one equilateral triangle in Fig 3.1. We choose various angles $\psi_c$ between the line joining the center of the inclusions and the $x_1$-axis and plot the free energy of the membrane and inclusions as a function of the distance $r$ separating the centers of the inclusions following methods described in Liang and Purohit [14]. We see that the free energy profile is mostly independent of $\psi_c$ and depends only on $r$, as expected for circular inclusions on an infinite membrane. The slight dependence on the angle $\psi_c$ arises due to the finite size of our membrane as well as the non-circular shape of both the membrane and inclusions among other causes [14]. The free energy has a maximum around $r/l = 20$ which corresponds to $r = 50$ nm and it decreases steeply for $r/l < 20$, while it is relatively flat for $r/l > 20$. Thus, an attractive force acts on the inclusions if the distance between them is smaller than a critical value which should lead to self-assembly. Here we note that when the center to center distance of two inclusions is small, typically $r/l < 6$, the formula Eq (3.2.1)-(3.2.2) given in [12] may not fit the results from our finite difference calculations. Keeping these limitations in mind, we will analyze the self-assembly process in the following.
3.2.2 Langevin dynamics

The motion of an inclusion on a membrane is driven both by the elastic and entropic interactions given above and due to Brownian motion (or diffusion). The inclusions are small enough that the motion can be considered over-damped. We use Langevin dynamics to study the stochastic process of a diffusing inclusion driven by entropic and elastic forces and Brownian motion. The Langevin equation for the motion of a single inclusion is,

\[
\frac{dr_i}{dt} = u_i, \tag{3.2.3}
\]

\[
m \frac{du_i}{dt} = -\nu u_i - \frac{\partial \phi}{\partial r_i} + \eta_i(t), \tag{3.2.4}
\]

where \( r_i \) is the \( i^{th} \) component of the position vector of the inclusion, \( u_i \) is \( i^{th} \) component of the velocity vector, \( \nu \) is translational drag coefficient, \( \phi \) is the potential representing elastic and entropic interactions, \( m \) is the mass of the inclusion and \( \eta_i(t) \) is a random force tensor with the following properties,

\[
\langle \eta_i(t) \rangle = 0, \quad \langle \eta_i(t)\eta_j(t') \rangle = 2\nu k_B T \delta_{ij} \delta(t - t'), \tag{3.2.5}
\]

We note here that the notation of any variables can also be regarded as a function of position in polar coordinates (E.g. \( \phi \) refers to both the potential itself and a function of \( r \) and \( \theta : \phi = \phi(r, \theta) \)). Since the mass of the inclusion is very small, we set LHS of Eq \( (3.2.4) \) to zero. Then, after approximating the delta-correlated random noise term \[76\], we get,

\[
dr_i = -\frac{1}{\nu} \frac{\partial \phi}{\partial r_i} dt + \sqrt{\frac{2k_B T dt}{\nu}} \xi_i, \tag{3.2.6}
\]

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where $\xi_i \sim \mathcal{N}(0, 1)$, a normally distributed random variable with mean 0 and variance 1. The translational drag coefficient $\nu$ of a circular inclusion is given by the Saffman–Delbrück model \[79\],

$$\nu = \frac{4\pi \eta_m \log(2\epsilon^{-1}) - \gamma}{\log(2\epsilon^{-1}) - \gamma},$$

(3.2.7)

where $\eta_m = 15.3 \times 10^{-9}$ Pa $\cdot$ s $\cdot$ m is the membrane viscosity (2D), $\eta_w = 8.5 \times 10^{-4}$ Pa $\cdot$ s is the bulk viscosity of water (3D), $l = 2.5$ nm is the radius of the circular cross section of the inclusion, $\epsilon = 2\eta_w l / \eta_m$ and $\gamma \approx 0.577$ is Euler’s constant \[80\].

Arroyo and DeSimone \[81\] calculated that for a lipid membrane of size about 500 nm membrane bending resistance and in-plane viscosity are the dominant driving and dissipative mechanisms. Here, membrane viscosity plays a crucial role in the dynamics of inclusions diffusing on a lipid membrane.

Our goal in doing the Langevin dynamic calculations is to determine the average time taken by the diffusing inclusion to coalesce with the stationary inclusion at the center of the membrane. This average time is usually referred to as a ‘mean first passage time’ in the literature of stochastic processes \[76\]. We aim to determine this first passage time as a function of the initial distance $r$ between the inclusions.

### 3.2.3 Fokker-Planck equation

Every Langevin equation can be represented by a Fokker-Planck equation \[76\]. We use the Fokker-Planck equation to study the first passage time of the diffusing inclusion because this problem is analytically tractable. Recall that we consider
two circular inclusions with radius $2l$ embedded in a square lipid membrane of side $2R_2$. One inclusion is fixed at the center $r = 0$ and the other is moving around. The diffusion coefficient is a scalar constant since the circular inclusion is isotropic and the lipid membrane is also isotropic. Then, the corresponding two-dimensional Fokker-Planck equation is a parabolic partial differential equation in radial symmetry and has a simple form,

$$\frac{\partial P}{\partial t} = -\nabla \cdot (fP) + D\Delta P,$$

(3.2.8)

where $f = -\frac{1}{\nu} \frac{\partial \phi}{\partial r} e_r$ is the drift velocity and $e_r$ is the unit vector in the radial direction (positive outward) and $P(r, t|y) = \int_0^{2\pi} p(r, t, \theta|y, \alpha)d\theta$ represents the probability density for a particle to be found at position $r$ at time $t$ in which $p$ denotes the probability density at separation $r$ between two inclusions and angle $\theta$ given initial condition $r = y, \theta = \alpha$. Note that $P$ is independent of $\alpha$ and is only dependent on $y$. Using the fact that $\Delta = \nabla \cdot \nabla$, Eq (3.2.8) can be rewritten as a conservation law:

$$\frac{\partial P}{\partial t} = -\nabla \cdot (fP) + D\nabla \cdot \nabla P = \nabla \cdot \left( \frac{1}{\nu} \frac{\partial \phi}{\partial r} Pe_r + D \frac{\partial P}{\partial r} \right)$$

$$= \nabla \cdot \left[ \left( \frac{1}{\nu} \frac{\partial \phi}{\partial r} P + D \frac{\partial P}{\partial r} \right) e_r \right] = \nabla \cdot J,$$

(3.2.9)

where $J = \left( \frac{1}{\nu} \frac{\partial \phi}{\partial r} P + D \frac{\partial P}{\partial r} \right) e_r$ is the flux of the probability density. The initial condition for Eq (3.2.8) is

$$P(r, 0|y) = \frac{1}{r} \delta(r - y).$$

(3.2.10)
The boundary condition for Eq (3.2.8) could be absorbing boundary conditions or reflecting boundary conditions or a mix of the two.

3.2.4 Steady-state solution

In order to obtain a non-vanishing steady-state solution to the Fokker-Planck equation Eq (3.2.8), we assume two reflecting boundary conditions. One of the inclusions can move freely, but it will be bounced back if it is sufficiently close to the fixed inclusion at the center (i.e. \( r = R_1 \)) or the outer boundary (i.e. \( r = R_2 \)), we assume the inclusion’s movement is restricted to the inscribed circle (orange) of the square membrane (purple) in Fig 3.2(d). Then, the two reflecting boundary conditions are given by [82],

\[
J_r(R_1, t) = 0, \quad J_r(R_2, t) = 0, \quad \forall t \geq 0.
\]

(3.2.11)

Eq (3.2.11) impiles that,

\[
\left( \frac{1}{\nu} \frac{\partial \phi}{\partial r} P + D \frac{\partial P}{\partial r} \right) \bigg|_{(R_2, t)} = 0
\]

\[
\left( k_B T \frac{\partial P}{\partial r} + \frac{\partial \phi}{\partial r} P \right) \bigg|_{(R_2, t)} = 0,
\]

(3.2.12)

where the Nernst-Einstein relation \( \nu D = k_B T \) [83] has been used in Eq (3.2.12) and \( \phi \) is the free energy. The steady-state solution of this Fokker-Planck equation can be obtained by setting \( \frac{\partial P}{\partial t} = 0 \) in Eq (3.2.8). Then, from Eq (3.2.9) we get,

\[
\frac{\partial J_r}{\partial r} + \frac{1}{r} J_r = 0 \implies J_r = \frac{B}{r},
\]

(3.2.13)
which means,

\[
\frac{\partial P}{\partial r} + \frac{1}{\nu D} \frac{\partial \phi}{\partial r} P = \frac{B}{Dr},
\]

(3.2.14)

or,

\[
k_B T \frac{\partial P}{\partial r} + \frac{\partial \phi}{\partial r} P = \frac{B k_B T}{Dr},
\]

(3.2.15)

where \(B\) is a constant with the same units as \(D\). The steady-state solution can be solved from the first order linear ODE Eq (3.2.14),

\[
P(r) = \exp \left( -\frac{\phi}{k_B T} \right) \left[ \int \exp \left( \frac{\phi}{k_B T} \right) \frac{B}{r D} dr + C \right],
\]

(3.2.16)

where \(C\) is a constant. We set \(t \to \infty\) in Eq (3.2.12) and compare it with Eq (3.2.15), then it immediately follows that \(B = 0\). Then, Eq (3.2.16) reduces to

\[
P(r) = C \exp \left( -\frac{\phi(r)}{k_B T} \right).
\]

(3.2.17)

This is nothing but the Boltzmann distribution expected from equilibrium statistical mechanics. If we take \(\phi = \frac{A_1}{r^4} + \frac{A_2}{r^6}\) with some constants \(A_1, A_2\), then \(C\) can be solved from the fact that the integral of the probability function in polar coordinates equals 1,

\[
1 = \int_{R_1}^{R_2} C \exp \left( -\frac{\phi}{k_B T} \right) r dr = \frac{C}{2} \int_{R_1}^{R_2} \exp \left( -\frac{A_1}{k_B T r^4} - \frac{A_2}{k_B T r^6} \right) r^2 dr
\]

\[
= \frac{C}{2} \int_{R_1^2}^{R_2^2} \exp \left( -\frac{A_1}{k_B T q^2} - \frac{A_2}{k_B T q^3} \right) dq,
\]

(3.2.18)

where we have changed variables in the last step \(q = r^2\). \(C\) can be numerically solved from Eq (3.2.18).

We use MATLAB to solve Eq (3.2.8) and compare the evolution of the solution with the steady-state solution Eq (3.2.17) with \(R_1 = 19.8\) nm and \(R_2 = 224.9\) nm.
We choose $R_1 = 19.8$ nm because below this value the analytical expression for the free energy in [12] might lose accuracy. We choose $R_2 = 224.9$ nm because of limitations of computational power on our desktop computers. In real cells and vesicles the radius $R_2$ would be on order of a few microns. The results in Fig 3.2 show that the Langevin method shown in Fig 3.2(a) can correctly predict the evolution of the probability function $P(r, t)$ (obtained from Eq (3.2.8) and shown in Fig 3.2(b)) to steady state. Fig 3.2(c) implies that the system consisting of membrane and two inclusions equilibrates by $t = 0.2$ s since the steady state solution nearly matches the solution at $t = 0.2$ s.
Figure 3.2: The evolution of $P(r, t)$ to the steady state distribution Eq (3.2.17). We divide the circular region into finitely many annuli with equal width $\Delta r$ and run 8000 simulations at each fixed time. If $s_j$ particles are found at the j-th annulus, then $P(r_j, t)$ can be computed from the probability that a particle is found at the j-th annulus: $P(r_j)r_j\Delta r = s_j/8000$. (a) $P(r, t)$ derived from Langevin dynamics. (b) $P(r, t)$ derived from Fokker-Planck equation. (c) Comparison between $P(r)$ at
Figure 3.2: $t = 0.2$ s computed from Fokker-Planck equation and its steady state distribution. Since we can compute the free energy only on a vertex of each triangular element, the free energy at the red point is approximated by the free energy at the green point. The orange circle shows the outer boundary $r = R_2$ for the first passage time problems studied next.

3.2.5 First passage time

Now that we have shown agreement between the results from the Fokker-Planck equation and the corresponding Langevin equation, we can proceed to an analysis of the first passage time for the self-assembly of two inclusions. An inclusion will typically be attracted to another inclusion when they are sufficiently close to each other. Hence, we will use absorbing boundary conditions at the inner boundary $r = R_1$. At the outer boundary, $r = R_2$ we use reflecting boundary conditions, but it is not possible to arrive at an analytical estimate of the mean first passage time under this boundary condition. However, the problem of the first passage time can be analytically solved if we use two absorbing boundary conditions at both sides. So, we proceed first with this calculation since it allows us to verify our first passage time estimated from the Langevin dynamic calculations in a rigorous fashion. Later, we will use more realistic boundary conditions in the Langevin dynamic simulations to estimate the rate of self-assembly. The time that the moving inclusion is absorbed to the absorbing walls is a random variable called ‘first passage time’ and we are
interested in the mean of the first passage time since it can describe the rate of self-assembly.

We rewrite the Fokker-Planck Eq (3.2.8) in the following form under two absorbing boundary conditions,

\[
\frac{\partial P}{\partial t} = \frac{\partial}{\partial r} \left[ \frac{1}{\nu} \frac{\partial}{\partial r} P + D \frac{\partial P}{\partial r} \right] + \frac{1}{r} \frac{\partial}{\partial r} \left[ \frac{1}{\nu} \frac{\partial}{\partial r} P + D \frac{\partial P}{\partial r} \right],
\]

(3.2.19)

with Dirichlet boundary condition,

\[ P(R_1, t) = 0, \quad P(R_2, t) = 0, \quad \forall t \geq 0. \]  

(3.2.20)

Define the survival probability \( S(y, t) \),

\[ S(y, t) = \int_{R_1}^{R_2} P(r, t|y) r dr. \]

(3.2.21)

Then, the first passage time density can be given as,

\[ f(y, t) = -\frac{\partial S(y, t)}{\partial t} = -\int_{R_1}^{R_2} \frac{\partial P(r, t|y)}{\partial t} r dr. \]

(3.2.22)

In [84], Itô constructed the fundamental solution for partial differential equations of parabolic type under Dirichlet boundary condition Eq (3.2.20) which also satisfies the initial condition Eq (3.2.10) in our problem. The form of the fundamental solution implies that \( tP(r, t|y) \to 0 \) as \( t \to \infty \). We use Eq (3.2.22) to compute the first moment of the first passage time \( T_1(y) \),

\[ T_1(y) = \int_0^\infty f(y, t) dt = -\int_0^\infty \int_{R_1}^{R_2} \frac{\partial P(r, t|y)}{\partial t} r dr dt \]

\[ = \int_{R_1}^{R_2} \int_0^\infty P(r, t|y) dt dr = \int_{R_1}^{R_2} g_1(r, y) r dr, \]

(3.2.23)
where $g_1$ is defined by,
\begin{equation}
  g_1(r, y) = \int_0^\infty P(r, t|y) dt.
\end{equation}

Using some technical but standard methods from the theory of stochastic process for the first passage time \cite{76, 82}, we can derive the following ODE for $T_1(y)$,

**Theorem 1:** The ODE for $T_1(y)$ with two absorbing walls at the outer and inner boundary is
\begin{equation}
  \frac{\partial^2 T_1(y)}{\partial y^2} \left( -\frac{1}{k_B T} \frac{\partial \phi}{\partial y} + \frac{1}{y} \right) \frac{\partial T_1(y)}{\partial y} + \frac{1}{D} = 0,
\end{equation}

with boundary conditions,
\begin{equation}
  T_1(R_1) = 0, \quad T_1(R_2) = 0.
\end{equation}

**Proof:** See Proof of Theorem 1.

We have solved the above ODE for the mean first passage time (with two absorbing boundary conditions) and plotted it as the red curve in Fig 3.3(a). The results for first passage time from Langevin dynamics is the blue curve in Fig 3.3(a). We find that the agreement between the two methods is excellent, suggesting that our Langevin dynamic simulations are predictive. The slight difference between these two methods is caused by (a) the analytical expression for the free energy Eq (3.2.1)-(3.2.2) used in Eq (3.2.25) is not accurate at small $r$, (b) we have to use the free energy of the nearest vertex of the triangular element to approximate the free energy of the moving particle at any time step (see Fig 3.2(d)), and (c) the
gradient of the free energy is computed by a finite difference method. In fact, the
distribution of first passage time in Fig 3.3(a) changes sharply if $R_1$ is changed by
a small amount in the region of $R_1 < 15$ nm. In Fig 3.3(b) we change to reflecting
boundary condition at $r = R_2$ and absorbing boundary condition at $r = R_1$ and
plot the first passage time obtained from Langevin dynamics as a function of the
distance between inclusions. This plot gives us an estimate of the time to self-
assembly for two inclusions under elastic and entropic interactions alone. The time
scale in Fig 3.3(b) is approximately one magnitude higher than in Fig 3.3(a), which
shows that the moving inclusion can exit the membrane much more rapidly when
there are two ‘doors’ than when there is only one ‘door’. Shnyrova et al. [29] have
shown that the time to self-assembly of a spherical bud formed by a viral protein
on vesicles about 4µm in diameter is a few seconds. Our estimates for time to self-
assembly in Fig 3.3(b) are about an order of magnitude lower, but the dimensions of
our membrane are also much smaller than the vesicles used in Shnyrova et al. [29].
Thus, our estimates of the time to self-assembly are not unreasonable. Fig 3.3(c)
shows how $P(r, t)$ evolves under absorbing boundary condition at $R_1$ and reflecting
boundary condition at $R_2$. As time progresses, we are more likely to find the moving
particle in a small neighborhood of the inner boundary. As $t \to \infty$, the probability
to find a particle exactly at $r = R_1$ will be 1. Fig 3.3(d) plots the first passage
time in the absence of Brownian motion ($T = 0$). The maximum in the curve near
$y = 70$nm happens because the free energy of the membrane-inclusion system has a
maximum at that separation. Thus, there is zero force acting on the inclusion when it is located at $y \approx 70\text{nm}$, so that the time to coalescence should tend to infinity. However, due to a finite grid size we cannot resolve this and we get a maximum in the curve. The time scales in Fig 3.3(a) and Fig 3.3(d) show that Brownian motion plays a dominant role in the model problem we are studying. In addition, the first passage time is hardly changed by removing interaction force (i.e. setting $\phi = 0$, see Fig 3.3(a) dashed line), showing again that Brownian motion plays a bigger role than interaction force in the self-assembly. The inset in Fig 3.3(d) is a comparison of the first passage time with $T = 0$ (red curve) and $T \neq 0$ (blue curve). The intersection of the two curves implies that the interaction force comes into play at small separations while the stochastic force is more significant when two particles are far away from each other. In Fig 3.3(a), Fig 3.3(b), Fig 3.3(c) we choose $R_1 = 19.84\text{nm}$ because the analytical expression for the free energy given in \cite{12} is not accurate at small separations. However, in Fig 3.3(d) we choose a smaller $R_1 = 12\text{nm}$ in order to demonstrate the effect of entropic and elastic force when two inclusions are sufficiently close. This choice of $R_1$ is reasonable as we do not use the formula in \cite{12} to make a comparison in Fig 3.3(d). We find that the first passage time will increase approximately linearly as the square of the radius of the outer boundary $R_2$ increases because Brownian motion dominates the self-assembly in most of the region.

An advantage of Eq (3.2.25) is that we can easily estimate the effects of chang-
ing various physical parameters on the first passage time (and hence the rate of self-assembly) without having to run lengthy Langevin dynamics simulations. For example, if we fix the temperature and increase diffusion coefficient $D$ (or decrease $\nu$), $T_1$ will decrease significantly. If we increase the bending modulus, $T_1$ will decrease because the attractive forces between the inclusions become stronger, but this is not a significant effect since the attractive forces come into play at short range while the problem is dominated by diffusion.

It is important to mention another subtle point. In computing the free energy of the membrane with two inclusions (i.e. $\phi(r)$) we are assuming equilibrium. This is a good assumption only if the longest wavelength vibration modes of the membrane have relaxed before an inclusion moves significantly. In \[85\], Lin and Brown computed the time over which the height correlation function \( \langle w(t)w(0) \rangle / \langle w^2 \rangle \) where $w$ is the out-of-plane deflection of the neutral plane) goes to zero. For a flat square membrane of side 112 nm, this time is 25 $\mu$s \[85\]. Since the relaxation time is proportional to $L^3$ where $L$ is the side of the square membrane \[85, 86\], the relaxation time in our problem can be computed as $25 \times 10^{-6} \times (500/112)^3 = 0.0022$ s, which is much smaller than the time scale in Fig 3.3(a) and Fig 3.3(b). Thus, by computing the free energy of the membrane-inclusion system and computing interaction forces from the gradient of the free energy we are ‘coarse-graining’ over the equilibrated vibration modes of the lipid membrane which allows us to focus entirely on the motion of the inclusions.
Figure 3.3: (a) Comparison of the first passage time derived from Fokker-Planck equation and Langevin dynamics. The inset shows two absorbing boundary condition at inner boundary $r = R_1$ and outer boundary $r = R_2$. (b) Prediction of the first passage time using Langevin dynamics. The inset shows an absorbing boundary condition at $R_1$ and a reflecting boundary condition at $R_2$. (c) The evolution of $P(r,t|y)$. The moving particle starts its kinetics at initial separation between two inclusions $y = 132.3$ nm (around the middle). As time is progressing, the probability of the moving particle being absorbed to the inner absorbing wall increases. (d) The black curve describes the first passage time without thermal fluctuation ($T = 0$) at initial angle $\alpha = 49.1^\circ$. The first passage time should go to infinity at
Figure 3.3: some point around 70 nm shown in this graph, which is close to the maximum point of the total free energy in Fig 3.1. The inset is a comparison of the first passage time between $T = 300$ K and $T = 0$ at $\alpha = 49.1^\circ$.

3.3 Self-assembly of elliptical inclusions

Our study of circular inclusions in the previous section showed that we can quantitatively describe their self-assembly on a lipid membrane. Most inclusions on a membrane (proteins involved in exo- and endo-cytosis, viral proteins) have more complex shapes \cite{9}. For this reason we consider cylindrical inclusions with elliptic cross-section. Three types of ellipses '$\phi_\frac{\pi}{6}$', '$\phi_\frac{\pi}{2}$', '$\phi_\frac{5\pi}{6}$' are considered here and are shown in Fig 3.4(a). They differ by how they are oriented with respect to our triangular grid. Some analytical results are available for these inclusions that can be used to verify our computations.
Figure 3.4: (a) Three types of elliptical inclusions in the finite difference grid based on the semi-analytic Gaussian integral method proposed in \[15, 14\]. (b) The interaction of two elliptical inclusions in a lipid membrane. $\psi_1, \psi_2$ are angles between the line connecting their centers and the major axis of the two elliptical inclusions. (c) Elliptical inclusion moving on the membrane without rotating. The green one is the initial position $(y, \alpha)$ and the red one is the current position $(r, \theta)$ of the moving elliptical inclusion. The purple one is the fixed inclusion located at the center.

### 3.3.1 Fitting of the interaction free energy

Kwiecinski et al., 2019 \[18\] proposed that the elastic energy of an infinite membrane with two identical elliptical inclusions depends on the inverse separation $r$ squared to lowest order,

$$E_{el} = \frac{c_1 \left( \cos 2\psi_1 + \cos 2\psi_2 \right)}{r^2} + \frac{c'_1}{r^4}. \quad (3.3.1)$$

This result is derived assuming elastic deformations of the lipid membrane only. It depends on the orientation of the inclusions through the angles $\psi_1$ and $\psi_2$ as
shown in figure Fig 3.4(b). Note that while the elastic interaction between two circular inclusions is always repulsive, Kwiecinski et al., 2019 [18] showed that one can have attractive interactions between elliptical inclusions even in the absence of thermal fluctuations. Thus, elliptical inclusions may have higher propensity to self-assembly.

The entropic interactions between elliptical inclusions have not been studied analytically, to the best of our knowledge. However, we can use our method based on Gaussian integrals to compute the entropic interactions [14]. We will use the form of expressions given in [16] to fit a simple analytical expression to the entropic part of the free energy.

\[ E_{en} = \frac{c'_2}{r^4} + \frac{c_3}{r^6} + O\left(\frac{1}{r^8}\right). \quad (3.3.2) \]

Ignoring \( O\left(\frac{1}{r^8}\right) \), we get an approximate expression for the total free energy,

\[ E = E_{el} + E_{en} = \frac{c_1(\cos 2\psi_1 + \cos 2\psi_2)}{r^2} + \frac{c_2}{r^4} + \frac{c_3}{r^6} + M(\varphi_\beta, \varphi_\eta), \quad (3.3.3) \]

where \( c_1, c_2, c_3, c'_1, c'_2 \) are constants and the function \( M(\varphi_\beta, \varphi_\eta) \), \( \eta, \beta \in \{\frac{\pi}{6}, \frac{\pi}{2}, \frac{5\pi}{6}\} \) coming from both the elastic part Eq (3.3.1) and entropic part Eq (3.3.2) depends on the orientations of the two elliptical inclusions, but not on \( r \).

Our first objective is to determine whether Eq (3.3.3) faithfully matches our computations based on the methods in [15, 14]. We have a square membrane and we place an elliptical inclusion at its center. We then place a second elliptic inclusion at various other positions on the membrane and compute the elastic and entropic
parts of the free energy of the membrane inclusion system. Fig 3.5 shows that our method agrees reasonably with Eq (3.3.3). However, the constants \((c_1, c_2, c_3)\) fitted from Eq (3.3.3) vary slightly among different quadrants (the major and minor axes of the ellipse in the center divides the square into four quadrants). This happens because we use triangular elements to mesh the square membrane which causes the mesh in each quadrant to be slightly different introducing some anisotropy. Also, we have a finite membrane while the analytical calculation in the elastic part of Eq (3.3.3) assumes an infinite membrane [18]. Nevertheless, in each quadrant Eq (3.3.3) is able to fit the data from our numerical calculations quite well. We choose \(\psi_e = \psi_{e1} = \psi_{e2}\). Our method captures the crossover from repulsive force at the smallest \(\psi_e = 0^\circ\) to attractive force at the largest \(\psi_e = 90^\circ\), which is likely to be important in studying the Brownian motion of anisotropic particles. The free energy function is fitted over all four quadrants since the analytical expression Eq (3.3.3) will be used in the Fokker-Planck equation to compute the first passage time.
Figure 3.5: Fitting of the total free energy by Eq (3.3.3). The two elliptical inclusions are chosen to be type \( \varphi_{90} \) (see Fig 3.4(a)) as shown in the panel on the top left. One inclusion is fixed at the center and the other is located at \((r, \psi_c)\) in a polar coordinate system. The remaining panels show the variation of the total free energy (elastic + entropic) of the membrane-inclusion system as a function of \( r \) for various \( \psi_c \). The symbols are obtained using our semi-analytic method based on Gaussian integrals [14] and the lines are fits using Eq (3.3.3). Note that some values of \( \psi_c \) result in repulsive interactions for small \( r \) (e.g. \( \psi_c = 0^\circ, 30^\circ \)), while others give an attractive interaction (e.g. \( \psi_c = 90^\circ, 270^\circ \)).
3.3.2 Langevin dynamics

When the inclusion is not circular, the translational drift (or drag) coefficient and diffusion coefficient should be characterized by second order tensors $\nu_{ij}, D_{ij}$ respectively where index $i, j = 1, 2$ represent two perpendicular directions $x_1$ and $x_2$. For an ellipsoid in 2D, the translational drift tensor is diagonal and described by $\nu_a, \nu_b$ for motion parallel and perpendicular to its major axis [87]. It is understood that the ellipsoid would diffuse more rapidly along its major axis, and this could lead to rotational diffusion due to particle anisotropy [87, 88, 89]. For simplicity, rotational diffusion of the inclusion is not considered in this subsection. The particle will diffuse along its major axis $x_1$ and minor axis $x_2$ independently with diffusion coefficient $D_\gamma = k_B T / \nu_\gamma$, for $\gamma$ either $a$ or $b$. To the best of our knowledge, many analytical expressions are available for the diffusion coefficient of ellipsoids in 3D, but little has been said about a cylindrical particle with elliptic cross section diffusing on a lipid membrane. Since we need diffusion and drag coefficients for such a particle on a lipid membrane, we combine the ideas of [79, 90] to add a geometric factor into the Saffman–Delbrück model Eq (3.3.7),

$$\nu_a = \frac{4\pi \eta_m}{\log(2\bar{\epsilon} - 1) - \gamma} G_a, \quad \nu_b = \frac{4\pi \eta_m}{\log(2\bar{\epsilon} - 1) - \gamma} G_b,$$

(3.3.4)

where $G_a, \bar{\epsilon}, G_b$ are given by,

$$G_a = \frac{8}{3} \left\{ \frac{1}{2\Gamma - 1} - \frac{2\Gamma^2 - 1}{(\Gamma^2 - 1)^2} \log \left( \frac{\Gamma + \sqrt{\Gamma^2 - 1}}{\Gamma - \sqrt{\Gamma^2 - 1}} \right) \right\}, \quad \bar{\epsilon} = \frac{2\eta_w \sqrt{l_a l_b}}{\eta_m},$$
\[ G_b = \frac{8}{3} \left[ \frac{1}{\Gamma^2 - 1} + \frac{2\Gamma^2 - 3}{(\Gamma^2 - 1)^2} \log \left( \Gamma + \sqrt{\Gamma^2 - 1} \right) \right]. \]  

where \( l_a, l_b \) are length of major axis, minor axis respectively and \( \Gamma = l_a/l_b \) is the ellipsoid aspect ratio. These expressions are not expected to be exact, but they do capture the anisotropic drag experienced by particles with elliptic cross-section.

Then, the Langevin equation Eq (3.2.3), Eq (3.2.4) are rewritten as,

\[
\frac{dr_i}{dt} = u_i, \quad (3.3.6)
\]

\[
m \frac{du_i}{dt} = -\nu_{ij} u_j - \frac{\partial \phi}{\partial r_i} + \eta_i(t), \quad (3.3.7)
\]

where \( \eta_i(t) \) here has the following properties,

\[
\langle \eta_i(t) \rangle = 0, \quad \langle \eta_i(t) \eta_j(t') \rangle = 2\nu_{ij} k_B T \delta(t - t'). \quad (3.3.8)
\]

Accordingly, Eq (3.2.6) is modified to,

\[
\frac{dr_i}{dt} = -\nu_{ij}^{-1} \frac{\partial \phi}{\partial r_j} dt + \sqrt{\frac{2k_B T dt}{\nu_{ii}}} \xi_i \text{ (no sum in the second term).} \quad (3.3.9)
\]

### 3.3.3 Fokker-Planck equation

Since the inclusions are elliptical, \( P \) is dependent both on \( y \) and the angle \( \alpha \) (see Fig 3.4(c)), and the potential \( \phi \) does not have radial symmetry. The drift velocity is accordingly also not isotropic. For these reasons, we consider \( p \) instead of \( P \), and then the Fokker-Planck equation is a partial differential equation of parabolic type,

\[
\frac{\partial p}{\partial t} = \frac{\partial}{\partial x_i} \left[ \nu_{ij}^{-1} \frac{\partial \phi}{\partial x_j} p \right] + \frac{\partial^2}{\partial x_i \partial x_j} \left[ D_{ij} p \right]
\]

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\[
\begin{align*}
&= - \left[ - \frac{\partial}{\partial x_i} \left( D_{ij} \frac{\partial p}{\partial x_j} \right) - \left( \nu_{ij}^{-1} \frac{\partial \phi}{\partial x_i} \right) \frac{\partial p}{\partial x_j} - \frac{\partial}{\partial x_i} \left( \nu_{ij}^{-1} \frac{\partial \phi}{\partial x_j} \right) p \right] \\
&= \frac{1}{\nu_a} \left( \frac{\partial^2 \phi}{\partial x_1^2} p + \frac{\partial \phi}{\partial x_1} \frac{\partial p}{\partial x_1} \right) + \frac{1}{\nu_b} \left( \frac{\partial^2 \phi}{\partial x_2^2} p + \frac{\partial \phi}{\partial x_2} \frac{\partial p}{\partial x_2} \right) + D_a \frac{\partial^2 p}{\partial x_1^2} + D_b \frac{\partial^2 p}{\partial x_2^2}.
\end{align*}
\]

### 3.3.4 First passage time

The first passage time of Eq (3.2.23) can be redefined as,

\[
T_1(y, \alpha) = \int_0^\infty f(y, \alpha, t) dt = - \int_0^\infty \int_{R_1}^{R_2} \int_0^{2\pi} \frac{\partial p(r, \theta, t|y, \alpha)}{\partial t} r dr d\theta dt
\]

\[
= \int_{R_1}^{R_2} \int_0^{2\pi} \int_0^\infty p(r, \theta, t|y, \alpha) dtdr dr = \int_{R_1}^{R_2} \int_0^{2\pi} \int_0^\infty q_1(r, \theta|y, \alpha) d\theta d\theta dt,
\]

where we have used \(tp(r, \theta, t|y, \alpha) \rightarrow 0\) as \(t \rightarrow \infty\) in the first equation of the second line and \(q_1\) is defined by,

\[
q_1(r, \theta|y, \alpha) = \int_0^\infty p(r, \theta, t|y, \alpha) dt.
\]

Then, we can derive a second order PDE for \(T_1(y, \alpha)\).

**Theorem 2:** The PDE for \(T_1(y, \alpha)\) with two absorbing walls at the outer and inner boundary is given below,

\[
\begin{align*}
(D_a \cos^2 \alpha + D_b \sin^2 \alpha) \frac{\partial^2 T_1}{\partial y^2} &+ \left( D_a \frac{\sin^2 \alpha}{y^2} + D_b \frac{\cos^2 \alpha}{y^2} \right) \frac{\partial^2 T_1}{\partial \alpha^2} + \left( -D_a \frac{\sin 2\alpha}{y} \right) \\
+ D_b \frac{\sin 2\alpha}{y} \frac{\partial^2 T_1}{\partial y \partial \alpha} &+ \left[ D_a \frac{\sin^2 \alpha}{y} + D_b \frac{\cos^2 \alpha}{y} + \frac{1}{\nu_a} \left( \frac{\sin 2\alpha \frac{\partial \phi}{\partial \alpha}}{2y} - \cos^2 \alpha \frac{\partial \phi}{\partial y} \right) \right] \\
- \frac{1}{\nu_b} \left( \sin 2\alpha \frac{\partial \phi}{\partial y} + \sin^2 \alpha \frac{\partial \phi}{\partial \alpha} \right) \frac{\partial T_1}{\partial y} &+ \left[ D_a \frac{\sin 2\alpha}{y^2} - D_b \frac{\sin 2\alpha}{y^2} + \frac{1}{\nu_a} \right] \\
&+ \left( \frac{\sin 2\alpha \frac{\partial \phi}{\partial y}}{2y} - \frac{\sin^2 \alpha \frac{\partial \phi}{\partial \alpha}}{y^2} \right) - \frac{1}{\nu_b} \left( \frac{\sin 2\alpha \frac{\partial \phi}{\partial y}}{2y} + \frac{\cos^2 \alpha \frac{\partial \phi}{\partial \alpha}}{y^2} \right) \frac{\partial T_1}{\partial \alpha} + 1 = 0.
\end{align*}
\]
with boundary conditions

\[ T_1(R_1, \alpha) = 0, T_1(R_2, \alpha) = 0, T_1(y, 0) = T_1(y, 2\pi). \quad (3.3.14) \]

**Proof:** See [Proof of Theorem 2](#).

Then, the first passage time solved from Eq (3.3.13) by finite difference method (see [Illustration of finite difference method](#)) can be compared with estimates from Langevin dynamics calculations as summarized in Fig 3.6.
Figure 3.6: The first passage time under different initial angle $\alpha = 90^\circ, 60^\circ, 30^\circ, 0^\circ$ derived from (a) Langevin dynamics and (b) Fokker-Planck equation. The insets of these two plots show two absorbing boundary conditions at inner boundary $r = R_1$ and outer boundary $r = R_2$. (c) gives a prediction using Langevin dynamics for a reflecting boundary condition at $R_2$ and an absorbing boundary condition at $R_1$ (as shown in the inset). (d) The black curve describes the first passage time without thermal fluctuation ($T = 0$) at $\alpha = 49.1^\circ$. The inset is a comparison of the first passage time between $T = 300$ K and $T = 0$ at $\alpha = 49.1^\circ$. 
The excellent agreement between the two methods demonstrates that our ideas can be applied to anisotropic inclusions. The curves in Fig 3.6(a) are not as smooth as those in Fig 3.3(a) due to our finite difference grid, finite time step $dt$ and our method to compute free energy (see Fig 3.2(d)). Nevertheless, it shows that the first passage time depends on the initial orientation of the moving inclusion if the initial center to center distance is fixed. An interesting result of Fig 3.6 is that the first passage time at initial angle $90^\circ$ under any fixed initial separation $y$ is the largest among the four cases while it has the strongest attractive entropic force in this direction (see Fig 3.5). On the other hand, the moving inclusion is getting absorbed most rapidly at initial angle $0^\circ$ even though the force in that direction is repulsive. This seems to contradict our intuition. In fact, if Brownian motion is prohibited by setting $T = 0$ in Eq (3.3.9), the first passage time will be in the reverse order (i.e. $T_1$ at $\alpha = 90^\circ$ is the smallest). The seeming anomaly in the presence of Brownian motion happens for the following reason. At $\alpha = 90^\circ$, the free energy reaches a local minimum in the $\theta$ direction that looks like a ‘trap’. It takes the moving particle more time to escape from this trap. By contrast, at $\alpha = 0^\circ$, the free energy is monotonic along $\theta$ direction. As a consequence, the moving particle is more likely to move in only one direction, and therefore reaches either of the two ‘doors’ at $r = R_1, r = R_2$ in less time. Fig 3.6(c) provides a prediction computed from Langevin dynamics under reflecting boundary condition at $R_2$ and absorbing boundary condition at $R_1$. The time scales are similar to those seen for
circular inclusions in Fig 3.3(c). The inset in Fig 3.6(d) indicates similar results as in the circular case, i.e. interaction force comes into play at small separations, while Brownian motion plays a bigger role when the two inclusions are far from each other.

3.3.5 Rotational diffusion of elliptical inclusions

If the rotational effects of elliptical inclusions are considered, the translational diffusion tensor $D_{ij}$ will be time-dependent [87]. Accordingly, the linear elliptic operator in Eq (B.0.2) will be time-dependent, too. For this reason, a PDE for the first passage time like Eq (3.3.13) cannot be derived from the Fokker-Planck equation. Nevertheless, we can still compute the first passage time from Langevin dynamics, which will now have an additional equation for the rotational motion of the elliptical inclusion [87],

$$\frac{d\Theta}{dt} = \omega,$$

$$I \frac{d\omega}{dt} = -\nu_\theta \omega - \frac{\partial \phi}{\partial \Theta} + \eta_\theta(t), \quad (3.3.15)$$

where $\Theta$ is an angle (see Fig 3.7(a)), $\omega$ is an angular velocity and $I$ is a moment of inertia of the elliptical inclusion. $\nu_\theta$ is a rotational drag coefficient, and $\eta_\theta(t)$ is a random torque given by [79] and with the following properties,

$$\nu_\theta = 16\pi \eta m l^2, \quad \langle \eta_\theta(t) \rangle = 0, \quad \langle \eta_\theta(t) \eta_\theta(t') \rangle = 2\nu_\theta k_B T \delta(t - t'). \quad (3.3.16)$$
Setting $I = 0$ in Eq (3.3.15) and combining with Eq (3.3.9), we get,

\[ d\Theta = -\frac{1}{\nu_{\theta}} \frac{\partial \phi}{\partial \Theta} dt + \sqrt{\frac{2k_B T dt}{\nu_{\theta}}} \xi_{\theta}, \]  

(3.3.17)

\[ dr' = -\nu^{-1}_{r'} \frac{\partial \phi}{\partial r_j'} dt + \sqrt{\frac{2k_B T dt}{\nu_{r'}}} \xi_{r'} \text{ (no sum in the second term).} \]  

(3.3.18)

where $\xi_{\theta}, \xi_{r'} \sim \mathcal{N}(0,1)$ and index $i', j'$ refer to body frame $x'_1, x'_2$ (see Fig 3.7(a)).

With the center of the moving inclusion fixed at each node on the grid, we can compute the total free energy in 6 directions ($\Theta_{1,2,3,4,5,6} = 30^\circ, 90^\circ, 150^\circ, 210^\circ, 270^\circ, 330^\circ$, see Fig 3.4(a)). We compute $\frac{\partial \phi}{\partial \Theta}$ in these six directions by a forward Euler method denoted as $\phi_{\Theta_1}, \phi_{\Theta_2}, \phi_{\Theta_3}, \phi_{\Theta_4}, \phi_{\Theta_5}, \phi_{\Theta_6}$, respectively. Then, for any $\Theta$ such that $\Theta_{m-1} < \Theta < \Theta_m$, we use linear interpolations of $\phi_{\Theta_{m-1}}$ and $\phi_{\Theta_m}$ to approximate $\frac{\partial \phi}{\partial \Theta}$ in the above equation.

The simulation result is shown in Fig 3.7(b) whose timescale is very similar to the one without rotational effects Fig 3.6(c). However, the first passage time in this case hardly depends on the initial angle $\alpha$. This is in good agreement with the results in [87] where the memory of initial angle $\alpha$ is washed out after long time if there is no external force ($\phi = 0$). We also study the distributions of the orientation of the moving particle when it crosses the inner boundary $r = R_1$. The result is shown in Fig 3.8. It seems that the moving particle crosses the inner boundary at angle around $60^\circ$ and $240^\circ$ with slightly higher probability, but this could be an artifact of our triangular grid. Rather, it is likely that all orientations are more or less equally probable when the particle crosses the $r = R_1$ boundary, but when the two particles coalesce they eventually adopt orientations corresponding to the
minimum free energy state.
Figure 3.7: (a) A cartoon of rotational motion. Initially both the moving inclusion and the centered inclusion are set to $\varphi_\pi$ types (i.e. $\Theta = 90^\circ$), but as time progresses the diffusing inclusion is allowed to rotate. (b) gives a prediction of the first passage time using Langevin dynamics. The inset shows a reflecting boundary condition at $R_2$ and an absorbing boundary condition at $R_1$. 
Figure 3.8: The probability distributions of the orientation of the moving particle when it crosses the inner boundary \( R_1 \) under four initial orientation \( \Psi_e = 0^\circ, 30^\circ, 60^\circ, 90^\circ \) and some appropriately chosen initial positions \( y \) such that the memory of the initial angle \( \Theta(0) \) is washed out when the inner boundary is crossed.
3.4 Hydrodynamic interactions of two circular inclusions

As a particle is moving along its trajectory, it will disturb the velocity field of the fluid around it. As a consequence, the viscous drag force exerted on other particles is also changed. This fluid-mediated hydrodynamic interaction between different particles is captured using the Oseen tensor \( \Omega(r_1 - r_2) \) which depends on the position of the particles \( r_1 \) and \( r_2 \). Let \( F_1 \) be the force acting on inclusion 1 and \( F_2 \) be the force acting on inclusion 2, then the velocity of the two inclusions are given by

\[
V_1 = \Omega(0) \cdot F_1 + \Omega(r_1 - r_2) \cdot F_2 + \sqrt{2} \, B(r_1 - r_2) \cdot \xi_1 \tag{3.4.1}
\]

\[
V_2 = \Omega(r_2 - r_1) \cdot F_1 + \Omega(0) \cdot F_2 + \sqrt{2} \, B(r_1 - r_2) \cdot \xi_2, \tag{3.4.2}
\]

where \( [\xi_k]_j, \ k \in \{0,1,2\} \) are random vectors with expectation values \( \langle [\xi_k]_j \rangle = 0 \), \( \langle [\xi_k]_i(t), [\xi_k]_j(t') \rangle = \delta_{ij} \delta(t-t') \), and a 2D version of the Oseen tensor \( \Omega(r) \) is given by

\[
\Omega(r) = \begin{cases} 
\frac{1}{4 \pi \eta m} \left[ \log \left( \frac{L}{r} - 1 \right) I + \frac{r \otimes r}{r^2} \right], & r \neq 0; \\
\frac{I}{\nu}, & r = 0.
\end{cases} \tag{3.4.3}
\]

In the above \( L \) is the side of the square membrane, \( r \) is the center to center distance between two inclusions. The weighting factors \( B \) in Eq (3.4.1)-(3.4.2) are the square root of the diffusion tensor \( D \) that is modified to include the hydrodynamic
interaction,
\[ D = \frac{k_B T}{\nu} (I + \nu \Omega), \quad D_{ij} = B_{ik} \cdot B_{jk}^T, \quad (3.4.4) \]
and \( I \) is second order identity tensor. In our model, since the inclusion 1 is always fixed, \( \mathbf{V}_1 = 0 \). We choose the center of inclusion 1 as the origin and use \( r = r_2 \) for brevity. Then,
\[ \mathbf{F}_1 = -\nu \Omega (r) \cdot \mathbf{F}_2 - \nu \sqrt{2} \mathbf{B}(r) \cdot \mathbf{\xi}_1. \quad (3.4.5) \]
\[ \implies \mathbf{V}_2 = (-\nu \Omega^2 (r) + \frac{1}{\nu}) \cdot \mathbf{F}_2 - \nu \sqrt{2} \Omega (r) \cdot \mathbf{B}(r) \cdot \mathbf{\xi}_1 + \sqrt{2} \mathbf{B}(r) \cdot \mathbf{\xi}_2. \quad (3.4.6) \]
Since the sum of two mutually independent normal random vectors is also a normal random vector, we can find a normal random vector \( [\mathbf{\xi}_0]_j \), such that,
\[ d\mathbf{r} = \mathbf{V}_2 dt = \mathbf{G}(r) \cdot \mathbf{F}_2 dt + \sqrt{2} \mathbf{\bar{B}}(r) \cdot \mathbf{\xi}_0 dt, \quad (3.4.7) \]
where the effective drift tensor \( \mathbf{G} \), effective diffusion tensor \( \bar{\mathbf{D}} \), and effective weighting factors \( \bar{\mathbf{B}} \) are given by,
\[ \mathbf{G}(r) = \left( -\nu \Omega^2 (r) + \frac{1}{\nu} \right), \quad \bar{\mathbf{D}}^2 = \nu^2 \Omega^2 \mathbf{B}^2 + \mathbf{B}^2. \quad (3.4.8) \]
Accordingly, the corresponding Fokker-Planck equation is a parabolic partial differential equation,
\[ \frac{\partial p}{\partial t} = \frac{\partial}{\partial x_i} \left[ G_{ij} \frac{\partial \phi}{\partial x_j} p \right] + \frac{\partial^2}{\partial x_i \partial x_j} \left[ \bar{D}_{ij} p \right] = - \left[ \frac{\partial}{\partial x_i} \left( \bar{D}_{ij} \frac{\partial p}{\partial x_j} \right) \right] - \left( G_{ij} \frac{\partial \phi}{\partial x_j} + \frac{\partial \bar{D}_{ij}}{\partial x_j} \right) \frac{\partial p}{\partial x_i} - \left( \frac{\partial}{\partial x_i} \left( G_{ij} \frac{\partial \phi}{\partial x_j} \right) + \frac{\partial^2 \bar{D}_{ij}}{\partial x_i \partial x_j} \right) p. \quad (3.4.9) \]
We transform Eq (3.4.9) into polar coordinates (see Computing the PDE for the first passage time under hydro-dynamic interaction), to get

\[
\frac{\partial p}{\partial t} = \frac{\partial^2 p}{\partial r^2} H_1(r, \theta) + \frac{\partial^2 p}{\partial \theta^2} H_2(r, \theta) + \frac{\partial^2 p}{\partial r \partial \theta} H_3(r, \theta) \\
+ \frac{\partial p}{\partial r} H_4(r, \theta) + \frac{\partial p}{\partial \theta} H_5(r, \theta) + p H_6(r, \theta).
\] (3.4.10)

In the above, \( H_1, H_2, H_3, H_4, H_5, H_6 \) are functions given in Computing the PDE for the first passage time under hydro-dynamic interaction. Using similar methods as before, a PDE for the first passage time can be derived (see Computing the PDE for the first passage time under hydro-dynamic interaction). We performed Langevin dynamic calculations as well as numerical integration of the PDE and the results are given in Fig 3.9.
Figure 3.9: The first passage time under different initial angle $\alpha = 90^\circ, 60^\circ, 30^\circ, 0^\circ$ derived from (a) Langevin dynamics and (b) Fokker-Planck equation. The insets of these two plots show two absorbing boundary conditions at $R_1$ and $R_2$. In addition, a comparison is made at $\alpha = 0^\circ$ between interaction force added ($\phi \neq 0$) and removed ($\phi = 0$). (c) gives a prediction for the first passage time using Langevin dynamics. The inset shows a reflecting boundary condition at $R_2$ and an absorbing boundary condition at $R_1$ by (d). The black curve describes the first passage time without thermal fluctuation ($T = 0$) at $\alpha = 49.1^\circ$. The inset is a comparison of the first passage time between $T = 300$ K (blue curve) and $T = 0$ (red curve) under hydrodynamic interactions and initial angle $\alpha = 49.1^\circ$. The black dashed curve
Figure 3.9: implies that when $T = 300 \text{ K}$ hydrodynamic interactions speed up self-assembly.

The excellent agreement between Fig 3.9(a) and Fig 3.9(b) again proves the effectiveness of our methods. From these two figures we also learn that the first passage time is not sensitive to the initial angle $\alpha$ for circular inclusions. Fig 3.9(b) shows that the Brownian motion is still dominant in the context of hydrodynamic interaction. Fig 3.9(c) predicts the first passage time when the outer boundary is replaced by reflecting boundary condition. A comparison of Fig 3.3(d) and Fig 3.9(d) shows that hydrodynamic interactions speed up self-assembly for two circular inclusions when $T = 300 \text{ K}$ (in agreement with [34]) and slow down self-assembly when $T = 0$. On the other hand, [94] show that hydrodynamic effects may not always speed up self-assembly of circular particles. However, our model is different from the one in [94] since (1) we fixed one inclusion in the center while they let both inclusions diffuse, and (2) we considered a flat lipid membrane while they performed computations on a spherical surface. Nevertheless, our results and those of [94] agree on the point that the coalescence time in the presence or absence of hydrodynamic interactions is of the same order of magnitude. The form of the black curve in Fig 3.9(d) is similar to Fig 3.3(d).
3.5 Self-assembly of multiple inclusions

In this section, we use Langevin dynamics to study the self-assembly of multiple inclusions. The inset of Fig 3.3(d) shows that self-assembly can be finished quickly at small separations where the interaction force is dominant. To verify this idea we study the self-assembly of three to six moving inclusions which are initially 16 nm away from the fixed inclusion at the center. In order to quantify the self-assembly of multiple inclusions, we compute probability distribution $n(r)$ (of finding an inclusion between $r$ and $r + dr$) after 30 time steps and compare between number of moving inclusions ($n_{\text{mov}} = 3, 4, 5, 6$). Due to limitations of computational power, we do not consider hydrodynamic interactions and run simulations 350 times for each case. Fig 3.10 shows that inclusions are slightly more likely to be absorbed to the center with a large number of moving inclusions ($n_{\text{mov}} = 5, 6$) than with small number. ($n_{\text{mov}} = 3, 4$). It seems that at small initial separations self-assembly could be finished sooner with more moving inclusions due to a stronger interaction force.
Figure 3.10: The probability distributions as a function of inclusion position after 30 time steps ($1.92 \times 10^{-5}$ s each time step). We divide radial direction into 43 evenly-spaced intervals of width 5 nm and count the number of moving inclusions in each interval after 30 time steps. Then, we use these numbers and divide by the total number of moving inclusions in 350 runs to get $n(r)$. E.g. if $n_{\text{mov}} = 3$ and the number of particles in the left most interval is 100, then the $n(r)$ for that interval is computed by $100/(350 \times 3)$. In each figure the inset is the total free energy of the membrane inclusion system as a function of separation (all inclusions are the same distance away from the center inclusion).
Our results in this section are limited by a lack of computational power. The calculation of the force on each inclusion in a cluster at every time-step is expensive since the equilibrium configuration of the membrane must be computed repeatedly and then numerical differentiation of the free energy of the whole system must be carried out. We were able to speed up this computation for two inclusions by tabulating the free energy of the membrane-inclusion system for every nodal position of the moving inclusion (remember that one of the inclusions is fixed at the center). This method is not practical if there are multiple moving inclusions, so we must resort to other techniques which we leave for future work.
Chapter 4

Kinetics of self-assembly of inclusions due to lipid membrane thickness-mediated interactions

This chapter is adapted from [95] “Xinyu Liao and Prashant K Purohit. Kinetics of self-assembly of inclusions due to lipid membrane thickness interactions. Soft Matter, 2021.”.

4.1 Introduction

Self-assembly of proteins on lipid membranes has been a topic of interest for at least the last three decades [34, 12, 23]. Proteins on membranes self-assemble because they interact with each other through forces that have their origins in membrane bending.
deformations\(^{13, 20}\), membrane thickness deformations\(^{96, 10, 11, 97, 19, 98, 13}\),
electrostatics\(^{17}\) and entropic interactions\(^{13, 16}\). There is a large literature on
this topic that we do not attempt to review here\(^{99, 25, 13, 71, 96, 20, 24, 70, 23, 21, 16, 22}\). Our interest is in self-assembly caused by membrane thickness mediated
interactions of proteins.

It is well known that lipid bilayers consist of two leaflets with the hydrophobic
tails of the lipid molecules spanning the membrane thickness. Proteins that are em-
bedded in the membrane have hydrophobic peptides placed in such a way as they
interact mostly with the hydrophobic tails of the lipid molecules. If the thickness
of the hydrophobic region of a protein is different from that of the lipid membrane
then the leaflets deform so that the membrane thickness in the vicinity of the pro-
tein changes (see Fig 4.1). The energy cost of the thickness deformation has been
estimated analytically by taking account of the lipid hydrocarbon chain entropy
\(^{97, 100}\). The result is an energy functional written in terms of the deformation
field \(u(x, y)\) of the half-membrane thickness and its gradients \(^{13, 97}\). The mem-
brane bending modulus \(K_b\), the membrane thickness modulus \(K_t\) and the isotropic
membrane tension \(F\) enter as parameters into this functional. The Euler-Lagrange
equation obtained by the minimization of this energy functional is a fourth order
linear partial differential equation (PDE). A series of papers by Phillips, Klug,
Haselwandter and colleagues \(^{10, 11, 96}\) start from this energy functional and uti-
lize the linearity of the PDE to computationally analyze allosteric interactions of
clusters of proteins of various shapes. The key idea is that the thickness deformation fields caused by distant proteins can overlap (superimpose) and give rise to interaction forces just as defects in elastic solids interact due to the overlap of deformation fields \[101\]. This idea has been in place since at least the mid-1990s \[97\], but it was computationally extended to complex protein shapes and large clusters by the above authors.

An important result that emerged from the research on clusters discussed above \[96\] is that the free energy has a maximum when plotted as a function of distance between individual proteins which form a lattice (the proteins on the lattice are all identical). To the left of the maximum there are strong attractive interactions between the proteins, while to the right there are weak repulsive forces which decay away as the proteins move far apart. The strong attractive forces should cause self-assembly if two (or more) proteins happen to come close together as they diffuse on the membrane. We are interested in the time scale of the self-assembly process. There are few experiments which focus on this time scale, but one by Shnyrova et al. \[29\] found that viral proteins (that did not interact electrostatically) on a micron-sized vesicle self-assemble in seconds.

Temporal evolution of the self-assembly of viral proteins on a lipid membrane has been analyzed in a few recent papers using simulations. Often these simulations can be computationally prohibitive, but they do give insight about time scales and intermediate states of the cluster of proteins assembling into a virus particle.
or nano-container [23, 94, 34, 12]. A drawback of these simulations is that they may not be able to tackle time scales of seconds over which self-assembly was seen to occur in experiments [29]. There is a large literature on phase-field approaches that can capture the evolution of particle concentrations on membranes (see for example [102, 72]), but these methods are not appropriate for computing the time to coalescence of a few particles diffusing on a membrane. We will take a different approach in this paper by analyzing self-assembly of differently shaped inclusions using Langevin dynamics and the corresponding Fokker-Planck equations. In recent work we viewed self-assembly of two inclusions as a first-passage time problem which can be quantitatively analyzed using the theory of stochastic processes [69]. We implemented this approach in the context of interactions based on membrane bending. The analytical calculations (using PDEs) in [69] were confined to absorbing boundary conditions on both boundaries. A novelty of this work is that we extend the PDE approach to include absorbing and reflecting boundary conditions.

This paper is arranged as follows. First, we quantify the interaction energy profile of hexagonal, rod- and star-shaped inclusions\(^1\). We show that our finite difference numerical method for computing energies agrees very well with analytical formulae (using Fourier-Bessel series) in most cases. After computing the interaction energies, we solve first-passage time problems to find the time scales over which two inclusions coalesce due to attractive interactions. We use both Langevin dynamics

\(^1\)We are limited in the shapes we can explore by the equilateral triangle grid used in our computations.
Table 4.1: List of parameters used in this chapter

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
<th>Units</th>
<th>Typical values</th>
</tr>
</thead>
<tbody>
<tr>
<td>ℓ</td>
<td>side length of triangular grid</td>
<td>nm</td>
<td>2.5</td>
</tr>
<tr>
<td>( K_b )</td>
<td>bending modulus</td>
<td>pN·nm</td>
<td>8.2 [69]</td>
</tr>
<tr>
<td>T</td>
<td>temperature</td>
<td>K</td>
<td>300</td>
</tr>
<tr>
<td>( k_B )</td>
<td>Boltzmann constant</td>
<td>N·m·K(^{-1})</td>
<td>1.38 \times 10^{-23}</td>
</tr>
<tr>
<td>( K_t )</td>
<td>thickness deformation modulus</td>
<td>pN·nm(^{-1})</td>
<td>248.4 [69]</td>
</tr>
<tr>
<td>r</td>
<td>separations between two inclusions</td>
<td>nm</td>
<td>9 – 125</td>
</tr>
<tr>
<td>( F )</td>
<td>applied tension</td>
<td>pN·nm(^{-1})</td>
<td>0.1 – 10</td>
</tr>
<tr>
<td>( a )</td>
<td>unperturbed bilayer half-thickness</td>
<td>nm</td>
<td>1.75 [69]</td>
</tr>
<tr>
<td>( R_1(\theta_1) )</td>
<td>shape function for the centered inclusion</td>
<td>nm</td>
<td></td>
</tr>
<tr>
<td>( R_2(\theta_2) )</td>
<td>shape function for the moving inclusion</td>
<td>nm</td>
<td></td>
</tr>
<tr>
<td>( \theta )</td>
<td>the angle between two inclusions and horizontal line (see Fig 4.2(b))</td>
<td>radian/degree</td>
<td></td>
</tr>
<tr>
<td>( u )</td>
<td>thickness deformation</td>
<td>nm</td>
<td></td>
</tr>
<tr>
<td>( u_i )</td>
<td>thickness deformation at node ( i )</td>
<td>nm</td>
<td></td>
</tr>
<tr>
<td>( V_i )</td>
<td>the area of the Voronoi cell at node ( i )</td>
<td>nm(^2)</td>
<td></td>
</tr>
<tr>
<td>( A_{ijk} )</td>
<td>the area of the triangle with vertices ( i, j, k )</td>
<td>nm(^2)</td>
<td></td>
</tr>
<tr>
<td>( R_1 )</td>
<td>radius of the inner boundary for diffusing inclusion</td>
<td>nm</td>
<td></td>
</tr>
<tr>
<td>( R_2 )</td>
<td>radius of the outer boundary for diffusing inclusion</td>
<td>nm</td>
<td></td>
</tr>
<tr>
<td>( \mathbf{u}_b )</td>
<td>the vector of all nodes determined by Eqs (4.2.15)-(4.2.16)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \mathbf{u}_a )</td>
<td>the vector of all nodes that are not in ( \mathbf{u}_b )</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \mathbf{u} )</td>
<td>( \mathbf{u} = [\mathbf{u}_a^T, \mathbf{u}_b^T]^T )</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \phi )</td>
<td>Energy of the system</td>
<td>pN·nm</td>
<td></td>
</tr>
<tr>
<td>( n )</td>
<td>ratio of side of triangle in the coarse grid to that in the fine grid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \nu(\nu_{ij}) )</td>
<td>translational drag coefficient (tensor for anisotropic inclusion)</td>
<td>s·pN·nm(^{-1})</td>
<td>2.32 \times 10^{-5} [69]</td>
</tr>
<tr>
<td>( D(D_{ij}) )</td>
<td>diffusion coefficient (tensor for anisotropic inclusion)</td>
<td>nm(^2·s^{-1})</td>
<td>1.76 \times 10^5 [69]</td>
</tr>
<tr>
<td>( T_n )</td>
<td>Chebyshev polynomials of the first kind</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( U_n )</td>
<td>Chebyshev polynomials of the second kind</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( K_n )</td>
<td>modified Bessel functions of the second kind</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

and the Fokker-Planck equation to obtain mean first passage times and study both isotropic and anisotropic problems with reflecting/absorbing boundary conditions. Finally, we summarize our results in the discussion and conclusion sections and point to various enrichments that can be implemented following our earlier work [69].

### 4.2 Energy landscape
4.2.1 Analytical solution based on Fourier-Bessel basis

Figure 4.1: (a) Schematic of bilayer deformations due to a thickness mismatch between hydrophobic region of a bilayer leaflet and an embedded protein. (b) The two types of boundary conditions that are used in this work. Dirichlet boundary
Figure 4.1: condition $U_i(\theta_i)$ gives the thickness deformation along the boundary of inclusion $i$, while the slope boundary condition $\nabla u \cdot \hat{n} = U_i''(\theta_i)$ determines the derivative along normal directions at each point along the boundary of inclusion $i$. The top view of the surrounding lipid molecules (green circles) is only shown along the horizontal line, but the lipids are everywhere on the plane. (c) A 3d plot of the thickness deformation field caused by one hexagonal inclusion. The thickness deformation is significant in the immediate vicinity of the inclusion and decays rapidly as one moves further away.
Figure 4.2: (a) The initial configuration of a system of two inclusions. The fixed
Figure 4.2: inclusion located at the center (blue) has local coordinate system \((r_1, \theta_1)\) and the moving inclusion (purple) has local coordinate system \((r_2, \theta_2)\). (b) The inclusion on the right moves to the green spot and forms an angle \(\theta\) with the horizontal line. (c) The energy of the configuration here is the same as the one in (b). Note that the hexagons in (c) are rotated when compared to hexagons in (a).

We consider a circular lipid membrane with radius \(R_2\) and two inclusions embedded in it. Our first goal is to compute the energy landscape seen by an inclusion interacting with another inclusion on a flat membrane. The interactions between the inclusions are a result of the overlap of membrane thickness deformation fields in their vicinity (see Fig 4.1(c) for the thickness deformation profile around one inclusion). The interaction energy will be computed by considering two inclusions, one fixed and the other moving as shown in Fig 4.2(a). The coordinate frame at the fixed inclusion (blue) denoted as inclusion 1 \((r_1, \theta_1)\) is set to be the default one. We assume that the moving inclusion (purple) denoted as inclusion 2 initially stays in the same orientation as inclusion 1 (see Fig 4.2(a)). To keep the analysis simple, when an inclusion moves we do not consider its rotational diffusion (see [69] where rotational diffusion was considered). As inclusion 2 moves from its initial position to the green spot and forms an angle \(\theta\) with the horizontal line (see Fig 4.2(b)), the energy of the system can be computed by rotating both inclusions anticlockwise by angle \(\theta\) from the initial configuration (see Fig 4.2(c)). This interaction energy will enter our analysis of the kinetics of the moving inclusion due to Brownian motion.
The elastic energy due to thickness deformation is given by \[19, 96, 10, 11\],

\[
\phi = \frac{1}{2} \int \left\{ K_b (\nabla^2 u)^2 + K_t \left( \frac{u}{a} \right)^2 + F \left[ \frac{2u}{a} + (\nabla u)^2 \right] \right\} \, dx dy, \tag{4.2.1}
\]

where \(K_b\) is the membrane bending modulus, \(K_t\) is the thickness deformation modulus, \(F\) is the applied tension and \(a\) is the unperturbed bilayer half-thickness. The integration is carried out over the area of the membrane. The Euler-Lagrange equation associated with Eq (4.2.1) is given by \[10\],

\[
K_b \nabla^4 u - F \nabla^2 u + \frac{K_t}{a^2} u + \frac{F}{a} = 0. \tag{4.2.2}
\]

Eq (4.2.2) can be reduced to the following form using the transformation \(\bar{u} = u + \frac{Fa}{K_t}\),

\[
(\nabla^2 - \nu_+)(\nabla^2 - \nu_-)\bar{u} = 0, \quad \nu_{\pm} = \frac{1}{2K_b} \left[ F \pm \left( F^2 - \frac{4K_bK_t}{a^2} \right)^{\frac{1}{2}} \right]. \tag{4.2.3}
\]

First, we consider the case of an infinitely large circular membrane with \(R_2 \to \infty\) without applied tension \((F = 0)\). We assume natural boundary condition which means that \(u = \bar{u} \to 0\) as \(R_2 \to \infty\). Let inclusion 2 be on the right side of inclusion 1. Then, a Fourier-Bessel series solution for the thickness deformation field around each inclusion \(i(i = 1, 2)\) can be obtained,

\[
\bar{u}_{i}^{\pm}(r_i, \theta_i) = A_{i,0}^{\pm} K_0(\sqrt{\nu_{\pm}} r_i) + \sum_{n=1}^{\infty} A_{i,n}^{\pm} K_n(\sqrt{\nu_{\pm}} r_i) \cos n\theta_i \\
+ B_{i,n}^{\pm} K_n(\sqrt{\nu_{\pm}} r_i) \sin n\theta_i \\
\approx A_{i,0}^{\pm} K_0(\sqrt{\nu_{\pm}} r_i) + \sum_{n=1}^{N} A_{i,n}^{\pm} K_n(\sqrt{\nu_{\pm}} r_i) \cos n\theta_i \\
+ B_{i,n}^{\pm} K_n(\sqrt{\nu_{\pm}} r_i) \sin n\theta_i, \tag{4.2.4}
\]

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where $K_n$ are modified Bessel functions of the second kind and we take the first $N$ terms in the series to approximate the sum. In [11] the authors used $N = 12$ to compute energy caused by thickness deformations and they showed that the series is almost converged. For small applied tension $F$ and large membrane size $R_2$, we follow [11] and use Eq (4.2.4) as an approximation for the solution of $\vec{u}_{1 \pm}^\pm$. Since the Euler-Lagrange equation (Eq (4.2.2)) is linear, the solution for Eq (4.2.3) is given by [11],

$$\vec{u} = \vec{u}_{1 \pm}^+(r_1, \theta_1) + \vec{u}_{1 \pm}^-(r_1, \theta_1) + \vec{u}_{2 \pm}^+(r_2, \theta_2) + \vec{u}_{2 \pm}^-(r_2, \theta_2), \quad (4.2.5)$$

in which we used the coordinate transformations,

$$r_2 = \sqrt{r^2 + r_1^2 - 2rr_1 \cos \theta_1} \equiv F_1(r_1, \cos \theta_1, r),$$

$$\cos \theta_2 = (-r + r_1 \cos \theta_1)/r_2, \quad \sin \theta_2 = r_1 \sin \theta_1/r_2;$$

$$r_1 = \sqrt{r^2 + r_2^2 + 2rr_2 \cos \theta_2} \equiv F_2(r_2, \cos \theta_2, r),$$

$$\cos \theta_1 = (r + r_2 \cos \theta_2)/r_1, \quad \sin \theta_1 = r_2 \sin \theta_2/r_1. \quad (4.2.6)$$

In order to efficiently apply the boundary conditions, we rewrite $\tilde{u}_{2 \pm}$ as a function of $r_1, \theta_1, r$ and $\tilde{u}_{1 \pm}$ as a function of $r_2, \theta_2, r$,

$$\tilde{u}_{1 \pm}^\pm(r_2, \theta_2, r) = A_{1 \pm}^{\pm} K_0(\sqrt{\nu \pm} F_2(r_2, \cos \theta_2, r))$$

$$+ \sum_{n=1}^{N} A_{1 \pm n}^{\pm} K_{n}(\sqrt{\nu \pm} F_2(r_2, \cos \theta_2, r)) T_n \left( \frac{r}{r_1} + \frac{r_2}{r_1 \cos \theta_2} \right).$$

The solution should include terms of the modified Bessel functions of the first kind when $F \neq 0$ and $R_2$ is finite. However, we show later that this approximation agrees quite well with the numerical solution of Eq (4.2.2).
\[
\frac{r_2}{r_1} \sin \theta_2, \quad (4.2.7)
\]

\[
\hat{u}_2^\pm(r_1, \theta_1, r) = A_{2,0}^\pm K_0(\sqrt{\nu} F_1(r_1, \cos \theta_1, r))
\]

\[
+ \sum_{n=1}^{N} B_{2,n}^\pm K_n(\sqrt{\nu} F_1(r_1, \cos \theta_1, r)) U_{n-1} \left( -\frac{r}{r_2} + \frac{r_1}{r_2} \cos \theta_1 \right)
\]

\[
\frac{r_1}{r_2} \sin \theta_1, \quad (4.2.8)
\]

where \(T_n, U_n\) are Chebyshev polynomials of the first kind and second kind, respectively. Let \(h_1 = \bar{u}_1^+ + \bar{u}_1^-, h_2 = \bar{u}_2^+ + \bar{u}_2^-, \hat{h}_1 = \hat{u}_1^+ + \hat{u}_1^-, \hat{h}_2 = \hat{u}_2^+ + \hat{u}_2^-\). We consider the following type of boundary conditions (see Fig 4.1(b)),

\[
(h_1 + \hat{h}_2)(\mathcal{R}_1(\theta_1), \theta_1, r) = U_1(\theta_1)
\]

\[
\hat{n} \cdot \left( \frac{\partial(h_1 + \hat{h}_2)}{\partial r_1}, \frac{1}{r_1} \frac{\partial(h_1 + \hat{h}_2)}{\partial \theta_1} \right)(\mathcal{R}_1(\theta_1), \theta_1, r) = U_1'(\theta_1) \quad (4.2.9)
\]

\[
(h_1 + h_2)(\mathcal{R}_2(\theta_2), \theta_2, r) = U_2(\theta_2)
\]

\[
\hat{n} \cdot \left( \frac{\partial(h_1 + h_2)}{\partial r_2}, \frac{1}{r_2} \frac{\partial(h_1 + h_2)}{\partial \theta_2} \right)(\mathcal{R}_2(\theta_2), \theta_2, r) = U_2'(\theta_2). \quad (4.2.10)
\]

We can solve for the \(4(2N + 1)\) coefficients \(A_{1,0}^\pm, A_{2,0}^\pm, A_{1,n}^\pm, A_{2,n}^\pm, B_{1,n}^\pm, B_{2,n}^\pm, n = 1, 2, \cdots, N\) because Eq (4.2.9)-(4.2.10) result in a linear system. This determines the full deformation field due to the overlap of the deformations caused by both inclusions. In the above expressions \(\mathcal{R}_1\) is the shape function for inclusion 1 which is defined as the boundary of inclusion 1 in the polar coordinates \((r_1, \theta_1)\), and similarly
for $R_2$ which is the shape function of inclusion 2. For a hexagon shaped inclusion $R_1$ is given by

$$R_1 = \frac{\sqrt{3} \ell}{2} \csc \left( \theta_1 - \frac{\pi}{3} \left( -1 + \text{floor} \left( \frac{3\theta_1}{\pi} \right) \right) \right), \quad 0 < \theta_1 \leq 2\pi, \quad (4.2.11)$$

where $\ell$ is the side length. The shape functions for rod shaped inclusion and star shaped inclusion are long and are given in the Appendix in the section named Shape functions for star and rod inclusion. The next step is to compute the energy $\phi(r)$ due to this deformation field. Note that the angular dependence of $\phi(r)$ appears through the shape functions of two inclusions, $R_1, R_2$.

Using the divergence theorem, the total energy expression in Eq (4.2.1) can be converted to the sum of line integrals over the boundary, i.e. $\phi = \phi_1 + \phi_2$ with $\phi_i$ given by,

$$\phi_i = \frac{1}{2} G_0 + \frac{1}{2} \int \nabla \cdot \left[ K_b (\nabla \bar{u}) \nabla^2 \bar{u} - K_b \bar{u} \nabla^3 \bar{u} + F \bar{u} \nabla \bar{u} \right] dxdy$$

$$= \frac{1}{2} G_1 - \frac{1}{2} \int_0^{2\pi} \hat{n} \cdot \left[ K_b (\nabla \bar{u}) \nabla^2 \bar{u} - K_b \bar{u} \nabla^3 \bar{u} + F \bar{u} \nabla \bar{u} \right]$$

$$\sqrt{R_i^2(\theta_i) + R_i^2(\theta_i)} d\theta_i$$

$$= \frac{1}{2} G_1 - \frac{1}{2} \int_0^{2\pi} \left[ U_i'(\theta) \left( K_b (\nu_+ \bar{u}_+ + \nu_- \bar{u}_-) + F \bar{u} \right) - K_b \bar{u} \hat{n} \cdot \nabla (\nu_+ \bar{u}_+ + \nu_- \bar{u}_-) \right] \sqrt{R_i^2(\theta_i) + R_i^2(\theta_i)} d\theta_i. \quad (4.2.12)$$

From the first line to the second line we assume the line integral along the outer boundary is a constant w.r.t $r$ (which works out to 0 as $R_2 \to \infty$ and $F \to 0$) and put it into the $G_1$ term (both $G_0$ and $G_1$ are constants). To compute Eq (4.2.12) we need to solve for the $4(2N+1)$ coefficients $A_{1,0}^\pm, A_{2,0}^\pm, A_{1,n}^\pm, A_{2,n}^\pm, B_{1,n}^\pm, B_{2,n}^\pm, n = 1, 2, \cdots, N$
from Eq (4.2.9)-(4.2.10) and use them to compute $\bar{u}_+, \bar{u}_-$. Then we can evaluate Eq (4.2.12) numerically. The energy $\phi(r)$ can be computed relatively efficiently using this technique. This is important since $\phi(r)$ must be computed repeatedly as inclusion 2 moves and $r$ changes due to Brownian motion when we solve the first passage time problem. We will also need the forces acting on inclusion 2 in our analysis later. Eq (4.2.12) gives an expression to compute the force analytically, which in the special case of an isotropic $\phi(r)$ (i.e., no angular dependence) works out to

$$
\phi'_i(r) = -\frac{R_i}{2} \int_0^{2\pi} \left[ U'_i(\theta) \left( K_b(\nu_+ \bar{u}'_+ + \nu_- \bar{u}'_-) + F \bar{u} \right) 
- K_b \bar{u}'_n \cdot \nabla (\nu_+ \bar{u}_+ + \nu_- \bar{u}_-)
- K_b \bar{u}'_n \cdot \nabla (\nu_+ \bar{u}'_+ + \nu_- \bar{u}'_-) \right] d\theta_i.
$$

When there is only one circular inclusion in the membrane, the thickness deformation field in Eq (4.2.5) has a closed form solution [10] which can be compared to the simulation result of Klingelhoefer et al. [103] who studied radial bilayer thickness profiles for the $G\alpha$ nanopore (among many others). We used the same parameters and boundary conditions as they did: $a = 34.19\text{Å}, U_1 = 0.81\text{Å}, U'_1 = 0.7, R_1 = 10\text{Å}$ for all $\theta_1$ and fit their curves by choosing $K_t = 120\text{pN}\cdot\text{nm}^{-1}, K_b = 2\text{pN}\cdot\text{nm}$ and $F = 0$. The black curve in Fig 4.3(a) (which comes from Eq (4.2.4) with $N = 8$) captures the overall trend and the magnitude of the bilayer thickness changes in the simulation done by Kingelhoefer et al. [103]. This is reasonable given that (a) the black curve is the result of a continuum approximation Eq (4.2.1) where as the lipids are discrete particles in the simulations of Kingelhoefer et al. [103], and (b)
the black curve assumes that the membrane is infinitely large where as it is finite in the simulations.

Figure 4.3: (a) Red squares are data from the simulation done by Klingelhofer et al. [103] and the black curve is fitted using the analytical method based on Fourier-Bessel function Eq (4.2.4). A reasonable agreement of the two profiles suggests
Figure 4.3: that the energy functional Eq (4.2.1) and the associated Euler-Lagrange equation are a good starting point for estimating interaction energies of inclusions. (b) The thickness deformation of one hexagon inclusion with shape function Eq (4.2.11), $K_b = 20k_B T$, $K_t = 60k_B T \cdot \text{nm}^{-2}$, boundary conditions $U_1 = -0.5 \text{ nm}$, $U'_1 = 0$ and under applied tension $F = 1\text{pN} \cdot \text{nm}^{-1}$ converges to the result computed by analytical method Eq (4.2.4) with $N = 8$ as $n$ increases. Recall that the side of the triangles in the fine grid is $1/n$ the side in the coarse grid.

4.2.2 Finite difference method based on refined grid

The above analysis gives us a semi-analytical technique to compute $\phi(r)$. This technique can be used when there are one or two inclusions whose shapes are simple. For a larger number of inclusions or those with complex shapes (see Fig 4.4(a)) we need a numerical method to compute the the thickness deformation field. Fortunately, Eq (4.2.1) can be minimized using a finite difference method. We discretize the membrane using equilateral triangle elements as shown in Fig 4.4(a) and Fig 4.4(b) following [15, 14]. We use a fine grid in the center of the domain and a coarse grid farther away for reasons explained below. Recall that one inclusion is fixed at the center of the domain and the other inclusion is allowed to move. The motion of the second inclusion is influenced by the change in interaction energy between the inclusions. This interaction energy depends on the gradients of $u(x, y)$ which changes rapidly when the two inclusions are nearby (which happens when the second inclu-
sion is near the center of the domain). Hence, to accurately compute the energy when the two inclusions are nearby (see Fig 4.4(b)) we use a fine grid in the center of the domain. When the inclusions are far away from each other there is hardly any interaction between them, so we use a coarse grid far away from the center of the domain to reduce computational cost. The side of the triangles in the fine grid is \(1/n\) of the side of the triangles in the coarse grid. The grid does not change as inclusion separation changes. The thickness deformation at node \(i\) is denoted by \(u_i\).
Figure 4.4: (a) Triangular discretization of the membrane in our finite difference method. The side of the triangles is $\ell$. Three types of inclusions are studied in this paper: hexagon(red), star(purple), rod(green). (b) A fine grid is implemented in a region near the center of the domain because the thickness deformation profile changes rapidly in that vicinity when two inclusions are present. The side of the triangles in the fine grid is $1/n$ that of the coarse grid. In this figure $n = 2$. (c) The blue regions are the Voronoi cells of purple node (in the coarse grid), yellow node (at the interface between fine and coarse grids) and white node (at the boundary of an inclusion), respectively.
Using methods similar to those in \[14, 15\] the energy is first written in a discrete form and then the thickness deformation field that minimizes this energy is computed. Finally, the minimizer is plugged back into the energy expression. Thus, the problem to be solved is

\[
\phi = \min_{u_i} K_V \sum_i \left( \sum_{c=1}^{6} u_i^c - 6u_i \right)^2 + \sum_i \frac{2}{9l^4} V_i \frac{K_t}{a} \left( \frac{u_i}{a} \right)^2 \\
+ \sum_i \frac{F u_i V_i}{a} + \sum_{i,j,k} \frac{F A_{ijk}}{3l^2} \left[ (u_i - u_j)^2 + (u_j - u_k)^2 + (u_k - u_i)^2 \right]
\]

\[
= \min_u u^T M u + \lambda u^T 1_u.
\]

(4.2.14)

For nodes \(i\) in the coarse or fine grid that are far away from the interface or the boundary of an inclusion (for example, see the purple node in Fig 4.4(c)) \(u_i^c, c = 1, 2, \ldots, 6\) are the thickness deformations at the nodes surrounding node \(i\). In the above \(V_i\) is the area of the Voronoi cell around node \(i\) and \(l\) is the side of the triangle. When node \(i\) is located at the boundary of an inclusion then the appropriate \(V_i\) is shown around the white node in Fig 4.4(c) and the sum over \(c\) in the first term is computed by assuming that the inclusion is flat. When node \(i\) is at the interface between the fine and coarse grid (see the yellow node in Fig 4.4(c)) then the appropriate \(V_i\) is shown around the yellow node in Fig 4.4(c) and we have to use different weights depending on where \(u_i^c\) is in the coarse grid or the fine grid\(^3\). In the final discrete version of the energy expression, \(1_u\) is a column vector of size \(\text{len}(u)\) with all entries 1 and \(\lambda = \frac{F V}{a} \). \(M\) is the stiffness matrix where \(M_{ij}\)

---

\(^3\)Slightly different weights for computing the energy contributions of the interface nodes do not change the final result for the profiles of \(u\) or the minimized energy.
multiplies $u_i u_j$. It has been shown\[11\] that the boundary condition can be written in the discrete form,

$u(r, \theta_i) = U(\theta_i), \ \forall i$ on the boundary

\[ u_k - \frac{1}{2} (u_i + u_j) \frac{1}{\sqrt{3l/2}} = U'(\theta_i'), \ \forall i', i, j, k \text{ pairs along the boundary} \]  

($i'$ is the midpoint of $i$ and $j$, see Fig 4.4(a)).  

(4.2.15)

(4.2.16)

Note that $u_i, u_j$ are given in Eq (4.2.15) and thus $u_k$ can be solved from Eq (4.2.16) immediately. We also assume that the inclusions are flat. Hence, Eq (4.2.14) can be rewritten as,

\[
\phi = \min_{\textbf{u}_a} \textbf{u}_a^T \begin{bmatrix} \textbf{M}_{aa} & \textbf{M}_{ab} \\ \textbf{M}_{ab}^T & \textbf{M}_{bb} \end{bmatrix} \begin{bmatrix} \textbf{u}_a \\ \textbf{u}_b \end{bmatrix} + \lambda \textbf{u}_a^T 1_a + \lambda \textbf{u}_b^T 1_b.
\]

(4.2.17)

In the above $\textbf{u}_b$ is the vector of all nodes determined by Eq (4.2.15)-(4.2.16) and $\textbf{u}_a$ is the vector of all nodes that are not in $\textbf{u}_b$. Taking $\frac{\partial (\textbf{u}^T \textbf{M} \textbf{u} + \lambda \textbf{u}^T 1_a)}{\partial \textbf{u}_a} = \textbf{0}$, we get $\bar{\textbf{u}}_a = -\text{M}_{aa}^{-1}(\text{M}_{ab}\textbf{u}_b + \frac{\lambda}{2} 1_a)$ at which Eq (4.2.17) is minimized where $1_a$ is a column vector of size $\text{len}(\textbf{u}_a)$ with all entries 1. Then, we can write the minimized total energy as,

\[
\phi = \left( \frac{\lambda}{2} 1_a^T + \textbf{u}_b^T \text{M}_{ab}^T \right) \text{M}_{aa}^{-1} \left( \text{M}_{ab}\textbf{u}_b + \frac{\lambda}{2} 1_a \right) - 2 \left( \frac{\lambda}{2} 1_a^T + \textbf{u}_b^T \text{M}_{ab}^T \right) \text{M}_{aa}^{-1} 1_a + \lambda \textbf{u}_b^T 1_b.
\]

(4.2.18)

In Fig 4.3(b) we compare the numerical solution of Eq (4.2.2) for the thickness deformation profile around one hexagon shaped inclusion using the above finite
difference technique with the analytical solution Eq (4.2.4) with $N = 8$, $K_b = 20k_B T$, $K_t = 60k_B T \cdot \text{nm}^{-2}$, $F = 1 \text{pN} \cdot \text{nm}^{-1}$, boundary conditions $U_1 = -0.5 \text{ nm}$, $U_1' = 0$ where $k_B$ is the Boltzmann constant and $T = 300 \text{K}$ is the absolute temperature and find excellent agreement as $n$ increases. Unless indicated otherwise, we use these parameter values in all calculations in this work. This shows that the analytical and numerical methods to compute the thickness deformation profiles are consistent with each other and with the results of molecular simulations documented in the literature. We use $n = 20$ in all the energy computations henceforth for each of the three different types of inclusions studied.

4.2.3 Applications to hexagon, rod and star shaped inclusions

We now focus on the interaction of two hexagon shaped inclusions on a lipid membrane which has a rotational periodicity of $\pi/3$. We use Eq (4.2.18) derived from our numerical method and Eq (4.2.12) derived from the analytical method, to compute the interaction energy of two inclusions separated by distance $r$ and then make comparisons. As shown in Fig 4.5(a), the energy computed using the analytical method for two inclusions separated by distance $r$ in two different orientations (shown in the inset differing by a rotation of $\pi/6$) are almost the same. Hence, we can simplify our model and consider the energy landscape generated by two hexagon inclusions as being almost isotropic (insensitive to rotation). In Fig 4.5(b), we fix the shape
of the two hexagons (see inset of the figure), and show that as \( n \) increases, the match between the energy computed from the numerical method and analytical method gets better, justifying our numerical approach of using a fine grid when the inclusions are nearby. From Fig 4.5(c) we learn that as applied tension increases, the attraction at small separations (around \( r = R_1 = 7 \) nm) becomes weaker, but the repulsive force at around 9 – 10 nm also becomes weaker. In Fig 4.5 we study short range interactions as small as center-to-center distance 7 nm for two hexagon inclusions. The side of the hexagon is 2.5 nm, so a 7 nm center-to-center distance corresponds to about 2 nm distance between their boundaries, which corresponds to two lipid molecules. At such small separations we do not expect that minimization of the continuum expression Eq (4.2.1) will capture molecular level deformations, although it has been shown previously that it captures many aspects of protein-induced bilayer perturbations[96, 104, 105]. For this reason, in later computations of the first passage time we take \( R_1 = 9 \) nm for both hexagon and star inclusions. Fig 4.6(a) and Fig 4.6(b) show that the computed energy hardly changes as we increase \( N \) from \( N = 8 \) to \( N = 9, 10 \) in Eq (4.2.12). For this reason we use \( N = 8 \) for all the computations in this work. In Fig 4.6(c), we compute the energy due to thickness deformations of a cluster of seven inclusions as shown in the inset using our finite difference method. We find a maximum in the energy around \( r = 9 \) nm similar to the findings in [15, 69] which studied interactions of inclusions due to membrane bending deformations in the presence of fluctuations and those in [96].
which studied thickness deformations of MscL lattice. We do not assume pairwise additivity of the energy in any of our computations (including those with 7 inclusions) because the thickness deformation field is solved by minimizing Eq (4.2.1) with boundary conditions applied at each inclusion.
Figure 4.5: (a) The energy computed by the analytical method using Eq (4.2.12) for two configurations of hexagon inclusions differing by a rotation of \( \pi/6 \) under \( F = 1 \text{pN} \cdot \text{nm}^{-1} \). (b) The energy of the configuration with \( F = 1 \text{pN} \cdot \text{nm}^{-1} \) computed
Figure 4.5: numerically using Eq (4.2.18) converged to the energy computed by Eq (4.2.12) as $n$ increases. Recall that the side of the triangles in the fine grid is $1/n$ that of the coarse grid. (c) A comparison of the energy profiles at three different applied tensions: 0.1pN·nm$^{-1}$, 1pN·nm$^{-1}$, 10pN·nm$^{-1}$. 
Figure 4.6: The energy computed using different number of terms in the series 
\( N = 8, 9, 10 \) in Eq (4.2.4) under applied tension (a) 1pNnm\(^{-1}\), (b) 10pNnm\(^{-1}\). The energy computed with different \( N \) are almost identical. For this reason we use
Figure 4.6: $N = 8$ in Eq (4.2.4) for all energy computations. The energy in these two plots is not scaled by subtracting the value at $r = R_2$. (c) The energy of a cluster of seven hexagons (one fixed at the center) as a function of separations $r$ under applied tension $F = 1\text{pN}\cdot\text{nm}^{-1}$. There is a maximum around $r = 9 \text{nm}$ which is similar to the findings in [15] that focuses on bending deformations and those in [96] that analyzes thickness deformations.
Figure 4.7:  (a) The energy of two rod shaped inclusions computed by numerical method Eq (4.2.18) with different $\theta$ under applied tension $1\text{pN}\cdot\text{nm}^{-1}$. (b) The energy computed by analytical method using Eq (4.2.12) fits the one computed by numerical method using Eq (4.2.18) both with $\theta = 0^\circ$ and $\theta = 90^\circ$ under applied tension $1\text{pN}\cdot\text{nm}^{-1}$.
Fig 4.7(a) shows that the energy landscape of two rod shaped inclusions is anisotropic - at small separations the force is repulsive at $\theta = 0^\circ$ and becomes attractive at some angle around $40^\circ < \theta < 50^\circ$. The attraction increases as $\theta$ goes up to $90^\circ$. This behavior of the energy of two rod shaped inclusions is reminiscent of the energy from out-of-plane deflection for two rods on a lipid membrane. Fig 4.7(b) shows that the energy computed by the numerical method and analytical method again agree very well which gives us confidence in the numerical method.
Figure 4.8: (a) The energy of two rod shaped inclusions computed by numerical method Eq (4.2.18) with different $\theta$ under applied tension $0.1\text{pN}\cdot\text{nm}^{-1}$. (b) The energy of two rod shaped inclusions computed by numerical method Eq (4.2.18) with different $\theta$ under applied tension $10\text{pN}\cdot\text{nm}^{-1}$.
Next we compute the interaction energy of rod shaped inclusions for tensions $F = 0.1\text{pN}\cdot\text{nm}^{-1}$ and $F = 10\text{pN}\cdot\text{nm}^{-1}$. The comparison between Fig 4.8 and Fig 4.7(a) shows that as applied tension increases, the interaction force becomes weaker at short separations, which implies that elastic interactions could be weakened by strong applied tension. Physically, this is reasonable, since high tension will tend to make the membrane flatter so that the thickness is more uniform everywhere.
Figure 4.9: (a) Solid lines are the energy of two star inclusions with different rotational angles computed by numerical method using Eq (4.2.18) and dashed lines are the energy computed by analytical method using Eq (4.2.12). The applied tension
Figure 4.9: is \(1 \text{pN} \cdot \text{nm}^{-1}\). (b) The energy of two star inclusions computed by numerical method under three applied tensions: \(0.1 \text{pN} \cdot \text{nm}^{-1}\), \(1 \text{pN} \cdot \text{nm}^{-1}\), \(10 \text{pN} \cdot \text{nm}^{-1}\). (c) The energy of one hexagon inclusion and one star inclusion under applied tension \(1 \text{pN} \cdot \text{nm}^{-1}\).

Next, we apply both methods to compute the interaction energy of two star shaped inclusions in Fig 4.9. Just as in the case of hexagons, we consider various orientations of the star shaped inclusions as shown in the inset of Fig 4.9(a). The match between the analytical method and numerical method is not as good in this case because the star shaped inclusion has 12 vertices at which the derivative along normal directions are discontinuous. Since in the analytical method we used Fourier-Bessel series to approximate the contour \((R_1, R_2)\) and the derivative along normal directions to the boundaries, it requires a large number of terms \(N\) to obtain a good approximation. This is computationally not feasible for symbolic operations in MATLAB. Thus, we have greater confidence in our finite difference numerical method to compute interaction energies in complex geometries. In Fig 4.9(b) we use our numerical method to compute the interaction energies for star shaped inclusions for various values of \(F\). The trends are similar to those seen for hexagon shaped inclusions. Finally, in Fig 4.9(c) we compute the energy of one hexagon and one star inclusion separated by distance \(r\) and find again that there is a maximum in the curve around \(r = 12\text{nm}\).

This completes our analysis of the interaction energy of inclusions due to bilayer
thickness interactions. In most cases we have shown that there is a maximum in the interaction energy of two inclusions around a separation $r = 10$ nm. For separations smaller than this value there is a strong attractive force between the inclusions which will cause them to coalesce. In the next section we will compute the time to coalescence as a function of the initial separation between the inclusions.

4.3 First passage time for isotropic inclusions under mixed boundary condition

Our main goal in this paper is to study the kinetics of an inclusion diffusing in an energy landscape resulting from elastic interactions with another inclusion. Efficient methods to compute the energy landscape developed above are a pre-requisite to this exercise. We will now use these methods to solve first passage time problems.

We consider a circular membrane of size $R_2 = 125$ nm with a circular inclusion of size 2.5 nm fixed at the center. Another circular inclusion of the same size is diffusing around driven by stochastic forces. Recall from the energy landscape that there are attractive interactions between inclusions when the separations are small. Hence, if the moving inclusion comes close enough to the static one at the center then it will be strongly attracted. Therefore, we assume that at $R_1 = 7$ nm there is an absorbing wall at which the moving inclusion will disappear by being attracted towards the center. We assume that at $R_2 = 125$ nm (far away) there is a reflecting
wall where the moving inclusion will be bounced back. Note that problems in which both boundaries are absorbing were solved elsewhere [69]. The exercise we will perform now is as follows. We place the second inclusion randomly on a circle of radius \( r = y \) at time \( t = 0 \) and let it diffuse around. At some time \( t = T_{in} \) when the inclusion hits the inner boundary for the first time we stop it from diffusing and record \( T_{in} \). We repeat this experiment a large number of times and record \( T_{in} \) for each repetition. The mean value of \( T_{in} \) is the mean first passage time \( T_1 \). Our goal is to find \( T_1(y) \) as a function of the initial condition \( r = y \). This can be done analytically or through a Langevin dynamics simulation. We will use both methods in the following.

To estimate \( T_1(y) \) analytically we first need to compute survival probabilities. Let \( p \) be the probability density (for finding the inclusion) at position \( r \) and angle \( \theta \) given initial condition \( r = y, \theta = \alpha \) and \( P(r, t|y) = \int_0^{2\pi} p(r, t, \theta|y, \alpha) d\theta \). The probability density \( p \) is independent of \( \theta \) since neither the energy landscape nor the diffusion (or drag) coefficient of the inclusion depends on it. As a result, the Fokker-Planck equation for the evolution of this probability is in the following isotropic form [69],

\[
\frac{\partial P}{\partial t} = \frac{\partial}{\partial r} \left[ \frac{1}{\nu} \frac{\partial \phi}{\partial r} P + D \frac{\partial P}{\partial r} \right] + \frac{1}{r} \left[ \frac{1}{\nu} \frac{\partial \phi}{\partial r} P + D \frac{\partial P}{\partial r} \right],
\]

(4.3.1)

with Dirichlet boundary condition at the inner boundary and Robin boundary condition at the outer boundary [82],

\[
P(R_1, t) = 0, \quad \left( k_B T \frac{\partial P}{\partial r} + \frac{\partial \phi}{\partial r} P \right) \bigg|_{(R_2, t)} = 0, \quad \forall t \geq 0.
\]

(4.3.2)
The first equation in Eq (4.3.2) is an absorbing boundary condition at $R_1$ which means that if the moving particle hits this wall it is absorbed and exits the kinetics. So, the probability to find such a particle at the absorbing wall is zero. The second equation in Eq (4.3.2) is the reflecting boundary condition at $R_2$. It means that when the moving particle hits the reflecting wall it will bounce back, hence the probability current at this point will be zero [82]. In the above $D$ is a diffusion coefficient of the inclusion in the lipid membrane and $\nu$ is a drag coefficient which are related by the Nernst-Einstein relation $\nu D = k_B T$ [69]. Let $S(y,t)$ be the survival probability,

$$S(y,t) = \int_{R_1}^{R_2} P(r,t|y)rdr.$$  \hspace{1cm} (4.3.3)

Then, we can get the first passage time density,

$$f(y,t) = -\frac{\partial S(y,t)}{\partial t} = -\int_{R_1}^{R_2} \frac{\partial P(r,t|y)}{\partial t} rdr.$$  \hspace{1cm} (4.3.4)

The existence of the first moment of $P(r,t|y)$ with respect to time $t$ can be shown from the fundamental solution constructed by Itô in [84]. Then, $tP(r,t|y) \to 0$ as $t \to \infty$. Accordingly, the first passage time $T_1(y)$ can be derived from Eq (4.3.4),

$$T_1(y) = \int_0^\infty f(y,t)dt = -\int_0^\infty \int_{R_1}^{R_2} \frac{\partial P(r,t|y)}{\partial t} rdr dt$$  
$$= \int_{R_1}^{R_2} \int_0^\infty P(r,t|y)drt = \int_{R_1}^{R_2} g_1(r,y)rdr,$$  \hspace{1cm} (4.3.5)

where $g_1$ is defined by,

$$g_1(r,y) = \int_0^\infty P(r,t|y)dt.$$  \hspace{1cm} (4.3.6)
**Theorem 3:** The ODE for $T_1(y)$ with a reflecting wall at the outer boundary and an absorbing wall at the inner boundary is

$$
\frac{\partial^2 T_1(y)}{\partial y^2} + \left( -\frac{1}{k_B T} \frac{\partial \phi}{\partial y} + \frac{1}{y} \right) \frac{\partial T_1(y)}{\partial y} + \frac{1}{D} = 0,
$$

(4.3.7)

with boundary conditions,

$$
T_1(R_1) = 0, \quad T'_1(R_2) = 0.
$$

(4.3.8)

**Proof:** See [Proof of Theorem 3](#).

Next, we describe how to estimate $T_1(y)$ using Langevin dynamics simulations. The overdamped version of the Langevin equation in an isotropic setting is given by [69],

$$
dr_i = -\frac{1}{\nu} \frac{\partial \phi}{\partial r_i} dt + \sqrt{\frac{2k_B T dt}{\nu}} \xi_i,
$$

(4.3.9)

where $i$ represents two perpendicular directions of the motion. $\nu$ is the translational drag coefficient of a circular inclusion which is calculated using the Saffman-Delbrück model,

$$
\nu = \frac{4\pi \eta_m}{\log(2\epsilon^{-1}) - \gamma},
$$

(4.3.10)

where $\eta_m = 15.3 \times 10^{-9}$ Pa $\cdot$ s $\cdot$ m is the membrane viscosity (2D), $\eta_w = 8.5 \times 10^{-4}$ Pa $\cdot$ s is the bulk viscosity of water (3D), $\ell = 2.5$ nm is the radius of the circular cross section of the inclusion, $\epsilon = 2\eta_w \ell / \eta_m$ and $\gamma \approx 0.577$ is Euler’s constant [80]. Then we use the Einstein relation $D = k_B T / \nu$ to compute the diffusion coefficient $D$. For more details we refer the readers to [69]. The drag and diffusion coefficient are both
given in Table 4.1. We have neglected rotational diffusion here because it is shown in [69] that it does not play a major role in determining the first passage time. \(\xi_i \sim \mathcal{N}(0, 1)\), a normally distributed random variable with mean 0 and variance 1, represents the stochastic force along direction \(i\). We initially put the moving particle somewhere at \(r = y\), and choose a time step \(dt\) that ensures convergence of the Lagenvin dynamics simulation. Then, for each time step \(dt\), we perform the calculation in Eq (4.3.9), updating the position of the moving inclusion. We record the time at which the moving particle hits the absorbing wall at \(R_1\). We run 8000 simulations and then take an average to estimate the first passage time. For more details the readers are referred to [69]. Fig 4.5(a) and Fig 4.9(a) show that the \(\phi(r)\) for hexagon and star inclusions can be regarded as nearly isotropic. For these we use Eq (4.3.7) to numerically solve for the first passage time and compare it with the results obtained from the Langevin simulations. Since inclusions have non-zero size and our computations of the interaction energy \(\phi(r)\) are not meaningful if the distance between their boundaries (not centers) is comparable to the size of a lipid headgroup (which is 1nm), so we choose \(R_1 = 9\) nm for isotropic inclusions (hexagon, star) and \(R_1 = 11\) nm for anisotropic inclusion (rod) in our first passage time calculations.
Figure 4.10: The first passage time for two hexagon inclusions is computed using
(a) Langevin dynamics simulations in Eq (4.3.9), (b) ODE in Eq (4.3.7) under three
applied tensions $0.1 \text{pN}\cdot\text{nm}^{-1}$, $1 \text{pN}\cdot\text{nm}^{-1}$, $10 \text{pN}\cdot\text{nm}^{-1}$.

Fig 4.10 shows that the first passage time for hexagonal inclusion derived from
the two methods are in good agreement. As the applied tension increases, the
first passage time is reduced at most $r$ that are not close to $R_1$. At first glance this might seem counter-intuitive because from Fig 4.5(c) we know that at small separations (close to $R_1$) the attraction force becomes weaker as applied tension increases. However, there is a stronger repulsive force at around $r = 9 - 10\text{nm}$ under large applied tension which slows the motion of the moving particle from a large starting separation.
Figure 4.11: The first passage time for two star inclusions is computed by (a) Langevin dynamics simulations in Eq (4.3.9), (b) ODE in Eq (4.3.7) under three applied tensions 0.1pN·nm⁻¹, 1pN·nm⁻¹, 10pN·nm⁻¹.
The first passage time computed by the two methods is also in good agreement when the inclusions are star shaped. The order of the first passage time is the same as the hexagonal inclusions and similar arguments for the shape of the curves can be made here.

Figure 4.12: The first passage time for hexagon inclusions with electrostatic potential $\phi = \frac{50}{r}$ estimated by Langevin equation Eq (4.3.9) with time step $dt = 10^{-6}$ (orange), with time step $dt = 10^{-7}$ (green), by ODE Eq (4.3.7) (blue) under applied tension 1pN·nm$^{-1}$. The purple curve is solved from Eq (4.3.7) by setting $\phi = 0$ as a comparison.

The first passage times in all the above computations are dominated by the Brownian motion because the membrane thickness mediated interactions play a significant role only for small separations $y$. To validate our methods for stronger
and longer range interactions between inclusions we choose $\phi = \frac{50}{r}$ to mimic electrostatic interactions between point charges and use Eq (4.3.9) and Eq (4.3.7) to compute the first passage times. The time step used in all previous Langevin simulations is $dt = 10^{-6}$ s. However, we did a small number of Langevin simulations with $dt = 10^{-7}$ to validate our results. From Fig 4.12 we see that as $dt$ decreases to $10^{-7}$ in Eq (4.3.9) the first passage time computed by Langevin simulations (green curves) converges to the one solved from the ODE in Eq (4.3.7). The purple curve in Fig 4.12 is derived by setting $\phi = 0$ in Eq (4.3.9) and Eq (4.3.7) for the purpose of comparison. It is clear that the repulsive electrostatic force slows down the kinetics of coalescence of the moving particle. These results show that our methods are also applicable in scenarios where deterministic forces play an important role in the interactions of the inclusions.

### 4.4 First passage time for anisotropic inclusions under mixed boundary condition

For two non-circular inclusions, the corresponding Fokker-Planck equation for the probability density $p$ is a partial differential equation of parabolic type [69],

$$
\frac{\partial p}{\partial t} = \frac{\partial}{\partial x_i} \left[ \nu_i \frac{\partial \phi}{\partial x_i} p \right] + \frac{\partial^2}{\partial x_i \partial x_j} \left[ D_{ij} p \right]
$$

$$
= \frac{1}{\nu_a} \left( \frac{\partial^2 \phi}{\partial x_1^2} p + \frac{\partial \phi}{\partial x_1} \frac{\partial p}{\partial x_1} \right) + \frac{1}{\nu_b} \left( \frac{\partial^2 \phi}{\partial x_2^2} p + \frac{\partial \phi}{\partial x_2} \frac{\partial p}{\partial x_2} \right) + D_a \frac{\partial^2 p}{\partial x_1^2} + D_b \frac{\partial^2 p}{\partial x_2^2}.
$$

(4.4.1)
Accordingly, we need to redefine the first passage time in Eq (4.3.5) which is now given by,

\[
T_1(y, \alpha) = \int_0^\infty f(y, \alpha, t)dt = -\int_0^\infty \int_{R_1}^{R_2} \int_0^{2\pi} \frac{\partial p(r, \theta, t|y, \alpha)}{\partial t} r dr d\theta dt
\]

\[
= \int_{R_1}^{R_2} \int_0^{2\pi} \int_0^\infty p(r, \theta, t|y, \alpha) dt r dr d\theta
\]

\[
= \int_{R_1}^{R_2} \int_0^{2\pi} q_1(r, \theta|y, \alpha) dr d\theta,
\]

(4.4.2)

where \(tp(r, \theta, t|y, \alpha) \to 0\) as \(t \to \infty\) is implemented in the first equation of the second line and \(q_1\) is defined by,

\[
q_1(r, \theta|y, \alpha) = \int_0^\infty p(r, \theta, t|y, \alpha) dt.
\]

(4.4.3)

**Theorem 2:** The PDE for \(T_1(y, \alpha)\) with a reflecting wall at the outer boundary and an absorbing wall at the inner boundary is given below,

\[
(D_a \cos^2 \alpha + D_b \sin^2 \alpha) \frac{\partial^2 T_1}{\partial y^2} + \left(\frac{D_a \sin^2 \alpha}{y^2} + \frac{D_b \cos^2 \alpha}{y^2}\right) \frac{\partial^2 T_1}{\partial \alpha^2} +
\]

\[
\left(-\frac{D_a \sin 2\alpha}{y} + \frac{D_b \sin 2\alpha}{y}\right) \frac{\partial^2 T_1}{\partial y \partial \alpha} + \left[\frac{D_a \sin^2 \alpha}{y} + \frac{D_b \cos^2 \alpha}{y}\right] +
\]

\[
\frac{1}{\nu_a} \left(\frac{\sin 2\alpha \frac{\partial \phi}{\partial \alpha}}{2y} - \cos^2 \alpha \frac{\partial \phi}{\partial y}\right) - \frac{1}{\nu_b} \left(\frac{\sin 2\alpha \frac{\partial \phi}{\partial \alpha}}{2y} + \sin^2 \alpha \frac{\partial \phi}{\partial y}\right)
\]

\[
+ \left[D_a \frac{\sin 2\alpha}{y^2} - D_b \frac{\sin 2\alpha}{y^2} + \frac{1}{\nu_a} \left(\frac{\sin 2\alpha \frac{\partial \phi}{\partial y}}{2y} - \sin^2 \alpha \frac{\partial \phi}{\partial \alpha}\right)\right]
\]

\[
- \frac{1}{\nu_b} \left(\frac{\sin 2\alpha \frac{\partial \phi}{\partial y}}{2y} + \cos^2 \alpha \frac{\partial \phi}{\partial \alpha}\right) \right] \frac{\partial T_1}{\partial \alpha} + 1 = 0,
\]

(4.4.4)

with boundary conditions

\[
T_1(R_1, \alpha) = 0, \quad \frac{\partial T_1}{\partial y}(R_2, \alpha) = 0, \quad T_1(y, 0) = T_1(y, 2\pi).
\]

(4.4.5)
Proof: See Proof of Theorem 4.

The overdamped Langevin equation in an anisotropic setting is given by \[ \text{dr}_i = -\nu_{ij} \frac{\partial \phi}{\partial r_j} dt + \sqrt{\frac{2k_B T}{\nu_{ii}}} \xi_i \] (no sum in the second term), \[(4.4.6)\]

where \(i\) represents two perpendicular directions of the motion and \(\nu_{ij}\) and \(\xi_i\) are the drag coefficient tensor and random force tensor. Note that for a rod shaped inclusion the drag coefficient along the longitudinal direction is smaller than that perpendicular to it. The details for how to compute these drag coefficients can be found in \[69\]. Fig 4.7(a) shows that the interaction energy \(\phi(r)\) for rod shaped inclusions depends on \(\theta\) (it is anisotropic). In the Langevin dynamics calculations, for each initial position \(y\) we use Eq (4.4.6) to run 8000 simulations with a reflecting wall at \(R_2\) and an absorbing wall at \(R_1\) for four \(\theta = 0^\circ, 30^\circ, 60^\circ, 90^\circ\) and then take an average (for each \(\theta\) separately) to estimate the first passage time. We also use Eq (3.3.13) to numerically solve the first passage time and compare the results derived from the two methods for \(F = 0.1, 1, 10\) pN/nm in Fig 4.13, Fig 4.14 and Fig 4.15, respectively.
Figure 4.13: The first passage time for two rod inclusions computed from (a) Langevin dynamics using Eq (4.4.6), (b) PDE using Eq (3.3.13) under applied tension 0.1pN·nm$^{-1}$. 

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The good agreement between the first passage time solved from the PDE in Eq (3.3.13) and estimated by Langevin equation once again shows that our methods work well. As shown in Fig 4.13, as the initial angle increases from 0° to 90°, the first passage time decreases at small separations, but increases at large separations. This can be explained by the fact that stronger attractive force near $R_1$ pulls the moving particle to be absorbed faster from smaller initial separations while stronger repulsive force around 12 – 16 nm leads to a larger first passage time when the particle is initially located at a large distance.
Figure 4.14: The first passage time for two rod inclusions computed from (a) Langevin dynamics using Eq (4.4.6), (b) PDE using Eq (3.3.13) under applied tension 1pN·nm$^{-1}$.
The result of the first passage time under 1pN/nm applied tension in Fig 4.14 is similar to the one under 0.1pN/nm applied tension.
Figure 4.15: The first passage time for two rod inclusions computed from (a) Langevin dynamics using Eq (4.4.6), (b) PDE using Eq (3.3.13) under applied tension $10\text{pN}\cdot\text{nm}^{-1}$.
Compared to the results under 0.11 pN·nm⁻¹ applied tension, the first passage time is reduced under 10 pN·nm⁻¹ applied tension. The order of magnitude of the first passage time under all three tensions is similar.

4.5 Discussion

This paper has two major parts. In the first part we use a finite difference method to compute the interaction energy of two inclusions due to membrane thickness deformations. In the second part we use the computed energy landscape to solve first passage time problems. Our method to compute energies is different from the analytical method in [10, 11] which uses perturbation theory to study thickness mediated interactions between two anisotropic inclusions; we implement an approach to compute the energy using the divergence theorem which is more general and can deal with strongly anisotropic inclusions. The advantage of analytical methods in both [10, 11] and this work is that they can compute the energy accurately at small applied tension $F$ if enough terms in the Fourier-Bessel series are used. However, it is time consuming to compute the coefficients in the Fourier-Bessel series and this becomes computationally infeasible when the inclusions are strongly anisotropic. On the other hand, our numerical method is able to handle arbitrary values of $F$ and can efficiently compute the interaction energy of two inclusions for different separations $r$ given a fixed set of parameters ($K_b, K_t, a$ etc.) which are stored in a pre-calculated stiffness matrix.
In the second part of the paper we compute the time to coalescence of two inclusions of various shapes as a function of the distance separating them. We use both Langevin dynamics and a PDE to arrive at our estimates. For two inclusions separated by about 125 nm we predict that the time to coalescence is hundreds of milliseconds irrespective of the shape of the inclusion. The time to coalescence with only membrane bending interactions was of similar magnitude as shown in [69]. The order of magnitude of the time to coalescence is the same even though the attractive force due to membrane thickness interactions is stronger than that due to membrane bending interactions in [69] at small separations. The reason is that even with membrane thickness interactions the attractive force decays to zero quickly and Brownian motion dominates the kinetics of the moving particle in most regions, just as in [69]. Therefore, at small separations the first passage time with thickness mediated interactions is smaller than that with out-of-plane bending interactions, but is not very different at large separations. The time to coalescence at large separations can be changed from that dictated by Brownian motion alone if longer range interactions (for instance, due to electrostatics) are taken into account as shown in Fig 4.12.
Chapter 5

Mechanics and interactions between proteins on DNA

5.1 Introduction

The mechanism of post-replicative mismatch repair (MMR) correcting errors in DNA is an important topic in biology as it could increase the accuracy of DNA replication up to 1000 times and prevent the onset of tumors that lead to cancer [35]. MutSα and MutLα are two important enzymes that carry out MMR in eukaryotes. The function of MutSα is to find a DNA mismatch, while MutLα is responsible for finding lesion-bound MutSα. In order to study the mechanisms of MutSα/MutLα target search, [35] used double-tethered DNA curtains with manipulated three G/T mismatches and found that MutSα targeted DNA mismatch sites either through 1D
sliding with diffusion coefficient $0.009 \mu m^2 s^{-1}$ and a chance of finding the mismatch at least 42.5% or 3D diffusion. MutL$\alpha$ finds mismatch-bound MutS$\alpha$ through 3D diffusion (with chance 55%) or 1D hopping (with chance 45%) \cite{35}. Different from 3D diffusion and limited by certain target association rates, the other three modes of motion (1D sliding, 1D hopping, inter-segmental transfer) are classified as \textit{facilitated diffusion} \cite{106}. However, in the absence of MutS$\alpha$, MutL$\alpha$ diffuses along the DNA individually and passes the G/T mismatches through 1D hopping \cite{107}. Moreover, MutS$\alpha$ and MutL$\alpha$ will not bind at sites that are not mismatched even at very large local concentration. Intersite transfer could happen before or after MutS$\alpha$/MutL$\alpha$ bind to their targets. In the former case it could help the mismatch search, while in the latter case it would cause damage while MutS$\alpha$/MutL$\alpha$ are scanning the flanking DNA. In this chapter, we focus on the 1D sliding of MutS$\alpha$ search for the mismatch site as 3D diffusion might be difficult if the lesion is blocked by a nucleosome \cite{108}.

CRISPR-Cas (clustered regularly interspaced short palindromic repeats and CRISPR-associated proteins) is a system of proteins that yields immune responses in bacteria \cite{36,37}. They are primarily divided into two classes: Class 1 uses effector complexes, while Class 2 utilizes a single effector protein \cite{38}. Cas9 and Cas12a are two types of Class 2 proteins that are widely used in genome editing \cite{36}. They have some distinguishing features. Cas9 works with two small RNAs, CRISPR RNA (crRNA), trans-activating crRNA (tracrRNA) and cleaves a DNA
double-strand producing blunt ends. Cas12a only requires one crRNA and produces staggered cleavage. Different from Cas9, Cas12a is able to process its pre-crRNA into crRNA \[109\], which helps multiplex genome editing. The DNA search and cleavage mechanism of Cas9 have been well studied \[110\] whereas the properties of Cas12a are largely unknown. In \[36\], the authors used single-molecule fluorescence imaging to study the dynamic features of Cas12a from Acidaminococcus sp. (AsCas12a). They found that AsCas12a ribonucleoproteins (RNPs) could promote target search through 1D diffusion and protospacer-adjacent motif (PAM) sites are favored places for AsCas12a RNPs to start 1D diffusion. In addition, PAM site is important in Cas9 that helps to bind the target DNA strand but does not play a crucial role in Cas12a on DNA cleavage. Using FIONA (fluorescence imaging with one nanometer accuracy), the authors also discovered that AsCas12a diffuses intermittently along the DNA with diffusion coefficient \(1.73 \mu m^2 s^{-1}\) and ionic strength could impact the rate of the diffusion \[111\]. Based on their experimental observations, the authors proposed a model for AsCas12a on DNA cleavage in Fig.6 of \[36\]. A remarkable feature in their model is that AsCas12a releases DNA fragment after cleavage, which is not the case for Cas9 \[112\]. They also illustrated that Cas12a cuts the non-target and target DNA strands sequentially and cleavage of the non-target strand is not a necessity for cleaving the target strand.

In the above we have given two examples of enzymes that carry out important biological functions on DNA using 1D diffusion based searches. Apart from these
experimental observations, some theoretical studies have analytically modeled the dynamics of DNA target search by CRISPR-associated Cas9 proteins. [113] proposed a discrete-state stochastic model for RNA-guided CRISPR-associated Cas9 protein that describes the physical-chemical interactions during its DNA target search. They showed that the dynamic processes can be cast as a first passage time problem. Their model can be expressed as a linear system of ordinary differential equations in terms of the probability density that the Cas9 protein reaches some PAM states at time $t$ for the first time, and analytical solutions can be derived using Fourier transform. Then, they can compute the mean search time analytically. Some parameters in their models like association/dissociation rates, and the rate of cleaving off-target DNA sequence can be adjusted in order to make different predictions. Their model is simple and fully analytically tractable and is in quantitative agreement with experiments from [112] in predicting the fraction of cleaved DNA as a function of time. The target search of the RNA-guided CRISPR-associated Cas9 protein is also modeled by another theoretical work [114] where the authors described the dynamics of DNA search as fractional Brownian motion. Starting from the generalized Langevin equation, the probability density distribution for the position of RNA-guided CRISPR-associated Cas9 protein can be analytically expressed. The 3D diffusion of the protein is assumed to start from the top of a cylinder while the target site is at the bottom of the cylinder and the motion is confined inside the cylinder due to the impact of many off-target interactions. This
can be accomplished using an absorbing boundary condition at the bottom and reflecting walls at the radial boundary. The survival probability can be computed from the probability density under the above boundary conditions and the mean target search time can be solved in a closed form. Using a different boundary condition with reflecting walls at the top, bottom and radial boundary, an analytical expression for the fluorescence intensity correlation function is obtained. Then, the authors compared their theoretical predictions on the survival probability, the mean square displacement and the intensity correlation for RNA-guided CRISPR-associated Cas9 protein with experiments in [115] and found good agreements (with experiment). Their model also shows that the 3D diffusion of Cas9 protein is an overdamped sub-diffusive process and the physically constrained 3D motion could facilitate the target search. These theoretical studies provide insight on the mechanism of DNA target search by RNA-guided CRISPR-associated Cas9 protein that could improve and promote genetic engineering technology.

An aspect that is missing from these studies is the allosteric interactions between the diffusing protein and the DNA mis-match. These allosteric interactions have their origin in the elasticity of DNA as explained in [116]. These allosteric interactions decay away after a few tens of base-pairs, but longer range allosteric effects can be produced by applying tension on the DNA. This was shown in [41] that developed a theoretical approach from the twisted worm-like-chain formula to study the elastic interactions between DNA-binding proteins under the applied tension.
The authors demonstrated the importance of the elastic interactions as its range can be up to hundreds of base-pairs. Their result shows that DNA twist plays a crucial role in the free energy which causes a damped oscillations even in the absence of applied torsion. These twist effects would impact the aggregation time between proteins in a qualitative manner. The authors also computed the mean first passage time for the self-assembly of two spherical DNA binding proteins where they fixed the initial linker-length as 200 base-pairs and plotted the mean first passage time as a function of applied tension with varying fraction wrapping parameter.

In this chapter, we focus on the 1D diffusion during DNA mismatch search by MutSα. The quantity we are interested in is the first and second moment of the first passage time which can capture the dynamics of the search process. We assume that the mismatch site is fixed at $x = 0$ and MutSα is diffusing along $x$ axis. For the translation motion along $x$ axis, there is a reflecting wall at $x = R_2$ representing the end of the DNA which means MutSα will be bounced back if it reaches the end and an absorbing wall at $x = R_1$ implies that MutSα will bind to the mismatch site if they are sufficiently close to each other. We will account for both short- and long-range allosteric effects in our calculations.

### 5.2 Energy Landscape

We first need an analytical expression for the free energy of the system (consisting of a length of DNA with a mis-match (and/or bound protein at the mis-matech)
and a weakly bound protein) which will be used in the computation of the first and second moment of the search time. We incorporate allosteric interactions into the free energy of the system which is not considered in most previous studies. The free energy of two DNA-binding proteins as a function of linker length $x$ is given in Fig. 2 of [41]. We assume the energy is in the following form,

$$
\phi(x) = \cos\left(\frac{2\pi(x-x_0)}{T_0}\right) h_1(f)e^{-\frac{(x-h_2(f))^2}{4h_3(f)^2}} + h_4(f) + h_5(x, f) + \left(e^{-\gamma_1 x} \sin\left(\frac{2\pi x}{T_0}\right) + e^{-\gamma_2 x}\right)^2,
$$

(5.2.1)

where $h_1, h_2, h_3$ are the magnitude, mean, standard deviation of the Gaussian distribution which is the magnitude of the cosine accounting for the oscillations at short separations. $h_4$ denotes the value of the free energy at very large separations. $h_5$ represents the dashed line in Fig. 2 of [41] which gives the free energy in the absence of twist resistance. The last term in Eq (5.2.1) accounts for the allosteric interactions discussed in [116]. We fit the energy in Fig. 2 of [41] using Eq (5.2.1) without the allosteric interactions term (last term), and get, $x_0 = 39.89$ bp, $T_0 = 10.46$ bp, $h_1(f) = -0.35 + 0.96f$, $h_2(f) = 86.7e^{-0.14f}$, $h_3(f) = 32.77e^{-0.11f}$, $h_4(f) = 76.9f^{0.1} + 3.25f$, $h_5(x, f) = (-10.87 - 4.77f)e^{-0.02 - 0.0066f}x$. Then we add the allosteric interactions into the energy expression by choosing $\gamma_1 = \gamma_2 = 10$ bp as discussed in [116]. The fit of the free energy is shown in Fig 5.1. Due to the scarcity of the data, the energy fitting shown in Fig 5.1 is not bad. The advantage of the fitted analytical expression in Eq (5.2.1) is that the force is also in a simple analytical form, which facilitates the computations for the first and second moment.
of the target search time (first passage time) in the following sections.

Figure 5.1: Free energy $\phi$ as a function of linker length $x$ and applied tension $f$. Diamond data are extracted from Fig.2 of [41]. Dashed lines are the fitted using Eq (5.2.1). The fraction wrapping is 0.5 and the twist resistance is 110 nm.

5.3 First Passage Time

The kinetics of MutS$\alpha$ binding to a DNA mismatch site can be described by a stochastic process $x(t)$, which is governed by the 1-Dimensional overdamped Langevin equation,

$$dx = -\frac{1}{\nu} \frac{\partial \phi}{\partial x} dt + \sqrt{\frac{2 k_B T}{\nu}} \xi_i,$$  (5.3.1)

where $\nu$ is the viscosity, $k_B$ is the Boltzmann constant, $T$ is the temperature, $\xi_i \sim \mathcal{N}(0, 1)$ is sampled from the standard normal distribution. Let $T(y)$ denote the time
that \( x(t) \) first exits \((R_1, R_2)\). Let \( T_n(y) \) be the \( n \)-th moment of \( T(y) \). The ODE for \( T_1(y) \) is given by

\[
\frac{k_B T}{\nu} T''_1(y) - \frac{1}{\nu} \frac{\partial \phi}{\partial x} T'_1(y) + 1 = 0. \tag{5.3.2}
\]

Using the boundary conditions \( T_1(R_1) = 0, \ T'_1(R_2) = 0 \), we can get

\[
T_1(y) = \int_{R_1}^{y} \int_{\eta}^{R_2} \frac{\nu}{k_B T} e^{-f(\xi)/k_B T} d\xi e^{f(\eta)/k_B T} d\eta \tag{5.3.3}
\]

The ODE for \( T_2(y) \) is given by

\[
\frac{k_B T}{\nu} T''_2(y) - \frac{1}{\nu} \frac{\partial \phi}{\partial x} T'_2(y) + 2T_1(y) = 0, \tag{5.3.4}
\]

with boundary conditions \( T_2(R_1) = 0, \ T'_2(R_2) = 0 \). We can get,

\[
T_2(y) = \int_{R_1}^{y} \int_{\eta}^{R_2} 2T_1(\xi)\nu/k_B T e^{-f(\xi)/k_B T} d\xi e^{f(\eta)/k_B T} d\eta. \tag{5.3.5}
\]

The diffusion coefficient used here is \( D = k_B T/\nu = 0.009 \mu m^2 s^{-1} = 8.65 \times 10^5 \text{bp}^2 \text{s}^{-1} \) given by the Einstein relation. On the other hand, \( T_1(y), T_2(y) \) can be estimated by running Langevin equation for multiple times under a given initial location \( y \) and then taking the average. We compute \( T_1(y), T_2(y) \) under three different diffusion coefficients \( D = 8.65 \times 10^4 \text{bp}^2 \text{s}^{-1}, 8.65 \times 10^5 \text{bp}^2 \text{s}^{-1}, 8.65 \times 10^6 \text{bp}^2 \text{s}^{-1} \) using Eq (5.3.3)-(5.3.5) and the Langevin equation Eq (5.3.1). The result is shown in Fig 5.2.

We can see that the computations of \( T_1(y), T_2(y) \) using the two methods are in excellent agreement (the lines from the analytical method and Langevin calculations are hardly distinguishable) and the increment of the magnitude of the mean first passage time is approximately proportional to the increment of the magnitude of
the diffusion coefficient. This is due to the fact that the DNA has a size of 1000 base-pairs while the free energy decays to 0 before 150 base-pairs so that Brownian motion dominates the target search in this scenario. For the second moment of the first passage time we observe a similar positive relation between the magnitude of the diffusion coefficient and the magnitude of \( T_2 \). We also compute the mean first passage time difference \( \Delta T_1 \) which is defined as the mean first passage time evaluated at \( y = 200 \)bp under applied tension \( f > 0 \) minus the mean first passage time evaluated at \( y = 200 \)bp under no applied tension \( (f = 0) \). We compare our computation of this quantity with the one done by \[41\] and the result is shown in Fig 5.3. The discrepancy is caused by the difference in the energy fitting using Eq (5.2.1) and consideration of allosteric interactions in our case. This can be verified by the fact that at \( f = 0 \) the mean first passage time \( T_1(200) \) derived by \[41\] is 0.20 s while using Eq (5.3.3) we get 0.2081 s, which are in good agreement and our energy fit is close to the free energy in \[41\] as \( f \to 0 \). Nevertheless, both of our computation and the one done in \[41\] demonstrate the fact that at small applied tension the target search process is facilitated while as applied tension further increases the impact of the applied tension decrease.
Figure 5.2: (a) Log of mean first passage time $\log T_1(y)$, (b) log of second moment of first passage time $\log T_2(y)$ under three different diffusion coefficients: $D = 8.65 \times 10^4$ bp$^2$s$^{-1}$, $8.65 \times 10^5$ bp$^2$s$^{-1}$, $8.65 \times 10^6$ bp$^2$s$^{-1}$. Scatter points are derived from Eq (5.3.3) and solid curves are estimated by Langevin dynamics Eq (5.3.1).
Figure 5.3: Mean first passage time difference evaluated at $y = 200$ bp under different applied tensions $f$. Red curve is computed using Eq (5.3.3). Blue points are the data extracted from [41]. The fraction wrapping is 0.5 and the twist resistance is 110 nm.

### 5.3.1 Comparison with experimental data

Gorman et al. [35] studied the rate of MutSα engaging the mismatches through 1D search. In order to compare with their experimental results, we set $R_1 = 10$, $R_2 = 13000$ and took diffusion coefficient $7.79 \times 10^4 \text{bp}^2/s$ as inferred in their experiment. The comparison is shown in Fig 5.4(a). We do not expect a perfect agreement because the quantity we computed in Fig 5.4(a) is the mean first passage time while the experimental data in Fig 5.4(a) are the time record of only one 1D mismatch search experiment given an initial position. Nevertheless, the result shows that the
mean first passage time computed by Eq (5.3.3) are of the same order of magnitude as the experimental data in [35], which justifies the model assumption behind our methods.

![Graph showing mean first passage time and experimental data](image)

Figure 5.4: Red curve is the mean first passage time computed by Eq (5.3.3) and each black point is one single experimental measurement by [35] measuring the time that MutSα finds the target site.

### 5.3.2 Exerting different applied tension \( f \)

We studied the mean and second moment of first passage time under different applied tension \( f = 0\text{pN}, 1\text{pN}, 2\text{pN}, 4\text{pN}, 6\text{pN}, 8\text{pN} \) with a fixed diffusion coefficient \( D = 8.56 \times 10^5\text{bp}^2/\text{s} \). The result in Fig 5.5 shows that the DNA target search is facilitated under all applied tension in the range from 1pN to 8pN. However,
the facilitation is of the most significance under small applied tension (1pN) and is gradually diminished as exerting force increases. This observation is an extension of Fig 5.3, showing that the same conclusion is also true for all initial location $y$. However, [117] found that the activity of DNA cleavage by Cas9 protein is prohibited in the presence of applied tension. This implies that applied tension would exert different influence on the actions of different biological objects.
Figure 5.5: (a) Mean first passage time $T_1(y)$, (b) second moment of first passage time $T_2(y)$ computed by Eq (5.3.3) under different applied tensions $f = 0, 1, 2, 4, 6, 8$ pN with diffusion coefficient $D = 8.56 \times 10^5$ bp$^2$/s.
Chapter 6

Conclusion

This thesis is mainly concerned with the mechanics of lipid membranes and inclusions in them. Here we briefly summarize the results of each chapter.

In Chapter 2 we investigated the mechanics of a vesicle subject to adhesion, micropipette aspiration and irradiation simultaneously. We computed the shapes of the vesicles at equilibrium and also studied the kinetics of competing processes such as adhesion and irradiation induced oxidation. An important aspect of our computations is that thermal undulations of the lipid membrane are taken into consideration so that they can be realistically compared to adhesion and irradiation experiments. The time variation of the mechanical moduli of irradiated vesicles is also taken into account in our computations. As such our results are in agreement with several experimental papers on oxidation induced structural changes in vesicles. For example, we predicted the kinetics of the area increment of a lipid membrane
sucked into a micro-pipette under different irradiation powers and the dynamics of
the contact radius during adhesion which are in agreement with experiments. We
have also made falsifiable predictions that can be tested in future experiments. Our
model may be extended to account for pore formation in oxidized membranes, but
we leave this to future work.

In Chapter 3, we have analyzed the self-assembly of inclusions on a lipid mem-
brane under the influence of elastic and entropic interaction forces that have their
origin in the bending deformation of the membrane. We have shown that the self-
assembly process is dominated by diffusion, but that attractive interaction forces
come into play at small separations. Unlike most earlier works that use various sim-
ulation techniques (such as Monte Carlo, molecular dynamics and various coarse-
grained methods that account for Brownian motion), we use both Langevin dy-
namics and the corresponding Fokker-Planck equation to cast the self-assembly as
a first passage time problem. We show that these two methods are in excellent
agreement for circular and elliptical inclusions and in the presence of hydrodynamic
interactions. Our ideas provide a different view of self-assembly that is based on
partial differential equations (for the first passage time) that could be leveraged
for creating fast computational methods. One such class of methods is based on
fluctuating hydrodynamics \[118\] which extends computational techniques for fluid-
structure interactions to problems with Brownian motion.

In Chapter 4, we have analyzed the temporal self-assembly of inclusions due to
interactions mediated by membrane thickness variations. We show that the results from Langevin dynamics simulations agree well with those obtained from a PDE for the first passage time. The approach based on the PDE is much faster than the Langevin dynamics simulation and could open new ways to study the process of self-assembly. This is a step beyond earlier studies which focused on the energy landscape of clusters of proteins, but did not look into kinetics. Some papers based on molecular simulation did consider the temporal process, but to the best of our knowledge most did not reach the time scales calculated in this paper. Phase-field methods can compute the evolution of particle concentrations on a membrane, but it is beyond their scope to track the motion of discrete particles as we have done here. We closed this chapter by mentioning some effects that we did not consider. First, hydrodynamic interactions between inclusions (based on the Oseen tensor) were shown to speed up self-assembly in [69] (see Chapter 3) and they are expected to have a similar effect here. Second, the temporal behavior of a cluster of inclusions are not studied in this paper due to limitations of computational power, but we expect the overall behavior to be similar to the clusters studied in our earlier work [69]. Third, only a limited set of inclusion shapes are considered in this paper, but it is found that the time to coalescence does not depend strongly on shape. We leave it to future work to add these refinements and extend this type of analysis to important functional proteins such as ion-channels [11].

In Chapter 5 we have attempted to show how 1D searches by various enzymes
on DNA can be modeled as first passage time problems. We can analytically calculate both the mean first passage time and its next higher moment. We have accounted for the effects of allosteric interactions between the DNA mismatch and the diffusing protein in our calculations. Since this type of allosteric interaction is rather short-ranged (few tens of base-pairs) it does not have a big effect on the search time. DNA tension leads to longer range allosteric interactions and this has a more noticeable effect on the search time. For low tensions the search time becomes shorter, but for high tensions the search time becomes longer.
Appendix A

Proof of Theorem 1

Integrate Eq (3.2.19) for $P$ over all $t \geq 0$,

$$
\int_0^\infty \frac{\partial P}{\partial t} dt = \frac{\partial}{\partial r} \left[ \frac{1}{\nu} \frac{\partial \phi}{\partial r} g_1 + D \frac{\partial g_1}{\partial r} \right] + \frac{1}{r} \left[ \frac{1}{\nu} \frac{\partial \phi}{\partial r} g_1 + D \frac{\partial g_1}{\partial r} \right]
$$

(A.0.1)

$$
-\frac{1}{r} \delta(r - y) = \mathcal{L}_r g_1(r, y),
$$

(A.0.2)

where $\frac{1}{r} \delta(r - y)$ is the initial condition and the second order linear differential operator $\mathcal{L}_r : \mathcal{D}(\mathcal{L}_r) \subset C^2([R_1, R_2]) \to C^2([R_1, R_2])$ is defined as,

$$
\mathcal{L}_r = \frac{\partial}{\partial r} \left[ \frac{1}{\nu} \frac{\partial \phi}{\partial r} + D \frac{\partial}{\partial r} \right] + \frac{1}{r} \left[ \frac{1}{\nu} \frac{\partial \phi}{\partial r} + D \frac{\partial}{\partial r} \right],
$$

(A.0.3)

with domain

$$
\mathcal{D}(\mathcal{L}_r) = \left\{ u \in C^2([R_1, R_2]) | u(R_1) = u(R_2) = 0 \right\},
$$

(A.0.4)

and the inner product is defined as,

$$
\langle u, v \rangle = \int_{R_1}^{R_2} uv dr, \quad \forall u, v \in \mathcal{D}(\mathcal{L}_r).
$$

(A.0.5)
It is useful to obtain the adjoint operator $\mathcal{L}_r^*$ (see Computing the PDE for the first passage time under hydro-dynamic interaction for the detailed procedure to derive the adjoint operator) which satisfies $\langle v, \mathcal{L}_r u \rangle = \langle \mathcal{L}_r^* v, u \rangle$, $\forall u, v \in \mathcal{D}(\mathcal{L}_r)$,

$$\mathcal{L}_r^* = -\frac{1}{\nu} \frac{\partial \phi}{\partial r} \frac{\partial}{\partial r} + D \frac{\partial^2}{\partial r^2} + \frac{1}{\nu} \frac{\partial \phi}{\partial r} \frac{1}{r} - D \frac{\partial^1}{\partial r}. \quad (A.0.6)$$

Proofs for the existence of the solutions of second order inhomogeneous linear ordinary differential equation are well known. Hence, we can find a $v_0 \in C^2([R_1, R_2])$ s.t. $\mathcal{L}_r^* v_0(r) = r$. Then, it follows from Eq $3.2.23$ that,

$$T_1(y) = \int_{R_1}^{R_2} \mathcal{L}_r^* v_0(r) g_1(r, y) dr = \int_{R_1}^{R_2} v_0(r) (\mathcal{L}_r g_1(r, y)) dr$$

$$= -\int_{R_1}^{R_2} v_0(r) \frac{1}{r} \delta(r - y) dr = -v_0(y) \frac{1}{y}, \quad (A.0.7)$$

$$\implies \mathcal{L}_y y T_1(y) = -y. \quad (A.0.8)$$

Using Eq $A.0.6$, we can derive a second order ODE for $T_1(y)$ Eq $3.2.25$.
Appendix B

Proof of Theorem 2

Transforming to polar coordinates, Eq (3.3.10) reads,

\[
\frac{\partial p}{\partial t} = \frac{1}{\nu_a} \left[ \begin{array}{c}
 p \cos^2 \theta \frac{\partial^2 \phi}{\partial r^2} + p \frac{\partial \phi}{\partial r} \frac{\sin^2 \theta}{r} - p \frac{\sin 2\theta}{r} \frac{\partial^2 \phi}{\partial r \partial \theta} + p \frac{\sin 2\theta}{r^2} \frac{\partial \phi}{\partial \theta} + p \frac{\sin^2 \theta}{r^2} \frac{\partial^2 \phi}{\partial \theta^2}
\end{array} \right]
\]

\[
+ \left( \cos \theta \frac{\partial \phi}{\partial r} - \frac{\sin \theta \partial \phi}{r} \right) \left( \cos \theta \frac{\partial p}{\partial r} - \frac{\sin \theta \partial p}{r} \right)
\]

\[
+ \frac{1}{\nu_b} \left[ \begin{array}{c}
 p \sin^2 \theta \frac{\partial^2 \phi}{\partial r^2} + p \frac{\partial \phi}{\partial r} \frac{\cos^2 \theta}{r} + p \frac{\sin 2\theta}{r} \frac{\partial^2 \phi}{\partial r \partial \theta} - p \frac{\sin 2\theta}{r^2} \frac{\partial \phi}{\partial \theta} + p \frac{\cos^2 \theta}{r^2} \frac{\partial^2 \phi}{\partial \theta^2}
\end{array} \right]
\]

\[
+ \left( \sin \theta \frac{\partial \phi}{\partial r} + \cos \theta \frac{\partial \phi}{\partial \theta} \right) \left( \sin \theta \frac{\partial p}{\partial r} + \cos \theta \frac{\partial p}{\partial \theta} \right)
\]

\[
+ D_a \left[ \begin{array}{c}
 \cos^2 \theta \frac{\partial^2 p}{\partial r^2} + \frac{\sin^2 \theta}{r} \frac{\partial p}{\partial r} - \frac{\sin 2\theta}{r} \frac{\partial^2 p}{\partial r \partial \theta} + \frac{\sin 2\theta}{r^2} \frac{\partial p}{\partial \theta} + \frac{\sin^2 \theta}{r^2} \frac{\partial^2 p}{\partial \theta^2}
\end{array} \right]
\]

\[
+ D_b \left[ \begin{array}{c}
 \sin^2 \theta \frac{\partial^2 p}{\partial r^2} + \frac{\cos^2 \theta}{r} \frac{\partial p}{\partial r} + \frac{\sin 2\theta}{r} \frac{\partial^2 p}{\partial r \partial \theta} - \frac{\sin 2\theta}{r^2} \frac{\partial p}{\partial \theta} + \frac{\cos^2 \theta}{r^2} \frac{\partial^2 p}{\partial \theta^2}
\end{array} \right] \quad \text{(B.0.1)}
\]

\[
= F_{r, \theta p} \quad \text{(B.0.2)}
\]
where the elliptic differential operator \( F_{r,\theta} : \mathcal{D}(F_{r,\theta}) \subset C^2([R_1, R_2] \times [0, 2\pi]) \to C^2([R_1, R_2] \times [0, 2\pi]) \) is in divergence form, with domain

\[
\mathcal{D}(F_{r,\theta}) = \{ u \in C^2([R_1, R_2] \times [0, 2\pi]) | u(R_1, \theta) = u(R_2, \theta) = 0, u(r, 0) = u(r, 2\pi) \},
\]

and the inner product is defined as,

\[
\langle u, v \rangle = \int_{R_1}^{R_2} \int_{0}^{2\pi} uv \, rdrd\theta, \quad \forall u, v \in \mathcal{D}(F_{r,\theta}).
\]

Then, we can derive \( F_{r,\theta}^* \), the adjoint operator of \( F_{r,\theta} \), satisfying

\[
\langle v, F_{r,\theta} u \rangle = \langle F_{r,\theta}^* v, u \rangle, \quad \forall u, v \in \mathcal{D}(F_{r,\theta}),
\]

\[
F_{r,\theta}^* = \frac{1}{\nu_a} \left[ \frac{\cos^2 \theta}{r} \frac{\partial \phi}{\partial r} - \frac{\sin 2\theta}{2r^2} \frac{\partial \phi}{\partial \theta} - \left( \frac{\cos^2 \theta}{r} \frac{\partial \phi}{\partial r} - \frac{\sin 2\theta}{2r} \frac{\partial \phi}{\partial \theta} \right) \frac{\partial}{\partial r} \right. \\
+ \left. \left( \frac{\sin 2\theta}{2r} \frac{\partial \phi}{\partial r} - \frac{\sin^2 \theta}{r^2} \frac{\partial \phi}{\partial \theta} \right) \frac{\partial}{\partial \theta} \right] + \frac{1}{\nu_b} \left[ \frac{\sin^2 \theta}{r} \frac{\partial \phi}{\partial r} + \frac{\sin 2\theta}{2r^2} \frac{\partial \phi}{\partial \theta} \right. \\
- \left. \left( \frac{\sin 2\theta}{2r} \frac{\partial \phi}{\partial r} + \frac{\sin^2 \theta}{r^2} \frac{\partial \phi}{\partial \theta} \right) \frac{\partial}{\partial r} \right] - \left( \frac{\sin 2\theta}{2r} \frac{\partial \phi}{\partial r} + \frac{\cos^2 \theta}{2r^2} \frac{\partial \phi}{\partial \theta} \right) \frac{\partial}{\partial \theta} \right]
+ D_a \left[ \frac{\cos^2 \theta}{r^2} \frac{\partial^2 \phi}{\partial r^2} + \frac{\sin^2 \theta}{r^2} \frac{\partial^2 \phi}{\partial \theta^2} - \frac{\sin 2\theta}{r} \frac{\partial}{\partial r} \frac{\partial^2 \phi}{\partial r \partial \theta} - \left( \frac{\sin^2 \theta + 2 \cos 2\theta}{r} \right) \frac{\partial}{\partial r} \right]
+ 2 \frac{\sin 2\theta}{r^2} \frac{\partial}{\partial \theta} + \frac{\sin^2 \theta + 2 \cos 2\theta}{r^2} \right]
+ D_b \left[ \frac{\sin^2 \theta}{r^2} \frac{\partial^2 \phi}{\partial r^2} + \frac{\cos^2 \theta}{r^2} \frac{\partial^2 \phi}{\partial \theta^2} + \frac{\sin 2\theta}{r} \frac{\partial}{\partial r} \frac{\partial^2 \phi}{\partial r \partial \theta} - \left( \frac{\cos^2 \theta - 2 \cos 2\theta}{r} \right) \frac{\partial}{\partial r} \right]
- \left. 2 \frac{\sin 2\theta}{r^2} \frac{\partial}{\partial \theta} + \frac{\cos^2 \theta - 2 \cos 2\theta}{r^2} \right].
\]

After \( q_1 \) is defined in Eq (3.3.12), we integrate Eq (B.0.2) for \( p \) over all \( t \geq 0 \),

\[
\int_{0}^{\infty} \frac{\partial p}{\partial t} \, dt = \frac{\partial}{\partial r} \left[ \frac{1}{\nu} \frac{\partial \phi}{\partial r} q_1 + Dq_1 \right] + \frac{1}{r} \left[ \frac{1}{\nu} \frac{\partial \phi}{\partial r} q_1 + Dq_1 \right]
+ \frac{1}{r} \frac{\partial}{\partial \theta} \left[ \frac{1}{\nu} \frac{\partial \phi}{\partial \theta} q_1 + \frac{Dq_1}{r} \right]
+ \frac{1}{\nu} \frac{\partial}{\partial \theta} \left[ \frac{1}{\nu} \frac{\partial \phi}{\partial \theta} q_1 + \frac{Dq_1}{r} \right].
\]

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\[-\frac{1}{r} \delta(r - y) \delta(\theta - \alpha) = F_{r,\theta} q_1, \quad \text{(B.0.6)}\]

$F^*_r$ is uniformly elliptic with certain boundary conditions the solution of which has been discussed in \[119\]. Then, we can find a $u_0 \in C^2([R_1, R_2] \times [0, 2\pi])$ s.t. $F^*_r u_0(r, \theta) = r$. It follows from Eq (3.3.11) that,

\[
T_1(y, \alpha) = \int_{R_1}^{R_2} \int_{0}^{2\pi} q_1 \left( F^*_r u_0(r, \theta) \right) d\theta dr
= \int_{R_1}^{R_2} \int_{0}^{2\pi} u_0(r, \theta) F_{r,\theta} q_1 d\theta dr
= -\int_{R_1}^{R_2} \int_{0}^{2\pi} u_0(r, \theta) \frac{1}{r} \delta(r - y) \delta(\theta - \alpha) d\theta dr
= -\frac{1}{y} u_0(y, \alpha), \quad \text{(B.0.7)}
\]

\[
\Rightarrow F^*_{y,\alpha} y T_1(y, \alpha) = -y. \quad \text{(B.0.8)}
\]

Using Eq (B.0.5), we can derive a second order PDE for $T_1(y)$ Eq (3.3.13).
Appendix C

Illustration of finite difference method

From [18], the free energy function in Eq (3.3.13) is given by,

\[ \phi(y, \alpha) = \frac{2c_1}{y^2} \cos 2\alpha + \frac{c_2}{y^4} + \frac{c_3}{y^6} + c_4. \]  

(C.0.1)

Then,

\[ \frac{\partial \phi}{\partial \alpha} = -\frac{4c_1}{y^2} \sin 2\alpha, \quad \frac{\partial \phi}{\partial y} = -\frac{4c_1}{y^3} \cos 2\alpha - \frac{4c_2}{y^5} - \frac{6c_3}{y^7}. \]  

(C.0.2)

Let \( i \) denote the index in \( \alpha \) direction and \( j \) in \( y \) direction. The finite difference scheme of Eq (3.3.13) then is:

\[
\begin{align*}
\kappa_1(i, j) \frac{T_{i,j+1} - 2T_{i,j} + T_{i,j-1}}{h_y^2} &+ \kappa_2(i, j) \frac{T_{i+1,j} - 2T_{i,j} + T_{i-1,j}}{h_\alpha^2} + \kappa_4(i, j) \frac{T_{i,j+1} - T_{i,j}}{h_y} \\
+ \kappa_3(i, j) \frac{T_{i+1,j+1} - T_{i+1,j-1} - T_{i-1,j+1} + T_{i-1,j-1}}{4h_\alpha h_y} + \kappa_5(i, j) \frac{T_{i+1,j} - T_{i,j}}{h_\alpha} & = -1,
\end{align*}
\]

(C.0.3)
for all inner points \((i,j)\) (see Fig C.1) where \(\kappa_1, \kappa_2, \kappa_3, \kappa_4, \kappa_5\) are given by,

\[
\kappa_1(i,j) = D_a \cos^2 \alpha_i + D_b \sin^2 \alpha_i
\]

\[
\kappa_2(i,j) = D_a \frac{\sin^2 \alpha_i}{y_j^2} + D_b \frac{\cos^2 \alpha_i}{y_j^2}
\]

\[
\kappa_3(i,j) = -D_a \frac{\sin 2\alpha_i}{y_j} + D_b \frac{\sin 2\alpha_i}{y_j}
\]

\[
\kappa_4(i,j) = D_a \frac{\sin^2 \alpha_i}{y_j} + D_b \frac{\cos^2 \alpha_i}{y_j} + \frac{1}{\nu_a} \left[ -4c_1 \sin^2 2\alpha_i \frac{4c_1}{2y_j^3} + \cos^2 \alpha_i \left( \frac{4c_1}{y_j^3} \cos 2\alpha_i \right. \\
+ \frac{4c_2}{y_j^5} + \frac{6c_3}{y_j^7} \left. \right) \right] + \frac{1}{\nu_b} \left[ \frac{4c_1 \sin^2 2\alpha_i}{2y_j^3} + \sin^2 \alpha_i \left( \frac{4c_1}{y_j^3} \cos 2\alpha_i + \frac{4c_2}{y_j^5} + \frac{6c_3}{y_j^7} \right) \right]
\]

\[
\kappa_5(i,j) = D_a \frac{\sin 2\alpha_i}{y_j^2} - D_b \frac{\sin 2\alpha_i}{y_j^2}
\]

\[
+ \frac{1}{\nu_a} \left[ - \sin 2\alpha_i \left( \frac{4c_1}{y_j^3} \cos 2\alpha_i + \frac{4c_2}{y_j^5} + \frac{6c_3}{y_j^7} \right) + \frac{4c_1 \sin 2\alpha_i \sin^2 \alpha_i}{y_j^4} \right] \\
+ \frac{1}{\nu_b} \left[ \sin 2\alpha_i \left( \frac{4c_1}{y_j^3} \cos 2\alpha_i + \frac{4c_2}{y_j^5} + \frac{6c_3}{y_j^7} \right) + \frac{4c_1 \sin 2\alpha_i \cos^2 \alpha_i}{y_j^4} \right].
\]

The boundary conditions can be easily implemented using the fact that \(T_1(R_1, \alpha) = T_1(R_2, \alpha) = 0\) and \(T_1(y, 0) = T_1(y, 2\pi)\).
Figure C.1: Finite difference method to solve Eq (C.0.3).
Appendix D

Computing the PDE for the first passage time under hydro-dynamic interaction

\[
\frac{\partial p}{\partial t} = \frac{\partial^2 p}{\partial r^2} \left[ \bar{D}_{11} \cos^2 \theta + \bar{D}_{22} \sin^2 \theta + \bar{D}_{12} \sin 2\theta \right]
\]
\[
+ \frac{\partial^2 p}{\partial \theta^2} \left[ \frac{\bar{D}_{22} \cos^2 \theta + \sin \theta (\bar{D}_{11} \sin \theta - 2 \cos \theta \bar{D}_{12})}{r^2} \right]
\]
\[
+ \frac{\partial^2 p}{\partial r \partial \theta} \left[ \frac{2 \cos 2\theta \bar{D}_{12} + (\bar{D}_{22} - \bar{D}_{11}) \sin 2\theta}{r} \right]
\]
\[
+ \frac{\partial p}{\partial r} \left[ \bar{D}_{22} \cos^2 \theta + 2 \frac{\partial D_{12}}{\partial \theta} \cos^2 \theta + 2r \frac{\partial D_{11}}{\partial r} \cos^2 \theta + r \frac{\partial \phi}{\partial r} \cos^2 \theta + 2 \frac{\partial p}{\partial \theta} \cos^2 \theta - 2 \bar{D}_{12} \sin \theta \cos \theta + 2 \sin \theta \frac{\partial D_{11}}{\partial \theta} \cos \theta - 2r \sin \theta \frac{\partial D_{12}}{\partial r} \cos \theta + 2rg_{12} \sin \theta \cos \theta + \bar{D}_{11} \sin^2 \theta - 2 \sin^2 \theta \frac{\partial D_{12}}{\partial \theta} + \sin 2\theta \frac{\partial D_{22}}{\partial \theta} \right]
\]
\[
\cdot \frac{1}{r} + \frac{\partial p}{\partial \theta} \left[ 2 \frac{\partial D_{22}}{\partial \theta} \cos^2 \theta + 2r \frac{\partial D_{12}}{\partial r} \cos^2 \theta \right]
\]
\[ -2\tilde{D}_{22}\sin\theta\cos\theta - 4\sin\theta\frac{\partial D_{12}}{\partial\theta}\cos\theta - 2r\sin\theta\frac{\partial D_{11}}{\partial r}\cos\theta \]
\[ -rg_{11}\sin\theta\frac{\partial\phi}{\partial r}\cos\theta + rg_{22}\sin\theta\frac{\partial\phi}{\partial r}\cos\theta - 2\cos2\theta\tilde{D}_{12} + \tilde{D}_{11}\sin2\theta \]
\[ + 2\sin^2\theta\frac{\partial D_{11}}{\partial\theta} - 2r\sin^2\theta\frac{\partial D_{12}}{\partial r} + r\sin2\theta\frac{\partial D_{22}}{\partial r} + rg_{12}\cos2\theta\frac{\partial\phi}{\partial r} \right] \cdot \frac{1}{r^2} \]
\[ + p \left[ \frac{\partial^2 D_{22}}{\partial\theta^2}\cos^2\theta + r\frac{\partial D_{22}}{\partial r}\cos^2\theta + r^2\frac{\partial^2 D_{11}}{\partial r^2}\cos^2\theta - 2\sin\theta\frac{\partial D_{22}}{\partial\theta}\cos\theta \right. \]
\[ - 2\sin\theta\frac{\partial^2 D_{11}}{\partial\theta^2}\cos\theta - 2r\sin\theta\frac{\partial D_{11}}{\partial r}\cos\theta + 2\theta\frac{\partial D_{11}}{\partial\theta} - 2\cos2\theta\frac{\partial D_{12}}{\partial\theta} \]
\[ + \sin^2\theta\frac{\partial^2 D_{11}}{\partial\theta^2} + r\sin^2\theta\frac{\partial D_{11}}{\partial r} - \left( \sin\theta\frac{\partial g_{11}}{\partial\theta}(r, \theta) - r\cos\theta\frac{\partial g_{11}}{\partial r}(r, \theta) \right) \]
\[ \cdot r\cos\theta\frac{\partial\phi}{\partial r} + \left( \cos\theta\frac{\partial g_{12}}{\partial\theta} + r\sin\theta\frac{\partial g_{12}}{\partial r} \right) \cdot r\cos\theta\frac{\partial\phi}{\partial r} \]
\[ - \left( \sin\theta\frac{\partial g_{12}}{\partial\theta} - r\cos\theta\frac{\partial g_{12}}{\partial r} \right) \cdot r\sin\theta\frac{\partial\phi}{\partial r} \]
\[ + \left( \cos\theta\frac{\partial g_{22}}{\partial\theta} + r\sin\theta\frac{\partial g_{22}}{\partial r}(r, \theta) \right) \cdot r\sin\theta\frac{\partial\phi}{\partial r} + 2\theta\frac{\partial^2 D_{22}}{\partial r^2} \]
\[ + r\left( -\sin2\theta\frac{\partial D_{12}}{\partial r} + 2\cos2\theta\frac{\partial^2 D_{12}}{\partial r^2} \right) + r^2\sin^2\theta\frac{\partial^2 D_{22}}{\partial r^2} \]
\[ + g_{11}r\left( r^2\frac{\partial^2 \phi}{\partial r^2}\cos^2\theta + \sin^2\theta\frac{\partial\phi}{\partial r} \right) + 2g_{12}r\left( -\cos\theta\sin\theta\frac{\partial\phi}{\partial r} \right) \]
\[ + g_{22}r\left( \frac{\partial\phi}{\partial r}\cos^2\theta + r\sin^2\theta\frac{\partial^2 \phi}{\partial r^2} \right) \right] \cdot \frac{1}{r^2} \]
\[ \triangleq \frac{\partial^2 p}{\partial r^2}H_1(r, \theta) + \frac{\partial^2 p}{\partial \theta^2}H_2(r, \theta) + \frac{\partial^2 p}{\partial r \partial \theta}H_3(r, \theta) + \frac{\partial p}{\partial r}H_4(r, \theta) + \frac{\partial p}{\partial \theta}H_5(r, \theta) + pH_6(r, \theta) = \mathcal{H}_{r, \theta}p \]  
(D.0.2)

where the second order linear differential operator \( \mathcal{H}_{r, \theta} : \mathcal{D}(\mathcal{H}_{r, \theta}) \subset H^1_0([R_1, R_2] \times [0, 2\pi]) \) is defined as,
\[
\mathcal{H}_{r, \theta} = H_1(r, \theta)\frac{\partial^2}{\partial r^2} + H_2(r, \theta)\frac{\partial^2}{\partial \theta^2} + H_3(r, \theta)\frac{\partial^2}{\partial r \partial \theta} + H_4(r, \theta)\frac{\partial}{\partial r} + H_5(r, \theta)\frac{\partial}{\partial \theta} + H_6(r, \theta), \tag{D.0.3}
\]
\[ \mathcal{D}(\mathcal{H}_{r,\theta}) = \{ u \in H^1_0([R_1, R_2] \times [0, 2\pi]) | u(R_1, \theta) = u(R_2, \theta) = 0, \ u(r, 0) = u(r, 2\pi) \}. \]  

(D.0.4)

It can be shown that there exists a positive lower bound on the minimum eigenvalues of \( \bar{\mathcal{D}} \), then \( \mathcal{H}_{r,\theta} \) is uniformly elliptic. To solve the adjoint operator \( \mathcal{H}^*_{r,\theta} \), we just start from the definition \( \forall u, v \in \mathcal{D} \),

\[
\int_0^{2\pi} \int_{R_1}^{R_2} v \mathcal{H}_{r,\theta} u r dr \, d\theta = \int_0^{2\pi} \int_{R_1}^{R_2} v \frac{\partial^2 u}{\partial r^2} H_1(r, \theta) + \frac{\partial u}{\partial r} \frac{\partial v}{\partial r} H_2(r, \theta) + \frac{\partial^2 u}{\partial \theta^2} H_3(r, \theta) \\
+ \frac{\partial u}{\partial r} \frac{\partial v}{\partial \theta} H_4(r, \theta) + \frac{\partial u}{\partial \theta} \frac{\partial v}{\partial \theta} H_5(r, \theta) + v H_6(r, \theta) u r dr \, d\theta \\
= \int_0^{2\pi} \int_{R_1}^{R_2} - \frac{\partial u}{\partial r} \frac{\partial v}{\partial r} H_1(r, \theta) - \frac{\partial u}{\partial \theta} \frac{\partial v}{\partial \theta} H_2(r, \theta) - \frac{\partial u}{\partial r} \frac{\partial v}{\partial \theta} H_3(r, \theta) \\
- \frac{\partial v H_4}{\partial r} - \frac{\partial v H_5}{\partial \theta} + v H_6(r, \theta) u r dr \, d\theta \\
= \int_0^{2\pi} \int_{R_1}^{R_2} u \left[ \frac{\partial^2 v}{\partial r^2} H_1 + 2 \frac{\partial v}{\partial r} \frac{\partial H_1}{\partial r} + \frac{\partial^2 H_1}{\partial r^2} \right] \\
+ u \left[ \frac{\partial^2 v}{\partial \theta^2} H_2 + 2 \frac{\partial v}{\partial \theta} \frac{\partial H_2}{\partial \theta} + \frac{\partial^2 H_2}{\partial \theta^2} \right] \\
+ u \left[ \frac{\partial^2 v}{\partial \theta \partial r} H_3 + \frac{\partial v}{\partial \theta} \frac{\partial H_3}{\partial r} + \frac{\partial v}{\partial r} \frac{\partial H_3}{\partial \theta} + \frac{\partial^2 H_3}{\partial \theta \partial r} \right] \\
- u \left[ \frac{\partial H_4}{\partial r} + H_4 \frac{\partial v}{\partial r} \right] - u \left[ \frac{\partial H_5}{\partial \theta} + H_5 \frac{\partial v}{\partial \theta} \right] + u H_6 v r dr \, d\theta \\
= \int_0^{2\pi} \int_{R_1}^{R_2} u \mathcal{H}^*_{r,\theta} v r dr \, d\theta, \tag{D.0.5}
\]

where we have used the fact that \( u, v, H_1, H_2, H_3, H_4, H_5, H_6 \) are periodic functions with period \( 2\pi \) and \( u(R_1, \theta) = u(R_2, \theta) = 0, v(R_1, \theta) = v(R_2, \theta) = 0 \) when we do
the integration by part. Then, it’s clear to see that,

\[ H^*_{r,\theta} = \frac{\partial^2}{\partial r^2} H_1 + 2 \frac{\partial}{\partial r} \frac{\partial H_1}{\partial r} + \frac{\partial^2 H_1}{\partial r^2} + \frac{\partial^2}{\partial \theta^2} H_2 + 2 \frac{\partial}{\partial \theta} \frac{\partial H_2}{\partial \theta} + \frac{\partial^2}{\partial \theta \partial r} H_3 \\
+ \frac{\partial}{\partial \theta} \frac{\partial H_3}{\partial r} + \frac{\partial}{\partial r} \frac{\partial H_3}{\partial \theta} + \frac{\partial^2 H_3}{\partial \theta \partial r} - \frac{\partial H_4}{\partial r} - \frac{\partial H_5}{\partial \theta} - H_6 \frac{\partial}{\partial \theta} + H_6. \]  

(D.0.6)

Then, using Eq (B.0.8) again for \( H^*_{y,\alpha} \),

\[ H^*_{y,\alpha} y T_1(y, \alpha) = -y, \]  

(D.0.7)

from which the first passage time ODE reads,

\[ H^*_{y,\alpha} \frac{\partial^2 T_1}{\partial y^2} + H^*_{y,\alpha} \frac{\partial^2 T_1}{\partial \alpha^2} + \frac{\partial^2 T_1}{\partial y \partial \alpha} + \left[ \frac{2 H_1}{y} + \frac{2}{\partial y} \frac{\partial H_1}{\partial y} + \frac{\partial H_3}{\partial \alpha} - H_4 \right] \frac{\partial T_1}{\partial y} + \left[ \frac{2}{\partial \alpha} \frac{\partial H_2}{\partial y} + \frac{\partial^2 H_1}{\partial y^2} + \frac{\partial^2 H_2}{\partial \alpha^2} \right] T_1 + 1 = 0. \]  

(D.0.8)

\( T_1 \) can be similarly solved by the method illustrated in Illustration of finite difference method.
Appendix E

Shape functions for star and rod inclusion

The shape function for star shaped inclusion of side \( \ell \) is given by:
\[ R_1 = \begin{cases} \frac{\sqrt{3} \ell}{2} \frac{1}{\sin(\theta_1 + \frac{\pi}{6})}, & 0^\circ < \theta_1 \leq 30^\circ, \\ \frac{\sqrt{3} \ell}{2} \frac{1}{\cos(\theta_1)}, & 30^\circ < \theta_1 \leq 60^\circ, \\ \frac{\sqrt{3} \ell}{2} \frac{1}{\sin(\theta_1 - \frac{\pi}{6})}, & 60^\circ < \theta_1 \leq 90^\circ, \\ \frac{\sqrt{3} \ell}{2} \frac{1}{\cos(\theta_1 - \frac{\pi}{3})}, & 90^\circ < \theta_1 \leq 120^\circ, \\ \frac{\sqrt{3} \ell}{2} \frac{1}{\sin(\theta_1 - \frac{\pi}{2})}, & 120^\circ < \theta_1 \leq 150^\circ, \\ \frac{\sqrt{3} \ell}{2} \frac{1}{\cos(\theta_1 - \frac{2\pi}{3})}, & 150^\circ < \theta_1 \leq 180^\circ, \\ \frac{\sqrt{3} \ell}{2} \frac{1}{\sin(\theta_1 - \frac{5\pi}{6})}, & 180^\circ < \theta_1 \leq 210^\circ, \\ \frac{\sqrt{3} \ell}{2} \frac{1}{\cos(\theta_1 - \pi)}, & 210^\circ < \theta_1 \leq 240^\circ, \\ \frac{\sqrt{3} \ell}{2} \frac{1}{\sin(\theta_1 - \frac{7\pi}{6})}, & 240^\circ < \theta_1 \leq 270^\circ, \\ \frac{\sqrt{3} \ell}{2} \frac{1}{\cos(\theta_1 - \frac{4\pi}{3})}, & 270^\circ < \theta_1 \leq 300^\circ, \\ \frac{\sqrt{3} \ell}{2} \frac{1}{\sin(\theta_1 - \frac{3\pi}{2})}, & 300^\circ < \theta_1 \leq 330^\circ, \\ \frac{\sqrt{3} \ell}{2} \frac{1}{\cos(\theta_1 - \frac{5\pi}{3})}, & 330^\circ < \theta_1 \leq 360^\circ, \end{cases} \] (E.0.1)

The shape function for rod inclusion with major axis length \(2\ell\) and minor axis

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length $\frac{\sqrt{3}\ell}{2}$ is given by:

$$R_1 = \begin{cases} 
\frac{1}{\sqrt{3}\ell} \frac{1}{\sin(\theta_1 + \frac{\pi}{3})}, & 0^\circ < \theta_1 \leq 30^\circ, \\
\frac{1}{2} \frac{1}{\sin(\theta_1)}, & 30^\circ < \theta_1 \leq 150^\circ, \\
\frac{1}{\sqrt{3}\ell} \frac{1}{\sin(\theta_1 - \frac{\pi}{3})}, & 150^\circ < \theta_1 \leq 180^\circ, \\
\frac{1}{\sqrt{3}\ell} \frac{1}{\sin(\theta_1 - \frac{2\pi}{3})}, & 180^\circ < \theta_1 \leq 210^\circ, \\
\frac{1}{2} \frac{1}{\sin(\theta_1 - \pi)}, & 210^\circ < \theta_1 \leq 330^\circ, \\
\frac{1}{\sqrt{3}\ell} \frac{1}{\sin(\theta_1 - \frac{4\pi}{3})}, & 330^\circ < \theta_1 \leq 360^\circ, 
\end{cases}$$

(E.0.2)
Appendix F

Proof of Theorem 3

Following techniques in [69, 82, 76], we integrate Eq (4.3.1) for $P$ over all $t \geq 0$,

$$
\int_0^\infty \frac{\partial P}{\partial t} \, dt = \frac{\partial}{\partial r} \left[ \frac{1}{\nu} \frac{\partial \phi}{\partial r} g_1 + D \frac{\partial g_1}{\partial r} \right] + \frac{1}{r} \left[ \frac{1}{\nu} \frac{\partial \phi}{\partial r} g_1 + D \frac{\partial g_1}{\partial r} \right] \quad (F.0.1)
$$

$$
- \frac{1}{r} \delta(r - y) = \mathcal{L}_r g_1(r, y), \quad (F.0.2)
$$

where $\frac{1}{r} \delta(r - y)$ is the initial condition and the second order linear differential operator $\mathcal{L}_r : \mathcal{D}(\mathcal{L}_r) \subset C^2([R_1, R_2]) \to C^2([R_1, R_2])$ is defined as,

$$
\mathcal{L}_r = \frac{\partial}{\partial r} \left[ \frac{1}{\nu} \frac{\partial \phi}{\partial r} + D \frac{\partial}{\partial r} \right] + \frac{1}{r} \left[ \frac{1}{\nu} \frac{\partial \phi}{\partial r} + D \frac{\partial}{\partial r} \right], \quad (F.0.3)
$$

with domain

$$
\mathcal{D}(\mathcal{L}_r) = \{ v_1 \in C^2([R_1, R_2]) \, | \, v_1(R_1) = 0, \quad k_B T v_1'(R_2) + \phi'(R_2) v_1(R_2) = 0 \}. \quad (F.0.4)
$$
Using the method in [69], we can get the adjoint operator $L_r^*$ which satisfies
\[ \langle v_2, L_r v_1 \rangle = \langle L_r^* v_2, v_1 \rangle, \quad \forall v_1 \in D(L_r), v_2 \in D(L_r^*), \]

\[ L_r^* = -\frac{1}{\nu} \frac{\partial \phi}{\partial r} \frac{\partial}{\partial r} + D \frac{\partial^2}{\partial r^2} + \frac{1}{\nu} \frac{\partial \phi}{\partial r} \frac{1}{r} - D \frac{\partial^1}{\partial r}. \] \hspace{1cm} (F.0.5)

with domain
\[ D(L_r^*) = \left\{ v_2 \in C^2([R_1, R_2]) \mid v_2(R_1) = 0, \frac{v_2(R_2)}{R_2} - \frac{v_2'(R_2)}{R_2} = 0 \right\}, \] \hspace{1cm} (F.0.6)

and the inner product is defined as,
\[ \langle v_1, v_2 \rangle = \int_{R_1}^{R_2} v_1 v_2 dr, \quad \forall v_1 \in D(L_r), v_2 \in D(L_r^*). \] \hspace{1cm} (F.0.7)

Then, we can find a $u_0 \in C^2([R_1, R_2])$ s.t. $L_r^* u_0(r) = r$. Then, it follows from Eq (4.3.5) that,
\[ T_1(y) = \int_{R_1}^{R_2} (L_r^* u_0(r)) g_1(r, y) dr = \int_{R_1}^{R_2} u_0(r) (L_r g_1(r, y)) dr \]
\[ = - \int_{R_1}^{R_2} u_0(r) \frac{1}{r} \delta(r - y) dr = -u_0(y) \frac{1}{y}, \] \hspace{1cm} (F.0.8)

\[ \implies L_y^* y T_1(y) = -y. \] \hspace{1cm} (F.0.9)

Using Eq (F.0.5), we can derive Eq (4.3.7), a second order ODE for $T_1(y)$. The boundary condition of $T_1(y)$ at the absorbing wall is straightforward [82, 76]: $T_1(R_1) = 0$. For the boundary condition at the reflecting wall, we appeal to the Langevin equation in Eq (4.3.9). If the particle sits at position $R_2$, decomposing the overdamped Langevin equation [69] into radial direction and angular direction, we have,
\[ dy = -\frac{1}{\nu} \frac{\partial \phi}{\partial y} dt + \sqrt{\frac{2 k_B T dt}{\nu}} \xi_y, \] \hspace{1cm} (F.0.10)
\[ d\theta = -\frac{1}{\nu y} \frac{\partial \phi}{\partial \theta} dt + \frac{1}{y} \sqrt{\frac{2k_B T dt}{\nu}} \xi_{\theta}. \]  

(F.0.11)

After time \( dt \), the particle can only move to \( R_2 + dy(dy < 0) \) along the radial direction because of the reflecting wall at \( R_2 \). The motion along the angular direction can be neglected because \( T_1(y) \) does not have dependence on angular direction. Note that \( dy \) is a random variable depending on \( \xi_y \) and \( dt \) with constraint \( R_1 \leq R_2 + dy \leq R_2 \). Then, we can write

\[
T_1(R_2) = dt + C_1(dt) \int_{C_2(dt)}^0 T_1(R_2 + dy) G(\xi_y) dy
\]

\[
= dt + C_1(dt) \int_{C_2(dt)}^0 (T_1(R_2) + T'_1(R_2 + \eta dy) dy) G(\xi_y) d\xi_y
\]

\[
= dt + T_1(R_2) + C_1(dt) \int_{C_2(dt)}^0 (T'_1(R_2) + \eta dy T''_1(R_2 + \beta dy)) dy G(\xi_y) d\xi_y
\]

\[
= dt + T_1(R_2) - \frac{1}{\nu} \frac{\partial \phi}{\partial y} T'_1(R_2) dt + C_1(dt) \int_{C_2(dt)}^0 T'_1(R_2) \sqrt{\frac{2k_B T dt}{\nu}} \xi_y G(\xi_y) d\xi_y
\]

\[
+C_1(dt) \int_{C_2(dt)}^0 \frac{\eta dy}{dy} T''_1(R_2 + \beta dy) \left( \frac{2k_B T dt}{\nu} \xi^2_y + o(dt) \right) G(\xi_y) d\xi_y
\]

where we used mean value theorem twice to reach to Eq (F.0.12) with \( R_2 + dy < R_2 + \eta dy < R_2 + \beta dy < R_2 \). Note that \( \beta dy \) depends on \( \eta dy \) and thus depends on \( dy \). \( C_2(dt) \) is the value to satisfy \( R_2 + dy = R_1 \) for given \( dt \) and \( \xi_y \). \( C_1(dt) \) is the scaling factor such that the integral of probability density equals 1: \( C_1(dt) \int_{C_2(dt)}^0 G(\xi_y) d\xi_y = 1 \) where \( G(\xi_y) = \frac{e^{-\xi^2_y/2}}{\sqrt{2\pi}} \). After some re-arrangements and dividing by \( dt \) on both sides,

\[
-1 = -\frac{1}{\nu} \frac{\partial \phi}{\partial y} T'_1(R_2) + T'_1(R_2) \sqrt{\frac{2k_B T dt}{\nu}} C_1(dt) \int_{C_2(dt)}^0 \xi_y G(\xi_y) d\xi_y
\]

\[
+C_1(dt) \int_{C_2(dt)}^0 \frac{\eta dy}{dy} T''_1(R_2 + \beta dy) \left( \frac{2k_B T dt}{\nu} \xi^2_y + o(1) \right) G(\xi_y) d\xi_y. \]  

(F.0.12)
As $dt \to 0$, $C_1 \to 2$, $C_2 \to -\infty$. Note that $\eta(dy) < 1$ and we have $|T_1''(R_2 + \beta dy)| < M$ for some $M$ because $T_1$ is $C^2$. Then if we take $t \to \infty$ the third term in RHS of Eq (F.0.12) can be bounded as,

$$\begin{align*}
\lim_{t \to \infty} & C_1(dt) \int_{C_2(dt)}^0 \eta(dy) T_1''(R_2 + \beta dy) \left( \frac{2k_B T}{\nu} \xi_y^2 + o(1) \right) G(\xi_y) d\xi_y \\
& \leq \lim_{t \to \infty} C_1(dt) \int_{-\infty}^0 \eta(dy) T_1''(R_2 + \beta dy) \left( \frac{2k_B T}{\nu} \xi_y^2 + o(1) \right) G(\xi_y) d\xi_y \\
& \leq 2M \lim_{t \to \infty} C_1(dt) \int_{-\infty}^0 \left( \frac{2k_B T}{\nu} \xi_y^2 \right) G(\xi_y) d\xi_y \\
& \leq 4M \int_{-\infty}^0 \left( \frac{2k_B T}{\nu} \xi_y^2 \right) G(\xi_y) d\xi_y \\
& < \infty.
\end{align*}$$

(F.0.13)

The first term in the RHS of Eq (F.0.12) is independent of $dt$ and thus is finite as $t \to \infty$. For the second term in RHS of Eq (F.0.12), $\lim_{t \to \infty} \left| C_1(dt) \int_{C_2(dt)}^0 \xi_y G(\xi_y) d\xi_y \right| < \infty$, but $\sqrt{\frac{2k_B T}{\nu dt}} \to \infty$ as $dt \to 0$. Since the LHS of Eq (F.0.12) is finite, we must have $T_1'(R_2) = 0$. 

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Appendix G

Proof of Theorem 4

We transform Eq (4.4.1) into polar coordinates,

\[ \frac{\partial p}{\partial t} = F_{r,\theta} p = \nabla \cdot S, \]  

where the elliptic differential operator \( F_{r,\theta} \) : \( D(F_{r,\theta}) \subset C^2([R_1, R_2] \times [0, 2\pi]) \rightarrow C^2([R_1, R_2] \times [0, 2\pi]) \) is in divergence form, with domain

\[ D(F_{r,\theta}) = \left\{ v_1 \in C^2([R_1, R_2] \times [0, 2\pi]) | v_1(R_1, \theta) = 0, \right. \]
\[ \left. \frac{\partial S(R_2, \theta)}{\partial \theta} = 0, \; v_1(r, 0) = v_1(r, 2\pi) \right\}, \]

and the inner product is defined as,

\[ \langle v_1, v_2 \rangle = \int_{R_1}^{R_2} \int_{0}^{2\pi} v_1 v_2 dr d\theta, \; \forall v_1, v_2 \in D(F_{r,\theta}). \]

The expression of \( F_{r,\theta} \) can be found in [69] and we ignore the expression of \( S \) for brevity. Then, it’s useful to derive \( F_{r,\theta}^* \) (see [69]), the adjoint operator of \( F_{r,\theta} \) that satisfies \( \langle v_1, F_{r,\theta} v_2 \rangle = \langle F_{r,\theta}^* v_1, v_2 \rangle, \; \forall v_1 \in D(F_{r,\theta}), \; v_2 \in D(F_{r,\theta}^*). \)
Next, we integrate Eq \( \text{(G.0.1)} \) for \( p \) over \( t \geq 0 \) and get,
\[
\frac{1}{r} \delta(r - y) \delta(\theta - \alpha) = \mathcal{F}_{r,\theta} q_1. \tag{G.0.4}
\]
Then, we can find a \( u_0 \in C^2([R_1, R_2] \times [0, 2\pi]) \) s.t. \( \mathcal{F}_{r,\theta}^* u_0(r, \theta) = r \). It follows from Eq \( \text{(4.4.2)} \) that,
\[
T_1(y, \alpha) = \int_{R_1}^{R_2} \int_0^{2\pi} q_1 \left( \mathcal{F}_{r,\theta}^* u_0(r, \theta) \right) d\theta dr
= \int_{R_1}^{R_2} \int_0^{2\pi} u_0(r, \theta) \mathcal{F}_{r,\theta} q_1 d\theta dr
= -\int_{R_1}^{R_2} \int_0^{2\pi} u_0(r, \theta) \frac{1}{r} \delta(r - y) \delta(\theta - \alpha) d\theta dr
= -\frac{1}{y} u_0(y, \alpha), \tag{G.0.5}
\]
\[
\Rightarrow \mathcal{F}_{y,\alpha}^* y T_1(y, \alpha) = -y. \tag{G.0.6}
\]
Then, we can derive a second order PDE for \( T_1(y) \) (Eq \( \text{(4.4.4)} \)). For boundary conditions, we just need to worry about the reflecting wall. For anisotropic case, \( dy < 0, \ d\theta \) could be either positive or negative. Similarly we can write,
\[
T(R_2, \theta) = dt + C_1(dt) \int_{C_2(dt)}^0 \int_{-\infty}^{\infty} T(R_2 + dy, \theta + d\theta) G(\xi_\theta) d\xi_\theta G(\xi_y) d\xi_y
= dt + C_1(dt) \int_{C_2(dt)}^0 \int_{-\infty}^{\infty} \left[ T(R_2 + dy, \theta) + T_\theta(R_2 + dy, \theta + \eta_{d_y,\theta}^*) d\theta \right]
\cdot G(\xi_\theta) d\xi_\theta G(\xi_y) d\xi_y
= dt + C_1(dt) \int_{C_2(dt)}^0 \left[ T_\theta(R_2 + dy, \theta + \eta_{d_y,\theta}^*) \right] G(\xi_\theta) d\xi_\theta G(\xi_y) d\xi_y
\]
\[
= dt + C_1(dt) \int_{C_2(dt)}^0 \left[ T_\theta(R_2 + dy, \theta + \eta_{d_y,\theta}^*) \right] G(\xi_\theta) d\xi_\theta G(\xi_y) d\xi_y
\]
\[ C_1(dt) \int_{C_2(dt)}^0 \int_{-\infty}^{\infty} [T_\theta(R_2 + dy, \theta)d\theta] G(\xi_\theta)d\xi_y G(\xi_\theta)d\xi_y \]
\[ + C_1(dt) \int_{C_2(dt)}^0 \int_{-\infty}^{\infty} \left[ T_\theta(R_2 + dy, \theta + \beta^*_d, d_y) \right] (d\theta)^2 \frac{\eta^*_{dy,d_0}}{d\theta} \]
\[ \cdot G(\xi_\theta)d\xi_y G(\xi_\theta)d\xi_y + T(R_2, \theta) \]
\[ = dt + T(R_2, \theta) + C_1(dt) \int_{C_2(dt)}^0 \frac{\eta_{dy}}{dy} T_{yy}(R_2 + \beta_{d}, \theta)(d\theta)^2 G(\xi_\theta)d\xi_y \]
\[ - \frac{1}{\nu} \frac{\partial \phi}{\partial y} T_y(R_2, \theta)d\theta + C_1(dt) \int_{C_2(dt)}^0 T_y(R_2, \theta) \sqrt{\frac{2k_B T d\theta}{\nu}} \xi_y G(\xi_\theta)d\xi_y \]
\[ - C_1(dt) \int_{C_2(dt)}^0 \int_{-\infty}^{\infty} \left[ T_\theta(R_2 + dy, \theta) \frac{1}{\nu} \frac{\partial \phi}{\partial \theta} d\theta \right] G(\xi_\theta)d\xi_y G(\xi_\theta)d\xi_y \]
\[ + C_1(dt) \int_{C_2(dt)}^0 \int_{-\infty}^{\infty} \left[ T_\theta(R_2 + dy, \theta + \beta^*_d) \right] \left( \frac{2k_B T d\theta}{R_2^2} \xi^2_\theta + o(1) \right) \frac{\eta^*_{dy,d_0}}{d\theta} G(\xi_\theta)d\xi_y G(\xi_\theta)d\xi_y \]
\[ + C_1(dt) \int_{C_2(dt)}^0 T_\theta(R_2 + dy, \theta) \frac{1}{R_2} \sqrt{\frac{2k_B T d\theta}{\nu}} \int_{-\infty}^{\infty} \xi_y G(\xi_\theta)d\xi_y G(\xi_\theta)d\xi_y \]

where in the process to Eq (G.0.7) we used mean value theorem three times with 
\[ \theta < \theta + \beta^*_d < \theta + \eta^*_{dy,d_0} < \theta + d\theta \] if \( d\theta > 0 \) and \( \theta + d\theta < \theta + \eta^*_{dy,d_0} < \theta + \beta^*_d < \theta \) if \( d\theta < 0 \). After some re-arrangements and dividing by \( dt \) on both sides, we get 

\[ -1 = C_1(dt) \int_{C_2(dt)}^0 \frac{\eta_{dy}}{dy} T_{yy}(R_2 + \beta_{d}, \theta) \left( \frac{2k_B T}{\nu} \xi^2_\theta + o(1) \right) G(\xi_\theta)d\xi_y \]
\[ + C_1(dt) \int_{C_2(dt)}^0 T_y(R_2, \theta) \sqrt{\frac{2k_B T}{\nu} \xi_y G(\xi_\theta)d\xi_y} - \frac{1}{\nu} \frac{\partial \phi}{\partial y} T_y(R_2, \theta) \]
\[ - C_1(dt) \int_{C_2(dt)}^0 \left[ T_\theta(R_2 + dy, \theta) \frac{1}{\nu} \frac{\partial \phi}{\partial \theta} d\theta \right] G(\xi_\theta)d\xi_y \]
\[ + C_1(dt) \int_{C_2(dt)}^0 \int_{-\infty}^{\infty} \left[ T_\theta(R_2 + dy, \theta + \beta^*_d) \right] \left( \frac{2k_B T}{\nu R_2^2} \xi^2_\theta + o(1) \right) \]
Using the continuity of $T_\theta$, $T_{yy}$ and $T_{\theta \theta}$ and the fact that $\eta dy, d\theta / d\theta < 1$, it is clear that the 1st, 3rd, 4th, 5th terms on RHS of Eq (G.0.8) are finite as $dt \to 0$. The 6th term is vanishing due to $\int_{-\infty}^{\infty} \xi_\theta G(\xi_\theta) d\xi_\theta = 0$. Since the LHS of Eq (G.0.8) is finite also, the 2nd term on RHS of Eq (G.0.8) must also be finite as $dt \to 0$. Accordingly, $T_y(R_2, \theta) = 0$ follows from $\lim_{dt \to \infty} \sqrt{\frac{2k_B T}{\nu dt}} \to \infty$. 

$$\frac{\eta_{dy,da}}{d\theta} \left[ G(\xi_\theta) d\xi_\theta G(\xi_y) d\xi_y + C_1(dt) \int_{C_2(dt)}^0 T_\theta(R_2 + dy, \theta) \right]$$ 

$$\frac{1}{R_2} \sqrt{\frac{2k_B T}{\nu dt}} \int_{-\infty}^{\infty} \xi_\theta G(\xi_\theta) d\xi_\theta G(\xi_y) d\xi_y.$$ (G.0.8)


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