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Peer reviewed|Thesis/dissertation

UNIVERSITY OF CALIFORNIA
RIVERSIDE

Multiscale Modeling of Cell Migration in Biological Systems

A Dissertation submitted in partial satisfaction
of the requirements for the degree of

Doctor of Philosophy

in

Mathematics

by

Austin Philip Hansen

March 2025

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To my family, friends, and mentors who taught me how to find acceptance even in the midst of the darkest moments; my heart is with each of you.

ABSTRACT OF THE DISSERTATION

Multiscale Modeling of Cell Migration in Biological Systems

by

Austin Philip Hansen

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Dr. Mark Alber, Co-Chairperson

Dr. Heyrim Cho, Co-Chairperson

In many different biological systems, the migration and distribution of specific cells, such as neural stem cells within the brain, has a large influence on the dynamics of the whole system. Furthermore, biological experiments are often costly and time consuming to perform and are unable to test certain hypotheses. For this reason, mathematical modeling is an invaluable tool which is able to make predictions as well as isolate specific variables in order to test and confirm a given biological hypothesis. In this thesis, we examine cell migration in three different biological systems. In each system, we develop a novel model based on underlying mechanistic assumptions and then use this model to test and investigate previous biological hypotheses. The modeling approaches in this thesis range from micro to macroscale models.

In the first section, a computational model of *Pseudomonas aeruginosa* migration is introduced and used to explore the impacts of bacterial reversals and wrap mode on efficiency of motion both with and without chemotaxis in different environments. Model simulations demonstrate that wrap mode can increase diffusion of *P. Aeruginosa* in a non-chemotactic environment. Wrap mode is shown to not only increase the chemotactic efficiency but also to reduce the metabolic expenditure of the bacteria that is required for migration. The experimentally observed frequency of wrap mode was used to demonstrate the existence of an optimal trade off between chemotactic efficiency and metabolic cost. It was also shown that the wrap mode can allow the bacteria to move along the Fungi and increases exploration of multimodal chemotactic profiles. Namely, respondents of *P. aeruginosa* to the chemotactic signals produced by a hypothetical fungal network were studied in a liquid environment. For cells undergoing a run-reverse pattern, it is shown that the bacteria are

likely to remain in the first local chemoattractant maximal production site on a fungi that they find. With wrap mode, however, the bacteria are more easily able to escape these local maximums to further explore their neighboring environment along the hyphae. Finally, we find that the dispersal of bacteria moving along a Fungi on agar is decreased when the network density of the fungi increases.

In the second section, we build an agent-based model of the migration of therapeutic neural stem cells in rodent brain. In the naïve mouse brain, the developed model based on generalized q-sampling imaging is shown to better account for the tissue microstructure over models based on diffusion tensor imaging. In calibrating to recent experimental data, we find that the model is able to fit the quantitative statistics of in-vivo experiments, however struggles to match the levels of distribution to the olfactory bulb seen in the experimental data. In addition, we show that both the quantitative and qualitative statistics are sensitive to the injection location. This highlights the need for additional experimental data. If distribution to the olfactory bulb is a persistent phenomenon, it suggests that future models of therapeutic neural stem cells in the naïve brain may need to include other forces such as chemotaxis or blood flow. If it is not a persistent phenomenon, it suggests that the experimental results may be a product of the injection location.

In the final section, we build a multiphase model of blood clot deformation under fluid flow. We explain some of the recent experimental literature that demonstrates the importance of red blood cells on the clot stability and then create a multiphase model which includes red blood cells, fibrin network, less and highly activated platelets, and platelet-rich plasma. The model couples together both macroscopic and microscopic forces and accounts for pairwise surface tensions between all phases.

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Chapter 1

Modeling Study of the Role of Wrap Mode and Reversals in *Pseudomonas aeruginosa* Motion in Different Environments

The basic model for gliding bacterial motion with reversals was originally developed in [7]. It was first extended and implemented in C++ to bacterial flagellated motion with reversals by Dr. Alireza Ramezani as described in his Ph.D. Thesis [83]. Simulation designs similar to 6.2.2 and 6.3 in [83] serve as a starting point for the study of the effect of wrap mode on *Pseudomonas aeruginosa* in liquid presented in this Thesis. However, the calibration to *P. aeruginosa* as well as the simulation designs including the motion of bacteria in the absence of chemotaxis and in response to a simulated chemoattractant profile from a fungal network, represent my own contributions and lead to novel conclusions. Furthermore, the model was extended in Section 1.9 in order to simulate the migration of *P. aeruginosa* along fungi on agar. The initial idea for this was generated in collaboration with Alireza Ramezani. However, the calibration, the liquid smoothing algorithm, the bacterial alignment algorithm as well as the model for bacterial growth and division was created and implemented by myself.

1.1 Introduction

Bacterial motility is a widely studied field of microbiology that intersects with many other disciplines such as bioremediation, pathogenesis, and agriculture. In many of these fields, both the long term bacterial population distributions as well as the individual bacterial trajectories provide crucial information about the dynamics of the colony and its subsequent interactions with the surrounding environment [15, 42, 86, 99, 115].

For example, a recent study showed that of 23 different strains of *Pseudomonas*, several strains promoted root colonization by mycorrhizal fungi of 3 different *Populus* species [61]. However, for bacteria to be present in adequate quantities, chemotaxis likely plays an important role in promoting the bacterial colonization of the fungal network [5, 30] and the bacteria’s ability to disperse along hyphae and transfer beneficial metabolites to the hyphae tips [1].

Although there is a wide range of motility strategies across different bacteria, flagellated motion is an important subcategory and is crucial to the migration of *Pseudomonas* in particular. For instance, it has been shown that both *Pseudomonas aeruginosa* and *Pseudomonas putida* use their flagella in three distinct modes of migration, namely push, pull, and wrap mode. In push and pull modes, a bacterium swims approximately in a straight line either pushed or pulled along by its flagellum. However, in wrap mode, the bacterium slows down and its cell body exhibits a prolonged change in orientation. Wrap mode is hypothesized to increase the chemotactic efficiency and existing model simulations seem to support this [4, 97]. However, it is not known under what conditions wrap mode offers the most benefit, and whether there are any trade-offs between the increased exploration that wrap mode is hypothesized to provide, and the overall ability to aggregate towards biologically realistic chemoattractant profiles like those produced by fungi.

To answer some of these questions we extend the Subcellular Element coarse grained particle approach developed in previous models of *P. aeruginosa* migration [9, 35, 36] to study the effects of wrap mode and reversals on bacterial motion in a fluid environment both with and without chemotaxis. In model simulations of bacterial chemotaxis we considered a simple point source with either linear or exponential gradients as well as a chemoattractant profile produced by a simulated fungal network. Without chemotaxis, our simulations suggest that wrap mode increases the diffusion of *P. aeruginosa*. However, this increase depends on the angular change in the direction of bacterial motion during wrap mode. With chemotaxis, we find that wrap mode enhances the ability of *P.*

aeruginosa to find the source, for a certain range of chemoattractant gradients, but hinders the ability for bacteria to remain close to this source. We show that wrap mode allows bacteria to move away from local maximums of the chemoattractant in order to explore more of the surrounding environment over time. We hypothesize that this may allow for an increased dispersion along hyphae within liquid environments. In addition, we note that any increase in chemotactic efficiency comes at a potentially lower metabolic cost for the bacteria suggesting another possible benefit of wrap mode. In the final sections, we extend the model to simulate bacterial migration along fungi on agar, as well as to include growth and division of the bacteria. We show that with increasing network densities the dispersal of the bacteria is decreased. Finally, we propose future directions and research questions for the presented model.

1.2 Biological and Modeling Background

Flagellated motion is a common form of bacterial migration that can lead to different swimming patterns, depending on the number of flagella and their location along the cell body. The motion of each flagellum is controlled by a motor which is responsible for changing the rotation of the flagellum from clockwise to counterclockwise. For example, in *Escherichia coli*, the flagella are positioned all along the cell body. When the flagella rotate counterclockwise, they bundle together behind the bacterium, propelling the cell body forward. However, when some of the flagella change directions and begin rotating clockwise, they spread out causing the bacterium to come to a stand still and reorient its cell body. This strategy of motility is called run-and-tumble [99]. In contrast, some bacteria, such as *Vibrio alginolyticus*, have a single flagellum located on one pole of its cell body and swims with a run-reverse-flick strategy. In this strategy, after each cycle of swimming forward and backward, *V. alginolyticus* undergoes a reorientation of around 90 degrees in a mode named flick before swimming forward again [99].

Within a number of polarly flagellated bacteria, a swimming mode called wrap mode has been discovered [52, 54, 57, 60, 73, 97]. In this mode, the flagellar filament wraps around the body of the bacterium and then unwraps when the flagellum switches its direction of rotation in a reversal. The most ubiquitous characteristics of wrap mode are a change in the swimming speed and angular velocity; however, the function and purpose is still debated and seems to vary heavily from bacteria to bacteria. For bipolar flagellated bacteria, wrap mode seems to be important for both efficient motion

and reorientation of the cell body. During runs, the front flagellum wraps around the cell body while the rear flagellum is extended behind, allowing for greater swimming speeds. In contrast, having both flagella wrap around the cell body seems to aid in reorientation of the cell body [28, 29, 73]. In *Shewanella putrefaciens* and *Burkholderia sp. RPE64*, some studies have observed a very different function. Here, wrap mode seems critically linked to a bacterium’s ability to escape from crevices in structured environments by allowing the bacterium to reverse and free itself in a method named screw thread motility [57, 60, 96]. For monopolar bacteria, such as *P. putida* and *P. aeruginosa*, wrap mode slows the bacteria, resulting in two distinct movement speeds and is suggested to improve aggregation/diffusion [4, 52, 97].

Several of these attempts to understand whether wrap mode serves a specific biological function have utilized computational modeling including models of the flagellum [56, 67, 78], as well as agent based and active particle models which predict long term bacterial dynamics both with and without chemotaxis [4, 54, 97]. Despite these studies, it is not fully understood whether wrap modes’ benefit is largely due to increased time before reversal events or increased angular exploration. Moreover, how wrap mode affects bacterial migration in multimodal chemotactic environments has largely been unstudied but is critical in understanding how bacteria may respond and aggregate around fungal networks or other more biologically relevant distributions.

In the following section, we investigate the role wrap mode plays in the migration of *P. aeruginosa*. The first part of the results section examines the effect of wrap mode in non chemotactic environments. Our simulation results show that wrap mode increases the diffusion coefficient of the bacteria, but that this increase is heavily dependent on the angular change during wrap mode. We then study the impact of wrap mode on the bacteria’s ability to locate and aggregate around a source of chemoattractant. The simulation comparisons confirm that wrap mode helps bacteria find the source by increasing the turn angle, allowing for more exploration of the local environment as well as quicker reorientation. The final sections involves modeling interactions between the bacterium *P. aeruginosa* with a hypothetical fungal network. Specifically, section 1.6 explores how bacteria respond to and aggregate around a fungal network within a liquid environment. Here the bacteria are able to freely swim in all directions and respond to the chemical profile generated by the fungal network. The model shows that wrap mode increases the potential of the bacteria to escape from local chemical maximums and explore the surrounding environment. In section 1.9 we model the dispersal, growth and division of bacteria along a hypothetical fungal network grown on agar. We show that the

topology of the network, such as the network density and ability to cross the hyphae, have a large impact on the overall dispersal.

1.3 Model Assumptions/Calibration

We developed a subcellular element model where each bacterium is described by five nodes connected by linear and rotational springs. The velocity of each cell is then described by an overdamped langevin equation. A description of all model parameters and values are provided in Table 1.1. The likelihood that bacteria enter wrap mode before reversing their direction of motion is governed

Table 1.1: Model Parameters

Parameter	Value	Units	Description
$L_{bacteria}$	2.0	μm	Length of bacteria
N_{nodes}	5.0	-	Number of nodes in each bacteria
l_{eq}	0.4	μm	Equilibrium length of linear spring
k_b	5.0	$\mu N \cdot \mu m^{-1}$	stiffness of linear spring
k_{bend}	0.5	$\mu N \cdot \mu m \cdot rad^{-1}$	stiffness of torsional spring
θ_{eq}	π	rad	Equilibrium angle of torsional spring
$f_{motor,run}$	.3795	μN	Motor force on each node in run mode
$f_{motor,wrap}$	.1445	μN	Motor force on each node in wrap mode
μ_{run}	.7144	s	Lognormal-Truncated Mean for run mode duration
$\sigma_{t,run}$	.7440	s	Lognormal-Truncated SD for run mode duration
$[a_1, b_1]$	[0, 9.6]	s	Range of durations for run mode
μ_{wrap}	-.3662	s	Lognormal-Truncated Mean for wrap mode duration
$\sigma_{t,wrap}$	.91784	s	Lognormal-Truncated SD for wrap mode duration
$[a_2, b_2]$	[0, 4]	s	Range of durations for wrap mode
t_{turn}	.2	s	Duration of reversal events
γ	.01	$\mu N \cdot s \cdot \mu m^{-1}$	Damping coefficient
t_{ζ}	.1	s	time between updates to chemotactic model
$L_x \times L_y$	*	-	domain size
dt	0.00001	s	Simulation time step
N_b	*	-	Number of Bacteria
T_{total}	*	s	Total simulation time
R_0	*	μm	Initial distance from the source
ζ	*	-	Chemotaxis sensitivity
P_{wrap}	*	-	Probability of Wrap Mode
Reaction Diffusion Parameters			
D	200	$\frac{\mu m^2}{a.u \ of \ time}$	Diffusion coefficient
p	1000	$a.u^{-1}$	Production rate of attractant
dt_{chem}	.05	$a.u \ of \ time$	Chemical model's time step
$\triangle x \times \triangle y$	5×5	μm^2	Chemical Grid Step Size
t_{chem}	10000	$a.u \ of \ time$	Total duration of reaction diffusion model

* Parameters varied in simulation runs

in the model by the wrapping probability P_{wrap} . Assuming the wrapping probability is zero, the bacteria will follow a run-reverse pattern. However, for nonzero wrapping probability, the bacteria will follow a run-wrap-reverse swimming pattern. Wrap mode is characterized in the model by a lower average velocity and higher angular velocity when compared to run mode. Both run and wrap durations in the absence of a chemoattractant gradient are calibrated using the experimental data for *P. aeruginosa* presented in [97]. The detailed description of calibration for run and wrap mode can be found in appendix A and further details on how each of these are modeled is described in the Methods section. Furthermore, when a chemical gradient is present and the bacteria are moving down the gradient, the durations are reduced, which leads to quicker reversals. The details of the chemotactic submodel are described in the section titled Submodel of Chemotactic motion. For simulations in sections 1.5 and 1.6, a point source of an arbitrary chemoattractant is located at the center of the domain. We use either a linear or exponential gradient and the details of both of these calculations can be found in A.2. For simulations in section ??, the chemoattractant profile is taken from a model of fungal growth.

1.4 Bacterial Diffusion Within Liquid

1.4.1 Wrap Mode Increases Diffusion Coefficient in Non-Chemotactic Environment

Previous studies have focused on the effect of wrap mode in a non chemotactic environment and found that wrap mode increases the diffusion of *P. putida* [86]. In addition, for bacteria following a simple run reverse pattern, it was shown that there was an optimal level of the angular noise during run mode which led to the highest diffusion coefficient [99]. From these two studies, two competing hypotheses emerge for why wrap mode may be beneficial without chemotaxis. First, wrap mode's primary function is to extend the time between reversals, thereby increasing diffusion. Second, the angular change during wrap mode coincides with an optimal level of the angular noise which yields an increased diffusion.

For *P. aeruginosa*, we show below that the angular change in direction of a bacterium's motion during wrap mode is more important than the increased time between reversals and is likely to be more of the reason for an increased diffusion. Furthermore, we show that the optimal angular

change during wrap mode is 180° , and while the experimentally observed average angle change of 90° produces less benefit, it still has a significant benefit. Within the following, simulations all durations, velocities, and reversal turn angles are chosen from the experimentally measured distributions.

1.4.2 Angular changes of 180° during wrap mode lead to the largest increase in the diffusion coefficient

In this set of simulations, we control the total angular change that occurs during wrap mode. In order to achieve this, we sample the wrap duration from the calibrated experimental distribution [97] and then select the direction of the force, F_{motor}^0 , applied to the frontier node that is required to achieve the desired total angular change during wrap mode. Six different simulations were run to compare no wrap mode vs. wrap mode with angular changes of $\{15^\circ, 45^\circ, 90^\circ, 135^\circ, 180^\circ\}$. The probability of wrap mode was set to 100%. 100 cells are initialized in the center of a 4000×4000 micron domain and their motion is simulated for 200 seconds. Figure 1.1 shows the results of the simulations. For

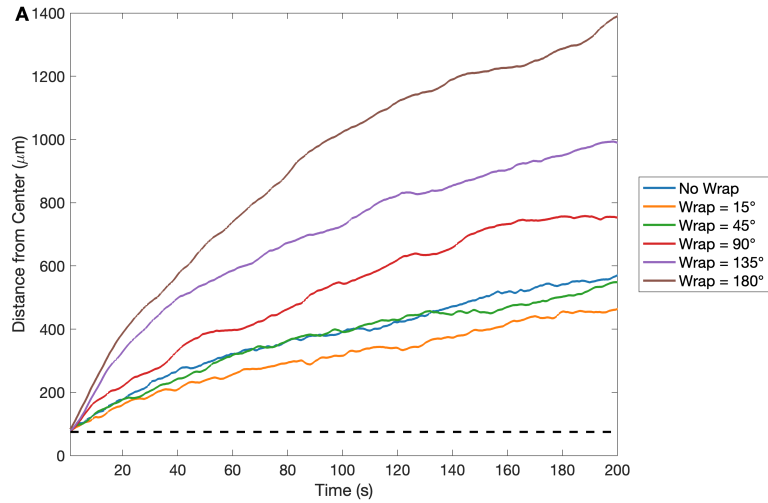


Figure 1.1: Plot showing average distance from the center of the domain over 200s of simulated migration for varying wrap angles. Here we see that for larger wrap angles we have a dramatic increase in the average distance.

smaller angles, such as 15° and 45° , there is actually a minor decrease in the average distance from the center at the end of the 200 seconds when compared to no wrap. However, for angle changes of 90° , 135° , and 180° , we see that there is a large increase in the average distance. We calculated the diffusion coefficient for each simulation using the mean squared displacement and found that the

diffusion coefficients at 200 seconds for each of the 6 simulations are 491.59, 326.19, 454.9, 902.89, 1495.53, and $2755.76 \frac{\mu m}{s}$ respectively. This demonstrates that an increase in the angular change leads to increased diffusion during wrap mode. Moreover, the bacteria with a 180° angular change during wrap mode had the largest diffusion coefficient overall, which represented around a 5.6-fold increase over the bacteria with no wrap mode present.

To better understand why 180° produces the largest increase in diffusion, we show a typical trajectory from this simulation in Figure 1.2. We can see that a complete 180° turn right before a reversal leads the bacterium to undergo multiple runs in the same direction. Thus, we conclude that this ability to have multiple runs in the same direction may be a crucial function of wrap mode and the primary driver of the increased diffusion.

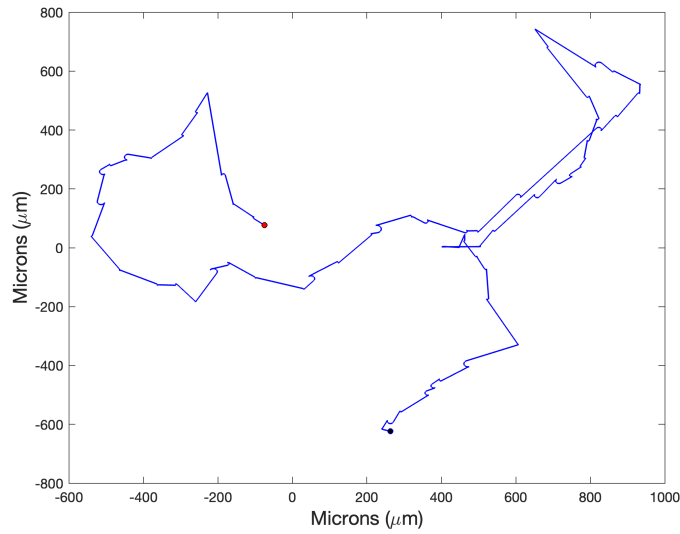


Figure 1.2: Plot of a single trajectory of a cell with 180° angular changes in wrap mode across the 200 simulated seconds. The red dot marks the start and the blue dot shows the ending location.

1.4.3 Increasing the probability of wrap mode increases the diffusion coefficient

Next we test to see how the diffusion coefficient changes with the probability of wrap mode. Since the experimental mean angular change during wrap mode is 90° , we control all angular changes to be 90° and vary the probability of wrap mode from 0% to 100%. In Figure 1.3 we plot the average distances from the center for 6 different probabilities. The calculated diffusion coefficients for wrap

probabilities $\{0\%, 20\%, 40\%, 60\%, 80\%, 100\%\}$ is 451.73, 449.02, 589.80, 566.18, 613.3, 855.44 $\frac{\mu m}{s}$ respectively. In general, we see that the larger the probability of wrap mode, the larger the diffusion coefficient. Moreover the diffusion coefficient is nearly twice as large for 100% wrap mode versus no wrap mode at all.

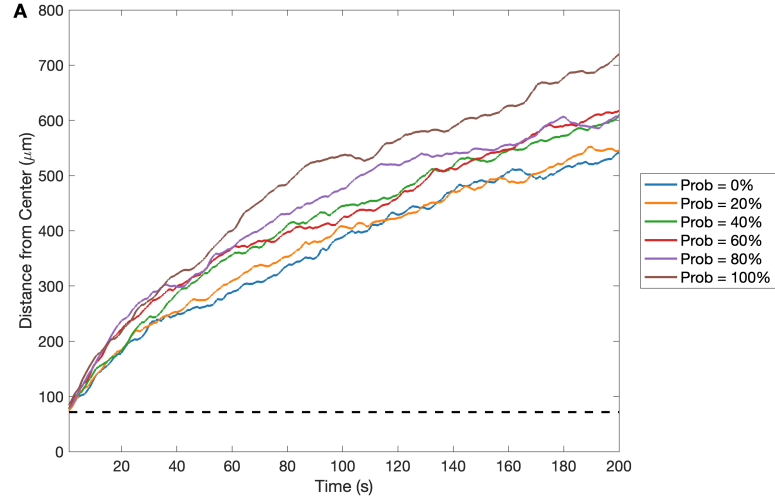


Figure 1.3: Average distance from center for 200 seconds of simulated migration for varying wrap probabilities. The angular changes during wrap are 90 degrees. We see that for higher wrap probabilities we have a greater increase in the average distance.

1.5 Chemotactic Model Parameter Exploration

This section explores the relationship between the chemosensitivity parameter, the gradient, and bacterial aggregation. We show that the equilibrium solution for bacteria in a linear gradient is dependent on the strength of the gradient while varying exponential gradients produce the same equilibrium solution given enough time.

1.5.1 Increase in chemosensitivity results in greater chemotactic efficiency.

We first examine bacterial migration using a run-reverse pattern with different bacterial sensitivities ζ to the chemoattractant. These simulations show that aggregation towards a point source increases with the sensitivity, ζ . We use 3 metrics for quantifying the chemotactic efficiency of the bacteria;

the average distance to the source $\bar{D}$, drift velocity defined as

$$v_d = \frac{\text{Starting Distance} - \text{Distance To Source}}{\Delta t} \quad (1.1)$$

and aggregation time τ_{ag} , which is defined to be the average time a bacteria spends within a given radius of the source. The radius here was chosen to be 50 microns. Figure 1.4. shows the simulation results for a fixed chemoattractant profile across sensitivity levels $\zeta = 1, 2, 5, 10, 20, 50$. The chemoattractant profile was generated using a reaction diffusion equation with the parameters listed in table 1.1. Each simulation was run with 200 cells, initialized in a circle around a point source. The total domain size was $1000\mu m \times 1000\mu m$.

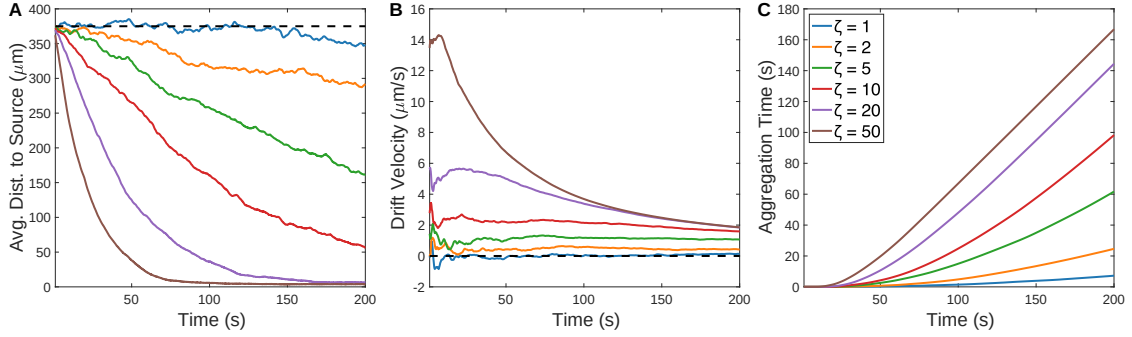


Figure 1.4: Simulations showing bacterial migration with a run-reverse pattern in the presence of a point source and varying chemo-sensitivities A) average distance to the source B) drift velocity C) aggregation time as a function of time.

Fig. 1.4A shows that as the sensitivity parameter ζ increases, the average distance to the source $\bar{D}$ decreases. This demonstrates that as the bacteria become more responsive to the chemoattractant gradient and modulate their reversal frequencies more aggressively, they are able to find the source faster. We see that when $\zeta = 1$, the overall decrease in average distance is very small, however for the largest sensitivity tested, $\zeta = 50$, the average distance decreases to below $50\mu m$ by around 45 seconds in the simulation. In Figure 1.4B we see that the drift velocity increases as the sensitivity increases. For $\zeta = 1$ we have a max drift velocity of $.96 \frac{\mu m}{s}$, however for $\zeta = 50$ there is a maximum drift velocity of $14.29 \frac{\mu m}{s}$. Since drift velocity measures how much migration towards the source has occurred relative to simulated time, we see that for larger sensitivities there is a spike at the beginning of the simulation and a gradual decrease as the simulation goes on. This is due to the fact that the bacteria travel as close to the source as possible very early on in the simulation and

then remain there for the rest of the simulated time. This is further reflected in the metric τ_{ag} . Here we see that for $\zeta = 1$, the bacteria spend on average 7 seconds in the center $50\mu m$ of the domain. However, for $\zeta = 50$ this increases all the way to an average of 166.98 seconds. This shows with larger sensitivities, the bacteria are not only able to find the source quicker, they remain here throughout the rest of the simulated time.

1.5.2 Chemoattractant gradient determines the average distance to the source at equilibrium

Next, we examine how varying the chemoattractant gradient between a linear and exponential profile impacts the chemotactic efficiency of bacteria with a fixed chemoattractant sensitivity. Simulations are run on the same domain size and initial conditions as section 1.5.1, except the total simulation time is 1600 seconds. The chemosensitivity is set to 1 and 200 cells are simulated for each simulation.

Linear Gradient

We turn our attention first to the simulated results of bacterial migration in the presence of a linear gradient. In Figure 1.5 we can see that as we increase the slope of the gradient we have a decrease in the average distance to the center $\bar{D}$, and an increase in drift velocity v_d and aggregation time τ_{ag} . We note however, that varying the strength of the gradient seems to yield a different equilibrium

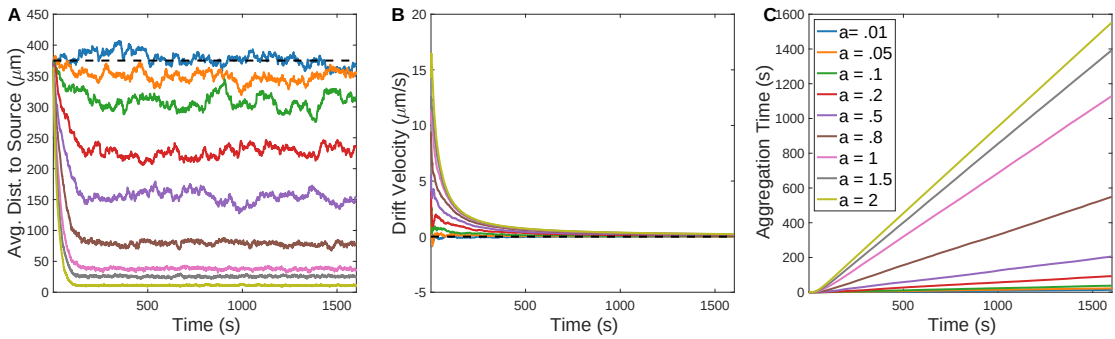


Figure 1.5: Simulations showing bacterial migration with a run-reverse pattern in the presence of a linear gradient for various slopes. A) shows the average distance to the source, B) shows the drift velocity, and C) shows the aggregation time.

solution. For example, given the largest gradient ($a = 2$), we see that $\bar{D}$ decreases rapidly until around 95s. The bacteria then roughly remains around $10\mu m$ for the rest of the simulation. This property can be seen across all of the different gradients. For example, for $a = .5$ we see that that

after the initial descent, the bacteria mostly stay around $150\mu m$. This behavior suggests that the value of the equilibrium solution is directly related to the strength of the gradient. Furthermore, we note that the variance in the fluctuations around each equilibrium solution is inversely related to the strength of the gradient, with the fluctuations being reduced as the gradient increases. Finally we see that if the gradient is too small, ($a < .01$), the bacteria have a hard time moving towards the source and are largely indistinguishable from purely random motion.

Exponential Gradient

In Figure 1.6 we look at how *Pseudomonas aeruginosa* responds to the presence of an exponential gradient. Here we see a much different property. For low production rates ($prod = 10$, $prod = 500$) the migration is relatively random. However, as we start to increase the production rate, we see that $\bar{D}$ steadily trends downwards throughout the whole simulation. In the case of the highest tested production rates, $\bar{D}$ only levels off when it has decreased to below $50\mu m$. This is likely because near the starting location the gradients are very weak, yet near the center the gradients become increasingly large. This difference causes the cells to move near randomly in the low gradient environment until it finds a region of the domain with a sufficient enough gradient to reliably guide the cells to the source. The smaller the source term the smaller this region will be in comparison to the whole domain. However, once a cell does find the source, the cell does not leave this region since the exponential gradients here have become so large that they reverse almost immediately when moving down the gradient.

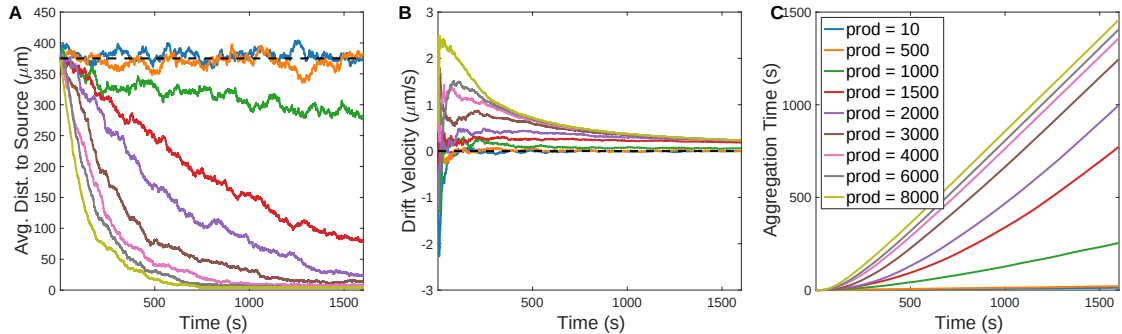


Figure 1.6: Simulations showing bacterial migration with a run-reverse pattern in the presence of an exponential gradient for various production rates. A) shows the average distance to the source, B) shows the drift velocity, and C) shows the aggregation time.

1.6 Bacterial Chemotaxis Within Liquid

1.6.1 Addition of wrap mode leads to a moderate increase in chemotactic efficiency at a lower metabolic cost compared to a run reverse strategy.

In section 1.4, we studied the impact of wrap mode on bacterial migration in a non chemotactic environment. We found that the increase in angular velocity had a large effect in increasing the diffusion coefficient. In this section, we test whether the benefits of wrap mode also apply in the presence of a chemoattractant gradient.

To do this, we simulate bacterial migration following a run-wrap-reverse pattern such that before each reversal there is a probability that a bacterium enters wrap mode. We first study the effects of wrap mode for bacteria exposed to a linear chemoattractant gradient. For all the following simulations, we set the chemotactic sensitivity to be the same in both modes $\zeta_{wrap} = \zeta_{run} = 1$ and compare the chemotactic efficiency of 0% chance of wrap mode vs. 38% chance of wrap mode. Here, 38% is the experimentally observed rate of wrap mode found in [97]. 100 bacteria are initialized in a circle centered around the source of the chemoattractant on a domain of 4000×4000 micrometers. The slope of the chemoattractant profile is set to 2 and we test three different scenarios with regards to the initial orientation of the cell body with respect to the chemoattractant gradient. This is done to help identify how much of the increased chemotactic efficiency comes from helping the bacteria reorient their direction of motion towards the source.

1.6.2 Random Orientations

We first initialize the bacteria starting with random orientations relative to the gradient. In Figure 1.7, we see that $\bar{D}$ decreases slightly faster for 38% chance when compared to 0% chance of wrap. In order to quantify this difference, we calculate the average slope for the first 80 seconds in the simulation. We find that 0% chance of wrap has a slope of $-13.39 \frac{\mu m}{s}$ while 38% chance of wrap has a slope of $-15.22 \frac{\mu m}{s}$. This sustained improvement of wrap mode leads to a maximum difference of $170.06 \frac{\mu m}{s}$ between the two average distances. This occurs at $97.6s$. We can also see that the standard deviation for 0% wrap is larger than for 38% chance of wrap. This is in part due to the fact that wrap mode slows down the bacteria and yields more lateral motion, which keeps the bacteria

more consistently moving up the gradient. Furthermore, by 140s we see that almost all of the cells for the 38% chance of wrap simulation have found the center of the source, which is depicted by the narrowing in the standard deviation. Figure 1.7C shows that τ_{ag} is greater for the 38% chance of wrap when compared to 0% chance of wrap. By the end of the simulation, the bacteria with a 38% chance of wrap mode spend on average 23.67 seconds more inside 50 micrometers of the center as opposed to the bacteria without wrap mode. Both of these things suggest that there is a moderate increase in the chemotactic efficiency for wrap mode.

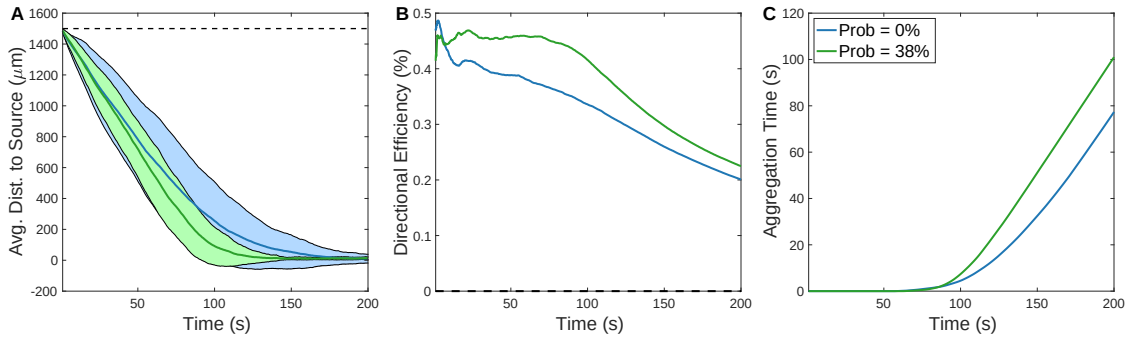


Figure 1.7: Simulations comparing bacterial migration with 0% chance of wrap and 38% chance of wrap. A) shows the average distance to the source, B) percentage of the total distance traveled directed towards the source and C) Aggregation Time.

However, in Figure 1.7B, we plot the percentage of the total distance traveled by the cell that is directed radially towards the source. We call this metric directional efficiency. We note that given two strategies with the same chemotactic efficiency, the strategy with less overall motion will have a larger directional efficiency, since the bacteria are able to achieve the same aggregation with less total motion. Thus, this metric helps to quantify any metabolic benefit afforded by the chemotactic strategy. We see that with a 38% chance of wrap, more of the bacteria's total motion is directed towards the source. The maximum difference occurs around 85s with the values being 44.25% and 35.47% for wrap vs no wrap respectively. This indicates that with wrap mode, bacteria are able to move up the gradient with nearly 10% more of their overall motion contributing to their movement towards the source. We point out that this increase in chemotactic efficiency only occurs within a limited range of chemoattractant gradients. In A.3, we show the same plots for gradient slopes of $\frac{4}{5}$, 1, 2, 4. From these results, we can see that for slopes less than 1 and greater than 4 there is not as much difference between 0% and 38% chance of wrap. This is the case because wrap mode actually increases the diffusion coefficient in low gradient environments. If the gradient is not large enough

to meaningfully impact the run and wrap durations, then wrap mode only serves to increase the diffusion coefficient of the bacteria which does not correspond to an increased chemotactic efficiency. Conversely if the gradient is very large, cells in run mode reverse very quickly when moving down the gradient. If this occurs quickly enough, then any increased angular exploration from wrap mode is able to be achieved by repeated reversal events.

In the next section, since increased angular momentum is an important part of wrap mode, we look at how the initial orientation of the bacteria affects the above results. To do this, we run the same set-up, but compare when the bacteria are all initially oriented perpendicular to the gradient versus when they are initially oriented parallel to the gradient.

1.6.3 Perpendicular Orientations

In Figure 1.8, we show the results for when bacteria are initially oriented perpendicularly. We see that when the bacteria are all facing away from the gradient, there is an increased separation between 0% and 38% chance of wrap for each of the three statistics discussed above. In particular, we see that 0% chance of wrap performs slightly worse than it did in Figure 1.7, especially in the beginning of the simulation. For example, the bacteria with 0% chance wrap have an average slope of $-12.79 \frac{\mu m}{s}$,

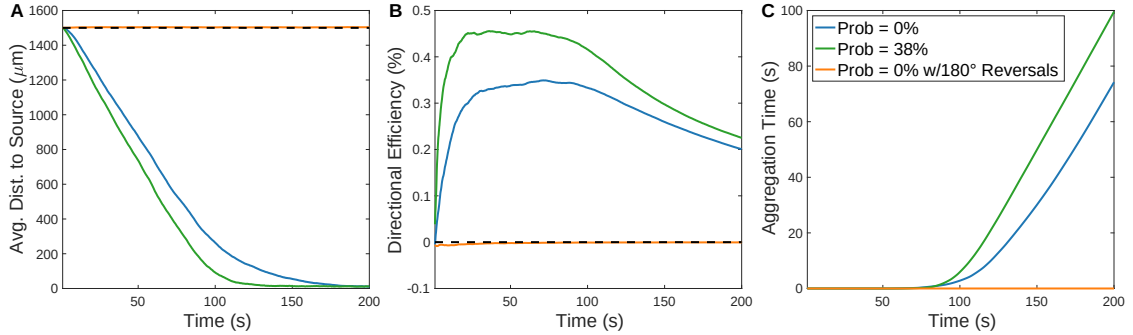


Figure 1.8: Simulations comparing bacterial migration with 0% chance of wrap and 38% chance of wrap. A) shows the average distance to the source, B) percentage of the total distance traveled directed towards the source and C) Aggregation Time.

while 38% chance of wrap have a slope of $-15.05 \frac{\mu m}{s}$ during the first 80s. This represents an increase of $.6 \frac{\mu m}{s}$ to the slope and only an increase of $.17 \frac{\mu m}{s}$ for 0% and 38% chance of wrap respectively. This difference shows the importance of wrap mode in reorientation. Since all the motion is directed away from the source at the start, the bacteria without wrap mode have a harder time reorienting and moving up the gradient. As a base comparison, we also plot the migration of bacteria that undergo a

run-reverse pattern, but where the angle change in their reversals are strictly 180° . Here, since the bacteria are oriented perpendicularly, they are not able to move any closer to the source and thus remain stuck throughout the whole simulation.

1.6.4 Parallel Orientations

In contrast, when we initially orient the bacteria towards the source, as in Figure 1.9, we see that the 0% chance of wrap performs slightly better up until 75-80 seconds. After this point, both the percentage of total distance traveled in the radial direction as well as the average distance from the source for 38% chance of wrap finally overtakes 0% chance of wrap. We again show the hypothetical 3rd motility strategy where the bacteria undergo 180° degree reversals. Here, we see that this strategy actually vastly outperforms either of the other two methods. This illustrates that when reorientation isn't required, the best strategy actually limits the angular variation. However, in biologically realistic settings, being able to reorient becomes crucial to efficient aggregation.

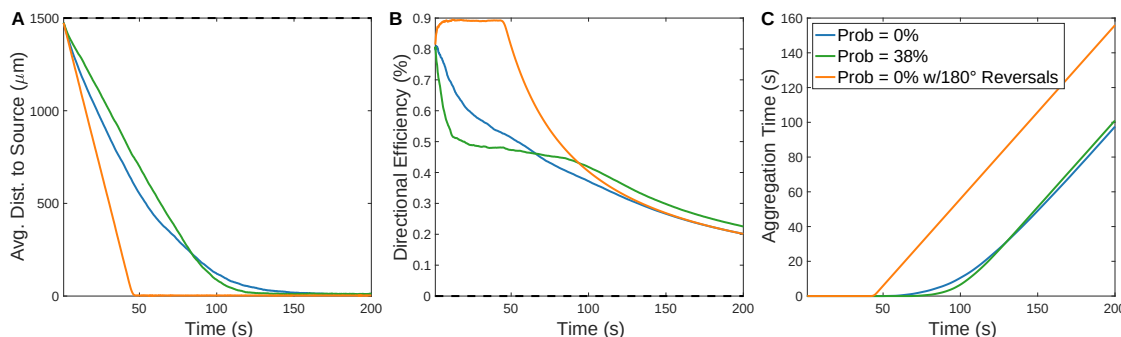


Figure 1.9: Simulations comparing bacterial migration with 0% chance of wrap and 38% chance of wrap. A) shows the average distance to the source, B) percentage of the total distance traveled directed towards the source and C) Aggregation Time.

1.6.5 The experimentally observed frequency of wrap mode may represent an optimal trade off between chemotactic efficiency and lower metabolic cost

We now turn our attention to bacterial migration in response to an exponential point source and further examine the two proposed benefits of wrap mode. Here, we set a point source in the center of a 2000×2000 domain and initialize bacteria in a circle of radius 375 micrometers with random

orientations with respect to the gradient. The source is run from a reaction diffusion equation with the parameters listed in Table 1.1. This time, the chemotactic sensitivity is set to $\zeta_{wrap} = \zeta_{run} = 30$ and 1000 cells are simulated for each percent chance of wrap mode $\{0\%, 20\%, 40\%, 60\%, 80\%, 100\%\}$. In Figure 1.10, we see that up until 52s, all percentages of wrap mode show a marginal improvement in chemotactic efficiency as measured by $\bar{D}$. This time in 1.10C, we plot the number of cells that are further than $50\mu m$ from the center. From this plot, we see that the number of cells are significantly fewer before the 80s mark, demonstrating an increased ability for cells to find the source. However, by the end of the simulated time, we see that 0% chance of wrap ultimately overtakes the wrap mode simulations in both A and C. Part of the reason for this is due to the nature of the exponential gradient yielding a low chemoattractant environment where the cells are initialized. As we studied in section 1.4, wrap mode actually increases the diffusion in low gradient environments. For this reason, some of the cells end up moving away from the source at the start, and get trapped in areas of low chemoattractant. This can be seen by the number of cells that are still farther than 50 microns from the center. At 200 seconds there are 5, 18, 12, 26, 28, and 31 cells for wrap probabilities 0%, 20%, 40%, 60%, 80%, 100% respectively. This shows that as wrap mode increases, the likelihood of being stuck in low gradient environments increases.

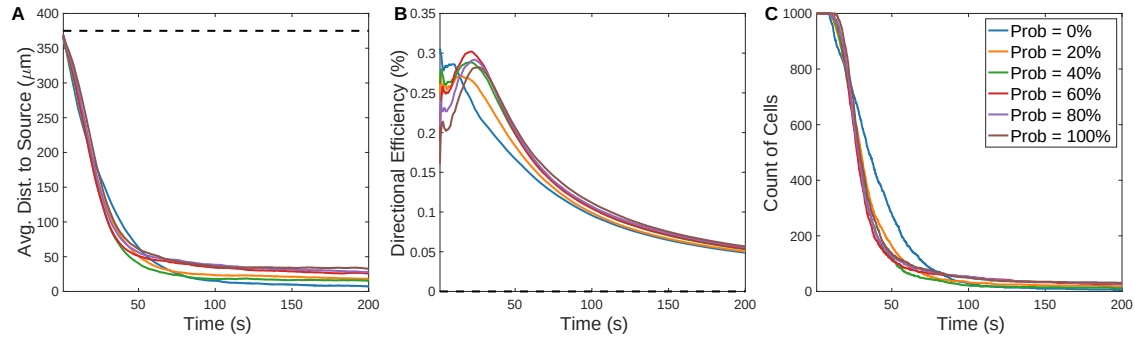


Figure 1.10: Simulations comparing bacterial migration with different percentages of wrap mode. A) shows the average distance to the source, B) percentage of the total distance traveled directed towards the source and C) Number of cells with distance from the source greater than 50 microns.

In 1.10B, we see that for the beginning of the simulation, 0% chance of wrap mode outperforms the wrap mode simulations very briefly. This again is likely due to the initial period of increased exploration with wrap mode in the low gradient regions. However, after the initial 20 seconds, the wrap mode simulations overtake the no wrap simulation. Furthermore, we see that the simulations with 40 – 100% chance of wrap mode perform very similarly with respect to the percentage of the

total distance traveled which is directed towards the source. This is likely due to two opposing outcomes of wrap mode. First, wrap mode slows down the bacteria, so an increased probability leads to less total distance traveled. However, wrap mode also increases the angular exploration which may be counterproductive at a high enough percentage. This suggests that the experimentally observed percentage of 38% may provide an optimal trade-off between a metabolic benefit and overall chemotactic efficiency. We again note that these benefits only occur within a range of gradient/sensitivities. For low and high gradients/sensitivities, wrap mode is likely not to provide as much added benefit.

1.6.6 Limiting the Distribution of Angle Changes in Reversal Events Drastically Reduces the Overall Chemotactic Efficiency

Finally, we examine how much angular variation is needed to yield efficient migration. In order to test this, we look at what happens to the chemotactic efficiency when we start to limit the angular changes that come from reversal events. Here we compare both 0% and 38% chance of wrap to the simulated motility from bacteria where the angle changes during reversals are limited to $180 \pm 5^\circ$, 10° , 15° , 20° . This is done by repeated sampling of the force on the frontier node to ensure that the chosen force produces an angle in the designated range. In Figure 1.11, we can see a clear trend. Here, 38% chance of wrap mode leads to the best aggregation, while the greater the reduction in angular variation during reversals, the worse the aggregation becomes. This further confirms that some of the benefit of wrap mode likely comes from increase in angular exploration. However, since wrap mode only marginally improves the average distance to the source and aggregation time, this also suggests that the distribution of angle changes in reversal events might be important to the overall migrational efficiency as well.

1.7 Bacterial Motion Along Fungi in Liquid

Next, we look at how bacteria migrate in response to fungi within a liquid environment. In this scenario, liquid occupies the entire domain except for where the fungal mass is. This allows the bacteria to freely swim towards and aggregate around the fungi by responding to the various chemoattractants excreted by the fungi. Furthermore, since the fungal mass is relatively small compared to the entire simulated domain, we make the simplifying assumption that the motion is

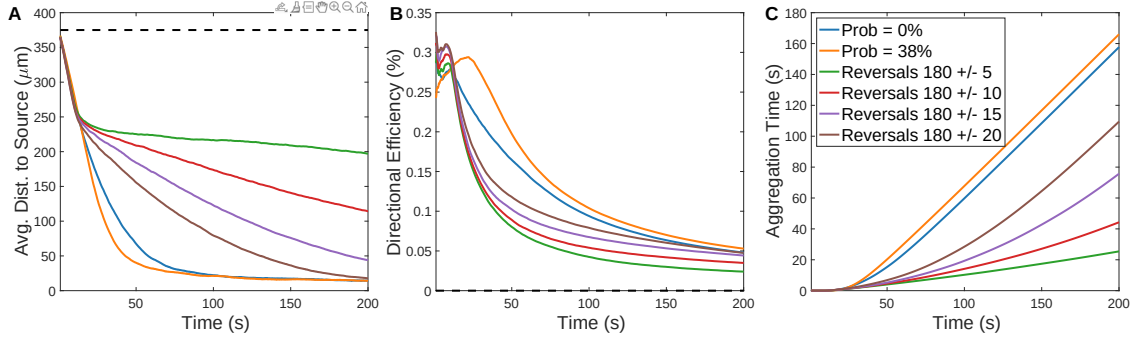


Figure 1.11: Simulations comparing bacterial migration with 38% chance of wrap and 0% chance of wrap with reduced distributions for angle changes during reversal events A) shows the average distance to the source, B) percentage of the total distance traveled directed towards the source and C) Aggregation Time.

driven entirely by chemotaxis rather than any mechanical interactions between the bacteria and fungi.

1.7.1 Wrap Mode Allows the Bacteria to Move Along the Fungi and Increases Exploration of the Multimodal Profile

In order to test the effect of wrap mode against a more biologically relevant chemoattractant profile, we use a snapshot of an external chemoattractant that was generated by a model of fungal growth [24]. The snapshot of the profile is taken after 10.01 hours of simulated fungal growth and we assume the profile is static during the entire duration of the simulations. Here the values of the chemoattractant profile range from $[1.236 \times 10^{-4} \text{ millimolar}, .0022 \text{ millimolar}]$. The bacterial sensitivity level is set to $\zeta_{wrap} = \zeta_{run} = 30$ while the domain is $4000 \times 4000 \mu m$. 100 bacteria are initialized in a circular domain surrounding the fungi and all simulations run for a total of 1600s.

In Figure 1.12, we see some interesting trends arise. First, we see in A that $\bar{D}$ decreases rapidly for all simulations up until around 200 seconds when the bacteria start to find regions of higher chemoattractant. However, after 200 seconds, the average distance to the center completely flattens for 0 and 20% chance of wrap. For higher percentages though, we see that as the chance of wrap mode increases, the bacteria have a greater ability to continue moving towards the center. In B we show the average chemoattractant level across all of the bacteria. Here we can clearly see that as we increase the chance of wrap mode, the bacteria's ability to find regions of higher metabolites increases as well.

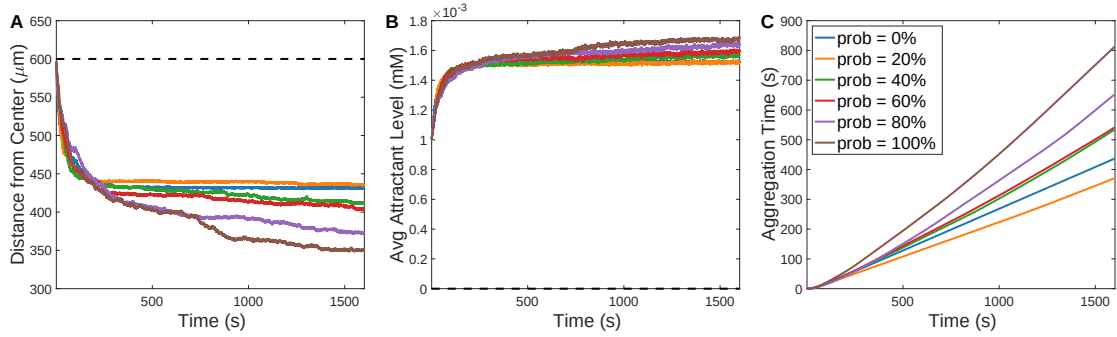


Figure 1.12: Simulations comparing bacterial migration with different probabilities of wrap mode in response to a chemoattractant profile simulated from fungal growth. A) shows the average distance to the center of the domain, B) Average chemoattractant level C) Aggregation Time.

In order to understand why this is, we plot the distribution of bacteria relative to chemoattractant profile in Figure 1.13. In A and B we show the distribution at 200s and 1600s for the simulation with 0% chance of wrap. Here we see that the initial maximums found by the bacteria at 200 seconds traps the bacteria at these locations for the entirety of the simulation. When we compare this to the distributions of the 100% wrap simulation in C and D, we see that the distribution is considerably different from 200 seconds to 1600 seconds.

This further supports the hypothesis that the increased angular exploration is one evolutionary benefit to wrap mode. As the bacteria enter wrap mode, they are able to move away from a local maximum and respond to other nearby gradients. This ultimately helps the bacteria travel along the hyphae and move towards higher regions of the chemoattractant, which may be an important part of efficient migration.

1.7.2 Conclusion

In this section, we examine how *P. aeruginosa* responds to the chemotactic signals produced by a hypothetical fungal network in a liquid environment. For cells undergoing a run-reverse pattern, we find that the bacteria are very likely to remain in the first local chemoattractant peak that they find. With wrap mode however, the bacteria are more easily able to escape these local maximums to further explore their neighboring environment along the hyphae.

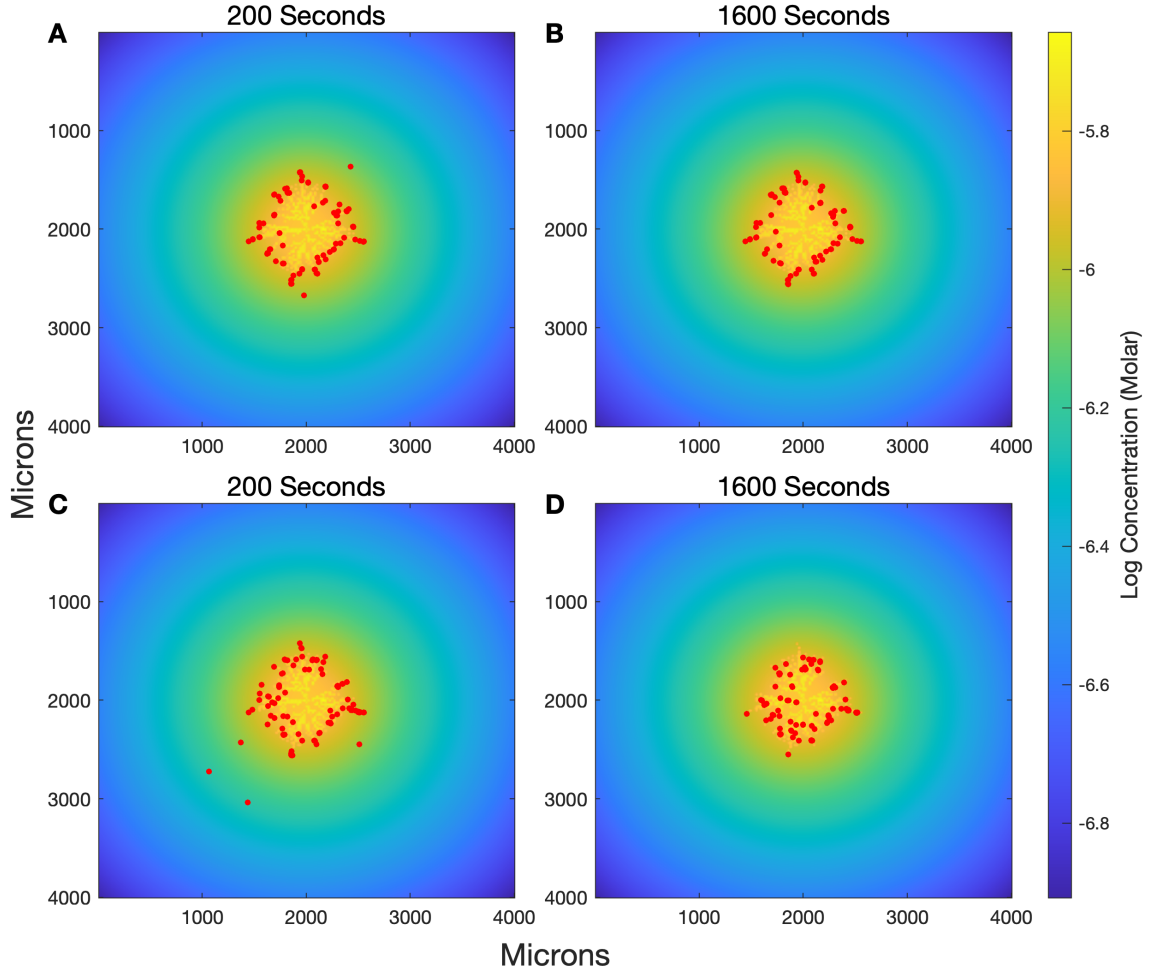


Figure 1.13: Distribution of bacteria in response to a chemoattractant profile generated from fungal growth. Bacteria shown as red dots overlaid on chemoattractant profile. A and B are from simulation with 0% chance of wrap and C and D are from 100% chance of wrap.

1.8 Methods

In this section, we describe a modification of the subcellular element (SCE) modeling approach, which is used to represent each individual bacterium and the mechanical forces on the bacterium. The model presented here is largely in alignment with a model of the bacterium *Myxococcus xanthus* presented in [7]. The SCE model is then coupled to a chemotactic submodel, which modulates the reversal frequency of each bacterium. This coupling simulates the ability of *Pseudomonas aeruginosa* to respond to various chemical profiles.

1.8.1 Subcellular element representation of *Pseudomonas aeruginosa*

In the subcellular element model used here, each cell is represented by a sequence of nodes connected by linear and rotational springs. Linear springs are used to model the bonding energy between consecutive nodes, which allows for some strain in the bacterium's length, while also maintaining the average length over time. Meanwhile, rotational springs are used between three consecutive nodes, which allows the cell body to turn and change directions over time. The average length of *Pseudomonas aeruginosa* is $2\mu m$. In order to maintain reasonable computational costs, while still accurately modeling the physical forces on each cell, we represent each bacterium by a sequence of five nodes. Thus the distance between each node is $l_{eq} = \frac{2}{N_{nodes}-1} = .5\mu m$. The bonding force $\mathbf{F}_{bond}^i$ on node i at location $\mathbf{x}_i$ is calculated by the expression

$$\mathbf{F}_{bond}^i = \sum_{j \in BN(i)} k_b (\|\mathbf{x}_i - \mathbf{x}_j\| - l_{eq}) \hat{\mathbf{r}}_i \quad (1.2)$$

where l_{eq} is the equilibrium distance between two consecutive nodes and is equal to the length of one bacterium divided by the $N_{nodes} - 1$, or the number of springs required to connect the nodes. The set of bonded neighbors of node i is denoted by $BN(i)$, k_b is the linear spring constant, and $\hat{\mathbf{r}}_i$ is the unit vector pointing from node i to node j . In order to determine the bending force on each joint comprised of 3 consecutive nodes, we first write down the bending energy U_{bend} between the nodes i , j and k .

$$U_{bend} = k_{bend} [1 - \cos(\theta_{ijk} - \theta_{eq})] \quad (1.3)$$

where k_{bend} is the rotational spring constant, θ_{ijk} is the angle between nodes i , j , and k , and $\theta_{eq} = \pi$ rad is the equilibrium angle. The bending forces of a joint ($\mathbf{F}_{bend}^i$, $\mathbf{F}_{bend}^j$, and $\mathbf{F}_{bend}^k$) can then be calculated by taking the derivative of the bending energy as follows.

$$\mathbf{F}_{bend}^i = -k_{bend} \left(\frac{\vec{\mathbf{r}}_{kj}}{|\vec{\mathbf{r}}_{ij}| |\vec{\mathbf{r}}_{kj}|} - \cos \theta_{ijk} \frac{\vec{\mathbf{r}}_{ij}}{|\vec{\mathbf{r}}_{ij}|^2} \right) \quad (1.4)$$

$$\mathbf{F}_{bend}^k = -k_{bend} \left(\frac{\vec{\mathbf{r}}_{ij}}{|\vec{\mathbf{r}}_{ij}| |\vec{\mathbf{r}}_{kj}|} - \cos \theta_{ijk} \frac{\vec{\mathbf{r}}_{kj}}{|\vec{\mathbf{r}}_{kj}|^2} \right) \quad (1.5)$$

$$\mathbf{F}_{bend}^j = -(\mathbf{F}_{bend}^i + \mathbf{F}_{bend}^k) \quad (1.6)$$

where $\mathbf{r}_{ij} = \mathbf{x}_i - \mathbf{x}_j$. Furthermore, in order to simulate the propulsive force applied by the flagellum on the cell body, we introduce the a constant force f_{motor} on node i . The magnitude of the force is calibrated to the velocity in both run and wrap mode. The details are found in appendix A. In order to smooth the motion of the bacterium, the propulsive force is distributed uniformly among all 5 nodes. Hence the force, F_{motor}^i , applied to node i is calculated by the equation

$$\mathbf{F}_{motor}^i = f_{motor} \hat{\mathbf{r}}_{i-1,i}, \quad 1 \leq i \leq 4 \quad (1.7)$$

In the absence of a reversal event or additional forces, the force on the head node is $\mathbf{F}_{motor}^0 = f_{motor} \hat{\mathbf{r}}_{0,1}$. The motion of a bacterium can be represented by a Langevin Equation. We make the assumption that the viscosity of the fluid is large enough such that the inertial forces can be discounted. In addition, we make the assumption that the liquid environment is completely homogeneous and without any flow; thus the propulsive force from the flagella will dominate the bacterium's motion. In addition, any stochastic elements from thermal fluctuations can be accounted for through the stochasticity in the reversal frequencies, as well as angle changes in reversal events and during wrap mode. Therefore we exclude the stochastic term and the equation of motion is written as

$$\frac{d\mathbf{x}_i(t)}{dt} = -\frac{1}{\gamma} \nabla_{\mathbf{x}} U(\mathbf{x}_i(t)) \quad (1.8)$$

where γ is the friction coefficient and $-\nabla_{\mathbf{x}} U(\mathbf{x}_i(t))$ is the net force acting on node i which can be written as

$$-\nabla_{\mathbf{x}} U(\mathbf{x}_i(t)) = \mathbf{F}(\mathbf{x}_i(t)) = \mathbf{F}_{bond}^i + \mathbf{F}_{bend}^i + \mathbf{F}_{motor}^i \quad (1.9)$$

In equation 1.8, the left hand side represents the velocity of node i at position $\mathbf{x}_i$, where as the right hand side accounts for the bonding, bending, and motor force applied to this node. Equation 1.8 is solved using a forward Euler method. Furthermore we impose periodic boundary conditions on the 2D simulated domain. This implemented by identifying opposite edges of the domain together, so that if a bacterium moves off of one side it reappears on the other. In this way, the total number of bacteria is maintained through the simulation.

1.8.2 Reversal Events and Mode Durations

Between pull and push mode, *Pseudomonas aeruginosa* performs a reversal in which the orientation of the cell body undergoes a rapid change, typically between $90^\circ - 180^\circ$. This process is modeled by first reversing the order of the nodes, such that the frontier node becomes the rear node, and then altering the direction of the force on the frontier node for .2 seconds. The direction is selected from a distribution such that the net angle changes for all reversal events match the experimentally observed distribution in [97]. Furthermore, the durations for both run and wrap mode can be written as $T_{run} = \frac{1}{\lambda_{run}}$ and $T_{wrap} = \frac{1}{\lambda_{wrap}}$ where λ_{run} and λ_{wrap} are the reversal frequencies in run and wrap mode, respectively. The initial durations T_{run} and T_{wrap} are selected from the calibrated distributions at the beginning of each mode. Each bacterium is then given an internal clock which increases with every time step. Once the mode duration is met, the bacteria either reverses or enters wrap mode. The details of the reversal angle and mode duration calibrations can be found in appendix A.

1.8.3 Wrap mode

The defining characteristics of wrap mode are a reduction in cell velocity, when compared to run mode, and an increase in the angular velocity. In accordance with [97], we define a chance for each bacterium to enter wrap mode before it reverses. Once in wrap mode, the propulsive force f_{motor} is reduced to match the experimentally observed velocity in wrap mode. Wrap mode also delays the reversal events described above. In wrap mode the internal clock will be reset, and once the selected duration for wrap mode has been met, the bacterium will undergo a reversal event. In order to obtain an angular rotation during wrap mode, the direction of F_{motor}^0 is changed from $\hat{r}_{1,0}$ to $\hat{w}$ where $\hat{w}$ is chosen from a distribution of possible directions. This distribution, as well as the durations of the wrap mode, are calibrated to match the statistics reported in [97] and the details can be found in appendix A.

1.8.4 Submodel of chemotactic motion

Each bacterium is able to modulate its motility in order to navigate towards regions of higher metabolites. This can be modeled in a number of ways, but in general the bacterium accomplishes this by adjusting its reversal frequency, which results in the bacterium spending more time moving up

the gradient of a chemoattractant, rather than down the gradient. We model the reversal frequency by the following equation which follows previous work on bacterial migration [11].

$$\lambda = \lambda_0 e^{-\zeta v \cdot \nabla s \Theta(-v \cdot \nabla s)} \quad (1.10)$$

$$\Theta(u) = \begin{cases} 0 & \text{for } u < 0 \\ 1 & \text{for } u > 0 \end{cases} \quad (1.11)$$

where λ_0 is the rate of reversal in absence of chemoattractant, v is the velocity of the bacteria. The concentration of the chemoattractant is represented by s , and ζ is a parameter which governs the sensitivity of the chemotactic response. Since Θ is the Heaviside function, the bacteria are considered pessimistic in their chemotactic response. This means that when a bacterium is moving up a gradient we have $-v \cdot \nabla s < 0$ and $\Theta = 0$. Therefore the reversal frequency $\lambda = \lambda_0$ or remains unchanged allowing the bacterium to continue moving up the gradient. On the other hand, when a bacterium is moving down the gradient we have that $-v \cdot \nabla s > 0$ and $\Theta = 1$. This results in an increase to λ which causes the bacteria to reverse quicker and move in another direction that may be more beneficial. We note that the durations of run and wrap mode are modulated by equation 1.10 since the duration is the inverse of the reversal frequency as discussed in section 1.8.2.

1.8.5 Implementation of Chemoattractant Profile

The profile of the chemoattractant is stored on a discretized domain with grid size of $1\mu m$. The size of this grid is chosen to be smaller than the distance traveled between consecutive updates in the chemotactic submodel. Since the gradient is checked every .1 seconds, and the bacterial velocity is $14.45 \frac{\mu m}{s}$, the grid size must be smaller than $1.45 \frac{\mu m}{s}$. Furthermore, we make the assumption that the bacteria sense the gradient by comparing their current environment to the environment at the previous time point. Thus, we calculate the term $v \cdot \nabla s$ used for a bacterium at time t and position $\mathbf{x}$ as

$$v \cdot \nabla s = s(\mathbf{X}, t + 1) - s(\mathbf{X}, t) \quad (1.12)$$

where $\mathbf{X}$ is the nearest grid point to the off lattice coordinate $\mathbf{x}$. We assume the time scale of diffusion of the chemoattractant to be larger than the migration of the bacteria. Therefore, the chemoattractant profile is assumed to be static throughout each simulation.

1.9 Bacterial Migration Around Fungi On Agar

In this section, we now turn our attention to modeling bacterial migration around fungi on soil or agar. In this environment, numerous studies have shown that in addition to the production of primary and secondary metabolites, fungi also produce a thin layer of liquid which bacteria swim in [1, 58, 81, 116]. Because of this, bacteria are able to use their flagellum within the liquid layer and spread along the hyphae. In order to understand the interactions between bacteria and fungi further, we extend the model presented in section 1.8 to model this context. We explain the model extensions in section 1.9.1. In section 1.9.2, we test the dispersal of *Pseudomonas aeruginosa* on different Network topologies and in section 1.9.3, we simulate the growth, division, and dispersal of *Pseudomonas aeruginosa* across a fixed network.

1.9.1 Model Components for Migration Around Fungi on Agar

For fungi growing in soil, the excreted liquid layer surrounding the hyphae would most likely completely encompass the fungal hyphae. In nature this would allow the bacteria to swim around the entire circumference of the hyphae, in addition to lengthwise. However, depending on the experimental setup, this liquid layer might not provide 360° coverage. In this case, the bacteria may not actually be able to cross the hyphae and would only be able to swim along the hyphae branches. We can account for both of these possibilities by modeling the fungi/agar environment with a pseudo 3D representation. This is done by dividing the two-dimensional domain into three distinct regions; agar, liquid, and the hyphae itself. Thus, by having these distinct environments we are both able to control the velocity in each and also implement some control in how likely the bacteria are to cross the hyphae. The velocity is controlled by adjusting the friction coefficient within the Langevin equation 1.8, for each region. The friction coefficients are listed in Table 1.2 In the next two sections we describe how the fungal environment is generated, as well as how the bacteria navigate along the fungi.

Table 1.2: Model Parameters

Parameter	Value	Units	Description
γ_a	.09	$\mu N \cdot s \cdot \mu m^{-1}$	Damping coefficient on Agar
γ_l	.01	$\mu N \cdot s \cdot \mu m^{-1}$	Damping coefficient in Liquid
γ_h	$\frac{.01}{.7}$	$\mu N \cdot s \cdot \mu m^{-1}$	Damping coefficient on Hyphae
τ	0.5	-	Smoothing Threshold
E_{Liquid}	0.8	-	Alignment Sensitivity to Liquid Layer
P_{cross}	*	-	Probability Bacterium Crossing Hyphae
S	.4	-	% of Hyphae in Viewing Region Needed for Crossing
P_{exit}	0	-	Probability Bacterium Exits Liquid
ϵ	0.0014	fJ	Strength of LJ interaction
σ_{ij}	$\frac{.6}{.122}$	μm	Repulsion Distance
L_{cutoff}	$2.5 \cdot \sigma_{ij}$	μm	LJ interaction distance cutoff
G_{spring}	*	-	Growth Rate of Spring
L_{new}	1.8	μm	Length of newly divided bacterium
L_{divide}	4.2	μm	Length bacterium that initializes division

* Parameter values varied in simulation runs

1.9.1.1 Modeling Fungal Liquid Environment

In order to have a realistic network composition, we take the coordinates from a simulated fungal network based on the model described and calibrated in [24]. We use coordinates taken from 40 – 80 hours of simulated growth depending on the simulations. The liquid layer is first approximated by using a combination of rectangles and semicircles, and then a smoothing algorithm is applied to smooth out the profile. We describe both of these in greater detail. Given a list of hyphal coordinates we first iterate through every hyphae and approximate each by a rectangle. More specifically, let (x_1, y_1) and (x_2, y_2) be the starting and ending coordinates of the hyphae respectively. We define two rectangles R_1 and R_2 where the central axis of each rectangle is formed by the line segment that connects these two points, and a height of H and $2L + H$, respectively. Height here refers to the length of the sides that are perpendicular to the central axis. Please refer to figure 1.14 for a graphical representation.

We then define any grid point $\mathbf{x} \in R_1$ as part of the hyphae itself, while any grid point $\mathbf{x} \in R_2 \setminus R_1$ is defined as part of the liquid layer surrounding the hyphae. Once the process has been applied to every hyphal segment, we then iterate through the entire list again and use a semi-circle to properly connect each segment. More specifically, for each ending segment coordinate (x_2, y_2) , we define 2 half circles S_1 and S_2 centered around this point with the base of the semi circle along the side of rectangle R_2 . S_1 and S_2 have a radius of $\frac{H}{2}$ and $\frac{H}{2} + L$ respectively. Then in a similar manner we define any grid point $\mathbf{x} \in S_1$ as part of the hyphae itself, while any grid point $\mathbf{x} \in S_2 \setminus S_1$ is

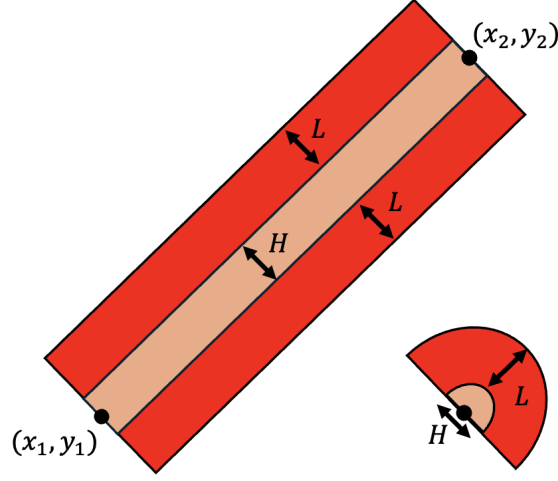


Figure 1.14: Figure showing both rectangular and Semi-Circle regions used to approximate the fungal-liquid environment. Here red is the liquid layer and pink is the fungal hyphae

defined as part of the liquid. Finally, for only the first 4 segments that lie at the origin, this process is applied to the start of the hyphae segment around (x_1, y_1) . This defines the first approximation. In order to complete the smoothing, the hyphae coordinates and the liquid coordinates are stored on two separate grids G_1 and G_2 . The smoothing algorithm is first applied to the hyphae coordinates in G_1 and then G_2 is updated with the smoothed hyphal coordinates in G_1 . Finally, the liquid is smoothed out on G_2 . This is done to both the hyphae points and the liquid layer independent to make navigating corners easier as well as to model the aggregation of liquid between 2 hyphae. We describe the smoothing algorithm in Algorithm 1 listed in appendix A. An example of a generated network can be seen in Figure 1.15.

1.9.1.2 Bacterial Alignment Algorithm

Although the exact mechanisms are unknown, multiple experimental videos show that bacteria within the liquid layer surrounding fungi, are able to navigate the topology of the network without many collisions. Thus, in order to model the motion of a bacterium within the liquid layer, we implement an alignment algorithm which attempts to align the bacterium in the direction of the liquid layer. This is done by adjusting the direction of the force applied to the frontier node, allowing the bacterium to successfully navigate the curves in the liquid layer. Thus the force on the frontier node can be

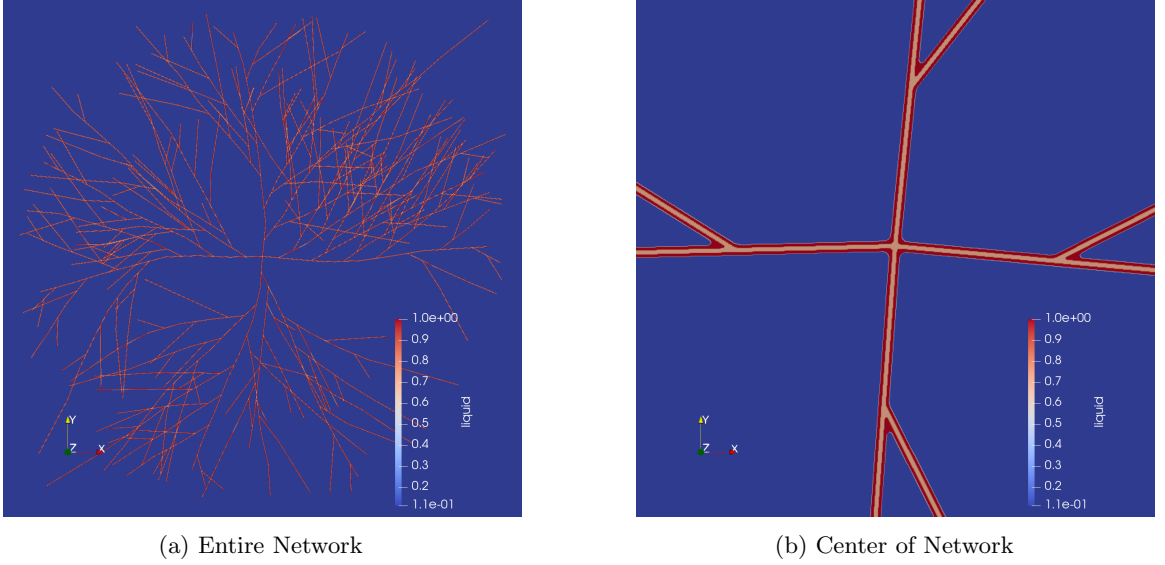


Figure 1.15: (A) Shows the entire network taken from fungal coordinates after 80.89 hours of fungal growth. (B) shows a zoomed in view of the center of the network. Here pink is the fungi itself, red is the surrounding liquid, and blue represent the agar/soil.

written as

$$\mathbf{F}_{motor}^0 = f_{motor} \left(\frac{1}{E_{liquid}} \hat{\mathbf{r}}_{1,0} + E_{liquid} \cdot \hat{\mathbf{L}}_T \right) \quad (1.13)$$

where $\hat{\mathbf{L}}_T$ is the unit normal vector in the direction chosen from the liquid alignment algorithm which seeks to align the bacteria with the liquid layer and E_{liquid} is a parameter which determines how sensitive the bacteria is to the liquid layer.

For modeling purposes, we make the assumption that *Pseudomonas Aeruginosa* is able to sense a small semicircular region in front of it and uses this information to navigate the liquid layer. We give the circular region a radius of $2\mu m$ which is the same as the length of the bacterium itself. The semicircle is then divided into 5 regions as shown in Figure 1.16 and the number of grid points that are liquid, fungi, and agar, is counted within each region. Finally, a force is applied in the direction of $\theta \text{ rad} = (R - 3) \cdot \frac{\pi}{5} + \sigma$ where R is the chosen region and σ is the current angle the cell body is rotated from the positive x-axis. Note that σ rotates the starting direction to align with the current direction of the bacteria. The algorithm for choosing the desired region is summarized in algorithm 2.

In addition to the alignment algorithm, we also allow for a probability that a bacterium leaves the liquid layer and that it crosses the hyphae. If the bacterium exits the liquid layer and moves onto the agar, then the alignment algorithm turns off, and the bacterium undergoes a sequence of runs

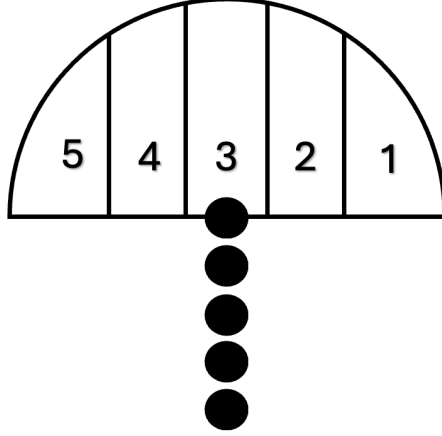


Figure 1.16: The viewing window in front of the bacteria which is used in the alignment algorithm by bacteria to navigate the liquid layer surrounding the fungi

and reversals as described in section 1.8. However, due to the greater friction force in this region, the velocity of the bacterium is much smaller than in liquid. A bacterium within the liquid layer perform reversals governed by their reversal frequencies, however the angular change that occurs during the reversal is only applied as long as the entire semicircular region in front of the bacterium is liquid. Otherwise, the bacterium will only perform 180° reversals. A bacterium is allowed only one chance to cross the hyphae each run. In addition, they must have enough hyphae within their viewing semicircle to be able to cross the hyphae. The probability defined in the model governs how likely they are to cross the hyphae during each run in which they encounter the hyphae.

1.9.1.3 Lennard-Jones Potential

Furthermore, since the liquid layer is a narrow region and collisions are possible, we model the interaction between individual bacterium with a Lennard-Jones type potential. Note that the standard Lennard-Jones potential includes both a repulsive and attractive force. However, for our case we only include the repulsive force as we make the assumption that there is no adhesion between bacterium in the liquid layer. Thus the potential force between nodes i and j on separate bacterium is written as

$$U_{LJ}(\mathbf{x}_i, \mathbf{x}_j) = 4 \cdot \epsilon \left(\frac{\sigma_{ij}}{\|\mathbf{x}_i - \mathbf{x}_j\|} \right)^{12} \quad (1.14)$$

where ϵ is a parameter that governs the strength of the interaction and σ_{ij} is the distance where the repulsive force starts. Thus, equation 1.9 can be written as

$$-\nabla_x U(\mathbf{x}_i(t)) = \mathbf{F}(\mathbf{x}_i(t)) = \mathbf{F}_{bond}^i + \mathbf{F}_{bend}^i + \mathbf{F}_{motor}^i + \mathbf{F}_{LJ}^i \quad (1.15)$$

where $\mathbf{F}_{LJ}^i$ is derived from by taking the gradient of the Lennard-Jones potential in equation 1.14

$$\mathbf{F}_{LJ}^i = \sum_{j \in NBN(i)} 4 \cdot \epsilon \left(12 \frac{\sigma_{ij}^{12}}{\|\mathbf{x}_i - \mathbf{x}_j\|^{13}} \right) \hat{\mathbf{r}}_{ij} \quad (1.16)$$

Here $NBN(i)$ is the set of nonbonded nodes, which includes all nodes j on different bacteria within a cutoff distance LJ_{cutoff} . In order to keep the bacteria from traveling through each other we also add an additional $N_{nodes} - 1$ nodes such that each node $\mathbf{x}_i^{LJ} = \frac{\mathbf{x}_{i+1} - \mathbf{x}_i}{2}$ for $0 \leq i \leq (N_{nodes} - 1)$. These nodes are only updated and considered in the calculation of equation 1.16

1.9.2 Increasing the Network Density Increases Bacterial Dispersal

With these added modeling components, we now test the effect of network density on the ability of the bacteria to disperse along a fungal network. This is done by initializing 100 bacteria in the center of 6 different networks of varying network density. Here, we define network density as the number of branch points per μm^2 . The primary metric we use to quantify dispersal ability is the average distance from the center of the fungal network over time. In order to isolate only the variable of network density, we note that if the fungal networks are of different sizes (as measured by the distance from center to the furthest point on the network), then the effect of the network density can not be accurately captured by this metric. Thus, to solve this, we generate 6 networks which all occupy the same spatial region.

To ensure that the networks are biologically relevant, we use the model described and calibrated in [24] to simulate the growth of a fungal network for 80.89 hours. This network is then used to generate 5 additional networks of different densities that occupy the same spatial domain. These 6 different networks as well as the branch points are depicted in figure 1.17. The 80.89 hour network shows the unaltered network simulated by the fungal model [24]. However, for each of the five previous networks, the network was created such that any branch that shows up at the specified time point is included, but no further branches are included in the network after the stated time. We do

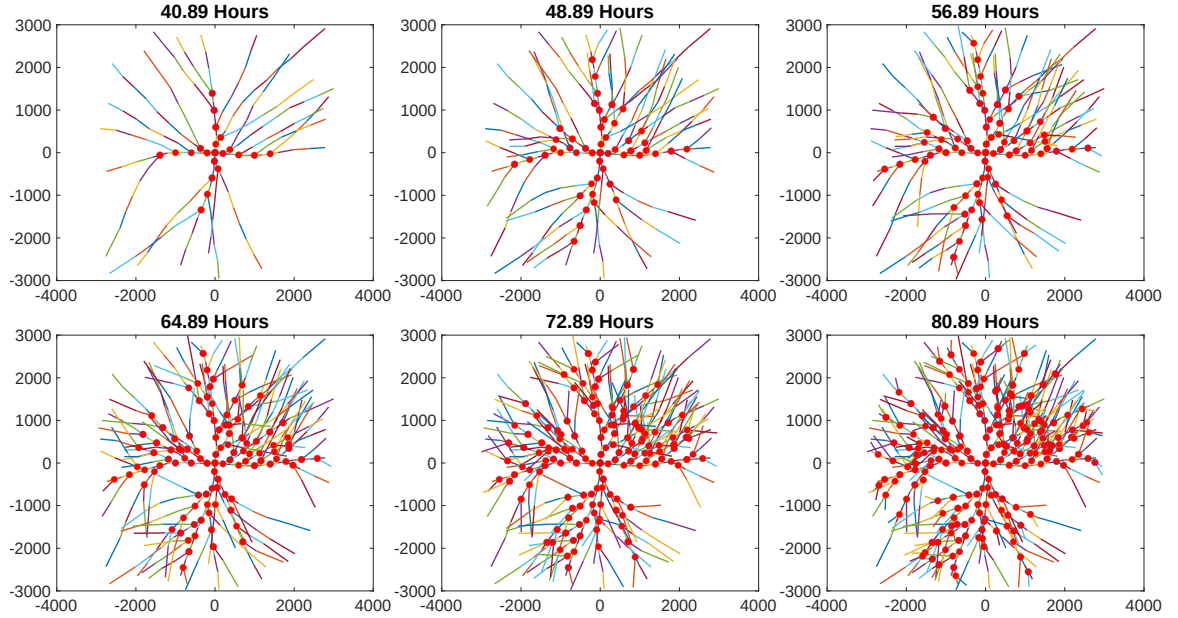


Figure 1.17: 6 Networks of varying densities. The fungal hyphae are depicted with varying colors for each individual hyphae segment. The branch points are indicated by red dots. Each network is a subset of the 80.89 hour network where the time points represent the time in which the fungi is no longer able to branch. The networks branches are all allowed to extend up until 80.89 hours, yielding networks that occupy the same physical space.

however, allow each branch that already exists on or before the specified time point to extend up to the 80.89 hour time point. In this manner, we can think of each network as the product of a fungi being able to extend its branches, but not being able to create new branches after the given time point. As seen in the plots, each of the first five networks is a proper subset of the sixth network, and all occupy the same physical size.

We quantify the network density in figure 1.18. In each plot, the branching density is shown for the network in six distinct regions. The six regions are created by looking at concentric circles of varying radii. In particular, let C_i be a circle of radius R_i . We then define the area corresponding to each distance D_i as $C_i - C_{i-1}$ given that $i > 0$. In our case $R_0 = 500\mu m$. The 6 different areas depicted in Figure 1.19

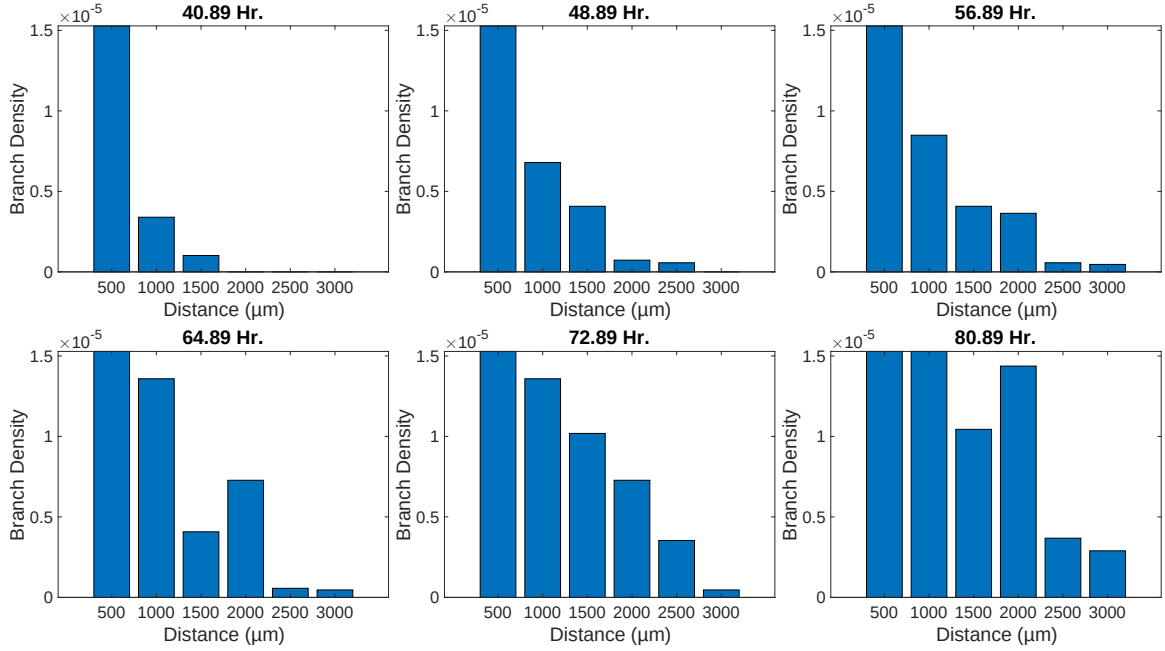


Figure 1.18: The branching density for each of the 6 networks shown in figure 1.17. For each plot the branching density is shown for 6 distinct regions of the domain where each region is the circular area with the stated radius excluding all previous regions with smaller radii. See figure 1.19 for a graphical depiction

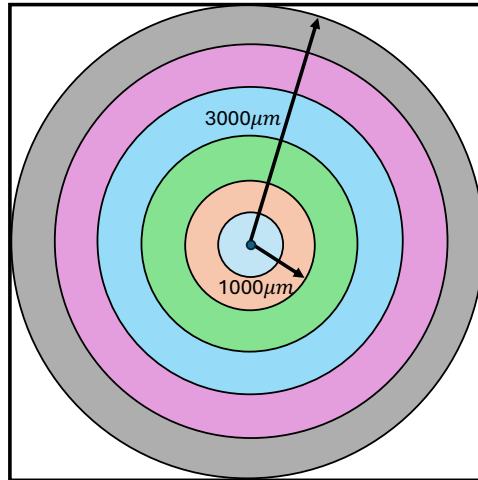


Figure 1.19: The 6 subdomains the branching density is calculated on for each network. Each region is a the circular area of a given radius in $[500\mu m, 1000\mu m, 1500\mu m, 2000\mu m, 2500\mu m, 3000\mu m]$ excluding all other circular regions of smaller radius We show two radii for reference

We now present the average distances across 100 bacteria randomly initialized in the liquid layer of each network. The bacteria start within a circular region of radius $200\mu m$ in the center of the network. Bacterial migration is simulated for $2400s$ and the crossing probability is set to 3% in each simulation. The bacteria are unable to leave the liquid layer that surrounds the fungi. On the left of figure 1.20 we see that as the network density increases, the average distance from the center decreases.

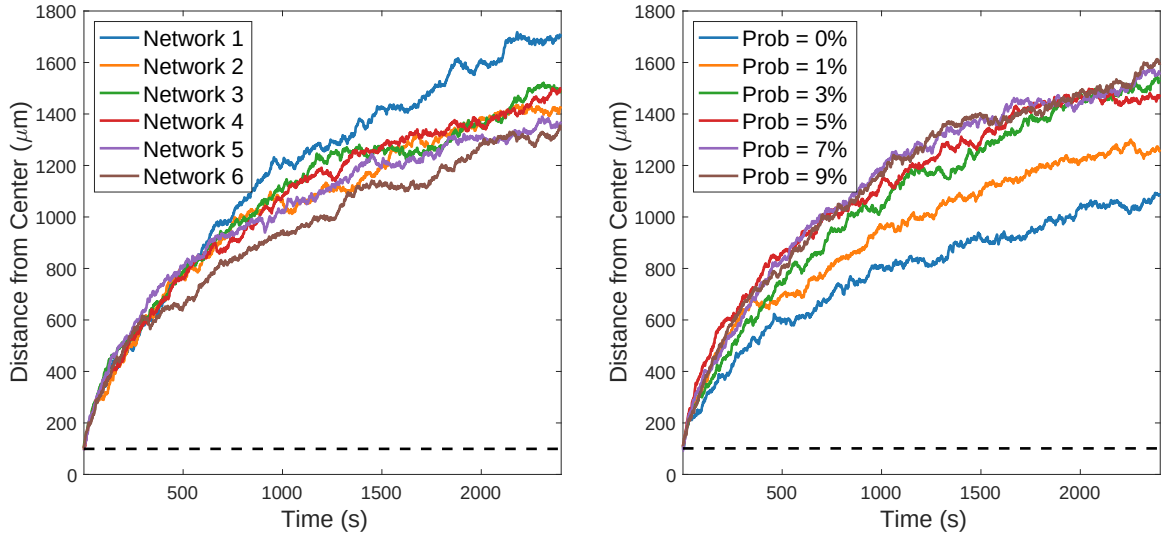


Figure 1.20: Left: The average distance across 100 bacteria initialized in the liquid layer of 6 different fungal networks of varying branching density. The bacteria start in the center $200\mu m$ of the domain and the total simulation time is $2400s$ each simulation. We see that as the network density increases the average distance to the center decreases. This illustrates that as the topology becomes more complicated the bacteria have a harder time dispersing to the tips of the network. Right: The average distance across 100 bacteria initialized in the liquid layer of a fixed network. The bacteria start in the center $200\mu m$ of the domain and the total simulation time is $2400s$ each simulation. Each simulation shows bacteria with a different crossing probability, 0%, 1%, 3%, 5%, 7%, 9% respectively. We see that as the crossing probability increases the average distance to the center also increases. However for crossing probabilities $> 3\%$ the benefits to the dispersal of the bacteria level out.

This shows that with more complicated topologies, the bacteria take longer to disperse across the network. This phenomenon directly relates to the accessibility of certain areas of the network given the branching structure. For instance, in order for a bacterium to get to the liquid layer on the other side of its current hyphae, it must travel around the tip of the hyphae or cross over the hyphae directly. Furthermore, if there are closed loops induced by the branch points or intersections, then there are some regions on the hyphae structure which only can be accessed by crossing the hyphae. Hence, if the crossing probability is very low, then the bacteria may struggle to reach certain

areas along the fungal network. On the contrary, if the crossing probability is very high, then the fungi does not serve as a barrier and the topology of the network is less impactful. However, since the crossing probability is set to 3% we see that even with some crossing probability, the branching density provides some resistance to the bacteria dispersal.

We can now examine the effects of the crossing probability on bacteria dispersal. On the right of figure 1.20 we show the dispersal of bacteria given different crossing probabilities. In each of the six simulations the network is now fixed and we vary the crossing probability. We test crossing probabilities of 0%, 1%, 3%, 5%, 7%, 9% respectively. We see that for probability 0%, 1%, and 3%, there is a significant increase in the average distance to the center as the crossing probability increases. However, we also note that for 3%, 5%, 7%, and 9% there is not a significant difference. This shows that for very low crossing probabilities the fungal hyphae act as a strong barrier impeding the dispersal of the bacteria, however given even a small crossing probability such as 3% the bacteria are still able to disperse at similar rates to simulations with higher crossing probabilities. The diminishing returns show that the crossing probability is only important in that it governs the accessibility of certain regions of the fungal network.

1.9.3 Bacterial Growth and Division

As bacteria move and explore their local environment, they grow and divide based on the available resources. In order to model this process we define a growth rate G_{spring} which is added to the equilibrium spring length l_{eq} at each time step. In this way, the distance between two consecutive nodes in each bacterium slowly increases, causing the length of the whole bacterium to grow over time. When the bacterium reaches a set length L_{divide} , a new bacterium is added to the simulation with length $1.8\mu m$ and the final node of the original bacteria serves as the final node of the new bacteria. In addition, the original bacterium is reduced to a length of $1.8\mu m$. In this process, the frontier node stays as the frontier node of the reduced bacterium.

The growth rate G_{spring} can be selected from an experimental distribution for each bacteria or can even be modulated based on the available nutrients by the use of a hill function. In this manner, we have

$$\hat{G}_{spring}(x) = \frac{C^n(x)}{K^n + C^n(x)} \cdot G_{spring}$$

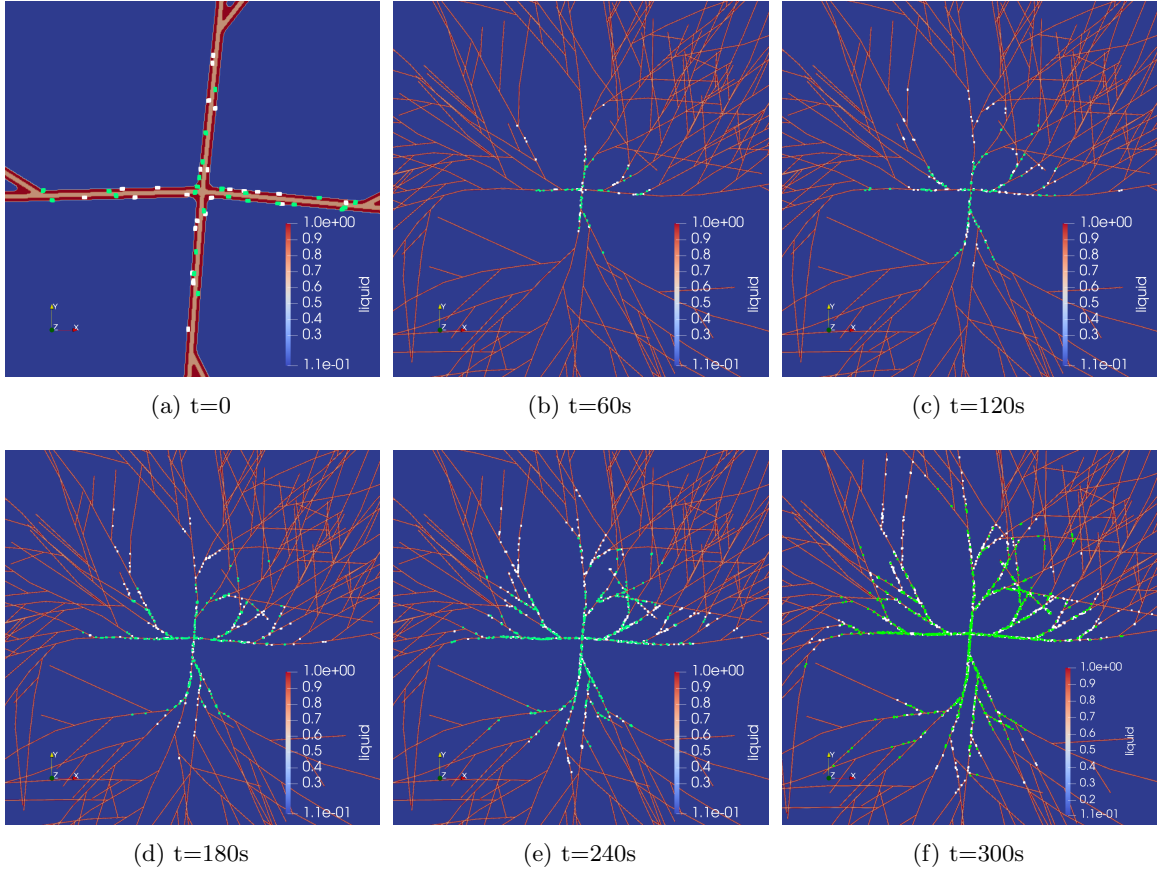


Figure 1.21: Images showing the progression of 2 species (white and green dots) growing and dividing across a fungal network for 300 seconds. We see that the green species stays more located in the center of the network while the white species has a few cells that were able to spread faster and start to populate the edges of the colony.

We demonstrate the model in Figure 1.21. In this simulation, we show the spread and growth of two different bacterial strains across a fungal network. We initialize 50 bacteria in the center of a fungal network, 25 in each strain, and let them spread and divide for 300 seconds. The growth rate for each bacterium is set to a constant $G_{spring} = 1 \cdot 10^{-7}$ and $L_{divide} = 4.2\mu m$, resulting in a doubling time of around 60 seconds. The crossing probability for both species is set to 9%. We see that the two species start off randomly distributed in the center of the network, however by 120 seconds we start to see some clustering emerge. For instance, more of the species colored white has migrated toward the upper center region of the fungi. This stochastic event later has downstream effects, as we see by 300 seconds, there are considerably more of this species in the upper region away from the center of the network. In general, we see that the green species looks more localized in the center while the white species has spread a bit farther out.

1.10 Discussion and Future Directions

In this chapter we developed a mathematical and computational model of *Pseudomonas aeruginosa* migration. In sections 1.4 - 1.7, we used the model to explore the effects of reversals and wrap mode both with and without chemotaxis in different environments. In the absence of chemotaxis, it was shown that wrap mode enhances the ability of the bacteria to diffuse across the environment. However, this was dependent on the angular change to the cell body that occurred during wrap mode. When wrap mode induced smaller angular changes to the bacteria's direction of motion, there was a minor decrease observed in the bacterial diffusion coefficient in model simulations. In contrast, larger angular changes in wrap mode (90° , 135° , and 180°) resulted in a significant increase in the diffusion coefficient. This finding suggests that wrap mode is instrumental in increasing the angular exploration of the bacteria which is the driving factor in the increased diffusion.

With chemotaxis present, we examined the effects of wrap mode in response to both linear and exponential chemoattractant profiles and found that there were three distinct regions where wrap mode had varying effects. With low chemoattractant gradients or low chemotactic sensitivities a run-wrap-reverse strategy showed similar chemotactic efficiency to a run-reverse pattern. Since wrap mode increases the angular exploration of the bacteria, the presence of wrap mode allows for the bacteria to reorient themselves much quicker than bacteria without wrap mode. If chemotaxis is relatively weak however, this reorientation isn't strongly biased towards the source of the chemoattractant

and therefore, it does not necessarily contribute to the overall chemotactic efficiency. In contrast, when the chemotactic force becomes strong enough, exploration away from the source is reduced causing wrap mode to increase the chemotactic efficiency. In this region we find that wrap mode both reduces the time it takes for bacteria to find the source as well as the overall motion that is needed to achieve this result. Thus wrap mode not only increases the chemotactic efficiency, but also reduces the metabolic expenditure of the bacteria that is required for migration.

Finally in very strong chemotactic environments we again see less difference between the run-reverse and run-wrap-reverse patterns. This is largely due to the way the model is constructed. Given that the bacteria update their reversal frequencies every .1 seconds, using equation 1.10 we see that as $v \cdot \nabla s < 0$ decreases $-\zeta v \cdot \nabla s \Theta(-v \cdot \nabla s) \rightarrow \infty$. Thus the reversal frequency becomes increasingly large inducing a reversal almost immediately when moving down the gradient. Since this behavior drastically reduces the time spent moving away from the source, the overall chemotactic efficiency is not aided as much by the increased angular exploration afforded by wrap mode.

With this understanding, we then explored the effect of wrap mode in response to a simulated chemoattractant profile from a fungal network. We found that increasing the chance of wrap mode led to a greater ability for bacteria to explore their local environment. When compared to a run-reverse strategy alone, a run-wrap-reverse strategy increased the likelihood that the bacteria could move away from local chemoattractant maximums, and find nearby regions with a greater chemoattractant concentration. Due to this, we hypothesize that the increased exploration of wrap mode may aid bacteria in exploring more of a given hyphal network.

In section 1.9 we extended the the model to do model the dispersal, growth, and division of bacteria along fungi grown on agar. We found that as the network density increased the overall dispersal of the bacteria decreased. Furthermore, we found that the probability of the bacteria to cross from one side of the hyphae to the other was crucial to the dispersal of the bacteria. Without any ability to cross over the hyphae, the overall dispersal was greatly decreased. However, we also found that increasing the crossing probability past 5% did not continue to provide dispersal benefits.

Finally, we demonstrated the model capabilities to simulate the growth, division, and dispersal of 2 different species along a fungi. This is important since the rhizosphere is known to be the home of a plethora of different microorganisms which all live together and form complex metabolic relationships. As the constituent organisms grow, divide and move around, new communities are created in a process called range expansion. Range expansion is a very common feature of diverse

communities and can either be driven by cell growth, cell motility, or microbial hitchhiking. In microbial hitchhiking non-motile cells attach themselves to motile ones to colonize new areas [65].

During the process of range expansion, various spatial patterns are formed between the different organisms. For instance, in range expansion driven by cell growth, cooperation between organisms tends to lead to greater spatial intermixing than competitive or antagonistic relationships. In range expansion driven by motility, often unique spatial patterns can form such as spot and stipe patterns [20], concentric rings [53,68], or in the case of *Pseudomonas aeruginosa*, branching patterns [46,98]. For a good review of these topics, we refer the reader to the following review [65].

Some diversity loss or ecological drift is a common phenomenon of range expansion, however many communities within the rhizosphere remain highly diverse. To better understand how diversity is maintained in many environments, a recent study examined the mixing coefficient of two competing strains of *Pseudomonas aeruginosa* on agar, and co-cultured with the fungi *Penicillium* [86]. They found that in the presence of *Penicillium* the mixing coefficient was greatly increased. In addition, it was proposed that the liquid layer generated by the fungi was one of the main drivers to this increased mixing, allowing more cells from the center of the colony to take part in range expansion.

Motivated by these results, we plan to test the hypothesis that the mechanical environment of the liquid layer is sufficient to explain the increased mixing. In order to do this, we plan to parallelize our code on GPUs and then test 4 distinct settings: the mixing of two species on agar, on agar with a fungal network present but without chemotaxis, and finally with a fungi present as well as the corresponding chemoattractant profile generated at either the tips of the hyphae or all along the hyphae branches. In addition to the parallelization, we will calibrate the growth rate and couple the growth and division to the metabolic profiles so as to be able to compare the mixing of both cooperative and competitive species.

1.11 Code availability

The implementation of the computational model presented in this chapter is available on request from the first author (Austin Hansen) or the faculty advisor (Mark Alber).

Chapter 2

Agent Based Modeling of Neural Stem Cell Migration

2.1 Introduction

Neurological disorders encompass a wide range of different ailments and are estimated to be one of the top contributors to the overall disease burden [93]. Despite the prevalence, for many of these disorders including Alzheimer's, Parkinson's, Huntington's, multiple sclerosis, glioblastoma, and traumatic brain injuries, the current treatments are only able to treat the symptoms and are unable to address fundamental drivers. For this reason, neural stem cell (NSC) therapies have emerged as a promising direction. Unlike many other treatments, neural stem cells are able to address multiple mechanisms of action ranging from cell replacement to immunomodulation, and can even be engineered to carry therapeutic agents to the injury sites [82, 110]. Because of the broad array of benefits, many trials have been completed or are currently underway testing the safety and efficacy of these treatments [16, 64, 84, 87, 101]. In some cases, treatments such as autologous hematopoietic stem cell transplantation have become more routinely used for patients with multiple sclerosis in relapse [71, 79]. However, additional safety and efficacy testing is still needed in order for neural stem cell therapies to be widely adopted.

One of the hurdles along this path that needs to be addressed is developing optimized delivery strategies which allow for an adequate number of viable cells to reach the target site. Since in vivo

experiments can often times be costly and time consuming, mathematical modeling can provide a platform to test injection strategies and ultimately inform both experimental design as well as clinical recommendations.

In the following chapter we build a computational model of NSC migration in two distinct settings. In the first part we extend the model in [85] for modeling the migration of NSC in naïve mouse brain. The proposed model accounts for crossing white matter fibers by creating a probabilistic model based on Generalized q-Sampling Imaging (GQI) as opposed to fractional anisotropy. The model is then calibrated to experimental data showing the median distance to the injection site as well as the percentage of neural stem cells on white matter over 9 months time. We show that although the model able to capture the quantitative data, it struggles to account for observed migration to the olfactory bulb shown in the experimental data. Through further testing of the simulated injection location we predict the possible routes from the corpus collasum to the olfactory bulb based on the modeling assumptions. Our results show that this qualitative behavior is either due to a specific experimental injection location or from another factor outside tissue structure. In the second part we turn our attention to NSC migration in response to traumatic brain injury (TBI). By extending the model presented in [47], we find that diffusion of the chemoattractant over time is not sufficient to explain the experimental data presented in [51]. We then propose two possible hypothesis's as well as their corresponding models which could explain the experimental data. We then show some initial simulations of these models and discuss our future plans.

2.2 Mathematical Modeling Background

Mathematical modeling has been used to great effect to understand neurological disorders, as well as tumor growth and more particularly, cancer cell migration and invasion [6, 66, 72, 76, 80]. In addition, a number of models have been developed for collective cell migration in general. For a few reviews of the various modeling techniques we refer the reader to the following [10, 19, 21, 23, 111]. Of these, the most relevant models are those of neural crest cells, which are particular to embryonic development. In [89] a partial differential equation model was used to study the migration of neural crest cells (NCCs) in the gut and found that the proliferation of a small number of cells at the leading edge was the main contributing force to their migration. Other continuous approaches have tried to include nonlocal interactions such as cell-cell contact inhibition or attraction within continuum approaches

by means of integro-partial differential equations as seen in [77]. Despite these successes, continuum models are often unable to capture more detailed cellular characteristics. For this reason, both individual-based and hybrid models have been employed. In [69], an agent-based model confirmed that efficient streaming of NC cells relied on the presence of two distinct phenotypes, with only a few cells near the front taking the role of leader cells and the rest being followers. In [41] the utilization of a random walk approach allowed the authors to account for the effects of the Rac1 enzyme on the motion of neural crest cells, while still providing the ability to derive continuous approximations of NCC migration. Despite this, very few models have been developed for the migration of therapeutic neural stem cells.

Of the few models of neural stem cell migration, both have been primarily based on tissue anisotropy [47,85]. This is in part due to the fact that many cells, including NSCs, show preferential movement along white matter tracts. Although the exact mechanism for this behavior is largely unknown, many studies have begun to take tissue structure into account as a force in cell migration. For example, computational models of glial cells which include the effects of tissue anisotropy, in addition to diffusion and proliferation, are better able to capture the geometry of glioma than those that exclude tissue anisotropy [95]. In addition, NSC clusters were shown to be oriented along the directions of white matter fibers [85] providing good evidence for this assumption. In the next section we outline these models as well as their corresponding assumptions below.

2.3 Modeling Assumptions and Basic Model Equations

The basic modeling equations that were proposed in [47,85], form the foundation for the extensions within this chapter. In [85] a simple model of NSC migration was proposed based on the assumption that the motion of a neural stem cell within an uninjured naïve mouse brain was primarily driven by the tissue structure in the brain. In particular, it was assumed that a NSC will demonstrate directed migration on white matter tracts and randomized migration in grey matter. The classification of white and grey matter here is decided by a threshold fractional anisotropy value which captures the degree of alignment within the brain fibers. Based on these assumptions, the discretized equation of motion is

$$\mathbf{x}_{i+1} = \mathbf{x}_i + \mathbf{b}(\mathbf{x}_i, \mathbf{x}_{i-1})\Delta t. \quad (2.1)$$

where $\mathbf{x}_i$ is the position of the neural stem cells at time t_i and

$$\mathbf{b}(\mathbf{x}_i, \mathbf{x}_{i-1}) = \begin{cases} [\text{sgn}((\mathbf{x}_i - \mathbf{x}_{i-1}) \cdot \mathbf{M}_{ev}(\mathbf{x}_i))] \cdot d_w \mathbf{M}_{ev}(\mathbf{x}_i), & M_{coh}(\mathbf{x}_i) \geq \epsilon_M \\ \xi(\omega) \cdot d_g, & M_{coh}(\mathbf{x}_i) < \epsilon_M. \end{cases} \quad (2.2)$$

Here point $\mathbf{x} = (x, y, z)$ is considered to be white matter if the fractional anisotropy $M_{coh}(\mathbf{x}) \geq \epsilon_M$ for some threshold value ϵ_M , and grey matter otherwise. In addition, $\mathbf{M}_{ev}(\mathbf{x}_i) = \frac{\mathbf{e}_1(\mathbf{x})}{\|\mathbf{e}_1(\mathbf{x})\|}$ is the normalized eigenvector $\mathbf{e}_1$ corresponding to the largest eigenvector at location $\mathbf{x}$ from diffusion tensor imaging, d_g and d_w are step sizes, and $\xi_i(\omega)$ is a random sample on the surface of a unit sphere such that $\|\xi_i\| = 1$. Since $\mathbf{e}_1$ is directed along the primary direction of the tissue alignment, based on equation 2.2, a NSC's motion will either be in the direction of $\mathbf{e}_1$ with a step size d_w or will be random with a step size d_g . The addition of $\text{sgn}((\mathbf{x}_i - \mathbf{x}_{i-1}) \cdot \mathbf{M}_{ev}(\mathbf{x}_i))$ chooses the direction of the motion along $\mathbf{e}_1$ which is most aligned with the previous chosen direction.

In [47] this model was extended based on the assumption that chemotaxis would be an additional driving force for the migration of neural stem cells in tumor bearing mice. In addition it was assumed that there would be some level of stochasticity in both the step sizes d_g , d_w , as well as the chemotaxis sensitivity. Hence the discretized equation of motion presented here is

$$\mathbf{x}_{i+1} = \mathbf{x}_i + \left[\mathbf{b}(\mathbf{x}_i, \mathbf{x}_{i-1}) + \hat{\lambda} \nabla C(\mathbf{x}_i) \right] \Delta t. \quad (2.3)$$

where $\hat{\lambda} = \eta(\omega) \cdot \lambda$ with λ being the chemotaxis sensitivity and $\eta(\omega) \sim \text{B}[\alpha_c, 1]$. Furthermore $\mathbf{b}$ is the same as equation 2.2 except the step sizes are made stochastic by multiplying both d_g and d_w by random sample $\psi(\omega)$ with $\psi(\omega) \sim \text{B}[1, \beta_w]$. $C(\mathbf{x})$ represents the concentration of the chemotattractant at position $\mathbf{x}$. In [47], it was assumed that the diffusion of chemoattractant occurred at a larger time scale than the migration of the neural stem cells and therefor was static throughout the tested simulations.

2.4 NSC Migration in Naïve Mouse Brain

2.4.1 Biological Background

One of the main assumptions of the previous NSC migration models is that tissue structure is a primary driving force in the direction of motion. In the naïve brain, tissue structure is the deciding factor, however, even when chemotaxis considered in equation 2.3, the effects due to white and grey matter can have a large impact on the motion of the cell. Furthermore, these effects are largely modeled in a deterministic manner, even though the complex tissue structure of the brain likely produces more random outcomes. In large part, this shortcoming is due to the reliance on Diffusion Tensor Imaging to quantify tissue structure, which is subject to a number of challenges stemming from its inability to resolve multiple fiber directions. Due to this, fractional anisotropy (FA) is unable to accurately capture specific microstructures in places of crossing fibers and is also insensitive to certain changes in white matter that come from damage or disease, which may be crucial in pathologies like glioma and traumatic brain injury. For example, it is well known that axonal diffusivity and radial diffusivity, both of which come from DTI, are often used to measure axonal and myelin damage. However, these measures can be affected by swelling and edema in ways that more advanced imaging techniques are not [112]. In one study, it was shown that for mild traumatic brain injury, DTI was not as sensitive as q-space imaging to changes in axonal volume that come from axonal shearing or twisting among other causes [108]. For a good review of the advantages and disadvantages of different imaging techniques see [112]. Despite these challenges with DTI imaging, Generalized Q-sampling imaging has been shown to accurately capture multiple crossing fibers in a single voxel, and was subject to less artifacts such as deletions or incompleteness when compared to DTI [55]. Moreover, in a study that examined 18 patients with eloquent gliomas, it was found that GQI was better able to capture metrics such as tract volume, density, and length [12]. It has also been suggested that GQI may aid in preoperative planning for patients with low-grade gliomas by helping to distinguish areas of the brain in which the glioma has infiltrated or displaced certain white matter tracts [25].

Thus, in order for NSC migration models to be adequately predictive for use in clinical settings, the utilization of more robust imaging techniques is likely a critical step. To address these limitations, we first focus on the naïve mouse brain and extend the model presented in equation 2.1 in two ways: first, through the use of GQI reconstruction we are able to create a probabilistic model which captures competing crossing fibers [37, 55, 113]; second, we alter the model to allow the motion of each cell to

be a continuous sum of directed and random directions based on the underlying anisotropy. Section 2.4.3 outlines the model equations and parameters, section 2.4.2 explains the experimental data to which the model is then calibrated to, while section 2.4.4 outlines the results from our parameter study, as well as the calibration and validation. Finally, in section 2.4.5 we discuss some of the limitations of the model and future directions for this work.

2.4.2 Experimental Data

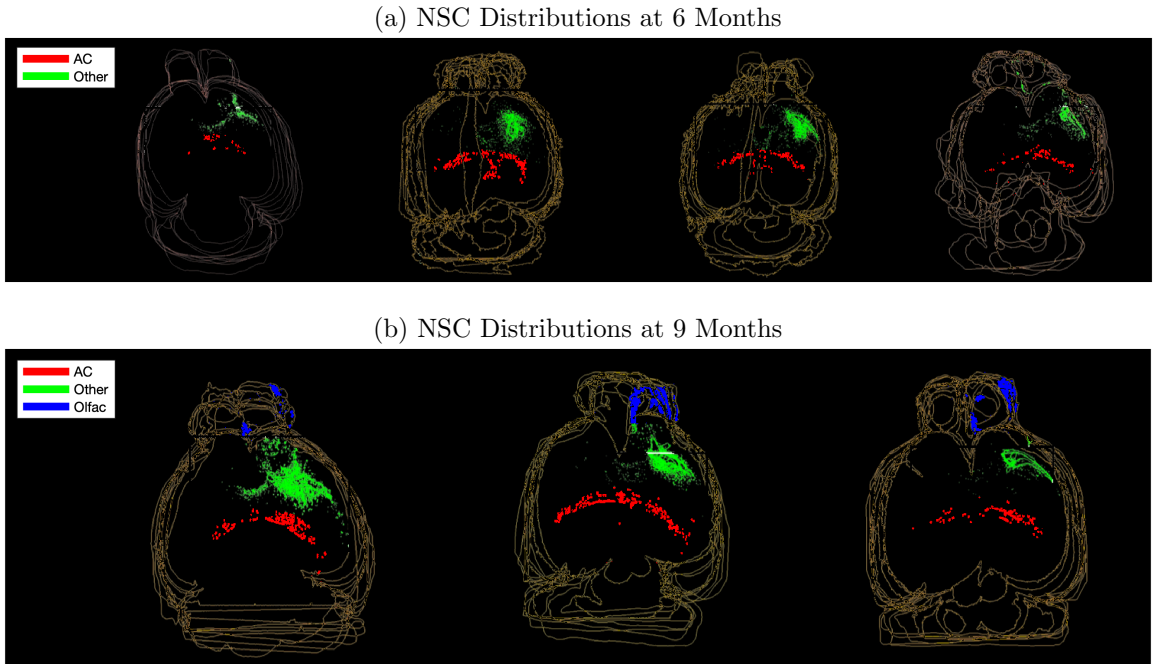


Figure 2.1: Experimental images from mice euthanized at 6 months (a) and 9 months (b). Figure shows the completed segmentation with red corresponding to the cells in the anterior commissure and blue within the olfactory bulb. The percentage of NSCs that arrived at anterior commissure at 6 months is estimated to be 4.6% , 12.2% , 5.5%, and 6.7% for each of the 4 samples with an average of 7.2%. Similarly the percentage of NSCs that arrived at olfactory bulb at 9 months is estimated to be 4.2% , 20.4% , and 33.4% for each of the 3 samples with an average of 19.3%. The percentages in the olfactory bulb demonstrates variability across different mice.

In order to calibrate the model we use time series data taken from previously published results examining the migration of neural stem cells in the naive mouse brain [85]. The authors experimentally injected NSC's into the frontal right part of the corpus callosum and then euthanized the mice at 3,6, and 9 months. Stainings were then completed for each mouse and the median distance from the injection site, as well as the percentage of cells on white matter was quantified for the 3 groups.

This process was completed for 5 mice at the 3 month mark, 4 mice at 6 months, and 3 mice at 9 months. Further details of the experimental design can be found here [85]. It was found that the median distance from the injection site was $437\mu m$, $1030\mu m$, and $902\mu m$ at 3, 6, and 9 months respectively, while the percentage on white matter was 66%, 72%, and 59%. In addition to this, it was observed that the NSC's remained largely in the corpus collosum at 3 months, but were able to migrate to the anterior commissure by 6 months, and to the olfactory bulb by 9 months. In order to get a rough quantification we take the 2D projected images of the mice at 6 and 9 months and quantify the percent of cells in the anterior commissure and olfactory bulb. Quantification's were done using Matlab's Color Thresholder as well as other built-in segmentation functions. The percentage calculations were performed by counting the number of pixels within the anterior commissure and olfactory bulb and weighting each pixel by its "value" or brightness from a HSV color map. The segmentations can be found in Figure 2.1. We estimate that there are an average of 7.2% of cells in the anterior commissure by 6 months, 8.5% of cells in the anterior commissure by 9 months, and 19.3% of cells in the olfactory bulb by 9 months.

2.4.3 Mathematical Model

We make the assumption that tissue structure is the primary driving force of neural stem cell migration in the absence of chemical signals. Furthermore, since white matter tracts are highly aligned while grey matter tracts are comparatively disorganized, the direction of a neural stem at a given time point will be a combination of directed motion along the white matter tract and random motion, with the relative proportion dependent on the degree of anisotropy. Thus, the equation for the position of a neural stem cell $\mathbf{x}_{k+1}$ given its position at time k is as follows:

$$\mathbf{x}_{k+1} = \mathbf{x}_k + \underbrace{w_{QA}(\mathbf{x}_k) \cdot d_w \cdot \mathbf{M}_{ev}(\mathbf{x}_k)}_{\text{Directed Motion}} + \underbrace{(1 - w_{QA}(\mathbf{x}_k)) \cdot d_g \cdot \sigma(\mathbf{x}_k) \cdot \boldsymbol{\xi}(\omega)}_{\text{Random Motion}} \quad (2.4)$$

where, $w_{QA}(\mathbf{x}_k)$ is a scaling factor dependent on the degree of anisotropy, d_w is the maximum step size along white matter, d_g is the maximum step size along grey matter, $\mathbf{M}_{ev}(\mathbf{x}_k)$ is the white matter fiber direction, $\sigma(\mathbf{x}_k)$ is the mean diffusivity, and $\boldsymbol{\xi}(\omega)$ is a random sample on a 3D unit sphere. Each of these is defined explicitly below.

2.4.3.1 Determination of Fiber Direction

The direction of the vector $\mathbf{M}_{ev}$ is probabilistically chosen based on the first three fiber directions resolved through generalized q-sampling imaging (GQI). In GQI reconstruction, each fiber direction is given a quantitative anisotropy value (QA), which measures the increased diffusion along a given direction over the background isotropic diffusion. The higher the QA value in a direction, the stronger the tissue alignment in this direction. We use GQI in conjunction with traditional DTI reconstruction to build a model that can accurately differentiate the following scenarios regarding fiber alignment and fiber density shown in Fig. 2.2, denoted as cases (A–D).

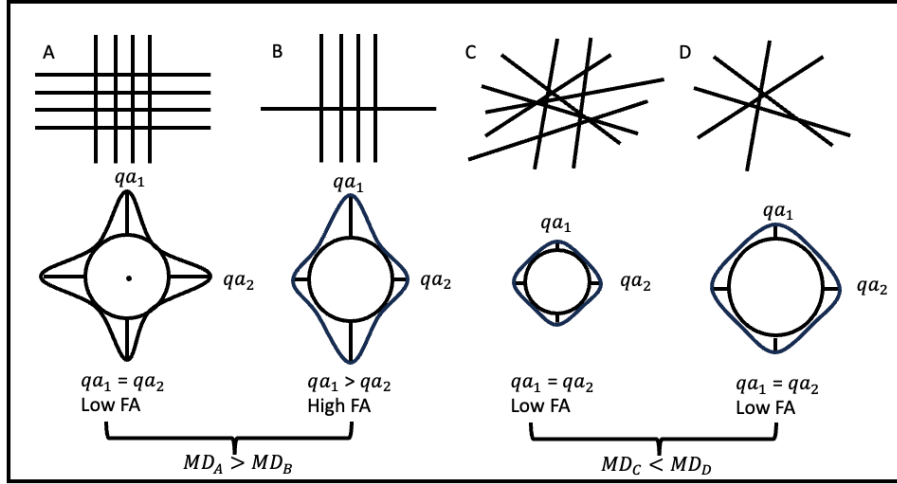


Figure 2.2: Different scenarios of tissue orientations in the top row and the corresponding resolution from GQI in the bottom row. For simplicity all of this is shown in 2D. Here, the acronyms are quantitative anisotropy (qa), fractional anisotropy (FA), and mean diffusivity (MD). (A) shows two equal crossing fibers which are highly aligned (B) shows a single white matter fiber (C) shows a densely packed region with little alignment, (D) shows a sparsely packed region of little alignment.

In the case of A and B, GQI is able to successfully distinguish between locations with multiple crossing fibers, as well as locations with only one major fiber direction. In A for example, GQI would yield two large QA values, qa_1 and qa_2 , for each of the major fiber directions. In contrast, B depicts a scenario in which $qa_1 > qa_2$ representing only one major fiber at this location.

For modeling purposes we only consider the first three QA values obtained from GQI reconstruction, denoted as qa_1 , qa_2 , and qa_3 , corresponding to the fiber directions, dir_1 , dir_2 , and dir_3 , respectively. Furthermore, in order to reduce the noise from any insignificant non-primary fiber directions, we eliminate secondary and tertiary QA values that are less than 0.1 by making them zero. Thus, when a NSC is at a location with multiple QA values greater than 0.1, the migration direction is

probabilistically chosen based on both the relative QA values and the momentum of the cell. Here we include some momentum within the direction selection so as to allow a NSC a greater ability to follow a secondary/tertiary fiber once it has been chosen. The probability to follow the direction of the i -th fiber based on anisotropy is defined as

$$\text{QAProb}_i = \frac{qa_i}{\sum_{j=1}^3 qa_j}, \quad i = 1, 2, 3. \quad (2.5)$$

To model the probability of choosing the i -th fiber due to momentum we compute the angle between the NSC's motion in the previous time step, $\text{NSC}_{\text{dir}} = \mathbf{x}_{k-1} - \mathbf{x}_{k-2}$, and the i -th fiber direction dir_i . Suppose n_i is the number of fiber directions at position $\mathbf{x}_k$.

Then we have

$$\text{DirProb}_i = \begin{cases} 1 & n_i = 1 \\ 1 - \frac{\text{Angle}_i}{\sum_j \text{Angle}_j} & n_i > 1 \text{ and } qa_i \neq 0 \end{cases} \quad (2.6)$$

Since it is unknown how much either of these factors contribute to motion independently, we assume that the overall probability is equally weighted between both. Hence the final equation for the probability of choosing the i -th fiber is

$$P(\text{NSC to choose the } i\text{-th fiber}) = P_i = \frac{1}{2}\text{QAProb}_i + \frac{1}{2}\text{DirProb}_i \quad (2.7)$$

The direction $\mathbf{M}_{ev} = \text{dir}_i$ is then selected at each time step based on the calculated probability for each cell at its current location.

2.4.3.2 Determination of Mean Diffusivity

In the case where there is little tissue alignment, quantitative anisotropy alone cannot distinguish those regions of the brain in which tissues are tightly packed from those in which there is less density due to factors such as myelin degradation or cerebrospinal fluid. In order to help distinguish between scenarios C and D of Fig. 2.2, we use mean diffusivity since it is a good measure of overall diffusion and can successfully quantify these differences. The mean diffusivity σ is defined as the average of the three eigenvectors resolved through DTI reconstruction, and is typically measured in $\frac{mm^2}{s}$. We only consider the effects of mean diffusivity within the randomized movement. This is done by multiplying the grey matter velocity by $\sigma(\mathbf{x}_k) = \frac{MD(\mathbf{x}_k)}{\max(MD)}$, where MD is the mean diffusivity. Thus,

the higher the mean diffusivity the more random motion will contribute to the overall motion. Note that this also has the effect of making the contribution from directed motion larger when both step sizes d_g and d_w are equal.

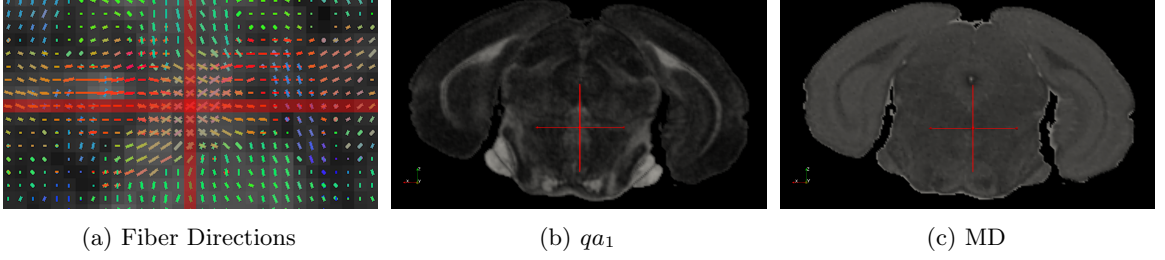


Figure 2.3: (A) shows the fiber directions as visualized in DSI-Studio for a given cross section (B) shows a cross section of the of the first quantitative anisotropy value (C) shows a cross section of the of the mean diffusivity. For B and C the lighter the color, the greater the value

2.4.3.3 Stochastic Migration Step Sizes

Both parameters d_w and d_g represent the step size that a neural stem cell can travel in a given time step along the white matter and grey matter, respectively. Due to heterogeneity in neural stem cell populations, not all cells have the same motility. We account for this stochasticity by modeling d_g and d_w as

$$d_w = \psi(\omega) \cdot \bar{d}_w$$

$$d_g = \psi(\omega) \cdot \bar{d}_g$$

where $\psi(\omega) \sim B[\alpha, \beta]$ is a random variable with beta distribution, and $\bar{d}_w$ and $\bar{d}_g$ are the maximum step sizes on white and grey matter, respectively. Furthermore, we take the parameters in the beta distribution as $\alpha, \beta \geq 1$. Note when $\beta > 1$ and $\alpha = 1$ the majority of cells move at a slower velocity, while only a few outliers move more quickly. In contrast, when $\alpha > 1$ and $\beta = 1$, most cells have a higher velocity and only a few move comparatively slow.

2.4.3.4 Proportion of Directed and Random Motion

The overall proportion of directed vs. random motion for a cell at position $\mathbf{x}$ is determined by the QA value of the chosen direction $\mathbf{M}_{ev}$. Thus, w_{QA} is represented by the hill function

$$w_{QA} = \frac{qa(\mathbf{x})^n}{qa(\mathbf{x})^n + K^n}$$

Parameter	Biological Meaning	Value
qa_i	Level of diffusion along the i th fiber resolved from GQI	*
dir_i	The i th fiber direction resolved from GQI	*
σ	Mean diffusivity	*
n	Hill coefficient	7
K	Level of anisotropy in which motion is half directed and half random	.18
d_g	Maximum step size of NSC migration on grey matter	.25
d_w	Maximum step size of NSC migration on white matter	.1
α_w	Stochasticity in migration speed parameter 1	1
β_w	Stochasticity in migration speed parameter 2	1

Table 2.1: Model parameters and their descriptions. * The first three parameters, qa_i , dir_i , and σ , are obtained from GQI reconstruction. The others are model parameters.

Here the closer w_{QA} is to 1 the more of its motion is directed by the tissue structure. In contrast, the closer w_{QA} is to 0, the more random the motion becomes. In addition, we point out that K represents the QA value in which half of the motion comes from the fiber direction and the other half is a random walk.

2.4.4 Results

2.4.4.1 Comparison between Single and Multi-Fiber Migration Models

In this section, we first illustrate the difference between a single fiber migration model using DTI and the probabilistic multi-fiber migration model based on GQI. For the single fiber migration model, the equation of motion is the same as in equation 2.4, except M_{ev} is set to the single direction resolved from DTI and $qa_1 = FA$ while $qa_2, qa_3 = 0$. Here FA is fractional anisotropy calculated from the eigenvalues. To accurately isolate the differences from the addition of multiple fibers, we remove the stochasticity parameters from the step sizes and set $d_g = 0.1$ and $d_w = 0.1$. The simulation is initialized with 100 NSCs in a single voxel located within a region with a high density of secondary fibers, shown in Figure 2.4 as a black dot. The simulations are run for 100 time steps equating to 1 day's time. Other parameters are set as $K = 0.18$, $n = 7$, and $\alpha = \beta = 1$.

In Figure 2.4(a) we see the migratory pathways from the primary fiber model based on DTI data. Here we see that all 100 cells roughly follow the same path and only near the very end do the cells start to show some hints of diverging into different trajectories. In contrast, the probabilistic multi-fiber model based on GQI reconstruction in Figure 2.4(b) shows multiple different pathways accessible to the cells. We can see that some cells follow the same white matter fiber as before, but

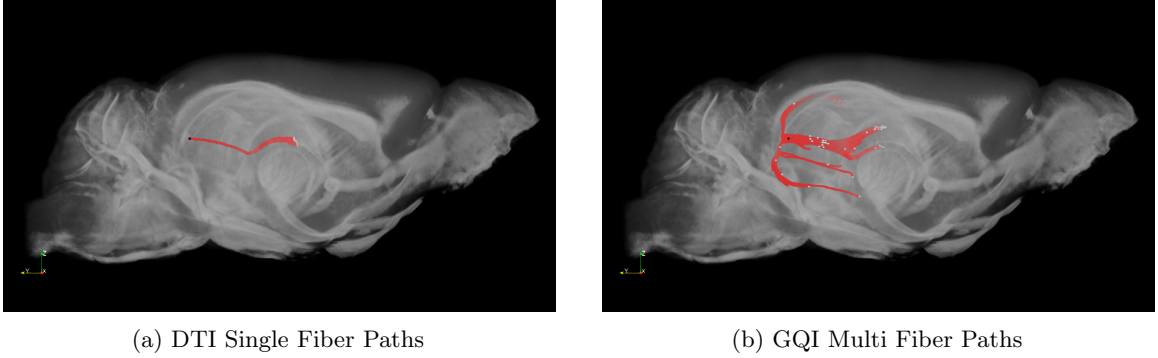


Figure 2.4: Comparison of simulated NSC migration trajectories. (a) 100 trajectories using DTI, which only resolves the primary fiber. (b) 100 trajectories using GQI reconstruction, which resolves multiple crossing fibers. In both plots, the black dot is the cell’s starting location and the white dot shows the final location.

we also have some secondary and tertiary fibers that lead vertically. This allows the cells to move up and down before finding fibers that are directed toward the front of the brain. The results of multi-fiber model demonstrate the NSCs’ enhanced ability to explore a broader range of regions within the brain.

2.4.4.2 Parameter Sensitivity Analysis

We now study the sensitivity of the model parameters $\bar{d}_w$, $\bar{d}_g$, K , n , α , and β . To do this, we run a set of 400 simulations where the parameter values were chosen by a Latin Hypercube Sampling with $\bar{d}_g$ and $\bar{d}_w \in [0, .6]$, $K \in [0, .5]$, $n \in [1, 30]$ and $\alpha, \beta \in [1, 100]$. The partial rank coefficient was then calculated for each parameter for the median distance and percent on white matter at 9 months. In Figure 2.5 we show the calculated PRCC values. We find that $\bar{d}_g$, $\bar{d}_w$, and K have the largest effect on the median distance from the injection site, as well as the percent on white matter over the course of the simulated 9 months. In particular, we find that K is inversely related to the median distance and percent on white matter, while n , α and β do not have a large impact on either of these quantities. However, we note that α and β govern the number of outliers in the distance from the injection site despite not having a large overall effect on the median distances.

In addition, we find that $\bar{d}_w$ is positively correlated with the median distances and is inversely related to the percent on white matter while $\bar{d}_g$ is positively related to both the median distance and the percent on white matter. However, we do observe some non-monotonicities for the parameter $\bar{d}_g$ when we plot the residuals $(x - \hat{x}_j)$ and $(y_j - \hat{y})$ where x and y are the rank transformed $\bar{d}_g$ values

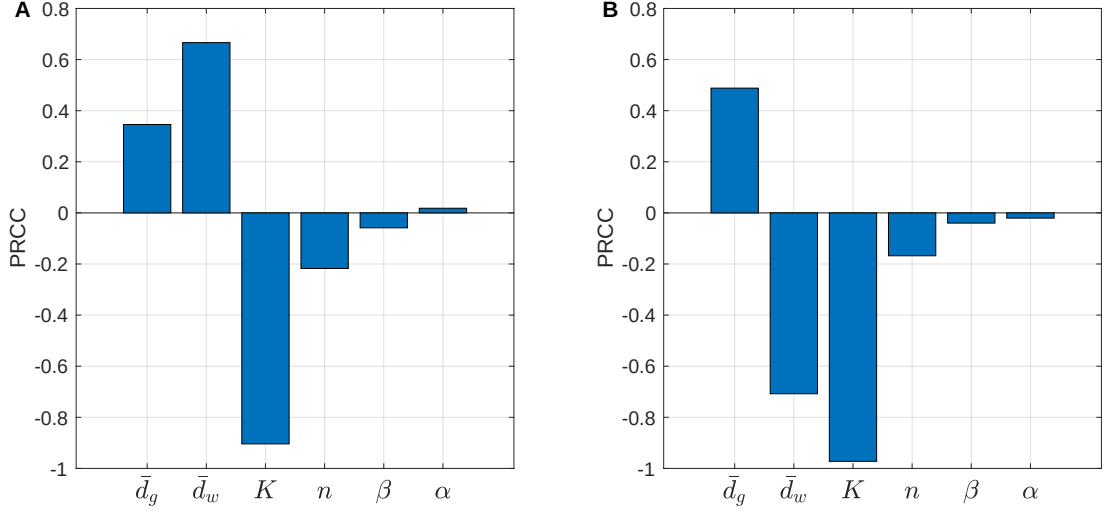


Figure 2.5: PRCC values shown for 6 parameters and their effect on (a) median distance at 9 months (b) percentage of NSC on WM at 9 months.

and 9 month median distances respectively and $\hat{x}$, $\hat{y}$ are from the linear regression models. The residual for $\bar{d}_g$ is depicted in Figure B.2 of appendix B. In order to identify the cause, we examined the parameter values that corresponded to these outliers and found that the majority of the outliers occurred with either a very low $\bar{d}_g$ or $\bar{d}_w$ value. Given this information, we calculated the PRCC for a set of simulations in which $\bar{d}_g$ and $\bar{d}_w$ were varied evenly in the range $[.0025, .15]$ and all other parameters were held constant with $K = .25$, $n = 7$, and $\alpha, \beta = 1$. In this we find that the PRCC value for d_g = and the corresponding residual plot shows a strong inverse relationship.

In addition to this change in PRCC for low step sizes, we also hypothesize that the ratio of $\bar{d}_g$ and $\bar{d}_w$ may play a crucial role given their opposite effects on the percentage of white matter. In order to explore this, we look at how the change in percent on white matter varies with the ratio of $\bar{d}_w$ to $\bar{d}_g$. In Figure 2.6(a), we plot the change in the percentage of NSCs on white matter measured by $\Delta W = W(t_{\text{end}}) - W(t_0)$, where $W(t)$ is the percentage of NSCs on white matter at time t . Here, t_0 is the initial time and $t_{\text{end}} = 27000$ or 9 months time. We see that the change in the percentage on white mater is positive when $\bar{d}_w/\bar{d}_g$ is very small, but as the ratio $\bar{d}_w/\bar{d}_g$ increases, the change in percentage becomes negative and decreases rapidly. In other words, when $\bar{d}_w/\bar{d}_g$ is large, NSCs tend to move out of the white matter. This implies that when the random movement, $\bar{d}_g$, dominates the migration, the cells collect on white matter, and when directed movement, $\bar{d}_w$, dominates the

migration, the cells tend to move to more grey matter locations. This depicts the inverse relation between the change in percent on white matter ΔW and $\bar{d}_w/\bar{d}_g$, and that ΔW may be an important measure in calibrating the step size parameters.

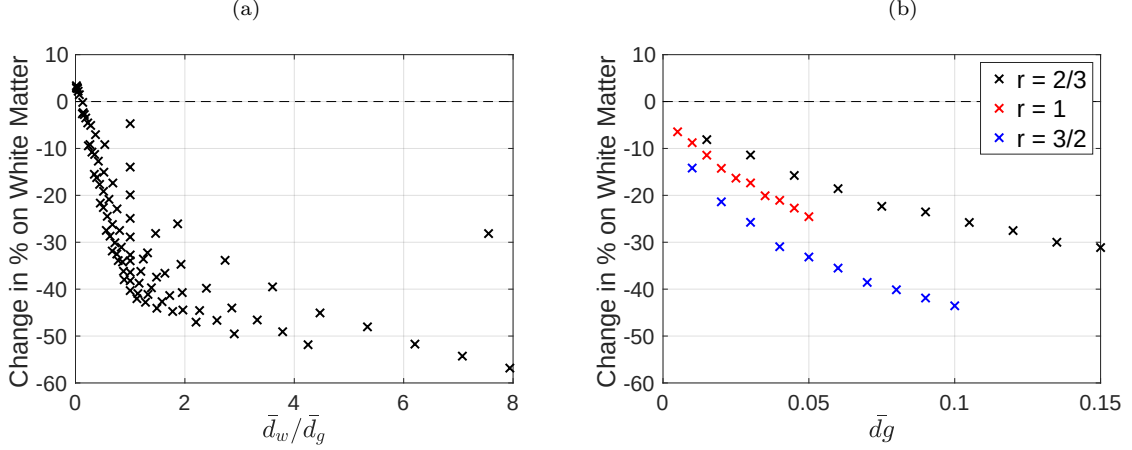


Figure 2.6: Change of percentage of NSCs on white matter depending on migration step sizes, $\bar{d}_w$ and $\bar{d}_g$. (a) The percent of NSCs on white matter decreases rapidly as the ratio of directed and random movement step sizes, $r = \bar{d}_w/\bar{d}_g$, increases. (b) The percent change on white matter is plotted for fixed step size ratios, $r = 2/3, 1, 3/2$. The percent on white matter also decays as the magnitude of step size $\bar{d}_g$ increases.

This however does not show the whole picture as we can see for each ratio there is some variance in the change in percentage on white matter. Thus, we consider three different fixed ratios $\bar{d}_w/\bar{d}_g = \frac{2}{3}, 1, \frac{3}{2}$, and observe how the change in percentage on white matter varies as the magnitude of the step sizes increases. To accomplish this, we set $\bar{d}_w = r_1 \cdot d_{\text{step}}$, $\bar{d}_g = r_2 \cdot d_{\text{step}}$ and vary $d_{\text{step}} \in [.005 : .05]$ for a fixed $r = \frac{r_1}{r_2} = \frac{2}{3}, 1, \frac{3}{2}$. Ten simulations are run for each ratio. Figure 2.6(b) shows that as the magnitude of the step sizes increases, the cells slowly occupy more grey matter. Thus not only does the ratio matter, but the velocity of the cells helps to determine how many cells are one white or grey matter by the end of the simulations. From this we hypothesize that for low step sizes the decrease in the median distance due to increasing $\bar{d}_g$ is likely due to an increased accumulation of cells on the local white matter tracts which traps cells and decreases their ability to travel to other regions of the brain. We conclude that both the migration distance and the percentage of NSCs on white matter is an important quantity for calibrating the model parameters, and that very low $\bar{d}_g$ and $\bar{d}_w$ values can likely be ruled out due to the reversed effects in this region.

2.4.4.3 Injection Location and Path Clustering Analysis toward Target Regions

In this section, we study the potential injection locations and migration paths to the specific target regions. An important qualitative trend found in the experiments reported in section 2.4.2 is the accumulation of NSCs in the anterior commissure at 6 months and the olfactory bulb at 9 months. In addition to the model parameters, a huge governing factor for this behavior is the injection location. Furthermore, since we estimated the percentage of NSCs in the olfactory bulb to be around 19% at 9 months, we hypothesize that cells must be traveling along major white matter paths to reach this region in sufficient quantities. To identify potential pathways to the anterior commissure and olfactory bulb, we inject 524,536 cells into the corpus callosum uniformly and simulate their migration for 9 months. The number of cells was chosen so that four cells were initialized at each lattice point within the corpus callosum. This was done for nine combinations of $\bar{d}_g$ and $\bar{d}_w$ values, with each being selected from the following set $\{0.1, 0.35, 0.6\}$. Figure 2.7 shows the heat maps of the NSC injection locations that made it to the anterior commissure and the olfactory bulb at any point during the simulation. The plotted quantity shows the number of NSCs that started at the corresponding injection location. We note that the plotted values are the maximum value of all the vertical Z slices within each X/Y bin, to visualize it from top view.

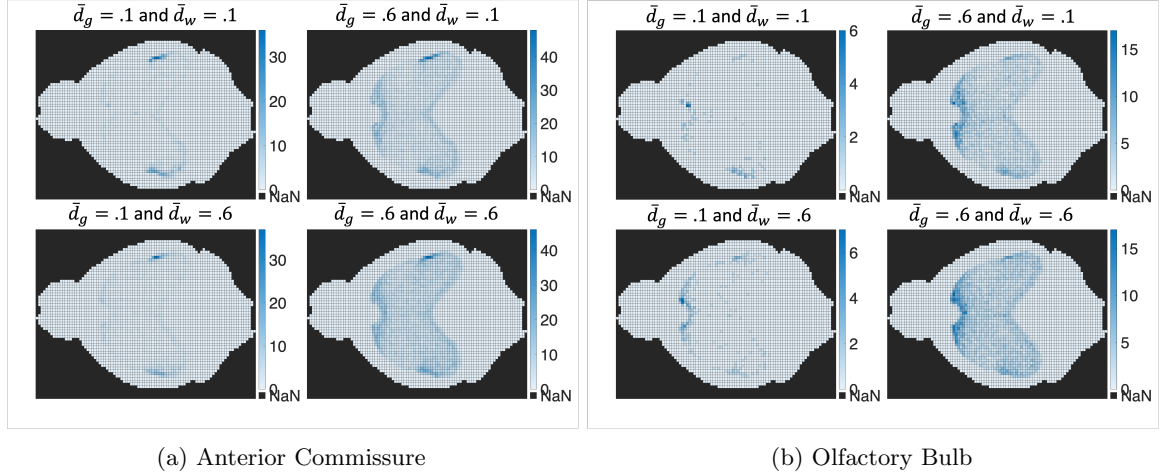


Figure 2.7: Heat maps showing the NSC injection locations for cells reaching the target regions, (a) anterior commissure and (b) olfactory bulb. Quantities show the maximum count for a vertical slice in the X/Y region.

In Figure 2.7(a), we notice that the injection locations that most reliably lead to the anterior commissure occur along the lateral edges of the corpus callosum. When the migration step sizes are

small, $\bar{d}_g = 0.1$ and $\bar{d}_w = 0.1$, this is one of the only locations that lead to the anterior commissure. However, as we increase both directed and random migration step sizes, more locations in the brain show up including all along the edges of the corpus callosum and in particular the front of the corpus callosum. In general, with larger step sizes more cells end up reaching the anterior commissure partly due to random motion. However, we note that persistent region across all parameter combinations is the lateral edges. For cells that reached the olfactory bulb shown in Figure 2.7(b), a similar behavior is observed, but the persistent region in this case occurs in the front part of the corpus callosum.

To identify robust pathways to the two target regions, we perform path clustering on the trajectories that made it to the anterior commissure and the olfactory bulb. This is done using k -medoids, where the distance between two trajectories is calculated as follows: First, for each day of the trajectory, the coordinates of the cell are binned by its position in the GQI image. Then a unique list of bins is created for each trajectory. Finally, the distance between two trajectories is calculated depending on the number of bins they have in common. In this way, if two trajectories share the exact same list of unique bins, the distance is zero.

In Figure 2.8, we plot the center of the k -medoids clustering with $k = 7$. Here, we notice some key findings. As shown in the heat maps above, the main trajectories leading to the anterior commissure start on the lateral sides and travel down to the temporal limb of the anterior commissure. In fact, 5 of the 7 trajectories shown in Figure 2.8(a) follow the white matter fibers in this region. In addition, we see two other paths, one which passes from the corpus callosum to the Fornix, and finally down to the anterior commissure, and one which passes from the front of the corpus callosum down to the temporal limbs of the anterior commissure. In contrast, for the trajectories that lead to the olfactory bulb shown in Figure 2.8(b), we see that cells either pass through the anterior commissure, the temporal limb, and finally to the olfactory bulb, or they follow a path down from the front of the corpus callosum directly to the olfactory bulb. We note that in the experiments described in section 2.4.2, the NSCs are injected at the front of the corpus callosum. Among the combined simulated paths leading to the anterior commissure and the olfactory bulb, the only injection site at the front of the corpus callosum is the one that follows a trajectory through the olfactory limb before migrating toward these regions. Therefore, we predict that the NSC injection site in the experiments would have overlapped with this identified location. Moreover, we emphasize that our model can identify potential injection sites and likely migration paths to any target location, offering a promising approach to improve the efficacy of NSC treatments.

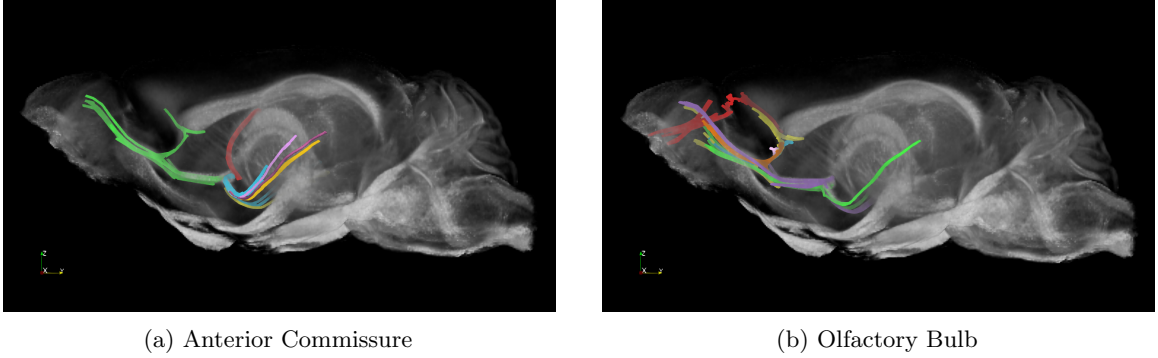


Figure 2.8: Predicted migration pathways of NSC trajectories reaching the target regions, (a) anterior commissure and (b) olfactory bulb. The paths are computed with k-medoids clustering with $k = 7$.

2.4.4.4 Significance of Injection Location in Target Arrival Rates and Model Calibration

Using the estimated experimental injection location and corresponding experimental data, we calibrate the model parameters, $\bar{d}_g, \bar{d}_w, K$. We calibrate only these three parameters since they are the most sensitive parameters to the experimental data. We estimate the best parameter set that minimizes the relative sum of squared errors of migration distance and percentage on white matter at 3, 6, and 9 months. The set of optimal parameters are $\bar{d}_w = 0.1$, $\bar{d}_g = 0.25$, $K = 0.18$. See Figure B.10 for the heat maps of the errors, which was narrowed down to $\bar{d}_g/\bar{d}_w \in [0.01, 0.35]$ and $K \in [0.22, 0.27]$ based on the error terms.

We now take the calibrated parameter set, $\bar{d}_w = 0.1$, $\bar{d}_g = 0.25$, $K = 0.18$, and look at the percentage of cells that arrive at the anterior commissure and olfactory bulb. Here we notice that the overall distribution of cells to the olfactory bulb is relatively small, with around 3.83% of the cells in the anterior commissure at 6 months and 7.7% of the cells being located in the olfactory bulb at 9 months. Since the experimental average is 7.23% and 19.9% respectively, we note that there is a difference between the experimental results and the simulation results. Furthermore, we note that the calibration of $\bar{d}_g, \bar{d}_w$, and K is effected by the injection location as well. To illustrate this, we take the optimal parameter set found above and run simulations in six alternate injection locations (2–6) which were of the most viable locations found in section 2.4.4.3. Figure 2.9 shows where these regions are located, while Table 2.2 shows the results of each simulation. We see that the original location (1) and adjacent locations (2, 6) have the highest percentage of cells in the anterior commissure and olfactory bulb, while all the other locations show significantly lower percentages here. Moreover, the error from the median distance and percentage on white matter also varies significantly.

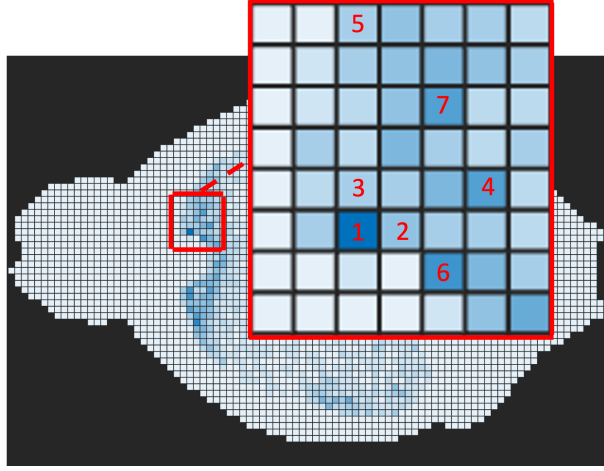


Figure 2.9: Heat map showing the injection sites for NSCs reaching the olfactory bulb at any point during the simulated 9 months and the selected seven injection locations (1–7) that were tested. The heat map shown is for $\bar{d}_g = .1$ and $\bar{d}_w = .35$

Location	Exp. Error	AC 6 Month%	AC 9 Month%	Olfac 6 Month%	Olfac 9 Month%
1	0.142	3.60%	6.73%	3.83%	7.77%
2	0.308	3.46%	7.48%	3.45%	7.97%
3	1.050	0.67%	0.97%	1.02%	1.55%
4	6.774	0.42%	0.18%	0.68%	0.32%
5	1.023	0.32%	0.05%	0.52%	0.12%
6	5.401	1.63%	3.44%	1.71%	3.89%
7	0.632	0.18%	0.08%	0.31%	0.16%

Table 2.2: Calibration error and percentages of NSCs arrived in anterior commissure (AC) and olfactory bulb (Olfac) at 6 and 9 months for simulations run at different injection locations (1–7) shown in Figure 2.9.

Since slight changes to the injection locations yield significant effects on the measured statistics, we hypothesize that such changes in the injection location could explain some of the variation we see between mice in the experimental data. For example, the estimates we found for the number of cells that made it to the olfactory bulb for each individual mouse were 33.3%, 20.3%, 4.2%. This illustrates a huge qualitative difference between the first two mice and the third. Our model similarly shows large differences in the percentage of cells that are able to make it to given regions; however, it is also suggestive of the fact that very few locations in the upper region of the corpus callosum lead to robust migration to the olfactory bulb. Hence we conclude that this behavior is either a product of injecting into a very specific location, or that tissue anisotropy alone is not adequate enough to explain the experimental results. We discuss this as well as some alternatives in the discussion section.

2.4.5 Discussion

In this section we present a probabilistic, agent-based model of neural stem cell migration in naive mouse brain that accounts for multiple crossing fibers. Using high-resolution GQI reconstruction, we are able to more accurately simulate the migration of NSCs along secondary and tertiary fibers within the brain. We demonstrate this ability by simulating the migration of 100 NSC's in a region of high crossing fibers and find that the cells are better able to traverse the resolved fiber directions when compared to a model which only uses DTI data. We show that the ratio $\bar{d}_w/\bar{d}_g$ is inversely related to the change in percentage on white matter demonstrating the usefulness of the temporal changes in white matter percentages for model calibration. However, we note that our work utilizes experimental data collected at three time points - 3, 6, and 9 months - and argue that additional temporal data not only would enhance the parameter calibration of this model, but might also uncover further mechanisms underlying NSC migration in the brain.

We also performed path clustering on NSCs injected uniformly throughout the corpus callosum and found that there was only one main white matter path that led to the anterior commissure and the olfactory bulb in the upper right region of the corpus callosum which corresponded to the injection location used experimentally. We further investigated the injection location and found that this had a significant effect on the migration path and overall distribution of the cells. For instance, the percentage of NSCs that arrived at the anterior commissure was significantly different by an order of magnitude with only a couple of hundred μm difference in the injection location. Our work emphasizes the importance of advanced imaging techniques, such as GQI, that can characterize individual brain tissue structures. This can be followed by a personalized injection location precisely determined to improve the efficacy of NSC treatments for specific target locations.

Finally, our model is calibrated to the experimental data for the migration of LM-NSC008 over 9 months. We found that the model is able to fit the quantitative data for the experimental median distance from injection site and percentage on white matter; however, it struggles to achieve the sufficient amount of NSC distribution to the olfactory bulb that was observed experimentally in some mice at 9 months. Again, we emphasize that the injection location of NSCs have a significant effect on the migration path and overall distribution of the cells suggesting that either this qualitative behavior is a product of injections being done in a specific location or other forces not included in the model. For example, blood vessels have been shown to play a role in neuronal migration both

in neonatal and adult rodents. Notably, some studies have shown that neuroblasts derived in the sub-ventricular zone of neonatal mice are able to travel to the cortex through the corpus callosum by use of the vasculature network [62]. In addition, VEGF helps to promote the growth of blood vessels that run parallel to the rostral migratory stream [17]. These vessels in adult mice, may provide neuroblasts with a scaffold as well as excrete various chemical signals that promote migration [90,104]. Due to findings like these, recent studies have begun to model the migration of neuroblasts from the subventricular zone to the olfactory bulb as a chemotactic process [2]. For a more in depth review of these topics see the following review [43]. Because of findings like this, chemotaxis as well as cell-cell adhesion may not only be an important driving force in NSC migration towards injury, but may also play a key role in migration in naive brain as well.

2.5 NSC Migration In Response to Traumatic Brain Injury

2.5.1 Biological Background and Experimental Data

We now turn our attention to developing a model of NSC migration in response to traumatic brain injury, where chemotaxis is known to be a major contributor to migration. In a recent study looking at the effects of neural stem cells on traumatic brain injury, it was found that rats administered with 7 doses of 1×10^6 human NSCs after a controlled cortical impact saw significant improvements to spatial learning as opposed to the control groups [51]. This was tested by means of a Morris water maze [94] which measures the rats ability to respond to distal cues, or landmarks that exist around the maze and allow the rat to learn the spatial structure. In addition to the cognitive improvements demonstrated in the paper, the neural stem cell distributions were also quantified on post-operative day 25 and day 46 for the TBI+NSC group. The imaging was done with confocal microscopy on a 1mm thick sections of the brain. In the following, we present both histograms as well as the cumulative distribution functions for the distance of the neural stem cells to the TBI at day 25 and day 46. These figures differ from the published results in that the quantification here is done over the entire cleared slice.

In figure 2.10 we see that on day 25, there is a large grouping of cells within 5mm of the TBI, while at day 46, the NSCs are more spread out. In addition, we also see a large reduction from day 25 to day 46 in the pixels identified as NSCs. In particular, there were 209,432 total pixels identified, while there were only 5,655 on day 46. This accounts for approximately a 97.3% reduction in the

total number of identified pixels and indicates either a large movement into other sections of the brain not encompassed by this slice, or a large decay rate of the viability of the NSCs.

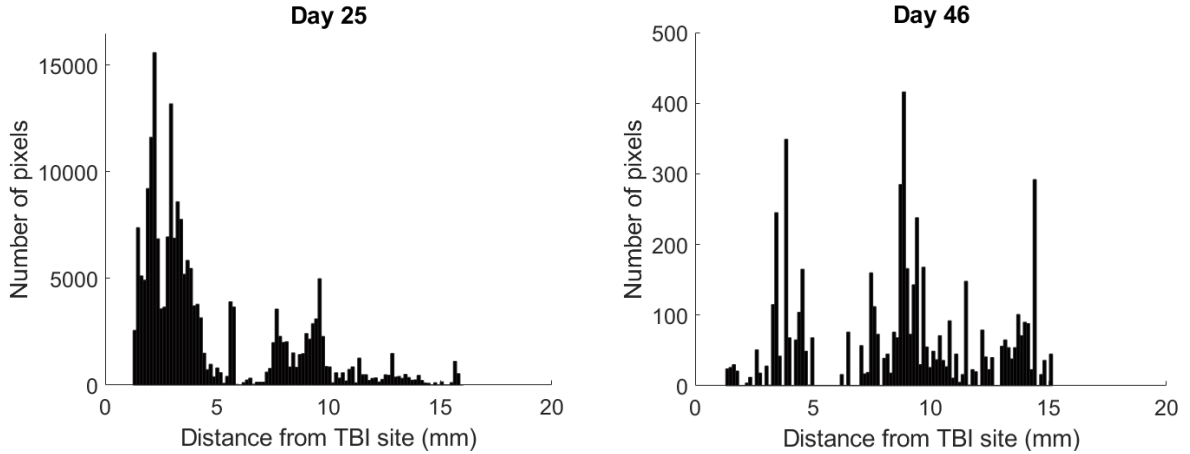


Figure 2.10: Histograms showing the number of pixels in identified NSCs found at distances ranging from 0 to 20mm away from the TBI site (a) shows the distribution at day 25 (R10) and (b) shows the distribution at day 46 (R30).

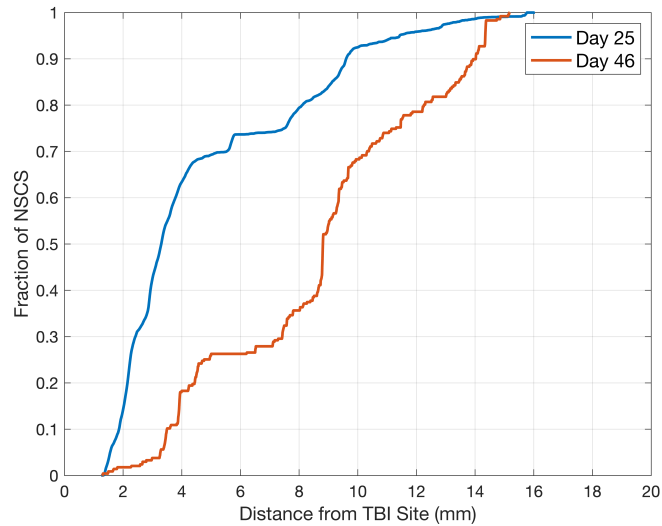


Figure 2.11: Cumulative Distribution Function showing the fraction of NSCs found within a certain distance from TBI site. Blue line represents day 25 while the orange line shows Day 46.

Looking at the corresponding CDF shown in Figure 2.11 we can see that there is a clear signal showing that the NSCs are much closer to the TBI site on day 25 than on day 46. This suggests that some further mechanism must be at play to account for this behavior. It is hypothesized that NSCs travel to distantly damaged axonal tracts at later time points, thus moving away from the TBI site

between day 25 and day 46. However, given the large amount of decay in NSCs, it cannot be ruled out that NSCs closer to the TBI site show an increased likelihood of decay over cells that are farther away.

In the next section, we show the limitations of diffusion alone to explain these findings. Next, we introduce two potential models based on the mechanisms of repair and preferential decay and show how these models can more adequately explain the data.

2.5.2 Preliminary Results

Traumatic brain injury, much like glioblastoma, produces a host of different cytokines and chemokines near the injury site, which are known to be chemoattractants for NSCs. Thus, we start with the model described by Equation 2.3. However, given the property that the NSCs were observed to be farther away on postoperative day 46 compared to day 25, we lift the condition that the concentration of the chemoattractant $C(\mathbf{x})$ is static. Thus, the ODE model of eq. 2.3 is coupled with a diffusion equation

$$\frac{\partial C(\mathbf{x}, t)}{\partial t} = D\Delta C$$

where D is the diffusion coefficient. In order to solve the diffusion equation, we use a forward Euler method with Neumann boundary conditions.

$$\frac{\partial C(\mathbf{x}, t)}{\partial \mathbf{n}} = 0 \quad \forall \mathbf{x} \in \delta\Omega$$

The initial concentration of the chemoattractant is given by the function $C(x, y, z, 0)$ which we define to take a value of 1 at the TBI site and gradually decays as the distance from the TBI site increases. Hence $C(x, y, z, 0)$ is given by

$$C(x, y, z, 0) = \left(1 + \sqrt{\frac{(x - x_c)^2}{\sigma_x^2} + \frac{(y - y_c)^2}{\sigma_y^2} + \frac{(z - z_c)^2}{\sigma_z^2}} \right)^{-p},$$

where (x_c, y_c, z_c) is the center of the injury site, $(\sigma_x, \sigma_y, \sigma_z)$ represents the distance from the TBI that the substance is halved in each x , y , and z direction, and p represents how gradually the substance decays. We make the simplifying assumption that $\sigma = \sigma_x = \sigma_y = \sigma_z$. A table of parameters for this model and model extensions can be found in Table 1.2

Parameter	Biological Meaning	Value
d_g	Maximum step size of NSC migration on grey matter	.1667
d_w	Maximum step size of NSC migration on white matter	.25
α_c	Stochasticity in chemotactic sensitivity	1
β_w	Stochasticity in migration speed parameter	1
λ	Chemotactic sensitivity	*
D	Diffusion Coefficient	*
σ	Distance from injury chemoattractant profile is halved	*
p	Steepness of chemoattractant decay	*
Diffusion Repair Parameters		
ζ_1	Strength of Source	*
ζ_2	Strength of Repair	*
Diffusion Decay Parameters		
M	Maximum Decay Rate	*
h	Half activation	*
γ	Hill Coefficient	*

Table 2.3: Model parameters and their descriptions. * these parameters are varied in different simulations.

We now attempt to calibrate the model by fitting the cumulative distribution functions depicted in [51] at day 25 and day 46. The optimization is done by minimizing the sum of squared error between the simulated and experimental CDFs. d_g , d_w , $\alpha_c = 1$, and β_w are fixed as in Table 1.2. We fit the parameters λ , σ , p and D . In figure 2.12 we show the simulation results from the optimal parameters. We see that the simulated CDF for day 25 shows cells considerably further away than the simulated CDF on day 46. This is because, given the model construction, as long as there is a

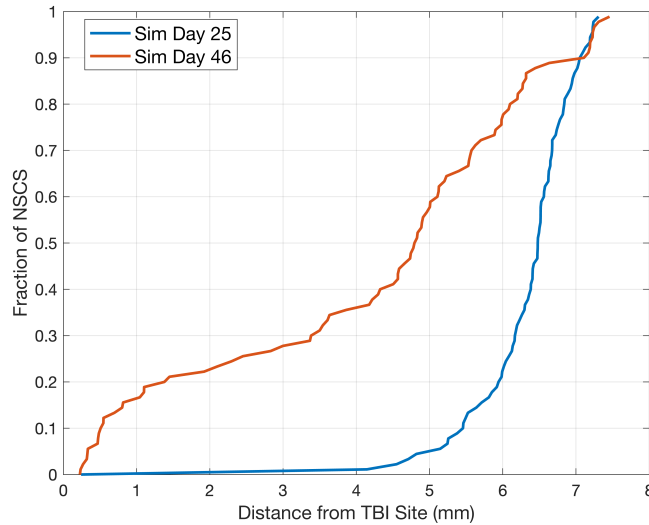


Figure 2.12: CDF of Optimal Simulation for

maximum chemoattractant level at the TBI site, the cells will continue to be biased towards this direction and over time make their way to the TBI location. Thus, in order to spread out by day 46 the diffusion coefficient has to be significantly large. However, with very large diffusion coefficients, the cells lose their ability to travel to the TBI site by day 25, and thus cannot capture the robust migration seen in the experimental results on day 25.

2.5.2.1 Diffusion Repair vs Decay

Based on the inability to capture the experimental phenomena presented in Section 2.5.2, we propose two potential hypotheses for what may be leading to this behavior. First, in [51], it was hypothesized that cells may repair the primary TBI site and then travel to secondary injury sites spread throughout the brain. Second, based on the results of figure 2.10 we see that there are considerably less NSCs at day 46, when compared to day 25. Based on this, we propose an alternative hypothesis that the NSCs may either die or differentiate and therefore not show up in the quantification at day 46. Since inflammation is higher at the primary TBI site, NSCs could travel to this region on day 25 and then differentiate or die between day 25 and day 46 at a higher rate than those far away. This could present the appearance that the NSCs are farther on day 46.

In order to test both of these hypotheses, we propose two different models. We first extend the base model in Equation 2.3 to model the hypothesis that NSC first travel to the primary damaged site before making their way to secondary damaged sites. In order to do this, we introduce 10 locations of secondary damage spread throughout the brain. These are selected in regions similar to those seen in the experimental data. Furthermore, instead of only modeling diffusion, we introduce a source term in the production of chemokines. Hence, the concentration of the chemoattractant is now modeled as a reaction diffusion equation $\frac{\partial C(\vec{x},t)}{\partial t} = D\Delta C + S$ where D is the diffusion coefficient and S is a source term that adds chemokines to the primary and secondary sites. Furthermore, a repair function can be introduced through the definition of S which accounts for the local effects of NSCs. The system can be modeled as follows:

$$\begin{cases} \frac{\partial C}{\partial t} = D\Delta C + \zeta_1 S \\ \frac{\partial S}{\partial t} = -\zeta_2 \sum_{j=1}^{\text{sites}} \sum_{i=1}^{\text{nseeds}} \mathbb{1}_{A_j} \cdot \mathbb{1}_{A_j}(x_i) \\ S(x, 0) = \mathbb{1}_{A_1} + \sum_{j=2}^{\text{sites}} \mathbb{1}_{A_j} \\ C(x, 0) = C_0 \end{cases} \quad (2.8)$$

where A_i is a damaged area with A_1 being the primary site and A_j for $j \neq 1$ secondary sites. Here ζ_1 accounts for the addition of source term, and ζ_2 represents the repair rate. Note that when a NSC is within a damaged site A_i , the amount of chemokine added to this region at future time points is reduced, thus emulating repair.

Next, we consider a model that accounts only for diffusion and preferential decay. Since there is approximately a 97.3% reduction in the number of NSCs seen in the staining from day 25 to day 46, we hypothesize that NSCs undergo higher rates of cell death or differentiation close to the primary TBI site. In order to model this behavior, every 4 days of the simulation, we update each cell's probability of death p_i based on the cell location $\mathbf{x}_i$. We then probabilistically determine the fate of the cell by generating a random sample ξ from a uniform distribution of $(0, 1)$, and mark the cell dead if $\xi > p$. The probability of death for each cell is higher when the cell is closer to the primary site. The following equation states how this probability is calculated

$$p_i(d_i) = M \cdot \left(1 - \frac{d_i^\gamma}{h^\gamma + d_i^\gamma}\right)$$

where γ and h are the hill coefficient and the half activation constant, respectively, and d_i is the distance between the cell location $\mathbf{x}_i$ and the primary injury site.

We now demonstrate some sample results from each of these models to show the initial promise of these two strategies. In Figure 2.13 we show the CDFs of both the diffusion repair model and the diffusion decay model. The left shows the results of the diffusion repair model and was run using the following parameters $d_g = .1667$, $d_w = .25$, $D = 1.5$, $\zeta_1 = .015$, and $\zeta_2 = 1 \times 10^{-4}$. The right shows a simulation of the diffusion decay model and was run with $d_g = .1667$, $d_w = .25$, $D = 1.5$, $M = .3$, $\gamma = 1$, $h = 20$. For each simulation, 30 cells were injected into the olfactory bulb for each of the 7 injections. Furthermore, the timing of the injections matched those found in the experiment. We see from the CDFs that the diffusion repair model is capable of capturing the qualitative dynamic of

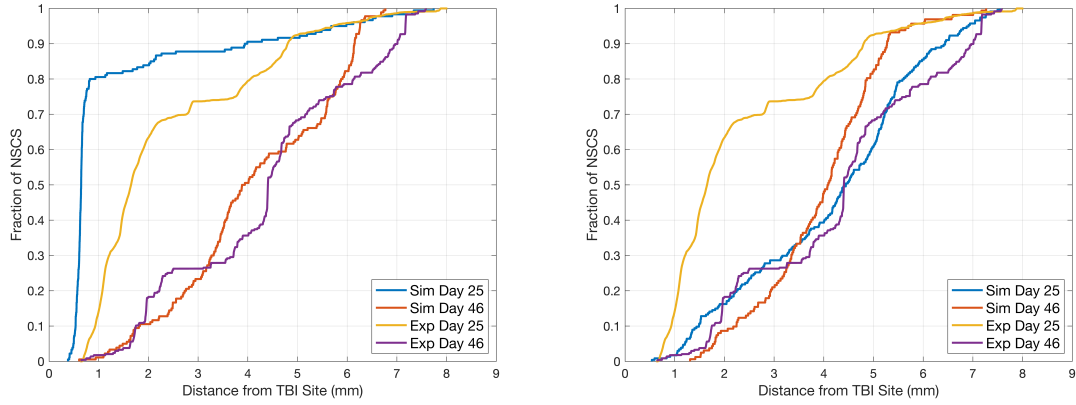


Figure 2.13: Cumulative distribution functions of the distance to the primary injury site from simulated migration of NSCs as well as from the experimental results. The left is taken from simulations following the diffusion repair model while the right is from a preferential decay model. Both are sample results and have not been optimized to fit the experimental data.

moving further away from the primary injury site on day 46 compared to day 25. Our simulated migration on day 25 shows a more robust movement to the primary site over the experimental data, as seen in blue and yellow. However, we see a very good fit by day 46 which can be seen by the orange and purple lines.

In figure 2.14 we plot the location of the NSCs at 6 different time points along with the chemoattractant profile and DTI image of the brain. Here we see that initially, during the first 21 days, the chemoattractant profile is largest at the primary site which is in the upper left part of the brain. However, at day 28 and beyond, the NSCs begin to repair the primary site while the secondary sites continue to produce chemoattractants. By day 35 we see that the gradient at the secondary sites has become large enough that the cells begin moving towards the opposite side of the brain. For the diffusion decay model, we see that we only capture the property of moving away in the $4mm$ region surrounding the primary injury site. In contrast, the CDF shows that there is a higher percentage of cells within distances larger than $4mm$ on day 46 than on day 25. We note that both of these results were taken from a sampling of the parameter space and do not necessarily represent the simulations that best fit the experimental data.

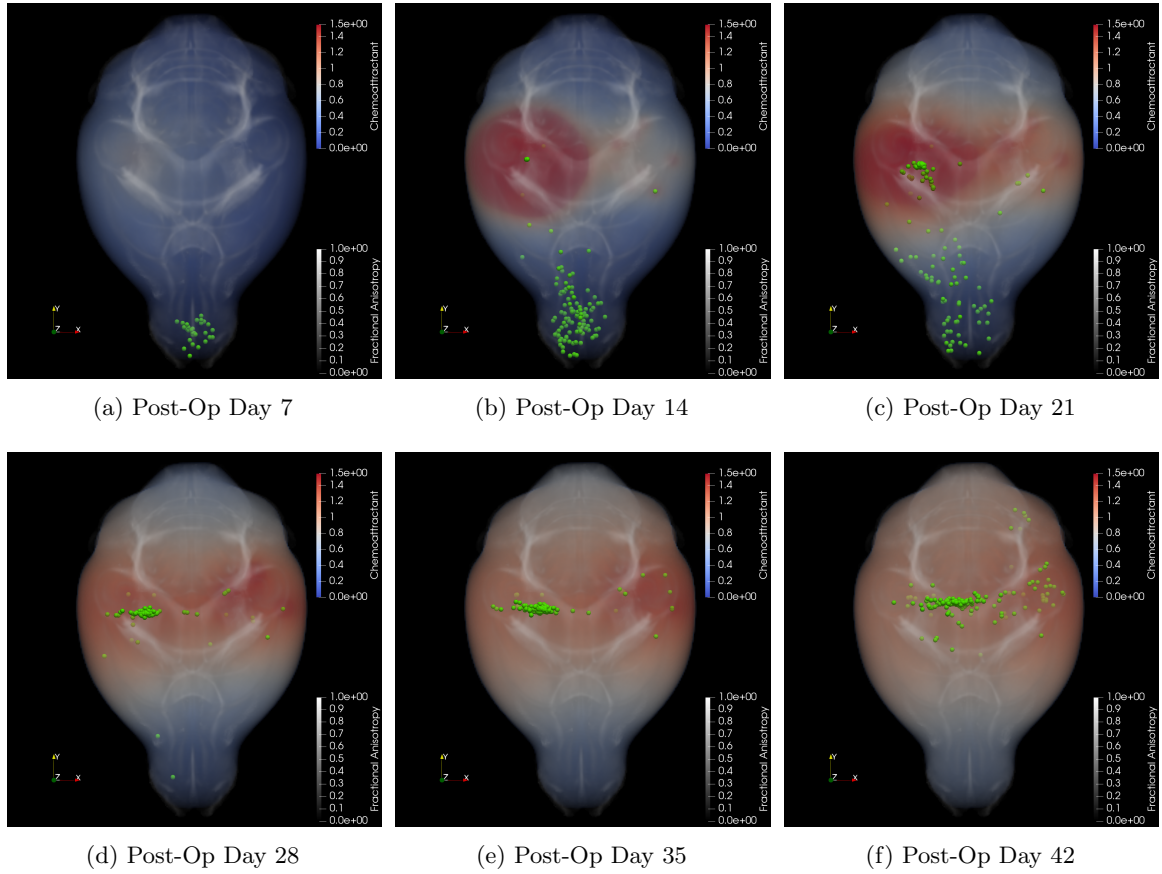


Figure 2.14: Images showing DTI (black-white) image overlayed with chemoattractant profile (blue-red) and NSC locations at 6 different post-operative days (green dots). We see most cells move to the primary injury site by day 28 and then move towards the secondary sites by day 42.

2.6 Discussion and Future Work

In the first part of this chapter, we developed a model of neural stem cell migration in the naïve mouse brain. Through the usage of generalized q-sampling imaging data, the developed model is better able to account for changes in the tissue microstructure as well as the crossing of white matter fibers. After a thorough sensitivity analysis, the model is then calibrated to experimental data for the migration of NSC in vivo. We find that the model is able to fit the quantitative statistics of median distance to injection site and percentage of cells on white matter at 3, 6, and 9 months. However, the model struggles to achieve similar levels of distribution to the olfactory bulb seen in the experimental results. We then show that the distribution to the olfactory bulb is very sensitive to the initial injection location. This finding outlines the need for additional experimental data and suggests that if the migration to the olfactory bulb is a persistent finding, then a mechanism other than tissue structure may need to be incorporated into the model. Some additional mechanisms are then discussed in Section

In the next section, we develop two preliminary models to explain the experimental distributions of NSCs in response to traumatic brain injury. Some initial results show that both models have the potential to explain the experimentally observed phenomenon of cells moving away from the primary injury site between postoperative day 25 and day 46. For future work, we plan to couple the preliminary models for both the diffusion repair and diffusion decay mechanisms with the multi-track fiber model based on GQI reconstruction. Once these models have been coupled, we will properly calibrate each of these to the experimental CDFs shown above. One key challenge in calibration may be the number of parameters in each model. To reduce the number of parameters we have to fit, we can fix those not related to chemotaxis by using the optimal parameters we found for the model in the naïve mouse brain. The distributions from the optimal simulations will then be compared, and any differences will be used to make predictions about which hypothesis is more biologically probable. Finally, with both calibrated models we can compare how different injection and dosing strategies affect the overall aggregation to the injury sites.

2.7 Data and Code Availability

The raw imaging data used for the GQI reconstruction in Section 2.4 was generously supplied by Duke Center for In Vivo Microscopy [100]. Thanks to James J Cook and Dr. Fang-Cheng Yeh for technical assistance during the reconstruction process in DSI-Studio.

The implementation of the computational model presented in this chapter is available on request from the first author (Austin Hansen) or the faculty advisor (Heyrim Cho).

Chapter 3

Multiphase Modeling of Blood Clot Formation Deformation

3.1 Biological Background and Modeling Background

The formation of blood clots are a crucial part of the wound healing process as they prevent further bleeding, and provide a scaffold for tissue repair and growth. However, when blood clots become too large and obstruct the flow of blood through veins or arteries, serious complications can occur. For example, myocardial infarctions occur when blood flow to the heart is obstructed, typically from clots formed in the nearby arteries. Over time, the lack of blood and oxygen results in the death of the heart tissue. Strokes are another very common example and is the fifth leading cause of death in the United States [3]. Another common complication of blood clots is the risk of embolization, or when a clot breaks off and travels to other areas such as the lungs. A common example of this is called pulmonary embolism and is another major cause of death in the US. Due to the wide range of complications and serious health impacts, developing a greater understanding of blood clot formation and embolization, as well as finding safe and effective treatments, is a major focus of research and medicine.

Blood clots consist of several main components, including platelets that can become active and stick to damaged areas, fibrin that forms a scaffold for the clot and is responsible for clot contraction, and red blood cells [26]. In addition, clot formation occurs in a variety of ways. In collagen-mediated

formation, collagen and platelets interact, causing the platelets to become active and adhere to the injury site while simultaneously recruiting other platelets and stimulating thrombin. Thrombin then leads to fibrinogen and the creation of the fibrin network. In tissue factor (TF) mediated thrombosis, thrombin is activated by active TF which is responsible for the generation of the fibrin network and the recruitment of active platelets [59].

In addition to clot formation, the environment in which clots are formed can have a large impact on the composition of the clots themselves. In arteries where the lumen is smaller and the shear flow is higher, arterial thrombi are often formed. In arterial thrombi, the overall percentage of platelets is higher and the volume fractions of the constituent clot components are 31.3% platelet, 43.3% fibrin, and 17.1% red blood cells. In venous thrombi, which are formed in lower shear flow, the volume fraction of platelets is considerably less, while the volume fraction of red blood cells is higher. The volume fractions in venous thrombi are .4%, 35% and 63.4% for platelets, fibrin, and red blood cells respectively [26].

Furthermore, many factors can affect the likelihood of embolization such as the permeability and viscosity of the clot. One underappreciated factor is the effect of red blood cells on the clot itself. Recent studies have begun to suggest that red blood cells play a much larger role than previously thought [22,102]. For instance, red blood cells embedded into the core of the clot increase the pore size of the fibrin network. Therefore, if red blood cells are washed out by fluid flow, the overall permeability of the clot increases. In contrast, during clot contraction, the fibrin network can squeeze around the red blood cells, forcing them into polyhedral shapes. The compressed red blood cells then act as a seal in the clot and reduce the overall permeability. These types of change can have large impacts on the likelihood of embolization and are not well understood [27,27].

Due to the complex relationships between the different components of the clot, many computational models have been developed to understand different aspects including clot formation, contraction, and deformation/embolization. The modeling approaches have ranged from continuous to particle-based models, as well as multiscale coupled models in both 2D and 3D. For example, a multiscale model of clot contraction composed of both fibrin and platelets was developed in [70]. In this modeling approach, fibrin was represented as a collection of nodes connected by non-linear elastic springs, linear bending springs, and cohesive forces. Meanwhile, platelets were represented by spheres that could interact by means of their filopodia to exert contractile forces on the neighboring fibrin network. Using this approach, it was found that the number of filopodia per platelet was positively correlated

with the time it took to undergo clot contraction, as well as the fibrin density. Furthermore, it was shown that platelet clustering was increased during clot contraction. In [117] a 3D multiphase model was developed where the initial volume fraction of the phase field model was defined by the output of a particle model of platelet aggregation and thrombus formation. The authors found that both clot permeability, as well as the elastic shear modulus, have a large effect on blood flow and clot deformation. In addition, steady-state versus pulsating flows were shown to lead to different embolization rates in abdominal aortic aneurysm. One of the drawbacks of the work in [117] is that the phase field model consists only of two phases and the differences between the core of the clot and shell regions are only accounted for through spatially dependent parameters such as permeability, rather than modeling the interactions directly. In a different multiphase model [109], a nested hierarchical approach was used to create a 3-phase model consistent with the clot structure seen in [91, 103]. Here, a dense core of fibrin network and less activated platelets is surrounded by an outer shell of highly activated platelets, while the flow of platelet-rich plasma around and through the thrombus is modeled through a coupled Navier-Stokes equation. The model in [109] predicted a nonlinear increase in clot deformation with an increase in shear rate. For a more in-depth review of different modeling approaches for blood clot formation, contraction and deformation see the following reviews [33, 38, 39, 48, 63, 114]

Due to the ability of multiphase models to produce thermodynamically consistent equations which can capture both the macroscopic and microscopic forces within the clot, as well as the effects from fluid flow on clot deformation and embolization, we look to extend the model presented in [109]. In light of the new understanding of the effect of red blood cells, we aim to do two things with the model. First, we get rid of the hierarchical approach and consider all pairwise surface tensions between phases. In addition, we add a separate phase for red blood cells so as to test how various distributions of red blood cells affect the overall clot stability. The key phases of the proposed model include red blood cells, fibrin network, less and highly activated platelets, as well as platelet-rich plasma. To introduce pairwise surface tensions, we rely on previous work done in Cahn-Hilliard systems, which considers all pairwise surface tensions [34, 107]. In the following section, we give an overview of the relevant modeling background. We then propose our model set up and derive a coupled Cahn-Hilliard Navier-Stokes equation which includes pair-wise surface tensions as well elastic energy. We then describe the strategy for the numerical implementation and discuss our future work.

3.2 Energy Dissipation Law and Derivation Strategy

A common approach to describe the motion of a particle x , is to write down all the forces acting on the given particle. Then Newton's 2nd law of motion $F = ma$ can be used to derive the equation of motion. Although this approach has a long fruitful history, energy variational methods offer a powerful alternative. In this approach, the equations of motion are built by considering all of the energies within the systems as the starting point. This ensures that the resulting equations are thermodynamically consistent. The key concepts of this approach include the least action principle, maximum dissipation principle, force balance, and energy dissipation laws. For a good presentation of these concepts we refer the reader to the following resource [40, 44].

Energy variational methods offer a powerful strategy and is built upon the first and second laws of thermodynamics. The first law of thermodynamics states that energy is conserved in a given system. It relates the kinetic energy $\mathcal{K}$ and the internal energy $\mathcal{U}$ to the work $\mathcal{W}$ and the heat $\mathcal{Q}$. The law is stated as

$$\frac{d}{dt}(\mathcal{K} + \mathcal{U}) = \dot{W} + \dot{Q} \quad (3.1)$$

The second law of thermodynamics can be thought of as saying that the entropy, or disorder in a system, must always increase with time. For systems with a constant temperature, the law is stated as

$$T \frac{d\mathcal{S}}{dt} = \dot{Q} + \Delta \quad (3.2)$$

where T is the temperature, $\mathcal{S}$ is the entropy, and Δ is the entropy production, which is equal to the energy dissipation during irreversible interactions. We note that $\Delta \geq 0$. By subtracting equations 3.1 and 3.2 we get

$$\frac{d}{dt}(\mathcal{K} + \mathcal{U}) - T \frac{d\mathcal{S}}{dt} = \dot{W} - \Delta$$

This can be simplified by noticing that $\mathcal{U} - T\mathcal{S}$ is the Helmholtz free energy $\mathcal{F}$, and the total energy is a sum of the kinetic and Helmholtz free energy $E^{tot} = \mathcal{K} + \mathcal{F}$. Thus we have that

$$\frac{d}{dt}E^{tot} = \dot{W} - \Delta$$

When $\dot{W} = 0$, or when no external work is done on the system, the resulting equation

$$\frac{d}{dt}E^{tot} = -\Delta \quad (3.3)$$

is called the energy dissipation law. This gives a foundational law which we can now use to derive our phase field model while ensuring the equations are thermodynamically consistent.

3.3 Model Derivation

3.3.1 Multiphase Representation of a Blood Clot

We represent the blood clot by combining phases of an inner core region consisting of fibrin, red blood cells, and highly activated platelets, an outer shell of less activated platelets, and platelet-rich plasma which can flow through and around the thrombus. For this we utilize five different phase functions $c_1, c_2, \dots, c_5$. In this case, c_i is the volume fraction of the i^{th} phase and takes a value of 1 where the phase is present and 0 where its absent. We allow each phase to interact with each other by considering pairwise surface tensions between each. A summary of the representations of each phase can be found below.

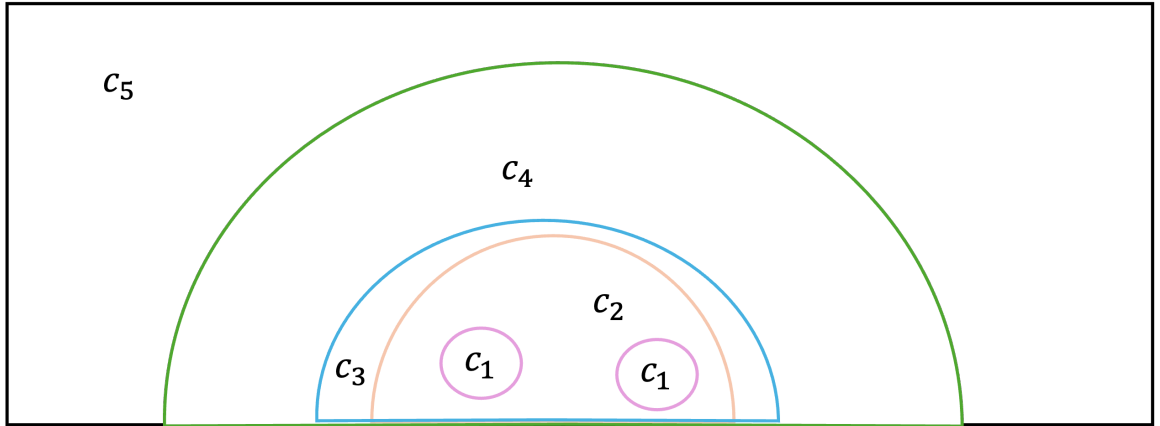


Figure 3.1: Graphic showing 5 phase model where c_1 represents the red blood cells, c_2 represents the Fibrin Network, c_3 represents the highly activated platelets, c_4 represents the less activated platelets, c_5 represents the platelet-rich plasma.

3.3.2 Free Energy Functionals

The energy within the system is composed of three components; the kinetic energy E_{kin} , the mixing energy E_{coh} , and the elastic energy E_{ela} . E_{kin} accounts for the macroscopic flow of the platelet-rich plasma as it flows through and around the thrombus, and leads to the Navier-Stokes portions of the coupled system. The kinetic energy is written as

$$E_{kin} = \int_{\Omega} (\frac{1}{2} \rho |\mathbf{u}|^2) dx \quad (3.4)$$

where ρ is the density local mixture while $\mathbf{u}$ is the velocity of the flow. On the other hand, the mixing energy E_{coh} and elastic energy E_{ela} encompass the microscopic elements of the system. The mixing energy is taken from [34] and is written as:

$$E_{coh} = \int_{\Omega} \sum_{i,j=1}^N \frac{\lambda_{ij}}{2} \nabla c_i \cdot \nabla c_j + \frac{\beta}{2} G(\vec{c}) dx \quad (3.5)$$

where λ_{ij} are the mixing energy density coefficients and are related to the pairwise surface tensions σ_{ij} by the equation $\lambda_{ij} = -\frac{3}{\sqrt{2}} \bar{\gamma} \sigma_{ij}$. Furthermore $\beta = \frac{3}{\sqrt{2}} \frac{1}{\bar{\gamma}}$ where $\bar{\gamma}$ represents the characteristic scale of the interfacial thickness between phases. We can view the mixing energy as representing a balance between the tendency for the phases to separate into pure components and preference against completely sharp interfaces. The first term $\nabla c_i \cdot \nabla c_j$ is responsible for reducing sharp interfaces by penalizing regions in the phases with very large gradients. In contrast the second term is responsible for phase separation as the function G is often given in the form of a multi-well potential. We can either use the form state in [34] which is written as

$$G(\vec{c}) = \sum_{i,j=1}^N \sigma_{ij} [g(c_i) + g(c_j) - g(c_i + c_j)]$$

where $g(c_i) = \frac{1}{4}c_i^2(1 - c_i)^2$ is a double well. Or we may take G to be as listed in [107] where $G(\vec{c}) = G_0(\vec{c}) + sG_1(\vec{c})$, and

$$G_0(\vec{c}) = \sum_{i,j=1}^N \sigma_{ij} [g(c_i) + g(c_j) - g(c_i + c_j)]$$

$$G_1(\vec{c}) = \sum_{i,j=1}^N \sigma_{ij} c_i^2 c_j^2 \left(\sum_{k \neq i,j} c_k^2 \right)$$

and s a stabilization parameter. For the derivation here, the choice does not effect the outcome.

Finally, the elastic energy E_{ela} accounts for the viscoelastic properties of the thrombus. Since the clot deforms and stretches under the forces of the fluid flow, the elastic forces are represented by the deformation gradient tensor $F = \frac{\partial \mathbf{x}}{\partial \mathbf{X}}$, where $\mathbf{x}$ are the current coordinates and $\mathbf{X}$ are the reference coordinates. Hence, the deformation gradient tensor describes how the mixture deforms from its original state. The energy term E_{ela} combines properties from Hookean elastic springs as well as a dissipation of energy through a Newtonian damper and can be written as

$$E_{ela} = \int_{\Omega} \frac{\lambda_e |\mathbf{F}|^2}{2} dx = \int_{\Omega} \frac{\lambda_e tr(\mathbf{F}\mathbf{F}^T)}{2} dx$$

where $\lambda_e = \sum_{i=1}^N c_i \lambda_{e,i}$ and $\lambda_{e,i}$ are the elastic energy densities of the i^{th} phase. By conservation of mass/volume principles we know that $\nabla \cdot \mathbf{F} = 0$ for all $t \geq 0$, since $\nabla \cdot \mathbf{F} = 0$ for $t = 0$. Thus one solution for $\mathbf{F}$ such that $\nabla \cdot \mathbf{F} = 0$ is

$$\mathbf{F} = \begin{bmatrix} -\partial_{x_2} \Psi_1 & -\partial_{x_2} \Psi_2 \\ -\partial_{x_1} \Psi_1 & -\partial_{x_1} \Psi_2 \end{bmatrix}$$

Hence, for simplicity, the elastic energy can be represented as follows where Ψ is a vector

$$E_{ela} = \int_{\Omega} \frac{\lambda_e |\nabla \Psi|^2}{2} d\mathbf{x} \quad (3.6)$$

Combining each of these components we have that

$$E_{tot} = E_{kin} + E_{coh} + E_{ela}$$

3.3.3 Macroscopic and Microscopic Dissipation

We now give the dissipation associated with the system, which combines the macroscopic dissipation associated to the kinetic energy and the microscopic dissipation associated to the mixing and elastic energies.

$$\Delta = \int_{\Omega} \frac{\eta}{2} |D|^2 + \frac{\eta(\vec{c})(1 - c_N)}{\kappa} |u|^2 + \sum_{i,j=1; j \neq i}^N \frac{1}{2} \tau_{ij} |\nabla \mu_i - \nabla \mu_j|^2$$

The parameter η represents the dynamic shear viscosity and is written as $\eta = \sum_i^N c_i \eta_i$ where η_i is the shear viscosity of the i^{th} phase. The term $D = \nabla \mathbf{u} + (\nabla \mathbf{u})^T$ is often written in terms of the strain tensor $\epsilon = \frac{1}{2} (\mathbf{u} + (\nabla \mathbf{u})^T)$ by setting $D = 2\epsilon$. The terms τ_{ij} serve as the phenomenological mobility coefficients and create a positive semi-definite matrix. Furthermore we impose the following conditions on τ_{ij} as shown in [34] to allow the system to satisfy given reduction consistency properties:

$$\sum_{j=1}^N \tau_{ij} = \sum_{j=1}^N \tau_{ji} = 0 \quad (3.7)$$

$$\tau_{ii} = - \sum_{j=1, j \neq i}^N \tau_{ij} \quad (3.8)$$

Finally, μ_i represents the chemical potentials and κ the permeability of the clot. Note that the chemical potentials μ_i are, by definition, the change in energy associated with a change in the particle number of a given phase. Hence, we have the property that $\mu_i = \frac{\delta E_{tot}}{\delta c_i}$ where δ represents the variation.

Using equations 3.7 and 3.8 associated to the mobility coefficients we can rewrite the dissipation term as follows:

$$\begin{aligned} \sum_{\substack{i,j=1 \\ j \neq i}}^N \frac{1}{2} \tau_{ij} |\nabla \mu_i - \nabla \mu_j|^2 &= \sum_{\substack{i,j=1 \\ j \neq i}}^N \frac{1}{2} \tau_{ij} [|\nabla \mu_i|^2 + |\nabla \mu_j|^2 - \nabla \mu_i \nabla \mu_j] \\ &= \sum_{i=1}^N \left[\sum_{\substack{j=1 \\ j \neq i}}^N \frac{1}{2} (\tau_{ij} + \tau_{ji}) \right] |\nabla \mu_i|^2 - \sum_{\substack{i,j=1 \\ j \neq i}}^N \frac{1}{2} \tau_{ij} \nabla \mu_i \nabla \mu_j \end{aligned} \quad (3.9)$$

Parameter	Biological Meaning
c_i	phase functions
ρ	local density of mixture
$\mathbf{u}$	velocity of flow
λ_{ij}	mixing energy density between phases i and j
σ_{ij}	surface tension between phases i and j
$\bar{\gamma}$	characteristic scale of interfacial thickness
$\lambda_{e,i}$	elastic energy density of i^{th} phase
η_i	shear viscosity of the i^{th} phase
τ_{ij}	mobility coefficient between phase i and phase j

Table 3.1: Model parameters and their descriptions.

By equation 3.8 we have that

$$\sum_{\substack{i,j=1 \\ j \neq i}}^N \frac{1}{2} \tau_{ij} |\nabla \mu_i - \nabla \mu_j|^2 = - \sum_{i=1}^N \tau_{ii} \nabla \mu_i \nabla \mu_i - \sum_{\substack{i,j=1 \\ j \neq i}}^N \frac{1}{2} \tau_{ij} \nabla \mu_i \nabla \mu_j$$

Since convention is to write the dissipation as positive our final dissipation Δ becomes

$$\Delta = \int_{\Omega} \frac{\eta}{2} |D|^2 + \frac{\eta(\bar{c})(1-c_5)}{\kappa} |u|^2 + \sum_{i=1}^N \tau_{ii} \nabla \mu_i \nabla \mu_i + \sum_{\substack{i,j=1 \\ j \neq i}}^N \frac{1}{2} \tau_{ij} \nabla \mu_i \nabla \mu_j \quad (3.10)$$

In table 3.1 we summarize all the parameters and their corresponding meaning

3.3.4 Derivation of N-Phase Coupled Cahn-Hilliard/Navier-Stokes System

In the following section, we derive a N-phase coupled Cahn-Hilliard/Navier-Stokes system which takes the form of:

$$\left\{ \begin{array}{l} \rho(\partial_t + \mathbf{u} \cdot \nabla) \mathbf{u} = \nabla \cdot \boldsymbol{\sigma} + \mathbf{H} \\ \nabla \cdot \mathbf{u} = 0 \\ (\partial_t + \mathbf{u} \cdot \nabla) \Psi = 0 \\ (\partial_t + \mathbf{u} \cdot \nabla) c_i = \nabla \cdot q_i \quad \text{for } 1 \leq i \leq N \end{array} \right. \quad (3.11)$$

where $\boldsymbol{\sigma}, \mathbf{H}, q_i$ are undetermined

To derive the correct form of the chemical potential μ_i we look at $\frac{\delta E_{Tot}}{\delta c_i}$. Recall for a functional $F[\rho] = \int f(\mathbf{r}, \rho(\mathbf{r}), \nabla \rho(\mathbf{r})) d\mathbf{r}$ the variational derivative can be shown to be

$$\frac{\delta F}{\delta \rho(r)} = \frac{\partial f}{\partial \rho} - \nabla \cdot \frac{\partial f}{\partial \nabla \rho}$$

Combining equations 3.4, 3.5, and 3.6 yields

$$\begin{aligned} E_{Tot} &= \frac{1}{2} \rho |\mathbf{u}|^2 + \frac{\lambda_e |\nabla \Psi|^2}{2} + \sum_{i,j=1}^N \frac{\lambda_{ij}}{2} \nabla c_i \cdot \nabla c_j + \frac{1}{\bar{\gamma}} G(\vec{c}) \\ &= \frac{1}{2} \rho |\mathbf{u}|^2 + \frac{\lambda_e |\nabla \Psi|^2}{2} + \sum_{\substack{i,j=1 \\ i \leq j}}^N \frac{(\lambda_{ij} + \lambda_{ji})}{2} \nabla c_i \cdot \nabla c_j + \frac{1}{\bar{\gamma}} G(\vec{c}) \end{aligned}$$

Thus, taking the variational derivative of E_{Tot} we have

$$\mu_i = \frac{\partial_{c_i} \lambda_e |\nabla \Psi|^2}{2} + \frac{\beta}{2} \partial_i G(\vec{c}) - \nabla \cdot \left(\sum_{j=1}^N \frac{(\lambda_{ij} + \lambda_{ji})}{2} \nabla c_j \right)$$

Or since $\lambda_{ij} = \lambda_{ji}$ we can write μ_i as

$$\mu_i = \frac{\partial_{c_i} \lambda_e |\nabla \Psi|^2}{2} + \frac{\beta}{2} \partial_i G(\vec{c}) + \sum_{j=1}^N -\lambda_{ij} \Delta c_j \quad (3.12)$$

Finally, we now solve for the unknowns $\boldsymbol{\sigma}, \mathbf{H}, q_1, q_2$ by applying the energy dissipation law. This is done by taking the time derivative of the total energy functional and then matching to the terms

in the dissipation. We first begin with the kinetic energy:

$$\begin{aligned}
\frac{dE_{kin}}{dt} &= \frac{d}{dt} \int_{\Omega} \frac{1}{2} \rho |\mathbf{u}|^2 d\mathbf{x} \\
&= \int_{\Omega} \frac{1}{2} \frac{\partial \rho}{\partial t} |\mathbf{u}|^2 d\mathbf{x} + \int_{\Omega} \rho \frac{\partial \mathbf{u}}{\partial t} \cdot \mathbf{u} d\mathbf{x} \\
&= \int_{\Omega} \frac{1}{2} \frac{\partial \rho}{\partial t} |\mathbf{u}|^2 d\mathbf{x} + \int_{\Omega} \rho \frac{d\mathbf{u}}{dt} \cdot \mathbf{u} d\mathbf{x} - \int_{\Omega} (\rho \mathbf{u} \cdot \nabla \mathbf{u}) \cdot \mathbf{u} d\mathbf{x} \\
&= \int_{\Omega} \frac{1}{2} \frac{\partial \rho}{\partial t} |\mathbf{u}|^2 d\mathbf{x} + \int_{\Omega} \rho \frac{d\mathbf{u}}{dt} \cdot \mathbf{u} d\mathbf{x} + \int_{\Omega} \nabla \cdot (\rho \mathbf{u}) \cdot \frac{|\mathbf{u}|^2}{2} d\mathbf{x} - \int_{\partial\Omega} \frac{|\mathbf{u}|^2}{2} \rho \mathbf{u} \cdot \mathbf{n} ds \\
&= \int_{\Omega} \rho \frac{d\mathbf{u}}{dt} \cdot \mathbf{u} d\mathbf{x} - \int_{\partial\Omega} \frac{|\mathbf{u}|^2}{2} \rho \mathbf{u} \cdot \mathbf{n} ds \\
&= \int_{\Omega} (\nabla \cdot \boldsymbol{\sigma}) \cdot \mathbf{u} d\mathbf{x} + \int_{\Omega} \mathbf{H} \cdot \mathbf{u} d\mathbf{x} - \int_{\Omega} P \nabla \cdot \mathbf{u} d\mathbf{x} - \int_{\partial\Omega} \frac{|\mathbf{u}|^2}{2} \rho \mathbf{u} \cdot \mathbf{n} ds \\
&= - \int_{\Omega} (\boldsymbol{\sigma} + PI) : \nabla \mathbf{u} d\mathbf{x} + \int_{\Omega} \mathbf{H} \cdot \mathbf{u} d\mathbf{x} + \int_{\partial\Omega} (\boldsymbol{\sigma} \cdot \mathbf{n}) \cdot \mathbf{u} ds - \int_{\partial\Omega} \frac{|\mathbf{u}|^2}{2} \rho \mathbf{u} \cdot \mathbf{n} ds
\end{aligned} \tag{3.13}$$

Where pressure P is introduced as Lagrange multipliers for fluid incompressibility and we make the substitution $\rho(\partial_t + \mathbf{u} \cdot \nabla) \mathbf{u} = \nabla \cdot \boldsymbol{\sigma} + \mathbf{H}$.

Next we write out the mixing energy:

$$\begin{aligned}
\frac{dE_{coh}}{dt} &= \frac{d}{dt} \int_{\Omega} \sum_{i,j=1}^N \frac{\lambda_{ij}}{2} \nabla c_i \cdot \nabla c_j + \frac{\beta}{2} G(\vec{c}) d\mathbf{x} \\
&= \int_{\Omega} \sum_{i,j=1}^N \frac{\lambda_{ij}}{2} \frac{\partial}{\partial t} (\nabla c_i \cdot \nabla c_j) d\mathbf{x} + \int_{\Omega} \frac{\beta}{2} G'(\vec{c}) \frac{\partial \vec{c}}{\partial t} d\mathbf{x} \\
&= \int_{\Omega} \sum_{i,j=1}^N \frac{\lambda_{ij}}{2} \left(\nabla \frac{\partial}{\partial t} c_i \cdot \nabla c_j + \nabla c_i \cdot \nabla \frac{\partial}{\partial t} c_j \right) d\mathbf{x} + \int_{\Omega} \frac{\beta}{2} G'(\vec{c}) \cdot \frac{\partial \vec{c}}{\partial t} d\mathbf{x} \\
&= \int_{\Omega} \sum_{i,j=1}^N \frac{\lambda_{ij}}{2} \left(\nabla \frac{\partial}{\partial t} c_i \cdot \nabla c_j \right) d\mathbf{x} + \int_{\Omega} \sum_{i,j=1}^N \frac{\lambda_{ij}}{2} \left(\nabla c_i \cdot \nabla \frac{\partial}{\partial t} c_j \right) d\mathbf{x} \\
&\quad + \int_{\Omega} \frac{\beta}{2} G'(\vec{c}) \cdot \frac{\partial \vec{c}}{\partial t} d\mathbf{x} \\
&= \int_{\partial\Omega} \sum_{i,j=1}^N \frac{\lambda_{ij}}{2} \left(\frac{\partial}{\partial t} c_i \cdot \nabla c_j \cdot n \right) ds - \int_{\Omega} \sum_{i,j=1}^N \frac{\lambda_{ij}}{2} \left(\frac{\partial}{\partial t} c_i \cdot \Delta c_j \right) d\mathbf{x} \\
&\quad + \int_{\partial\Omega} \sum_{i,j=1}^N \frac{\lambda_{ij}}{2} \left(\nabla c_i \cdot \frac{\partial}{\partial t} c_j \cdot n \right) ds - \int_{\Omega} \sum_{i,j=1}^N \frac{\lambda_{ij}}{2} \left(\Delta c_i \cdot \frac{\partial}{\partial t} c_j \right) d\mathbf{x} \\
&\quad + \int_{\Omega} \frac{\beta}{2} G'(\vec{c}) \cdot \frac{\partial \vec{c}}{\partial t} d\mathbf{x} \\
&= \int_{\partial\Omega} \sum_{i,j=1}^N \left(\lambda_{ij} \frac{\partial}{\partial t} c_i \cdot \nabla c_j \cdot n \right) ds - \int_{\Omega} \sum_{i,j=1}^N \lambda_{ij} \left(\frac{\partial}{\partial t} c_i \cdot \Delta c_j \right) d\mathbf{x} \\
&\quad + \int_{\Omega} \frac{\beta}{2} G'(\vec{c}) \cdot \frac{\partial \vec{c}}{\partial t} d\mathbf{x}
\end{aligned} \tag{3.14}$$

Note that we can rewrite the following terms as

$$\begin{aligned}
\int_{\Omega} - \sum_{i,j=1}^N \lambda_{ij} \left(\frac{\partial}{\partial t} c_i \cdot \Delta c_j \right) d\mathbf{x} + \frac{\beta}{2} G'(\vec{c}) \cdot \frac{\partial \vec{c}}{\partial t} d\mathbf{x} &= \int_{\Omega} - \sum_{i=1}^N \left[\sum_{j=1}^N \lambda_{ij} \left(\frac{\partial}{\partial t} c_i \cdot \Delta c_j \right) \right] \\
&\quad + \left(\sum_{i=1}^N \frac{\beta}{2} \partial_{c_i} G(\vec{c}) \cdot \frac{\partial c_i}{\partial t} \right) d\mathbf{x} \\
&= \int_{\Omega} \sum_{i=1}^N \left[\sum_{j=1}^N (-\lambda_{ij} \Delta c_j) + \frac{\beta}{2} \partial_{c_i} G(\vec{c}) \right] \frac{\partial c_i}{\partial t} d\mathbf{x}
\end{aligned} \tag{3.15}$$

Finally, we turn the elastic energy:

$$\begin{aligned}
\frac{dE_{ela}}{dt} &= \frac{d}{dt} \int_{\Omega} \frac{\lambda_e |\nabla \Psi|^2}{2} d\mathbf{x} \\
&= \int_{\Omega} \sum_{i=1}^N \frac{\partial_{c_i} \lambda_e}{2} |\nabla \Psi|^2 \frac{\partial c_i}{\partial t} d\mathbf{x} + \int_{\Omega} \lambda_e \nabla \Psi : \nabla \left(\frac{\partial \Psi}{\partial t} \right) \\
&= \int_{\Omega} \sum_{i=1}^N \frac{\partial_{c_i} \lambda_e}{2} |\nabla \Psi|^2 \frac{\partial c_i}{\partial t} d\mathbf{x} \\
&\quad - \int_{\Omega} (\nabla \cdot (\lambda_e \nabla \Psi)) \cdot \frac{\partial \Psi}{\partial t} d\mathbf{x} + \int_{\partial\Omega} (\lambda_e \nabla \Psi \cdot \mathbf{n}) \cdot \frac{\partial \Psi}{\partial t} ds \\
&= \int_{\Omega} \sum_{i=1}^N \frac{\partial_{c_i} \lambda_e}{2} |\nabla \Psi|^2 \frac{\partial c_i}{\partial t} d\mathbf{x} \\
&\quad + \int_{\Omega} (\nabla \cdot (\lambda_e \nabla \Psi)) \cdot (\mathbf{u} \cdot \nabla \Psi) d\mathbf{x} + \int_{\partial\Omega} (\lambda_e \nabla \Psi \cdot \mathbf{n}) \cdot \frac{\partial \Psi}{\partial t} ds \\
&= \int_{\Omega} \sum_{i=1}^N \frac{\partial_{c_i} \lambda_e}{2} |\nabla \Psi|^2 \frac{\partial c_i}{\partial t} d\mathbf{x} \\
&\quad + \int_{\Omega} (\nabla \Psi^T (\nabla \cdot (\lambda_e \nabla \Psi))) \cdot \mathbf{u} d\mathbf{x} + \int_{\partial\Omega} (\lambda_e \nabla \Psi \cdot \mathbf{n}) \cdot \frac{\partial \Psi}{\partial t} ds
\end{aligned} \tag{3.16}$$

We now collect all the terms from equations 3.13, 3.14, and 3.16 and apply the rewriting done in the mixing term from equation 3.15.

$$\begin{aligned}
\frac{d(E_{kin} + E_{ela} + E_{coh})}{dt} &= - \int_{\Omega} (\boldsymbol{\sigma} + P\mathbf{I}) : \nabla \mathbf{u} d\mathbf{x} + \int_{\Omega} \mathbf{H} \cdot \mathbf{u} d\mathbf{x} + \int_{\partial\Omega} (\boldsymbol{\sigma} \cdot \mathbf{n}) \cdot \mathbf{u} dS - \int_{\partial\Omega} \frac{|\mathbf{u}|^2}{2} \rho \mathbf{u} \cdot \mathbf{n} ds \\
&\quad + \int_{\Omega} \sum_{i=1}^N \frac{\partial_{c_i} \lambda_e}{2} |\nabla \Psi|^2 \frac{\partial c_i}{\partial t} d\mathbf{x} \\
&\quad + \int_{\Omega} (\nabla \Psi^T (\nabla \cdot (\lambda_e \nabla \Psi))) \cdot \mathbf{u} d\mathbf{x} + \int_{\partial\Omega} (\lambda_e \nabla \Psi \cdot \mathbf{n}) \cdot \frac{\partial \Psi}{\partial t} ds \\
&\quad + \int_{\Omega} \sum_{i=1}^N \left[\sum_{j=1}^N (-\lambda_{ij} \Delta c_j) + \frac{\beta}{2} \partial_{c_i} G(\vec{c}) \right] \frac{\partial c_i}{\partial t} d\mathbf{x} + \int_{\partial\Omega} \sum_{i,j=1}^N \left(\lambda_{ij} \frac{\partial}{\partial t} c_i \cdot \nabla c_j \cdot \mathbf{n} \right) ds
\end{aligned}$$

Rearranging the terms so as to collect the bulk terms in front and the boundary terms at the end we get:

$$\begin{aligned}
\frac{dE_{tot}}{dt} = & - \int_{\Omega} (\boldsymbol{\sigma} + PI) : \nabla \mathbf{u} dx + \int_{\Omega} \mathbf{H} \cdot \mathbf{u} dx \\
& + \int_{\Omega} \sum_{i=1}^N \frac{\partial_{c_i} \lambda_e}{2} |\nabla \Psi|^2 \frac{\partial c_i}{\partial t} d\mathbf{x} + \int_{\Omega} (\nabla \Psi^T (\nabla \cdot (\lambda_e \nabla \Psi))) \cdot \mathbf{u} d\mathbf{x} \\
& + \int_{\Omega} \sum_{i=1}^N \left[\sum_{j=1}^N (-\lambda_{ij} \Delta c_j) + \frac{\beta}{2} \partial_{c_i} G(\vec{c}) \right] \frac{\partial c_i}{\partial t} d\mathbf{x} \\
& + \int_{\partial\Omega} (\boldsymbol{\sigma} \cdot \mathbf{n}) \cdot \mathbf{u} dS - \int_{\partial\Omega} \frac{|\mathbf{u}|^2}{2} \rho \mathbf{u} \cdot \mathbf{n} ds + \int_{\partial\Omega} (\lambda_e \nabla \Psi \cdot \mathbf{n}) \cdot \frac{\partial \Psi}{\partial t} ds \\
& + \int_{\partial\Omega} \sum_{i,j=1}^N \bar{\gamma} \left(\lambda_{ij} \frac{\partial}{\partial t} c_i \cdot \nabla c_j \cdot \mathbf{n} \right) ds
\end{aligned} \tag{3.17}$$

Further factoring out $\frac{\partial c_i}{\partial t}$ from lines 2 and 3 of equation yields:

$$\begin{aligned}
\frac{dE_{tot}}{dt} = & - \int_{\Omega} (\boldsymbol{\sigma} + PI) : \nabla \mathbf{u} dx + \int_{\Omega} \mathbf{H} \cdot \mathbf{u} dx + \int_{\Omega} (\nabla \Psi^T (\nabla \cdot (\lambda_e \nabla \Psi))) \cdot \mathbf{u} d\mathbf{x} \\
& + \int_{\Omega} \sum_{i=1}^N \left[\frac{\partial_{c_i} \lambda_e}{2} |\nabla \Psi|^2 + \sum_{j=1}^N (-\lambda_{ij} \Delta c_j) + \frac{\beta}{2} \partial_{c_i} G(\vec{c}) \right] \frac{\partial c_i}{\partial t} d\mathbf{x} \\
& + \int_{\partial\Omega} (\boldsymbol{\sigma} \cdot \mathbf{n}) \cdot \mathbf{u} dS - \int_{\partial\Omega} \frac{|\mathbf{u}|^2}{2} \rho \mathbf{u} \cdot \mathbf{n} ds + \int_{\partial\Omega} (\lambda_e \nabla \Psi \cdot \mathbf{n}) \cdot \frac{\partial \Psi}{\partial t} ds \\
& + \int_{\partial\Omega} \sum_{i,j=1}^N \bar{\gamma} \left(\lambda_{ij} \frac{\partial}{\partial t} c_i \cdot \nabla c_j \cdot \mathbf{n} \right) ds
\end{aligned} \tag{3.18}$$

Recall from our assumption in 3.11 $\frac{\partial c_i}{\partial t} = \nabla \cdot \mathbf{q}_i - u \cdot \nabla c_i$. Hence,

$$\begin{aligned}
\frac{dE_{tot}}{dt} = & - \int_{\Omega} (\boldsymbol{\sigma} + PI) : \nabla \mathbf{u} dx + \int_{\Omega} \mathbf{H} \cdot \mathbf{u} dx + \int_{\Omega} (\nabla \Psi^T (\nabla \cdot (\lambda_e \nabla \Psi))) \cdot \mathbf{u} d\mathbf{x} \\
& + \int_{\Omega} \sum_{i=1}^N \left[\frac{\partial_{c_i} \lambda_e}{2} |\nabla \Psi|^2 + \sum_{j=1}^N (-\lambda_{ij} \Delta c_j) + \frac{\beta}{2} \partial_{c_i} G(\vec{c}) \right] (\nabla \cdot \mathbf{q}_i - u \cdot \nabla c_i) d\mathbf{x} \\
& + \int_{\partial\Omega} (\boldsymbol{\sigma} \cdot \mathbf{n}) \cdot \mathbf{u} dS - \int_{\partial\Omega} \frac{|\mathbf{u}|^2}{2} \rho \mathbf{u} \cdot \mathbf{n} ds + \int_{\partial\Omega} (\lambda_e \nabla \Psi \cdot \mathbf{n}) \cdot \frac{\partial \Psi}{\partial t} ds \\
& + \int_{\partial\Omega} \sum_{i,j=1}^N \bar{\gamma} \left(\lambda_{ij} \frac{\partial}{\partial t} c_i \cdot \nabla c_j \cdot \mathbf{n} \right) ds
\end{aligned} \tag{3.19}$$

Now recalling the definitions of the chemical potential terms μ_i we can substitute equation 3.12 in to line 2 of equation 3.19. Thus we have

$$\begin{aligned}
\frac{dE_{tot}}{dt} = & - \int_{\Omega} (\boldsymbol{\sigma} + PI) : \nabla \mathbf{u} dx + \int_{\Omega} \mathbf{H} \cdot \mathbf{u} dx + \int_{\Omega} (\nabla \Psi^T (\nabla \cdot (\lambda_e \nabla \Psi))) \cdot \mathbf{u} d\mathbf{x} \\
& + \int_{\Omega} \sum_{i=1}^N \mu_i (\nabla \cdot \mathbf{q}_i - \mathbf{u} \cdot \nabla c_i) d\mathbf{x} \\
& + \int_{\partial\Omega} (\boldsymbol{\sigma} \cdot \mathbf{n}) \cdot \mathbf{u} dS - \int_{\partial\Omega} \frac{|\mathbf{u}|^2}{2} \rho \mathbf{u} \cdot \mathbf{n} ds + \int_{\partial\Omega} (\lambda_e \nabla \Psi \cdot \mathbf{n}) \cdot \frac{\partial \Psi}{\partial t} ds \\
& + \int_{\partial\Omega} \sum_{i,j=1}^N \bar{\gamma} \left(\lambda_{ij} \frac{\partial}{\partial t} c_i \cdot \nabla c_j \cdot \mathbf{n} \right) ds
\end{aligned} \tag{3.20}$$

Finally we solve for $\boldsymbol{\sigma}, \mathbf{H}, q_1, q_2$ by matching terms with the dissipation energy in equation 3.9. First we collect terms related to the flow $\mathbf{u}$

$$\begin{aligned}
- \int_{\Omega} \frac{\eta(\vec{c})(1 - c_N)}{\kappa} |\mathbf{u}|^2 d\mathbf{x} &= \int_{\Omega} \mathbf{H} \cdot \mathbf{u} - \sum_{i=1}^N \mu_i (\mathbf{u} \cdot \nabla c_i) + (\nabla \Psi^T (\nabla \cdot (\lambda_e \nabla \Psi))) \cdot \mathbf{u} d\mathbf{x} \\
\int_{\Omega} \mathbf{H} \cdot \mathbf{u} d\mathbf{x} &= \int_{\Omega} \sum_{i=1}^N \mu_i (\mathbf{u} \cdot \nabla c_i) - (\nabla \Psi^T (\nabla \cdot (\lambda_e \nabla \Psi))) \cdot \mathbf{u} \\
&\quad - \frac{\eta(\vec{c})(1 - c_N)}{\kappa} |\mathbf{u}|^2 d\mathbf{x} \\
\mathbf{H} &= \sum_{i=1}^N \mu_i \nabla c_i - (\nabla \Psi^T (\nabla \cdot (\lambda_e \nabla \Psi))) - \frac{\eta(\vec{c})(1 - c_N)}{\kappa} \cdot \mathbf{u}
\end{aligned} \tag{3.21}$$

Next we solve for q_i . To do this we collect the terms related to the chemical potentials μ_i .

$$\int_{\Omega} \mu_i (\nabla \cdot \mathbf{q}_i) d\mathbf{x} = - \int_{\Omega} \tau_{ii} |\nabla \mu_i|^2 + \sum_{\substack{j=1 \\ j \neq i}}^N \frac{1}{2} \tau_{ij} \nabla \mu_i \nabla \mu_j + \sum_{\substack{j=1 \\ j \neq i}}^N \frac{1}{2} \tau_{ji} \nabla \mu_j \nabla \mu_i d\mathbf{x}$$

Integrating by parts we have

$$- \int_{\Omega} \tau_{ii} |\nabla \mu_i|^2 + \sum_{\substack{j=1 \\ j \neq i}}^N \frac{1}{2} \tau_{ij} \nabla \mu_i \nabla \mu_j + \sum_{\substack{j=1 \\ j \neq i}}^N \frac{1}{2} \tau_{ji} \nabla \mu_j \nabla \mu_i d\mathbf{x} = \int_{\Omega} -\nabla \mu_i \cdot \mathbf{q}_1 + \int_{\partial\Omega} \mu_i \cdot (\mathbf{q}_1) \cdot \mathbf{n} ds$$

Using the symmetry of our mobility matrix we get

$$\begin{aligned}
-\int_{\Omega} \tau_{ii} |\nabla \mu_i|^2 + \sum_{\substack{j=1 \\ j \neq i}}^N \tau_{ij} \nabla \mu_i \nabla \mu_j d\mathbf{x} &= \int_{\Omega} -\nabla \mu_1 \cdot (q_1) + \int_{\partial\Omega} \mu_1 \cdot (q_1) \cdot \mathbf{n} ds \\
q_i &= \tau_{ii} \nabla \mu_i + \sum_{\substack{j=1 \\ j \neq i}}^N \tau_{ij} \nabla \mu_j \\
q_i &= \sum_{j=1}^N \tau_{ij} \nabla \mu_j
\end{aligned} \tag{3.22}$$

Finally we collect the terms related to $\nabla \mathbf{u}$

$$\begin{aligned}
-\int_{\Omega} [\frac{\eta}{2} |\nabla \mathbf{u} + (\nabla \mathbf{u})^T|^2] &= -\int_{\Omega} (\boldsymbol{\sigma} + PI) : \nabla \mathbf{u} dx \\
\boldsymbol{\sigma} &= \eta D - PI
\end{aligned} \tag{3.23}$$

Hence our system combining equations 3.11 with equations 3.21, 3.22, and 3.23

$$\begin{cases} \rho(\partial_t + \mathbf{u} \cdot \nabla) \mathbf{u} &= \nabla \cdot \boldsymbol{\sigma} + \mathbf{H} \\ \nabla \cdot \mathbf{u} &= 0 \\ (\partial_t + \mathbf{u} \cdot \nabla) \Psi &= 0 \\ (\partial_t + \mathbf{u} \cdot \nabla) c_i &= \nabla \cdot q_i \quad \text{for } 1 \leq i \leq N \end{cases} \tag{3.24}$$

where

$$\begin{aligned}
\mathbf{H} &= \sum_{i=1}^N \mu_i \nabla c_i - (\nabla \Psi^T (\nabla \cdot (\lambda_e \nabla \Psi))) - \frac{\eta(\vec{c})(1 - c_N)}{\kappa} \cdot \mathbf{u} \\
q_i &= \sum_{j=1}^N \tau_{ij} \nabla \mu_j
\end{aligned} \tag{3.25}$$

$$\boldsymbol{\sigma} = \eta D - PI$$

$$\mu_i = \frac{\partial_{c_i} \lambda_e |\nabla \Psi|^2}{2} + \frac{\beta}{2} \partial_i G(\vec{c}) + \sum_{j=1}^N (-\lambda_{ij} \Delta c_j)$$

3.4 Equation in Vector Form

In order to implement the numerical algorithm our general strategy will be to use an implicit scheme to solve for the first $N - 1$ phases and then use the relationship $c_N = 1 - \sum_{i=1}^{N-1} c_i$ to solve for c_N . Since we are only concerned with the first $N - 1$ phases we can rewrite the equations $(\partial_t + \mathbf{u} \cdot \nabla) c_i = \nabla \cdot \mathbf{q}_i$ for $1 \leq i \leq N - 1$ into vectorized system entirely in terms of the first $N - 1$ phase variables.

Using the condition that $\tau_{ij} = \tau_{ji}$ the i^{th} equation can be written as

$$\frac{\partial c_i}{\partial t} + (\mathbf{u} \cdot \nabla) c_i = \sum_{j=1}^N \nabla \cdot \left[\tau_{ij} \nabla \left(- \left(\sum_{l=1}^N \lambda_{jl} \Delta c_l \right) + \frac{\beta}{2} \partial_{c_j} G(\vec{c}) + \frac{\partial_{c_j} \lambda_e |\nabla \Psi|^2}{2} \right) \right] \quad (3.26)$$

Substituting $c_N = 1 - \sum_{k=1}^{N-1} c_k$ and $\tau_{iN} = -\sum_{j=1}^{N-1} \tau_{ij}$ into 3.26 and noting that $\Delta c_N = \Delta \left(1 - \sum_{k=1}^{N-1} c_k \right) = -\Delta \sum_{k=1}^{N-1} c_k$ the right hand side becomes

$$\begin{aligned} &= \sum_{j=1}^{N-1} \nabla \cdot \left[\tau_{ij} \nabla \left(- \left(\sum_{l=1}^{N-1} \lambda_{jl} \Delta c_l - \lambda_{jN} \Delta \sum_{k=1}^{N-1} c_k \right) + \frac{\beta}{2} \partial_{c_j} G(\vec{c}) + \frac{\partial_{c_j} \lambda_e |\nabla \Psi|^2}{2} \right) \right] \\ &+ \nabla \cdot \left[\left(- \sum_{j=1}^{N-1} \tau_{ij} \right) \nabla \left(- \left(\sum_{l=1}^N \lambda_{jl} \Delta c_l \right) + \frac{\beta}{2} \partial_{c_j} G(\vec{c}) + \frac{\partial_{c_j} \lambda_e |\nabla \Psi|^2}{2} \right) \right] \end{aligned} \quad (3.27)$$

We now make the following substitutions for ease of computation. Let,

$$L_j = - \left(\sum_{l=1}^{N-1} \lambda_{jl} \Delta c_l - \lambda_{jN} \Delta \sum_{k=1}^{N-1} c_k \right) \quad (3.28)$$

$$E_j = \frac{\beta}{2} \partial_{c_j} G(\vec{c}) + \frac{\partial_{c_j} \lambda_e |\nabla \Psi|^2}{2} \quad (3.29)$$

Hence equation 3.27 can be written as

$$\begin{aligned} \frac{\partial c_i}{\partial t} + (\mathbf{u} \cdot \nabla) c_i &= \sum_{j=1}^{N-1} \nabla \cdot [\tau_{ij} \nabla (L_j + E_j)] \\ &+ \nabla \cdot \left[\left(- \sum_{i=1}^{N-1} \tau_{1i} \right) \nabla (L_N + E_N) \right] \end{aligned} \quad (3.30)$$

Factoring out like mobility terms we get

$$\frac{\partial c_i}{\partial t} + (\mathbf{u} \cdot \nabla) c_i = \sum_{j=1}^{N-1} \nabla \cdot [\tau_{ij} \nabla (L_j - L_N + E_j - E_N)] \quad (3.31)$$

We can now define a term $\tilde{L}_j$ written as

$$\begin{aligned} \tilde{L}_j &= L_j - L_N \\ &= - \left(\sum_{l=1}^{N-1} \lambda_{jl} \Delta c_l - \lambda_{jN} \sum_{l=1}^{N-1} \Delta c_l \right) + \left(\sum_{l=1}^{N-1} \lambda_{Nl} \Delta c_l - \lambda_{NN} \sum_{l=1}^{N-1} \Delta c_l \right) \\ &= \sum_{l=1}^{N-1} (-\lambda_{jl} + \lambda_{jN} + \lambda_{Nl} - \lambda_{NN}) \Delta c_l \end{aligned} \quad (3.32)$$

Thus substituting equation 3.32 into 3.31 yields

$$\frac{\partial c_i}{\partial t} + (\mathbf{u} \cdot \nabla) c_i = \sum_{j=1}^{N-1} \nabla \cdot [\tau_{1j} \nabla (\tilde{L}_j + E_j - E_5)] \quad (3.33)$$

Where

$$\tilde{L}_j = \sum_{l=1}^{N-1} (-\lambda_{jl} + \lambda_{jN} + \lambda_{Nl} - \lambda_{NN}) \Delta c_l \quad (3.34)$$

Given the above calculations we can now write out the vectorized system for $N = 5$ as in our proposed model set up

$$\begin{aligned} & \left\{ \begin{aligned} & \frac{\partial}{\partial t} \begin{bmatrix} c_1 \\ c_2 \\ c_3 \\ c_4 \end{bmatrix} + \begin{bmatrix} (\mathbf{u} \cdot \nabla) c_1 \\ (\mathbf{u} \cdot \nabla) c_2 \\ (\mathbf{u} \cdot \nabla) c_3 \\ (\mathbf{u} \cdot \nabla) c_4 \end{bmatrix} = \nabla \cdot \begin{bmatrix} \tau_{11} & \tau_{12} & \tau_{13} & \tau_{14} \\ \tau_{21} & \tau_{22} & \tau_{23} & \tau_{24} \\ \tau_{31} & \tau_{32} & \tau_{33} & \tau_{34} \\ \tau_{41} & \tau_{42} & \tau_{43} & \tau_{44} \end{bmatrix} \begin{bmatrix} \nabla \mu_1 \\ \nabla \mu_2 \\ \nabla \mu_3 \\ \nabla \mu_4 \end{bmatrix} \\ & \begin{bmatrix} \mu_1 \\ \mu_2 \\ \mu_3 \\ \mu_4 \end{bmatrix} = \nabla \cdot \tilde{\Lambda} \begin{bmatrix} \nabla c_1 \\ \nabla c_2 \\ \nabla c_3 \\ \nabla c_4 \end{bmatrix} + \frac{\beta}{2} \begin{bmatrix} \partial_{c_1} G(\vec{c}) - \partial_{c_5} G(\vec{c}) \\ \partial_{c_2} G(\vec{c}) - \partial_{c_5} G(\vec{c}) \\ \partial_{c_3} G(\vec{c}) - \partial_{c_5} G(\vec{c}) \\ \partial_{c_4} G(\vec{c}) - \partial_{c_5} G(\vec{c}) \end{bmatrix} + \frac{\lambda_e}{2} \begin{bmatrix} \partial_{c_1} |\nabla \Psi|^2 - \partial_{c_5} |\nabla \Psi|^2 \\ \partial_{c_2} |\nabla \Psi|^2 - \partial_{c_5} |\nabla \Psi|^2 \\ \partial_{c_3} |\nabla \Psi|^2 - \partial_{c_5} |\nabla \Psi|^2 \\ \partial_{c_4} |\nabla \Psi|^2 - \partial_{c_5} |\nabla \Psi|^2 \end{bmatrix} \end{aligned} \right. \end{aligned}$$

Where

$$\tilde{\Lambda}_{ij} = -\lambda_{ij} + \lambda_{i5} + \lambda_{5j} - \lambda_{55}$$

Thus the final proposed model system for the complete 5 phase coupled Cahn-Hilliard Navier-Stokes equations can be written as:

$$\left\{ \begin{array}{l} \rho(\partial_t + \mathbf{u} \cdot \nabla) \mathbf{u} = \nabla \cdot (\eta D - P) - \sum_i^5 c_i \nabla \mu_i - (\nabla \Psi^T (\nabla \cdot (\lambda_e \nabla \Psi))) - \frac{\eta(\vec{c})(1-c_3)}{\kappa} \cdot \mathbf{u} \\ \nabla \cdot \mathbf{u} = 0 \\ (\partial_t + \mathbf{u} \cdot \nabla) \Psi = 0 \\ \frac{\partial}{\partial t} \begin{bmatrix} c_1 \\ c_2 \\ c_3 \\ c_4 \end{bmatrix} + \begin{bmatrix} (\mathbf{u} \cdot \nabla) c_1 \\ (\mathbf{u} \cdot \nabla) c_2 \\ (\mathbf{u} \cdot \nabla) c_3 \\ (\mathbf{u} \cdot \nabla) c_4 \end{bmatrix} = \nabla \cdot \begin{bmatrix} \tau_{11} & \tau_{12} & \tau_{13} & \tau_{14} \\ \tau_{21} & \tau_{22} & \tau_{23} & \tau_{24} \\ \tau_{31} & \tau_{32} & \tau_{33} & \tau_{34} \\ \tau_{41} & \tau_{42} & \tau_{43} & \tau_{44} \end{bmatrix} \begin{bmatrix} \nabla \mu_1 \\ \nabla \mu_2 \\ \nabla \mu_3 \\ \nabla \mu_4 \end{bmatrix} \\ \begin{bmatrix} \mu_1 \\ \mu_2 \\ \mu_3 \\ \mu_4 \end{bmatrix} = \nabla \cdot \tilde{\Lambda} \begin{bmatrix} \nabla c_1 \\ \nabla c_2 \\ \nabla c_3 \\ \nabla c_3 \end{bmatrix} + \frac{\beta}{2} \begin{bmatrix} \partial_{c_1} G(\vec{c}) - \partial_{c_5} G(\vec{c}) \\ \partial_{c_2} G(\vec{c}) - \partial_{c_5} G(\vec{c}) \\ \partial_{c_3} G(\vec{c}) - \partial_{c_5} G(\vec{c}) \\ \partial_{c_4} G(\vec{c}) - \partial_{c_5} G(\vec{c}) \end{bmatrix} + \frac{\lambda_e}{2} \begin{pmatrix} \partial_{c_1} |\nabla \Psi|^2 - \partial_{c_5} |\nabla \Psi|^2 \\ \partial_{c_2} |\nabla \Psi|^2 - \partial_{c_5} |\nabla \Psi|^2 \\ \partial_{c_3} |\nabla \Psi|^2 - \partial_{c_5} |\nabla \Psi|^2 \\ \partial_{c_4} |\nabla \Psi|^2 - \partial_{c_5} |\nabla \Psi|^2 \end{pmatrix} \end{array} \right.$$

3.5 Discussion and Future Work

In this chapter we derived a novel 5 phase coupled Cahn-Hilliard/Navier-Stokes system to describe the deformation of a blood clot under fluid flow. The phases include an inner core consisting of red blood cells, fibrin network, and highly activated platelets, an outer core of less activated platelets, and the flow of platelet-rich plasma. The model is thermodynamically consistent and accounts for both macroscopic and microscopic forces within the system. Furthermore, relying on recent advances in [34] and [107], the model proposed here includes all pairwise surface tensions between phases.

For future work, we plan to use the same numerical algorithm proposed in [109]. The implementation of this is currently ongoing using FEniCSx. Once the numerical implementation has been successfully completed, we plan to calibrate the model to recent experimental data on the mechanical properties of blood clots such as viscosity, elastic energy density, mobility coefficients, and surface

tensions for each of the 5 phases. Finally, we will use the calibrated model to test the deformation and embolization of the thrombus under different flow conditions, permeabilities, and distributions of red blood cells.

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Appendix A

Supplementary Materials for Chapter 1

For calibration to *Pseudomonas aeruginosa* we use the experimental data found in [97]. However, we note that pull and push mode statistics for *P. aeruginosa* are very similar. Furthermore, other experimental data from *P. putida* suggests that pull mode takes up less than 2 percent of the total swimming time [4]. Thus we model push and pull mode as only one distinct mode which we refer to as “run” throughout this text.

A.1 Model Calibration

A.1.1 Velocity

We fix a constant velocity for both run mode and wrap mode respectively. For both we choose the average velocity found for push and wrap mode as follows:

$$\text{Push Mode: } 37.95 \frac{\mu m}{s}$$

$$\text{Wrap Mode: } 14.45 \frac{\mu m}{s}$$

This is done by adjusting the force F_{motor}^i . Given the overdamped Langevin equation

$$\frac{dx_i(t)}{dt} = -\frac{1}{\gamma} \nabla_i U^i(x, t) \quad (\text{A.1})$$

we make the assumption that $F_{bond}^i + F_{bend}^i$ do not contribute to the velocity of the bacteria. Hence, we have that

$$\begin{aligned} \frac{dx_i(t)}{dt} &= -\frac{1}{\gamma} \nabla U^i(x, t) = \frac{1}{\gamma} F^i(x, t) \\ &= \frac{1}{\gamma} (F_{bond}^i + F_{bend}^i + F_{motor}^i) = \frac{1}{\gamma} F_{motor}^i \end{aligned}$$

Given $\gamma = .01$ we have that in run mode:

$$\begin{aligned} \frac{F_{motor}^i}{.01} &= \frac{dx_i(t)}{dt} = 37.95 \\ F_{motor}^i &= .3795 \end{aligned}$$

Similarly in wrap mode we have

$$\begin{aligned} \frac{F_{motor}^i}{.01} &= \frac{dx_i(t)}{dt} = 14.45 \\ F_{motor}^i &= .1445 \end{aligned}$$

A.1.2 Durations

We fit the durations of both wrap and run modes in the following manner. First the experimental histograms in Figure 5A and Figure S6D in [97] were extracted using the function GRABIT in MATLAB. Then both μ and σ are found such that the error

$$\sum_{i=1}^{\text{nbins}} \left(f(x_i, \mu, \sigma) \frac{\text{max duration}}{\text{nbins}} - y_i \right)^2 \quad (\text{A.2})$$

is minimized. Here f is the pdf for a truncated lognormal distribution, max duration is the maximum mode duration observed from experiments, and y_i is the experimental pdf for a given duration x_i . For run durations we choose to calibrate to the push durations found in Figure S6D and exclude the first 2 bins from the calibration to get a more accurate fit. The following figures show both the

extracted experimental data as well as the fit distribution. Here we found for run duration $\mu = .7144$ and $\sigma = .7440$.

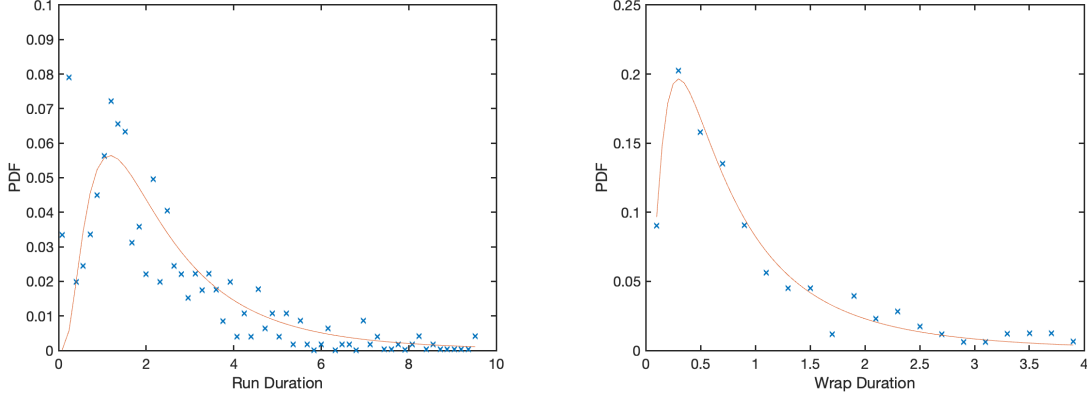


Figure A.1: Calibrated pdfs for both run durations (left) and wrap durations (right). The blue shows the experimental data while the red shows the pdf of the truncated log-normal distribution.

A.1.3 Angular Changes

Finally in order to calibrate both the angle change in reversals events as well as the angle change during wrap mode we refer to Figure 5D and Figure 5C in [97] respectively.

Reversals

In our model, since the time during a reversal event is constant, the resulting angle is entirely determined by the force placed on the frontier node. In order to calibrate the force needed to match the experimental distribution we first plot the relationship between the frontier force and the total angle change during the reversal event. Furthermore, we limit the forces to only those which produce angle changes between $90^\circ - 180^\circ$. Line of best fit for Figure A.2 is

$$y = -254.40x + 179.65 \quad (\text{A.3})$$

where y is the change in orientation and x is the force applied to the frontier node.

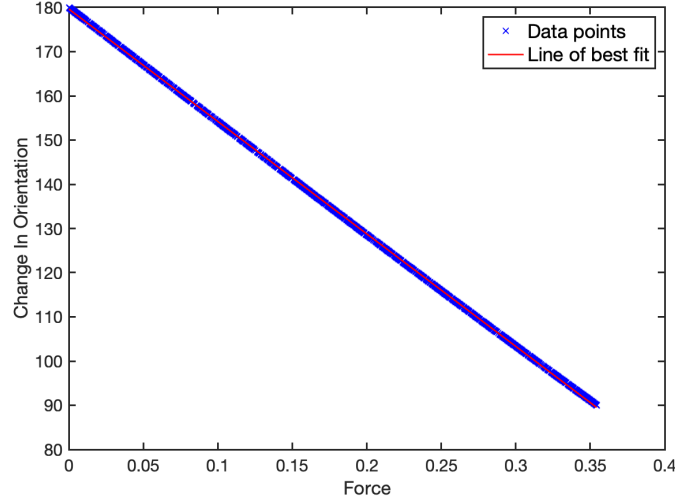


Figure A.2: Plot showing the relationship between the force applied to the frontier node during reversal events and the change in orientation of the bacteria after the initial 180 degree reversal. The blue shows all instances of reversal events that occurred in 200s of simulated bacterial migration. In the simulations bacteria do not enter wrap mode and chemotaxis is turned off.

Given this relationship in order to limit our angular changes between $90^\circ - 180^\circ$ we solve for the force that leads to an angular change of 90°

$$\begin{aligned}
 90 &= -254.40x + 179.65 \\
 -89.65 &= -254.40x \\
 x &= \frac{89.65}{254.40}
 \end{aligned}$$

In contrast, when $x = 0$, the total angular change is 180° since we reverse the order of nodes before applying the force during the reversal process.

Now using the linear function found above we plot the experimental pdf which can be made a function of the force applied to the frontier node. Figure A.3 shows this data along with the line of best fit. Here the data was fit to an exponential and the equation found is

$$E = 0.4495e^{-18.11x} \quad (\text{A.4})$$

Finally, in order to convert this into a usable distribution, we create a CDF from this function using the fact that all of the possible forces should be between 0 and $\frac{89.65}{254.40}$. To find the CDF, we

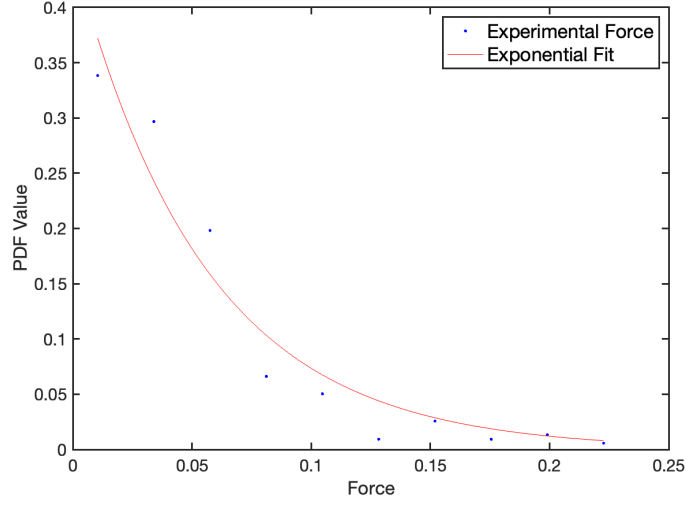


Figure A.3: Plot of the experimental pdf for the force and the line of best fit. The force is found by determining the force necessary for a given turn angle using the relationship found in Figure 2.

first solve the following equation for a

$$1 = \int_0^{\frac{89.65}{254.40}} \frac{0.4495}{a} e^{-18.11x} dx \quad (\text{A.5})$$

$$a \approx 0.0247785 \quad (\text{A.6})$$

Then the desired CDF is

$$F = \int_0^x \frac{0.4495}{0.0247785} e^{-18.11x} dx = 1.0017 - 1.0017e^{-18.11x} \quad (\text{A.7})$$

while the inverse of this is

$$x = \frac{\ln(\frac{F-1.0017}{-1.0017})}{-18.11} \quad (\text{A.8})$$

Then we can use inverse transform sampling to generate a force sampled from the fit distribution. Now that we have the inverse CDF, F^{-1} , we can generate a random sample from a uniform distribution within $[0,1]$ and then calculate the force γ that such $\gamma = F^{-1}(u)$. This force γ is the force that is applied to the frontier node. By using this method we plot the histogram from the calibrated parameters within our model. In Figure A.4, we can see the histogram is a good fit to the experimental data.

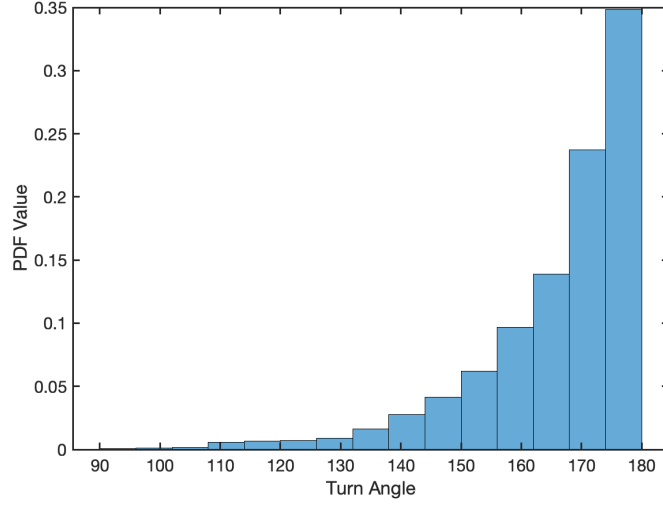


Figure A.4: Simulated pdf of the turn angles during reversal events with calibrated forces.

Wrap Mode

In order to calibrate the turn angle for wrap mode, we note that the angles presented in Figure 5A and 5E of [97] refer to the angle change made between pull and push modes. Thus this includes both the angle change from wrap mode itself as well as the change from a reversal event. Henceforth, we use the term “turn angle” to refer to the total angle change between the beginning of wrap mode and the end of the reversal event, while “wrap angle” will refer to the angle change only during wrap itself.

Data for figure 5E was extracted and fit to an exponential function of the form $ae^{bx} + c$. Figure A.5 shows both the extracted data and the exponential of best fit,

$$y = 51.08e^{-1.439d} + 87.02 \quad (\text{A.9})$$

where d is the duration and y is the turn angle.

Since both the duration and the force affect the turn angle, we first sample a duration from the truncated log-normal distribution shown in Figure A.6, and then use equation A.9 to find the corresponding turn angle that that event should abide by. We make note that reversals are on average centered around 180° . Furthermore, given that the turn angle is always less than 180° we have that

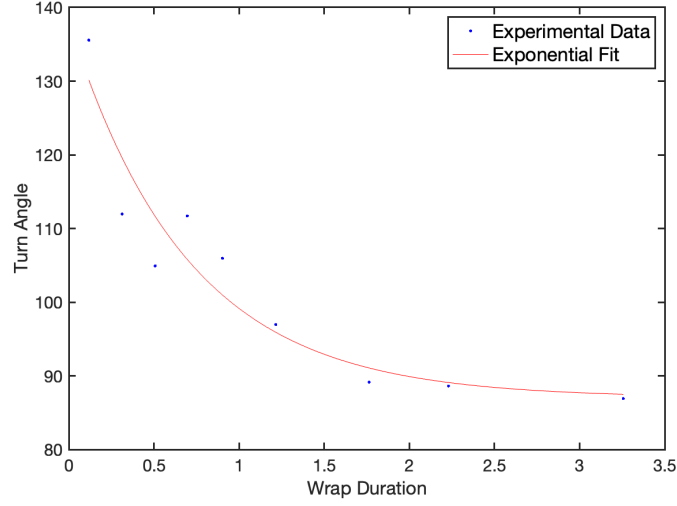


Figure A.5: Plot showing wrap duration vs turn angle. Blue is extracted from experimental figures and red is the line of best fit.

TurnAngle = Reversal – WrapAngle. Hence, the wrap angle for this given turn angle is

$$W = 180 - (51.08e^{-1.439d} + 87.02) \quad (\text{A.10})$$

Finally we are left with finding the force to apply which yields a wrap angle of W . This however, can be done by noting that there is a linear relationship between the force and wrap angle for a fixed duration. In order to deal with this relationship changing for each duration we can normalize all the equations by dividing the wrap angle by the duration. For example, for durations of .5, 2, and 3.5 seconds we have

$$\begin{aligned} \frac{W}{d} &= 403.27 \cdot F + 0.03, \\ \frac{W}{d} &= 397.12 \cdot F + 0.03, \\ \frac{W}{d} &= 396.23 \cdot F + 0.03 \end{aligned}$$

respectively. Thus we approximate this relationship by the equation

$$\frac{W}{d} = 398.67 \cdot F \quad (\text{A.11})$$

where F is the force applied during wrap mode. This finally allows us to find the necessary force by solving for the equation

$$F = \frac{W}{398.67d} = \frac{180 - (51.08e^{-1.439d} + 87.02)}{398.67d} \quad (\text{A.12})$$

For this calibration we have the following calibrated figures resembling 5E and 5A:

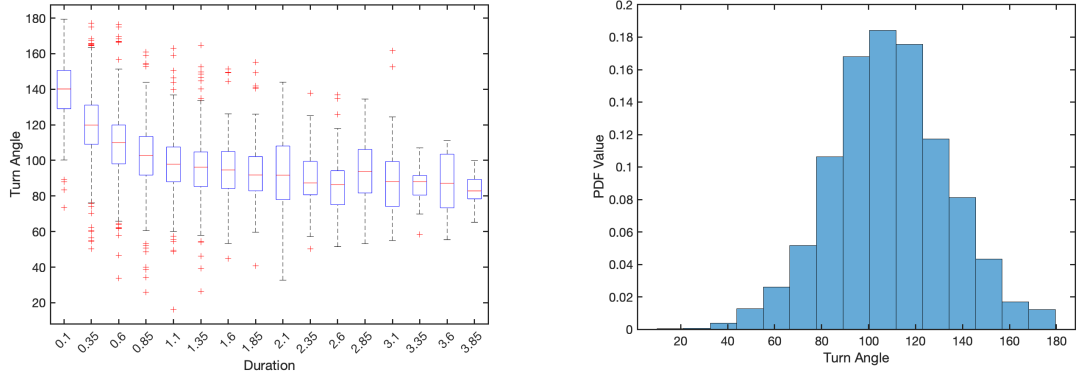


Figure A.6: (Left) shows boxplots of the turn angles during wrap mode for given durations. (Right) shows the pdf of the turn angles during wrap mode. Both figures were generated from simulated bacterial migration across 200s with no chemotaxis

A.2 Chemoattractant Profile Generation

Here we describe how the concentration for linear and exponential gradients are calculated.

A.2.1 Linear

For linear gradients, we use the following analytical expression to generate gradients with different slopes. C is the concentration of the chemoattractant and is calculated by

$$c_{i,j} = a \left(\sqrt{\left(\frac{\text{domain}X}{2}\right)^2 + \left(\frac{\text{domain}Y}{2}\right)^2} - \sqrt{(i - \text{cntr}X)^2 + (j - \text{cntr}Y)^2} \right) \quad (\text{A.13})$$

where (i, j) is a grid point in the domain and $(cntrX, cntrY)$ is the center of the domain. This yields a linear gradient with slope a at every point. Figure S10 shows the profile and a plot of the concentrations on the 1-dimensional diagonal cross section.

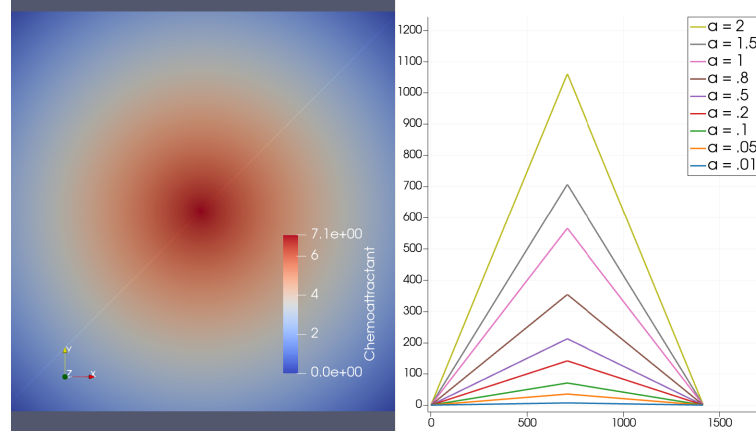


Figure A.7: Plot showing wrap duration vs turn angle. Blue is extracted from experimental figures and red is the line of best fit.

A.2.2 Exponential

In order to generate an exponential profile we use a forward euler method to solve a reaction diffusion equation for an arbitrary chemoattractant. The domain is discretized to squares with length of $5 \mu m$ and the chemoattractant can diffuse from one square into the 4 neighboring squares. We assume the time scale of diffusion for the chemoattractant is faster than the motion of the bacteria. Therefore we calculate the profile before initializing the bacteria colony. The reaction-diffusion equation is solved with no-flux boundary conditions and is run for 10,000 steps. The resultant profile is then interpolated to a $1 \mu m$ grid and taken to be the static profile used throughout the whole simulation. Below we show the resultant profiles for various production rates when the diffusion coefficient is set to 200.

A.3 Wrap Mode Benefit is Dependent on Strength of Gradient

The following section outlines how the benefits of wrap mode only occur within a certain range of gradients. Since the bacteria are made more responsive by increasing the chemosensitivity parameter

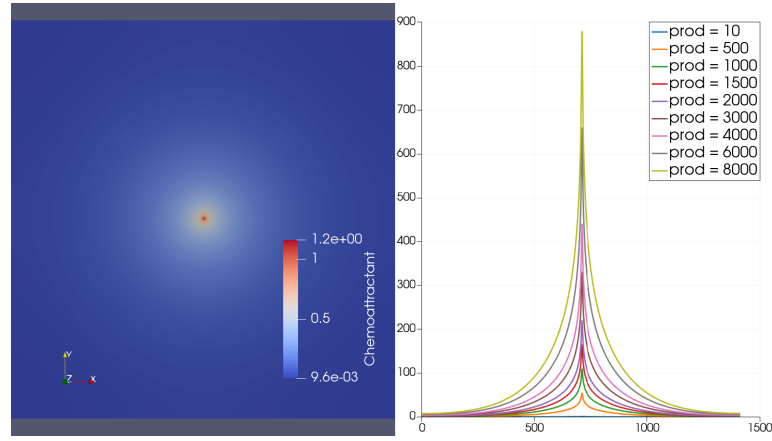


Figure A.8: Plot showing wrap duration vs turn angle. Blue is extracted from experimental figures and red is the line of best fit.

as well as increasing the gradient, this result can also be framed by saying that wrap mode only shows benefit within a set range of chemotactic sensitivities. For both smaller and larger gradients we see that 0% and 38% chance of wrap mode show very similar results. This is the case because wrap mode actually increases the diffusion coefficient in low gradient environments. If the gradient is not large enough to meaningfully impact the run and wrap durations, then this increased diffusion does not correspond to an increased aggregational efficiency. Conversely if the gradient is very large, cells in run mode reverse very quickly when moving down the gradient. If this occurs quick enough then any increased angular exploration from wrap mode is able to be achieved by repeated reversal events.

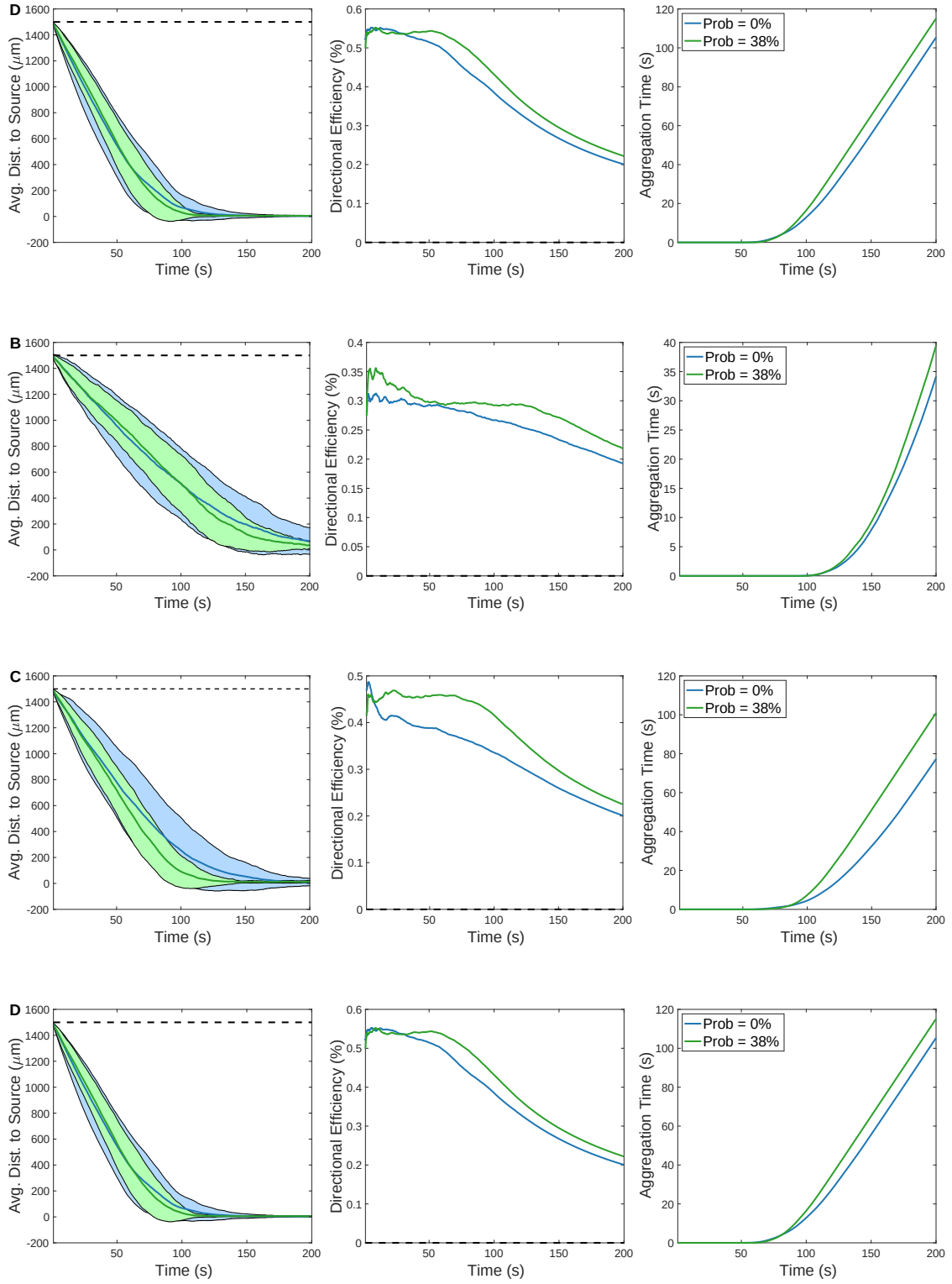


Figure A.9: Bacterial migration in response to different linear gradient profiles (A) Slope of $\frac{4}{5}$ (B) Slope of 1 (C) Slope of 2 (D) Slope of 4. We see that wrap mode gives the most benefit for intermediate chemoattractant gradients.

A.4 Smoothing Algorithm

Algorithm 1 Smoothing Algorithm

Require: Grid $G[nx][ny]$, Threshold $\tau = 0.5$, Viewing Window with dimensions $W_x \times W_y$, and ROI is the region to smooth

Ensure: List of points to update

```
1: Initialize empty list PointsToUpdate
2: for  $p = W_x$  to  $nx - W_x$  do
3:   for  $q = W_y$  to  $ny - W_y$  do
4:     if  $G[p][q]$  is a background point then
5:       ROICount  $\leftarrow 0$ 
6:       TotalCount  $\leftarrow 0$ 
7:       for  $i = -W_x$  to  $W_x$  do
8:         for  $j = -W_y$  to  $W_y$  do
9:           if  $(i, j) \neq (0, 0)$  and  $G[p+i][q+j]$  is in ROI then
10:            ROICount  $\leftarrow$  ROICount + 1
11:            TotalCount  $\leftarrow$  TotalCount + 1
12:          else
13:            TotalCount  $\leftarrow$  TotalCount + 1
14:          end if
15:        end for
16:      end for
17:      ratio  $\leftarrow$  ROICount / TotalCount
18:      if ratio  $\geq \tau$  then
19:        Add  $(p, q)$  to pointsToUpdate
20:      end if
21:    end if
22:  end for
23: end for
24: return pointsToUpdate
```

A.5 Alignment Algorithm

Algorithm 2 Alignment Algorithm

Require: GridsWithLiquid[5] - Number of grids that is liquid within in each sector (Sectors 1-5),
percentage[i] is percentage of non liquid in region i

Ensure: region which the bacteria should move towards

```
1: if If every grid point is liquid in regions 4 and 5 but there is non-liquid in regions 1 and 2 then
2:   if percentage[1]  $\geq$  50% then
3:     if percentage[2]  $\leq$  30% then
4:       region  $\leftarrow$  2
5:     else if percentage[2] in (30%, 60%] then
6:       region  $\leftarrow$  3
7:     else if percentage[2] in (60%, 80%] then
8:       region  $\leftarrow$  4
9:     else
10:      region  $\leftarrow$  5
11:    end if
12:  else
13:    region  $\leftarrow$  1
14:  end if
15: else if If every grid point is liquid in regions 1 and 2 but there is non-liquid in regions 4 and 5
    then
16:   Similar logic for regions 4 and 5
17: else
18:   Find region with maximum liquid concentration
19:   Create list of eligible regions with a liquid concentration at least 80% of the maximum liquid
    concentration
20:   if eligible regions exist then
21:     Randomly select region from eligible ones
22:   end if
23: end if
24: return region
```

Appendix B

Supplementary Materials for Chapter 2

B.1 Parameter Exploration

In this section we explore the relationship between the model parameters $\bar{d}_w$, $\bar{d}_g$, K , n , α , and β . We expand on the observations concluded from the Partial Rank Correlation Coefficients (PRCC) shown in section 4.2, by running three additional sets of 100 simulations each. In each set, 2 parameters are varied over a stated range, and all other parameters remain constant. We then plot the effect on the median distance from injection site, as well as the percentage of cells on white matter over the course of the simulated 9 months. For all simulations, 10,000 cells were injected into a spherical region of radius $.135mm$ in the upper right part of the Corpus Callosum, so as to approximate to the experimental injection site in [85].

B.1.1 Step Size Analysis

We first turn our attention to the effects of $\bar{d}_g$ and $\bar{d}_w$. As shown through the PRCCs, $\bar{d}_w$ is positively correlated to the median distance and is inversely related to the percent on white matter. This is further illustrated in figure B.1. Here we compare 2 simulations where $\bar{d}_w$ is varied between .01888 and .10078, while $n = 7$, $K = .24$, $\alpha = \beta = 1$, and $\bar{d}_g = 0.05164$. A and B show the box plots for the start of each simulated month, as well as the experimental distances at months 3, 6, and 9 shown in

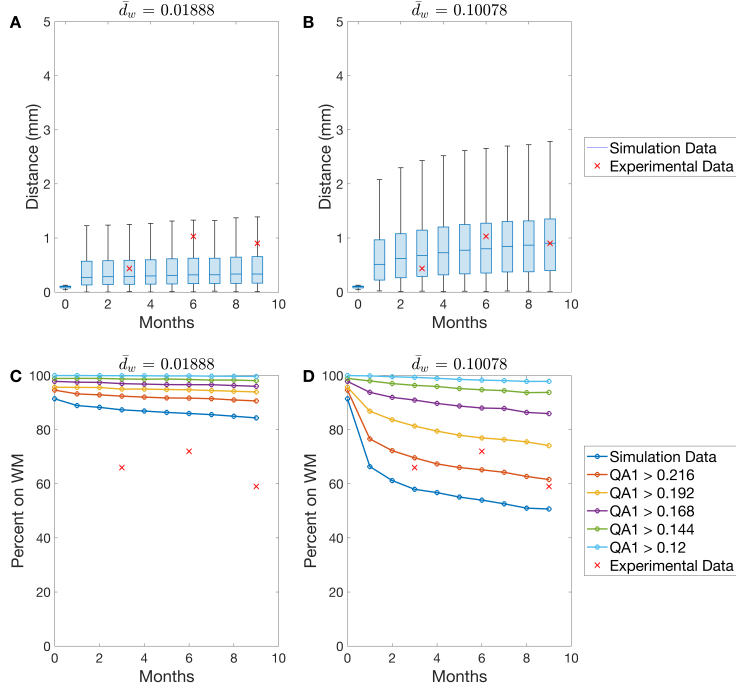


Figure B.1: Box plots of the distances from injection site as well as the percent on white matter across the 9 months of simulated NSC migration. Here $\bar{d}_g = .05164$ is fixed. A and C show $\bar{d}_w = .01888$. While B and D show $\bar{d}_w = .10078$. We see as $\bar{d}_w$ increases the median distance increases while the percent on white matter decreases.

red. We clearly see an increase in the median distance from the injection site as we increase $\bar{d}_w$. In order to quantify the percentage of NSCs on white matter, we consider a NSC at location $\mathbf{x}$ to be on white matter if $QA_1(\mathbf{X}) > K$ and grey matter otherwise. Here $\mathbf{X}$ is the nearest lattice point to the point $\mathbf{x}$ which may be off lattice. This designates every point in the brain with a primary QA value greater than K as white matter. Since QA is relatively smooth we also plot the interface around the white matter tracts by considering $\mathbf{x}$ to be on white matter when $QA_1(\mathbf{X}) > (K - \frac{K}{10} \cdot i)$ for $i \in [0, 1, 2, 3, 4, 5]$. Looking at plots C and D of Figure 4, we can again clearly see that increasing $\bar{d}_w$ leads to a decrease in the percentage on white matter.

In contrast, $\bar{d}_g$ is positively related to both the median distance and the percent on white matter. The positive correlation between both step sizes and the median distance from the injection site is intuitive, as the larger the step sizes, the faster the cells travel in the brain. However, as mentioned in the main text, the effect of $\bar{d}_g$ on the median distance is partially dependent on the range of step sizes considered when calculating the PRCC. In figure B.2 we plot the residuals $(x - \hat{x}_j)$ and $(y_j - \hat{y})$ where x and y are the rank transformed $\bar{d}_g$ values and 9 month median distances respectively,

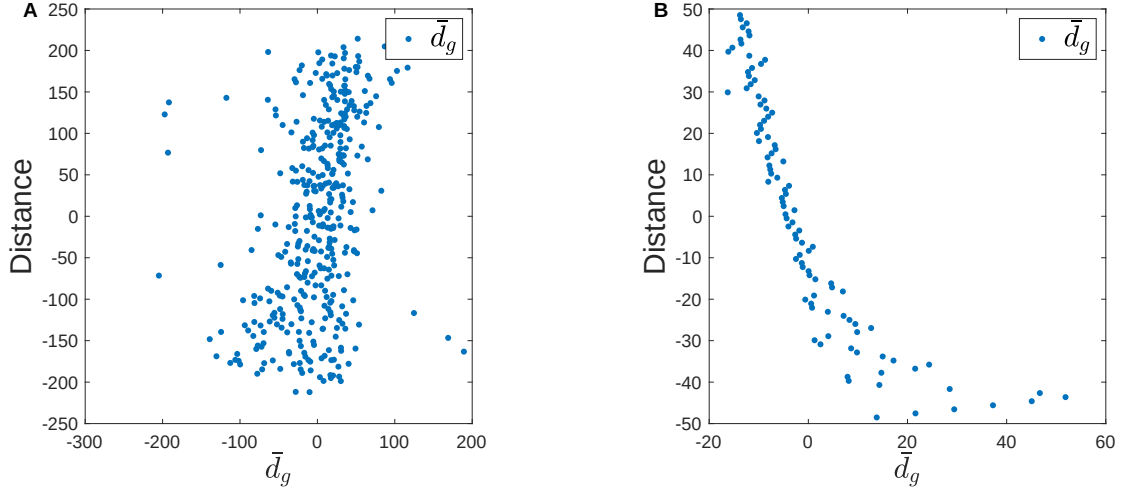


Figure B.2: Plot showing the residuals $(x - \hat{x}_j)$ and $(y_j - \hat{y})$ where x and y are the rank transformed $\bar{d}_g$ values and 9 month median distance respectively and $\hat{x}$, $\hat{y}$ are from the linear regression models. (a) shows the residuals for sensitivity analysis run across the entire relevant parameter space while (b) shows the residuals for simulations with $\bar{d}_g \in [.0025, .15]$

and $\hat{x}$, $\hat{y}$ are from the linear regression models. In A we depict the residuals from the full Latin hyper-cube sampling of 400 simulations with parameter values sampled from the following ranges: $\bar{d}_g$ and $\bar{d}_w \in [0, .6]$, $K \in [0, .5]$, $n \in [1, 30]$ and $\alpha, \beta \in [1, 100]$. In B, the depicted residuals come from a set of 100 simulations where $\bar{d}_g$ and $\bar{d}_w$ are selected from the range $[.0025 : .01638 : .14992]$ while $n = 7$, $K = .24$, $\alpha = \beta = 1$ are held constant.

In comparing the residuals we notice that for smaller $\bar{d}_g$ and $\bar{d}_w$ values, the correlation is actually negative. We illustrate this in figure B.3. Here $\bar{d}_g$ is varied between .01888 and .10078 while $n = 7$, $K = .24$, $\alpha = \beta = 1$, and $\bar{d}_w = 0.05164$. Figure A and B show that as $\bar{d}_g$ increases, the median distance decreases. This is true for all 9 simulated months. Furthermore, in C and D, we can see the inverse relationship between the percent on white matter and $\bar{d}_g$. As discussed in section 4.2, the accumulation of the NSC's in white or grey matter is governed by the ratio $\frac{\bar{d}_w}{\bar{d}_g}$. Thus, an increase in $\bar{d}_g$ actually increases the tendency of the cells to accumulate on white matter. We hypothesize that when the step sizes are small, the increased accumulation on white matter may lead to a disproportionate decrease in the ability of cells to leave their local white matter tracts. This could lead the NSCs to become trapped in one area of the brain, ultimately lowering the median distance from the injection site. However, when the step sizes are larger, this increased accumulation may not

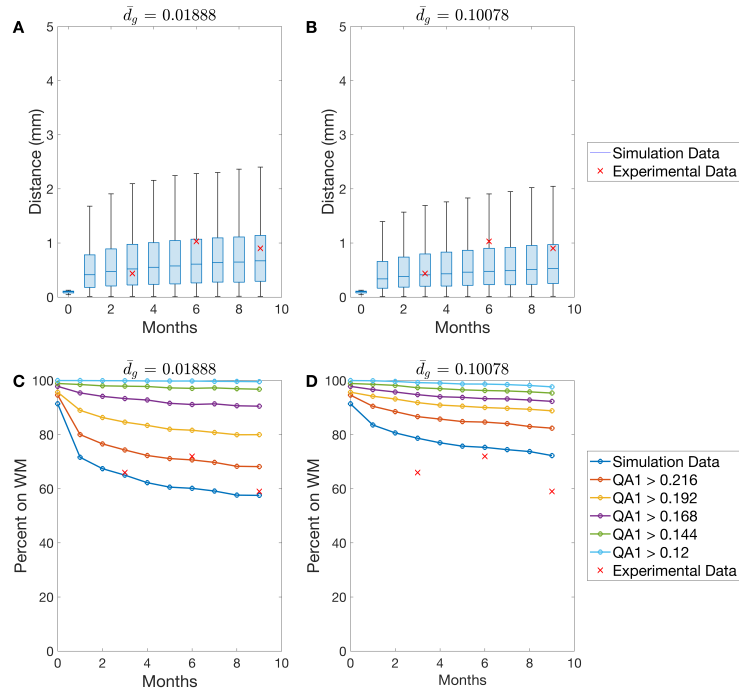


Figure B.3: Box plots for the distances from injection site as well as the percent on white matter across the 9 months of simulated NSC migration. Here $\bar{d}_w = .05164$ is fixed. A and C show $\bar{d}_g = .01888$. While B and D show $\bar{d}_g = .10078$. We see as $\bar{d}_g$ increases the median distance decreases while the percent on white matter increases.

be as impactful, as the cells will move fast enough to traverse the grey matter areas of the brain before finding other white matter fibers to follow.

B.1.2 Hill Parameters Analysis

In this section we illustrate the effects of the parameters K and n on the median distances from the injection site and percent on white matter. The simulations shown in figure B.4 are taken from a set of 100 simulations with $K \in [.1 : .04 : .46]$ and $n \in [3 : 2 : 21]$. The rest of the parameters are fixed with $dg_m = dw_m = .1$ and $\alpha = \beta = 1$. The first observation we make is that as we increase K , the median distance from the injection site decreases. Since K is the point where half of the cell velocity comes from directed motion and half is random, this implies that as cells move with more randomized motion, the lower the median distance from injection site will be. Since K also directly controls the QA values that are considered white matter, we see that increasing K decreases the percent on white matter as expected. This happens because less regions in the brain are considered as white matter with increased K .

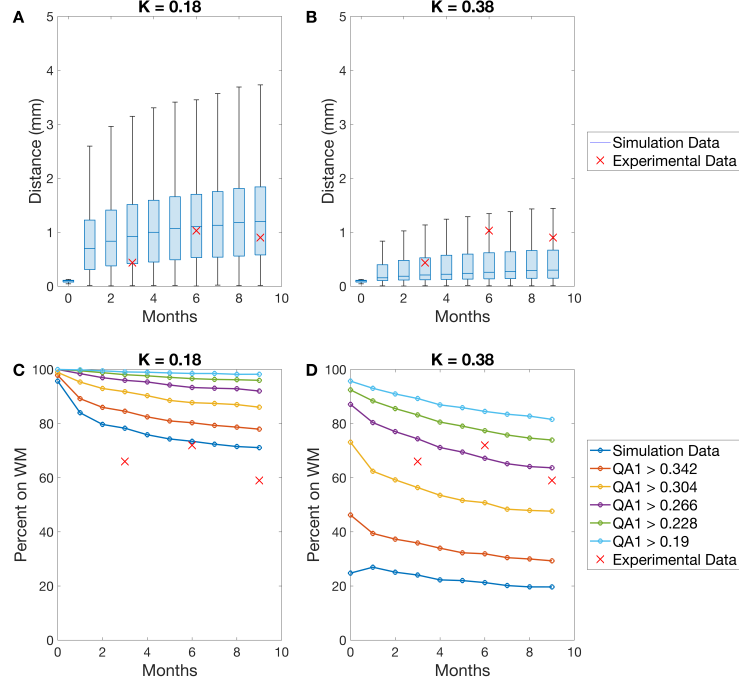


Figure B.4: Box plots for the distances from injection site as well as the percent on white matter across the 9 months of simulated NSC migration. Here $n = 7$ is fixed. A and C show $K = .18$. While B and D show $K = .38$. In We see as K increases the median distance as well as percent on white matter decreases.

Now, considering a fixed K and a variable n , we find that the parameter n does not make much difference. The only notable trend shows up when we have a large fixed value of $K > .42$, which is

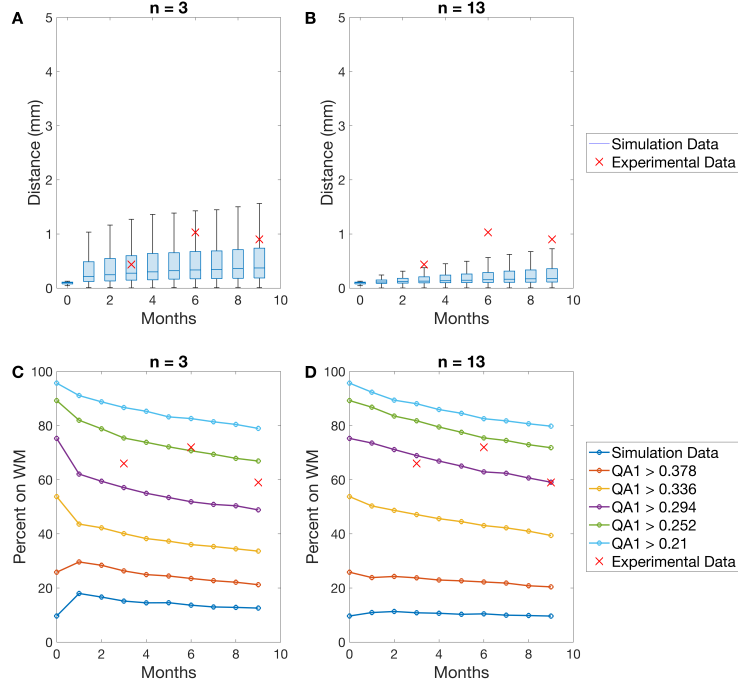


Figure B.5: Box plots for the distances from injection site as well as the percent on white matter across the 9 months of simulated NSC migration. Here $K = .42$ is fixed. A and C show $n = 3$. While B and D show $n = 13$. We see as n increases the median distance as well as the variance also decreases. The percent on white matter is relatively unaffected.

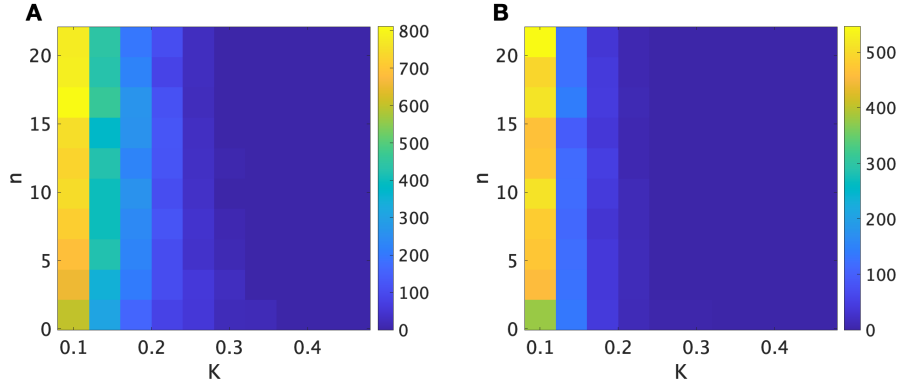


Figure B.6: Heat map showing the number of cells that are within the Anterior Commissure at 6 months (Top Left), the AC at any point in the simulation (Top Right), the Olfactory bulb by 9 months (Bottom Left) and the Olfactory bulb at any point (Bottom Right). We see that for lower K values the great amount of cells make it to the AC and CC respectively.

depicted in figure B.5. Here we see that when n increases, the median distance decreases. This is because cells are unable to leave the local white matter tract. Since K is large, most parts of the brain are considered grey matter and thus an increase in n causes this grey matter motion to be

completely random. This causes it to become increasingly less likely that cells are able to travel across large portions of grey matter and find other white matter tracts to explore.

Finally, if we consider how many cells make it to the AC and olfactory bulb at any point in the simulation, we note one more interesting trend. In figure B.6 we plot heat maps showing the number of cells that made it to the anterior commissure and the olfactory bulb at any point in the simulation. Again, n does not play a big role in either of these behaviors; however, we see that as K decreases the amount of cells that make it to these structures increases. This suggests that as more motion comes from the tissue structure, the more migration we get to the AC and the olfactory bulb.

B.1.3 Stochasticity Analysis

Finally, we illustrate the effects of varying the stochasticity parameters α and β . Here we fix $K = .24$, $n = 7$, $dg_m = dw_m = .1$. We point out that for a given beta distribution, $X \sim Beta(\alpha, \beta)$, as β increases, the distribution skews towards the left, and thus more cells have smaller step sizes. Inversely as α increases, the distribution skews toward the right, and more cells have a larger step size. In order to isolate only the effects of stochasticity we compare the results for the extreme case when X is uniform with $\alpha = \beta = 1$ and when $X(x) = .5$ for all x . Note that both have the same expected value of .5. Furthermore, in order to eliminate the noise from starting locations, we initialize the NSC's at a single location within the brain. In Figure B.7 we see that there is little effect on the median distance and percent on white matter when the expected value is fixed. However, this stochasticity parameter does effect the number of outliers. We see that in A there are considerably more outliers for the uniform distribution in comparison to the fixed value in B. This suggests that the expected value is the biggest contributor to the median distance and percent on white matter.

We can again look at the heat map for the number of cells that made it to the AC and Olfactory Bulb coming from 100 simulation with $\alpha, \beta \in [1, 10]$. We see in figure B.8 that the number of cells that reach the AC and olfactory bulb is highest when β is low and α is large. Since varying α, β changes the expected value of the distributions, the average velocities of the NSC will have the highest velocity in this region. Therefore, this result can be interpreted as just showing that increased cell velocity increases the probability that the NSCs arrive at the AC and olfactory bulb.

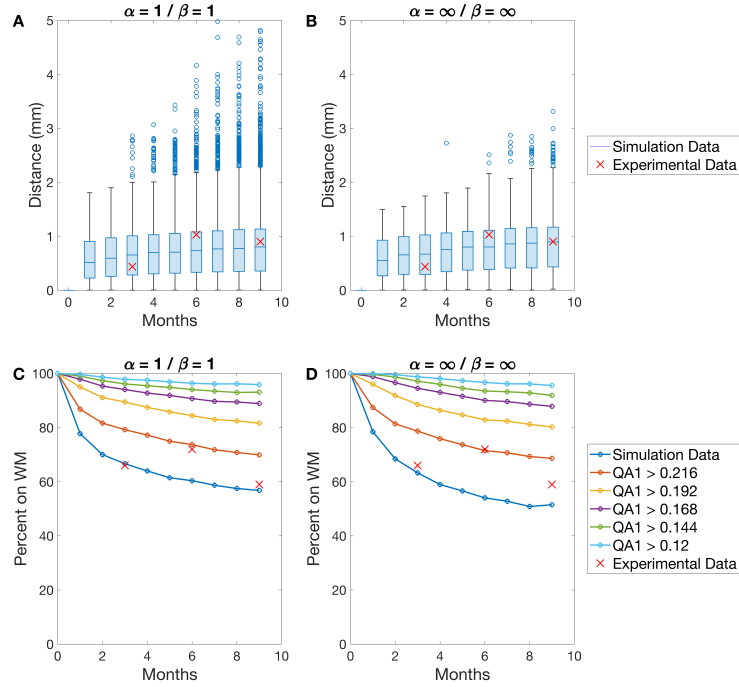


Figure B.7: Box plots for the distances from injection site as well as the percent on white matter across the 9 months of simulated NSC migration. A and C show a uniform distribution with $\alpha = \beta = 1$. B and D show the case when $X(x) = .5$ for all x . We see that with the stochasticity parameters only effects the outliers and not the median distances.

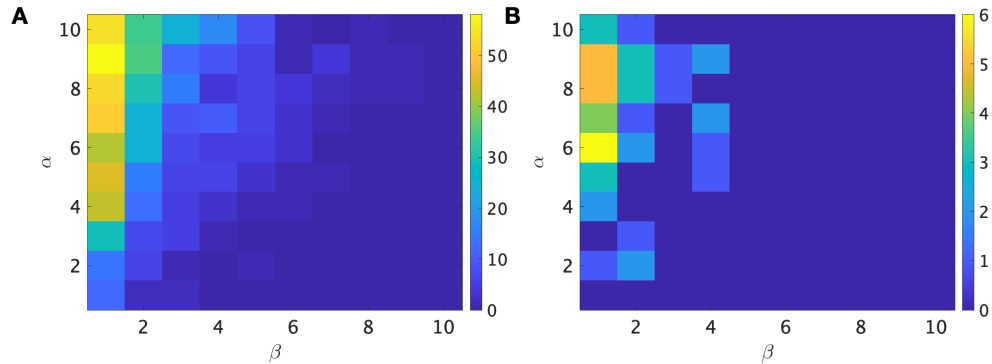


Figure B.8: Heat map showing the number of cells that are within the Anterior Commissure at 6 months (Top Left), the AC at any point in the simulation (Top Right), the Olfactory bulb by 9 months (Bottom Left) and the Olfactory bulb at any point (Bottom Right). We see that for lower K values the great amount of cells make it to the AC and CC respectively.

B.2 Experimental Calibration

In this section we explain the details of the model calibration. From the above parameter analysis we can conclude that the parameters $\bar{d}_g$, $\bar{d}_w$, and K have the largest influence on the simulated trajectories. Since α and β only effect the amount of outliers, we fix $\alpha = \beta = 1$. Furthermore, we set $n = 7$. We make this selection since for $K \approx .25$, a value of $n = 7$ yields $QA_H \in [.2, .8]$ for around 20% of the primary QA values. This means 20% of locations in the brain will lead to motion which is a combination of directed and random, such that each is weighted between 20% – 80%, while the rest of the QA values will lead to motion which is either mostly random, or directed along the white matter tracts. To depict the relationship between K , n , and the values QA in the brain, we plot the hill function for $n = 7$ and $K \in [.1, .46]$. In addition, for each function we show the percentage of cells within the brain that have a primary QA such that $QA_H \in [.2, .8]$. Here we can see that given $n = 7$ the maximum number of cells in this range occurs for $K = .18$ and is a unimodal distribution around this maximum.

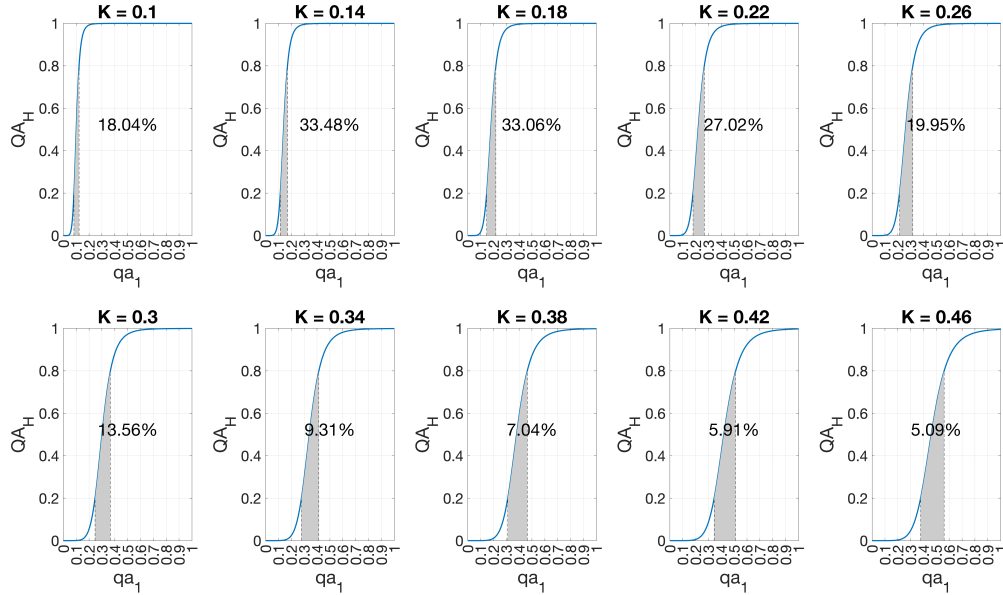


Figure B.9: Plots showing the Hill function for $n = 7$ and $K \in [.1, .46]$. the shaded area represents the portion of the function in which $QA_H \in [.2, .8]$. The percentage stated shows the percentage of cells in the brain such that QA_1 lies in this region

We then ran a host of simulations varying $\bar{d}_g$, $\bar{d}_w$, and K and calculated the sum of the squared error term between the simulated and experimental values at 3,6,9 months. The following set of heat

maps shows a narrowed-down range for this analysis. The simulation outlined in green represents the simulation with the smallest error term showing a value of .132.

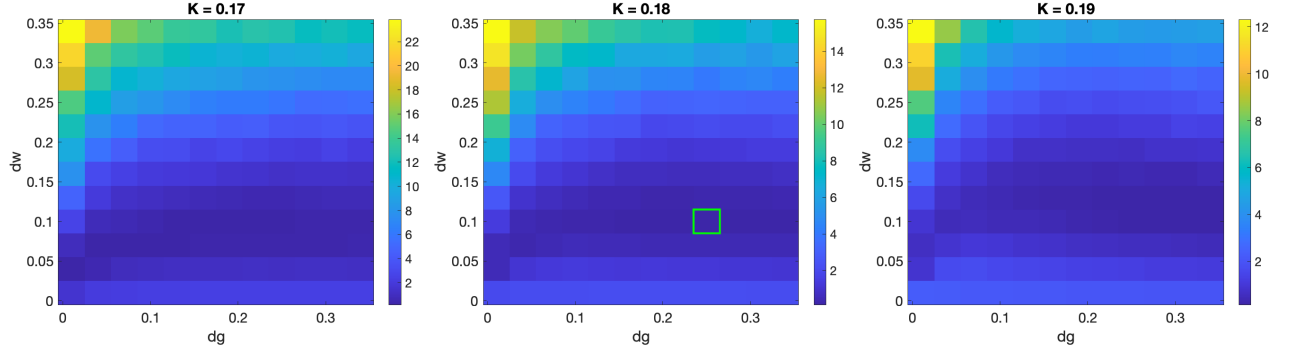


Figure B.10: Heat showing the sum of squared error between the simulated and experimental median distances from injection and percentage on white matter at 3,6,9 months. The green highlight show the simulations with the best error which occurs with parameter values $\bar{d}_g = .25$, $\bar{d}_w = .1$, and $K = .18$