Analytical and Computational Modeling of Membrane Nanotubes

Sina Mirzaeifard
University of Tennessee - Knoxville, smirzaei@vols.utk.edu

Follow this and additional works at: https://trace.tennessee.edu/utk_gradthes
Part of the Biochemical and Biomolecular Engineering Commons, Computational Engineering Commons, and the Polymer Science Commons

Recommended Citation
https://trace.tennessee.edu/utk_gradthes/3194

This Thesis is brought to you for free and open access by the Graduate School at TRACE: Tennessee Research and Creative Exchange. It has been accepted for inclusion in Masters Theses by an authorized administrator of TRACE: Tennessee Research and Creative Exchange. For more information, please contact trace@utk.edu.
To the Graduate Council:

I am submitting herewith a thesis written by Sina Mirzaeifard entitled "Analytical and Computational Modeling of Membrane Nanotubes." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Chemical Engineering.

Steven M. Abel, Major Professor

We have read this thesis and recommend its acceptance:

Brian J. Edwards, Paul M. Dalhaimer

Accepted for the Council:

Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)
Analytical and Computational Modeling of Membrane Nanotubes

A Thesis Presented for the Master of Science Degree
The University of Tennessee, Knoxville

Sina Mirzaeifard
December 2014
ACKNOWLEDGEMENTS

Foremost, I would like to express my sincere gratitude to my advisor Dr. Steve Abel for the continuous support of my M.Sc. study and research, for his patience, motivation, enthusiasm, and immense knowledge. He helped me like a brother in all the time of research and writing of this project. I could not have imagined having a better advisor and mentor for my M.Sc. study.

Besides my advisor, I would like to thank the rest of my thesis committee: Prof. Brian Edwards, and Dr. Paul Dalhaimer, for their encouragement, insightful comments, and hard questions.

Last but not the least, I would like to thank my parents for giving birth to me at the first place and supporting me spiritually throughout my entire life.
ABSTRACT

This thesis investigates the interplay between cell membranes and the actin cytoskeleton in cellular structures known as membrane nanotubes. Membrane nanotubes are slender membrane structures that physically connect cells over long distances, and experiments suggest that they play a role in transferring material and information between cells. Disrupting the actin cytoskeleton disrupts membrane nanotubes. Although recent studies have revealed insight into the physical properties and functions of membrane-actin systems, further research is needed to understand their behavior in biological contexts. Membrane nanotubes provide a novel system with which to investigate interactions between the cell membrane and actin.

In this thesis, we use analytical theory and computer simulations to better understand actin filaments enclosed in membrane nanotubes. We begin by describing a theoretical framework based on continuum models of membranes and actin polymers. Using analytical theory, we calculate the energies of various polymer-membrane configurations. Although confined biopolymers are often assumed to adopt helical configurations, we demonstrate that an alternative configuration is energetically favorable in a wide range of parameter space. We then employ Monte Carlo simulations to investigate the equilibrium behavior of a semiflexible polymer confined within spatial regions characteristic of membrane nanotube dimensions. To investigate flexible membranes, we use Monte Carlo simulations of discrete, triangulated elastic surfaces. We begin by studying a tubular membrane in isolation and investigate the influence of bending rigidity and tube dimensions on characteristics of the membrane. Finally, we use computer simulations to study a system in which a semiflexible polymer is placed inside of a membrane tube, which serves as a model of a membrane nanotube. We find that the presence of the polymer has small effect on membrane properties for typical cell parameters.
# TABLE OF CONTENTS

Chapter 1:  **Introduction** ........................................................................................................... 1
  1.1. Biology background ................................................................................................. 1
  1.2. Theoretical and computational framework ......................................................... 2
    1.2.1. Semiflexible polymer ....................................................................................... 3
    1.2.2. Membrane ....................................................................................................... 4
    1.2.3. Computer simulation ......................................................................................... 4
  1.3. Review of previous work ......................................................................................... 5
  1.4. Thesis outline ......................................................................................................... 7

Chapter 2:  **Problem formulation** .............................................................................................. 8
  2.1. Goals of our study .................................................................................................. 8
  2.2. Details of simulation models ................................................................................. 9
  2.3. System quantities of interest ................................................................................ 11

Chapter 3:  **Results and discussion** ........................................................................................ 12
  3.1. Analytical calculations ......................................................................................... 12
  3.2. Polymer behavior at equilibrium ......................................................................... 18
  3.3. Membrane behavior at equilibrium ..................................................................... 24
    3.3.1. Polymerized membrane .................................................................................. 24
    3.3.2. Influence of fluidity on membrane ................................................................. 28
  3.4. Membrane nanotubes ........................................................................................... 33

Chapter 4:  **Summary and conclusions** ................................................................................... 38

List of References ....................................................................................................................... 40

Vita ............................................................................................................................................. 43
LIST OF FIGURES

Figure 1. Two T cells connected by a membrane nanotube. ................................. 2

Figure 2. Sketch of filopodia by Faix et al. (2006). ................................................... 6

Figure 3. Polymer with helical wrappings inside of a tube. ........................................ 6

Figure 4. A simulated polymer with $M = 100$ and $\lambda = 20 \text{kBT}$. Image shown after $10^5$ Monte Carlo steps. ................................................................. 9

Figure 5. Flat triangulated surface consisting of 100 particles. ................................. 10

Figure 6. Bond flip mechanism. ................................................................. 10

Figure 7. A simulated polymerized membrane nanotube with a membrane consisting of 840 particles and a polymer consisting of 160 particles. Image shown after $10^4$ Monte Carlo steps................................................................. 11

Figure 8. For polymers longer than a confining membrane, the polymer energy decreases as the radius or length of the membrane increases. In the figure, the radius increases from 0.050 µm (top) to 0.300 µm (bottom) with an increment of 0.025 µm. ....................................................... 12

Figure 9. Circular cap configuration of a polymer inside of a confining tube. ............. 13

Figure 10. Polymer winding in the horizontal direction with $L<2R$. ......................... 14

Figure 11. Polymer winding in the vertical direction with $L<2R$. ......................... 14

Figure 12. Energy as a function of radius and ratio of membrane to polymer length for (a) membrane tube with helical polymer and (b) membrane tube with circular cap polymer. ...... 14

Figure 13. Polymer energy as a function of membrane radius for circular cap (left) and helical (right) polymers. Red circles show a range of radii ($R \approx 100$ nm) that provide lower energies for different membrane lengths (increasing lower to upper). The membrane length increases from 0.6 µm to 1.5 µm (left) and from 0 µm to 1.5 µm (right) with an increment of 0.1 µm. .......... 15

Figure 14. a) Energy difference between helical and circular cap configurations as a function of membrane length with fixed radius and polymer length. The red circle shows the small range of $L$ over which the helical polymer provides lower energy than the circular one. The stepwise behavior is due to employing a floor function for the circular case. b) Energy difference as a function of membrane radius with fixed membrane and polymer lengths. As $R$ increases, the difference vanishes. ................................................................. 16

Figure 15. a,d) Energy difference as a function of membrane length with fixed radius and polymer length. b,e) Energy difference as a function of membrane radius with fixed membrane
and polymer lengths. c,f) Energy difference as a function of polymer length with fixed radius and length membrane. The red circle shows the region of polymer lengths in which that energy difference behaves differently. .......................................................... 17

Figure 16. Polymers with random initial configurations (M=100) are shown in black and green. Polymers with straight initial configurations are shown in blue and red. a,b) Energy, end-to-end distance, and radius of gyration plots of two polymers with $\lambda = 1000$. Average number of accepted moves is 22%. .......................................................... 18

Figure 17. Polymers with random initial configurations (M=100) are shown in black and green. Polymers with straight initial configurations are shown in blue and red. a,b) Energy, end-to-end distance, and radius of gyration plots of two polymers with $\lambda = 20$. Average number of accepted moves is 73%. c,d) Energy, end-to-end distance, and radius of gyration plots of two polymers with $\lambda = 1$. Average number of accepted moves is 87%. e,f) Energy, end-to-end distance, and radius of gyration plots of two polymers with $\lambda = 0.5$. Average number of accepted moves is 88%. .......................................................... 19

Figure 18. Energy (units of $k_B T$) of free polymers with different persistence lengths: $\lambda = 10$ (dark green), 50 (red), 200 (turquoise), 2000 (purple). Mean energies for above persistence lengths are 297.8, 299, 307.6, 452.5, respectively. ........................................................................................................ 20

Figure 19. Energy (units of $k_B T$) of free polymers ($\lambda = 15$) with different total lengths: $M = 10$ (blue), 100 (dark green), 200 (red), 300 (turquoise), 400 (purple), 500 (yellow). ....................... 20

Figure 20. Correlation functions of energy for polymers (M=100, $\lambda = 1$ (left), and $\lambda = 20$ (right)), with the independent variable measuring number of Monte Carlo steps. ......................... 21

Figure 21. Polymer initial configurations: linear, helical, jagged (from left to right). ........... 21

Figure 22. Results show energy and end-to-end distance plots for three distinct trajectories starting with the same jagged configuration ($M = 100, \lambda = 20$, tube radius = 2). ..................... 22

Figure 23. Results show energy and end-to-end distance plots for two individual polymers (single trajectory for each) starting with linear (top) and helical (bottom) configurations ($M = 100, \lambda = 20$, tube radius = 2). .......................................................... 22

Figure 24. Energy (units of $k_B T$) of a polymer ($M = 300$) with (a) $\lambda = 90$ and (b) 15. Tube radius = 1 (dark green), 1.5 (red), 2 (turquoise), 2.5 (purple), 3 (khaki), 3.5 (brown), 4 (blue), 4.5 (yellow), 5 (black). .......................................................... 23

Figure 25. Mean energy (units of $k_B T$) for unconfined and confined polymerized membrane tubes with $\kappa = 40\ k_B T$. Red and blue colors correspond to increasing diameter and length, respectively. Red circles represent a cylinder with 20 layers and 9, 14, 19, 24, and 29 particles in each layer. Blue circles represent a cylinder with 10, 15, 20, 25, and 30 layers with 19 particles in each layer. .......................................................................................................................... 25
Figure 26. Initial configuration for a cylindrical membrane with 1596 particles (84 layers with 19 particles in each layer).

Figure 27. Mean energy (units of $k_B T$) for polymerized tubular membranes in both confined and unconfined cases.

Figure 28. Configurations of polymerized membranes with $\kappa = 0$ (upper) and 1000 (lower) in both confined (left) and unconfined (right) cases. Images shown after $2 \times 10^6$ Monte Carlo steps.

Figure 29. Total membrane length for a confined polymerized tubular membrane with movable confinement boundaries ($\kappa = 40$).

Figure 30. Mean energy (units of $k_B T$) of unconfined and confined fluid membrane tubes with $\kappa = 40 \ k_B T$. Red and blue colors show increasing in diameter and length, respectively. Red triangles represent a cylinder with 20 layers and 9, 14, 19, 24, and 29 particles in each layer. Blue triangles represent a cylinder with 10, 15, 20, 25, and 30 layers with 19 particles in each layer.

Figure 31. Energy (units of $k_B T$) of fluid membranes in both confined and unconfined cases.

Figure 32. Specific heat ($C$) for confined fluid membranes. A crumpling transition occurs near $k = 1.25$.

Figure 33. Configurations of confined fluid membranes with $\kappa = 0$ (left) and 1000 (right). Images shown after $2 \times 10^6$ Monte Carlo steps.

Figure 34. Configurations of an unconfined fluid membrane with $\kappa = 40$. Image shown after $2 \times 10^6$ Monte Carlo steps.

Figure 35. Total length of the membrane tube for a fluid membrane with Monte Carlo trial moves that allow a change in length ($\kappa = 40$).

Figure 36. Mean energy (units of $k_B T$) and specific heat for spherical fluid membranes. Numbers of particles are 42 (blue), 92 (dark green), 162 (red), 252 (turquoise).

Figure 37. Configurations of a spherical fluid membrane with a semiflexible polymer inside under different conditions. The membrane has $N = 1442$ and $\lambda = 10$. The polymer has $M=300$, with persistence lengths of (a) 0, (b)13, and 65 (c and d). For (c) and (d) two snapshots (left and right) from different views are shown. Images shown after $5 \times 10^6$ Monte Carlo steps.

Figure 38. Configurations of a spherical fluid membrane with a semiflexible polymer inside under different conditions. The membrane has $N = 1442$ and $\lambda = 10$. The polymer has $M=300$, with persistence lengths of (a) 260 and (b and c) 2600. Excluded volume is considered for the polymer in case c. For cases: a, b, and c two snapshots (left and right) from different views are shown. Images shown after $5 \times 10^6$ Monte Carlo steps.
Figure 39. Energy (units of $k_B T$) for semiflexible polymers ($M = 300$, $l_p = 15$, helical initial configuration) confined in a hard tube with radius = 3 (black), a fluid membrane tube (green), and a polymerized membrane tube (purple). .................................................. 35

Figure 40. Energy (units of $k_B T$) of polymerized membrane tubes (left) and fluid membrane tubes (right) ($N = 1596$, $\kappa = 40$) with a semiflexible polymer inside ($M = 300$, $l_p = 15$, helical initial configuration). Blue and green colors represent a confined membrane tube with and without polymer, respectively. .................................................. 36

Figure 41. Mean energy (units of $k_B T$) of polymerized membrane tubes (left) and fluid membrane tubes (right) ($N = 1596$, $\kappa = 40$) as a function of polymer length ($l_p = 15$, helical initial configuration). .................................................. 36

Figure 42. Energy (units of $k_B T$) of polymerized membrane tubes (left) and fluid membrane tubes (right) ($N = 1596$, $\kappa = 40$) with a helically initiated polymer inside. The persistence lengths of the polymers are 15 (blue) and 90 (green). .................................................. 36
Chapter 1: Introduction

1.1. Biology background

All living organisms are composed of one or more cells. A typical eukaryotic cell contains a variety of components like proteins, lipids, and organelles that are enclosed within a cell membrane. The function of a cell is governed by the physical interactions of such components, which are organized across multiple time- and length-scales. This thesis investigates the interaction between membranes and the actin cytoskeleton, two key components of eukaryotic cells, in cellular structures known as membrane nanotubes.\(^1,2\)

The cell membrane consists of a thin lipid bilayer and associated protein molecules. The lipid molecules are mostly phospholipids, and their amphipathic character (i.e., they have hydrophilic and hydrophobic regions) drives the formation of a lipid bilayer with a thickness of about 5 nm. This lipid bilayer avoids free edges in order to minimize free energy, forming a closed and sealed configuration that helps the cell isolate and protect its interior parts from the external environment. This cell membrane is dynamic and mediates communication with the cell’s environment. The cell membrane is relatively impermeable and utilizes transmembrane proteins to transport material into and out of the cell.\(^1\)

The cytoskeleton consists of a network of filamentous proteins that supports cell shape and facilitates cell movement. The cytoskeleton in eukaryotic cells has three main filaments: actin filaments (also known as microfilaments), intermediate filaments, and microtubules. Actin filaments are responsible for shaping the cell’s surface and facilitating cell movement. Actin filaments are thin (5-9 nm) and dynamic polymers comprised of monomeric actin proteins. Actin filaments play many important roles in cells and can form two- and three-dimensional networks as well as linear bundles.\(^1\)

Recent experimental studies have found that many types of cells can physically link to each other over long distances by means of structures known as membrane nanotubes (e.g., see Fig. 1).\(^2-6\) Membrane nanotubes are long and slender tubes formed from the plasma membrane, and can vary in size, structure, and formation processes. It is speculated that cells use membrane
nanotubes as a means of intercellular communication, and viruses can propagate from one cell to another by means of membrane nanotubes.\textsuperscript{5,7,8} Membrane nanotubes require the cytoskeleton for stability, and their length can extend over several hundred microns. Membrane nanotubes with a diameter less than 700 nm contain only actin and carry portions of plasma membrane between cells in both directions, whereas thicker membrane tubes contain both actin and microtubules and can carry components of the cytoplasm such as organelles and vesicles between cells.\textsuperscript{9} Two distinct processes can contribute to the formation of membrane nanotubes. In the first, an actin-driven protrusion extends from one cell to connect to a nearby cell, and in the second, cells adhere to each other while in close proximity, and a nanotube forms as they move apart. It is also feasible to observe the combination of these two processes (i.e., an actin-mediated protrusion extends from one cell to another and elongation of the nanotube occurs as the cells move apart).\textsuperscript{7}

![Figure 1. Two T cells connected by a membrane nanotube.\textsuperscript{9}](image)

### 1.2. Theoretical and computational framework

A small number of theoretical and computational studies have investigated long tubular extensions from cells.\textsuperscript{8,10,11} However, the physics of tubular membranes confined between two cells remains to be studied, and the recent discovery of membrane nanotubes may provide an interesting model system that can provide a greater understanding of membrane-cytoskeleton interactions.

We study membrane nanotubes using both analytical theory and computer simulations. On sufficiently long length scales, such as those relevant to membrane nanotubes, an actin filament can be modeled as a semiflexible polymer (also known as a worm-like chain). The polymer is represented as a continuous curve in which curvature is energetically unfavorable, with a term
known as persistence length characterizing the polymer flexibility. Membranes can also be modeled as continuous surfaces at sufficiently long length scales, with an energy that depends on bending rigidity ($\kappa$) and surface tension ($\gamma$) terms. The bending rigidity is a constant that characterizes membrane flexibility. Membranes can be characterized as fluid or polymerized, depending on the ability to resist in-plane shear. The continuum models of both polymers and membranes are amenable to computer simulation by describing them as discrete structures consisting of particles (nodes) connected by bonds (edges).

In this section, we introduce the continuum models of actin and membranes that form the theoretical basis for our work. We also outline the Monte Carlo computer simulation methods that allow us to explore properties of polymer-membrane systems at thermal equilibrium.

1.2.1. Semiflexible polymer

Actin filaments can be modeled as semiflexible polymers (worm-like chains) on length scales of interest in this study. The configuration of a polymer is mathematically parameterized by curve $r(s)$ with inextensibility condition $\left| \frac{\partial r(s)}{\partial s} \right| = 1$ (i.e., it has a unit tangent vector or velocity) and a total length of $l$ (called the contour length). The energy of such a polymer is given by

$$E_{\text{polymer}} = \frac{l_p k_B T}{2} \int_0^l ds \left| \frac{\partial^2 r(s)}{\partial s^2} \right|^2,$$

where $l_p$ denotes the persistence length and $k_B T$ is the thermal energy. Here, persistence length is a function of the size of a bundle of actin, which is a small number of cross-linked actin filaments, and the term within the integral is the square of the local curvature. As a result, highly curved polymers have high energy while a completely straight polymer has zero curvature and consequently zero energy.
1.2.2. Membrane

Membranes can be modeled as continuum elastic sheets on length scales of interest in this study. For many membranes, the shape of the membrane can be mathematically parameterized by the surface $z(x_1, x_2)$. The energy associated with such a surface consists of surface tension and bending rigidity terms and is given by

$$E_{\text{membrane}}(z) = \frac{1}{2} \int d^2 x [\gamma (\nabla z)^2 + \kappa (\nabla^2 z)^2],$$

where $z$ denotes surface function, $\gamma$ denotes surface tension, and $\kappa$ denotes membrane rigidity. The surface tension term penalizes membranes with high surface area and the bending rigidity term penalizes high local curvature.

In our analytical calculations, we model the cell membrane as a thin elastic cylinder. The energy of an ideal cylindrical membrane can be written:

$$E_{\text{membrane}} = E_{\text{tension}} + E_{\text{bending}} = (2\pi RL)\gamma + \frac{\kappa\pi L}{R},$$

where $R$ denotes the tube radius, and $L$ denotes the tube length.

1.2.3. Computer simulation

While analytical calculations can provide much insight, it is often hard to generalize analytical results and to account for thermal fluctuations. Computer simulations allow exploration of equilibrium configurations. We use Monte Carlo methods to simulate discretized representations of both polymers and membranes in which particles are connected by edges to represent the objects. Particles (nodes) occupy different positions, are connected to each other by bonds (edges), and can change their positions randomly. The length between connected particles is constrained to a minimum and maximum length. The angles between bonds, which may vary during a simulation, are related to the curvature and hence, bending energy. Monte Carlo simulations are a well-known technique in statistical physics to sample configurations at thermal equilibrium. Typical simulation steps are as follows:
• Generate a trial configuration: Pick a vertex at random and move it by a random displacement: \( \vec{r}_i' = \vec{r}_i + \Delta \vec{x} \)

• Test whether the new configuration is allowed by checking length constraint.
  • If so, calculate the change in energy, \( \Delta u = u_{\text{trial}} - u_{\text{initial}} \)
  • If \( \Delta u \leq 0 \), accept the trial move.
  • If \( \Delta u > 0 \), accept with probability \( e^{-\frac{\Delta u}{k_B T}} \).

• If the trial move is not accepted, keep the previous configuration.

• Repeat

A single Monte Carlo step (MCS) is completed after each particle attempts to move one time on average. This Monte Carlo technique generates equilibrium configurations that are sampled from the canonical ensemble.

1.3. Review of previous work

Previous work by other research groups has suggested approaches we can take to study membrane nanotubes. Work by Pronk et al. considered membrane protrusions known as filopodia, which are finger-like cellular projections with actin inside.\(^{13}\) They consider an ideal cylindrical tube with a helically wrapped polymer and use analytical calculations to investigate the energy of the system. The helical configuration of the polymer is described mathematically by

\[
r(s) = \begin{pmatrix}
R \cos(2\pi ns) \\
R \sin(2\pi ns) \\
s\sqrt{1 - 4n^2\pi^2R^2}
\end{pmatrix},
\]

\( n \)
with \( n \) the number of helix windings per unit contour length (i.e., \( n = \frac{\# \text{ windings}}{l} \)). One can readily calculate \( \left| \frac{\partial^2 r(s)}{\partial s^2} \right| = 4R\pi^2 n^2 \), and it can be shown that the total vertical length is:

\[
L = l\sqrt{1 - 4n^2\pi^2 R^2},
\]

which allows us to write \( \int_0^l ds \left( \frac{\partial^2 r(s)}{\partial s^2} \right)^2 = \frac{l}{R^2} \left( 1 - \frac{l^2}{l^2} \right)^2 \), and thus,

\[
\frac{E_{\text{polymer}}}{k_B T} = \frac{l l_p}{2R^2} \left( 1 - \frac{l^2}{l^2} \right)^2.
\]

Note that this equation holds only when the membrane length is less than the polymer length. There is no energy of confinement when the membrane is as long or longer than the polymer, since the polymer can fit in the tube without any curvature. Physically, the polymer energy should decrease as the membrane length increases since the polymer requires fewer windings to fit.

The total energy of the semiflexible polymer confined in an elastic tube is the summation of the polymer and membrane energies,

\[
E = \frac{l_p k_B T}{2} \int_0^l ds \left( \frac{\partial^2 r(s)}{\partial s^2} \right)^2 + \left( \frac{\pi \kappa}{R} + 2\pi \gamma R \right) L.
\]

The authors demonstrate that the presence of a bundle of actin in the system can stabilize a filopodium against collapse. When actin is not present, energetics favor collapse of the filopodium. According to their results, a global energy minimum always occurs at a tube of zero length and infinite radius with a straight parallel polymer inside. However, in certain parameter regimes, a local energy minimum is possible at nonzero tube length and radius.
Recent work by Fosnaric et al. considered a single semiflexible polymer confined within a fluid vesicle. The authors use Monte Carlo (MC) computer simulations to study the effects of changing the persistence length of a polymer in a lipid vesicle. At equilibrium, interactions between the polymer and membrane result in the membrane forming a disk-like shape, which is in contrast with the spherical shape of an empty fluid membrane. Also, the polymer tends to adopt an ordered coil shape at equilibrium.

1.4. Thesis outline

Although recent studies have revealed insight into the structure, physical properties, and functions of membrane-actin systems, further research is needed to understand their behavior in a wide variety of situations. Membrane nanotubes provide a novel system in which to investigate interactions between the cell membrane and actin. As such, we use analytical theory and computer simulations to better understand actin filaments enclosed in membrane nanotubes. In this thesis, we begin by describing the theoretical framework and presenting analytical results. We demonstrate that a helical configuration is not the only likely configuration for a bundle of actin filaments in a tube and show that an alternative configuration is energetically favorable in a wide range of parameter space. We then employ Monte Carlo simulations to model a semiflexible polymer at thermal equilibrium. Polymer quantities such as energy and end-to-end distance are calculated to study the polymer behavior in both confined and unconfined spatial regions. Confining a polymer in space gives insight into the effects of flexible membranes and allows us to estimate equilibration times for the simulations. To investigate flexible membranes, we use Monte Carlo simulations of discrete triangulated surfaces. We begin by studying the membrane without a polymer and investigate effects of various parameters and membrane shapes (e.g., spherical and cylindrical). Finally, we consider a system in which a semiflexible polymer is placed inside of an enclosed membrane. This serves as a model of a membrane nanotube. We conclude by giving an overview of the physics and behavior of membrane nanotube models studied in this thesis and by discussing future directions and open questions remaining.
Chapter 2: Problem formulation

2.1. Goals of our study

To gain insight into the behavior of membrane nanotubes, we begin by studying polymers and membranes in isolation and then consider a polymer confined within a membrane. In this study, we model membrane nanotubes as an elastic cylindrical membrane with a semiflexible polymer inside.

We first seek to investigate the behavior of a semiflexible polymer without a membrane present. Our initial studies will confine a polymer in geometries such as hard cylinders. We will study polymer quantities such as end-to-end distance, radius of gyration, and energy, which will provide useful comparison for understanding effects of flexible membranes in subsequent simulations and will also allow us to estimate equilibration times of the simulations. We will study the effect of changing the initial configuration (e.g., helical, straight, and random in both confined and unconfined to geometries). Moreover, we will independently vary persistence length \(l_p\) and polymer length \(l\) to investigate the effects on the system at equilibrium. Adding excluded volume between particles is another subject of interest, as it may change features of the polymer behavior. Performing the outlined tests will allow us to investigate the polymer without complications from the membrane, and we can characterize how fixed and bounded geometries affect the polymer behavior.

We will follow a similar procedure for the membrane in isolation. We will study a cylindrical, polymerized membrane with two cases governing the ends of the cylinder: free ends (the membrane can freely move in space) and fixed ends (the cylinder ends are confined to lateral movements only, which mimics an empty membrane nanotube confined between two cells). We will systematically change the bending rigidity \(\kappa\), the size of the cylinder (radius and length), the number of triangles, and the triangulation method. In addition to the polymerized membrane, we will incorporate membrane fluidity and redo the previous tests to investigate the effects of fluidity on the membrane. We will also consider other membrane shapes found in cell biology such as spherical vesicles.
We will conclude by simulating a polymer confined within a membrane tube, which represents our model of a membrane nanotube. We will study the interplay between the membrane and polymer, including the effects of thermal fluctuations. We seek to understand the influence of physical properties of the system such as persistence length, polymer length, bending rigidity, and membrane diameter. We will attempt to model biological conditions, and our ultimate goal is to provide insight into the fascinating biology of membrane nanotubes by elucidating physical processes that govern their behavior.

2.2. Details of simulation models

We employ Monte Carlo simulations to study a discrete model of a semiflexible polymer. The polymer consists of $M$ vertices (nodes $(r_i)$) connected by $M-1$ bonds (edges $(E_i)$). The bond length can range from $l_{\text{min}} = 1$ to $l_{\text{max}} = 1.67$, and the total length of polymer is $l = \sum_{i=1}^{M-1} l_{E_i}$. The energy of the polymer is given by

$$E_{\text{polymer}} = \lambda \sum_{i=1}^{M-2}(1 - \cos \theta_i),$$

where $\theta$ denotes the angle between two successive bonds and $\lambda = k_B T \left( \frac{\xi}{l} \right)$, with $\xi$ the persistence length and $l$ the average of the maximum and minimum bond lengths.

![Figure 4. A simulated polymer with $M = 100$ and $\lambda = 20 \ k_B T$. Image shown after $10^5$ Monte Carlo steps.](image)

We also use Monte Carlo simulations to study discrete representations of membranes. The membrane consists of $N$ vertices that are connected by bonds forming a triangular mesh (see Fig.
5). Two connected vertices must be separated by an edge length between $l_{\text{min}} = 1$ and $l_{\text{max}} = 1.67$, and particles have excluded volume and cannot occupy the same space at a given time (i.e., particles are considered rigid spheres).\textsuperscript{15} The bending energy for these discrete, triangulated surfaces used by Nelson, Kantor, and Kardar,\textsuperscript{15}

$$E = \lambda \sum_{(i,j)} (1 - n_i \cdot n_j),$$

The sum extends over all triangles sharing an edge, $\lambda$ depends on the bending rigidity, and $n_i$ denotes a normal vector to a triangle.\textsuperscript{16}

Polymerized membranes, which resist in-plane shear, maintain a fixed connectivity of particles. Baumgartner and Ho pioneered a model of a fluid membrane in which a randomly triangulated surface can break bonds between particles and create new bonds.\textsuperscript{17} Therefore, membrane particles can move throughout the membrane, in contrast with polymerized membranes.\textsuperscript{17,18}

\textbf{Figure 5.} Flat triangulated surface consisting of 100 particles.

This additional type of Monte Carlo move is known as a bond flip. This mechanism provides fluidity for the membrane, allowing each vertex to escape its original neighbors. In this model, during each Monte Carlo step, $N$ bonds are randomly chosen to be cut and replaced by new bonds (see Fig. 6). New bonds must satisfy the bond length constraint and each particle must have at least 3 bonds to other particles to prevent either bonds or triangles from “dangling”.\textsuperscript{19,20}

\textbf{Figure 6.} Bond flip mechanism.
2.3. System quantities of interest

We calculate quantities such as energy, end-to-end distance, and radius of gyration to investigate the behavior of the polymer. End-to-end distance ($\vec{r}_e^i$) is the distance between the first ($\vec{r}_1^i$) and last particles ($\vec{r}_M^i$), $\vec{r}_e^i = \vec{r}_M^i - \vec{r}_1^i$. The radius of gyration is given by

$$R_g^2 = \frac{1}{2M^2} \sum_{i,j}(r_{ij} - \bar{r}_j)^2.$$  

The time correlation function of the energy (where time denotes the number of Monte Carlo steps), is defined as

$$C_E(\tau) = \frac{1}{T_{\text{max}} - \tau} \sum_{t \text{ (MCS) = 0}}^{T_{\text{max}} - \tau} \bar{E}(t) \bar{E}(t + \tau),$$

where $[\bar{E}(t) = E(t) - \langle E \rangle]$. This function is applied to measure how long correlations in energy fluctuations persist. A similar correlation function can be defined for the radius of gyration. Specific heat, which can be used to discern transitions, is another quantity of interest and is calculated by

$$C = \frac{1}{N} [\langle E^2 \rangle - \langle E \rangle^2].$$

Figure 7. A simulated polymerized membrane nanotube with a membrane consisting of 840 particles and a polymer consisting of 160 particles. Image shown after $10^4$ Monte Carlo steps.
Chapter 3: Results and discussion

3.1. Analytical calculations

In this section, we study the energetics of a system consisting of a semiflexible polymer confined within an ideal cylindrical membrane. We use analytical theory and employ the previously mentioned formula,

$$E = \frac{1}{2}l_p k_B T \int_0^l ds \left( \frac{\partial^2 r(s)}{\partial s^2} \right)^2 + \left( \frac{\pi \kappa}{R} + 2\pi \gamma R \right) L,$$

to calculate the energy of such a system. Physically, the polymer energy should decrease as the membrane length increases, with no energy of confinement when the membrane is longer than the polymer (if the membrane length is equal to or greater than the polymer length, the polymer can fit in the tube without any curvature).

![Figure 8](image-url) For polymers longer than a confining membrane, the polymer energy decreases as the radius or length of the membrane increases. In the figure, the radius increases from 0.050 μm (top) to 0.300 μm (bottom) with an increment of 0.025 μm.

Previous studies have commonly assumed helical configurations for many confined biopolymers such as ds-DNA and actin filaments. For a polymer in a confined tube, the helix has a specific radius, total length, and number of windings. We wish to investigate other well-
behaved configurations that may be energetically favorable when compared with the helical configuration.

We consider a specific alternative conformation for the polymer in a tubular membrane, which we call the circular cap configuration. The polymer in this configuration follows the length of the membrane tube without curvature and turns at the top and bottom of the membrane by making semicircles.

Therefore, the polymer contributes to the energy only in the cap areas. We have shown by analytical and computational methods that this is an energetically favorable configuration in some ranges of membrane radii and length. The shape of a circularly wrapped polymer at its turning points can be described by

\[ r(s) = \begin{pmatrix} R \cos(as) \\ R \sin(as) \end{pmatrix}, \]

The inextensibility condition, \( \left| \frac{\partial r(s)}{\partial s} \right| = 1 \), allows us to find \( \alpha = \frac{1}{R} \). If we have a polymer with \( n \) turns (semicircles), the energy associated with the polymer is \( \frac{n \pi l_p}{2R} \). One can calculate the number of turns using:

\[ l = (L - R) + \pi R + (L - 2R) + \pi R + \cdots = (L - R) + n\pi R + (n - 1)(L - 2R) \]

Neglecting the last uncompleted turn so that \( n \) is an integer, we have \( n = \left\lfloor \frac{l - R}{\pi R + L - 2R} \right\rfloor. \)

This past scenario only holds for \( L > 2R \). For cases in which \( L < 2R \), we consider two other cases: (i) Winding only in the horizontal direction (see Fig. 10, \( n = \frac{l}{\pi R} \)) and (ii) winding in the vertical direction with a smaller radius (see Fig. 11). In case (ii), the new radius for polymer
winding is \( L/2 \), giving \( n = \frac{2L-L}{\pi L} \). Clearly, when \( L \) approaches 0, the energy associated with polymer tends to infinity. Here, the polymer energy (like the helical scenario) is zero when the polymer length is less than or equal to the membrane length.

If we calculate the total energy of the polymer-membrane system with physical properties for a typical cell (\( \kappa = 40 \, k_B T \), \( \gamma = 0.0025 \, \frac{k_B T}{nm^2} \), \( l_p = 15 \, \mu m \)), the global minimal energy that represents the most likely condition varies from helical to circularly wrapped case. As shown in Figure 12, this minimum for a polymer with helical configuration occurs at the largest radius (\( R=300 \, nm \)) and shortest membrane length (\( L=0 \, nm \)) considered. In addition to this energy minimum, a local energy minimum exists at a radius of 100 nm and a length ratio of approximately 0.9. This local energy minimum can stabilize a filopodium against collapse. When \( L < 2R \), a circular cap configuration with vertical windings has a minimum energy in a narrow range of membrane lengths and radii (\( L \approx 411 \, nm \), \( R \approx 156 \, nm \)). Here, the polymer significantly contributes to the total energy of the system, in contrast with the helical case.

Generally, there are two approaches to find the local and global energies, and hence the associated membrane radius. When the polymer length is shorter than the membrane length,
there is no contribution to the energy by the polymer. In this case, after taking the derivative of total energy with respect to $R$, the global minimum in energy involves a balance of contributions from bending rigidity and surface tension. When we are in the regime in which the polymer length is larger than the membrane length, the previous minimum no longer exists and the analytical method does not lead to an explicit solution. For filopodia, we apply numerical methods to find a range of radii, around $R \approx 100$ nm, that minimize the energy in a range of membrane lengths for both helical and circular cap shapes. If we now calculate the radius of an empty tube, which includes only surface tension and bending rigidity, we obtain the equation:

$$
\left( \frac{\partial E}{\partial R} \right)_L = -\frac{\kappa n L}{R^2} + 2 \pi \gamma L = 0 \quad \Rightarrow \quad R = \frac{\kappa}{\sqrt{2 \gamma}}.
$$

The radius is equal to 89.4 nm. Since a bundle of polymers trigger radial forces, an empty tube results in a smaller radius.

![Figure 13](image)

**Figure 13.** Polymer energy as a function of membrane radius for circular cap (left) and helical (right) polymers. Red circles show a range of radii ($R \approx 100$ nm) that provide lower energies for different membrane lengths (increasing lower to upper). The membrane length increases from 0.6 $\mu$m to 1.5 $\mu$m (left) and from 0 $\mu$m to 1.5 $\mu$m (right) with an increment of 0.1 $\mu$m.

We now consider helical and circular cap polymer configurations and compare the energy difference $\Delta E = E_{\text{helix}} - E_{\text{cap}}$. For $l \leq L$, $\Delta E = 0$, since there is no energy associated with polymer in both cases. Figure 14 shows that actin in an elastic tube is more energetically favored in a circular cap shape than in a helical shape for much of parameter space. If we increase the membrane radius, $\Delta E$ approaches zero. However, the helical shape is energetically favorable in a small region where the polymer length is close to that of the membrane.
In addition to this region, if we consider the case in which \( L < 2R \), a helical polymer configuration is energetically favorable compared with a circular configuration with windings in the horizontal or vertical direction. For a case which the polymer winds in the horizontal direction, as \( L \) increases, the energy of the helical polymer decreases while the energy of the polymer in the circular configuration does not change (see Fig. 15 a). On the contrary, increasing membrane radius leads to zero \( \Delta E \) (see Fig. 15 b). Changing the polymer length results in a more complicated relationship. When the polymer length is close to the membrane length, with polymer length increase, \( \Delta E \) increases since the energy of the helical case is approximately constant while increasing the length of polymer causes energy to increase for a polymer with horizontal windings. For long polymers, the ratio of membrane to polymer length is nearly zero. Consequently, \( \Delta E \) decreases until the helical polymer matches with the horizontal windings case (see Fig. 15 c). With vertical wrapping, as \( L \) increases \( \Delta E \) decreases. For a region around \( L=0 \), a significant difference can be observed due to the large number of windings (see Fig. 15 d). If one extends radius size, \( \Delta E \) decreases to a constant value (the energy of the circular cap shape) because of quadratic energy reduction for the helical polymer with fixed energy for the circular windings (see Fig. 15 e). Moreover, as the polymer length increases, \( \Delta E \) decreases due to a significant number of windings for the circular cap shape. A short region may be seen in which

Figure 14. a) Energy difference between helical and circular cap configurations as a function of membrane length with fixed radius and polymer length. The red circle shows the small range of \( L \) over which the helical polymer provides lower energy than the circular one. The stepwise behavior is due to employing a floor function for the circular case. b) Energy difference as a function of membrane radius with fixed membrane and polymer lengths. As \( R \) increases, the difference vanishes.
ΔE increases for the same reason as in the case of a polymer with horizontal wrapping (see Fig. 15 f).

**Figure 15.** a,d) Energy difference as a function of membrane length with fixed radius and polymer length. b,e) Energy difference as a function of membrane radius with fixed membrane and polymer lengths. c,f) Energy difference as a function of polymer length with fixed radius and length membrane. The red circle shows the region of polymer lengths in which that energy difference behaves differently.
3.2. Polymer behavior at equilibrium

We systematically change the persistence length of an unconfined (free) polymer and run simulations for $10^8$ Monte Carlo steps in order to allow the system to reach equilibrium. Starting with straight and random configurations yields fluctuations around a similar value of various quantities.

The following figures (Fig. 16 and 17) indicate that as persistence length increases, the polymer tends to reach relatively straight configurations more rapidly, starting from a random configuration. The number of accepted moves also decreases. Consequently, polymers with greater persistence lengths, which show less flexibility in their behaviors, require more MCS (i.e., Monte Carlo Steps) to equilibrate.

Figure 16. Polymers with random initial configurations (M=100) are shown in black and green. Polymers with straight initial configurations are shown in blue and red. a,b) Energy, end-to-end distance, and radius of gyration plots of two polymers with $\lambda = 1000$. Average number of accepted moves is 22%.
Figure 17. Polymers with random initial configurations (M=100) are shown in black and green. Polymers with straight initial configurations are shown in blue and red. a,b) Energy, end-to-end distance, and radius of gyration plots of two polymers with $\lambda = 20$. Average number of accepted moves is 73%. c,d) Energy, end-to-end distance, and radius of gyration plots of two polymers with $\lambda = 1$. Average number of accepted moves is 87%. e,f) Energy, end-to-end distance, and radius of gyration plots of two polymers with $\lambda = 0.5$. Average number of accepted moves is 88%.
We perform similar simulations using a larger polymer with 300 particles and also extend the simulations up to $2 \times 10^8$ to compare the effects of different persistence lengths. Figure 18 shows that as persistence length increases, the mean energy associated with the polymer configuration slightly increases. Increasing the total length (number of monomers) of the polymer is of interest. The mean energy increases in proportion to the number of particles (see Fig. 19).

**Figure 18.** Energy (units of $k_BT$) of free polymers with different persistence lengths: $\lambda = 10$ (dark green), 50 (red), 200 (turquoise), 2000 (purple). Mean energies for above persistence lengths are 297.8, 299, 307.6, 452.5, respectively.

**Figure 19.** Energy (units of $k_BT$) of free polymers ($\lambda = 15$) with different total lengths: $M = 10$ (blue), 100 (dark green), 200 (red), 300 (turquoise), 400 (purple), 500 (yellow).
We calculate correlation functions for energy data associated with polymers with $\lambda = 1$ and 20. The relaxation time differs from trajectory to trajectory for the energy correlation function (see Fig. 20).

Confined polymers provide useful comparisons for understanding effects of flexible membranes and estimating equilibrium time for individual polymers. We study the behavior of a polymer confined in a hard tube. Its behavior depends on the initial configuration, the persistence length $\lambda$ of polymer, and the radius of the tube. We consider three different initial configurations: linear, helical, and jagged (see Fig. 21) With jagged initial configuration, we observe sudden energy level jumps that are likely associated with the removal of a loop in the polymer shape (see Fig. 22). In contrast with jagged case, helical and linear polymers appear to equilibrate during the simulation (see Fig. 23).
Figure 22. Results show energy and end-to-end distance plots for three distinct trajectories starting with the same jagged configuration ($M = 100, \lambda = 20$, tube radius = 2).

Figure 23. Results show energy and end-to-end distance plots for two individual polymers (single trajectory for each) starting with linear (top) and helical (bottom) configurations ($M = 100, \lambda = 20$, tube radius = 2).
As mentioned above, another important quantity that affects the system is the radius of tube. We systematically increase the radius of a tube that surrounds a long polymer with relatively high persistence length. Figure 24 (a) shows that as the radius decreases, more Monte Carlo steps are required to allow the system to equilibrate, since the number of rejected moves for all cases dramatically increases. For instance, if radius changes from 5 to 1, the number of rejected moves increases by a factor of ~12. If we decrease the persistence length of the polymer, the effect of confinement decays (see Fig. 24 b).

![Figure 24](image.png)

**Figure 24.** Energy (units of \(k_B T\)) of a polymer (\(M = 300\)) with (a) \(\lambda = 90\) and (b) 15. Tube radius = 1 (dark green), 1.5 (red), 2 (turquoise), 2.5 (purple), 3 (khaki), 3.5 (brown), 4 (blue), 4.5 (yellow), 5 (black).
We consider excluded volume for individual polymers, finding that the energy of the polymers with excluded volume is similar to those without excluded volume at equilibrium with a tube radius of 2. The equilibration time is slightly shorter for the case with excluded volume. However, we ignore excluded volume for polymers in the rest of simulations to save simulation run-time while still capturing key physical details.

3.3. Membrane behavior at equilibrium

We start with polymerized tubular membranes and then consider the addition of fluidity. As mentioned earlier in this thesis, there are two main quantities of interest in the study case of isolated membrane: bending rigidity and membrane tube dimensions (length and radius). We change the dimensions of the cylinder (length and radius) by increasing and decreasing the length between confining planes, the number of particles, the number of layers, and, as a consequence of previous choices, the number of triangles. Then, we systematically increase the bending rigidity from small values to large values.

3.3.1. Polymerized membrane

In this thesis, a cylinder with 20 layers and 19 particles per layer is chosen to be the starting point of the investigation. We made this choice, since increasing the number of layers or the number of particles in each layer yield the same number of surface triangles, which makes it easier to understand the compare the of changing tube size by either length or radius. Therefore, we first systematically increase and decrease the number of layers with 19 particles in each layer. Second, we add or remove particles from each layer with the same number of layers (= 20). We run simulation trajectories for each case. Figure 25 shows that increasing diameter expectedly decreases the membrane energy due to decreasing curvature on the membrane surface. It also demonstrates that increasing membrane length leads to a linear increase in the membrane energy. We performed this test for both unconfined and confined membranes, obtaining similar results.
We also increase the bending rigidity for the polymerized membrane with 1596 particles (84 layers with 19 particles in each later (see Fig. 26)). For both confined and unconfined cases, with increasing $\kappa$, the membrane energy increases, as we expected (See Fig. 27). However, the energy of the unconfined membrane is slightly higher than that of the confined membrane for small $\kappa$, since the unconfined membrane can shorten and reach a more crumpled shape than the confined membrane. On the contrary, unconfined membranes with relatively large $\kappa$ can grow longitudinally while confined membranes find a curvier shape, which results in increased membrane energy (See Fig. 28).
Figure 26. Initial configuration for a cylindrical membrane with 1596 particles (84 layers with 19 particles in each layer).

Figure 27. Mean energy (units of $k_B T$) for polymerized tubular membranes in both confined and unconfined cases.
Figure 28. Configurations of polymerized membranes with $\kappa = 0$ (upper) and 1000 (lower) in both confined (left) and unconfined (right) cases. Images shown after $2 \times 10^6$ Monte Carlo steps.
Before moving to a fluid membrane, we perform another test to check the total membrane length in the confined case. Starting from an initial configuration with equilateral triangles, we allow the membrane move in longitudinal direction in addition to lateral direction. Figure 29 shows that a polymerized membrane appears to fluctuate around a specific value.

![Figure 29. Total membrane length for a confined polymerized tubular membrane with movable confinement boundaries (κ = 40).](image)

### 3.3.2. Influence of fluidity on membrane

Here, we simulate fluid membranes and perform the same tests performed for polymerized membrane in order to study the effect of fluidity in membranes. As before, we systematically change the dimensions of the membrane. The fluid membrane energy follows a similar trend when compared with the polymerized case. However, the mean energy of fluid membranes are less than polymerized membranes due to the ability to sample a larger set of configurations because of the bond flip mechanism. For the unconfined case, fluid membranes tend to adopt a spherical shape in order to minimize curvature, thus lowering the energy state. Fluid membranes with smaller radius adopt more spherical configurations.
We now increase the bending rigidity for fluid membranes to investigate the membrane behavior as $\kappa$ changes. It is clear from Figure 31 that increasing the bending rigidity does not lead to a monotonic increase in the membrane energy. As bending rigidity increases from small values, the membrane energy increases until a local maximum in the mean membrane energy occurs. After this peak, the energy decreases with increasing bending rigidity and eventually starts to increase again. To investigate this unexpected behavior, we calculate the specific heat of these systems. As Figure 32 shows, a local maximum exists near $\kappa = 1.25$. This is indicative of a crumpling transition, which has been discussed in literature related to membrane sheets.\textsuperscript{19} This transition can be observed by a change in the membrane shape from a polymer branched membrane to a locally flat membrane (see Fig. 33). An interesting behavior in the unconfined case is that at large bending rigidities, the membrane adopts a spherical shape to minimize the energy level (see Fig. 34).
Figure 31. Energy (units of $k_B T$) of fluid membranes in both confined and unconfined cases.

Figure 32. Specific heat (C) for confined fluid membranes. A crumpling transition occurs near $k = 1.25$. 
We again incorporate Monte Carlo trial moves that allow the confined membrane tube to change its total length. Figure 35 shows that the total length shows little variation for a fluid membrane. Fluidity allows the membrane to adopt lower energy states than in the polymerized case, and the fluid membrane can stabilize itself at shorter lengths by having particles migrate to the middle, leading to less curvature. Therefore, we will ignore longitudinal fluctuations later in this thesis for fluid membrane tubes.

Figure 33. Configurations of confined fluid membranes with $\kappa = 0$ (left) and 1000 (right). Images shown after $2\times10^6$ Monte Carlo steps.

Figure 34. Configurations of an unconfined fluid membrane with $\kappa = 40$. Image shown after $2\times10^6$ Monte Carlo steps.
We also consider starting with a spherical shape for the fluid membrane and increase the sphere size by adding particles. The membrane behavior is similar to the cylindrical cases. Hence, the crumpling transition occurs around the same region of bending rigidity ($\kappa = 1.25$).

Figure 35. Total length of the membrane tube for a fluid membrane with Monte Carlo trial moves that allow a change in length ($\kappa = 40$).

Figure 36. Mean energy (units of $k_B T$) and specific heat for spherical fluid membranes. Numbers of particles are 42 (blue), 92 (dark green), 162 (red), 252 (turquoise).
3.4. Membrane nanotubes

Thus far, we independently studied the behavior of isolated polymers and isolated membranes. Here, we investigate the behavior of a system that consists of a polymer confined within a membrane tube using conditions described previously. We perform tests in which we independently change persistence length, total length of polymer, initial configuration, and membrane bending rigidity. We also consider different polymer tethering conditions to mimic the effect of the polymer originating from one side of the membrane tube.

To begin, we attempt to reproduce results obtained by Fosnaric et al.\textsuperscript{15} This serves as a check of the simulations and also allows us to study vesicles, which are another ubiquitous type of lipid bilayer in cell biology. The entire system, including the membrane with a semiflexible polymer inside, behaves in analogous manner for different parameters.

*Figure 37.* Configurations of a spherical fluid membrane with a semiflexible polymer inside under different conditions. The membrane has $N = 1442$ and $\lambda = 10$. The polymer has $M=300$, with persistence lengths of (a) 0, (b)13, and 65 (c and d). For (c) and (d) two snapshots (left and right) from different views are shown. Images shown after $5 \times 10^6$ Monte Carlo steps.
Figure 38. Configurations of a spherical fluid membrane with a semiflexible polymer inside under different conditions. The membrane has $N = 1442$ and $\lambda = 10$. The polymer has $M = 300$, with persistence lengths of (a) 260 and (b and c) 2600. Excluded volume is considered for the polymer in case c. For cases: a, b, and c two snapshots (left and right) from different views are shown. Images shown after $5 \times 10^6$ Monte Carlo steps.
We find that our results are consistent with those reported by Fosnaric et al. Here, we return to the membrane tube case. We model the system as a membrane with a confined semiflexible polymer. The polymer is tethered to at one membrane end with physical parameters that mimic a realistic biological system. The results obtained for a confined polymer in fluid and polymerized membrane tube, along with the previous results for confined polymer in hard tube, indicate that membrane flexibility allows the polymer equilibrate more quickly (see Fig. 39).

![Energy vs. MCS](image)

**Figure 39.** Energy (units of $k_B T$) for semiflexible polymers ($M = 300, l_p = 15$, helical initial configuration) confined in a hard tube with radius $= 3$ (black), a fluid membrane tube (green), and a polymerized membrane tube (purple).

In the model of the membrane tube in the biological regime for cell parameters, the polymer apparently has minimal effect on membrane energy (see Fig. 40). We then vary the total length of the polymer in order to investigate the effect of polymer length on the system. Figure 41 shows that the polymer length has negligible effect on membrane energy and that polymers with different sizes tend to follow a similar trend with isolated polymers with no surrounding membrane. Increasing the persistence length slightly increases the membrane energy for polymerized membrane (see Fig. 42), although the membrane behavior exhibits small changes with changing persistence length.
Figure 40. Energy (units of $k_B T$) of polymerized membrane tubes (left) and fluid membrane tubes (right) ($N = 1596, \kappa = 40$) with a semiflexible polymer inside ($M = 300, l_p = 15$, helical initial configuration). Blue and green colors represent a confined membrane tube with and without polymer, respectively.

Figure 41. Mean energy (units of $k_B T$) of polymerized membrane tubes (left) and fluid membrane tubes (right) ($N = 1596, \kappa = 40$) as a function of polymer length ($l_p = 15$, helical initial configuration).

Figure 42. Energy (units of $k_B T$) of polymerized membrane tubes (left) and fluid membrane tubes (right) ($N = 1596, \kappa = 40$) with a helically initiated polymer inside. The persistence lengths of the polymers are 15 (blue) and 90 (green).
We also perform simulations of membrane tube model under different initial and boundary conditions: fixing two sides of the polymer at the membrane ends, allowing the polymer to move freely inside of the membrane, confining the polymer at one side but allowing it to leave the membrane from the other side (i.e., open ended tube), starting with circularly wrapped configuration for polymer, and allowing the membrane to move in longitudinal direction in addition to lateral movement. In all these cases, the polymer appears to have a small effect on the membrane behavior. It might be of interest to mention that a circular initial configuration for polymer allows the polymer equilibrate more quickly in comparison with a helical initial configuration. However the membrane behavior remains almost unchanged.
Chapter 4: Summary and conclusions

In this thesis, we investigated physical systems motivated by membrane nanotubes. At length scales of interest, the properties of actin bundles and cell membranes can be well-characterized as semiflexible polymers and thin elastic sheets, respectively. We used this continuum perspective as a theoretical basis along with a combination of analytical theory and computer simulations to investigate a number of physical scenarios that give insight into equilibrium properties of membrane nanotubes.

We started our investigation by using analytical calculations to consider two different polymer configurations confined within an elastic tube: helically wrapped and circularly wrapped polymers. The helical configuration was considered because semiflexible biopolymers are often assumed to adopt a helically wrapped configuration in confinement. Our calculations indicate that over a wide parameter range, the circularly wrapped polymer configuration is energetically favorable when confined within an elastic membrane tube. We then employed Monte Carlo simulations to explore equilibrium configurations of various polymer systems in confined geometries. As expected, initial conditions of the polymer can dramatically influence the equilibration time for the system, with an unphysical “jagged” configuration leading to loops in the polymer that are difficult to remove when the polymer is confined.

We then introduced a simulation model for elastic membranes in which the membrane is represented as a triangulated surface. Two types of membranes were considered: polymerized and fluid membranes. To gain insight into equilibrium properties, we began by studying the membrane in isolation. Varying the dimensions, we observed that boundary conditions placed on the polymerized membrane had little effect on the mean energy as a function of the surface area (number of triangles in the triangulation). However, when considering a fluid membrane, unconfined tubes yielded much smaller energies than confined tubes. We also considered effects of the bending rigidity, and at small values, we observed a peak in the specific heat of the membrane, as calculated by equilibrium energy fluctuations. Characterizing the membrane shapes sampled in simulations indicated that this was associated with a transition from
crumpled membrane to one that is locally smooth. This transition had been observed in flat and spherical membranes, but to our knowledge, this is the first observation in tubular shapes.

Our final physical model for a membrane nanotube was one in which we confined a semiflexible polymer, representing a bundle of actin, within a fluctuating elastic tube. As a test of our simulation methods, we compared results for semiflexible polymers within spherical vesicles to recently published results. As expected, polymers with large persistence length dramatically deformed the vesicle into a flat, disc-like shape. In the model of membrane nanotubes, we found that the presence of a polymer within the membrane had a small effect on properties of the membrane, using parameters motivated by biological systems. It is interesting to note that fluctuating tubes allow enclosed polymers to equilibrate quickly when compared with polymers confined within rigid tubes.

Membrane nanotubes provide an interesting system from the perspective of studying membrane-cytoskeleton interactions. The modeling in this thesis provides a first step toward understanding their behavior. Future studies will likely fall along two lines. In the first, it will be interesting to investigate the effect of enclosed polymers on the properties of membranes near the transition from flat to crumpled behavior. In the second study, it will be interesting to investigate the processes by which membrane nanotubes initially form. This will necessitate the modeling of cell-cell interactions through receptor-binding. These receptor binding events will then drive deformation of the cell membrane, which can then facilitate the extension of membrane tubes. This will likely involve cooperative effects between receptor clustering, membrane deformation, and actin polymerization. Such modeling is also likely to provide insight into other biological systems, including T cells, B cells, and NK cells.
List of References


Vita

Mr. Sina Mirzaeifard was born in Isfahan, Iran but immediately made his way to Tehran, the capital. He studied at Soroosh high school, a high school for talented students. After graduation, he got admitted to Iran University of Science and Technology (ranked among top five outstanding technical universities in Iran). He obtained his Bachelors in Chemical Engineering in September 2010. Later, he pursued his success by working in Oil and Gas industries for two years. Thereafter, he joined Dr. Abel’s group at the University of Tennessee, Knoxville where he got valuable knowledge on analytical theory and computational simulation of membrane nanotubes in cell biology. Sina graduated with Masters of Science in Chemical Engineering from the University of Tennessee, Knoxville in December 2014.