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STATEMENT OF DISSERTATION APPROVAL

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ABSTRACT

We formulate and analyze three spatio-temporal models for cell polarization in budding yeast, fission yeast, and the neuronal growth cone, respectively. We focus on the roles of diffusion and active transport of cytosolic molecules along cytoskeletal filaments on the establishment of a polarized distribution of membrane-bound molecules. Our first model couples the diffusion equation on a finite interval to a pair of delay differential equations at the boundaries. The model is used to study the oscillatory dynamics of the signaling molecule Cdc42 in fission yeast. We explore the effect of diffusion by performing a bifurcation analysis and find that the critical time delay for the onset of oscillations increases as the diffusion coefficient decreases. We then extend the model to a growing domain and show that there is a transition from asymmetric to symmetric oscillations as the cell grows. This is consistent with the experimental findings of “new-end-take-off” in fission yeast. In our second model, we study the active transport of signaling molecules along a two-dimensional microtubule (MT) network in the neuronal growth cone. We consider a Rac1-stathmin-MT pathway and use a modified Dogterom–Leibler model for the microtubule growth. In the presence of a nonuniform Rac1 concentration, we derive the resulting nonuniform length distribution of MTs and couple it to the active transport model. We calculate the polarized distribution of signaling molecules at the membrane using perturbation analysis and numerical simulation. We find the distribution is sensitive to the explicit Rac1 distribution and the stathmin-MT pathway. Our third model is a stochastic active transport model for vesicles containing signaling molecules in a filament network. We first derive the corresponding advection-diffusion model by a quasi-steady-state analysis. We find the diffusion is anisotropic and depends on the local density of filaments. The stability of the homogeneous steady state is sensitive to the geometry of filaments. For a parallel MT network, the homogeneous steady state is linearly stable. For a network with filaments nucleated from the membrane (actin cytoskeleton), the homogeneous steady state is linearly unstable and a polarized distribution can occur.
To my parents.
## CONTENTS

ABSTRACT .................................................................................. iii

LIST OF FIGURES ................................................................. viii

LIST OF TABLES ................................................................. xii

ACKNOWLEDGEMENTS .................................................... xiii

CHAPTERS

1. INTRODUCTION ................................................................. 1

   1.1 Rho GTPase and the cytoskeleton ........................................ 2
     1.1.1 Rho GTPases ......................................................... 2
       1.1.1.1 Cdc42 ......................................................... 4
       1.1.1.2 Rac1 .......................................................... 5
     1.1.2 Actin polymerization and assembly ............................... 5
     1.1.3 Microtubules ....................................................... 6
       1.1.3.1 Dogterom–Leibler model .................................. 7

   1.2 Cell polarization in budding yeast ........................................ 8
     1.2.1 Positive feedback loops for Cdc42 ............................... 8
       1.2.1.1 Bem1-dependent positive feedback loop ............... 8
       1.2.1.2 Actin-dependent positive feedback loop ............... 9
     1.2.2 Mathematical models for budding yeast ....................... 9
       1.2.2.1 Turing-type model ......................................... 10
       1.2.2.2 Active transport model ................................... 10
       1.2.2.3 Stochastic model ......................................... 11

   1.3 Cell polarization in fission yeast ......................................... 12
     1.3.1 Oscillatory Cdc42 ............................................... 12
     1.3.2 Negative feedback loop ......................................... 13
     1.3.3 Microtubule-dependent positive feedback ................... 13
     1.3.4 Mathematical models for fission yeast ....................... 14

   1.4 Neuronal polarization .................................................... 16
     1.4.1 Growth cone in axon elongation ................................ 16
     1.4.2 Growth cone in axon guidance ................................ 17
     1.4.3 Feedback loops for axon formation and guidance ........... 19
       1.4.3.1 A schematic mechanism for axon specification ...... 19
       1.4.3.2 Simplified signals for axon turning .................... 20
     1.4.4 Mathematical models for neuronal polarization .......... 20

   1.5 Outline and overview of results ....................................... 24

2. A PDE–DDE MODEL FOR CELL POLARIZATION IN FISSION YEAST .......... 26

   2.1 PDE–DDE model ......................................................... 28
2.2 Analysis of the model in the fast diffusion limit ........................................... 30
  2.2.1 Bifurcation analysis of steady states with zero time delay ....................... 30
  2.2.2 Delay-induced oscillations with fixed length ........................................... 31
  2.2.3 Delay-induced oscillations with varying length ....................................... 37
2.3 Analysis of the full PDE–DDE model ............................................................ 40
  2.3.1 Linear stability analysis ........................................................................ 40
  2.3.2 Hopf bifurcation .................................................................................... 45
     2.3.2.1 Bifurcation from the symmetric steady state ...................................... 45
     2.3.2.2 Bifurcation from the asymmetric steady state ................................. 45
2.4 NETO ........................................................................................................... 49
2.5 Discussion ....................................................................................................... 53
3. NEURONAL GROWTH CONE MEMBRANE POLARITY VIA MICROTUBULE LENGTH REGULATION ................................................................. 55
  3.1 Model for growth cone membrane polarization via microtubule length .......... 58
     3.1.1 Active transport of signaling molecules .............................................. 59
     3.1.2 MT model ......................................................................................... 60
     3.1.3 Stathmin model ................................................................................. 62
     3.1.4 Coupling between active transport and MT growth models ............... 64
  3.2 Analysis of the active transport model ......................................................... 65
     3.2.1 Uniform distribution of MT lengths .................................................... 65
     3.2.2 Nonuniform distribution of MT lengths .............................................. 66
  3.3 MT polarization generated by Rac1 ............................................................... 70
     3.3.1 Uniform Rac1 .................................................................................... 71
     3.3.2 Nonuniform Rac1 .............................................................................. 74
  3.4 Polarization of membrane signaling molecules by Rac1 ............................. 79
  3.5 Discussion ....................................................................................................... 80
4. A STOCHASTIC ACTIVE-TRANSPORT MODEL ................................................ 83
  4.1 Deterministic model ..................................................................................... 85
  4.2 Stochastic active transport model ................................................................. 86
  4.3 Quasi-steady-state analysis ......................................................................... 91
  4.4 Linear stability analysis and dispersion relation ........................................... 96
     4.4.1 Case (i): Parallel filaments ................................................................. 96
     4.4.2 Case (ii): Nucleation at the cell membrane ......................................... 99
  4.5 Discussion ....................................................................................................... 107
5. FUTURE DIRECTIONS ..................................................................................... 109
  5.1 Noise in active transport ............................................................................. 109
  5.2 Selective transport into dendrites or axon ..................................................... 112
  5.3 Motor-driven force in growth cone turning ................................................... 114

APPENDICES
A. ACTIVE COMPARTMENTS WITH DELAYS COUPLED THROUGH BULK DIFFUSION ..................................................................................................................... 116
B. NUMERICS ................................................................. 142
REFERENCES .............................................................. 146
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1</td>
<td>Cell polarization</td>
<td>2</td>
</tr>
<tr>
<td>1.2</td>
<td>The Rho GTP cycle</td>
<td>4</td>
</tr>
<tr>
<td>1.3</td>
<td>Cdc42 and Rac1 downstream pathways</td>
<td>5</td>
</tr>
<tr>
<td>1.4</td>
<td>Actin polymerization and assembly</td>
<td>6</td>
</tr>
<tr>
<td>1.5</td>
<td>Dynamic instability of microtubules</td>
<td>7</td>
</tr>
<tr>
<td>1.6</td>
<td>Polarized yeast cells during budding and mating</td>
<td>9</td>
</tr>
<tr>
<td>1.7</td>
<td>Positive feedback loops in budding yeast</td>
<td>9</td>
</tr>
<tr>
<td>1.8</td>
<td>Schematic diagrams of mathematical models for budding yeast</td>
<td>11</td>
</tr>
<tr>
<td>1.9</td>
<td>New-end take off in fission yeast</td>
<td>13</td>
</tr>
<tr>
<td>1.10</td>
<td>Positive feedback loops in fission yeast</td>
<td>14</td>
</tr>
<tr>
<td>1.11</td>
<td>Axon-dendrites polarity in cultured neurons</td>
<td>17</td>
</tr>
<tr>
<td>1.12</td>
<td>Neuronal growth cone</td>
<td>18</td>
</tr>
<tr>
<td>1.13</td>
<td>Growth cone turning toward to an attractant</td>
<td>19</td>
</tr>
<tr>
<td>1.14</td>
<td>A schematic mechanism for axon specification</td>
<td>20</td>
</tr>
<tr>
<td>1.15</td>
<td>Simplified model of positive and negative signals</td>
<td>21</td>
</tr>
<tr>
<td>1.16</td>
<td>Schematics of neuronal polarization models</td>
<td>22</td>
</tr>
<tr>
<td>1.17</td>
<td>Schematic model of GABA receptor and microtubules</td>
<td>23</td>
</tr>
<tr>
<td>2.1</td>
<td>Compartmental model of NETO based on Cdc42 oscillations.</td>
<td>29</td>
</tr>
<tr>
<td>2.2</td>
<td>Steady-state solution of $X_1$ vs. the parameter $C_{\text{tot}}$ (in units of $C_{\text{sat}}$) for unit cell length and zero time delay</td>
<td>31</td>
</tr>
<tr>
<td>2.3</td>
<td>Bifurcation diagrams in the $(L, X_1)$ plane for the DDE model (2.6) with $C_{\text{tot}}/L = 6$ and zero delays</td>
<td>32</td>
</tr>
<tr>
<td>2.4</td>
<td>Numerical solutions of the DDE model for different time delays and initial conditions</td>
<td>32</td>
</tr>
<tr>
<td>2.5</td>
<td>Hopf bifurcation curves for the symmetric steady state (green) and the asymmetric steady state (blue) as a function of $C_{\text{tot}}$ for fixed cell length $L_0 = 1$</td>
<td>35</td>
</tr>
<tr>
<td>2.6</td>
<td>Amplitude and period of the periodic solution as a function of the time delay with $C_{\text{tot}} = 6.5$ and $L_0 = 1$</td>
<td>36</td>
</tr>
<tr>
<td>2.7</td>
<td>Switch from a periodic solution branching from the symmetric steady state to a periodic solution near the asymmetric steady state</td>
<td>36</td>
</tr>
</tbody>
</table>
2.8 Numerical solutions of the DDE model with $C_{\text{tot}}(t) = 6(1 + \gamma t)$ and $L(t) = 1 + \gamma t$ ................................................................. 38
2.9 Effect of the growth rate on the timing of the switch from asymmetric to symmetric oscillations for the DDE model .................................................. 38
2.10 Hopf bifurcation curves for the symmetric and asymmetric steady states .... 39
2.11 Frequency along the Hopf curve branching from the asymmetric steady state as a function of cell length $L$ and critical delay .............................. 39
2.12 Plot of the real part of the function $F_{\text{in}}(\omega)$ with different diffusion coefficients . 44
2.13 Effects of diffusion on Hopf bifurcations from the symmetric steady state ... 46
2.14 Effects of diffusion on Hopf bifurcations from the asymmetric steady state ... 47
2.15 Hopf curves along the symmetric and asymmetric steady states as $C_{\text{tot}}$ changes for $D = 2, 20$ ................................................................. 48
2.16 Numerical solution with different time delays ........................................ 48
2.17 Comparison of effects of diffusion on Hopf bifurcations for a large diffusion coefficient ................................................................. 49
2.18 Switch from asymmetric to symmetric oscillations with $C_{\text{tot}}$ and $L$ slowly increasing functions of time .................................................. 52
2.19 Effect of the growth rate and diffusion coefficient on the timing of the switch from asymmetric to symmetric oscillations ........................................ 53
3.1 Sketch of the Rac1-stathmin-MT pathway ........................................... 56
3.2 A simplified 2D model of a growth cone with a nonuniform distribution of MT lengths as specified by the interface $z = \phi(x)$ ..................................... 59
3.3 Two-dimensional stathmin-regulated MT growth model .......................... 61
3.4 Numerical solutions of equations (3.1a) and (3.1b) vs. perturbative solutions ... 70
3.5 Numerical solutions of equations (3.1a) and (3.1b) ................................ 71
3.6 Steady-state solutions for the average MT length as a function of the normalized stathmin concentration and the active Rac1 concentration .......... 74
3.7 Numerical and asymptotic solution of average MT length with catastrophe-promoting stathmin ................................................................. 77
3.8 Average MT lengths for sinusoidal and piecewise Rac1 concentration profiles . 78
3.9 Numerical plots of MT length and steady-state membrane concentration $u(x)$ for piecewise Rac1 distribution .................................................. 80
3.10 Parameter dependence of steady-state membrane concentration $u(x)$ ...... 81
4.1 Schematic illustration of filament geometry in model of Hawkins et al. [68] ... 85
4.2 Two-dimensional hybrid active transport model ...................................... 88
4.3 Dispersion curves in the case of parallel filaments for various values of $\xi$ ... 99
4.4 Construction of filament density due to nucleation of asters at membrane .... 100
4.5 Dispersion curves for nucleation model ........................................ 106
4.6 Dispersion curves for different values of speed and unbinding rate ........ 106
4.7 Plots of wavenumber and inverse growth rate for the faster growing eigen-
mode as a function of motor speed and unbinding rate ......................... 107
4.8 Comparison of dispersion curves generated from our model and the model
of Hawkins et al. ........................................................................... 108
4.9 Plots of wavenumber and inverse growth rate as a function of
ξ ................................................................................................. 108
5.1 Polarized membrane protein trafficking in neurons .............................. 113
5.2 Molecular motors in growth cone turning .......................................... 115
A.1 Hopf bifurcation curves in (D, τ) plane for different values of the coupling
parameter β ................................................................................. 121
A.2 Hopf bifurcation curves in (β, τ) plane for D = 0.2 and D = 1 ............. 123
A.3 Phase diagram in (D, τ) plane with different time delays ................... 123
A.4 Instability of the in-phase oscillation in the coexistence region ............ 124
A.5 Stable antiphase solutions solutions for D = 0.1, β = 1.0 and different delays . 126
A.6 Stable in-phase solutions solutions for D = 0.6, β = 1.0 and different delays . 127
A.7 Stability of the antiphase oscillation for D = 0.5 and sufficiently large time
delay ............................................................................................... 128
A.8 Switch from an antiphase oscillation to an in-phase oscillation as D increases
from 0.1 to 0.6 with a fixed time delay τ = 3.2 .................................. 129
A.9 Switch in oscillation mode occurs when D increases from 0.1 to 0.4 with a
fixed time delay τ = 2.5 ................................................................. 129
A.10 Counterclockwise contour Γ consisting of the semi-circle Γ_R and the imaginary
axis Γ_I ............................................................................................. 130
A.11 Sketch of possible trajectories of F(iy) as y changes from R to 0 ............ 131
A.12 Numerical plots of F(iy) (in-phase) as y changes from R to 0 for different
values of the delay ......................................................................... 133
A.13 Numerical plots of F(iy) (antiphase) as y changes from R to 0 for different
values of the delay ......................................................................... 133
A.14 Contour plots of max(ℜ(λ), 0) in the (D, τ) plane ................................ 134
A.15 Plot of cos(τ√R^2 – β^2) (blue) and −β/r (red) as a function of r ......... 135
A.16 Ref(λ) and ImF(λ) as λ travels along Γ_{I*} = {iy} with y changes from 100 to
0.01 ............................................................................................... 135
A.17 Hopf bifurcation in (β, r) plane .................................................... 136
A.18 Change of the phase and amplitude of the oscillations with different values
of the coupling parameter β_1 ......................................................... 138
A.19 Hopf bifurcation curves in the (β_1, τ) plane for β_2 = 1 and different D . 139
A.20 Numerical solution with different $\tau$ and $\beta_1$ close to $\beta_2$ ................. 140
A.21 Numerical result of PDE–DDE model with Mackey–Glass model ............... 141
B.1 Finite volume grid in two space dimension $\Omega = [0, L] \times [0, R]$. ............ 143
LIST OF TABLES

1.1 Mathematical models for cell polarization in budding yeast. .................... 3
1.2 Mathematical models for cell polarization in fission yeast. ...................... 3
1.3 Mathematical models for neuronal polarization. ................................. 3
3.1 Parameter values used for simulation ............................................. 75
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CHAPTER 1

INTRODUCTION

Cell polarization occurs in many types of cells during different intracellular processes such as cell movement, cell division, cell differentiation, and neuronal development. Among different cell types, budding yeast, fission yeast, and neuronal cells are the most extensively studied. As a common feature in different cells, cell polarization depends crucially on the regulation of signaling molecules and the cytoskeleton; see Figure 1.1. During cell polarization, signaling molecules break their uniform distribution and become localized at a particular site. Meanwhile, cytoskeletal structures such as F-actin and microtubules are redistributed to localize at the site with a higher concentration of signaling molecules.

There are several differences among cell polarization in budding yeast, fission yeast, and neurons. First, these cells display different polarized shapes. During mating, budding yeast breaks its spherical symmetry and forms a bud (shmoo). Fission yeast maintains a rod shape while it undergoes polarized growth by tip extension. A neuron has a soma, several short dendrites, and a long axon with a highly motile tip, the growth cone. The distinct lengths of dendrites and axons suggest that neuronal polarization is possibly regulated in a length-dependent manner. Second, the type of cytoskeleton which actively regulates cell polarization is different. Microtubules play a more important role in cell polarization of fission yeast and neurons while the actin cytoskeleton plays the major role in budding yeast.

A major question in cell polarization is how the asymmetric distribution of signaling molecules and the cytoskeleton are established. Generally speaking, cell polarization can be triggered by intracellular or extracellular signals. For example, budding cells use intrinsic spatial landmarks (bud scars) from previous cell divisions, while mating cells polarize in the direction of their mating partner as defined by gradients of mating pheromones. In the absence of spatial cues, budding yeast cells can also polarize in a random direction,
namely, they exhibit *spontaneous symmetry breaking*. The detailed molecular mechanisms for cell polarization are complex, and often include multiple positive and (or) negative feedback loops. The specific details of feedback loops vary with respect to cell types.

As an important tool for examining possible mechanisms of cell polarization, mathematical modeling and analysis has been widely applied; see the reviews [41, 43, 59, 81, 108]. For budding yeast, various models have been developed, which include Turing-type models, active transport models, and stochastic models; see Table 1.1. However, less work has been done for cell polarization in neurons and fission yeast; see Table 1.2 and Table 1.3. In this chapter, we start with a general review of cytoskeleton and a specific class of signaling molecules, Rho GTPase. We then review the main features of cell polarization in different cell types and related mathematical models. We finish the chapter with an overview of our work.

### 1.1 Rho GTPase and the cytoskeleton

In this section, we present a brief review of the Rho GTPase, actin polymerization and assembly, and dynamic instability of microtubules.

#### 1.1.1 Rho GTPases

One major class of signaling molecules is the Rho GTPases, which are best known for their effects on the actin cytoskeleton. Most Rho GTPases cycle between two forms: an active, guanosine triphosphate (GTP)-bound form and an inactive, guanosine diphosphate (GDP)-bound form. The active form diffuses slowly and is located in the membrane. The inactive form diffuses rapidly and can either be in the membrane or the cytoplasm.
### Table 1.1. Mathematical models for cell polarization in budding yeast.

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<thead>
<tr>
<th>Model</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Turing model</td>
<td>Goryachev and Pokhilko (2008) [60]</td>
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<td>Rätz and Röger (2012) [131]</td>
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<td>Rubinstein et al. (2012) [135]</td>
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<td>Lo et al. (2012, 2013, 2014) [98]</td>
</tr>
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<td>Active transport model</td>
<td>Marco et al. (2007) [102]</td>
</tr>
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<td>Slaughter et al. (2009) [139]</td>
</tr>
<tr>
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<td>Hawkins et al. (2009, 2012) [25, 68]</td>
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<td></td>
<td>Layton et al. (2011) [94]</td>
</tr>
<tr>
<td>Stochastic model</td>
<td>Áltschuler et al. (2008) [3]</td>
</tr>
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<td>Jilkine et al. (2011) [80]</td>
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<td>Lawson et al. (2011) [93]</td>
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<td>Freisinger et al. (2013) [50]</td>
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<td>Muller et al. (2016) [109]</td>
</tr>
</tbody>
</table>

### Table 1.2. Mathematical models for cell polarization in fission yeast.

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<thead>
<tr>
<th>Model</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>MT and Tea</td>
<td>Csikász-Nagy et al. (2008) [34]</td>
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<td>Cerone et al. (2012) [28]</td>
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<td>MT</td>
<td>Foethke et al. (2009) [49]</td>
</tr>
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<td>Cdc42 oscillation</td>
<td>Das et al. (2012) [36]</td>
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<td>Actin and For3</td>
<td>Wang and Vavylonis (2008) [151]</td>
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</tbody>
</table>

### Table 1.3. Mathematical models for neuronal polarization.

<table>
<thead>
<tr>
<th>Model</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Axon-dendrites</td>
<td>Samuels et al. (1996) [136]</td>
</tr>
<tr>
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<td>Fivaz et al. (2008) [48]</td>
</tr>
<tr>
<td></td>
<td>Toriyama et al. (2010) [147]</td>
</tr>
<tr>
<td></td>
<td>Pearson et al. (2011) [126]</td>
</tr>
<tr>
<td>GABA receptor</td>
<td>Dahan et al. (2010) [15]</td>
</tr>
<tr>
<td>Axon guidance</td>
<td>Hentschel and Ooyen (1999) [72]</td>
</tr>
<tr>
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<td>Kobayashi et al. (2010) [89]</td>
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<td>Mahajan and Athale (2012) [101]</td>
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<td>Takaki et al. (2015) [144]</td>
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<td>Najem et al. (2015) [112]</td>
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<td>Goodhill et al. (2016) [118]</td>
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</tbody>
</table>
Figure 1.2. Cycling between Rho-GDP and Rho-GTP. Redrawn from [70].

The cycling between these two states is regulated by guanine nucleotide exchange factors (GEFs), GTPase-activating proteins (GAPs), and nucleotide dissociation inhibitors (GDIs); see Figure 1.2. Specifically, the GEFs activate Cdc42 by stimulating the release of GDP to promote GTP binding, while the GAPs promote inactivation by accelerating hydrolysis of GTP. On the other hand, the modulator GDIs regulate the cycling by binding to the inactive form and retaining it in the cytosol. Among different Rho GTPase family members (about 20 in mammalian cells), Cdc42 and Rac1 have been extensively studied.

1.1.1.1 Cdc42

The small Rho GTPase Cdc42 acts as the center of polarization [45]. As a member of the Rho family, Cdc42 cycles between the active GTP-bound form and inactive GDP-bound form. The diffusion rate of Cdc-GTP is estimated to be at least one order of magnitude slower than that of Cdc42-GDP. Activated Cdc42 at the membrane regulates multiple processes including the polarization of actin polymerization, microtubule dynamics, and membrane trafficking through multiple pathways; see Figure 1.3. For example, activated Cdc42 regulates actin polymerization via at least two pathways. In the first pathway, activated Cdc42 binds to and activates the Wiskott-Aldrich syndrome protein (WASp), which in turn recruits and activates the actin-related protein 2/3 (Arp2/3) complex. The second pathway involves the p21-activated kinases PAKs which regulate actin polymerization indirectly. Activation of PAKs also leads to the phosphorylation and inhibition of the microtubule-destabilizing protein stathmin. In addition to the activation of WASp and PAKs, activated Cdc42 also binds to Par6 and activate aPKC, which participates in the
regulation of microtubules and membrane traffic.

1.1.1.2 Rac1

The Rho GTPase Rac1 is another key regulator of cytoskeleton. Activated Rac1 regulates actin dynamics by activating PAK and WASp family verprolin-homologous WAVE proteins. In particular, PAK signals to the actin dynamics through cofilin while WAVE directly interacts with Arp2/3. Rac1 also regulates microtubule dynamics through PAKs by inhibiting the microtubule destabilizer stathmin.

In addition to their regulation of actin and microtubules, recent research in hippocampal neurons indicates that there is a positive feedback between Rac1 and Cdc42, which involves the GEFs of Rac1 and Cdc42 [120].

1.1.2 Actin polymerization and assembly

Actin filaments are helical, polar polymers with a barbed end and a pointed end; see Figure 1.4 (a). At the barbed end, actin filament undergoes polymerization by adding subunit actin monomers, globular actin (G-actin). At the pointed end, monomers disassociate. Actin polymerization depends on the availability of G-actin and free barbed ends, and is regulated by capping proteins, actin-nucleating proteins, and severing proteins. On the one hand, capping proteins inhibit actin polymerization by binding to the barbed ends and blocking monomer addition. This barbed end capping is inhibited by barbed end protector proteins such as ENA and VASP. On the other hand, actin polymerization is
Figure 1.4. Actin polymerization and assembly. (a) Actin filament. (b) Two distinct actin structures: filopodium with parallel bundles of F-actin and lamellipodium with branched actin networks. Redrawn from [37].

enhanced by actin-nucleating proteins, including actin-related protein (Arp2/3) complex and formins. However, Arp2/3 complex and formins create new barbed ends in different ways. In particular, Arp2/3 complex binds at the side of an existing filament to initiate a new filament that branches from the mother filament, whereas formins nucleate a new filament and remain bound to the barbed end to facilitate polymerization. In contrast to the positive regulation of Arp2/3 and formins, actin severing protein cofilin acts to sever F-actin and block the barbed end elongation.

Individual actin filaments are assembled to form networks with different spatial organizations. There are two types of actin structures: actin bundles and branched actin networks. In actin bundles, the actin filaments are closely cross-linked into parallel arrays. In actin networks, the actin filaments are loosely cross-linked in orthogonal arrays that form three-dimensional meshworks. Actin bundles can be found in filopodia of the neuronal growth cone and in actin cables of budding yeast. Actin networks exist in lamellipodia of the growth cone and actin patches of budding yeast. For the sake of illustration, we plot the filopodium and lamellipodium in Figure 1.4 (b).

1.1.3 Microtubules

Microtubules are hollow cylinders built of alternating α- and β-tubulin heterodimers. The α/β heterodimers are arranged in a head to tail manner to form a protofilament. 13
protofilaments are packed side by side to form a tubular structure (microtubule). Similar to actin filaments, microtubules also have two distinct ends: a rapidly growing plus-end and a slowly growing minus-end. The minus-end of a microtubule shrinks unless it is stabilized by minus-end capping proteins. At the plus-end, microtubules undergo rapid switches between growth and shrinkage, a behavior termed dynamic instability; see Figure 1.5. The dynamic instability is characterized by four parameters: the growth and shrinking rates, the catastrophe rate, and the rescue rate. Here catastrophe refers to the transition from growth to shrinkage, while rescue refers to the transition from shrinkage to growth.

1.1.3.1 Dogterom–Leibler model

The dynamic instability has been modeled by Dogterom and Leibler using a stochastic two-state model, the Dogterom–Leibler model [40]. Microtubules randomly switch between two states: a growth state with a constant velocity $v_+$ and a shrinkage state with a constant velocity $v_-$. The catastrophe rate is denoted by $\omega_c$ and the rescue rate is denoted by $\omega_r$. Let $p_+(x,t)$ and $p_-(x,t)$ be the probabilities of finding a MT with length $x$ at time $t$ in a growing or shrinking state, respectively. Then the probabilities evolve according to the master equations

\begin{align}
\frac{\partial p_+}{\partial t}(x,t) &= -\frac{\partial(v_+p_+)}{\partial x}(x,t) + \omega_r p_-(x,t) - \omega_c p_+(x,t), \\
\frac{\partial p_-}{\partial t}(x,t) &= \frac{\partial(v_-p_-)}{\partial x}(x,t) - \omega_r p_-(x,t) + \omega_c p_+(x,t).
\end{align}

Figure 1.5. Dynamic instability of microtubules. Redrawn from [32].
1.2 Cell polarization in budding yeast

The budding yeast *Saccharomyces cerevisiae* is a single-celled organism, whose cells are round to ovoid with 5 – 10µm in diameter. There are three cell types in yeast: haploid a and α cells and diploid a/α cells. Haploid cells can divide vegetatively until they encounter a cell of opposite mating type, in which case they mate to form an a/α diploid. Diploid a/α cells can also divide vegetatively by budding. During budding and mating, cells are highly polarized; see Figure 1.6.

As a major component of the polarity machinery, activated Cdc42 localizes and forms a cluster at a site on the cortex that later becomes the front of the cell. In the absence of Cdc42, cell growth gives rise to large, round, unbudded cells [1]. The other major component of cell polarization in yeast is the actin cytoskeleton, which consists of actin patches and actin cables. Actin cables are long, bundled filaments which act as tracks for cargo transport by myosin motors. Actin patches are branched actin networks, which are sites of endocytosis.

1.2.1 Positive feedback loops for Cdc42

Two major positive feedback loops are proposed as a base for the machinery of Cdc42 clustering. One is an actin-dependent positive feedback [46, 102, 129, 154] and the other is an actin-independent Cdc42-Cdc24-Bem1 loop [24, 91, 141, 155]. Recent studies also suggest that negative feedback and competition serve to control the number of polarization sites [74, 104, 156, 157]. In the following, we briefly describe the two positive feedback loops.

1.2.1.1 Bem1-dependent positive feedback loop

The actin-independent positive feedback loop requires the Cdc42 GEF, Bem1, and a PAK-family kinase. The special GEF for Cdc42 in budding yeast is Cdc24. Bem1 is a scaffold protein which increases local concentration of signaling molecules by bringing together two or more additional proteins. We illustrate the feedback loop in Figure 1.7. In this loop, local activation of the GEF Cdc24 produces Cdc42-GTP which then recruits Bem1. In turn, Bem1 stabilizes Cdc24 at the site of polarization by binding to Cdc24 and forming a complex, which includes a PAK kinase [91, 155].
Figure 1.6. Polarized yeast cells during budding and mating. Redrawn from [30].

**Figure 1.7.** Two positive feedback loops in budding yeast. (a) Actin-independent, Cdc42-Cdc24-Bem1 loop. Cdc42-GTP recruits Bem1 which in turn recruits or activates the GEF Cdc24. (b) Actin-dependent loop. Cdc42-GTP stimulates the nucleation of actin cables through the formin nucleator. Transport of vesicles carrying cytosolic Cdc42 to the polarization site. Redrawn from [154].

### 1.2.1.2 Actin-dependent positive feedback loop

The actin-dependent positive feedback depends on the transport along actin cables [153]; see Figure 1.7 (b). At the membrane, Cdc42-GTP promotes the formation of actin cables through the formin nucleator. On the other hand, molecular motors carry vesicles containing cytosolic Cdc42 along actin cables to the polarization site, which in turn enhance the activation of Cdc42 near the membrane.

### 1.2.2 Mathematical models for budding yeast

Mathematical models based on the above two mechanisms have been widely proposed; see the reviews [59, 81, 108]. For the sake of illustration, we review several models below.
**1.2.2.1 Turing-type model**

For budding yeast, the Bem1-dependent positive feedback of Cdc42 has been modeled by Turing-type reaction-diffusion models [60, 98, 131, 135]. The Turing mechanism [148] for symmetry breaking is driven by diffusion and generally involves a slowly diffusing molecule and a rapidly diffusing molecule. In the case of Cdc42, the GTP-bound active form diffuses more slowly than the GDP-bound inactive form.

As a classic example of the Turing-type model for cell polarization, Goryachev and Pokhilko [60] used the following reaction-diffusion equations to model the cycling of Cdc42 between inactive and active states:

\[
\frac{\partial X}{\partial t} = D_m \nabla X + E_c \alpha X^2 Y + E_c \beta X Y - \gamma X, \quad (1.2a)
\]
\[
\frac{\partial Y}{\partial t} = D_c \nabla Y - E_c \alpha X^2 Y - E_c \beta X Y + \gamma X, \quad (1.2b)
\]

Here \(X\) is the concentration of the slowly diffusing, inactive Ccd42, and \(Y\) is the concentration of active Cdc42. The first terms on the right-hand side represent the diffusion with diffusion coefficients \(D_m\) and \(D_c\), respectively. The last terms represent the deactivation at a constant rate \(\gamma\). The quadratic term \(E_c \beta X Y\) and the cubic term \(E_c \alpha X^2 Y\) represent two possible forms of autocatalytic activation. In particular, the cubic term represents a mechanism in which the activation requires the formation of Cdc24/Bem1/Cdc42 while the quadratic term only requires Cdc24/Bem1. \(E_c\) is the total amount of Cdc24/Bem1 and \(\alpha\) and \(\beta\) are constant. Simulations of the model show that the homogeneous steady state of the model (1.2) exhibits Turing-type instability to spatial perturbation.

**1.2.2.2 Active transport model**

For the actin-dependent positive feedback, Marco et al. [102] proposed a model of actin-dependent transport, lateral diffusion, and endocytosis. They assumed that there is a directed transport from the cytoplasm to a specific window \(\chi\) at the membrane at a rate \(h\). Moreover, endocytosis of membrane-bound proteins in the window occurs at a different rate from the rate of endocytosis outside the window. These rates are represented by \(e_a, e_a/\alpha\), respectively. In addition, membrane-bound proteins undergo lateral diffusion with a diffusion coefficient \(D\). The schematic model is illustrated in **Figure 1.8** (a). Denote the density distribution of the protein in the membrane by \(f\) and the homogeneous
Figure 1.8. Schematic diagrams of mathematical models for budding yeast. (a) Active transport model by Marco et al. [102]. Parameters: lateral diffusion $D_f$, directed transport rate $h$ in the directed window $\chi$, endocytosis rate $e_a$ inside the window, endocytosis rate $e_a/\alpha$ outside the window. Redrawn from [102]. (b) A stochastic model for spontaneous cell polarization by Altschuler et al. [3]. Purple arrow: spontaneous membrane association with rate $k_{on}$. Red arrow: positive feedback with rate $k_{fb}$. Black arrow: spontaneous dissociation with rate $k_{off}$. Green arrow: lateral diffusion. Redrawn from [3].

cytoplasmic distribution by $F_{cyto}$, the equation of $f$ is given by

$$\frac{\partial f}{\partial t} = D_f \nabla f - (e_a \chi + \frac{e_a}{\alpha} (1 - \chi)) f + h F_{cyto} \frac{\chi}{\int \chi}. \tag{1.3}$$

Numerical simulation shows that a balance between directed transport, lateral diffusion, and endocytosis is sufficient for accurate establishment of polarized distribution of membrane proteins. Moreover, there exists an optimal endocytosis rate for the height and sharpness of the polarized distribution of membrane proteins.

The model by Marco et al. has been further studied by Slaughter et al. [139] and Layton et al. [94]. Slaughter et al. considered both an actin-dependent and an actin-independent feedback loops. With these two parallel pathways, the authors investigated how the related parameter determines different Cdc42 polarized distributions which are corresponding to different morphogenetic fates (bud and shmoo). On the other hand, Layton et al. [94] explicitly modeled the vesicular transport of Cdc42, instead of considering Cdc42 traffic as a membrane-free flux. Interestingly, Layton et al. find that without the membrane-free assumption, the actin-based transport model would dissipate the polarization, unless Cdc42 diffuses very slowly and is concentrated into both exocytic and endocytic vesicles.

1.2.2.3 Stochastic model

Altschuler et al. [3] proposed a stochastic model of positive feedback for spontaneous cell polarization in budding yeast. The positive feedback represents the self-recruitment
of membrane-bound signaling molecules from a cytoplasmic pool. Specifically, four main transport mechanisms of signaling molecules are considered: (1) recruitment of cytoplasmic molecules to the location of membrane-bound molecules with rate $k_{fb}$; (2) spontaneous membrane association with rate $k_{on}$; (3) spontaneous membrane disassociation with rate $k_{off}$; and (4) lateral diffusion of membrane-bound molecules with a diffusion coefficient $D$; see Figure 1.8 (b). If the total number of signaling molecules is large, analysis of the model shows that the distribution of signaling molecules on the membrane converges to a homogeneous steady state and no cluster persists. However, if the total number of signaling molecules is small, the model with a positive feedback alone is sufficient to create and maintain a localized distribution of membrane-bound molecules.

1.3 Cell polarization in fission yeast

The fission yeast *Schizosaccharomyces pombe* is also a well-studied model organism for cell polarization. Fission yeast cells are rod-like with a constant diameter around 4 µm. Cells grow in length by tip extension. Shortly after cell division, new-born cells grow at the ‘old’ end, the preexisting end before cell division. During the G2 phase, cells initiate growth at the ‘new’ cell tip, the cell division site. This transition from growing at one tip (mono-polar growth) to growing at both tips (bipolar growth) is named *new-end-take-off* (NETO); see Figure 1.9.

1.3.1 Oscillatory Cdc42

As in budding yeast, activated Cdc42 accumulates at the growth site and regulates the polarized growth of fission yeast. However, there is a major difference between the polarized distribution in budding and fission yeast. In fission yeast, Cdc42 is localized at the two growing tips during bipolar growth, while in budding yeast, Cdc42 is localized at a single budding site. In addition, experimental results show that activated Cdc42 at the two tips oscillates in an out-of-phase manner with an average period of 5 min during the bipolar growth [36]. Oscillatory dynamics of Cdc42 is also observed in budding yeast [74, 92].
1.3.2 Negative feedback loop

The oscillatory behavior of Cdc42 indicates that negative feedback is involved in cell polarization. In contrast to positive feedback loops, which serve to amplify small signals, negative feedback loops lead to adaptive behavior and promote stability of the system. However, negative feedback mechanisms for Cdc42 oscillation are less well understood. One possibility is that negative feedback could result from the activation of inhibitors such as the Cdc42 GAPs (Rga6 and Rga4) [36, 134].

1.3.3 Microtubule-dependent positive feedback

Similar to budding yeast, there exist a cytoskeleton-independent positive feedback and a cytoskeleton-dependent pathway. The cytoskeleton-independent pathway depends on the scaffold protein Scd2 and on a second GEF Gef1 (Bem1 homolog); see Figure 1.10 (a). This positive feedback loop works in a similar way to the Bem1-dependent feedback loop in budding yeast.

A major difference between cell polarization of budding and fission yeast lies in the cytoskeleton-dependent pathway. In fission yeast, the cytoskeleton-dependent pathway depends crucially on microtubules. The schematic diagram of the microtubule-dependent pathway is illustrated in Figure 1.10 (b). Microtubules are organized in bundles oriented along the long axis of the cells with their growing ends pointing to the cell tips. At the growing ends, microtubules transport the Tea complex (Tea1/Tea4) to the cell tip. The deposited Tea complex is then anchored at the cortex by Tea3 and Mod5. At the cell tip,
the Tea complex actively organizes polarized growth and actin assembly. Specifically, the Tea1-Tea4 complex activates Cdc42 by recruiting the kinasin Pom1 which inhibits the GAP (Rag4) of Cdc42. Tea4 can also bind directly to the actin nucleator formin For3 and form a complex. The activation of Cdc42 and recruitment of For3 lead to the assembly of polarized actin cables. There is likely a positive feedback between Tea1/Tea4 complex and Mod5/Tea3 as the localization of Mod5/Tea3 is dependent on Tea.

Independent of the microtubule-Tea pathway, the shape of the cell itself also provides a positive feedback signal. The rod shape of the cell helps to orient the microtubules along the long axis of the cell. This directs the MT plus ends and its cargoes to the cell tips, and thus induces polarized growth that maintains cell shape.

### 1.3.4 Mathematical models for fission yeast

Csikász-Nagy et al. [34] proposed a model to study the polarized distribution of actin polymers and the role of the Tea complex in regulating actin polymerization. In this model, microtubules transport the Tea complex to the cortex and stimulate the actin polymerization via a positive feedback dependent on the Tea complex. The concentration of the Tea complex $u(x, t)$ is modeled by an advection-reaction-diffusion equation

$$
\frac{\partial u}{\partial t} = D_u \frac{\partial^2 u}{\partial x^2} + v_u \frac{\partial u}{\partial x} - k_{du} u + k_{su} - \frac{u}{L} \frac{\partial L}{\partial t},
$$

(1.4a)

where $D_u$ is the diffusion constant, $v_u$ is the transport rate along microtubules, $k_{su}$ is the syndissertation rate of the complex and $k_{du}$ is the degradation rate. The last term represents the dilution due to linear growth of the cell length $L$. On the other hand, actin monomers $G$ and polymers $F$ are modeled by a pair of reaction-diffusion equations
\[ \frac{\partial G}{\partial t} = D_G \frac{\partial^2 G}{\partial x^2} + k_s - k_d G - (k'_3 + k''_3 u F^2)G + k_4 F - \frac{G L}{L} \frac{\partial L}{\partial t} \], (1.4b)
\[ \frac{\partial F}{\partial t} = D_F \frac{\partial^2 F}{\partial x^2} + \left(k'_3 + k''_3 u F^2\right)G - k_4 F - d_d F - \frac{F L}{L} \frac{\partial L}{\partial t}. \] (1.4c)

Here \( D_G \) and \( D_F \) are diffusion coefficients, \( k_s \) is the actin syndissertation rate, \( k_d \) is the actin degradation rate, and \( k_4 \) is actin depolymerization rate. \( k'_3 \) is the background actin polymerization rate and \( k''_3 \) is the autocatalytic polymerization rate, which is promoted by the tea complex. Numerical result shows that there are both bipolar and monopolar growth modes as cell elongates. Here bipolar growth refers to a stable equilibrium with the same value at two tips while bipolar growth refers to a stable equilibrium with one larger value at one tip than the other.

Assuming that the diffusion of a polarity protein complex (for3, tea, etc.) is sufficiently fast, Cerone et al. [28] reduced the model (1.4) to a pair of ordinary differential equations

\[ \frac{dx_1}{dt} = (k_0 + k_{cat} x_1^2) (x_{T1} - x_1) (y - x_1 - x_2) - k_d x_1, \] (1.5a)
\[ \frac{dx_1}{dt} = (k_0 + k_{cat} x_1^2) (x_{T2} - x_2) (y - x_1 - x_2) - k_d x_2. \] (1.5b)

These two equations represent the activation and deactivation of the complex at the two tips. The active complex at both tips are denoted by \( x_{1,2} \) and the inactive forms are represented by \( x_{T1,2} - x_{1,2} \). Here \( x_{T1,2} \) are the total amounts of the complex at two tips. The activation is regulated by autocatalytic process with a coefficient \( k_{cat} \) and is dependent on a limiting component \( y \). The particular limiting component is assumed to increase as cell length increases. Bifurcation analysis of the ODE model showed that there is a transition from monopolar to bipolar growth via a saddle-node bifurcation, as cell length changes.

Recently, Das et al. [36] presented a model consisting of a pair of delay differential equations for the oscillatory dynamics of Cdc42. In their model, the activation of Cdc42 at the two tips is regulated by a positive feedback with saturation, while the deactivation rate is regulated by a negative feedback. Moreover, the slow diffusion of membrane-bound Cdc42 is ignored and the Cdc42 in the cytoplasm is taken to be well mixed due to fast diffusion. Let \( C_{cito} \) be the concentration in the cytoplasm and \( C_{tip1}(t) \) and \( C_{tip2}(t) \) be the concentrations of Cdc42 at two tips, respectively. The model by Das et al. is given by
\[
\begin{align*}
\frac{dC_{\text{tip}1}}{dt} &= \frac{k^+ (C_{\text{tip}1})}{V} C_{\text{cyto}} - k^- (C_{\text{tip}1}) C_{\text{tip}1}, \\
\frac{dC_{\text{tip}2}}{dt} &= \frac{k^+ (C_{\text{tip}2})}{V} C_{\text{cyto}} - k^- (C_{\text{tip}1}) C_{\text{tip}2}, \\
C_{\text{tot}} &\equiv C_{\text{tip}1} + C_{\text{tip}2} + C_{\text{cyto}}.
\end{align*}
\]

The total amount of Cdc42 changes as the length of the cell increases. The association rate \(k^+\) is regulated by positive feedback with saturation and the dissociation rate \(k^-\) is controlled by negative delayed feedback. Simulation result shows that the concentrations of Cdc42 at two tips changes from asymmetric oscillation to symmetric oscillation, as the cell length increased.

There are also mathematical models for actin and microtubules. For example, Wang and Vavylonis [151] presented an ordinary differential equation model for formin for3 mediated actin cable assembly. Foethke et al. [49] used computer simulation to study the spatial organization of microtubules.

### 1.4 Neuronal polarization

A neuron with a single axon but multiple dendrites is highly polarized. The axon is a single, long process that transmits signals to other neurons. Dendrites consist of multiple branched processes and dendrite spines, which contain neurotransmitter receptors to receive signals from other neurons.

Experiments in cultured hippocampal neurons have revealed that there are different stages during the development of axon and dendrites; see Figure 1.11. At Stage 1, several filopodia (thin, finger-like protrusions of bundled actin filaments) form at the leading edge of the cell. At Stage 2, several immature neurites emerge, which are cylindrical extensions with a growth cone at the distal tip. At Stage 3, one neurite grows much more rapidly than other neurites. This rapidly growing neurite then develops to form an axon at a later stage while other neurites become dendrites. The process of specification of a future neuron, characterized by its enlarged growth cone, establishes the neuronal polarization.

#### 1.4.1 Growth cone in axon elongation

The axon elongation is directed by its motile tip, the axonal growth cone. The growth cone by itself is highly polarized. In vitro, the growth cones are characterized by three
**Figure 1.11.** Axon-dendrite polarity in cultured neurons. Stage 1: formation of filopodia. Stage 2: formation of immature neurites. Stage 3: one neurite grows at a much more rapid rate, starts to break the initial morphological symmetry, and establishes the polarity. Redrawn from [7].

Domains: the peripheral (P)-domain, the central (C)-domain, and the transition (T)-zone between P- and C-domain; see **Figure 1.12.** The P-domain has two actin-rich structures: filopodia and lamellipodia. Filopodia are formed by actin bundles while lamellipodia are formed by branched actin networks. In these structures, actin filaments face their barbed (growing) end toward the membrane. The C-domain is rich in organelles, vesicles, and microtubules. In particular, there is a subset of microtubules which are more stable, restricted in the C-domain and a subset of pioneer dynamic microtubules that can extend into the P-domain; see **Figure 1.12.** In the T-zone, there is another type of actin structure consisting of actin of arcs, which are oriented perpendicular to the radial filopodia [138].

As a major engine for axon elongation, actin polymerization in the P-domain produces a protrusion force on the membrane that leads to the elongation of filopodia. Simultaneously, the polymerization generates a retrograde flow which pushes the actin filaments and the adjacent membrane backwards. The retrograde flow also acts to hinder the invasion of microtubules into the P-domain. The outcome of the protrusion and the retrograde flow is dependent on the adhesion of the filopodium to the substratum and the action of myosin molecular motors. While myosin motors enhance the retrograde flow by dragging the actin back towards the C-domain [107], signals from surface adhesion receptors bound to a substrate can suppress the retrograde flow of actin filaments [10].

### 1.4.2 Growth cone in axon guidance

The growth cone guides the axon to its target by responding to extracellular attractive and repulsive cues. In the case of an attractive guidance cue, localized guidance receptors
regulate actin binding proteins and increase actin polymerization or decrease retrograde actin flow. As a result, the protrusion and adhesion in the P-domain nearest the guidance cue is promoted, which leads to the turning of the growth cone toward the attractant cue; see Figure 1.13. In contrast, growth cones turn away from a negative cue due to the decreased protrusion and (or) adhesion on the side that is closer to the repulsive cue.

Recent studies have shown that microtubules also play major roles in growth cone motility [21, 58, 96, 99, 142]. First, individual microtubules actively explore the P-domain by the dynamic instability at the plus ends. These dynamic, pioneer microtubules may act as guidance sensors via two possible processes: (1) carrying signals to and from the membrane or (2) acting as a scaffold for the localized recruitment of signaling molecules [142]. Second, during growth cone steering, the stable microtubules in the C-domain enter the new growth region and fix the axonal direction.

Furthermore, it is also reported that the actin cytoskeleton and microtubules undergo active interactions in growth cone motility [26, 99]. On the one hand, microtubules which successfully enter the P-domain may follow the trajectories of actin bundles in filopodia. It is also suggested that these microtubules may couple to actin bundle retrograde flow, which in turn inhibits the protrusion activities of microtubules. In the C-domain, actin arcs form a barrier which regulates the microtubule advance. In addition, stabilized microtubules on one side of the growth cone enhance lamellipodial protrusion on that side before turning occurs [21].

Figure 1.12. Neuronal growth cone. Redrawn from [99].
1.4.3 Feedback loops for axon formation and guidance

We briefly describe a mechanism for axon specification and signaling pathways for axon turning.

1.4.3.1 A schematic mechanism for axon specification

A possible mechanism for the formation of one single axon has been proposed by Andersen and Bi [5]. In their schematic model, axon formation is regulated by both positive and negative feedback; see Figure 1.14. Positive feedback regulation leads to the enhancement of actin polymerization, microtubule assembly, intracellular protein transport, and membrane recruitment. Negative feedback regulation that acts simultaneously as positive feedback leads to opposite reactions such as decreased actin dynamics. More importantly, each neurite generates a negative feedback signal which propagates throughout the cell and interacts with positive feedback signals in other neurites. This is termed long-range inhibition.

Before the symmetry breaking, the positive and negative feedback are balanced. Between Stage 2 and Stage 3 of neuronal development, the balance is broken by a positive cue. The positive cue activates a local positive feedback loop at a particular neurite, which elongates rapidly to become an axon. Meanwhile, this neurite generates a strong negative signal which propagates to other neurites and prevents the formation of multiple axons. The molecular details for this schematic model are still emerging. Positive regulators such
as Rac1 and Cdc42 contribute to the local activation by accumulation at one single neurite and regulating the actin dynamics and intracellular trafficking. Although the negative regulator RhoA may contribute to a local negative feedback signal in the cell body and (or) all minor neurites, the global inhibition feedback signaling from the axon remains largely unknown.

1.4.3.2 Simplified signals for axon turning

In the presence of guidance signals, membrane receptors read the signal and initiate intracellular signaling pathways which target at the actin cytoskeleton; see Figure 1.15. Depending on the type of membrane receptors they interact with, guidances cues (netrins, semaphorins, ephrins, and slits) can either attract or repel growth cones by promoting actin polymerization or depolymerization [63, 76]. In general, attractive guidance cues typically activate Cdc42 and Rac1, but inhibit RhoA through GEFs and GAPs. The small Rho GTPase RhoA is generally associated with inhibition of neurite outgrowth. Activated Rac and Cdc42 induces the formation of lamellipodia and filopodia and promote neurite outgrowth and extension, respectively. On the other hand, repulsive cues are generally thought to activate RhoA and inhibit Rac1 and Cdc42.

1.4.4 Mathematical models for neuronal polarization

Fivaz et al. [48] proposed a model based on a local positive feedback between the small Rho GTPase HRas and PI3K at the neurite tip. There are HRas in active and inactive form at each neurite $i(1 \leq i \leq N)$, denoted by $R_i^T$ and $R_i^D$. The activation rate of HRas is
Figure 1.15. Simplified model of positive and negative signals. Guidance cues activate membrane receptors, which lead to the activator of GTPase regulators including Rho-GEFs, and Ras. Redrawn from [99].

enhanced by the local PIP3, $P_i$. The dynamics of PIP3 is regulated by local PI3K activity, which is stimulated by active HRas. In addition to the positive feedback, there is transport of HRas between the neurite and the soma. Moreover, the transport from the soma to the neurite is regulated by PIP3; see Figure 1.16 (a). The concentrations of HRas at each neurite and the soma are modeled by

$$
\frac{dR_i^T}{dt} = k_1(P_i + P_b)R_i^D - (\alpha + \beta)R_i^T, \quad (1.7a)
$$

$$
\frac{dR_i^D}{dt} = k_0(P_i + P_b)S - \beta R_i^D + \alpha R_i^T, \quad (1.7b)
$$

$$
\frac{dS}{dt} = \beta \sum_{i=1}^{N}(R_i^D + R_i^T) - k_0S(NP_b + \sum_{i}^{N}P_i). \quad (1.7c)
$$

Here $k_0, k_1$ are constant, $P_b$ is the basal concentration of PIP3, $\alpha$ is the deactivation rate, and $\beta$ is the constant transport rate from the neurite to the soma. The equation for $P_i$ is given by

$$
\frac{dP_i}{dt} = \frac{k_p(R_i^T + R_b)}{K_M + R_i^T + R_b} - \phi P_i, \quad (1.7d)
$$

where $k_p, R_b, K_M,$ and $\phi$ are constant. The first term on the right-hand side represents the production rate that is enhanced by active HRas. The second term represents the degradation. Finally, the neurite growth rate is given by

$$
\frac{dL_i}{dt} = k_L(P_i + P_b) - \frac{\lambda L_i}{K_L + L_i}. \quad (1.7e)
$$
Figure 1.16. Schematics of neuronal polarization models. (a) Positive feedback model of Fivaz et al. There is a positive feedback between HRas and PI3K at the tip. The feedback also enhances the transport of HRas from cell body to the neurite. Moreover, the total amount HRas is conserved which guarantees the formation of one single axon. (b) Length-dependent diffusion model of Toriyama et al. Polarity protein shootin1 accumulates and enhances neurite outgrowth. The retrograde diffusion from the neurite to cell body is regulated by neurite length. Diffusion is more slow in longer neurites. This length-dependent diffusion together with the positive regulation of shootin1 on neurite outgrowth establishes a positive feedback loop. Redrawn from [77, 147].

Numerical simulation of the system (1.7) shows that the local feedback between HRas and PI3K coupled to polarized transport of a limited pool of HRas is sufficient to give rise to a single axon.

Toriyama et al. [147] proposed an alternative model which involves length-dependent retrograde diffusion of Shootin1. Shootin1 is another polarity regulator which accumulates at one neurite and promotes the neurite outgrowth. Shootin1 molecules are transported stochastically from the cell body and diffuse back (retrograde) to the cell body. More importantly, diffusion of shootin1 in a shorter neurite is faster than that in a longer neurite. This length-dependent diffusion of shootin1 together with the activation of neurite outgrowth by shootin1 establishes a positive feedback; see Figure 1.16 (b).

Let $C(t)$ and $C_0(t)$ be the concentrations of shootin1 in the growth cone and the cell body, respectively. Let $L$ be the length of neurite, then the concentration of shootin1 in the growth cone changes according to

$$\frac{dC}{dt} = -\frac{AD}{VL}(C - C_0) + w(t) \quad (1.8a)$$

where $A$ is the cross-sectional area of the neurite, $D$ is the diffusion coefficient, and $V$ is the volume of the growth cone. The second term $w(t)$ represents a stochastic anterograde transport. The neurite outgrowth rate is modeled by the ODE

$$\frac{dL}{dt} = \delta \kappa_{on} M - \delta \kappa_{off} \exp[-A_s(C) + A_1(L)] \quad (1.8b)$$
Figure 1.17. Schematic model for the redistribution of GABA receptor and microtubules in response to the GABA gradient. Redrawn from [15].

where $M = 15$ is fixed, $\delta$ is the size of the monomer molecule such as tubulin, $\kappa_{on}$ and $\kappa_{off}$ are the rates of polymerization and depolymerization. The functions $A_s(C)$ and $A_1(L)$ are given by

$$A_s(C) = \frac{a_s C^h}{K_s^h + C^h}, \quad A_1(L) = \frac{a_1 \ln(L/L_0)}{\ln(K_1/L_0) + \ln(L/L_0)}.$$

Numerical simulation result shows that this system (1.8) can amplify stochastic fluctuations of shootin1 signals and generate an asymmetric signal for axon specification.

In [15], Bouzigues et al. present a computational model for the redistribution of GABA receptors and microtubules in the growth cone in response to a GABA concentration gradient. The model is based on interactions between microtubules and GABA receptors [16]. Activated receptors stimulate microtubule growth. On the other hand, receptors can be transiently trapped at the end of microtubules due to the interaction with microtubules. The growth cone is modeled by a half disk containing a constant number of $k$ microtubules and $n$ identical independent receptors. Initially, the microtubules have a uniform length and receptors are distributed symmetrically. In the presence of a GABA gradient, GABA receptors and microtubules are redistributed toward the direction of the gradient; see Figure 1.17.

In addition to the above models, mathematical modeling of neuronal polarization is still emerging [89, 101, 112, 118, 126, 144]. For example, Pearson et al. [126] presented a stochastic model for axon growth; Mahajan and Athale [101] proposed a computational model for polarized microtubule polarization in a turning growth cone; Najem et al. [112] constructed a phase-field model to couple actin dynamics with neuronal growth.
1.5 Outline and overview of results

In this dissertation, we formulate and analyze three types of spatio-temporal models for cell polarization in budding yeast, fission yeast, and neuronal growth cone, respectively, with an emphasis on the latter two cell types. Our work focuses on the establishment of a polarized distribution of signaling molecules at the membrane. In particular, we examine the effects of cytosolic diffusion, and the density and length distribution of cytoskeletal filaments on the polarized distribution of signaling molecules. Motivated by the different geometries of cells and their cytoskeletal structures, we formulate three different models including (1) a partial differential equation coupled with delay differential equations for fission yeast, (2) a two-dimensional active transport model coupled with microtubule growth for the neuronal growth cone membrane polarization, and (3) a stochastic active transport model for budding yeast and neuronal growth cone. To carry out the analysis of these models, we use mathematical tools including perturbation analysis, bifurcation analysis, spectral analysis, and numerical simulation. We summarize our main results and present the outline of the dissertation below.

In Chapter 2, we consider a compartmental model consisting of a diffusion equation and a pair of delay differential equations (DDEs) for Cdc42 in fission yeast. The DDEs represent the binding of Cdc42 to the cell membrane and rerelease into the cytoplasm via unbinding. The diffusion equation and the DDEs are coupled via boundary conditions. For this model, we use analytical tools and numerical simulation to explore the effect of diffusion and length growth on the oscillation of Cdc42 at the two tips. Using linear stability analysis and bifurcation analysis, we find that the critical time delay for the onset of oscillations via a Hopf bifurcation increases as the diffusion coefficient decreases. We then extend the model to a growing domain and solve it numerically. Our numerical results show that the system undergoes a transition from asymmetric to symmetric oscillations as the cell grows, consistent with experimental findings. Moreover, the critical length at which the transition occurs depends on the diffusion coefficient. From a mathematical perspective, our PDE–DDE model provide a new framework to study synchronization of coupled oscillators. Depending on the explicit form of DDE, the model can give rise to complex bifurcations and different types of oscillation modes. We thus further study a PDE–DDE model for two classic DDEs including the delayed logistic equation and the
The Mackey–Glass equation in Appendix A.

In Chapter 3, we consider a two-dimensional active transport model of signaling molecules coupled with a modified Dogterom-Liebler model for microtubule growth in the neuronal growth cone. The microtubule growth is regulated by a Rac1-stathmin-Microtubule pathway, in which active Rac1 inhibits activation of stathmin and thus indirectly enhances microtubule growth. Assuming that the dynamics of microtubules occur much faster than the change of signaling molecules, we derive a formula for the length distribution of microtubules when the Rac1 is uniformly distributed. For a weakly nonuniform distribution of Rac1, we use regular perturbation and numerical simulation to find the resulting nonuniform length distribution of microtubules. Our results suggest that the lateral diffusion of stathmin tends to weaken the effects of Rac1 on the distribution of mean MT lengths. Finally, we couple the microtubule length to an active transport model of signaling molecules. For different choices of Rac1 distributions, we use numerical simulation to compare the corresponding nonuniform distribution of membrane-bound molecules.

In Chapter 4, we present a stochastic active transport model for cell polarization in neuronal cells and budding yeast. We start with a Chapman–Kolmogrov equation for active transport and then derive the effective reaction diffusion equations for the concentration of cytosolic signaling molecule using a quasi-steady-state analysis. We find that the effective diffusion of cytosolic molecules is anisotropic and depends on the local density of cytoskeleton. Finally, we perform a linear stability analysis of the homogeneous steady state of the effective reaction diffusion equations. We thus show that the stability of the homogeneous steady state is sensitive to the geometry of the cytoskeleton. The steady state is linearly unstable if filaments are nucleated at sites on the cell membrane (cortical actin), whereas it is linearly stable if the filaments nucleate from organizing sites within the cytoplasm (microtubule asters).

In Chapter 5, we propose several future directions for our work.
CHAPTER 2
A PDE–DDE MODEL FOR CELL POLARIZATION IN FISSION YEAST

In this chapter, we focus on the oscillatory dynamics of the signaling molecule Cdc42 during cell polarization in fission yeast. As described in Section 1.3, the active Cdc42 located at the cell tips oscillate in an out-of-phase manner with an average period of 5 min. Moreover, as the cell elongates, the mean amplitudes of the oscillations at the two tips change. In the case of longer cells exhibiting bipolar growth, the mean amplitudes of the oscillations were the same at both ends (symmetric, anticorrelated oscillations). On the other hand, for shorter, less mature cells exhibiting monopolar growth, the amplitude was significantly larger at the growing end (asymmetric, anticorrelated oscillations). The observed dynamics suggests that there is competition for active Cdc42 (or associated regulators) at the two ends and indicates the presence of some form of delayed feedback.

Das et al. model Cdc42 oscillations in terms of a system of delayed differential equations (DDEs) with positive feedback and delayed negative feedback [36]. The DDE model was able to account for the transition from oscillating monopolar (asymmetric) to oscillating bipolar (symmetric) states during cell elongation. However, one of the potential limitations of the Das et al. model is that the effects of cytosolic diffusion were ignored. That is, the concentration of Cdc42 in the bulk of the cell was assumed to be spatially uniform and could thus be determined by imposing conservation of total Cdc42 within the cell at a given length.

We extend the Das et al. model, in order to investigate the effects of cytosolic bulk diffusion and cell length on Cdc42 oscillations. Exploiting the rod-like geometry of fission yeast, we treat the cell as a finite one-dimensional (1D) domain of length $L$. Cdc42 diffuses within the interior of the domain, $x \in (0, L)$, and can bind to the cell membrane at the
ends $x = 0, L$. Moreover, membrane-bound Cdc42 can unbind and reenter the cytosol. The nontrivial nature of the dynamics arises from the fact that both the binding and unbinding rates at each end are taken to depend nonlinearly on the local membrane concentration. In particular, the association rate is regulated by positive feedback and the dissociation rate is regulated by delayed negative feedback along identical lines to the model of Das et al. [36]. The resulting dynamical system takes the form of a coupled PDE–DDE, where the bulk dynamics is described by a simple diffusion partial differential equation (PDE), and the exchange of Cdc42 between the cytosol and the end membrane compartments is modeled in terms of flux boundary conditions that involve both the positive and delayed negative feedback (DDE).

From a mathematical perspective, our model is a new example of a class of models recently formulated and studied by Ward and collaborators [61, 62]. These consist of spatially segregated dynamically active units, such as cells or localized signaling compartments, that are coupled with each other via a signaling molecule that diffuses in the bulk domain between the active units. Gou et al. [61, 62] considered the particular case in which each isolated compartment is a conditional oscillator. That is, in isolation, a compartment’s dynamics is at a stable fixed point, but can exhibit sustained oscillations in a different parameter regime. For the sake of illustration, each isolated compartment was modeled in terms of a planar dynamical system (without delays). Using linear stability analysis, the authors showed that diffusive coupling can induce in-phase or antiphase oscillations for a pair of active compartments. There are, however, a number of differences between our model and those studied by Gou et al. [61, 62]. First, our membrane compartments are not conditional oscillators, that is, the existence of oscillations depends crucially on the delayed coupling between the compartments and the bulk diffusion. Second, there is only one chemical species in our model, that is, the signaling molecule is the same molecule as in each active compartment. Third, the size of the domain changes during cell elongation.

The rest of this chapter is organized as follows. In Section 2.1, we present our PDE–DDE model. In Section 2.2, we show that our model reduces to the DDE model of Das et al. [36] in the fast diffusion limit. In order to provide a baseline for comparisons with the full model, we solve the steady states numerically and investigate conditions for a Hopf bifurcation using a mixture of numerical simulations and linear stability analysis. In
Section 2.3, we turn to the analysis of the full PDE–DDE model and demonstrate that for biophysical values of diffusivity, bulk diffusion can have a significant affect on the critical time delay and amplitude of Cdc42 oscillations for a cell of fixed length. In Section 2.4, we solve the diffusion equation on a growing domain under the additional assumption that the total amount of the signaling molecule increases as the cell length increases. We show that the system undergoes a transition from asymmetric to symmetric oscillations as the cell grows, consistent with experimental findings of “near-end-take-off” in fission yeast. We also show that the critical length where the switch occurs depends on both the diffusion and the growth rate.

2.1 PDE–DDE model

Consider a one-dimensional compartmental model consisting of a PDE for the substrate concentration in the cytosol and a DDE for the concentration at each end compartment; see Figure 2.1. The length of the domain is taken to be \( L \). Let \( C(x, t) \) be the cytosolic concentration of Cdc42 at \( x \) and \( X_i(t) \), \( i = 1, 2 \), the concentration of Cdc42 at the i-th compartment, where \( t, t > 0 \), denotes time. (Concentrations are defined as the number of molecules per unit cross-section of the cell, which is assumed to be fixed.) The PDE–DDE model is taken to be

\[
\frac{\partial C(x, t)}{\partial t} = D \frac{\partial^2 C(x, t)}{\partial x^2}, \quad 0 < x < L, \quad t > 0, \tag{2.1a}
\]

with flux boundary conditions

\[
-D \partial_x C(0, t) = -k^+(X_1(t))C(0, t) + k^-(X_1(t), X_1(t - \tau))X_1(t), \tag{2.1b}
\]

\[
-D \partial_x C(L, t) = k^+(X_2(t))C(L, t) - k^-(X_2(t), X_2(t - \tau))X_2(t), \tag{2.1c}
\]

and

\[
\frac{dX_1}{dt} = k^+(X_1(t))C(0, t) - k^-(X_1(t), X_1(t - \tau))X_1(t), \tag{2.1d}
\]

\[
\frac{dX_2}{dt} = k^+(X_2(t))C(L, t) - k^-(X_2(t), X_2(t - \tau))X_2(t). \tag{2.1e}
\]

The association rate \( k^+ \) is regulated by positive feedback with saturation in the form of an exponential [36]:

\[
k^+(X) = (k_0^+ + k_n^+ (X/C_{sat})^n) \exp(-X/C_{sat}), \quad n \geq 2. \tag{2.2}
\]
The dissociation rate $k_-$ is controlled by negative delayed feedback according to

$$k^-(X(t), X(t - \tau)) = k_0^- \left[ 1 - \frac{\epsilon}{2} + \epsilon \frac{X(t - \tau)^h}{X(t)^h + X(t - \tau)^h} \right], \quad (2.3)$$

where $\tau$ is the time delay, $k_0^-$ is the baseline dissociation rate in the absence of the delayed negative feedback, $\epsilon$ represents the strength of the delayed negative feedback, and $h$ is the Hill coefficient. Equations (2.1a)-(2.1e) are supplemented by the conservation equation

$$\int_0^L C(x, t) \, dx + X_1(t) + X_2(t) = C_{\text{tot}}, \quad (2.4)$$

where $C_{\text{tot}}$ is the total number of Cdc42 molecules per unit area.

In the above formulation of the model, we have assumed that $L$ and $C_{\text{tot}}$ are fixed. This simplification is based on the observation that cell elongation is much slower than any of the processes associated with the Cdc42 dynamics. In Section 2.4, we will explicitly incorporate elongation of the cell by taking $L$ and $C_{\text{tot}}$ to grow linearly in time along the lines to Das et al. [36]. In particular, we will determine how diffusion affects the transition from oscillating monopolar (asymmetric) to oscillating bipolar (symmetric) states during cell elongation. We fix the units of concentration by setting $C_{\text{sat}} = 1$. The unit of time is taken to be minutes (the typical time-scale of binding/unbinding and the delay $\tau$) and the unit of length is taken to be $5\mu m$ (comparable to the initial length of a fission yeast cell immediately following cell division). It follows that after nondimensionalization, a diffusion coefficient of $D = 1$ corresponds to $D \approx 0.5 \mu m^2/s$ in physical units. The other parameters of the model are taken to be similar to those of Das et al. [36].
2.2 Analysis of the model in the fast diffusion limit

We now show how to recover the DDE model of Das et al. [36] from our PDE–DDE model by taking the diffusion coefficient $D \to \infty$. If we introduce the small parameter $\epsilon = L^2 k^- / D$ for large $D$, then the leading order terms of the diffusion equation (2.1a) and the boundary conditions give $C(x, t) = C_0(t)$. Using the conservation equation (2.4), we can rewrite $C_0(t)$ as

$$C_0(t) = \frac{C_{\text{tot}} - X_1(t) - X_2(t)}{L}. \quad (2.5)$$

The DDE model by Das et al. (see equation (1.6)) can then be recovered by substituting equation (2.5) into the DDE of $X_i(t)$. That is,

$$\frac{dX_1}{dt} = \frac{k^+(X_1)}{L} \left( C_{\text{tot}} - X_1(t) - X_2(t) \right) - k^- (X_1(t), X_1(t - \tau)) X_1(t), \quad (2.6a)$$

$$\frac{dX_2}{dt} = \frac{k^+(X_2)}{L} \left( C_{\text{tot}} - X_1(t) - X_2(t) \right) - k^- (X_2(t), X_2(t - \tau)) X_2(t). \quad (2.6b)$$

In the following, we carry out a detailed analysis of the resulting DDE (2.6). This provides the baseline behavior which will be compared with the behavior of the full model for finite $D$ in Section 2.3.

2.2.1 Bifurcation analysis of steady states with zero time delay

In the absence of a time delay, $\tau = 0$, we numerically determine the steady-state solution of the DDE (2.6) as a function of the total Cdc42 concentration $C_{\text{tot}}$; see Figure 2.2. For small values of $C_{\text{tot}}$, there exists only a stable symmetric steady state. At $C_{\text{tot}} \approx 1.8$, the symmetric steady-state solution loses its stability via a supercritical pitchfork bifurcation, resulting in the formation of a pair of stable asymmetric steady states. However, for $C_{\text{tot}} \approx 6.1$, the symmetric state regains stability due to a subcritical pitchfork bifurcation. This leads to a range of values of $C_{\text{tot}}$ over which a pair of stable asymmetric steady states coexists with a stable symmetric steady state. As $C_{\text{tot}}$ is further increased, the asymmetric steady states disappear via saddle node bifurcations so that one returns to a single stable symmetric state. Following Das et al. [36], suppose that we vary the cell length $L$ and take the total substrate concentration $C_{\text{tot}}$ to be a linearly increasing function of cell length $L$. (The only explicit dependence of cell-length in the DDE model (2.6) is that it scales the rates $k_0^\pm$ and $k_n^\pm$.) In Figure 2.3 (a), we plot the resulting bifurcation diagram in the $(X_1, L)$-plane.
Figure 2.2. Steady-state solution of $X_1$ vs. the parameter $C_{\text{tot}}$ (in units of $C_{\text{sat}}$) for unit cell length and zero time delay ($\tau = 0$). The bifurcation diagram suggests that there is always a symmetric steady-state solution and a pair of asymmetric steady-state solutions for the parameter $C_{\text{tot}}$ in a certain range. As $C_{\text{tot}}$ increases, the symmetric steady-state solution loses its stability and the asymmetric steady-state solution becomes stable. For large value of $C_{\text{tot}}$, the asymmetric steady state does not exist and the symmetric steady state is stable. There is also a parameter regime for which stable symmetric and asymmetric steady states coexist. Parameters: $L = 1, k_0^+ = 2.25, k_n^+ = 6.467, n = 4, k_0^- = 1, \epsilon = 0.5375, h = 40$.

Without time delay, the asymmetric steady state is linearly stable for $L \in (0.25, 1.285)$ while the symmetric steady state is linearly stable for $L > 1.014$. Moreover, at $L_{sd} \approx 1.285$, the asymmetric steady state vanishes through a saddle-node bifurcation. Only the symmetric steady state exists and it is linearly stable for $L > L_{sd}$. This suggests that the cell can undergo a transition from monopolar (asymmetric) growth to bipolar (symmetric) growth as the cell length increases. The critical value of $L$ at the saddle-node point is dependent on $C_{\text{tot}} = C_0$ at $L = L_0 = 1$ and the strength of the positive feedback $k^+(x)$. If we assume there is no saturation in the positive feedback by dropping the exponential term in equation (2.2), that is, $k^+(X) = (k_0^+ + k_n^+ (X/w)^n)$ with $n \geq 2$, then the saddle-node point is not observed and the symmetric steady state cannot be stabilized as $L$ increases; see Figure 2.3 (b).

2.2.2 Delay-induced oscillations with fixed length

We now consider the DDE (2.6) with a nonzero time delay and a fixed value of $C_{\text{tot}}$. We first simulate the DDE using MATLAB’s builtin solver dde23. In Figure 2.4, we plot the numerical solution of the DDE (2.6) for different choices of the time delay $\tau$. For the sake
Figure 2.3. Bifurcation diagrams in the $(L, X_1)$ plane for the DDE model (2.6) with $C_{\text{tot}}/L = 6$ and zero delays. (a) Full model. The stable symmetric and asymmetric steady states coexist for a small range of $L \in [1.014, 1.285]$. For $L > 1.285$, the asymmetric steady state vanishes through a saddle-node bifurcation. (b) Nonsaturating positive feedback $k^+(x) = k_{0}^+ + k_{n}^+ x^n$. The saddle-node point where the stable asymmetric state vanishes is no longer observed. Other parameters are the same as Figure 2.3.

Figure 2.4. Numerical solutions of the DDE model for different time delays and initial conditions. (a-c) Initial conditions: $X_1(t) = 1.1, X_2(t) = 1, -\tau \leq t \leq 0$. (d-e) Initial conditions: $X_1(t) = 5, X_2(t) = 1.1, -\tau \leq t \leq 0$. Here $C_{\text{tot}} = 6.5, L = 1$, and other parameters are the same as Figure 2.2.
of illustration, we choose $C_{tot} = 6.5$ so that the symmetric and asymmetric states coexist when $\tau = 0$; see Figure 2.2. In Figure 2.4 (a-c), the initial condition is taken to be close to the symmetric steady-state solution with $X_1(t) = 1, X_2(t) = 1$ for all $-\tau \leq t \leq 0$. For small time delays, the symmetric steady-state solution remains stable. However, as $\tau$ is increased, the steady state destabilizes, and a periodic, symmetric antiphase solution emerges. When the delay is further increased, the system switches to an asymmetric antiphase solution. One possible reason is that the basin of the attraction of the symmetric steady state is small for small $\tau$. In Figure 2.4 (d,e), we plot the corresponding numerical solution when the initial condition is taken to be near the asymmetric steady-state solution. For a range of values $C_{tot}$, the stable asymmetric branch can also go unstable to oscillations if the delay $\tau$ crosses a threshold from below.

In order to determine the critical time delay at a Hopf bifurcation point, we perform a linear stability analysis of the DDE model for a fixed length $L = L_0$. Setting

$$F(X_1, X_2) = \frac{1}{L_0} k^+(X_1)(C_{tot} - X_1(t) - X_2(t)) - k^-(X_1(t), X_1(t - \tau))X_1(t),$$

with $L_0 = 1$, the linearized system near the steady state $(\overline{X}_1, \overline{X}_2)$ is

$$\frac{d}{dt} \begin{pmatrix} y_1(t) \\ y_2(t) \end{pmatrix} = \begin{pmatrix} F_{11} & F_{12} \\ F_{21} & F_{22} \end{pmatrix} \begin{pmatrix} y_1(t) \\ y_2(t) \end{pmatrix} - \frac{eh}{4} \begin{pmatrix} y_1(t - \tau) \\ y_2(t - \tau) \end{pmatrix},$$

(2.7)

where

$$F_{11} = \frac{\partial F}{\partial X_1}(\overline{X}_1, \overline{X}_2) = \frac{nk^+_n\overline{X}_1^{n-1}}{L_0} (C_{tot} - \overline{X}_1 - \overline{X}_2)e^{-\overline{X}_1},$$

$$- \frac{k^+_n + k^+_n\overline{X}_1^m}{L_0} (C_{tot} - \overline{X}_1 - \overline{X}_2 + 1)e^{-\overline{X}_1} - (1 - \frac{eh}{4}),$$

$$F_{12} = \frac{\partial F}{\partial X_2}(\overline{X}_1, \overline{X}_2) = -\frac{k^+_n + k^+_n\overline{X}_1^m}{L_0} e^{-\overline{X}_1},$$

and

$$F_{21} = \frac{\partial F}{\partial X_1}(\overline{X}_2, \overline{X}_1), \quad F_{22} = \frac{\partial F}{\partial X_2}(\overline{X}_2, \overline{X}_1).$$

Consider the perturbation

$$X_1 = \overline{X}_1 + \sigma_1 e^{\lambda t}, \quad X_2 = \overline{X}_2 + \sigma_2 e^{\lambda t}.$$

Substituting it into the linearized DDE gives

$$\lambda \begin{pmatrix} \sigma_1 \\ \sigma_2 \end{pmatrix} = \begin{pmatrix} F_{11} - \frac{eh}{4} e^{-\lambda \tau} & F_{12} \\ F_{21} & F_{22} - \frac{eh}{4} e^{-\lambda \tau} \end{pmatrix} \begin{pmatrix} \sigma_1 \\ \sigma_2 \end{pmatrix}. $$
For a symmetric steady state, we have $F_{11} = F_{22}$ and $F_{12} = F_{21}$, so that the matrix is cyclic and symmetric with the eigenvectors $\sigma_1 = -\sigma_2$ (antiphase) and $\sigma_1 = \sigma_2$ (in-phase). The corresponding eigenvalue equations are

$$\left(\lambda - F_{11} + \frac{eh}{4}e^{-\lambda \tau}\right) + F_{12} = 0, \quad \left(\lambda - F_{11} + \frac{eh}{4}e^{-\lambda \tau}\right) - F_{12} = 0,$$

(2.8)

In order to identify a Hopf bifurcation point, we set $\lambda = i\omega$ in equation (2.8) and equate real and imaginary parts. This yields the pair of equations

$$\omega - \frac{eh}{4} \sin(\omega \tau) = 0, \quad \cos(\omega \tau) = \frac{4}{eh}(F_{11} \pm F_{12}),$$

(2.9)

corresponding to the eigenvectors $\sigma_1 = \pm \sigma_2$. The existence of the solution $(\omega, \tau)$ requires that

$$\left|\frac{4}{eh}(F_{11} \pm F_{12})\right| \leq 1.$$  

(2.10)

In the asymmetric case, we have the characteristic equation

$$\left(\lambda - F_{11} + \frac{eh}{4}e^{-\lambda \tau}\right)\left(\lambda - F_{22} + \frac{eh}{4}e^{-\lambda \tau}\right) - F_{12}F_{21} = 0.$$  

(2.11)

Again we set $\lambda = i\omega$ in equation (2.11) and equate real and imaginary parts:

$$\begin{bmatrix} \frac{eh}{4} \cos(\omega \tau) - F_{11} \\ \frac{eh}{4} \cos(\omega \tau) - F_{22} \end{bmatrix} \begin{bmatrix} \frac{eh}{4} \cos(\omega \tau) - F_{11} \\ \frac{eh}{4} \cos(\omega \tau) - F_{22} \end{bmatrix} - (\omega - \frac{eh}{4} \sin(\omega \tau))^2 - F_{12}F_{21} = 0,$$

(2.12a)

$$\begin{bmatrix} \omega - \frac{eh}{4} \sin(\omega \tau) \\ \frac{eh}{2} \cos(\omega \tau) - F_{11} - F_{22} \end{bmatrix} \begin{bmatrix} \frac{eh}{4} \cos(\omega \tau) - F_{11} - F_{22} \end{bmatrix} = 0.$$  

(2.12b)

Noting that $F_{12} < 0$ and $F_{21} < 0$, we have

$$\begin{bmatrix} \frac{eh}{4} \cos(\omega \tau) - F_{11} \\ \frac{eh}{4} \cos(\omega \tau) - F_{22} \end{bmatrix} \begin{bmatrix} \frac{eh}{4} \cos(\omega \tau) - F_{11} \\ \frac{eh}{4} \cos(\omega \tau) - F_{22} \end{bmatrix} = (\omega - \frac{eh}{4} \sin(\omega \tau))^2 + F_{12}F_{21} > 0.$$  

It follows that

$$\frac{eh}{2} \cos(\omega \tau) - F_{11} - F_{22} \neq 0,$$

and equation (2.12a,b) can be simplified as

$$\omega - \frac{eh}{4} \sin(\omega \tau) = 0,$$

(2.13a)

$$\begin{bmatrix} \frac{eh}{4} \cos(\omega \tau) - F_{11} \\ \frac{eh}{4} \cos(\omega \tau) - F_{22} \end{bmatrix} \begin{bmatrix} \frac{eh}{4} \cos(\omega \tau) - F_{11} \\ \frac{eh}{4} \cos(\omega \tau) - F_{22} \end{bmatrix} = F_{12}F_{21}.$$  

(2.13b)
Finally, we solve the different systems (2.9) and (2.13) numerically. In Figure 2.5 (a), we plot the Hopf bifurcation curves in the \((C_{\text{tot}}, \tau)\)-plane for the stable symmetric and asymmetric steady states. Note that for the parameter set chosen in Figure 2.2, there is no solution \((\omega, \tau)\) of equations (2.13a, b) such that the eigenvector \(\sigma_1 = \sigma_2\). In Figure 2.5 (b), we check the necessary condition (2.10) for the existence of in-phase solution for the symmetric steady state. The result shows that \(4(F_{11} + F_{12})/h\epsilon < -1\) and hence the condition (2.10) for the existence of in-phase solution is not satisfied. It follows that oscillations bifurcating from the symmetric and asymmetric steady states are antiphase. (We find, however, that in-phase oscillations can exist if the strength of the negative feedback \(\epsilon\) is sufficiently strong or the nonlinearity \(h\) is sufficiently high.)

In Figure 2.6, we take \(C_{\text{tot}} = 6.5\) and plot the amplitude and period of the periodic solutions branching from the symmetric and asymmetric steady states as a function of time delay. For the periodic solution branching from the symmetric steady state, the period increases dramatically at \(\tau \approx 0.26\). This suggests that there is a homoclinic orbit resulting from the collision of the symmetric limit cycle with the unstable asymmetric fixed point; see Figure 2.2. Indeed, as the time delay crosses \(\tau = 0.263\), the numerical solution evolves to the periodic solution branching from the asymmetric steady state. For the sake of illustration, we plot the numerical solution as a function of time with time delay \(\tau = 0.263\) and \(\tau = 0.2632\); see Figure 2.7.
Figure 2.6. Amplitude and period of the periodic solution as a function of the time delay with $C_{tot} = 6.5$ and $L_0 = 1$. Blue: result of the periodic solution branching from the symmetric steady state. Green: result of the periodic solution branching from the asymmetric steady state. At time delay $\tau \approx 0.263$, the period of the periodic solution branching from the symmetric steady state increases dramatically. It suggests that there is possibly a homoclinic or heteroclinic bifurcation. Other parameters are the same as Figure 2.2.

Figure 2.7. Switch from a periodic solution branching from the symmetric steady state to a periodic solution near the asymmetric steady state at time delay $\tau \approx 0.263$. (a,b) $X_{1,2}$ vs. $t$. (c,d) periodic solution in $(X_1, X_2)$ plane. Other parameters are the same as Figure 2.2.
2.2.3 Delay-induced oscillations with varying length

We now use the simplified DDE model of Das et al. [36] to investigate the effects of cell elongation. For the sake of illustration, suppose that both $L$ and $C_{tot}$ increase linearly with time at a rate $\gamma$. That is, we modify equations (2.6) by taking

$$L(t) = L_0[1 + \gamma t], \quad C_{tot}(t) = C_0[1 + \gamma t], \quad (2.14)$$

where $L_0, C_0$ are the initial length and total Cdc42 concentration, respectively. It is important to emphasize, however, that in the case of growing cells, one can no longer treat the DDE model as the fast diffusion limit of the full PDE–DDE model (2.1a). Indeed, integrating the diffusion equation (2.1a) over the growing interval $[0, L(t)]$ and imposing the boundary conditions gives

$$\int_0^{L(t)} \partial_t C(x, t) dx = -\frac{dX_1}{dt} - \frac{dX_2}{dt}. \quad (2.15)$$

Differentiating the conservation condition

$$C_{tot}(t) = \int_0^{L(t)} C(x, t) dx + X_1(t) + X_2(t)$$

then implies that

$$\frac{dC_{tot}(t)}{dt} = C(L(t), t) \frac{dL}{dt}. \quad (2.16)$$

This last equation holds in the fast diffusion limit for which $C(L(t), t) \rightarrow C_0(t)$. However, it is not satisfied by the growth law of equation (2.14) for which $C_0(t)/L(t) = C_0/L_0$. Such a law is consistent if one takes proper account of diffusion in a linearly growing domain, which involves adding a convection term to the diffusion model (see Section 2.3).

Consistent with the numerical results of Das et al. [36], we find that for moderate delays (e.g. $\tau = 0.5$), the numerical solution changes from an asymmetric to a symmetric oscillation; see Figure 2.8. Moreover, the switch to the symmetric state occurs at a smaller value of $L$ and $C_{tot}$ (smaller times $t$) for larger $\tau$. Also, for larger delays, the oscillations become less sinusoidal, the frequency of oscillations decreases, and we observe an additional transition from large amplitude to low amplitude symmetric oscillations; see Figure 2.8 (c,f). Finally, as shown in Figure 2.9, the transition between asymmetric and symmetric oscillations occurs at larger cell lengths when the growth rate $\gamma$ is increased. In conclusion, as previously shown by Das et al. [36], the DDE model reproduces the switch from asymmetric to symmetric Cdc42 oscillations observed experimentally during NETO.
Figure 2.8. Numerical solutions of the DDE model with $C_{\text{tot}}(t) = 6(1 + \gamma t)$ and $L(t) = 1 + \gamma t$. (a) $C_{\text{tot}}$ vs. time $t$ for $\gamma = 0.005$. (b,c) Numerical solutions of $X_{1,2}(t)$ for $\gamma = 0.005$ and $\tau = 0.5, 3$, respectively. (d-f) Corresponding figures for $\gamma = 0.01$.

Figure 2.9. Effect of the growth rate on the timing of the switch from asymmetric to symmetric oscillations for the DDE model. As $\gamma$ is increased, the switch from asymmetric to symmetric oscillation occurs at longer cell lengths. Here $\tau = 0.5$ and other parameters are the same as in Figure 2.2.

To detect the basic mechanism for the switch, we choose the length $L$ as the bifurcation parameter and plot the Hopf bifurcation curves in the $(L, \tau)$-plane for the stable symmetric and asymmetric steady states; see Figure 2.10. The corresponding Hopf frequency of the Hopf curve branching from the asymmetric fixed point is plotted as a function of the cell length $L$, or equivalently the critical time delay $\tau_c$ in Figure 2.11. It can be seen that the frequency vanishes at the Hopf bifurcation point, indicative of a Bogdanov–Takens bifurcation. The termination of the asymmetric Hopf branch at a critical cell length $L_c$ is
Figure 2.10. Hopf curves bifurcation curves for the symmetric steady state (green) and the asymmetric steady state (blue) in the \((L, \tau)\) plane with \(C_{\text{tot}} = 6L\). The Hopf curve for the asymmetric steady state exists for \(L \in (0.25, 1.28)\) while the Hopf curve for the symmetric steady state is plotted for \(L > 1.014\). Other parameters are the same as Figure 2.2.

Figure 2.11. Plot of frequency along the Hopf curve branching from the asymmetric steady state as a function of (a) cell length \(L\) and (b) critical delay \(\tau\). Near \((\tau, L) \approx (0.18, 1.285)\), the frequency is zero indicative of a Bogdanov-Takens bifurcation. Other parameters are the same as Figure 2.2.

the basic mechanism underlying the switch from an asymmetric oscillation to a symmetric oscillation. It is also consistent with the vanishing of the asymmetric steady states via a saddle-node bifurcation when \(\tau = 0\); see Figure 2.3 (a). The same basic mechanism holds when bulk diffusion is included using the full PDE–DDE model (2.1a). However, as we will show in Section 2.4, the critical length where the switch occurs is sensitive to the value of the diffusion coefficient.
2.3 Analysis of the full PDE–DDE model

In this section, we return to the full PDE–DDE model given by equations (2.1a)-(2.3), in order to investigate how the switch from asymmetric to symmetric oscillations depends on bulk diffusion. For the moment, we take $L$ and $C_{\text{tot}}$ to be fixed with $C_{\text{tot}}$ treated as a bifurcation parameter. We also perform the rescalings $x \rightarrow \tilde{x} = x/L$, $C \rightarrow \tilde{C} = CL$, and $D \rightarrow \tilde{D} \equiv D/L^2$. The only dependence on $L$ then appears through the association rate $k_+$, as in the pure DDE model. The steady-state solution for the bulk concentration $C$ satisfies (after dropping the tilde)

$$C''(x) = 0, \quad C'(0) = C'(1) = 0.$$ 

Hence $C(x)$ is homogeneous, i.e., $C(x) = \overline{C}$. The conservation equation of the total amount of substrate requires that

$$\int_0^1 \overline{C} dx + X_1 + X_2 = C_{\text{tot}}.$$ 

It follows that $\overline{C} = C_{\text{tot}} - X_1 - X_2$. The steady-state solution $(\overline{X}_1, \overline{X}_2)$ satisfies

$$0 = \frac{k^+(\overline{X}_1)}{L} (C_{\text{tot}} - \overline{X}_1 - \overline{X}_2) - k^-(\overline{X}_1, \overline{X}_1) \overline{X}_1,$$

$$0 = \frac{k^+(\overline{X}_2)}{L} (C_{\text{tot}} - \overline{X}_1 - \overline{X}_2) - k^-(\overline{X}_2, \overline{X}_2) \overline{X}_2.$$ 

Note that the PDE–DDE has the same steady-state solution as the DDE model and there exist a symmetric steady-state solution $(\overline{X}_1 = \overline{X}_2)$ and an asymmetric steady-state solution $(\overline{X}_1 \neq \overline{X}_2)$ for different choices of total substrate concentration $C_{\text{tot}}$; see Figure 2.1.

2.3.1 Linear stability analysis

Consider the perturbation near the steady state $(C_0, \overline{X}_1, \overline{X}_2)$,

$$C(x,t) = \overline{C} + e^{\lambda t} \eta(x), \quad X_i(t) = \overline{X}_i + e^{\lambda t} \phi_i.$$ 

Substituting into the linearized PDE–DDE model near the steady-state solution gives

$$D \frac{\partial^2 \eta(x)}{\partial x^2} = \lambda \eta(x), \quad 0 < x < 1,$$

$$D \eta(0) = \frac{k^+(\overline{X}_1)}{L} \eta(0) + \left[ \frac{k'_+(\overline{X}_1)}{L} \overline{C} - \left( 1 - \frac{eh}{4} + \frac{eh}{4} e^{-\lambda \tau} \right) \right] \phi_1 = \lambda \phi_1,$$

$$-D \eta(1) = \frac{k^+(\overline{X}_2)}{L} \eta(1) + \left[ \frac{k'_+(\overline{X}_2)}{L} \overline{C} - \left( 1 - \frac{eh}{4} + \frac{eh}{4} e^{-\lambda \tau} \right) \right] \phi_2 = \lambda \phi_2.$$ 

and

\[
\begin{align*}
\left[ \lambda + 1 - \frac{eh}{4}(1 - e^{-\lambda \tau}) - \frac{k'_+(\overline{X}_1)}{L} \right] \phi_1 &= \frac{k^+(\overline{X}_1)}{L} \eta(0), \\
\left[ \lambda + 1 - \frac{eh}{4}(1 - e^{-\lambda \tau}) - \frac{k'_+(\overline{X}_2)}{L} \right] \phi_2 &= \frac{k^+(\overline{X}_2)}{L} \eta(1).
\end{align*}
\] (2.16a, 2.16b)

where \( k'_+(\overline{X}) = \frac{dk^+(X)}{dX}_{X=\overline{X}}. \) Rewriting equation (2.16) gives

\[
\phi_1 = B_1(\lambda, \tau) \eta(0), \quad \phi_2 = B_2(\lambda, \tau) \eta(1),
\] (2.17)

with

\[
B_i(\lambda, \tau) = \frac{k^+(\overline{X}_i)/L}{\lambda + 1 - \frac{eh}{4}(1 - e^{-\lambda \tau}) - \frac{k'_+(\overline{X}_i)}{L}}.
\]

Substituting equation (2.17) into the boundary conditions for \( \eta(x) \) yields the following boundary-value problem:

\[
\begin{align*}
D \frac{\partial^2 \eta(x)}{\partial x^2} &= \lambda \eta(x), \quad 0 < x < 1, \ t > 0, \\
D \partial_x \eta(0) &= \lambda B_1(\lambda, \tau) \eta(0), \\
-D \partial_x \eta(1) &= \lambda B_2(\lambda, \tau) \eta(1).
\end{align*}
\] (2.18)

The solution \( \eta(x) \) can be expressed in the form

\[
\eta(x) = \frac{\eta_0 + \eta_1 \cosh(\sqrt{\lambda/D}(x - \frac{1}{2}))}{2 \cosh(\frac{1}{2}\sqrt{\lambda/D})} + \frac{\eta_1 - \eta_0 \sinh(\sqrt{\lambda/D}(x - \frac{1}{2}))}{2 \sinh(\frac{1}{2}\sqrt{\lambda/D})},
\] (2.19)

where \( \eta_0 = \eta(0) \) and \( \eta_1 = \eta(1). \) The boundary conditions then require

\[
\begin{pmatrix}
A_+(&\lambda) + &\lambda B_1(\lambda, \tau) \\
A_-(&\lambda) & A_+(&\lambda) + &\lambda B_2(\lambda, \tau)
\end{pmatrix}
\begin{pmatrix}
\eta_0 \\
\eta_1
\end{pmatrix} = 0,
\] (2.20)

where

\[
A_\pm(\lambda) = \sqrt{\lambda D} \frac{\tanh(\frac{1}{2}\sqrt{\lambda/D}) \pm \coth(\frac{1}{2}\sqrt{\lambda/D})}{2}.
\] (2.21)

Setting the determinant to zero and dividing through by \( \lambda D \) gives

\[
\frac{\lambda}{D} B_1 B_2 + (B_1 + B_2) \sqrt{\frac{\lambda}{D}} \frac{\tanh(\frac{1}{2}\sqrt{\lambda/D}) + \coth(\frac{1}{2}\sqrt{\lambda/D})}{2} + 1 = 0.
\] (2.22)

The presence of terms involving \( \sqrt{\lambda/D} \) means that we have to introduce a branch cut in the complex \( \lambda \)-plane along \(( -\infty, 0]\). Fortunately, for finite \( D, L \), this does not affect the eigenvalue relation (2.22) since, as \( \lambda \to 0 \), we have \( \tanh(\sqrt{\lambda/4D}) \to \sqrt{\lambda/4D} \) and
\[ \coth(\sqrt{\lambda/4D}) \to \sqrt{4D/\lambda}, \] that is, any square roots in (2.22) cancel. However, care has to be taken in the limit \( D \to 0 \) \((D/(k_0^2 L^2) \to 0 \) in dimensionless units) since, up to exponentially small errors, \( \eta(x) \approx e^{-\sqrt{\lambda/4D}x} \) such that equation (2.22) reduces to
\[ \frac{\lambda}{D} B_1 B_2 + (B_1 + B_2) \sqrt{\frac{\lambda}{D}} + 1 = 0. \]

One can no longer eliminate the square roots and there is a continuous spectrum in addition to a discrete spectrum. We will avoid these complexities here by taking \( D > 0 \).

Note that equations (2.17) and (2.22) are well-defined provided that the denominators of the function \( B_1,2(\lambda, \tau) \) are nonzero. Therefore, we need to check that the boundary value problem still makes sense in the singular limit. Let
\[ A(\lambda, \tau, X_i) = \lambda + \frac{eh}{4} e^{-\lambda \tau} + 1 - \frac{eh}{4} - k'_+ (X_i) \frac{C}{L}, \]
and consider the eigenvalue problem associated with the symmetric steady-state solution \( X_1 = X_2 > 0 \). If \( A(\lambda, \tau, X_1) = 0 \), then equation (2.17) requires that
\[ \eta(0) = \eta(1) = 0. \]

It is known that the Dirichlet boundary value problem (2.15a) of \( \eta(x) \) only has a trivial solution, i.e., \( \eta(x) = 0 \). To solve for \((\phi_1, \phi_2)\), we substitute \( \eta(x) = 0 \) into the boundary conditions (2.15b) and (2.15c). It follows that
\[ 0 = \left[ 1 - \frac{eh}{4} + \frac{eh}{4} e^{-\lambda \tau} - k'_+ (X_i) \frac{C}{L} \right] \phi_i = -\lambda \phi_i, \quad i = 1, 2, \]
with the first identity holding since \( A(\lambda, \tau, X_i) = 0 \). Noting that \( \lambda = 0 \) is not a solution of \( A(\lambda, \tau, X_i) = 0 \), it follows that \( \phi_1 = \phi_2 = 0 \). Therefore, if \( A(\lambda, \tau, X_i) = 0 \), the associated solution \((\eta(x), \phi_1, \phi_2)\) is trivial. Hence, we can assume that \( A(\lambda, \tau, X_i) \neq 0 \) for the symmetric steady state, and equation (2.22) holds. For the symmetric steady state \( X_1 = X_2 \), we have \( B_1 = B_2 \) and the resulting cyclic matrix (2.20) has the eigenvectors \((1, 1)^T, (1, -1)^T\). It follows that \( \lambda_+ (\lambda) \pm \lambda_- (\lambda) + \lambda B_1 (\lambda, \tau) = \) for the in-phase (+) and antiphase (−) solutions, respectively, with corresponding eigenvalue equations
\[ B_1 + \sqrt{\frac{D}{A}} \tanh \left( \frac{1}{2} \sqrt{\frac{\lambda}{D}} \right) = 0 \text{ (in-phase)}, \quad (2.23a) \]
\[ B_1 + \sqrt{\frac{D}{A}} \coth \left( \frac{1}{2} \sqrt{\frac{\lambda}{D}} \right) = 0 \text{ (antiphase)}. \quad (2.23b) \]

For the DDE model, the linear stability analysis indicates that the oscillation mode is sensitive to the parameters \( \epsilon \) and \( h \) of the negative feedback. With the same parameter
set as the DDE model, numerical results of our PDE-DDE model show that the oscillations are also antiphase for different diffusion coefficients (see below). In fact, we can check numerically that the eigenvalue equation (2.23a) does not have a purely imaginary solution \( \lambda = i\omega \). To compare with the result of DDE model, we rewrite equation (2.23a) as following

\[
\frac{-1}{B_1} = \sqrt{\frac{\lambda}{D}} \coth\left(\frac{1}{2} \sqrt{\frac{\lambda}{D}}\right) \Rightarrow -A(\lambda, \tau, X_1) = \sqrt{\frac{\lambda}{D}} \coth\left(\frac{1}{2} \sqrt{\frac{\lambda}{D}}\right).
\]

Also, noting that

\[
F_{12} = -k^+(\bar{X}_1)/L, \quad A(\lambda, \tau, \bar{X}_1) = \lambda + \frac{eh}{4} e^{-\lambda \tau} - F_{11} + F_{12},
\]

we have

\[
\frac{\lambda + \frac{eh}{4} e^{-\lambda \tau} - F_{11} + F_{12}}{F_{12}} = \sqrt{\frac{\lambda}{D}} \coth\left(\frac{1}{2} \sqrt{\frac{\lambda}{D}}\right).
\]

(2.24)

Since \( \sqrt{\frac{\lambda}{D}} \coth\left(\frac{1}{2} \sqrt{\frac{\lambda}{D}}\right) \to 2 \) as \( D \to \infty \). It follows that equation (2.24) is reduced to

\[
\frac{\lambda + \frac{eh}{4} e^{-\lambda \tau} - F_{11} + F_{12}}{F_{12}} = 2 \Rightarrow \lambda + \frac{eh}{4} e^{-\lambda \tau} - F_{11} - F_{12} = 0.
\]

This is the eigenvalue equation (in-phase) of the DDE model; see equation (2.8). As shown in Figure 2.5 (b), there is no in-phase solution for the given parameters since \( 4(F_{11} + F_{12})/eh < -1 \). For a finite \( D \), we can check numerically that there is no solution of \( \lambda = i\omega \) by comparing the real parts of equation (2.24). Let

\[
F_{in}(\omega) = \sqrt{\frac{\lambda}{D}} \coth\left(\frac{1}{2} \sqrt{\frac{\lambda}{D}}\right), \quad \omega > 0,
\]

numerical plot of the real part of \( F_{in} \) indicates that \( Re(F_{in}) > 2 \); see Figure 2.12. Suppose that there exists a solution \( (\lambda, \tau) \) of equation (2.24) with \( \lambda = i\omega \), then the real part of the left-hand side of equation (2.24) must satisfy

\[
\frac{eh}{4} \cos(\omega \tau) - F_{11} + F_{12} > 2.
\]

Since \( F_{12} < 0 \), we have

\[
\cos(\omega \tau) < 4(F_{11} + F_{12})/eh < -1.
\]

By contradiction, there is no solution of equation (2.24) with \( \lambda = i\omega \). In other words, there is no in-phase oscillation emerging from a Hopf point along the symmetric steady state.

Next, we consider the eigenvalue problem for the asymmetric steady-state solution \( \bar{X}_1 \neq \bar{X}_2 \) where at least \( B_1 \) or \( B_2 \) is singular. Since \( k^+(X) \) is monotonically increasing, it
Figure 2.12. Plot of the real part of the function $F_{\text{in}}(\omega)$ with different diffusion coefficients. Baseline parameters: $k^+_0 = 2.25$, $k^+_n = 6.467$, $n = 4$, $C_{\text{tot}} = 6.5$, $k^-_0 = 1$, $\epsilon = 0.5375$, $h = 40$ and $L = 1$.

follows that $A(\lambda, \tau, X_1)$ and $A(\lambda, \tau, X_2)$ cannot attain zero simultaneously. Without loss of generality, we assume that

$$A(\lambda, \tau, X_1) = 0, \quad A(\lambda, \tau, X_2) \neq 0.$$  

It follows that $\eta(0) = 0$ and $\phi_2 = B_2(\lambda, \tau)\eta(1)$. The solution for $\eta(x)$ can be rewritten as

$$\eta(x) = \frac{\eta_1}{2} \left( \frac{\cosh(\sqrt{\lambda/D}(x - \frac{1}{2}))}{\cosh(\frac{1}{2}\sqrt{\lambda/D})} + \frac{\sinh(\sqrt{\lambda/D}(x - \frac{1}{2}))}{\sinh(\frac{1}{2}\sqrt{\lambda/D})} \right).$$

The boundary conditions (2.15b) and (2.15c) require that

$$\phi_1 = \frac{\eta_1}{2} \sqrt{\frac{D}{\lambda}}[\coth(\frac{1}{2}\sqrt{\lambda/D}) - \tanh(\frac{1}{2}\sqrt{\lambda/D})],$$

$$0 = \left( \sqrt{\frac{\lambda}{AD}} \frac{\tanh(\frac{1}{2}\sqrt{\lambda/D}) + \coth(\frac{1}{2}\sqrt{\lambda/D})}{2} + \lambda B_2(\lambda, \tau) \right) \eta_1.$$  

Hence, in order to have a nontrivial solution, we require

$$A(\lambda, \tau, X_1) = 0, \quad \frac{\tanh(\frac{1}{2}\sqrt{\lambda/D}) + \coth(\frac{1}{2}\sqrt{\lambda/D})}{2} + \sqrt{\frac{\lambda}{D}} B_2(\lambda, \tau) = 0.$$

The second equation also arises from taking the limit $B_1 \to \infty$ in equation (2.22). Note that the root $\lambda$ of the second equation is dependent on the parameter $D$ while the root of the first equation is independent of $D$. For any fixed $\tau$ and $D$, these two equations can be solved numerically.
2.3.2 Hopf bifurcation

In the following, we numerically solve the eigenvalue relations for the symmetric and asymmetric steady states in order to determine Hopf bifurcation curves, and we show the results of various numerical simulations. Our main goal is to investigate how bulk diffusion can change the critical time delay for the onset of oscillations.

2.3.2.1 Bifurcation from the symmetric steady state

In Figure 2.13 (a,b), we plot the critical delay for a Hopf bifurcation from the symmetric steady state as a function of the diffusion coefficient $D$ and total substrate concentration $C_{\text{tot}}$, respectively. Compared to the parameter $C_{\text{tot}}$, the critical time delay is more sensitive to the value of the diffusion coefficient. For small $D$, the critical time delay is relatively large but decreases as $D$ increases. There exists an asymptote of the critical time delay as $D \to \infty$, which agrees with the critical delay for the DDE model by Das et al. [36]; see Section 2.2. For an arbitrarily chosen point A $((D, \tau) = (2, 1.5))$ on the Hopf curve in Figure 2.13 (a), we determine the direction of the Hopf bifurcation by solving the PDE–DDE (2.1a) numerically. For a parameter set $(D, \tau) = (2, 1.4)$ below the Hopf point, the numerical solution indicates that the steady-state solution is stable; see Figure 2.13(c). For a parameter set $(D, \tau) = (2, 1.6)$ above the Hopf point, the steady state loses its stability and the numerical solution oscillates near the symmetric steady state; see Figure 2.13(d). This suggests that the Hopf bifurcation at the point A is supercritical.

2.3.2.2 Bifurcation from the asymmetric steady state

For the asymmetric steady state, we plot the Hopf curves and numerical solutions of $X_{1,2}$ in Figure 2.14. Again the critical time delay decreases as the diffusion coefficient increases, but the critical time delay tends to be smaller than for the symmetric steady state.

Recall that from the bifurcation diagram of the DDE model (see Figure 2.2), there exists a parameter domain $C_{\text{tot}} \in [6.1, 7.6]$ where the symmetric steady state and asymmetric steady state coexist for $\tau = 0$. In Figure 2.15, we plot the Hopf branches corresponding to the symmetric and asymmetric steady states in this parameter domain for different values of $D$. Unlike the Hopf curves of the DDE model where the two branches intersect (see Figure 2.5 (a), no intersection of the Hopf branches is observed for $D = 2$ and $D = 20$. 
Figure 2.13. Effects of diffusion on Hopf bifurcations from the symmetric steady state. (a) Critical delay \( \tau \) vs. diffusion coefficient \( D \). As \( D \to \infty \), the critical time delay approaches a horizontal asymptote, which is the same as the DDE model. (b) Critical delay \( \tau \) vs. the total substrate concentration \( C_{\text{tot}} \). (c,d) Numerical solution of \( X_{1,2} \) with \((D, \tau)\) below and above the Hopf point A, respectively, indicating that the Hopf bifurcation at A is supercritical and the oscillation mode is antiphase. Baseline parameters: \( k^+ = 2.25, k^- = 6.467, n = 4, C_{\text{tot}} = 6.5, k^0_0 = 1, \epsilon = 0.5375, h = 40 \) and \( L = 1 \). Initial conditions: \( X_1(t) = 3.1, X_2(t) = 3.3, C(x, t) = 0.1 \) for \(-\tau \leq t \leq 0\). Instead, the two Hopf curves separate the parameter domain \((C_{\text{tot}}, \tau)\) into three different regions. In region 1, both of the steady-state solutions are linearly stable. In region 2, the asymmetric steady state is linearly unstable and a small perturbation near the asymmetric steady state generates oscillatory solutions. In region 3, we expect to find both symmetric and asymmetric oscillations. In order to explore this possibility, we plot the numerical solutions for different initial conditions in Figure 2.15 (c,d). The initial conditions are either near the symmetric steady state

\[
C(x, t) = 0.1, \quad X_1(t) = 3.1, \quad X_2(t) = 3.3, \quad -\tau \leq t \leq 0;
\]
Figure 2.14. Effects of diffusion on Hopf bifurcations from the asymmetric steady state. (a) Critical delay $\tau$ vs. diffusion coefficient $D$. (b) Critical delay $\tau$ vs. the total substrate concentration $C_{\text{tot}}$. (c,d) Numerical solution of $X_{1,2}$ with $(D, \tau)$ below and above the Hopf point B, respectively, indicating that the Hopf bifurcation at B is supercritical. Baseline parameters: $k_0^+ = 2.25$, $k_n^+ = 6.467$, $n = 4$, $C_{\text{tot}} = 6.5$, $k_0^- = 1$, $\epsilon = 0.5375$, $h = 40$. Initial conditions: $X_1(t) = 1$, $X_2(t) = 5.3$, $C(x, t) = 0.2$ for $-\tau \leq t \leq 0$.

or near the asymmetric steady state

$$C(x, t) = 0.5, \quad X_1(t) = 1, \quad X_2(t) = 5, \quad -\tau \leq t \leq 0.$$ 

In both cases, oscillations occur around the asymmetric steady state. On the other hand, for smaller time delays in region 3, the first initial condition can lead to symmetric oscillations; see Figure 2.16. This suggests that the time delay could give rise to spontaneous symmetry breaking.

Figure 2.15 (a,b) implies that for a finite diffusion coefficient, $D = 2$ and $D = 20$, the critical time delay for the symmetric branch is larger than that for the asymmetric branch. This is different from the result of critical time delays for the two branches for the DDE model; see Figure 2.5 (a). To compare the critical time delay in the fast diffusion limit with the DDE model given by (2.6), we plot the Hopf curves in the $(D, \tau)$ plane for large
Figure 2.15. Hopf curves in \((\tau, C_{\text{tot}})\) plane and numerical solutions with different initial conditions. (a,b) Hopf curves along the symmetric and asymmetric steady states as \(C_{\text{tot}}\) changes for \(D = 2, 20\), respectively. (c) Numerical solution of \(X_{1,2}\) with initial conditions: \(C(x,t) = 0.1, X_1(t) = 3.1, X_2(t) = 3.3, -\tau \leq t \leq 0\). (d) Numerical solution of \(X_{1,2}\) with initial conditions: \(C(x,t) = 0.5, X_1(t) = 1, X_2(t) = 5, -\tau \leq t \leq 0\). Both of the solutions oscillate near the asymmetric steady state. Baseline parameters: \(D = 2, C_{\text{tot}} = 6.5, L = 1, \tau = 2, k_0^+ = 2.25, k_n^+ = 6.467, n = 4, k_0^- = 1, \epsilon = 0.5375, h = 40\).

Figure 2.16. Numerical solution with different time delays. As the time delay increases, the amplitude of the oscillation increases. At \(\tau = 1.9\), the numerical solution changes from symmetric to asymmetric oscillations. Parameters: \(D = 2, k_0^+ = 2.25, k_n^+ = 6.467, n = 4, k_0^- = 1, \epsilon = 0.5375, h = 40\). Initial conditions: \(X_1(t) = 3.1, X_2(t) = 3.3, C(x,t) = 0.1\) for \(-\tau \leq t \leq 0\).
Figure 2.17. Comparison of effects of diffusion on Hopf bifurcations from the symmetric and asymmetric steady states for a large diffusion coefficient. (a) $C_{\text{tot}} = 6.5$. (b) $C_{\text{tot}} = 7.45$. For a wide range of values of diffusion coefficients, the critical time delay at a Hopf bifurcation from the symmetric steady state is larger than that for the asymmetric steady state. This still holds for large $D$ when $C_{\text{tot}} = 7.45$. However, for a smaller value of $C_{\text{tot}} = 6.5$ and a sufficiently large $D (> 190)$, the critical time delay at a Hopf bifurcation from the symmetric steady state is smaller than that for the asymmetric steady state. This is consistent with the results for the critical time delay in the DDE model; see Figure 2.2.

Baseline parameters: $k^+_0 = 2.25$, $k^+_n = 6.467$, $n = 4$, $C_{\text{tot}} = 6.5$, $k^- = 1$, $\epsilon = 0.5375$, $h = 40$.

$D$ with $C_{\text{tot}} = 6.5, 7.45$, respectively; see Figure 2.17. For the smaller value $C_{\text{tot}} = 6.5$ and sufficiently large $D$, the Hopf curve for the symmetric branch decreases faster than that for asymmetric steady state and reaches a smaller asymptote. On the other hand, if $C_{\text{tot}} = 7.45$, then the asymptote of the Hopf curve for the symmetric branch is larger than that for the asymmetric branch. The result is consistent with the result of the DDE model; see Figure 2.5 (a).

2.4 NETO

In the DDE model of Cdc42 oscillations by Das et al. [36] and the ODE model of NETO by Cerone, Novák, and Neufeld [28], it is crucial to assume that the total amount of substrate increases as the cell length increases. One possible explanation for this assumption is that a typical cell must double its mass and duplicate its contents so that the new daughter cells can contain the components needed for independent growth and eventual division. Recall that the total amount $C_{\text{tot}}$ of Cdc42 per unit area, for fixed length $L$, is defined by the conservation equation

$$C_{\text{tot}} = \int_0^L C(x, t) dx + X_1(t) + X_2(t).$$
In order to reproduce the experimentally observed switch from asymmetric to symmetric oscillations during NETO, we now modify our PDE–DDE model to take into account diffusion in a growing domain $\Omega_t = [0, L(t)]$, in which the total substrate concentration $C_{\text{tot}}$ increases explicitly with respect to time $t$. We will extend our analysis of diffusion in a growing 1D domain along similar lines to Crampin, Gaffney, and Maini [33], who studied the particular problem of spontaneous pattern formation for a reaction-diffusion equation on a growing domain. We begin by expressing the resulting conservation equation in the form

$$\frac{d}{dt} \int_{\Omega_t} C(x,t) dx + \int_{\Omega_t} \frac{\partial J(x,t)}{\partial x} dx = \frac{dC_{\text{tot}}(t)}{dt}, \quad (2.25)$$

with

$$J(x,t) = -D \frac{\partial C(x,t)}{\partial x}, \quad \int_{\Omega_t} \frac{\partial J(x,t)}{\partial x} dx = \frac{dX_1}{dt} + \frac{dX_2}{dt}. \quad (2.26)$$

Using the Reynolds transport theorem to evaluate the first term on the left–hand side,

$$\frac{d}{dt} \int_{\Omega_t} C(x,t) dx = \int_{\Omega_t} \left[ \frac{\partial C(x,t)}{\partial t} + \frac{\partial [\phi(x,t)C(x,t)]}{\partial x} \right] dx, \quad (2.26)$$

where $\phi(x,t)$ is the flow of the domain at time $t$. If we take

$$C_{\text{tot}}(t) = \frac{C_0}{L_0} L(t),$$

with $C_0 = C_{\text{tot}}(0)$, then we obtain the evolution equation

$$\frac{\partial C(x,t)}{\partial t} + \frac{\partial [\phi(x,t)C(x,t)]}{\partial x} = D \frac{\partial^2 C(x,t)}{\partial x^2} + \frac{C_0 \dot{\rho}}{L_0 \rho}, \quad 0 < x < L(t), \quad t > 0. \quad (2.27)$$

Let $X \in [0, L_0]$ be the local coordinate system at the initial length $L_0$. Using a Lagrangian description, we can represent spatial position at time $t$ as

$$x = \Gamma(X,t) = X \rho(t), \quad \rho(0) = 1,$$

with corresponding flow

$$\phi(x,t) = X \dot{\rho} = x \frac{\dot{\rho}}{\rho}. \quad (2.28)$$

Consistent with the analysis of the DDE model, we take $\rho(t) = 1 + \gamma t$ so that

$$L(t) = L_0 (1 + \gamma t).$$

Substitution into equation (2.27) gives

$$\frac{\partial C(x,t)}{\partial t} + \left( \frac{\dot{\rho}}{\rho} \right) \left( x \frac{\partial C(x,t)}{\partial x} + C(x,t) \right) = D \frac{\partial^2 C(x,t)}{\partial x^2} + \frac{C_0 \dot{\rho}}{L_0 \rho}. \quad (2.29)$$
Following Crampin, Gaffney, and Maini [33], we transform equation (2.29) to the fixed interval \([0, L_0]\) by performing the change of variables
\[
(x, t) \rightarrow (\bar{x}, \bar{t}) = \left( \frac{x}{\rho(t)}, t \right).
\] (2.30)

Under this transformation, the advection term in equation (2.29) is eliminated and, on dropping the overbars, we obtain the modified evolution equation
\[
\frac{\partial C(x, t)}{\partial t} = \frac{D}{L(t)^2} \frac{\partial^2 C(x, t)}{\partial x^2} - \left( \frac{\dot{\rho}}{\rho} \right) C(x, t) + \frac{C_0 \dot{\rho}}{L_0 \rho}.
\] (2.31a)

The boundary conditions are
\[
-\frac{D}{L(t)} \frac{\partial C(0, t)}{\partial x} = -\frac{k^+ (X_1(t))}{L(t)} C(0, t) + k^- (X_1(t), X_1(t - \tau)) X_1(t),
\] (2.31b)
\[
-\frac{D}{L(t)} \frac{\partial C(L_0, t)}{\partial x} = \frac{k^+ (X_2(t))}{L(t)} C(L_0, t) - k^- (X_2(t), X_2(t - \tau)) X_2(t).
\] (2.31c)

The DDE for \(X_{1,2}\) are
\[
\frac{dX_1}{dt} = \frac{k^+ (X_1(t))}{L(t)} C(0, t) - k^- (X_1(t), X_1(t - \tau)) X_1(t),
\] (2.31d)
\[
\frac{dX_2}{dt} = \frac{k^+ (X_2(t))}{L(t)} C(L_0, t) - k^- (X_2(t), X_2(t - \tau)) X_2(t).
\] (2.31e)

In the following, we take \(L_0 = 1\) and solve the PDE–DDE (2.31a)-(2.31e) numerically on the domain \([0, 1]\).

From our analysis of the pure DDE model in Section 2.2, we expect there to be a switch from an asymmetric oscillation to a symmetric oscillation as the cell grows. However, one major difference between the PDE–DDE model and the DDE model is that in the former case, the Hopf curve for symmetric bifurcations lies above the Hopf curve for asymmetric bifurcations, as illustrated in Figure 2.15 for fixed \(L\). This suggests that when the asymmetric Hopf branch disappears, the symmetric state may be below its Hopf bifurcation point so that no symmetric oscillations are observed. This is indeed found to be the case as illustrated in Figure 2.18 for a small constant growth rate \(\gamma = 0.01\). The initial condition is chosen to be near the asymmetric steady state when \(C_{\text{tot}} = 6\). For a fixed time delay \(\tau = 1.4\) and \(D = 2\), the numerical solution of \(X_{1,2}\) starts oscillating near the asymmetric steady state and evolves to the symmetric steady state as \(C_{\text{tot}}\) increases. However, oscillations near the symmetric steady state are not observed, since the time delay is below the critical
Figure 2.18. Switch from asymmetric to symmetric oscillations with $C_{tot}$ and $L$ slowly increasing functions of time $t$. For a smaller diffusion coefficient $D = 2$, the time delay $\tau = 1.4$ is not large enough to give rise to symmetric oscillations. On the other hand, for $\tau = 2$ and either $D = 2$ or $D = 3$, the numerical solution changes from asymmetric to symmetric oscillations consistent with the pure DDE model. As the diffusion coefficient increases, the amplitude of the symmetric oscillations also increases. The numerical solution is for the PDE–DDE model (2.31a)-(2.31e). Parameters: $L_0 = 1$, $\gamma = 0.01$, $k_0^+ = 2.25$, $k_n^+ = 6.467$, $n = 4$, $k_0^- = 1$, $\epsilon = 0.5375$, $h = 40$. Initial conditions: $X_1(t) = 1$, $X_2(t) = 4.5$, $C(x,t) = 0.5$ for $-\tau \leq t \leq 0$.

The time delay for $D = 2$. That is, for changing $L$, one can construct Hopf curves similar to those of Figure 2.15 (a) for fixed cell length, and show that the system is in region 2. This is different from the behavior of the DDE model shown in Figure 2.8 (b), for which a small time delay (e.g. $\tau = 0.5$), can give rise to both symmetric and asymmetric oscillations. On the other hand, for larger $\tau$ and $D$, the PDE–DDE model does exhibit a transition from asymmetric to symmetric oscillations as $L$ increases. Moreover, the amplitude of the symmetric oscillations increases as $D$ increases; see Figure 2.18 (c,d). In Figure 2.19, we plot the numerical solution as a function of $L/L_0$ for different $D$ and growth rates $\gamma$. Interestingly, as the growth rate increased from 0.005 to 0.01, we find that the switch
Figure 2.19. Effect of the growth rate and diffusion coefficient on the timing of the switch from asymmetric to symmetric oscillations. As $\gamma$ is increased, the switch from asymmetric to symmetric oscillation occurs later; see (a,b). On the other hand, as $D$ is increased, the switch occurs earlier; see (c,d). The numerical solution is for the PDE–DDE model (2.31a)-(2.31e). Baseline parameters: $D = 3$, $\tau = 3$, $\gamma = 0.01$, $L_0 = 1$. Other parameters are the same as in Figure 2.18. Initial conditions: $X_1(t) = 1$, $X_2(t) = 4.5$, $C(x,t) = 0.5$ for $-\tau \leq t \leq 0$.

from an asymmetric to symmetric oscillations occurs at a relatively larger value of $L/L_0$, again reflecting a breakdown of the adiabatic approximation. On the other hand, as $D$ is increased, the switch occurs at a smaller value of $L/L_0$.

2.5 Discussion

We studied a one-dimensional PDE–DDE model for the signaling molecule Cdc42 during cell polarization in fission yeast. Using linear stability analysis and numerical simulations, we investigated Hopf bifurcations of the symmetric and asymmetric steady states. We showed that the critical time delay at the Hopf point is sensitive to the diffusion coefficient; as the diffusion coefficient increases, the critical delay decreases and reaches an asymptote. This suggests that the DDE model by Das et al. [36] underestimates the
critical time delay. Finally, we solved the diffusion equation on a growing domain under the additional assumption that the total amount $C_{\text{tot}}$ of the signaling molecule increases as the cell length increases. We showed that the system undergoes a transition from asymmetric to symmetric oscillations as the cell grows, consistent with experimental findings of “near-end-take-off” in fission yeast. We also found that the critical length where the switch occurs depends on both $D$ and the growth rate.

We note that the particular result concerning the effect of diffusion on the critical time delay for a Hopf bifurcation has previously been shown for a genetic control model by Busenberg and Mahaffy [22, 23]. However, there are several major differences between our model and theirs. First, the genetic control model involves two species, mRNA and a repressor protein, whereas there is only a single chemical component in our model (signaling protein Cdc42). Second, in our model, the delayed negative feedback is incorporated into the boundary conditions, while the delayed feedback in the genetic control model occurs in a reaction term of the repressor protein. Third, the genetic control model consists of two compartments, one of which is well-mixed. Most significantly, our model has both symmetric and asymmetric steady states, while the genetic model has a unique steady state.

There are a number of possible extensions of our work. First, we followed Das et al. [36] and took the basic mechanism for generating Cdc42 oscillations to be a negative feedback loop with a discrete delay. Since the precise cause of Cdc42 oscillations is not currently known, it would be worthwhile exploring alternative mechanisms as highlighted by Novak and Tyson [122]. Second, our model could be modified to study other signaling molecules that are involved in the polarization of fission yeast such as formin for3p [106]. Third, it would be interesting to consider the full 3D geometry of fission yeast, where bulk diffusion occurs in the cylindrical interior of the cell and the end compartments are treated as hemispherical caps.

From a more general mathematical perspective, our study suggests that it would be worthwhile extending the class of diffusion models considered by Gou et al. [61, 62] to the case where diffusively active compartments evolve according to DDEs. As a preliminary study, we analyze a PDE–DDE model with the delayed logistic equation and the Mackey–Glass equation; see Appendix A.
CHAPTER 3

NEURONAL GROWTH CONE MEMBRANE POLARIZATION VIA MICROTUBULE LENGTH REGULATION

In this chapter, we present a mathematical model of membrane polarization in growth cones. We proceed by coupling an active transport model of cytosolic proteins along a two-dimensional microtubule (MT) network with a modified Dogterom–Leibler model of MT growth. In particular, we consider a Rac1-stathmin-MT pathway in which the growth and catastrophe rates of MTs are regulated by cytosolic stathmin, while the stathmin is regulated by Rac1 at the membrane. Our goal is to determine how the length distribution of MTs affects the distribution of membrane-bound proteins.

Before introducing our model, we present some details regarding Rac1, stathmin, and MT. Stathmin is known as an important family of soluble phosphoproteins found in growth cones that can regulate MT growth [31, 55, 128]. It is known that stathmin can indirectly inhibit MT through promoting catastrophe rate of microtubules or sequestering the free tubulins, thus lowering the local tubulin concentration, reducing the MT growth velocity, and increasing the catastrophe rate. There is also experimental evidence of an alternative mechanism for reducing the MT growth rate, which occurs at high pH values, in which stathmin increases the MT catastrophe rate, but not the growth velocity, by direct interaction with the MT filaments [64]. Stathmin itself can be regulated through a Rac1-Pak pathway [27]; see Figure 3.1. The signaling molecule Rho GTPase Rac1 is found to be active (phosphorylated) when membrane-bound at the leading edge of the growth cone [13]. Active Rac1 can deactivate stathmin via the intermediate protein Pak [152]. Moreover, because the active form of Rac1 is located at the leading edge of the growth cone, it can induce a spatial gradient of stathmin phosphorylation and thus stathmin MT/tubulin interactions [119]. Finally, because the distribution of active Rac1 within the leading edge
Figure 3.1. Sketch of the Rac1-stathmin-MT pathway. Rac1 proteins (circles) are located at the leading edge of the growth cone in active (solid circles) or inactive form (open circles). The active region of Rac1 generates a gradient in stathmin phosphorylation such that the concentration of active stathmin increases with distance from the active Rac1 domain. Active stathmin inhibits the growth of MTs. There is also a potential feedback pathway involving the interaction between Rac1 and MT tips, which we neglect in our model.

can be modified by extracellular guidance cues [14], it follows that the Rac1-stathmin-MT pathway provides one possible mechanism for growth cone steering via MT polarization.

The above mechanism has been explored in a computational model of a two-dimensional (2D) growth cone by Mahajan and Athale [101]. These authors consider a reaction-diffusion model of receptor-driven activation (dephosphorylation) and inactivation (phosphorylation) of stathmin, and modulate the MT dynamics by increasing the local catastrophe rate according to the local stathmin concentration. One major conclusion of their study is that the stathmin-based regulation of MT dynamics is sufficient to generate growth-cone turning, without the need for amplification from positive feedback in which MT tips promote the inactivation of stathmin. Indeed, their modeling study suggests that the feedback from MTs can amplify noise and generate spurious polarization in the absence of external cues.
Recently, Zeitz and Kierfeld [159] have analyzed a more biophysically detailed model of MT regulation based on the signaling proteins Rac1 and stathmin. In contrast to the 2D growth cone model of Mahajan and Athale [101], they consider a one-dimensional (1D) model consisting of an ensemble of parallel MTs growing within a 1D concentration gradient of stathmin. The latter is generated by the Rac1-based dephosphorylation of stathmin at one end of the domain. Zeitz and Kierfeld [159] consider both catastrophe-promoting and tubulin-sequestering mechanisms of stathmin regulation, and find that the latter exhibits a stronger dependence on the level of active Rac1. Moreover, the inclusion of feedback between MT tips and activation of Rac1 has a much more significant effect on the tubulin-sequestering mechanism, resulting in bistability between a state of high Rac1 activation and a state of low Rac1 activation.

In this work, we consider a different aspect of growth cone steering via MT polar- ization, namely, how a nonuniform distribution of MT lengths generated by the Rac1-stathmin-MT pathway can support membrane polarization in the leading edge of the growth cone. Under the assumption that all MTs are nucleated from the same source, a variation in MT length translates into a variation in the distance of MT plus ends from the cell membrane. The latter causes a corresponding nonuniformity in the active transport of signaling proteins (or lipids) along the MT filament tracks, resulting in a nonuniform distribution of membrane-associated molecules. The point of our modeling study is to determine whether the mechanism of MT regulation considered by previous authors is sufficient to generate a significant variation in the concentration of membrane-bound molecules. To compare the tubulin-sequestering and catastrophe-promoting mechanisms of stathmin-based MT regulation, we adopt the model of Zeitz and Kierfeld [159]. A major result of our modeling study is to establish that only the tubulin-sequestering mechanism appears to support a significant variation in membrane-bound proteins, and this is sensitive to the precise form of the Rac1 distribution and parameters such as the tubulin association rate. This is partly due to the fact that the lateral diffusion of stathmin within the growth cone reduces the spatial variation of MT lengths compared to the results of the 1D model considered by Zeitz and Kierfeld [159].

The rest of the chapter is organized as follows. In Section 3.1, we present the three main components of our model: (1) an advection-diffusion model for cytosolic proteins, (2)
a two-dimensional model for stathmin-regulated MT growth, and (3) a reaction-diffusion model for the Rac1-regulated stathmin. In Section 3.2, for a nonuniform MT length distribution, we use regular perturbation theory and numerical simulations to determine the resulting distribution of membrane-bound proteins. In Section 3.3 and Section 3.4, we consider different nonuniform Rac1 distributions and determine the resulting nonuniform mean MT length distribution and the membrane-bound proteins, respectively.

3.1 Model for growth cone membrane polarization via microtubule length

Our model treats the growth cone as a rectangular two-dimensional (2D) domain \( \Omega = \{(x,z); 0 \leq x \leq L, 0 \leq z \leq R\} \) with the leading edge of the growth cone at \( z = R \) and the MTs parallel to the z-axis; see Figure 3.2. Based on experimental measurements of growth cones of cultured neurons, we take \( L = R = 10\mu m \) [11]. We list the three components of our model below.

1. An advection-diffusion model for the active transport of cytosolic proteins along a fixed MT network, which takes into account the binding and unbinding of molecules at the membrane surface. The distribution of MT lengths is specified by an interface function \( z = \phi(x) \), where \( z \) is the mean distance of MTs from the cell membrane at a given transverse coordinate \( x \).

2. A 2D version of the modified Dogterom–Leibler model [159] of MT growth, which takes into account the stochastic switching between catastrophe and rescue. Following Zeitz and Kierfeld [159], both the catastrophe rate and growth rate can be modified by the local concentration of stathmin in the cytosol, resulting in a nonuniform distribution of MT lengths.

3. A 2D version of the reaction-diffusion model of the Rac1-stathmin signaling pathway recently introduced by Zeitz and Kierfeld [159]. This determines the stathmin concentration gradient within the cytosol for a given active Rac1 distribution in the membrane. Thus a nonuniform Rac1 concentration ultimately leads to a nonuniform distribution of proteins in the cell membrane when the latter are actively transported along the Rac1-stathmin-regulated MT network.
3.1.1 Active transport of signaling molecules

We start with the model for the active transport of signaling molecules along the MTs. We assume that the MTs form a uniformly distributed bundle of filaments orthogonal to the cell membrane. Let $\phi(x)$ denote the mean distance of the plus ends of the local MT population at $x$ from the cell membrane - the corresponding mean length is $\phi(x)$. (The resulting interface $z = \phi(x)$ need not be continuous.) Suppose that a given configuration of MTs acts as a system of filament tracks for the active transport of some signaling protein that is targeted for delivery to the cell membrane (we leave the identity of the actively transported cytosolic molecules open, but candidates are signaling proteins such as Cdc42 and Rac1 or membrane lipids). Let $c(x,z,t)$ denote the concentration of the signaling protein within the cytosol of the growth cone and $u(x,t)$ denote the concentration at the cell membrane. The protein molecules in the cytosol undergo alternating sequences of diffusion and active transport by molecular motors along microtubules while molecules at the membrane undergo diffusion along the membrane. At the cell membrane $z = R$, molecules can attach and detach from the membrane with rates $k_+$ and $k_-$, respectively. The concentrations
$c(x,z,t)$ and $u(x,t)$ evolve according to the advection-reaction-diffusion equations (note that the use of advection–diffusion equations to model active intracellular transport can be justified from first principles under certain assumptions regarding the rates of switching between different motile states of a motor-cargo complex [18, 53, 117, 133])

$$\frac{\partial c(x,z,t)}{\partial t} = -v(x,z) \frac{\partial c(x,z,t)}{\partial z} + D \frac{\partial^2 c(x,z,t)}{\partial x^2} + D \frac{\partial^2 c(x,z,t)}{\partial z^2}, \quad (3.1a)$$

$$\frac{\partial u(x,t)}{\partial t} = D_m \frac{\partial^2 u(x,t)}{\partial x^2} + k_+u(x,t) - k_-c(x,R,t). \quad (3.1b)$$

where

$$v(x,z) = \begin{cases} v_0, & \text{if } 0 < z < \phi(x), \\ 0, & \text{if } \phi(x) < z < R. \end{cases} \quad (3.2)$$

Here the velocity $v(x,z)$ has a jump discontinuity at the interface $\Gamma = \{(x,z), z = \phi(x)\}$. Equations (3.1a, 3.1b) are supplemented by the reflecting boundary conditions at $x = 0, L$ and $z = 0,$

$$\frac{\partial c}{\partial x}(0,z,t) = \frac{\partial c}{\partial x}(L,z,t) = 0, \quad \frac{\partial u}{\partial x}(0,t) = \frac{\partial u}{\partial x}(L,t) = 0, \quad (3.3)$$

$$D \frac{\partial c}{\partial z}(x,0,t) - v_0 c(x,0,t) = 0, \quad 0 < x < L, \quad (3.4)$$

and a flux conservation condition at $z = R$

$$-D \frac{\partial c}{\partial z}(x,R,t) = k_+c(x,R,t) - k_-u(x,t), \quad 0 < x < L. \quad (3.5)$$

At the interface $\Gamma = \{(x,z), z = \phi(x)\}$, we impose continuity of $c(x,z,t)$ and the corresponding flux, which leads to the jump conditions

$$c(x,z,t)_{|z=\phi_+(x)} = 0, \quad [v(x,z)c(x,z,t) - D \frac{\partial c(x,z,t)}{\partial z}]_{|z=\phi_+(x)} = 0, \quad (3.6)$$

where $\phi_\pm = \lim_{\epsilon \to 0} [\phi \pm \epsilon], \epsilon > 0.$

### 3.1.2 MT model

Our main aim is to calculate the steady-state concentration of membrane-bound signaling proteins for an interface determined by a stathmin-based model of MT polarization. We proceed by constructing a 2D version of the reaction-diffusion model of Zeitz and Kierfeld [159], in which there is a fixed distribution of Rac1 in the leading edge of the growth
Figure 3.3. Two-dimensional stathmin-regulated MT growth model. Active Rac1 in the leading edge ($z = R$) generates a gradient of phosphorylated stathmin. As $z$ increases, the concentration of active (dephosphorylated) stathmin becomes larger, thus increasing the likelihood that an MT undergoes catastrophe.

cone, and feedback interactions between Rac1 and MT tips that reach the membrane are ignored. The schematic diagram is shown in Figure 3.3. The basic assumptions of the stathmin-regulated MT growth model are thus as follows.

1. The concentration of active Rac1 in the cell membrane is given by the prescribed function $r_{on}(x)$, $0 \leq x \leq L$.

2. Stathmins in both the active (dephosphorylated) and inactive (phosphorylated) states diffuse in the cytosol with the same diffusion coefficient $D$. Activation of stathmin takes place in the cytosol with a constant rate $k_{on}$ while deactivation only occurs at the leading edge under the regulation of the active Rac1 at a rate $k_{off}$.

3. MTs stochastically switch between a growth state and a shrinkage state at a catastrophe rate $\omega_c$ and a rescue rate $\omega_r$. MTs polymerize in the positive $z$-direction at an average velocity $v_+$ in the growth state and depolymerize at an average velocity $-v_-$ in the shrinkage state with $v_+ > 0$.

4. The growth of MTs is regulated by the local stathmin concentration either by directly increasing the catastrophe rate or by sequestering tubulin (see below).
Let \( p_\pm(x,z,t) \) denote the density of MTs at lateral position \( x \) at time \( t \) with length \( z \) and in the growth (+) or shrinkage (−) phase. Here length is determined by the vertical distance \( z \) of an MT’s plus end from the trailing edge of the growth cone at \( z = 0 \). The densities \( p_\pm \) evolve according to the extended Dogterom–Leibler model [40],

\[
\frac{\partial p_\pm}{\partial t}(x,z,t) = -\frac{\partial[v_\pm(x,z,t)p_\pm(x,z,t)]}{\partial z} - \omega_c(x,z,t)p_+(x,z,t) + \omega_r p_r(x,z,t), \tag{3.7a}
\]

\[
\frac{\partial p_-}{\partial t}(x,z,t) = v_- \frac{\partial p_-}{\partial z}(x,z,t) + \omega_c(x,z,t)p_+(x,z,t) - \omega_r p_r(x,z,t). \tag{3.7b}
\]

where the space-time dependence of the catastrophe rate \( \omega_c \) and growth velocity \( v_+ \) arises from their dependence on the stathmin concentration; see below. We impose reflecting boundary conditions at \( x = 0, L, \)

\[
\left. \frac{\partial p_\pm}{\partial x}(x,z,t) \right|_{x=0,L} = 0, \tag{3.8}
\]

and at \( z = 0, R, \)

\[
v_+(x,0)p_+(x,0,t) - v_- p_-(x,0,t) = v_+(x,R)p_+(x,R,t) - v_- p_-(x,R,t) = 0. \tag{3.9}
\]

### 3.1.3 Stathmin model

We now construct the final component of the full model. Let \( S_{\text{on}}(x,z,t) \) and \( S_{\text{off}}(x,z,t) \) denote the concentration of active and inactive stathmin, respectively, at position \( (x,z) \) at time \( t \). The stathmin concentrations are taken to satisfy the reaction-diffusion equations

\[
\frac{\partial S_{\text{off}}}{\partial t}(x,z,t) = D_s \nabla^2 S_{\text{off}}(x,z,t) - k_{\text{on}} S_{\text{off}}(x,z,t) \tag{3.10a}
\]

\[
\frac{\partial S_{\text{off}}}{\partial t}(x,R,t) = -\frac{D_s}{\delta} \frac{\partial S_{\text{off}}}{\partial z}(x,R,t) \bigg|_{z=R} - k_{\text{on}} S_{\text{off}}(x,R,t) + r_{\text{on}}(x) k_{\text{off}} S_{\text{on}}(x,R,t), \tag{3.10b}
\]

and

\[
\frac{\partial S_{\text{on}}}{\partial t}(x,z,t) = D_s \nabla^2 S_{\text{on}}(x,z,t) + k_{\text{on}} S_{\text{off}}(x,z,t) \tag{3.11a}
\]

\[
\frac{\partial S_{\text{on}}}{\partial t}(x,R,t) = -\frac{D_s}{\delta} \frac{\partial S_{\text{on}}}{\partial z}(x,R,t) \bigg|_{z=R} + k_{\text{on}} S_{\text{off}}(x,R,t) - r_{\text{on}}(x) k_{\text{off}} S_{\text{on}}(x,R,t). \tag{3.11b}
\]

Following Zeitz and Kierfeld [159], we are assuming that there exists a boundary layer of width \( \delta \) at the leading edge \( z = R \), within which stathmin molecules deactivate (phosphorylate) at a rate \( r_{\text{on}}(x) k_{\text{off}} \) and activate (dephosphorylate) at a rate \( k_{\text{on}} \). Outside this
boundary layer, only dephosphorylation occurs. Equations (3.10) and (3.11) are supplemented by the following boundary conditions at \( x = 0, L \) and \( z = 0 \):

\[
\left. \frac{\partial S_{\text{off, on}}}{\partial x} \right|_{x=0,L} = 0, \quad \left. \frac{\partial S_{\text{off, on}}}{\partial z} \right|_{z=0} = 0. \tag{3.12}
\]

The stathmin model is coupled to the MT growth model by taking the catastrophe rate, and possibly, the growth velocity, to depend on the local concentration of active stathmin. We consider two forms of coupling [159]:

**A** One suggested pathway for stathmin to suppress MT growth is by direct interaction with an MT filament, resulting in an increase in the catastrophe rate. Experimental data suggest a linear increase of the catastrophe rate with the concentration of active stathmin, so we take

\[
\omega_c(x, z, t) = \omega_c^0 + k_c S_{\text{on}}(x, z, t). \tag{3.13}
\]

with the baseline catastrophe rate \( \omega_c^0 = 7 \times 10^{-4} \text{s}^{-1} \) and the catastrophe-promoting constant \( k_c = 0.005 \text{s}^{-1} \mu \text{M}^{-1} \) [75].

**B** Another possible mechanism involves sequestering of free tubulin by stathmin, which leads to an increase of the catastrophe rate and a decrease of the growth rate. It turns out that a single active stathmin protein sequesters two tubulin proteins [75],

\[
2T + S \rightleftharpoons ST_2.
\]

If this is combined with the kinetics of activation/deactivation of stathmin, then at chemical equilibrium, the normalized concentration of free tubulin \( t \equiv [T]/[T_0] \), where \([T_0]\) is the total tubulin concentration, can be expressed as a nonlinear function of the normalized active stathmin concentration \( s_{\text{on}} = S_{\text{on}}/[T_0] \) [159]:

\[
t(s_{\text{on}}) = \frac{1}{3} \left[ 1 - 2s_{\text{on}} + \frac{k(1 - 2s_{\text{on}})^2 - 3}{k\alpha(s_{\text{on}})} + \alpha(s_{\text{on}}) \right]
\]

with \( k \equiv K_0[T_0]^2 \), where \( K_0 \) is the equilibrium constant for the stathmin activation reaction,

\[
\alpha(s) = \left[ (1 - 2s)^3 + \frac{9}{k}(1 + s) + \beta(s) \right]^{1/3},
\]

and

\[
\beta(s) = 3 \sqrt{\frac{3}{k^3}} \left[ 1 + k^2(1 - 2s)^3 + k(2 + 10s - s^2) \right].
\]
Because the microtubule growth velocity \( v_+ \) depends on the local tubulin concentration, it follows that a spatial variation in active stathmin concentration leads to a spatial variation in the growth velocity. That is,

\[
[T](x,z) = [T_0]t(s_{on}(x,z))
\]

and

\[
v_+(x,z) = (\kappa_{on}[T](x,z) - \kappa_{off})d,
\]

(3.14)

where \( d \approx 0.6 \text{ nm} \) is the effective tubulin dimer size and \( \kappa_{on}, \kappa_{off} \) are binding and unbinding rates. Experimentally one finds that the average time spent in the growing state, \( \langle \tau_+ \rangle = 1/\omega_c \) is a linear function of the growth velocity, so that the catastrophe rate also becomes space-dependent:

\[
\omega_c(x,z) = [a + b v_+(x,z)]^{-1},
\]

(3.15)

for constant coefficients \( a = 20 \text{ s} \) and \( b = 138 \times 10^{10} \text{ s}^{-2} \text{ m}^{-1} \) [79].

3.1.4 Coupling between active transport and MT growth models

The last component of our model is specifying how we couple the stathmin-regulated MT growth model with the active transport model. Suppose that the MT length distributions \( p_\pm \) have reached a steady state before the active transport of membrane-bound signaling molecules. We will assume that the total number \( N \) of MTs is fixed and that they are uniformly distributed in the interval \( x \in [0,L] \). Setting \( p(x,z) = p_+(x,z) + p_-(x,z) \), we have

\[
\int_0^R p(x,z)dz = \frac{N}{L},
\]

(3.16)

and the average MT length at \( x \) is

\[
\bar{z}(x) = \frac{L}{N} \int_0^R zp(x,z)dz.
\]

(3.17)

We then make the identification \( \phi(x) = \bar{z}(x) \) for all \( 0 \leq x \leq L \).

Finally, there are a few assumptions of the Zeitz-Kierfeld model [159] that need to be highlighted with regard to its incorporation into our active transport model. These authors consider a 1D model consisting of an ensemble of parallel MTs aligned along the \( z \) axis, and determine the distribution of MT lengths in response to a Rac1-induced stathmin concentration gradient \( S(z) \). In our continuum 2D model, we are assuming that at each point \( x \), there is an ensemble of MTs along the lines of Zeitz and Kierfeld [159], which
sample the local concentration gradient $S(x, z)$ for the given $x$. We consider a continuum model, because we can then use analytical and numerical methods from the theory of partial differential equations. However, the validity of a continuum model is based on the assumption that the number of MTs is sufficiently large. In the case of relatively few MTs, one would need to consider a stochastic model, in which one keeps track of the growth and shrinkage of individual MTs (see also Mahajan and Athale [101]). One would also have to consider a stochastic version of the active transport model. Another assumption of the Zeitz-Kierfeld model is that the tubulin concentration is either uniform or is regulated by the stathmin concentration via fast tubulin-sequestering. Thus it ignores possible changes in the tubulin concentration due to the polymerization/depolymerization of the MTs; the latter would introduce an effective interaction between the MTs [40]. For simplicity, we assume that tubulin-sequestering is the dominant process. A third major assumption is that both the stathmin concentration and MT length distribution have sufficient time to reach steady state before significant turning of the growth cone and consequent changes in the Rac1 distribution along the leading edge. This is reasonable given the fast diffusivity of stathmin [119] and the experimental observation that MT dynamical instabilities are at least an order-of-magnitude faster than translocation speeds of a growth cone [56].

3.2 Analysis of the active transport model

In this section, we analyze the active transport model with a uniform length distribution and a specific nonuniform distribution of MT lengths.

3.2.1 Uniform distribution of MT lengths

Suppose that the MTs have the same length $\xi$ so that $\phi(x) = \xi > 0$. The corresponding velocity function is then $v(x, z) = v_0 H(\xi - z)$, where $H$ is the Heaviside function. The steady-state solutions $c(x, z)$ and $u(x)$ satisfy equations (3.1a) and (3.1b) with all time derivatives set to zero, and the jump conditions reduce to

$$c(x, \xi^+) = c(x, \xi^-), \quad D \frac{\partial c}{\partial z}(x, \xi^-) - v_0 c(x, \xi^-) = D \frac{\partial c}{\partial z}(x, \xi^+).$$

(3.18)

where $\xi^\pm = \lim_{\epsilon \to 0} [\xi \pm \epsilon]$, $\epsilon > 0$. After imposing the various boundary conditions, we obtain the $x$-independent solutions
\[ c(z) = \begin{cases} 
  c_0 e^{-\frac{z}{D}(R-z)}, & \text{if } 0 < z < \xi, \\
  c_0 e^{-\frac{\xi}{D}(R-\xi)}, & \text{if } \xi < z < R. 
\end{cases} \quad (3.19) \]

and

\[ u = \frac{k_+}{k_-} c(R) = c_0 \frac{k_+}{k_-} e^{-\frac{\xi}{D}(R-\xi)}. \quad (3.20) \]

The coefficient \( c_0 \) is determined by the conservation equation

\[ \int_0^L u(x) \, dx + \int_0^R \int_0^L c(x,z) \, dx \, dz = M, \quad (3.21) \]

where \( M \) is the total number of proteins. Hence,

\[ c_0 = \frac{M/L}{e^{-\frac{\xi}{D}(R-\xi)} / (k_+/k_- + R - \xi + D/v_0) - (D/v_0)e^{-\frac{\xi}{D}R}}. \]

### 3.2.2 Nonuniform distribution of MT lengths

Let us now consider steady-state solutions of the active transport model given by equations (3.1a) and (3.1b) with a nonuniform distribution of MT lengths as specified by an interface function of the form

\[ \phi(x) = z_0 [1 + \sigma \psi(x)], \quad (3.22) \]

When \( \sigma \) is small, we can use regular perturbation theory to obtain an approximate solution of the steady-state membrane concentration of the active transport model given by equations (3.1a) and (3.1b). Suppose the steady-state solution is of the form

\[ c(x,z) = \begin{cases} 
  c_0 e^{-\frac{z}{D}(R-z) + \sigma \Psi_{-}(x,z)}, & \text{if } z < z_0[1 + \sigma \psi(x)], \\
  c_0 e^{-\frac{\xi}{D}(R-z_0) + \sigma \Psi_{+}(x,z)}, & \text{if } z_0[1 + \sigma \psi(x)] < z < R. 
\end{cases} \quad (3.23) \]

Introduce the domains

\[ \Omega_- = \{(x,z), z < z_0[1 + \sigma \psi(x)]\}, \quad \Omega_+ = \{(x,z), z_0[1 + \sigma \psi(x)] < z < R\}. \]

In region \( \Omega_- \), the steady-state solution satisfies

\[ 0 = -\frac{v_0}{D} \frac{\partial c}{\partial z} (x,z) + D \nabla^2 c(x,z) \]

\[ = v_0 \left( -\frac{v_0}{D} - \sigma \frac{\partial \Psi_-}{\partial z} \right) + D \left( \frac{v_0^2}{D^2} + 2\sigma \frac{v_0}{D} \frac{\partial \Psi_-}{\partial z} + \sigma \frac{\partial^2 \Psi_-}{\partial z^2} \right) + \sigma D \frac{\partial^2 \Psi_-}{\partial x^2} + O(\sigma^2) \]

\[ = \sigma v_0 \frac{\partial \Psi_-}{\partial z} + \sigma D \nabla^2 \Psi_- + O(\sigma^2). \]
Collecting the $O(\sigma)$ term gives

\[ \frac{v_0}{\partial z} \nabla^2 \Psi - D \frac{\partial \nabla^2 \Psi}{\partial z} = 0. \] \hspace{1cm} (3.24)

Similarly, we have

\[ D \nabla^2 \Psi = 0. \] \hspace{1cm} (3.25)

The reflecting boundary conditions of $c(x,z)$ at $x = 0, L$ yield

\[ \left. \frac{\partial \Psi}{\partial x} \right|_{x=0,L} = 0, \quad \left. \frac{\partial \Psi}{\partial x} \right|_{x=0,L} = 0. \] \hspace{1cm} (3.26)

At $z = R$, we have the flux conservation condition of equation (3.5):

\[ -D \left. \frac{\partial c}{\partial z} \right|_{z=R} = k_+ c(x, R) - k_- c_0 e^{v_0 (R-z_0)/D} u_1(x). \] \hspace{1cm} (3.27)

Suppose the steady-state solution of $u$ is of the form

\[ u(x) = u_0 + \sigma u_1(x), \]

then the order $\sigma$ terms in the boundary condition (3.27) are

\[ -D \frac{\partial \Psi}{\partial z}(x, R) = k_+ \Psi(x, R) - k_- \frac{\Psi_0 e^{v_0 (R-z_0)/D} u_1(x)}{\rho_0}. \] \hspace{1cm} (3.28)

For simplicity, we take the trailing edge to be at $z = -\infty$ rather than $z = 0$ and impose the Dirichlet boundary condition at $z = -\infty$. That is,

\[ c(x, -\infty) = 0. \]

This implies that $\Psi_-$ is bounded.

Equations (3.24) and (3.25) can be solved by separation of variables. A standard calculation yields

\[ \Psi_+(x, z) = \sum_{n=0}^{\infty} A_n \cos(\sqrt{\lambda_n} x) e^{\sqrt{\lambda_n}(R-z)} + \bar{A}_n \cos(-\sqrt{\lambda_n} x) e^{-\sqrt{\lambda_n}(R-z)}, \] \hspace{1cm} (3.29a)

\[ \Psi_-(x, z) = \sum_{n=0}^{\infty} B_n \cos(\sqrt{\lambda_n} x) e^{-\rho_n(R-z)}, \] \hspace{1cm} (3.29b)

where

\[ \lambda_n = \left( \frac{n\pi}{L} \right)^2, \quad \rho_n = \frac{-v_0 + \sqrt{v_0^2 + 4D^2 \lambda_n}}{2D} > 0. \] \hspace{1cm} (3.30)
The coefficients $A_n, \tilde{A}_n$ and $B_n$ can be determined by the boundary condition at $z = R$ and the jump condition at the interface $\Gamma = \{(x,z)|z = z_0[1 + \sigma \psi(x)]\}$. The jump conditions (3.6) yield

\[ -\frac{z_0 v_0}{D} \psi(x) + \Psi_+(x,z_0) - \Psi_-(x,z_0) = 0, \quad \frac{\partial \Psi_+(x,z_0)}{\partial z} - \frac{\partial \Psi_-(x,z_0)}{\partial z} = 0. \]  

(3.31)

Using the cosine series expansions of $\Psi_+$ and $\Psi_-$, we have

\[ \sum_{n=0}^{\infty} \left( A_n e^{\sqrt{\lambda_n}z_R} + \tilde{A}_n e^{-\sqrt{\lambda_n}z_R} - B_n e^{-\rho_n z_R} \right) \cos(n\pi x/L) = \frac{z_0 v_0}{D} \psi(x), \]

\[ \sum_{n=0}^{\infty} \left( \tilde{A}_n e^{\sqrt{\lambda_n}z_R} - A_n e^{-\sqrt{\lambda_n}z_R} + B_n \rho_n e^{-\rho_n z_R} \right) \cos(n\pi x/L) = 0, \]

where $z_R = R - z_0$. Since $\{\cos(n\pi x/L)\}_{n \in \mathbb{Z}}$ is complete in $C^1([0,L])$, we can express $\psi$ as

\[ \psi(x) = \frac{a_0}{2} + \sum_{n=1}^{\infty} a_n \cos(n\pi x/L), \]

where

\[ a_n = \frac{1}{L} \int_{0}^{L} \psi(x) \cos(n\pi x/L) dx. \]

The uniqueness of the representation of the cosine series requires that

\[ A_n e^{\sqrt{\lambda_n}z_R} + \tilde{A}_n e^{-\sqrt{\lambda_n}z_R} - B_n e^{-\rho_n z_R} - \frac{z_0 v_0}{D} (a_n - \frac{\delta_{0,n}}{2} a_n) = 0, \]  

\[ A_n e^{\sqrt{\lambda_n}z_R} - \tilde{A}_n e^{-\sqrt{\lambda_n}z_R} + B_n \rho_n e^{-\rho_n z_R} = 0. \]  

(3.32)

The boundary condition (3.28) at $z = R$ is coupled to the steady-state solution $u(x)$ of equation (3.1b), that is,

\[ D_m \frac{d^2 u}{dx^2} + k_+ c(x,R) - k_- u(x) = 0 \]  

(3.33)

with $c(x,0) = \sigma_0 e^{-v_0/D(R-z_0)} + c\Psi_+(x,R)$. Substituting $u(x) = u_0 + \sigma u_1(x)$ into equation (3.33) gives

\[ k_+ c_0 e^{-v_0/D(R-z_0)} - k_- u_0 = 0, \]  

\[ D_m \frac{d^2 u_1}{dx^2} - k_- u_1(x) = -k_+ c_0 e^{-v_0/D(R-z_0)} \Psi_+(x,R). \]  

(3.34)

Noting that

\[ \Psi_+(x,R) = \sum_{n=0}^{\infty} (A_n + \tilde{A}_n) \cos(\sqrt{\lambda_n}x). \]
equation (3.34) has the particular solution
\[ u_1(x) = \sum_{n=0}^{\infty} c_n \cos(\sqrt{\lambda_n}x), \]  
(3.35)
with
\[ c_n = k_+ c_0 e^{-\frac{\pi}{L} (R-z_0)} \frac{A_n + \tilde{A}_n}{k_- + D_m \lambda_n}, \]  
(3.36)
Substituting the cosine series expressions of \( \Psi \) and \( u_1 \) into the boundary condition (3.28) gives
\[ \sum_n D (A_n - \tilde{A}_n) \sqrt{\lambda_n} \cos \sqrt{\lambda_n}x \]
\[ = \sum_n \left( k_+ - \frac{k_- k_+}{k_- + D_m \lambda_n} \right) (A_n + \tilde{A}_n) \cos \sqrt{\lambda_n}x. \]
It follows that
\[ D (A_n - \tilde{A}_n) \sqrt{\lambda_n} = \frac{k_+ D_m \lambda_n}{k_- + D_m \lambda_n} (A_n + \tilde{A}_n). \]
Hence
\[ \tilde{A}_n = C_n A_n, \]  
(3.37)
where
\[ C_n = D \sqrt{\lambda_n} - \frac{k_+ D_m \lambda_n}{k_- + D_m \lambda_n}. \]  
(3.38)
Substituting equation (3.37) into equation (3.32b) gives
\[ B_n = \frac{\sqrt{\lambda_n}}{\rho_n} [C_n e^{(\rho_n - \sqrt{\lambda_n})z_R} - e^{(\rho_n + \sqrt{\lambda_n})z_R}] A_n, \]  
(3.39)
and substituting equation (3.38) into equation (3.32a) yields
\[ A_n = \frac{z_0 v_0}{D} \frac{a_n (1 - \frac{1}{2} \delta_{0,n})}{(1 + \sqrt{\lambda_n} / \rho_n) e^{\sqrt{\lambda_n} x_0} + C_n (1 - \sqrt{\lambda_n} / \rho_n) e^{-\sqrt{\lambda_n} x_0}}, \]  
(3.40)
As an illustration of the above analysis, suppose that
\[ \phi(x) = z_0 [1 + \sigma \psi(x)], \quad \psi(x) = \cos(\frac{\pi}{L}x). \]  
(3.41)
Then \( A_n = \tilde{A}_n = B_n = 0, \) for all \( n \neq 1, \) and the steady-state concentration of membrane signaling protein is
\[ u(x) = \frac{k_+ c_0}{k_-} e^{-v_0 / D (R-z_0)} + \sigma u_1(x), \]  
(3.42)
Figure 3.4. Numerical solutions of equations (3.1a) and (3.1b) vs. perturbative solutions for (a) $\sigma = 0.05$ (b) $\sigma = 0.1$. Other parameters are as follows: $R = 10\mu m$, $L = 10\mu m$, $D = 0.1\mu m^2 s^{-1}$, $D_m = 0.01\mu m^2 s^{-1}$, $k_- = 0.1 s^{-1}$, $k_+ = 1\mu ms^{-1}$, $v_0 = 1\mu ms^{-1}$, $z_0 = R/2$.

where

$$u_1(x) = \frac{A_1(1 + C_1)k_+c_0}{k_- + D_m/2}\frac{1}{L^2}e^{-v_0(R-z_0)/D}\cos\left(\frac{\pi}{L}x\right),$$

and

$$A_1 = \frac{z_0\rho_0}{D} \left(1 + \sqrt{\lambda_1}/\rho_1 e^{\sqrt{\lambda_1}(R-z_0)} + C_1(1 - \sqrt{\lambda_1}/\rho_1)e^{-\sqrt{\lambda_1}(R-z_0)}\right)^{-1},$$

$$C_1 = \frac{D\sqrt{\lambda_1} - (k_+D_m\lambda_1)/(k_- + D_m\lambda_1)}{D\sqrt{\lambda_1} + (k_+D_m\lambda_1)/(k_- + D_m\lambda_1)}.$$

This establishes that a nonuniform distribution of MT lengths supports a nonuniform concentration of a membrane protein that is actively transported along the MTs.

In Figure 3.4, we compare the approximate perturbative solution with a numerical solution of the full equations for $\sigma = 0.1, 0.05$. It can be seen that there is good agreement, but the amplitude of the inhomogeneity is small. However, the same type of behavior is obtained as the amplitude $\sigma$ of the inhomogeneity is increased. This is illustrated in Figure 3.5.

### 3.3 MT polarization generated by Rac1

Next we turn to steady-state solutions of the 2D MT/stathmin model given by equations (3.7), (3.10), and (3.11).
3.3.1 Uniform Rac1

We first consider the case of a uniform Rac1 distribution, \( r_{on}(x) = r_0 \), for which we can directly apply the steady-state analysis of Zeitz and Kierfeld [159]. Setting \( \omega_c = \omega_c(z) \) and \( v_+ = v_+(z) \), adding equations (3.7a) and (3.7b), and setting time-derivatives to zero yields the steady-state equation

\[
\partial_z [v_+(z)p_+(z) - v_-p_-(z)] = 0.
\]

This implies that

\[
v_+(z)p_+(z) - v_-p_-(z) \equiv J.
\]

The boundary condition at \( z = 0, R \) requires that \( J(x) = 0 \). It follows that

\[
p_-(z) = \frac{v_+(z)}{v_-}p_+(z).
\] (3.44)
Substituting equation (3.44) into equation (3.7a) gives
\[
\frac{\partial v_+(z)p_+(z)}{\partial z} + \left[ \frac{\omega_r}{v_-} - \frac{\omega_c(z)}{v_+(z)} \right] v_+(z)p_+(z) = 0.
\]
Hence
\[
v_+(z)p_+(z) = v_+(0)p_+(0) \exp \left( \int_0^z \lambda(z') dz' \right),
\]
where
\[
\lambda(z) = \frac{\omega_r}{v_-} - \frac{\omega_c(z)}{v_+(z)}. \tag{3.45}
\]
It follows that the total density of MTs with length \( z \) is
\[
p(z) \equiv p_+(z) + p_-(z) = \left( 1 + \frac{v_+(z)}{v_-} \right) p_+(z)
= \mathcal{N} \left( 1 + \frac{v_-}{v_+(z)} \right) \exp \left( \int_0^z \lambda(z') \, dz' \right). \tag{3.46}
\]
The normalization factor \( \mathcal{N} \) is determined by equation (3.16) which gives
\[
\mathcal{N} = \frac{N}{L} \left[ \int_0^R \left( 1 + \frac{v_-}{v_+(z)} \right) \exp \left( \int_0^z \lambda(z') \, dz' \right) \, dz \right]^{-1}. \tag{3.47}
\]
It remains for us to determine the \( z \) dependence of the functions \( v_+(z) \) and \( \omega_c(z) \) by finding the steady-state solution of the expressions in equations (3.10) and (3.11) for stathmin. In the case of a uniform Rac1 concentration, there then exists an \( x \)-independent steady-state solution for \( S_{\text{off}} \) of the form
\[
S_{\text{off}}(z) = \Lambda_0 \cosh(\nu_0 z), \quad \nu_0 = \sqrt{\frac{k_{\text{on}}}{D_s}}. \tag{3.48}
\]
The coefficient \( \Lambda_0 \) depends on the steady-state boundary condition at the leading edge \( z = R \), where deactivation of stathmin takes place:
\[
\left. \frac{D_s}{\delta} \frac{\partial S_{\text{off}}}{\partial z} \right|_{z=R} = k_{\text{off}} r_{\text{on}} S_{\text{on}}(R) - k_{\text{on}} S_{\text{off}}(R). \tag{3.49}
\]
Let \( S(z) = S_{\text{on}}(z) + S_{\text{off}}(z) \) be the total stathmin concentration at \( z \). Because \( S(z) \) evolves according to the 1D steady-state diffusion equation with reflecting boundaries, it follows that \( S(z) = S_{\text{tot}} = \text{constant} \). Hence
\[
S_{\text{on}}(z) = S_{\text{tot}} - S_{\text{off}}(z) = S_{\text{tot}} - \Lambda_0 \cosh(\nu_0 (z - R)). \tag{3.50}
\]
Substituting equation (3.50) into the boundary condition equation (3.49) gives

\[ \Lambda_0 = \frac{S_{tot} k_{off} r_0}{(D_s / \delta) v_0 \sinh(v_0 R) + (r_0 k_{off} + k_{on}) \cosh(v_0 R)}. \] \tag{3.51} 

Finally, one can determine the average MT length by substituting for \( S_{on} \) into either model of stathmin-MT coupling: equation (3.13) (direct interactions) or equations (3.14) and (3.15) (indirect interactions via tubulin-sequestering). For example, in the former case, we have

\[ \omega_c(z) = \omega_c^0 + k_c \left[ S_{tot} - \Lambda_0 \cosh(v_0 z) \right]. \] \tag{3.52} 

Substituting equation (3.52) into the steady-state density of MT lengths, equation (3.46), and using equation (3.45), gives

\[ p(z) = N \left( 1 + \frac{v_+}{v_-} \right) \exp \left[ \gamma z + \frac{k_c \Lambda_0}{v_+ v_0} \sinh(v_0 z) \right], \] \tag{3.53} 

where

\[ \gamma = \frac{\omega_r}{v_-} - \frac{\omega_c^0 + k_c S_{tot}}{v_+}, \] \tag{3.54} 

and

\[ N \left( 1 + \frac{v_+}{v_-} \right) = \frac{N}{L} \left[ \int_0^R \exp \left[ \gamma z + \frac{k_c \Lambda_0}{v_+ v_0} \sinh(v_0 z) \right] dz \right]^{-1}. \]

We conclude that for a uniform distribution of active Rac1 within the membrane, the profile of MT lengths in Figure 3.3 is \( \phi(x) = \xi \) with

\[ \xi = \frac{L}{N} \int_0^R z p(z) dz. \]

In Figure 3.6, we plot the mean MT length distribution \( \bar{z} = \bar{\zeta} \) as a function of \( S_{tot} \) for both forms of MT-stathmin interactions, recovering previous results in [159]. Note that we use very similar parameter values to those of Zeitz and Kierfeld [159] (see Table 3.1). It follows that in the full 2D model, a spatial variation in active Rac1 concentration, \( r(x) \), will result in a spatial variation in the mean length \( \bar{z} = \bar{z}(x) \) and, hence, a spatially varying interface function \( \phi(x) = z(x) \) for the active transport model.
Figure 3.6. Steady-state solutions for the average MT length $\bar{z} = \bar{\zeta}$ as a function of the normalized stathmin concentration $s = S_{\text{tot}}/[T_0]$, where $[T_0]$ is the total tubulin concentration, and the active Rac1 concentration for fixed $s$. (a,c) Tubulin-sequestering stathmin. (b,d) Catastrophe-promoting stathmin. For sufficiently large $s$, the model acts like a switch, jumping from a small $\bar{z}$ in the absence of active Rac1 ($r_0 = 0$) to a large $\bar{z}$ for constitutively active Rac1 ($r_0 = 1$). In our 2D model, a spatial variation in active Rac1 concentration, $r(x)$, will result in a spatial variation in the mean length $\bar{z} = \bar{z}(x)$ and, hence, a spatially varying interface function $\phi(x) = \bar{z}(x)$. Parameters: $v_+ = 0.06 \mu m s^{-1}, v_- = 0.18 \mu m s^{-1}, w_r = 0.18 s^{-1}, k_c = 0.005 s^{-1} \mu M^{-1}$.

3.3.2 Nonuniform Rac1

Now suppose we have a nonuniform Rac1 concentration given by

$$r_{\text{on}}(x) = r_0 + r_1 \cos(\pi x/L).$$

When $r_1$ is small ($0 \ll r_1 \ll 1$), we can again use perturbation theory to obtain an approximate solution of the steady-state stathmin concentration in the cytosol. Consider a steady-state solution for the inactive stathmin concentration of the form

$$S_{\text{off}}(x,z) = S_{\text{off}}(z) + r_1 S_1(x,z),$$
Table 3.1. Parameter values used for simulation

<table>
<thead>
<tr>
<th>Description</th>
<th>Parameter</th>
<th>Value/Reference</th>
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<tbody>
<tr>
<td>Time step</td>
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<tr>
<td>Spatial step</td>
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<td>Growth cone</td>
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<td>depth</td>
<td>10 $\mu$m</td>
</tr>
<tr>
<td></td>
<td>cell edge region</td>
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<tr>
<td>Active-transport model</td>
<td>Diffusion coefficient</td>
<td>0.1 $\mu$m$^2$s$^{-1}$ [68]</td>
</tr>
<tr>
<td></td>
<td>Diffusion coefficient(membrane)</td>
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</tr>
<tr>
<td></td>
<td>Advection coefficient</td>
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</tr>
<tr>
<td></td>
<td>Detachment rate</td>
<td>0.1s$^{-1}$ [68]</td>
</tr>
<tr>
<td></td>
<td>Attachment rate</td>
<td>1 $\mu$m$^{-1}$ [68]</td>
</tr>
<tr>
<td>Microtubule</td>
<td>Tubulin concentration</td>
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</tr>
<tr>
<td></td>
<td>Effective dimer length</td>
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</tr>
<tr>
<td></td>
<td>Tubulin associate rate</td>
<td>$\omega_{on} = \kappa_{on}[T_0]$ 143 s$^{-1}$[150]</td>
</tr>
<tr>
<td></td>
<td>Dissipation velocity</td>
<td>$\kappa_{off}d$ 3.6 nms$^{-1}$ [78]</td>
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<td>Growth velocity ($S_{on} = 0$)</td>
<td>$v_+$ 0.06 $\mu$m$^{-1}$ [42, 57, 78, 150]</td>
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<tr>
<td></td>
<td>Shrinkage velocity</td>
<td>$v_-$ 0.18 $\mu$m$^{-1}$ [42, 57, 150]</td>
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<td>Rescue rate</td>
<td>$\omega_r$ 0.18 s$^{-1}$ [113, 130, 150]</td>
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<td>Catastrophe rate ($S_{on} = 0$)</td>
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<tr>
<td></td>
<td>Catastrophe rate ($\omega_c = 1/(a + bv_+)$)</td>
<td>$a$ 20 s</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$b$ 1.38 $\times$ 10$^{10}$ s$^2$m$^{-1}$[79]</td>
</tr>
<tr>
<td>Stathmin</td>
<td>Activation rate</td>
<td>$k_{on}$ 1 s$^{-1}$[119]</td>
</tr>
<tr>
<td></td>
<td>Deactivation rate</td>
<td>$k_{off}$ 300 s$^{-1}$</td>
</tr>
<tr>
<td></td>
<td>Diffusion coefficient</td>
<td>$D_s$ 15 $\mu$m$^2$s$^{-1}$ [119]</td>
</tr>
<tr>
<td></td>
<td>Sequestering equilibrium constant</td>
<td>$K_0$ 25 $\mu$M$^{-2}$ [4, 35, 73, 82, 119]</td>
</tr>
<tr>
<td></td>
<td>Catastrophe promotion constant</td>
<td>$k_c$ 0.005 s$^{-1}$µM$^{-1}$ [75]</td>
</tr>
</tbody>
</table>

with $S_{off}(z)$ satisfying equation (3.48) and $S_1(0, z) = 0$. Substituting into equations (3.10a) and (3.10b), and collecting the order $O(r_1)$ term gives

$$D_s \nabla^2 S_1(x, z) - k_{on} S_1(x, z) = 0$$  \hspace{1cm} (3.55)

supplemented by reflecting boundary conditions at $x = 0, L$ and $z = 0$, and

$$- \frac{D_s \partial S_1}{\delta} \frac{\partial S_1}{\partial z} \bigg|_{z=R} = k_{on} S_1(x, R) - r_0 k_{off} S_1(x, R) + \cos(\pi x / L) k_{off} (S_{tot} - S_{off}(R)) = 0. \hspace{1cm} (3.56)$$

Here we have used the identity $S_{tot} = S_{on}(x) + S_{off}(x)$. Equation (3.55) can be solved by the method of separation of variables to give
\[ S_1(x,z) = \sum_{n=1}^{\infty} \Lambda_n \cos(\mu_n x) \cosh(\nu_n z), \quad (3.57) \]

where
\[ \mu_n = \frac{n\pi}{L}, \quad \nu_n = \sqrt{\frac{k_{\text{on}}}{D_s} + \mu_n^2}, \]

and the coefficients \( S_n \) are determined by the boundary condition at \( z = R \). Substituting the solution into equation (3.56) gives
\[ 0 = -\frac{D_s}{\delta} \sum_{n=1}^{\infty} \Lambda_n \cos(\mu_n x) \nu_n \sinh(\nu_n R) + \cos(\pi x / L)[S_{\text{tot}} - S_{\text{off}}(R)] \]
\[ - [r_0k_{\text{off}} + k_{\text{on}}] \sum_{n=1}^{\infty} \Lambda_n \cos(\mu_n x) \cosh(\nu_n R). \quad (3.58) \]

Matching the coefficients of \( \cos(\mu_1 x) \), we find
\[ \Lambda_n = \frac{[S_{\text{tot}} - S_{\text{off}}(R)]k_{\text{off}}\nu_n}{(D_s/\delta)\nu_n \sinh(\nu_n R) + [k_{\text{on}} + r_0k_{\text{off}}] \cosh(\nu_n R)}, \quad n \geq 1. \]

Then the steady-state concentration of active stathmin is
\[ S_{\text{on}}(x,z) = S_{\text{tot}} - \Lambda_0 \cosh(\nu_0 z) - r_1 \Lambda_1 \cos(\mu_1 x) \cosh(\nu_1 z). \]

with \( \Lambda_0 \) given by equation (3.51) and
\[ \Lambda_1 = \frac{[S_{\text{tot}} - S_{\text{off}}(R)]k_{\text{off}}}{(D_s/\delta)\nu_1 \sinh(\nu_1 R) + [k_{\text{on}} + r_0k_{\text{off}}] \cosh(\nu_1 R)}. \]

The steady-state analysis of MT lengths under catastrophe-promoting stathmin (see equations (3.45) and (3.46)) can be extended to the case of \( x \)-dependent stathmin concentration gradient. We find that the steady-state density of MT lengths is given by
\[ p(x,z) = \mathcal{N}(x) \exp \left[ \gamma z + \frac{k_c \Lambda_0}{\nu_0} \sinh(\nu_0 z) \right] \]
\[ + r_1 \frac{k_c \Lambda_1}{\nu_1} \cos(\mu_1 x) \sinh(\nu_1 z) \right] \]
\[ \approx \mathcal{N}(x) q_0(z) \left[ 1 + r_1 \eta(z) \cos(\mu_1 x) \right], \]

with \( \gamma \) and \( \Lambda_0 \) given by equations (3.54) and (3.51), respectively, and
\[ q_0(z) = \exp \left[ \gamma z + \frac{k_c \Lambda_0}{\nu_0} \sinh(\nu_0 z) \right], \]
\[ \eta(z) = \frac{k_c \Lambda_1}{\nu_1} \sinh(\nu_1 z). \]
Figure 3.7. Numerical and asymptotic solution of average MT length with catastrophe-promoting stathmin. (a) Concentration of active Rac1 $r_{\text{on}}(x) = 1 + r_1 \cos(\pi x / L)$ for $r_1 = 0, 0.2$. (b) Corresponding steady-state distribution of average MT lengths as a function of membrane coordinate $x$ using numerical simulation (solid lines) and perturbation theory (markers). The normalized stathmin concentration $s = S_{\text{tot}} / [T_0] = 0.71$. Other parameters are as in Figure 3.6.

The normalization factor $N(x)$ is determined by the condition

$$N(x) = \frac{N}{L} \left[ \int_0^R (q_0(z) + r_1 q_1(z) \cos(\mu_1 x)) dz \right]^{-1} \approx \frac{N}{Lq_0} \left[ 1 - r_1 \frac{\bar{q}_1}{q_0} \cos(\mu_1 x) \right],$$

with $q_1(z) = q_0(z) \eta(z)$,

$$\bar{q}_j = \int_0^R q_j(z) dz.$$

It follows that

$$p(x,z) = \frac{N}{Lq_0} \left[ q_0(z) + r_1 \left( q_1(z) - q_0(z) \frac{\bar{q}_1}{q_0} \right) \cos(\mu_1 x) \right]. \quad (3.59)$$

We conclude that a weakly $x$-dependent Rac1 membrane concentration generates a weakly $x$-dependent MT interface $z = \phi(x) = z_0 + r_1 z_1 \cos(\pi x / L)$ with

$$z_0 = \frac{1}{q_0} \int_0^R z q_0(z) dz, \quad z_1 = \frac{1}{q_0} \int_0^R z \left( q_1(z) - q_0(z) \frac{\bar{q}_1}{q_0} \right) dz.$$

For catastrophe-promoting stathmin, the numerical solution of the mean length of MTs agrees well with the asymptotic expansion solution we have derived in the supporting material; see Figure 3.7. Because of the strongly nonlinear dependence of the MT growth velocity on the active stathmin, it is difficult to find an asymptotic expansion solution of the mean MT length for tubulin-sequestering stathmin.

The numerical solutions of the mean MT length for both tubulin-sequestering stathmin and catastrophe-promoting stathmin are plotted in Figure 3.8. In the case of the sinusoidal
Figure 3.8. Average MT lengths for sinusoidal and piecewise Rac1 concentration profiles. (a) Plot of Rac1 distribution $r_{\text{on}}(x) = 0.5 + r_1 \cos(\pi x / L)$ for different values of $r_1$. (b,c) Corresponding MT length distributions for tubulin-sequestering and catastrophe-promoting stathmin, respectively. (d). Plot of piecewise Rac1 distribution. (e,f) Corresponding MT length distributions for tubulin-sequestering and catastrophe-promoting stathmin, respectively. The normalized stathmin concentration $s = S_{\text{tot}} / [T_0] = 0.46$ for tubulin-sequestering stathmin and $s = 0.71$ for catastrophe-promoting stathmin. Other parameters are as in Figure 3.6.
Rac1 distribution \( r_{\text{on}}(x) = r_0 + r_1 \cos(\pi x / L) \), the corresponding MT length distribution exhibits only a weak spatial variation even for large amplitude Rac1 inhomogeneities. This holds for both tubulin-sequestering stathmin and catastrophe-promoting stathmin, and is mainly due to the lateral diffusion of the stathmin in the two-dimensional growth cone. A more significant spatial variation in MT lengths is obtained using a piecewise Rac1 distribution, \( r_{\text{on}}(x) = H(3 - x) \) where \( H \) is the Heaviside function. Moreover, the mean MT length changes more dramatically when it is regulated by the tubulin-sequestering mechanism rather than the catastrophe-promoting mechanism. This is consistent with the results of the 1D model in [159]; see also Figure 3.6. However, due to the diffusion of stathmin in the two-dimensional growth cone, the regulation of MT length by Rac1 is weakened compared to the 1D model. As the active Rac1 concentration \( r_{\text{on}} \) decreases from 1 to 0, the mean MT length decreases with a upper bound smaller than the MT mean length with \( r_{\text{on}} = 1 \) and a lower bound larger than the MT mean length with \( r_{\text{on}} = 0 \).

3.4 Polarization of membrane signaling molecules by Rac1

Recall that the main goal of our modeling study is to investigate to what extent stathmin-regulated MT polarization provides a possible substrate for membrane polarization via the active transport of signaling molecules along the polarized MT network. This can now be investigated by coupling the Rac1-stathmin model of MT length regulation given by equations (3.7), (3.10), and (3.11) with the active transport model of equations (3.1a) and (3.1b), and (3.2-3.6). This is achieved by setting the interface function \( \phi(x) = \bar{z}(x) \) with \( \bar{z}(x) \) defined according to equation (3.17). For the sake of illustration, we consider the piecewise Rac1 distribution. The numerical solution of the resulting steady-state mean MT length distribution is shown in Figure 3.9 (a) and the corresponding membrane concentration \( u(x) \) is shown in Figure 3.9 (b) for tubulin-sequestering stathmin (solid curves) and catastrophe-promoting stathmin (shaded curves). In both cases, there exists a stable inhomogeneous distribution of the membrane concentration \( u(x) \). However, tubulin-sequestering stathmin generates a significantly larger spatial variation in the membrane concentration \( u(x) \).

In Figure 3.10, we show plots of \( u(x) \) with different parameters for tubulin-sequestering stathmin and catastrophe-promoting stathmin, respectively. For tubulin-sequestering stath-
Figure 3.9. Numerical plots of (a) mean MT length and (b) steady-state membrane concentration $u(x)$ for piecewise Rac1 distribution. (Solid curves) Membrane polarization for MTs regulated by tubulin-sequestering stathmin. The normalized stathmin concentration is $s = S_{\text{tot}}/[T_0] = 0.46$. (Shaded curves) Membrane polarization for MTs regulated by catastrophe-promoting stathmin. The normalized stathmin concentration is $s = S_{\text{tot}}/[T_0] = 0.71$. Other parameters are as in Figure 3.5 and Figure 3.6.

min, increasing the shrinkage velocity $v_-$ or reducing the rescue rate $\omega_r$ reduces the membrane concentration as well as its spatial variation. We explore how the membrane concentration changes with respect to the growth velocity $v_+$ by changing the tubulin association rate $\omega_{\text{on}} = \kappa_{\text{on}}[T_0]$; see equation (3.14). As the tubulin association rate increases, the growth velocity of MTs increases and thus the average MT length also increases, resulting in an increase in membrane concentration. For the catastrophe-promoting stathmin, the membrane concentration is sensitive to the choice of shrinkage velocity, rescue rate $\omega_r$, and catastrophe-promoting constant $k_c$. It is less sensitive to the catastrophe rate $\omega_0 = S_{\text{on}} = 0$. Unlike the tubulin-sequestering stathmin mechanism, the degree of spatial variation of $u(x)$ is relatively insensitive to the choice of parameters.

3.5 Discussion

In this chapter, we studied an advection-diffusion model for the active transport of cytosolic signaling proteins along a two-dimensional MT network in a growth cone. The model was coupled to a modified Dogterom–Leibler model of MT growth, with the growth rate and catastrophe rate regulated by stathmin and Rac1 as proposed in [159]. The active
Rac1 located in the membrane inhibits active stathmin near the membrane, while stathmin in the active state inhibits the growth of MTs via two possible pathways, tubulin-sequestering and catastrophe-promoting.

We first showed that a nonuniform MT network results in a spatially varying concentration of signaling molecules on the membrane. We then explored the MT length distribution under the regulation of stathmin for different choices of the Rac1 distribution on the membrane. For a nonuniform Rac1 distribution, we showed that the MTs grow
towards the location with a higher Rac1 concentration for both tubulin-sequestering and
catastrophe-promoting stathmin, thus resulting in a polarized distribution of membrane
proteins. The spatial variation of the MT length depends on the precise form of the Rac1
distribution and parameters such as the catastrophe-promoting constant and the tubulin
association rate. For a piecewise constant Rac1 distribution, tubulin-sequestering stathmin
generates a more significant membrane polarization than catastrophe-promoting stathmin.
However, due to the lateral diffusion of the stathmin in the two-dimensional domain, the
spatial variation of the MT length is smaller compared to the results of the one-dimensional
model considered by Zeitz and Keifeld [159]. Which of the two stathmin-based regulatory
mechanism dominates appears to depend on the pH level, suggesting that perhaps there
is some form of pH regulation of stathmin in the growth cone.

One possible extension of our work would be to consider the closed feedback loop
of Rac1-Stathmin-MT as proposed in Zeitz and Kierfeld [159], whereby MTs that reach
the membrane surface activate Rac1—in particular, to determine whether or not such a
feedback mechanism can enhance the inhomogeneity of MT growth by counteracting the
effects of the lateral diffusion of stathmin. More generally, it would be interesting to
explore how the role of Rac1 in microtubule growth relates to another well-known sig-
naling pathway for growth-cone steering, namely, Ca$^{2+}$ [143, 145, 146]. It is known that
extracellular guidance cues cause an asymmetric elevation of Ca$^{2+}$ across the growth cone,
which then mediates an imbalance in exocytosis-endocytosis. This in turn redirects lipids,
adhesion molecules, and cytoskeletal elements asymmetrically across the growth cone,
resulting in growth cone steering. In the case of an attractive (repulsive) cue, Ca$^{2+}$ en-
hances exocytosis (endocytosis) at the leading edge of the growth cone, resulting in turning
the growth cone towards (away from) the extracellular signal. The downstream effects of
Ca$^{2+}$ appear to depend on the amplitude of the Ca$^{2+}$ signal. There is also experimental
evidence that Rac 1 modulates the stimulus-evoked release of Ca$^{2+}$ in growth cones. This
occurs via two parallel mechanisms [160]: (i) enhancing MT assembly along the lines out-
lined in our work, which subsequently promotes the spread of the endoplasmic reticulum
(ER)-based Ca$^{2+}$ release machinery into the growth cone; (ii) increasing so-called reactive
oxygen species production, which facilitates inositol 1,4,5-triphosphate (IP$_3$)-dependent
Ca$^{2+}$ release.
CHAPTER 4

A STOCHASTIC ACTIVE-TRANSPORT MODEL

In this chapter, we present a stochastic model of the cytoplasmic transport of vesicles on a two-dimensional filament network, in which a vesicle containing signaling molecules can randomly switch between a diffusing state and a state of directed motion along a filament. To explore the role of the geometry of a filament network, we consider two types of filament networks: (i) filaments that grow from a nucleating center in the cytoplasm and (ii) filaments that nucleate from sites on the membrane. The first corresponds to microtubules which grow radially from the central microtubule organizing center towards the cell periphery. The second case corresponds to actin filaments which are nucleated near the plasma membrane.

A recent modeling study by Hawkins et al. [68] has demonstrated that the geometry of the organization of cytoskeletal filaments plays a crucial role in determining whether the cell is capable of spontaneous cell polarization or only polarizes in response to an external chemical gradient [68]. More specifically, the authors showed that the former holds if filaments are nucleated at sites on the cell membrane, whereas the latter applies if the filaments nucleate from organizing sites within the cytoplasm (microtubule asters). The model thus captures differences in experimental studies of cell polarization in budding yeast [102, 139] and neuronal growth cones [16]. However, the model involves two major simplifying assumptions: (i) the cytoplasmic signaling molecules are treated as free particles rather than bound to vesicles; (ii) the transport of molecules in the cytoplasm is represented in terms of an advection-diffusion process with isotropic, homogeneous diffusion. As highlighted by Layton et al. [94, 137], one potential problem with assumption (i) is that vesicular transport makes cell polarization more difficult to sustain, since fusion of vesicles leads to the release of membrane lipids as well as signaling molecules. This
suggests that either there is some active mechanism for increasing the concentration of signaling molecules in the membrane following vesicle fusion, or that some other factor is transported by vesicles that maintains cell polarization. Interestingly, experimental evidence for the former mechanism has recently been obtained by establishing that vesicles deliver Cdc42 to sites of polarized growth in yeast [39].

In this work, we relax both assumptions (i) and (ii), and show that a more biophysically realistic model of active vesicular transport in the cytoplasm leads to an advection-diffusion equation with anisotropic and space-dependent diffusion. Our starting point is a stochastic model of active transport on a two-dimensional cytoskeletal network, in which a vesicle containing signaling molecules can randomly switch between a diffusing state and a state of directed motion along a cytoskeletal filament. Using a quasi-steady-state analysis, we show how the resulting stochastic hybrid system can be reduced to an advection-diffusion process for the concentration of vesicles in the cytoplasm. We thus derive an explicit expression for the anisotropic diffusion tensor and show how it depends on cytoskeletal geometry. We then use linear stability analysis to derive conditions for the growth of a precursor pattern for cell polarization, and thus demonstrate that our more realistic model of active transport supports spontaneous polarization in the case of nucleation at the cell membrane but not from asters. The effects of spatially varying/anisotropic diffusion and the dependence on various biophysical parameters of the stochastic model are also highlighted. Finally, note that although we do take into account the vesicular nature of active transport (assumption (i)), we do not address the particular issue of lipid transport. However, our more realistic model provides a framework for exploring this issue in future work.

Note that this model differs from our previous model (Chapter 3) of the role of active transport in cell polarization, which considers spatial inhomogeneities in the length distribution of MTs in the neuronal growth cone. Instead, we now assume all filaments reach the membrane surface and consider spatial inhomogeneities in the density distribution of MTs. We also explore the effect of orientations of filaments by comparing the result of MT networks with that of the actin cytoskeleton.
4.1 Deterministic model

We begin by describing the deterministic model of Hawkins et al. [68]. For simplicity, the cell is taken to be two-dimensional and curvature effects are ignored; see Figure 4.1. The cell boundary is given by the $x$-axis, and the cytoplasm given by the half-plane $(x,z), z > 0$. Let $u(x,t)$ denote the concentration of signaling molecules in the membrane and let $c(x,z,t)$ denote the corresponding concentration in the cytoplasm. Hawkins et al. [68] consider a deterministic reaction-diffusion model of the form

\begin{align}
\frac{\partial u(x,t)}{\partial t} &= D_m \frac{\partial^2 u(x,t)}{\partial x^2} + k_{on} c(x,0,t) - k_{off} u(x,t), \tag{4.1a} \\
\frac{\partial c(x,z,t)}{\partial t} &= D \nabla^2 c(x,z,t) - \mathbf{v} \cdot \nabla c(x,z,t). \tag{4.1b}
\end{align}

The first equation represents diffusion of signaling molecules within the membrane together with transfer between the membrane and cytoplasm, where $k_{on}$ and $k_{off}$ are the binding and unbinding rates. The second equation is an advection-diffusion equation that describes the hybrid transport dynamics of molecules in the cytoplasm, which randomly switch between diffusive motion and ballistic motion along the filaments. It is assumed that the switching rates are sufficiently high so that the underlying stochastic process can be reduced to the given advection-diffusion process with isotropic diffusion - we will carry out this reduction explicitly in Section 4.2, and show that there are additional terms reflecting space-dependent and anisotropic diffusion. The above equations are supplemented by the conservation equation

\begin{equation}
M = \int_{-L/2}^{L/2} u(x,t) dx + \int_{-L/2}^{L/2} \int_0^{\infty} c(x,z,t) dz. \tag{4.2}
\end{equation}
with $M$ the total number of signaling molecules. Since the concentration profile decays exponentially in the $z$ direction, the range of $z$ is taken to be the half-line. Finally, there is conservation of flux at the membrane:

$$k_{on} c(x, 0, t) - k_{off} u(x, t) = D \frac{c(x, z, t)}{\partial z} \bigg|_{z=0} - v_z(x, 0, t)c(x, 0, t). \quad (4.3)$$

The velocity field $v(x, z, t)$ depends on the geometry of the filaments, which is itself determined by the concentration of signaling molecules on the membrane. Hawkins et al. [68] distinguish between two cases; see Figure 4.1:

(i) Filaments that grow from a nucleating center in the cytoplasm (microtubule aster) are approximately perpendicular to the membrane surface. Assuming that the speed of active transport at $(x, z)$ is proportional to the local density of parallel filaments, and that the latter is proportional to the concentration of surface signaling molecules $u(x, t)$, we have

$$v(x, z, t) = -\kappa_0 u(x, t)e_z, \quad (4.4)$$

where $\kappa_0$ is a constant that specifies the coupling between the signaling molecules and filaments. This type of geometry holds for the distribution of microtubules in neuron growth cones; see Figure 1.12, where gamma-aminobutyric acid (GABA) receptors appear to associate with and regulate the growing microtubule ends [16].

(ii) Filaments that nucleate from sites on the membrane can be approximated by a superposition of asters. Assuming that the velocity field at $r = (x, z)$ is determined by the local density of filaments, and this decreases with distance from each nucleation site $r' = (x, 0)$, then

$$v(r, t) = -\kappa_0 \int_{-L/2}^{L/2} \frac{r - r'}{|r - r'|^2} u(x', t)dx', \quad (4.5)$$

where $L$ is the circumference of the cell. This geometry reflects the organization of the actin cytoskeleton in budding yeast, as illustrated in Figure 1.6.

### 4.2 Stochastic active transport model

In order to construct a stochastic version of the Hawkins et al. model, we first have to consider active transport processes in a little more detail [17]. The main types of active intracellular transport involve the molecular motors kinesin and dynein carrying resources along microtubular filament tracks, and myosin V motors transporting cargo along actin
filaments. Microtubules and actin filaments are polarized polymers with biophysi-
ically distinct plus and minus ends, and this polarity determines the preferred direction in which
an individual molecular motor moves. For example, kinesin moves towards the plus end
whereas dynein moves towards the minus end of a microtubule. Each motor protein un-
dergoes a sequence of conformational changes after reacting with one or more adenosine
triphosphate (ATP) molecules, causing it to step forward along a filament in its preferred
direction. Thus, ATP provides the energy necessary for the molecular motor to do work
in the form of pulling its cargo along a filament in a biased direction. The movement of
molecular motors occurs over several length and time scales [20, 83, 88, 90, 95]. In the case
of a single motor, there are at least three levels of modeling:

(a) the mechanico-chemical energy transduction process that generates a single step of
the motor;

(b) the effective biased random walk along a filament during a single run;

(c) the alternating periods of directed motion along the filament and diffusive or sta-
tionary motion when the motor is unbound from the filament.

We will consider level (c) by treating a motor/cargo complex as a particle that randomly
switches between a free diffusion state and a ballistic motion state with velocity $V(\theta)$, $\theta \in
[0, \pi]$, where the direction arg$[V] = \theta$ is determined by the orientation $\theta$ of the cytoskeletal
filament at $(x, z)$ to which the complex is bound. Following Hawkins et al. [68], we take
$x \in [-L/2, L/2]$ and $z \in R^+$. The orientation $\theta$ is defined as the angle subtended at the
boundary $z = 0$; see Figure 4.1, and we assume that a particle moves towards the plus end
of the filament (toward the boundary) with a constant speed $v_0$. Thus

$$V(\theta) = -v_0 \cos \theta e_x - v_0 \sin \theta e_z. \quad (4.6)$$

The stochastic transport process is illustrated in Figure 4.2 in the case of parallel filaments
with $\theta = \pi/2$ and $V = -v_0 e_z$. At a sufficiently small spatial scale, the filaments are
discrete objects and one would need to specify the spatial location of each filament. We
will consider a simplified continuum model under the “homogenization” assumption that
the cytoskeletal network is sufficiently dense and ordered. Thus at each point $(x, z)$, there
is a density $\rho(x, z, \theta)$ of filaments with the given orientation $\theta$ – the probability of binding to any one of these filaments will then be proportional to $\rho(x, z, \theta)$. Ultimately, we will take $\rho$ to depend on the concentration of signaling molecules on the membrane surface, so that $\rho$ becomes time-dependent. For the moment, however, we will treat $\rho$ as time-independent.

Let $p_0(r, t)$ denote the probability density that the particle is at position $(x, z)$ at time $t$ and is in the diffusive state. Similarly, let $p(r, t)$ be the corresponding probability that it is bound to a microtubule and moving with velocity $V(\theta)$. Transitions between the diffusing state and the ballistic state are governed by a discrete Markov process. The transition rate $\beta$ from a ballistic state with velocity $V(\theta)$ to the diffusive state is taken to be constant, whereas the reverse transition rate is taken to depend on the local density of filaments, $\alpha \rho(r, \theta)$. We then have the following Chapman–Kolmogorov (CK) equations describing the evolution of the probability densities for $t > 0$:

$$\frac{\partial p(r, \theta, t)}{\partial t} = -V(\theta) \cdot \nabla p(r, \theta, t) - \frac{\beta}{\epsilon} p(r, \theta, t) + \frac{\alpha \rho(r, \theta)}{\epsilon} p_0(r, t), \quad (4.7a)$$

$$\frac{\partial p_0(r, t)}{\partial t} = \epsilon D_0 \nabla^2 p_0(r, t) + \frac{\beta}{\epsilon} \int_0^{\pi} p(r, \theta, t) d\theta - \frac{\alpha \overline{\rho}(r)}{\epsilon} p_0(r, t), \quad (4.7b)$$

with $r = (x, z)$ and

$$\overline{\rho}(r) = \int_0^{\pi} \rho(r, \theta) d\theta.$$

We have introduced a small dimensionless parameter $\epsilon$, $0 < \epsilon \ll 1$, in order to incorporate the assumption that the switching rates are very fast and diffusion is slow compared to typical motor velocities (on the length scale of a cell). That is, specifying space and time
measurements in units of $L$ and $L/v_0$, we take $aL/v_0 = O(1)$ and $D_0/Lv_0 = O(1)$. Note that in the limit $\epsilon \to 0$, the total probability density at each point $r$ is conserved, that is,

$$\int_0^\pi p(r, \theta, t) d\theta + p_0(r, t) = c(r, t), \quad \int c(r, t) d\mathbf{r} = 1,$$

where $c(\mathbf{r})$ is determined by the initial conditions. It follows that the system rapidly converges to steady-state distributions

$$(p(\mathbf{r}, \theta, t), p_0(\mathbf{r}, t)) \to c(\mathbf{r})(p^*(\mathbf{r}, \theta), p^*_0(\mathbf{r}))$$

with

$$p^*_0(\mathbf{r}) = \frac{\beta}{a\rho(\mathbf{r}) + \beta}, \quad p^*(\mathbf{r}, \theta) = \frac{a\rho(\mathbf{r}, \theta)}{a\rho(\mathbf{r}) + \beta}. \quad (4.8)$$

Since equations (4.7) are defined in the semi-infinite rectangular domain $x \in [-L/2, L/2]$, $z \in R^+$, it is necessary to specify boundary conditions along the edges $x = \pm L/2$ and along the cell membrane at $z = 0$. First, we impose periodic boundary conditions with respect to $x$ so that $p(-L/2, z, \theta, t) = p(L/2, z, \theta, t)$ and $p_0(-L/2, z, t) = p_0(L/2, z, t)$. However, it will be convenient to take the limit $L \to \infty$ when modeling the distribution of microtubules generated by nucleation from the cell membrane, which is reasonable given the exponential decay of the concentrations with respect to $z$ (see below). Second, we impose a no-flux condition along $z = 0$, which requires constructing a probabilistic version of the flux conservation equation (4.3) in order to take into account binding and unbinding of vesicles to the cell membrane. Suppose that if a motor-bound vesicle is in the diffusive state and at the membrane ($z = 0$), then the vesicle can transition to a membrane-bound state and immediately release its contents by fusing with the membrane (exocytosis). Furthermore, we assume that new vesicles form on the membrane and are subsequently released back into the cytoplasm (endocytosis) at a rate that depends on the local density $u(x, t)$ of signaling molecules within the membrane. This is motivated by the observation in yeast that the density of actin patches varies with the membrane density of Cdc42. We then have the following equation for conservation of vesicular flux at the boundary $z = 0$:

$$\epsilon D_0 \frac{\partial p_0(x, z, t)}{\partial z} \bigg|_{z=0} + v_0 \int_0^\pi \sin(\theta) p(x, 0, \theta, t) d\theta = \bar{k}_{on} p_0(x, 0, t) - \bar{k}_{off} u(x, t) \quad (4.9)$$

where $\bar{k}_{on}$ and $\bar{k}_{off}$ are the rates of vesicular binding and unbinding, respectively. (Note that $\bar{k}_{on}$ has units of speed as $p_0$ is density per unit area.) For simplicity, we neglect any
time delays associated with the fusion and budding of membrane-bound vesicles. We also
assume that there is a dynamic equilibrium of vesicular transport so that the total number
of vesicles \( M_{\text{ves}} \) is fixed:

\[
1 = \int_{-L/2}^{L/2} \int_0^\infty \int_0^\pi p(x, z, \theta, t) d\theta + p_0(x, z, t) \right] dz \ dx. \tag{4.10}
\]

Assuming that each vesicle contains \( n_{\text{ves}} \) signaling molecules and that protein degrada-
tion can be ignored, the total number of signaling molecules \( M \) satisfies the conservation
equation

\[
M = \int_{-L/2}^{L/2} u(x, t) dx + n_{\text{ves}} M_{\text{ves}} = \text{constant}. \tag{4.11}
\]

The concentration \( u(x, t) \) of Cdc42 in the membrane (not contained in vesicles) thus evolves
according to

\[
\frac{\partial u(x, t)}{\partial t} = \epsilon D u \frac{\partial^2 u(x, t)}{\partial x^2} + \gamma n_{\text{ves}} M_{\text{ves}} \left[ k_{\text{on}} p_0(x, 0, t) - k_{\text{off}} u(x, t) \right], \tag{4.12}
\]

where \( \gamma \) is a dimensionless conversion factor that determines the change in membrane
concentration following endocytosis/exocytosis of a single vesicle. Equation (4.12) is sup-
plemented by the periodic boundary condition \( u(-L/2, t) = u(L/2, t) \).

A subtle aspect of constructing the above stochastic model is how to couple the stochas-
tic vesicular dynamics to the distribution \( u(x, t) \) of signaling molecules such as Cdc42
within the cell membrane. This raises one of the potential difficulties with the active
transport models considered in [68, 102], namely, these models effectively treat transport
as a continuous flux of proteins. That is, they do not explicitly take into account the
vesicular nature of motor transport. As highlighted by Layton et al. [94, 137], vesicular
transport makes cell polarization more difficult to sustain. A simple argument for this
proceeds as follows. First, it is clear that if the concentration of signaling molecules within
a vesicle is the same as a local region of membrane, then fusion of the vesicle releases both
signaling molecules and additional lipid membrane so the concentration does not change,
in contrast to a continuous flux of signaling molecules alone. Hence, exocytic vesicles
need to have higher concentrations of the signaling molecule than the polarization site in
order to enhance the concentration. A dynamic equilibrium of recycling can be maintained
only if endocytic vesicles also have an enhanced concentration of signaling molecules.
There are various active mechanisms for enhancing the concentration of proteins within
vesicles, and although evidence for such processes within the context of cell polarization is currently lacking, it has recently been observed that vesicles do indeed deliver Cdc42 to sites of polarized growth in yeast [39]. We will ignore these issues here and simply assume that each vesicle contains $n_{\text{ves}}$ signaling molecules and the membrane concentration increases/decreases by an amount $\gamma n_{\text{ves}}$ given a unit flux of vesicles undergoing exo/endocytosis.

Another simplification of our model (and the model of Hawkins et al. [68]) is that the distinction between active/inactive states of Cdc42 is not made in the case of yeast. As highlighted in the introduction, there is also an actin-independent positive feedback mechanism that can establish cell polarity, in which Bem1, an adaptor protein with multiple binding sites, forms a complex with Cdc42 that enables recruitment of more Cdc42 to the plasma membrane. However, it is thought that each mechanism is by itself sufficient to establish cell polarization, suggesting that the presence of two distinct but connected mechanisms leads to greater robustness [140, 154]. In this work, we focus on the actin-dependent mechanism and show that it alone can support spontaneous polarization.

### 4.3 Quasi-steady-state analysis

In order to derive a diffusion approximation of the CK equations (4.7), we will use a quasi-steady-state (QSS) approximation. This was first developed from a probabilistic perspective by Papanicolaou [125]; see also [54]. It has subsequently been applied to a wide range of problems in biology [17], including cell movement [123, 124], wave-like behavior in models of slow axonal transport [51, 52, 133], molecular motor-based models of random intermittent search [116, 117], and stochastic neural networks [19]. The QSS approximation is based on the assumption that for $0 < \epsilon \ll 1$, solutions remain close to the steady-state solution. Hence, we set

\begin{align*}
  p(r, \theta, t) &= c(r, t)p^*(r, \theta) + \epsilon w(r, \theta, t) \\
  p_0(r, t) &= c(r, t)p_0^*(r) + \epsilon w_0(r, t),
\end{align*}

with $r = (x, z)$ and

\begin{align*}
  c(r, t) &= \int_{0}^{\pi} p(r, \theta, t)d\theta + p_0(r, t), \quad \int_{0}^{\pi} w(r, \theta, t)d\theta + w_0(r, t) = 0.
\end{align*}
Furthermore, the initial conditions are taken to be
\[ c(\mathbf{r}, 0) = \delta(\mathbf{r} - \mathbf{X}), \quad w(\mathbf{r}, \theta, 0) = w_0(\mathbf{r}, 0) = 0, \]
which are equivalent to the following initial conditions for the full probability densities:
\[ p(\mathbf{r}, \theta, 0) = \delta(\mathbf{r} - \mathbf{X})p^*(\mathbf{X}, \theta), \quad p_0(\mathbf{r}, 0) = \delta(\mathbf{r} - \mathbf{X})p_0^*(\mathbf{X}). \]
That is, we assume that the system starts on the slow manifold so fast transients can be ignored. One could equally well assume a uniform initial condition for \( c \) with respect to position \( \mathbf{r} \).

Perturbation and projection methods can now be used to derive a closed equation for the scalar component \( c(\mathbf{r}, t) \) [18]. First, integrating equation (4.7a) with respect to \( \theta \) and adding to equation (4.7b) yields (to first order in \( \epsilon \))
\[ \frac{\partial c(\mathbf{r}, t)}{\partial t} = \epsilon D_0 \nabla^2 p_0(\mathbf{r}, t) - \int_0^{\pi} \mathbf{V}(\theta) \cdot \nabla p(\mathbf{r}, \theta, t) d\theta 
- \epsilon \int_0^{\pi} \mathbf{V}(\theta) \cdot \nabla w(\mathbf{r}, \theta, t) d\theta. \tag{4.15} \]
Next, substituting equations (4.13) and (4.13b) into equations (4.7a) and (4.7b) yields
\[ p^*(\mathbf{r}, \theta) \frac{\partial c(\mathbf{r}, t)}{\partial t} + \epsilon \frac{\partial w(\mathbf{r}, \theta, t)}{\partial t} = -\mathbf{V}(\theta) \cdot \nabla [p^*(\mathbf{r}, \theta)c(\mathbf{r}, t) + \epsilon w(\mathbf{r}, \theta, t)] - \beta w(\mathbf{r}, \theta, t) 
+ \alpha \rho(\mathbf{r}) w_0(\mathbf{r}, t). \tag{4.16} \]
and
\[ p_0^*(\mathbf{r}) \frac{\partial c(\mathbf{r}, t)}{\partial t} + \epsilon \frac{\partial w_0(\mathbf{r}, t)}{\partial t} = \epsilon D_0 \nabla^2 (p_0^*(\mathbf{r})c(\mathbf{r}, t) + \epsilon w_0(\mathbf{r}, t)) \beta \int_0^{\pi} w(\mathbf{r}, \theta, t) d\theta 
- \alpha \beta \rho(\mathbf{r}) w_0(\mathbf{r}, t). \tag{4.17} \]
Now substitute (4.15) into (4.16) and (4.17) and introduce the asymptotic expansion
\[ w \sim w^{(0)} + \epsilon w^{(1)} + \ldots \]
Collecting terms to leading order in \( \epsilon \) and using equation (4.14) then gives
\[ w_0^{(0)}(\mathbf{r}, t) \sim \frac{p_0^*(\mathbf{r})}{\beta \rho(\mathbf{r})} \int_0^{\pi} \mathbf{V}(\theta) \cdot \nabla (p^*(\mathbf{r}, \theta)c(\mathbf{r}, t)) d\theta \tag{4.18} \]
Finally, substituting equations (4.18) and (4.19) into (4.15) yields to $O(\varepsilon)$ the Fokker-Planck equation

$$
\frac{\partial c}{\partial t} = - \int_0^{\pi} V(\theta) \cdot \nabla \left[ p^*(r, \theta)c(r, t) \right] d\theta + \varepsilon D_0 \nabla^2 [p_0^*(r)c(r, t)] + \varepsilon \sum_{i,j=x,z} \frac{D_{ij}(r)}{\vec{a}(r)} \frac{\rho(r, \theta)}{\bar{\rho}(r)} \int_0^{\pi} V(\theta) \cdot \nabla \left[ p^*(r, \theta)c(r, t) \right] d\theta',
$$

(4.20)

where

$$
a(r) = \frac{\alpha \bar{\rho}(r)}{\alpha \bar{\rho}(r) + \beta}, \quad b(r) = \frac{\beta}{\alpha \bar{\rho}(r) + \beta}.
$$

(4.21)

Dropping $O(\varepsilon)$ corrections to the effective drift velocity, this simplifies to

$$
\frac{\partial c}{\partial t} = - \int_0^{\pi} V(\theta) \cdot \nabla \left[ p^*(r, \theta)c(r, t) \right] d\theta + \varepsilon \sum_{ij=x,z} D_{ij}(r) \frac{\partial^2 c(r, t)}{\partial r_i \partial r_j},
$$

(4.22)

where

$$
D_{ij}(r) = b(r)D_0 \delta_{ij} + Q_{ij}(r)
$$

(4.23)

with

$$
Q_{ij}(r) = \frac{1}{\beta} \int_0^{\pi} \int_0^{\pi} \frac{\rho(r, \theta)}{\bar{\rho}(r)} a(r) V_i(\theta) V_j(\theta') \left[ \delta(\theta - \theta') - [1 + b(r)] a(r) \frac{\rho(r, \theta)}{\bar{\rho}(r)} \right] d\theta d\theta' \geq 0.
$$

(4.24)

We now make the simplifying assumption that the density of filaments is sufficiently small so that $\alpha \bar{\rho}(r) \ll \beta$ for all $r$, which implies that

$$
a(r) \approx \frac{\alpha \bar{\rho}(r)}{\beta} \ll 1, \quad b(r) = 1 - a(r).
$$

(4.25)

This approximation allows us to greatly simplify the analysis without losing the basic structure of the equations and to link up with the analysis of Hawkins et al. [68]. (A biophysical justification of such an approximation would require a more detailed analysis of the interaction between a diffusing motor/cargo complex and a cytoskeletal network.)
It is motivated by the idea that the unbinding rate from a single filament is independent of other filaments, whereas binding to a filament involves competition with other neighboring filaments. From a mathematical prospective, one would need to take into account the discrete nature of the cytoskeletal network using homogenization theory, for example.)

Carrying out a perturbation expansion of equation (4.22) with respect to $a(r)$ and keeping only leading order terms gives

$$\frac{\partial c}{\partial t} = -\nabla \cdot [v(r)c(r,t)] + \epsilon \sum_{i,j=x,z} D_{ij}(r) \frac{\partial^2 c(r,t)}{\partial r_i \partial r_j}, \quad (4.26)$$

where

$$v(r) = \frac{\alpha}{\beta} \int_0^\pi V(\theta) \rho(r, \theta) d\theta, \quad (4.27)$$

and $D_{ij} = D_0 [1 - a(r)] \delta_{ij} + Q_{ij}$ with

$$Q_{ij}(r) = \frac{\alpha}{\beta} \int_0^\pi \rho(r, \theta) V_i(\theta) V_j(\theta) d\theta. \quad (4.28)$$

Note that, for simplicity, we ignore $O(\epsilon)$ corrections to the drift term and take the nonuniform diffusion matrix to be outside the second-order derivatives.

Finally, it is necessary to determine the boundary condition for $c(r,t)$ by applying the QSS approximation to the flux conservation equation (4.9). In order to relate our QSS approximation to the Hawkins et al. model (4.1), we rescale $c(x,t)$ according to $c(r,t) \to n_{ves} M_{ves} c(r,t)$ so that it can be reinterpreted as the concentration of Cdc42 in the cytosol.

Using equations (4.13) and keeping only lowest order terms in $\epsilon$ then gives

$$\epsilon \sum_j D_{2j}(x,z) \frac{\partial c(x,z,t)}{\partial r_j} \bigg|_{z=0} - v_z(x)c(x,0,t) = k_{on}(x)c(x,0,t) - k_{off} u(x,t), \quad (4.29)$$

with

$$v_z(x) = -\frac{\alpha v_0}{\beta} \int_0^\pi \rho(x,0,\theta) \sin \theta d\theta, \quad (4.30)$$

and

$$k_{on}(x) = \gamma \tilde{k}_{on} [1 - a(x,0)], \quad k_{off} = \gamma M_{ves} n_{ves} \tilde{k}_{off}. \quad (4.31)$$

Moreover, equation (4.12) becomes

$$\frac{\partial u(x,t)}{\partial t} = \epsilon D_m \frac{\partial^2 u(x,t)}{\partial x^2} + [k_{on}(x)c(x,0,t) - k_{off} u(x,t)]. \quad (4.32)$$

and the conservation equations (4.10) and (4.11) reduce to

$$M = \int_{-L/2}^{L/2} \int_0^\infty c(x,z,t) dz + \int_{-L/2}^{L/2} u(x,t) dx. \quad (4.33)$$
In the above QSS analysis, we have determined the diffusion coefficients to $O(\epsilon)$ and all other coefficients to $O(1)$. This approximation still holds if we take the density of filaments $\rho$ to depend on the membrane-bound concentration $u(x, t)$ of Cdc42, even though $\rho$ becomes time-dependent, since the evolution of $u$ is much slower than the transition rates between mobile states. Hence, starting from a biophysically detailed stochastic model of motor transport, we have derived an effective reaction diffusion equation given by equations (4.26) and (4.32) together with the conservation equations (4.29) and (4.33). Our model is similar in structure to the more phenomenological model of Hawkins et al. [68]; see equations (4.1), (4.2), and (4.3), with some significant differences:

(i) The velocity field $v(r)$ in the drift term of equation (4.26) has a direct biophysical interpretation in the terms of the distribution of polymer filaments and the rates of binding/unbinding of molecular motors. As we show below, this requires modifying the aster velocity field introduced by Hawkins et al. [68].

(ii) The effective diffusion of cytosolic Cdc42, which reflects the stochastic nature of motor transport, is anisotropic. If we set $D_{ij} = D_0 \delta_{i,j}$ then equation (4.26) is identical in form to equation (4.1) of the Hawkins et al. model.

In the following section, we will use our modified reaction-diffusion model to determine conditions for spontaneous pattern formation in the two geometric configurations shown in Figure 4.2, extending the previous analysis of Hawkins et al. [68]. Recall that in order to derive the deterministic reaction-diffusion model from our stochastic model, we introduced a small parameter $\epsilon$, based on the assumption that transitions between diffusive and motor-driven state are fast relative to diffusion in the cytosol, which is itself faster than membrane-bound diffusion. In our analysis of pattern formation, we reabsorb the factors of $\epsilon$ and deal with physical parameters. Finally, following standard treatments of cell polarization, we assume that membrane diffusion is slower than cytoplasmic diffusion by taking $D_m \ll D_0$. However, this inequality could be mitigated by the fact that we are modeling the diffusion of vesicles in the cytoplasm, which tends to be slower than that of single molecules due to molecular crowding.
4.4 Linear stability analysis and dispersion relation

In the following, we perform a linear stability analysis for the reaction-diffusion model and derive the dispersion relation.

4.4.1 Case (i): Parallel filaments

In the particular example of parallel fibers that are orthogonal to the cell membrane and have a density proportional to $u(x, t)$ (case (i) in Figure 4.1), we have

$$\rho(r, \theta) \rightarrow \rho(r, \theta, t) = \kappa u(x, t) \delta(\theta - \pi / 2),$$

which implies that $\bar{\rho}(r, t) = \kappa u(x, t)$. Substituting into equation (4.27) shows that this choice of fiber density recovers the velocity distribution (4.4) of Hawkins et al.:

$$v(r) = -\frac{\alpha \kappa}{\beta} v_0 u(x, t) \int_0^{\pi} \cos(\theta) e_x + \sin(\theta) e_z] \delta(\theta - \pi / 2) d\theta = -\frac{\alpha \kappa}{\beta} v_0 u(x, t) e_z,$$

which is identical to equation (4.4) on setting $\kappa_0 = \eta v_0, \quad \eta = \frac{\alpha \kappa}{\beta}$. (4.35)

For the given density, equations (4.26) and (4.32) then reduce to

$$\frac{\partial c}{\partial t} = \eta v_0 u(x, t) \frac{\partial c(r, t)}{\partial z} + D(x, t) \nabla^2 c(r, t) + Q_{zz}(x, t) \frac{\partial^2 c(r, t)}{\partial z^2},$$

and

$$\frac{\partial u(x, t)}{\partial t} = D_m \frac{\partial^2 u(x, t)}{\partial x^2} + [k_{on}(x) c(x, 0, t) - k_{off} u(x, t)].$$

where

$$D(x, t) = D_0 [1 - \eta u(x, t)], \quad Q_{zz}(x, t) = \frac{\eta v_0^2}{\beta} u(x, t), \quad k_{on}(x) = \gamma \tilde{k}_{on} [1 - \eta u(x, t)].$$

Equations (4.36) and (4.37) have an $x$ independent steady-state solution $c(x, z, t) = c(z), u(x, t) = u^*$ satisfying the pair of equations

$$0 = k_{on}^* c(0) - k_{off} u^*, \quad 0 = D_{zz}^* \frac{d^2 c(z)}{dz^2} + \eta v_0 u^* \frac{d c(z)}{dz},$$

with

$$D_{zz}^* = D_0 [1 - \eta u^*] + Q_{zz}^*, \quad Q_{zz}^* = \frac{\eta v_0^2}{\beta} u^*, \quad k_{on}^* = \gamma \tilde{k}_{on} [1 - \eta u^*].$$

After imposing a zero flux boundary condition at the membrane surface, we see that

$$u^* = \frac{k_{on}^*}{k_{off}} c(0), \quad c(z) = c(0) e^{-\xi z}, \quad \xi = v_0 \eta u^*/D_{zz}^*.$$  

Note that the condition $a(r) \ll 1$ implies that $\xi D_{zz}^* \ll v_0$. (4.41)
The stability of the steady state is determined by substituting

\[ u(x,t) = u^* + U(k) e^{i k x + \lambda t}, \quad c(x,z,t) = c(z) + C(k,z) e^{i k x + \lambda t} \]

into equations (4.36) and (4.37), and Taylor expanding to first-order in \( U(k) \) and \( C(k,z) \). The resulting linear equations are

\[
\begin{align*}
(\lambda + D_m k^2 + k_{\text{off}} [1 + \eta u^*]) U(k) &= k_{\text{on}} C(k,0), \\
(\lambda + D_0^* k^2) C(k,z) &= D_{zz}^* \frac{d^2 C(k,z)}{dz^2} + \eta v_0 u^* \frac{dC(k,z)}{dz} \\
&+ \left[ \frac{\eta v_0^2}{\beta} - \eta D_0 \right] \frac{d^2 c(z)}{dz^2} U(k) + \eta v_0 \frac{dC(z)}{dz} - U(k),
\end{align*}
\]

with \( D_0^* = D_0 (1 - \eta u^*) \). On eliminating \( U(k) \) and using equation (4.41), equation (4.42b) becomes

\[
(\lambda + D_0^* k^2) C(k,z) = D_{zz}^* \frac{d^2 C(k,z)}{dz^2} + \eta v_0 u^* \frac{dC(k,z)}{dz} \\
+ e^{-\xi z} \frac{F(\xi)}{\lambda + D_m k^2 + [1 + \eta u^*] k_{\text{off}}} C(k,0),
\]

with

\[ F(\xi) = k_{\text{off}} (\eta u^* \xi) \left( \left[ \frac{v_0^2}{\beta} - D_0 \right] \xi - v_0 \right) = k_{\text{off}} D_{zz}^* \xi^2 \left( \left[ \frac{v_0^2}{\beta} - D_0 \right] \xi - v_0 \right). \]

This has a solution of the form

\[ C(k,z) = A e^{-\xi z} + [C(k,0) - A] e^{-\rho z}, \]

where \( \rho \) is the positive real root of the equation\(^1\)

\[
\rho^2 - \xi \rho - \frac{\lambda}{D_{zz}^*} - \frac{D_0^*}{D_{zz}^*} k^2 = 0,
\]

which gives

\[ \rho = \frac{1}{2} \left[ \xi + \sqrt{\xi^2 + 4(D_0^* k^2 + \lambda) / D_{zz}^*} \right]. \]

and

\[ A = \frac{1}{(\lambda + D_0^* k^2) \lambda + D_m k^2 + k_{\text{off}} [1 + \eta u^*]} C(k,0). \]

\(^1\)For sufficiently negative \( \lambda \), it is possible for \( \rho \) to become complex-valued. However, this case does not play a role in the construction of the dispersion relation. It is also physically irrelevant, since it does not represent an instability of the steady state.
In order to derive the dispersion relation $\lambda = \lambda(k)$, we impose the zero-flux condition 
(4.29), which has the specific form
\[
- (D(x, t) + Q_{zz}(x, t)) \frac{\partial c(x, 0, t)}{\partial z} - \eta v_0 u(x, t)c(x, 0, t) 
+ k_{on}(x)c(x, 0, t) - k_{off}u(x, t) = 0. \tag{4.44}
\]
Substituting the linearized solution gives
\[
D_{zz}^* [(\xi - \rho)A + \rho C(k, 0)] + \eta \bar{\xi} \left( \frac{v_{0}^{2}}{b} - D_0 \right) c(0)U(k) - \eta v_0 u^* C(k, 0) - \eta v_0 c(0)U(k) 
+ k_{on}^* C(k, 0) - k_{off}^*(1 + \eta u^*)U(k) = 0. \tag{4.45}
\]
Combining equations (4.43) and (4.45) and using equations (4.41) yields the characteristic equation
\[
0 = \left( \frac{F(\bar{\xi})}{k_{off} \bar{\xi}} - k_{on}^* (1 + \eta u^*) \right) \frac{k_{off}}{\lambda + D_m k^2 + k_{off}^*[1 + \eta u^*]} 
+ k_{on}^* - D_{zz}^* (\xi - \rho) + \frac{D_{zz}^* (\xi - \rho)}{(\lambda + D_0^* k^2)} \cdot \frac{F(\bar{\xi})}{\lambda + D_m k^2 + k_{off}^*[1 + \eta u^*]}, \tag{4.46}
\]
which can be rearranged as
\[
0 = \left( \frac{F(\bar{\xi})}{k_{off} \bar{\xi}} - (1 + \eta u^*)D_{zz}^* (\xi - \rho) \right) \frac{k_{off}}{D_{zz}^*} 
+ \left( \frac{k_{on}^*}{D_{zz}^*} - (\xi - \rho) \right) \left[ \frac{F(\bar{\xi})}{\lambda + D_m k^2} \right] + \frac{(\xi - \rho)}{\lambda + D_0^* k^2} F(\bar{\xi}). \tag{4.47}
\]
Note that the characteristic equation of Hawkins et al. [68] is recovered by taking $v_{0} \bar{\xi}/\beta \to 0$ and $\eta u^* \to 0$ (all other terms fixed). Then $D_0^* = D_{zz}^* = D_0$, $k^* = \bar{k}_{on}$ and $F(\bar{\xi}) = -D_0 \bar{\xi} z^2 k_{off}$ in equation (4.47):
\[
0 = (\lambda + D_0 k^2) \left[ k_{off}(\rho - 2\bar{\xi}) + \left( \frac{\bar{k}_{on}}{D_0} - (\xi - \rho) \right) \left[ \frac{1}{\lambda + D_m k^2} \right] \right] 
- \bar{\xi}^2 D_0 k_{off}(\xi - \rho). \tag{4.48}
\]
Following Hawkins et al. [68], we express $k$ and $\bar{\xi}$ in units of $R^{-1}$ and choose the following parameter values
\[
D_0 = 0.1 \mu m^2 s^{-1}, D_m = 0.01 \mu m^2 s^{-1}, R = 10 \mu m, k_{off} = 0.1 s^{-1}, \bar{k}_{on} = 1 \mu ms^{-1},
\]
with $\gamma = 1$. There is then one free parameter in equation (4.48), namely, the spatial decay rate $\bar{\xi}$. On the other hand, the full dispersion relation (4.47) depends on the additional
parameters $\eta u^*, v_0$, and $\beta$. Equation (4.41) shows that $u^*$ is proportional to $c(0)$, and the latter is determined by the conservation equation (4.33). Moreover, $\eta = \kappa \alpha / \beta$ is proportional to the strength of coupling $\kappa$ between the membrane-bound signaling molecules and the distribution of filaments. Equation (4.41) also implies that $\xi$ is related to the other free parameters according to

$$\xi = \frac{v_0 \eta u^*}{D_0 [1 - \eta u^*] + \eta u^* (v_0^2 / \beta)}.$$ 

Recall that we are working in a parameter regime for which $\eta u^*$ is small - this then puts constraints on the allowed range of $\xi$, i.e. $\xi$ cannot be too large. However, this is not a major restriction given the parameter regime we are considering. (One could also relax the constraint on $\eta u^*$, although this introduces several additional terms into the dispersion relation and greatly complicates the analysis.) In Figure 4.3, we show typical dispersion curves $\lambda = \lambda(k)$ in the case of parallel filaments for various values of $\xi$ and $v_0 = 1 \mu m s^{-1}, \beta = 1 s^{-1}$. Consistent with the findings of Hawkins et al. [68], the parallel configuration does not support spontaneous cell polarization.

### 4.4.2 Case (ii): Nucleation at the cell membrane

Now suppose that the probability density of a filament of orientation $\theta$ at $(x, z)$ depends on the concentration of nucleated asters at location $(x', 0)$ on the membrane, such that
The trigonometric construction is illustrated in Figure 4.4. Assume that the concentration of asters at \((x', 0)\) is proportional to the density of Cdc42 at that location. The density of filaments from a single aster at \((x', 0)\) will vary as the inverse of the Euclidean distance \(r = \sqrt{(x - x')^2 + z^2}\) from the aster. On the other hand, the density of asters contributing to fibers passing through \((x, z)\) and subtending an angle \(\theta\) will vary as \(\sin \theta dx'\) so that

\[
\rho(x, z, \theta, t) = \frac{\kappa}{\pi} \frac{u(x - x', t)}{\sqrt{(x - x')^2 + z^2}} \sin \theta dx'.
\]

(4.49)

Since

\[
\sin \theta = \frac{z}{\sqrt{(x - x')^2 + z^2}},
\]

and \(dx' = (z/ \sin^2 \theta) d\theta\), we see that

\[
\rho(x, z, \theta, t) = \frac{\kappa}{\pi} u(x - x', t) = \frac{\kappa}{\pi} u(x - z \cotan \theta, t).
\]

(4.50)

Note that the range of \(\theta\) will depend on \(L\). That is, \(\theta \in [\theta_-(r), \theta_+(r)]\) such that

\[
\theta_+(r) = \pi - \tan^{-1} \frac{z}{L/2 - x}, \quad \theta_-(r) = \tan^{-1} \frac{z}{x + L/2}.
\]

Substituting into equation (4.27) shows that the fiber density (4.50) generates a similar mean velocity distribution (4.5) to Hawkins et al. [68]. That is

\[
v(r, t) = -\frac{\alpha \kappa}{\beta \pi} \frac{u_0}{\theta_+(r)} \int_{\theta_-(r)}^{\theta_+(r)} \left[ \cos \theta \mathbf{e}_x + \sin \theta \mathbf{e}_z \right] u(x - z \cotan \theta, t) d\theta
\]

\[
= -\frac{\alpha \kappa}{\beta \pi} \frac{u_0}{\theta_+(r)} \int_{-L/2}^{L/2} \left[ \frac{x - x'}{(x - x')^2 + z^2} \mathbf{e}_x + \frac{z}{(x - x')^2 + z^2} \mathbf{e}_z \right] \frac{zu(x', t)}{\sqrt{(x - x')^2 + z^2}} dx'
\]

\[
= -\frac{\alpha \kappa}{\beta \pi} u_0 \int_{-L/2}^{L/2} \left[ \frac{r - r'}{|r - r'|^2} \left[ \frac{z}{|r - r'|^2} \right] u(x', t) \right] dx',
\]

(4.51)
where \( \mathbf{r} = (x, z) \) and \( \mathbf{r}' = (x', 0) \). The main difference from the aster-based velocity distribution considered by Hawkins et al. [68] is the additional factor in square brackets. This is necessary so that the total density \( \bar{\rho}(\mathbf{r}) \) averaged over all orientations is finite. For simplicity, we will take \( L \to \infty \) and \( \theta \in [0, \pi] \) in the following. It follows from equations (4.50) and (4.28) that

\[
\bar{\rho}(\mathbf{r}, t) = \frac{\kappa}{\pi} \int_0^\pi u(x - z \cos \theta / \sin \theta, t) d\theta \tag{4.52}
\]

and

\[
Q_{ij}(\mathbf{r}, t) = \frac{\kappa \alpha \beta}{\pi^2} \int_0^\pi u(x - z \cotan(\theta), t) V_i(\theta) V_j(\theta) \frac{d\theta}{\pi} \tag{4.53}
\]

where \( (i, j) \in \{ (x, x), (x, z), (z, z) \} \).

Let us first calculate the steady-state solution. Equations (4.32) and (4.52) have the \( x \) and \( t \)-independent solutions

\[
\bar{\rho} = \frac{\kappa u^*}{\pi} \int_0^\pi d\theta = \kappa u^*, \quad u^* = \frac{k^*_on}{k^*_off} c(0),
\]

Substituting these constant solutions into the expressions for \( Q_{ij} \) gives

\[
Q^*_{xx} = \frac{v_0^2 \eta u^*}{\beta} \left[ \int_0^\pi \cos^2 \theta \frac{d\theta}{\pi} \right] = \frac{v_0^2 \eta u^*}{2\beta},
\]

\[
Q^*_{zz} = \frac{v_0^2 \eta u^*}{\beta} \left[ \int_0^\pi \sin^2 \theta \frac{d\theta}{\pi} \right] = \frac{v_0^2 \eta u^*}{2\beta},
\]

\[
Q^*_{xz} = 0.
\]

Equation (4.26) thus becomes

\[
0 = \frac{2v_0 \eta u^*}{\pi} \frac{dc}{dz} + D^*_zz \frac{d^2c(z)}{dz^2},
\]

with \( D^*_zz = D_0[1 - \eta u^*] + Q^*_{zz} \). It follows that

\[
c(z) = c(0)e^{-\xi z}, \quad \xi = 2v_0 \eta u^* / \pi D^*_zz. \tag{4.54}
\]

Following along similar lines to case (i), the stability of the steady state is determined by substituting

\[
u(x, t) = u^* + U(k)e^{ikx + \lambda t}, \quad c(x, z, t) = c(z) + C(k, z)e^{ikx + \lambda t} \tag{4.55}
\]

into equations (4.26) and (4.32), and Taylor expanding to first-order in \( U(k) \) and \( C(k, z) \).

The linear equation corresponding to equation (4.32) is identical in form to case (i):

\[
(\lambda + D_mk^2 + k_{off}[1 + \eta u^*])U(k) = k^*_on C(k, 0). \tag{4.56}
\]
The linearized version of equation (4.26) takes the form

\[ \lambda C(k, z) e^{ikx} = -\frac{\eta}{\pi} \int_0^\pi \mathbf{V}(\theta) \cdot \nabla \left[ u^* C(k, z) e^{ikx} + c(z) e^{-ik\cot(\theta)} U(k) e^{ikx} \right] d\theta \]

\[ + \left[ D_{zz}^* \frac{d^2C(k, z)}{dz^2} + 2ikD_{xz}^* \frac{dC(k, z)}{dz} - k^2 D_{xx}^* C(k, z) \right] e^{ikx} \]

\[ + \Delta D_{zz}(k, z) \frac{U(k)}{u^*} \frac{d^2c(z)}{dz^2} e^{ikx}, \]  \hspace{1cm} (4.57)

where we have canceled common factors of \( e^{\lambda t} \),

\[ D_{ij}^* = D_0 [1 - \eta u^*] \delta_{ij} + Q_{ij}^*, \]  \hspace{1cm} (4.58)

and

\[ \Delta D_{zz}(k, z) = -\frac{\kappa a u^*}{\beta} D_0 \int_0^\pi e^{-ik\cot(\theta)} \frac{d\theta}{\pi} + \frac{\kappa a u^* v_0^2}{\beta^2} \int_0^\pi e^{-ik\cot(\theta)} \sin^2(\theta) \frac{d\theta}{\pi}. \]  \hspace{1cm} (4.59)

Equation (4.57) reduces to the form

\[ \lambda C(k, z) = D_{zz}^* \frac{d^2C(k, z)}{dz^2} + 2v_0 \eta u^* \frac{dC(k, z)}{dz} - k^2 D_{xx}^* C(k, z) \]

\[ + \eta U(k) \left( v_0 \frac{dc}{dz} g_s(kz) + \left[ \frac{v_0^2}{\beta} f_s(kz) - D_0 f(kz) \right] \frac{d^2c(z)}{dz^2} \right) \]  \hspace{1cm} (4.60)

where

\[ f(q) \equiv \int_0^\pi e^{-iq\cot(\theta)} \frac{d\theta}{\pi} = e^{-|q|}, \]  \hspace{1cm} (4.61a)

\[ g_s(q) \equiv \int_0^\pi e^{-iq\cot(\theta)} \sin \theta \frac{d\theta}{\pi} = \frac{2}{\pi} |q| K_1(|q|), \]  \hspace{1cm} (4.61b)

\[ f_s(q) \equiv \int_0^\pi e^{-iq\cot(\theta)} \sin^2 \theta \frac{d\theta}{\pi} = \frac{1 + |q|}{2} f(q), \]  \hspace{1cm} (4.61c)

with \( K_1 \) a modified Bessel function of order \( n = 1 \). In fact, the integrals \( f(q), f_s(q), \) and \( g_s(q) \) can be evaluated as follows:

\[ f(q) = \int_0^\pi e^{-iq\cot(\theta)} \frac{d\theta}{\pi} = \frac{1}{\pi} \int_{-\infty}^{\infty} e^{-iqr} \frac{dr}{1 + r^2} = e^{-|q|}, \]  \hspace{1cm} (4.62)

and

\[ f_s(q) = \int_0^\pi e^{-iq\cot(\theta)} \sin^2 \theta \frac{d\theta}{\pi} = \frac{1}{\pi} \int_{-\infty}^{\infty} e^{-iqr} \frac{dr}{1 + r^2} \frac{1}{1 + r^2} \]

\[ = -\frac{1}{2a} \frac{d}{da} \frac{1}{\pi} \int_{-\infty}^{\infty} e^{-iqr} \frac{dr}{a^2 + r^2} \bigg|_{a=1} = -\frac{1}{2a} \frac{d}{da} \frac{e^{-a|q|}}{a} \bigg|_{a=1} = \left( \frac{1}{2a} \frac{e^{-a|q|}}{a} + \frac{|q| e^{-a|q|}}{2a} \right)_{a=1} = \frac{1 + |q|}{2} f(q). \]  \hspace{1cm} (4.63)
Similarly,
\[
\int_{0}^{\pi} e^{-i q \cot(\theta)} \sin \theta \frac{d\theta}{\pi} = \frac{1}{\pi} \int_{-\infty}^{\infty} e^{-i q r} \frac{1}{\sqrt{1 + r^2}} \frac{dr}{1 + r^2} = \frac{1}{\pi} \int_{-\infty}^{\infty} \cos(qr) \frac{dr}{\sqrt{1 + r^2}} 1 + r^2
\]
\[
= -\frac{1}{\pi} \int_{0}^{\infty} \frac{\cos(qr)}{\sqrt{a^2 + r^2}} dr \bigg|_{a=1}.
\]

Noting that the modified Bessel function of the second kind of order \( n = 0 \) has the integral representation
\[
K_0(q) = \int_{0}^{\infty} \cos(qr) / \sqrt{1 + r^2} dr,
\]
we have
\[
g_s(q) = -\frac{1}{\pi} \int_{0}^{\infty} \frac{\cos(qr)}{\sqrt{a^2 + r^2}} dr \bigg|_{a=1} = -\frac{2}{\pi} |q| K_0(|q|) = -\frac{2}{\pi} |q| K_1(|q|).
\]

Equation (4.64) can be rewritten as the linear, inhomogeneous equation
\[
\hat{L}_z C(k, z) = -h(k, z) C(k, 0),
\]
with \( C(k, \infty) = 0 \),
\[
\hat{L}_z C = D_{zz}^* \frac{d^2 C}{dz^2} + \frac{2v_0 \eta u^*}{\pi} \frac{dC}{dz} - [\lambda + k^2 D_{xx}^*] C,
\]
and
\[
h(k, z) \equiv \Theta(k) e^{-\xi z} [C_1 g_s(kz) + C_2 f_s(kz) + C_3 f(kz)]
\]
Note that we have substituted for \( U(k) \) using equation (4.56), written in the form
\[
\frac{U(k)}{C(k, 0)} = \Theta(k), \quad \Theta(k) \equiv \frac{k_{\text{off}}}{\lambda + D_{mj} k^2 + k_{\text{off}} [1 + \eta u^*]}',
\]
and set
\[
C_1 = -\eta u^* \xi \Xi, \quad C_2 = \frac{\eta u^* \xi^2 v_0^2}{\beta}, \quad C_3 = -\eta u^* \xi^2 D_0.
\]
Equation (4.65) can be solved using Green’s functions. That is, let \( G(z, z') \) satisfy the equation
\[
\hat{L}_z G(z, z') = \delta(z - z'), \quad G(0, z') = 0, \quad G(\infty, z') = 0.
\]
A standard calculation yields

\[
G(z, z') = \begin{cases} 
\frac{e^{-\rho_1 z + \rho_2 z'} - e^{-\rho_2 (z - z')}}{D^*_{zz} (\rho_1 - \rho_2)}, & \text{if } z < z', \\
\frac{e^{-\rho_1 z + \rho_2 z'} - e^{-\rho_1 (z - z')}}{D^*_{zz} (\rho_1 - \rho_2)}, & \text{if } z > z'.
\end{cases}
\] (4.71)

where

\[
\rho_1 = \frac{v_0 \eta u^* / \pi + \sqrt{(v_0 \eta u^* / \pi)^2 + D^*_{zz} [\lambda + D^*_{xx} k^2]}}{D^*_{zz}} > 0,
\]

\[
\rho_2 = \frac{v_0 \eta u^* / \pi - \sqrt{(v_0 \eta u^* / \pi)^2 + D^*_{zz} [\lambda + D^*_{xx} k^2]}}{D^*_{zz}} < 0.
\]

Taking into account the boundary conditions, we find that the solution to equation (4.65) is

\[
\frac{C(k, z)}{C(k, 0)} = -\int_0^\infty G(z, z') h(k, z') dz' + e^{-\rho_1 z}.
\] (4.72)

The final step is to derive the dispersion equation \( \lambda = \lambda(k) \) by considering the linearized version of the flux conservation equation (4.29):

\[
D^*_{zz} \frac{\partial C(k, z)}{\partial z} \bigg|_{z=0} - \xi \Delta D_{zz}(k, 0)c(0) \frac{U(k)}{u^*} + \frac{2\eta v_0}{\pi} [u^* C(k, 0) + c(0) U(k)]
\]

\[
= k^* C(k, 0) - k_{off} (1 + \eta u^*) U(k),
\]

where \( \Delta D_{zz} \) is given by equation (4.59) for \( z = 0 \),

\[
\Delta D_{zz}(k, 0) = -\eta u^* D_0 + \frac{\eta u^* v_0^2}{2\beta},
\]

which is independent of \( k \). Substituting for \( C(k, z) \) using equation (4.72) and noting that

\[
D^*_{zz} \frac{\partial G(z, z')}{\partial z} \bigg|_{z=0} = -e^{\rho_2 z'},
\]

we have

\[
\frac{D^*_{zz}}{C(k, 0)} \frac{\partial C(k, z)}{\partial z} \bigg|_{z=0}
\]

\[
= -D^*_{zz} \rho_1 + \Theta(k) \int_0^\infty e^{-[\xi - \rho_2] z'} [C_1 g_s(kz') + C_2 f_s(kz') + C_3 f(kz')] dz'.
\] (4.74)

We can evaluate the integrals involving the functions \( f(kz) \) and \( f_s(kz) \) explicitly:

\[
\int_0^\infty e^{-\rho_1 z} f_s(kz) dz = \frac{1}{2} \int_0^\infty e^{-(\rho_1 + k) z} (1 + kz) dz
\]

\[
= \frac{1}{2(\rho_1 + k)} + \frac{k}{2(\rho_1 + k)^2} = \frac{2k + \rho_1}{2(k + \rho_1)^2},
\]

\[
\int_0^\infty e^{-\rho_1 z} f(kz) dz = \int_0^\infty e^{-(\rho_1 + k) z} dz = \frac{1}{k + \rho_1}
\]
where we have used the identity \(\rho_1 + \rho_2 = 2v_0\eta u^N/(\pi D^+_zz) = \xi\). In the case of \(g_s(kz)\), \(k \neq 0\), we have

\[
G_s(k, \rho_1) = \int_0^\infty e^{-\rho_1 z}g_s(kz)dz = \frac{1}{k} \int_0^\infty e^{-\rho_1 z'/k}g_s(z')dz' = \frac{2}{k\pi} \int_0^\infty e^{-\rho_1 z'/k}K_1(z')dz' = \frac{2}{k\pi} \left[ \frac{\rho_1}{k} - \frac{\rho_1}{(\rho_1/k)^2 - 1 - \arccosh(\rho_1/k)} \right].
\]

On the other hand, if \(k = 0\), then

\[
G_s(0, \rho_1) = \int_0^\infty e^{-\rho_1 z}g_s(0)dz = \frac{2}{\pi} \int_0^\infty e^{-\rho_1 z}dz = \frac{2}{\pi \rho_1}.
\]

Finally, combining equations (4.73) and (4.74) and (4.68), we can eliminate a common factor of \(C(k, 0)\) to obtain the equation

\[
(D^+_zz[\xi - \rho_1 - k^*_0] + \Theta(k) \int_0^\infty e^{-\rho_1 z'} [C_1 g_s(kz') + C_2 f_s(kz') + C_3 f(kz')] dz' = [\xi \Delta D_{zz}(k, 0) - D^+_zz \xi - (1 + \eta u^N)k^*_0] \Theta(k).
\]

Substituting for \(\Theta(k)\), this reduces to (see also (4.47))

\[
0 = [\xi \Delta D_{zz}(k, 0) - D^+_zz \xi - D^+_zz(\xi - \rho_1)(1 + \eta u^N)] \frac{k_{off}}{D^+_zz} + \left[ k^*_0 \frac{D^+_zz}{D^+_zz} - (\xi - \rho_1) \right] [\lambda + D_m k^2] - \frac{k_{off}}{D^+_zz} \int_0^\infty e^{-\rho_1 z'} [C_1 g_s(kz') + C_2 f_s(kz') + C_3 f(kz')] dz'.
\]

Example dispersion curves \(\lambda = \lambda(k)\) for the nucleation configuration (case (iii)) are plotted in Figure 4.5 (a). Consistent with the results of Hawkins et al. [68], we find that there is a finite range of wave numbers \((0, k_c)\) for which \(\lambda(k) > 0\), with a maximum at \(k = k_{max}\). This maximum corresponds to a dominant (fastest growing) instability of finite characteristic length \(2\pi/k_{max}\). We also see that \(k_{max}\) increases with \(\xi\), as illustrated in Figure 4.5 (b). For ease of presentation, we treat \(k\) as a continuous variable, which holds when \(L \rightarrow \infty\). For finite \(L\) and periodic boundary conditions with respect to \(x\), the dispersion curves are discretely sampled at the points \(k = 2\pi m/L\) for integer \(m\). Given a particular cell circumference \(L = 2\pi R\), no cell polarization patches will be observed if \(2\pi/k_{max} > L\), that is, \(k_{max}R < 1\). However, as \(\xi\) increases, it crosses a sequence of thresholds given by \(k_{max}R = n_c\) for positive integers \(n_c\), beyond which \(n_c\) patches...
Figure 4.5. Dispersion curves and $k_{\text{max}}$. (a) Dispersion curves for nucleation model for various $\xi$. Same parameter values as Figure 4.3. (b) Variation of $k_{\text{max}}$ with $\xi$. Same parameter values as (a) except $v_0 = 0.1\mu m s^{-1}, \bar{k}_{\text{on}} = 0.5\mu m s^{-1}$. Both $\xi$ and $k$ are in units of $R^{-1}$.

Figure 4.6. Dispersion curves for different values of (a) speed $v_0$ with $\beta = 1 s^{-1}$ and (b) unbinding rate $\beta$ with $v_0 = 1\mu m s^{-1}$. Other parameters: $D_0 = 0.1\mu m^2 s^{-1}, D_m = 0.01\mu m^2 s^{-1}, R = 10\mu m, k_{\text{off}} = 0.1 s^{-1}, \bar{k}_{\text{on}} = 1\mu m s^{-1}, \xi = 2$. Both $\xi$ and $k$ are in units of $R^{-1}$.

form. The occurrence of the most common polarization pattern ($n_c = 1$) is illustrated in Figure 4.5 (b).

One major feature of our model is that there are additional biophysical parameters such as the motor speed $v_0$ and unbinding rate $\beta$, both of which affect the dispersion curves and the value of $k_{\text{max}}$; see Figure 4.6. In Figure 4.7, we show plots of the wavenumber $k_{\text{max}}$ and inverse growth rate $\tau = \lambda(k_{\text{max}})^{-1}$ of the fastest growing eigenmode as functions of $v_0$ and $\beta$. It can be seen, for example, that $k_{\text{max}}$ is a decreasing function of $v_0$ and an increasing
function of $\beta$. Hence, our model identifies parameters associated with motor transport that can be tuned to generate spontaneous cell polarization, beyond the total number of molecules $M$ and the coupling parameter $\kappa$. Finally, note that it is not possible to reduce our model to case (ii) of Hawkins et al. [68], since we take a different expression for the aster velocity field (see equation (4.51)), which is necessary in order to generate the velocity field from a normalizable distribution of filaments. Nevertheless, one can obtain a good match between the two models by slightly changing the baseline parameters in our model. This is illustrated in Figure 4.8 and Figure 4.9.

4.5 Discussion

We presented a stochastic model of active vesicular transport, which takes into account positive feedback between membrane-bound signaling molecules and cytoskeletal filaments. In particular, we considered the cytoplasmic transport of vesicles on a two-dimensional cytoskeletal network, in which a vesicle containing signaling molecules can randomly switch between a diffusing state and a state of directed motion along a filament. Using a quasi-steady-state analysis, we showed how the resulting stochastic hybrid system can be reduced to an advection-diffusion equation with anisotropic and space-dependent diffusivity. We used linear stability analysis to derive conditions for the growth of a precursor pattern for cell polarization. The effects of cytoskeletal geometry on cell polarization identified in Hawkins et al. [68] persist when biophysical details regarding motor-driven
vesicular transport are taken into account. Our more biophysically detailed model of motor transport allows us to determine how the conditions for spontaneous cell polarization depend on motor parameters such as mean speed and the rate of unbinding from filament tracks. One outstanding challenge is to understand how the vesicular transport of signaling proteins such as Cdc42 [39] can enhance the membrane-bound concentration of the proteins. This will require a more detailed model of the fusion and budding of vesicles with the cell membrane.
CHAPTER 5

FUTURE DIRECTIONS

We conclude this dissertation with a discussion of future directions with an emphasis on active transport of vesicles by motor proteins. In Section 5.1, we focus on the active transport of vesicles by motors along the cytoskeleton and discuss potential effects of the underlying noise on cell polarization. In Section 5.2, we describe a selective transport of vesicles carrying axonal (dendritic) membrane proteins into axons (dendrites). In Section 5.3, we present a recent finding on growth cone turning, which depends on microtubule-based motor proteins.

5.1 Noise in active transport

Most of our models are formulated by deterministic partial differential equations. One possible extension of our work is to explore the effect of intrinsic noise. From the aspect of active transport involving cytoskeletal filaments and molecular motors, the intrinsic noise may come from the discrete nature of actin (MT) networks, dynamic movement or growth of filaments, and the intermittent nature of motor/cargo transport in a filament network. The spatial organization of the cytoskeleton is highly complex. In budding yeast, there are randomly oriented cables in addition to the cables which extend from the bud along the mother-bud axis. These randomly oriented cables move around the cortex of cells at a rate of $0.59 \pm 0.14 \mu m/s$ [158]. In neuronal growth cones, microtubules display different lengths. On the other hand, the motion of molecular motors along filaments is also complex. For example, cargoes can be transported by teams of motors moving in different directions. The diversity of motors attached to one cargo enables the cargo to switch between actin-based and microtubule-based transport. Second, the spatial heterogeneity of the underlying filament network may affect the binding and unbinding rate of motors.

One question of interest is: how would the noise from the complex spatial organization of cytoskeleton affect the efficiency of vesicle transport to the membrane? Would it lead to
a polarized transport toward a particular site at the membrane? What structural property of filament network (such as length and orientation) is important? How would these answers depend on the cell geometry (sphere or rod)? For spherical cells, a recent study by Hafner and Rieger [66] suggests that spatial organization of the cytoskeleton enhances cargo delivery to specific areas on the plasma membrane.

Computational and analytical studies on the efficiency of active transport in cytoskeletal networks include those focusing on first passage time and anomalous diffusivity [6, 66, 67, 84]. Ando et al. use computer simulation to explicitly model the randomly ordered actin filament network [6]. They investigate the filament length and orientation, polarity organization, and binding/unbinding rates on the mean first passage time (MFPT). Interestingly, they find that filament polarity is more important than filament length and filament orientation in reducing average time. In particular, for a network with a large MFPT, there was a hotspot of greater than 10-folder higher residence times near the nucleus in a region with multiple filaments point inward. Reversing the orientation of filaments leads to a drop of MFPT and the disappearance of the hotspot.

Hafner et al. develop an analytical framework for the stochastic dynamics of motor/cargo transport in the cytoskeleton [67]. Their main result concerns anomalous diffusivity. In their model, the motors are assumed to switch between a motion (moving) and pausing (waiting) states at constant rates. The constant transition rate from moving to pause (waiting) is denoted by $\kappa_w$ and vise versa with $\kappa_m$. Motors in the motion state move with a step of length $l$ taken from a probability distribution $F(l)$. The simplified model for the motion of motors on one filament is given by

$$P_{n+1}^{M}(x) = \int dl F(l) \left[ \kappa_m p_n^{W}(x-l) + (1 - \kappa_w) p_n^{M}(x-l) \right],\quad (5.1a)$$

$$P_{n+1}^{W}(x) = \kappa_w p_n^{M}(x-l) + (1 - \kappa_m) p_n^{W}(x).\quad (5.1b)$$

Here $P_n^{M}(x)$ and $P_n^{W}(x)$ are the probability densities of finding a motor at position $x$ and time step $n$ in the motion and waiting states, respectively. A more detailed two-dimensional model which takes into account the structure of filament network can be generalized from the simplified model (5.1). However, some modification and additional assumptions are required. In addition to the position of the motor $(x,y)$, the moving direction $\theta$ is also recorded. Also, it is assumed that motors moving along a filament can
switch to a neighboring filament at the next time step. This switch of moving direction occurs at a probability $1 - p$. The choice of the new direction of motion depends on the structure of the filament network. Denote the angle between two intersecting filaments by $\phi$, a probability function $R(\phi)$ is chosen to represent the structural property. The detailed two-dimensional model which takes into account the switch between filaments is given by

\[
P_{n+1}^M(x, y|\theta) = p \int dl F(l) \left[ \kappa_m P_n^W (x', y'|\theta) + (1 - \kappa_w) P_n^M (x', y'|\theta) \right] \\
+ (1 - p) \int dl F(l) \int_{-\pi}^{\pi} d\gamma R(\phi) \left[ \kappa_m P_n^W (x', y'|\gamma) + (1 - \kappa_w) P_n^M (x', y'|\gamma) \right],
\]

\[
P_n^W (x, y|\theta) = \kappa_w P_n^M (x, y|\theta) + (1 - \kappa_m) P_n^W (x, y|\theta).
\]

Here $x' = x - l \cos \theta$, $y' = y - l \sin \theta$ and $\phi = \theta - \gamma$.

Using a Fourier-z-transform, Hafner et al. derive an analytical expression for the mean squared displacement of the displacement of motors, which is dependent on the parameters of structural properties of the filament network and transition probabilities between different phases of motor motion. They show that there are transitions between different types of anomalous diffusive dynamics. The crossover time to the asymptotic diffusive or ballistic motion varies by several orders of magnitude.

Despite the above models on active transport in a complex filament network, how the noise from the intermittent motor/cargo transport and/or the structure of a filament network would contribute to a polarized distribution of membrane-bound molecule is not clear. Previous stochastic models on cell polarization by Layton [94] and Slaughter [139] neglect the underlying structure of filament network and the intermittent nature of motor transport. In a recent study for cell polarization in fission yeast, Recouvreux et al. [132] proposed that the lateral diffusion together with microtubule tip affinity may be sufficient to establish a polarized distribution. Their model is a simplification of the complex MT-Tea pathway (see Figure 1.10). They take the cortex as a one-dimensional discrete lattice where particles undergo lateral diffusion. A microtubule tip in contact with a cell pole is represented by a static binding site (“trap”). Membrane bound proteins can bind or unbind to the trap. Moreover, these traps (MT tips) can randomly disappear due to catastrophe. Monte Carlo simulations show that there is accumulation of proteins at the cell poles where MT density is higher. The finding based on such a simple mechanism is exciting. One can then develop an explicit analytic model (either deterministic or stochastic) to check their
proposed mechanism. A more realistic two-dimensional model with a MT network is also worthwhile.

5.2 Selective transport into dendrites or axon

Compared to the intensive studies on the establishment of neuronal polarization, studies on the maintenance of neuronal polarity have begun recently; see [12] for an overview. One relevant question concerns the maintenance of polarized distributions of membrane proteins in the axon and dendrites. In particular, the types of polarized membrane proteins in the axon are different from those in dendrites. However, both the axonal and dendritic proteins are synthesized together in the common compartment within the somatodendritic domain. This gives rise to a question: how is each type of protein distributed to its distinct destination (either the axon or dendrites)?

In general, the accurate delivery of membrane proteins to axon or dendrites is achieved through a sequence of processes including vesicle budding, transport, and fusion; see Figure 5.1. Newly synthesized proteins bud from the trans-Golgi network and are then packaged into a distinct set of vesicles. That is, vesicles carrying axonal proteins do not contain dendritic proteins. The sorted vesicles are transported into the axons and dendrites where they deliver their contents to the plasma membrane by exocytic fusion. Several questions regarding the trafficking arise: Can vesicles carrying dendritic proteins enter axons? How and where does the trafficking of axonal and dendritic proteins diverge? In the following, we briefly review some recent experimental findings [2, 87].

For a better illustration, we describe the organization of microtubules and related motor activities, which are different in dendrites and axons. In mature neurons, 90% axonal microtubules are oriented with their plus ends pointing toward the axonal growth cone, whereas dendritic microtubules have mixed orientations [8, 9]. Vesicles carrying dendritic proteins are transported bidirectionally along microtubules by motor proteins (dynein) [87]. These vesicles are observed to have an equal frequency to enter either dendrites or the axon [2]. However, dendritic vesicles entering the axon can rarely go beyond the axon initial segment (actin-rich, see [71, 127]). They halt or reverse their moving direction (toward the cell body), which is possibly facilitated by the interaction of vesicles with Myosin Va. The exact sorting location for dendritic and axonal vesicles remains unknown.
Figure 5.1. Trafficking of polarized membrane proteins in neurons. Newly synthesized dendritic and axonal membrane proteins are sorted and loaded into different sets of vesicles. Vesicles are then transported along microtubules into dendrites or axons. Although vesicles carrying dendritic proteins can enter axons, they halt or reverse their moving direction (toward the cell body) near the axon initial segment. Vesicles carrying axonal proteins can be transported and delivered to both dendrites and axons. However, the transport is biased toward axons. Green: dendritic proteins (vesicles). Red: axonal proteins (vesicles). Redrawn from [12, 47, 87].

A recent study by Faiás et al. [47] proposed that the sorting occurs at a location defined as the pre-axonal zone, which is between the cell body and the axon initial segment; see Figure 5.1.

In contrast to the dendritic proteins, axonal proteins are transported into dendrites as well as the axon, but the transport is biased toward the axon. One possible pathway for the bias is that microtubules leading from the cell body into the axon may be biochemically modified so that kinesins can walk on them preferentially [114, 115].

Mathematical modeling regarding the polarized transport into axons and dendrites is lacking. In [87], Kapitein et al. use numerical and mathematical modeling to reveal how bidirectional movement on mixed microtubule arrays results in efficient dendritic targeting with a limited number of vesicles. It is also interesting to explore the specific sorting site in the axon where dendritic vesicles are sorted. The formation of distinct subdomains in axons is by itself interesting (see the review [121] for subcellular patterning events in axons).
5.3 Motor-driven force in growth cone turning

The importance of microtubules and microtubule-based motors in neuronal polarity has been demonstrated in a recent study by Kahn and Baas [85]. They propose a motor-regulated model for microtubule reorganization in growth cone turning. The related MT-based motors include cytoplasmic dynein, kinesin-5 (also called Eg5 or kif11), and kinesin-12 (Kif15). Cytoplasmic dynein generates a force to drive some microtubules into the peripheral domain of growth cone. Cytoplasmic dynein also transports short microtubules into axon. On the other hand, kinesin-5 and kinesin-12 impose a brake on this dynein-driven force and thus prevent the invasion of microtubules into the peripheral domain. During growth cone turning, kinesin-5 become localized on the side of the growth cone which is opposite to the direction of turn; see Figure 5.2.

Kinesin-5 is a slow homotetrameric motor which moves approximately 100 times slower than cytoplasmic dynein. This enables kinesin-5 to act as a brake that can halt the movement of microtubules by other motors. It is essential for growth cone turning. Inhibition or depletion of kinesin-5 causes growth cones to fail to turn in response to guidance signals [110, 111]. The association of kinesin-5 with microtubules is regulated by its phosphorylation. The relevant kinase is cyclin-dependent kinase 5 (CDK5) [86]. When a growth cone encounters a guidance cue, kinesin-5 becomes locally phosphorylated on the side of the growth cone which is opposite to the direction of turn. Similar effect of kinesin-12 inhibition is observed [97]. In contrast to kinesin-5, which imposes its activity mainly in the transition zone, kinesin-12 acts mainly in the peripheral domain and filopodia.

Based on their findings on motor-driven forces, Khan and Baas proposed a model for microtubule behaviors in growth cone advance. In particular, the growth cone advance is characterized by four different phases: growing, pausing, resuming growth, and turning. At each phase, microtubules undergo growth or reorganization. During the growing phase, dynein-driven forces permit a portion of microtubules to enter the peripheral domain. The growth cone remains unpolarized throughout the peripheral domain. When the growth cone pauses, a part of the advancing microtubules form a curved bundle. Later when the axon resumes its growth, this bundle becomes rapidly unbundled. Meanwhile, microtubule severing occurs more frequently. As a result of the unbundling and increased severing, more short microtubules are propelled away from the bundle. Finally, during
Figure 5.2. Molecular motors in growth cone turning. During the growth cone turning, kinesin-5 and/or kinesin-12 forces become localized at the side of the growth cone opposite to the direction of turn. Black arrow: direction of growth cone turn. Gray arrow: actin-retrograde flow. Small blue arrow: direction of movement of short microtubules by cytoplasmic motors. Redrawn from [85].

In the turning phase, kinesin-5 (kinesin-12) becomes polarized at the side of growth cone opposite to the direction of turn, and thus the dynein-driven microtubule invasion becomes dominant at the side of the direction of turn.

Although many details regarding the activation of motor proteins remain unknown, the mechanism of motor-driven force imposed on microtubules provides evidences of the importance of microtubules and motors. It would be interesting to model the localized phosphorylation of kinesin-5 motors.
APPENDIX A

ACTIVE COMPARTMENTS WITH DELAYS COUPLED THROUGH BULK DIFFUSION

In this appendix, we analyze a PDE–DDE model for the delayed logistic equation. In Section A.1, we introduce the one-dimensional PDE–DDE model for the delayed logistic equation. We then use linear stability analysis to derive a characteristic equation that depends nonlinearly on the associated eigenvalue. We use the characteristic equation to derive necessary conditions for a Hopf bifurcation. We then numerically calculate the critical time delay as a function of various model parameters, including the time delay, the bulk diffusivity, and the strength of diffusive coupling at the boundaries. In Section A.2, we use numerical simulations to explore the switching between in-phase and antiphase oscillations with changes in parameters. We also use the bifurcation tool DDE-BIFTOOL [44] to plot the amplitude of the periodic solutions. One limitation of our linear stability analysis is that we do not check that the pair of pure imaginary eigenvalues crosses over to the right-half complex plane above the Hopf bifurcation point nor determine whether or not there are already other eigenvalues in the right-half complex plane. Therefore, in Section A.3, we apply the winding-number method to detect the number of eigenvalues in the right-half plane for different parameter regimes. In Section A.4, we briefly explore extensions of our analysis to (i) asymmetric coupling and (ii) a PDE–DDE model based on the Mackey–Glass equation [100].

A.1 PDE–Logistic model

Consider the concentration of a single chemical species in a one-dimensional finite domain $[0, L]$. The boundaries $x = 0$ and $x = L$ represent a dynamically active membrane whose dynamics is governed by a DDE. For concreteness, we take the DDE to be the delayed logistic equation; we consider the Mackey–Glass DDE in Section A.4. The bulk region $(0, L)$ represents the cytoplasm of a cell within which molecules undergo diffusion.
with diffusion coefficient $D$. The bulk region and the two end compartments are coupled by a linear diffusive flux with coupling parameter $\beta$. Denoting the concentration at the boundaries as $X_1(t), X_2(t)$ and the concentration in the bulk as $C(x, t)$, the model is given by

$$ \frac{\partial C}{\partial t}(x, t) = D \frac{\partial^2 C}{\partial x^2}, \quad 0 < x < L, \; t > 0,$$

$$ D \partial_x C(0, t) = \beta(C(0, t) - X_1(t)),$$

$$ -D \partial_x C(L, t) = \beta(C(L, t) - X_2(t)), \quad (A.1)$$

and

$$ \frac{dX_1}{dt} = \beta(C(0, t) - X_1(t)) + f(X_1(t), X_1(t-\tau)),$$

$$ \frac{dX_2}{dt} = \beta(C(L, t) - X_2(t)) + f(X_2(t), X_2(t-\tau)), \quad (A.2a)$$

$$ (A.2b)$$

where

$$ f(x(t), x(t-\tau)) = rx(t)(1 - x(t-\tau)/M). \quad (A.3)$$

Here $\tau$ is the time delay, $r$ is the growth rate, and $M$ is the carrying capacity.

A steady-state solution satisfies

$$ C(x) = \frac{C_L - C_0}{L} x + C_0, \quad D \frac{C_L - C_0}{L} = \beta(C_0 - X_1) = -\beta(C_L - X_2). \quad (A.4)$$

Rewriting $C_0$ and $C_L$ in terms of $X_1$ and $X_2$ gives

$$ C_0 = \frac{(1 + \alpha)X_1 + \alpha X_2}{1 + 2\alpha}, \quad C_L = \frac{\alpha X_1 + (1 + \alpha)X_2}{1 + 2\alpha},$$

with $\alpha = D/(L\beta)$. It follows that

$$ C_0 - X_1 = \frac{\alpha(X_2 - X_1)}{1 + 2\alpha}, \quad C_L - X_2 = \frac{\alpha(X_1 - X_2)}{1 + 2\alpha}. \quad (A.5)$$

Suppose $X_1 > X_2$, then $C_0 - X_1 < 0$ and $C_L - X_2 > 0$. Thus $f(X_1, X_1) = -\beta(C_0 - X_1) > 0$. Hence $0 < X_1 < M$. Similarly, we have $f(X_2, X_2) = -\beta(C_L - X_2) < 0$. Hence $X_2 < 0$ or $X_2 > M$. Since $X_1 > X_2$, we have $X_2 < 0$. This implies that any nonnegative steady-state solution $(X_1, X_2)$ must satisfy $X_1 = X_2$. It then follows that

$$ C(x) = C_0 = X_1 = X_2, \quad f(X_1, X_1) = 0.$$

The function $f$ has an unstable trivial root, and a stable positive root, $x = M$. 
A.1.1 Linear stability analysis

We investigate the occurrence of oscillations in the PDE–Logistic model by carrying out a linear stability analysis of the positive steady state,

\[ C(x) = C_0 = X_1, \quad X_1 = X_2 = M, \]

and deriving conditions for a Hopf bifurcation. An investigation of linear stability means that we want to determine the spectrum of the linear operators obtained by linearizing about the fixed point. Therefore, we consider perturbed solutions of the form

\[ C(x) = C_0 + e^{\lambda t} \eta(x), \quad X_i = M + \phi_i e^{\lambda t}, \quad i = 1, 2, \]

substituting it into the linearized system near the steady state gives

\[
\begin{cases}
D\eta''(x) = \lambda \eta(x) \\
D\eta'(0) = \beta(\eta(0) - \phi_1) \\
-D\eta'(L) = \beta(\eta(L) - \phi_2)
\end{cases}
\] (A.6)

and

\[
\begin{cases}
\lambda \phi_1 = \beta(\eta(0) - \phi_1) - re^{-\lambda \tau} \phi_1 \\
\lambda \phi_2 = \beta(\eta(L) - \phi_2) - re^{-\lambda \tau} \phi_2.
\end{cases}
\] (A.7)

Using equation (A.7), we can rewrite \((\phi_1, \phi_2)\) in terms of \(\eta(0)\) and \(\eta(L)\). That is,

\[
\phi_1 = \frac{\beta \eta(0)}{\lambda + \beta + re^{-\lambda \tau}}, \quad \phi_2 = \frac{\beta \eta(L)}{\lambda + \beta + re^{-\lambda \tau}}.
\] (A.8)

Substituting it into the boundary conditions of \(\eta(x)\) gives a nonlinear eigenvalue problem

\[
\begin{cases}
D\eta''(x) = \lambda \eta(x) \\
D\eta'(0) = B(\lambda, \tau) \eta(0) \\
-D\eta'(L) = B(\lambda, \tau) \eta(L)
\end{cases}
\] (A.9)

where

\[
B(\lambda, \tau) = \beta \left[ 1 - \frac{\beta}{\lambda + \beta + re^{-\lambda \tau}} \right].
\] (A.10)

The eigenvector \(\eta(x)\) can be expressed in the form

\[
\eta(x) = \frac{\eta_0 + \eta_1}{2} \cosh \left( \frac{\sqrt{\lambda} / D(x - \frac{L}{2})}{2} \right) + \frac{\eta_1 - \eta_0}{2} \sinh \left( \frac{\sqrt{\lambda} / D(x - \frac{L}{2})}{2} \right),
\] (A.11)

where \(\eta_0\) and \(\eta_1\) are unknown. The boundary conditions require

\[
\begin{pmatrix}
A_+(\lambda) + B(\lambda, \tau) \\
A_-(\lambda)
\end{pmatrix}
\begin{pmatrix}
\eta_0 \\
\eta_1
\end{pmatrix} = 0.
\] (A.12)
where
\[ A_\pm(\lambda) = \frac{\sqrt{\lambda D}}{2} \left[ \tanh\left( \frac{L}{2} \sqrt{\lambda/D} \right) \pm \coth\left( \frac{L}{2} \sqrt{\lambda/D} \right) \right]. \]

Since the matrix is cyclic and symmetric, it follows that equation (A.12) has the solutions \( \eta_0 = \eta_1 \) (in-phase) and \( \eta_0 = -\eta_1 = 1 \) (antiphase) with \( \lambda \) satisfying the corresponding pair of equations
\[
\begin{align*}
A_- + A_+ + B &= 0 \quad \text{(in-phase),} \\
A_+ - A_- + B &= 0 \quad \text{(antiphase).}
\end{align*}
\]
(A.13a) \hspace{1cm} (A.13b)

Note that the presence of terms involving \( \sqrt{\lambda/D} \) means that we have to introduce a branch cut in the complex \( \lambda \)-plane along \((-\infty, 0] \) with \(-\pi < \text{Arg}(\lambda) < \pi \). Fortunately, for finite \( D, L \), we have \( \tanh(\lambda/4D) \to \lambda/4D \) and \( \coth(\lambda/4D) \to 4D/\lambda \), that is, any square roots in (A.13) cancel. However, care has to be taken in the limit \( D \to 0 \), since one can no longer eliminate the square roots and there is a continuous spectrum in addition to a discrete spectrum. We will avoid these complexities here by taking \( D > 0 \).

We now use equations (A.13) to derive necessary conditions for a Hopf bifurcation. That is, we look for pure imaginary solutions \( \lambda = i\omega \) with \( \omega \) real and construct Hopf bifurcation curves as a function of \( D, \tau \) and \( \beta \). Note, however, that in order to ensure the emergence of limit cycle oscillations via a primary Hopf bifurcation, one also has to check that a pair of eigenvalues cross over to the right-half complex plane as one crosses the Hopf curve and that there are no other eigenvalues already in the right-half plane. One way to keep track of the number of eigenvalues in the right-half complex plane is to use a winding-number argument (see Section A.3). One issue that cannot be addressed using linear stability analysis is whether or not a Hopf bifurcation is supercritical. However, all of our numerical simulations suggest that the bifurcation is indeed supercritical (see Section A.2).

Although we take \( D > 0 \) throughout, we first briefly consider the discrete spectrum when \( D \to 0 \) for \( \lambda \neq 0 \). Noting that
\[ \lim_{D \to 0} \sqrt{\lambda D} \tanh\left( \frac{L}{2} \sqrt{\frac{\lambda}{D}} \right) = \lim_{D \to 0} \sqrt{\lambda D} \coth\left( \frac{L}{2} \sqrt{\frac{\lambda}{D}} \right) = 0, \]
we have
\[ B(\lambda, \tau) = \beta \left[ 1 - \frac{\beta}{\lambda + \beta + re^{-\lambda \tau}} \right] = 0. \]
It follows that

$$\lambda + re^{-\lambda \tau} = 0. \quad \text{(A.14)}$$

This is the characteristic equation of the delayed logistic equation

$$\frac{dx}{dt} = f(x(t), x(t - \tau)) = rx(t)(1 - x(t - \tau)/M).$$

At a Hopf bifurcation point, the critical time delay $\tau$ and the frequency $\omega$ satisify

$$\cos(\omega \tau) = 0, \quad \omega - r \sin(\omega \tau) = 0.$$

It follows that

$$\tau = \frac{\pi}{2r} + \frac{2n \pi}{r}, \quad \omega = r > 0, \quad n = 0, 1, 2, \cdots \quad \text{(A.15)}$$

In the slow diffusion limit, the Hopf point occurs at $\tau = \pi/(2r)$, and is independent of the coupling parameter $\beta$. The diffusion is too slow to affect the concentration at the membrane.

Next, we consider the solution of equation (A.13) as $D \to \infty$. As $D \to \infty$, assuming $|\lambda| = O(1) \ll D$, we have

$$\lim_{D \to \infty} \sqrt{\lambda \alpha} \tanh \left( \frac{L}{2} \sqrt{\frac{\lambda}{2D}} \right) = \frac{\lambda L}{2},$$

and

$$\sqrt{\lambda \alpha} \coth \left( \frac{L}{2} \sqrt{\frac{\lambda}{D}} \right) \approx \frac{2D}{L} = O(D) \to \infty.$$  \(\text{(A.16)}\)

Hence the solution $(\tau, \omega)$ of equation (A.13) in the fast diffusion limit satisfies

$$B(\lambda, \tau) = -\frac{\lambda L}{2}, \text{ or } B(\lambda, \tau) = O(D) \to \infty.$$  \(\text{(A.16)}\)

The first equation in equation (A.16) gives the characteristic equation

$$\beta \left[ 1 - \frac{\beta}{\lambda + \beta + re^{-\lambda \tau}} \right] = -\frac{\lambda L}{2}. \quad \text{(A.17)}$$

Noting that for any $\lambda > 0$ real and $\tau > 0$,

$$1 - \frac{\beta}{\lambda + \beta + re^{-\lambda \tau}} > 0 > -\frac{\lambda L}{2\beta}.$$
Figure A.1. Hopf bifurcation curves in $(D, \tau)$ plane for different values of the coupling parameter $\beta$. (a,c) Critical time delay $\tau$ vs. $D$ with $\beta = 1, 0.5$, respectively. Solid curves show Hopf bifurcation points, whereas dashed curves are continuations through a double Hopf point (H-H). Below the curves, the steady state is stable. (b, d) Frequency $\omega$ (imaginary eigenvalue) vs. $D$ with $\beta = 1, 0.5$, respectively. As $D$ increases, the primary bifurcation switches from an antiphase to an in-phase limit cycle oscillation. Blue: in-phase. Green: antiphase. Other parameters: $L = 1$, $r = 1$, $M = 1$.

thus equation (A.17) has no positive real root. In fact, there is a negative real root. Hopf bifurcation can occur as the eigenvalue crosses the imaginary axis. Setting $\lambda = i\omega$, the critical time delay and frequency at a Hopf point satisfy

$$1 + i\frac{\omega L}{2\beta} = \frac{\beta}{i\omega + \beta + re^{-i\omega\tau}} = \frac{\beta}{\beta + r \cos \omega \tau + i(\omega - r \sin(\omega \tau))}.$$  

(A.18)

The equation can be solved numerically and the critical time delay is the same as the asymptote of the Hopf curve (in-phase) in Figure A.1 (a).

The second equation in (A.16) does not have a solution unless

$$\lambda + \beta + re^{-\lambda \tau} = 0.$$  

(A.19)
This type of characteristics equation has been studied in detail in [65, 69]. Setting $\lambda = i\omega$, and taking $\omega > 0$ without loss of generality gives

$$\beta + r \cos \omega \tau = 0, \quad \omega - r \sin \omega \tau = 0.$$  \hspace{1cm} (A.20)

It follows that

$$\cos \omega \tau = \frac{-\beta}{r}, \quad \omega = r \sin \omega \tau = \sqrt{r^2 - \beta^2}.$$  

If $\beta > r$, there is no solution of $(\omega, \tau)$. This implies that the critical time delay (associated with the antiphase oscillation) does not exist for strong coupling $\beta$ in the fast diffusion limit. On the other hand, if $\beta < r$, then there are denumerably many solutions

$$\omega = \sqrt{r^2 - \beta^2} > 0, \quad \tau_n = \frac{(2n - 1) \pi - \arccos(\beta/r)}{\omega}, \quad n \in \mathbb{N}.$$  

**A.1.2 Hopf bifurcation curves**

Using the above linear stability analysis, we now construct Hopf bifurcation curves with respect to different model parameters. In Figure A.1, Hopf bifurcation curves for the critical time delay $\tau_c$ are plotted as a function of the diffusion coefficient $D$ for fixed coupling $\beta$. The critical time delay is computed by taking the real and imaginary parts of the two eigenvalue relations in (A.13) and solving the resulting system using Maple. There are two branches of Hopf curves corresponding to in-phase and antiphase oscillations; they intersect at Hopf–Hopf points, which act as organizing centers for more complex oscillatory solutions. It is difficult to resolve which branch is dominant as $D \to 0$, since both the in-phase and antiphase branches approach the critical time delay of the uncoupled delayed logistic equation. However, away from the origin, we find that as the diffusion coefficient $D$ increases, the critical time delay increases, and the primary bifurcation switches from an antiphase to an in-phase oscillation. It can be checked that the asymptotic limit of the in-phase branch agrees with the solution $(\lambda, \tau) = (i\omega, \tau)$ of equation (A.17). In Figure A.2, we plot the corresponding Hopf bifurcation curves in the $(\beta, \tau)$ plane for fixed $D$. Now there is a switch from antiphase to in-phase oscillations as $\beta$ is increased away from zero.

Next, we take the time delay to be fixed and plot the phase diagram in $(\beta, D)$ plane; see Figure A.3. This is computed by numerically solving the eigenvalue equation (A.13) for the solution $\lambda = i\omega$ and $D$ with a varying $\beta$. For $\tau = 2$, antiphase (in-phase) oscillations exist
Figure A.2. Hopf bifurcation curves in \((\beta, \tau)\) plane for (a) \(D = 0.2\) and (b) \(D = 1\). Solid curves show Hopf bifurcation points, whereas dashed curves are continuations through a double Hopf point (H-H). As \(\beta\) increases, the primary bifurcation switches from an antiphase to an in-phase limit cycle oscillation. Blue: in-phase. Green: antiphase. Other parameters: \(L = 1, r = 1, M = 1\).

Figure A.3. Phase diagram in \((D, \tau)\) plane with different time delays. Antiphase (in-phase) oscillations exist for \((\beta, D)\) below the green (blue) line. (a) \(\tau = 2\), antiphase oscillation exists in region 2. In region 3, both in-phase and antiphase oscillations exist, however, numerical result suggests in-phase oscillation is unstable; see Figure A.4 (a). (b) \(\tau = 2.4\). The blue and green curves intersect. Our numerical plot in Figure A.4 (b) suggests there is possibly an unstable torus bifurcation. (c) \(\tau = 2.45\). Antiphase oscillation exists for parameters below the green line. In-phase oscillations exist for any choice of \((\beta, D)\). In the coexistence region, i.e., below the green line, we find in-phase oscillation can possibly evolve to antiphase oscillation; see Figure A.4 (c). (d) The asymptote of critical time delay (solution of equation (A.18)) as a function of the coupling strength as \(D \to \infty\). The two points \((\beta, \tau) = (0.25, 2.0)\) and \((0.6, 2.4)\) imply the asymptote of \(\beta\) for \(\tau = 2, 2.4\); see (a, b).
for parameters below the green (blue) line. Above these lines, i.e., region 1, the steady state is stable. This implies that for sufficiently large $(\beta, D)$, the fixed time delay $\tau = 2$ is below a Hopf bifurcation. The result is consistent with the numerical result shown in Figure A.1 and Figure A.2. In the coexistence region 3, the in-phase oscillation is observed to be unstable; see Figure A.4 (a). For $\tau = 2.4$, similar result is observed. One difference between $\tau = 2$ and $\tau = 2.4$ is that the blue and green curves intersect in the latter case. Numerical solution for $(\beta, D)$ chosen within the coexistence region shows there is an unstable torus bifurcation; see Figure A.4 (b). For $\tau = 2.45$, the time delay is sufficiently large so that in-phase oscillations exist for any $(\beta, \tau)$. This can be explained by the plot of the asymptote of critical time delay which has a maximum around 2.43; see Figure A.3 (d). On the other hand, antiphase oscillations exist for parameters below the green line. Again, we find that the in-phase oscillation can lose its stability in the coexistence region; see Figure A.4 (c).

In summary, our linear stability analysis suggests that the existence of oscillations depends on the diffusion coefficient, coupling strength, and time delay. First, the critical
time delay at a Hopf bifurcation increases as the diffusion coefficient or coupling strength increases. Second, for a fixed time delay, the effect of diffusion or coupling on the stability of steady state is sensitive to the value of the time delay. In particular, for a small time delay ($\tau < \pi/2$), changing the diffusion coefficient or coupling strength will not destabilize the steady state. For a sufficiently large time delay, oscillations always exist for any $(D, \beta)$. For a moderate time delay, decreasing the diffusion coefficient or coupling strength can give rise to in-phase or antiphase oscillations.

### A.2 In-phase oscillation vs. antiphase oscillation

In this section, we numerically explore the occurrence of in-phase and antiphase oscillations. The full PDE–DDE system given by equations (A.1) and (A.2) is simulated by discretizing the PDE into a system of ODEs using a method of line approach with a spatial step size $h = 1/20$. The resulting ODE–DDE system is solved using the DDE solver `dde23` in MATLAB. The amplitude plots and Floquet multipliers are computed for the DDE–ODE system using DDE-BIFTOOL.

First, suppose that $\beta = 1$ as in Figure A.1 (a,b). For $D = 0.1$ and the initial condition

$$ C(x, t) = 1, \quad X_1(t) = 1 + 0.1, \quad X_2(t) = 1 - 0.1, \quad -\tau \leq t \leq 0, $$

the numerical solution with different time delays is shown in Figure A.5 (a, b). We find that the primary Hopf bifurcation to an antiphase solution is supercritical at the predicted critical time delay $\tau = \tau_{hp} \approx 1.99$ (see the inset in Figure A.1 (a)). As $\tau$ increased to 2.3, although in-phase oscillation is observed for the initial condition $X_1 = X_2 = 1.1$, we find that it is unstable under perturbation near the initial condition. For the sake of illustration, we choose the initial condition

$$ C(x, t) = 1, \quad X_1(t) = 1 + .1, \quad X_2(t) = 1 + .09, \quad -\tau \leq t \leq 0. $$

The numerical solution starts near an in-phase oscillation but evolves to an antiphase oscillation; see Figure A.5 (c). The instability of in-phase oscillation is also observed when we plot the amplitude of the periodic solutions using DDE-BIFTOOL; see Figure A.5 (d). The stability is determined by computing the Floquet multipliers using DDE-BIFTOOL. We find that the in-phase oscillations have Floquet multipliers outside the unit circle. On the other hand, the antiphase oscillations have Floquet multipliers inside the unit circle.
Figure A.5. Stable antiphase solutions solutions for $D = 0.1$, $\beta = 1.0$ and different delays $\tau$. Corresponding bifurcation curves are shown in Figure A.1 (a). (a) $\tau = 1.9$. The steady-state solution is stable. (b) $\tau = 2.1$. Antiphase oscillations occur given the initial condition $C(x,0) = 1$, $X_1(0) = 1.1$, $X_2(0) = 0.9$. (c) $\tau = 2.3$. An in-phase oscillation changes to an antiphase oscillation for the initial condition $C(x,0) = 1$, $X_1(0) = 1.1$, $X_2(0) = 1.09$. (d) Amplitude of the periodic solutions as a function of time delay. Numerical result shows that the in-phase periodic solution has Floquet multipliers outside the unit circle and thus it is unstable. On the other hand, the antiphase periodic solution has Floquet multiplier inside the unit circle. Other parameters: $L = 1$, $r = 1$, $M = 1$. Spatial step size $h = 0.05$.

Plotting the amplitude as a function of delay for $\beta = 1$ and fixed $D$ corresponds to taking a vertical slice through Figure A.1 (a).

Now, suppose that the diffusion coefficient is increased to $D = 0.6$. Consistent with Figure A.1 (a), (b) we now find that the antiphase oscillation is unstable and the in-phase oscillation is stable; see Figure A.6. This feature was also observed in the PDE–ODE model of [61]. More complex behavior can be obtained if the system operates close to the double-Hopf point in Figure A.1 (a), as has also been found for a PDE–ODE system [62]. For example, if $D = 0.5$, then the antiphase oscillation can change from unstable to stable as the time delay $\tau$ is increased from 2.7 to 2.9; see Figure A.7 (b), (c). The numerical stability analysis of the antiphase periodic solution suggests that there exists a pair of complex
Figure A.6. Stable in-phase solutions solutions for $D = 0.6$, $\beta = 1.0$ and different delays $\tau$. Corresponding bifurcation curves are shown in Figure A.1 (a). (a) Amplitude of the periodic solutions as a function of time delay. Numerical result shows that the antiphase periodic solution has Floquet multipliers outside the unit circle and thus it is unstable. On the other hand, the in-phase periodic solution has Floquet multiplier inside the unit circle. (b, c) $\tau = 2.3, 3.2$, respectively. Initial conditions: $C(x, 0) = 1$, $X_1(0) = 1.1$, $X_2(0) = 0.9$. Other parameters are $L = 1$, $r = 1$, $M = 1$.

Floquet multipliers outside the unit circle for $\tau > \tau_{tr} \approx 2.8$; see Figure A.7 (a). This implies there is a torus bifurcation near $(D, \tau) \approx (0.5, 2.8)$.

A change of oscillation modes is also observed when diffusion coefficient changes with a fixed time delay. For $\tau = 3.2$, as the diffusion coefficient $D$ changes from 0.1 to 0.6, the oscillation switches from antiphase to in-phase; see Figure A.8. A similar result is observed for $\tau = 2.5$, as the diffusion coefficient $D$ changes from 0.1 to 0.4; see Figure A.9.

### A.3 Winding-number argument

In this section, we use the winding-number argument studied in [61] to count the number $N_0$ of roots of the characteristic equation (A.13) with $\text{Re} \lambda > 0$. If $N_0 = 0$, then the steady state is linearly stable. Otherwise, it is linearly unstable. Moreover, there is a
Figure A.7. Stability of the antiphase oscillation for $D = 0.5$ and sufficiently large time delay. (a) Amplitude of the periodic solutions with the number of unstable Floquet multipliers. Blue: number of unstable Floquet multiplier (# unst)=0, Red: number of unstable Floquet multiplier (# unst)=2. (b) Plots of Floquet multipliers (antiphase branch) with $\tau = 2.7$ and $\tau = 2.8$. There are a pair of complex Floquet multipliers (red markers) outside the unit circle for $\tau = 2.7$. (c) For $\tau = 2.7$, the antiphase oscillation is unstable. (d) For $\tau = 2.9$, the antiphase oscillation is stable. Initial conditions: $C(x, 0) = 1$, $X_1(0) = 1.1$, $X_2(0) = 0.9$. Other parameters: $L = 1$, $\beta = 1$, $r = 1$, $M = 1$.

Hopf point if there exists a root on the imaginary axis. We start with the eigenvalue $\lambda$ associated with the in-phase oscillation and consider the function

$$F(\lambda) = B(\lambda, \tau) + \sqrt{\lambda D} \tanh \left( \frac{L}{2} \sqrt{\frac{\lambda}{D}} \right)$$

$$= \beta \left[ 1 - \frac{\beta}{\lambda + \beta + re^{-\lambda \tau}} \right] + \sqrt{\lambda D} \tanh \left( \frac{L}{2} \sqrt{\frac{\lambda}{D}} \right). \quad (A.21)$$

Recall that we are taking the primary branch of $\sqrt{\lambda}$, whilst the first term on the right-hand side is analytic except for a countable set of simple poles when $D > 0$. Replacing the tanh function by coth gives the eigenvalue $\lambda$ associated with the antiphase oscillation. To find the number of roots of $F$ on the right-half complex plane $\{z, \Re(z) > 0\}$, we construct the
Figure A.8. Switch from an antiphase oscillation to an in-phase oscillation as $D$ increases from 0.1 to 0.6 with a fixed time delay $\tau = 3.2$. (a) $D = 0.1$, (b) $D = 0.5$, (c) $D = 0.6$. As the diffusion coefficient increases from 0.1 to 0.6, the solution changes from antiphase oscillations to in-phase oscillations; see (a) and (d). For $D = 0.5$, a new type of oscillatory mode (torus) emerges due to the double Hopf bifurcation. However, the new mode disappears at a sufficiently large time ($t \approx 300$, see (b)), and the oscillations are again antiphase synchronous. For $D = 0.6$, the oscillation starts as antiphase but ends up as in-phase synchronous. Initial conditions: $C(x,0) = 1$, $X_1(0) = 1.5$, $X_2(0) = 0.5$.

Figure A.9. As in Figure A.8, a switch in oscillation mode occurs when $D$ increases from 0.1 to 0.4 with a fixed time delay $\tau = 2.5$. Initial conditions: $C(x,0) = 1$, $X_1(0) = 1.1$, $X_2(0) = 1.09$. 

Figure A.10. Counterclockwise contour $\Gamma$ consisting of the semi-circle $\Gamma_R$ and the imaginary axis $\Gamma_I$. Here $\Gamma_R = \{ z = Re^{i\theta}, -\pi/2 < \theta < \pi/2 \}$ and $\Gamma_I = \Gamma_I^- \cup \Gamma_I^+ = \{ z = iy, -R \leq y \leq R \}$.

counterclockwise contour $\Gamma$ consisting of the semi-circle $\Gamma_R = \{ z = Re^{i\theta}, -\pi/2 < \theta < \pi/2 \}$ and the imaginary axis $\Gamma_I = \{ z = iy, -R \leq y \leq R \}$; see Figure A.10. Applying the argument principle to the function $F(\lambda)$ on the contour $\Gamma$ gives

$$N_0 - N_\infty = \frac{1}{2\pi} \Delta_{\Gamma} \text{Arg} F(\lambda), \quad (A.22)$$

where $N_0(N_\infty)$ is the number of zeros (poles) of $F$ inside $\Gamma$ and $\Delta_{\Gamma} \text{Arg} F(\lambda)$ is the change of the argument of $F$ along the contour $\Gamma$. In the following, we perform the classical analysis to calculate $N_\infty$ and $\Delta_{\Gamma} \text{Arg} F(\lambda)$ and determine $N_0$.

For the path $\Gamma_R$, as $R = |\lambda| \rightarrow \infty$, we have

$$|B(\lambda, \tau)| = \beta|1 - \frac{\beta}{\lambda + \beta + re^{-\lambda\tau}}| \leq \beta + \frac{\beta^2}{|\lambda| - \beta - r} = \beta + O\left(\frac{1}{R}\right), \quad \text{Re}(\lambda) > 0,$$

and $\sqrt{\lambda D} \tanh \left( \frac{\lambda}{2} \sqrt{\frac{A}{D}} \right) \approx \sqrt{\lambda D}$. Hence

$$F(\lambda) \approx \beta + \sqrt{\lambda D} \Rightarrow |F(\lambda)| \approx \sqrt{RD} \neq 0, \quad \text{for } |\lambda| = R \gg 1. \quad (A.23)$$

So there are no zeros nor poles on $\Gamma_R$. Furthermore, we have

$$\Delta_{\Gamma_R} \text{Arg} F(\lambda) = \text{Arg} F(iR) - \text{Arg} F(-iR)$$

$$= 2[\text{Arg} F(iR) - \text{Arg} F(0)] \approx \frac{\pi}{2}, \quad R \gg 1. \quad (A.24)$$
Figure A.11. Sketch of possible trajectories of $F(iy)$ as $y$ changes from $R$ to 0. (a) Three examples of trajectories that do not pass through the negative real axis and $\Delta \text{Arg} F = 0 - \pi/4$. (b) Hopf bifurcation. There exists a $y_0 \in (0, R)$ such that $F(iy_0) = 0$. (c) $F(iy)$ crosses the negative real axis, $\Delta_{i\gamma} \text{Arg} F = (\pi - \pi/4) + 0 - (-\pi) = 7\pi/4$. Noting that the square root function does not appear in the function $B(iy, \tau)$, it is possible that $F(iy)$ crosses the negative $x$-axis.

It follows that

$$N_0 = N_{\infty} + \frac{1}{2\pi} \Delta_{i\gamma} \text{Arg} F(\lambda) + \frac{1}{2\pi} \Delta_{i\gamma} \text{Arg} F(\lambda)$$

$$= N_{\infty} + \frac{1}{4} + \frac{1}{2\pi} \Delta_{i\gamma} \text{Arg} F(\lambda)$$

$$= N_{\infty} + \frac{1}{4} + \frac{1}{\pi} \Delta_{i\gamma} \text{Arg} F(\lambda)$$

(A.25)

where $\Gamma_{i\gamma} = \{z = iy, 0 \leq y \leq R\}$. It remains to calculate $\Delta_{i\gamma} \text{Arg} F(\lambda)$. Noting that

$$F(iR) \approx \sqrt{DR} = \sqrt{DR}e^{\pi i/4}, \quad F(0) = \frac{\beta r}{\beta + r} > 0,$$

(A.26)

the change of argument along the half imaginary axis $\Gamma_{i\gamma}$ can be $\pi/4 + 2k\pi$ or $-\pi/4 + 2k\pi$. In Figure A.11, we sketch some possible images of $F(\Gamma_{i\gamma})$. For a given set of parameter values, we need to determine numerically whether or not the image $F(\Gamma_{i\gamma})$ crosses the negative real axis, and if it does, then the number of times it crosses.

We also need to check if there are any poles or zeros on the path $\Gamma_{i\gamma}$. The number of poles or zeros is dependent on the parameters $(\beta, \tau, r)$. Note that if $F(\lambda)$ has a zero $\lambda = iy$, then there is a Hopf bifurcation. Since we are interested in the parameter regime above the Hopf curve, where an oscillatory solution has already emerged, we choose parameters so that there are no zeros on the imaginary axis. It can be checked that $F$ has a pole where $B(\lambda, \tau)$ is singular. That is, $B(\lambda, \tau)$ has a pole on the imaginary axis $\Gamma_{i\gamma}$ if there exists $\lambda = iy$ such that

$$H(\lambda) = \lambda + \beta + re^{-\lambda \tau} = 0.$$

(A.27)
Setting $\lambda = iy$ and separating the imaginary and real parts of $H(\lambda)$ gives

\begin{align}
  y - r \sin(y\tau) &= 0, \quad (A.28a) \\
  \beta + r \cos(y\tau) &= 0. \quad (A.28b)
\end{align}

It follows that $\cos(y\tau) = -\beta/r$ and $\sin(y\tau) = \pm \sqrt{1 - \beta^2/r^2}$.

If $r < \beta$, there is no solution of $y$ to equation (A.28b). That is, there are no poles on $\Gamma_I$ provided $r < \beta$.

If $r \geq \beta$, we can eliminate $y$ from equation (A.28b) using $y = r \sin(y\tau) = \pm r \sqrt{1 - \beta^2/r^2}$.

That is,

\begin{equation}
  \beta + r \cos(\tau \sqrt{r^2 - \beta^2}) = 0. \quad (A.29)
\end{equation}

For any fixed $\beta, \tau > 0$, and $r \geq \beta$, equation (A.29) has denumerably many positive solutions $\{r_i(\beta)\}$. For any $r \neq r_i(\beta)$, equation (A.29) does not have a solution, i.e., there are no zeros of $H(\lambda)$ on the imaginary axis. That is, there are no poles of $F$ on $\Gamma_I$.

In order to find the number of poles $N_\infty$ inside the contour $\Gamma$, we cite the Lemma 3 in [65] below.

**Lemma 1.** (Hadeler and Tomiuk [65]) Let $\nu > 0$ be given. Then equation

\begin{equation}
  \nu + \alpha \cos \sqrt{\alpha^2 - \nu^2} = 0
\end{equation}

has denumerably many positive solutions

\begin{equation}
  \alpha_1(\nu) < \alpha_2(\nu) < \alpha_3(\nu) < \ldots
\end{equation}

with $\sin \sqrt{\alpha^2 - \nu^2} > 0$, such that $\alpha_k(\nu) \to \infty$ as $k \to \infty$. For $\alpha \in (\alpha_k(\nu), \alpha_{k+1}(\nu)]$, equation

\begin{equation}
  H(\lambda; \alpha, \nu) = \nu + \lambda + \alpha e^{-\lambda} = 0
\end{equation}

has exactly $k$ solutions $\lambda^1, \lambda^2, \ldots, \lambda^k$ in $\text{Re} \lambda > 0, \text{Im} \lambda > 0$, and

\begin{equation}
  \text{Re} \lambda^l > 0, \quad (2l - 3/2)\pi < \text{Im} \lambda^l < (2l - 1)\pi, \quad l = 1,\ldots,k.
\end{equation}

### A.3.1 Winding number for $\beta \geq r$

Set $\alpha = r\tau$ and $\nu = \beta\tau$ in Lemma 1, if $\beta \geq r$, then there are no poles on $\Gamma_I$ nor inside the contour $\Gamma$. That is

\begin{equation}
  N_\infty = 0. \quad (A.30)
\end{equation}
Figure A.12. Numerical plots of $F(iy)$ (in-phase) as $y$ changes from $R$ to $0$ for different values of the delay $\tau$. (a) Below the Hopf point. $\tau = 2.3 < \tau_{hp}$, $\Delta_{\Gamma^+} ArgF = -\pi/4$. (b) $\tau = \tau_{hp} = 2.435$. (c) $\tau = 2.5 > \tau_{hp}$, $\Delta_{\Gamma^+} ArgF = 7\pi/4$. Other parameters: $L = 1$, $D = 1$, $\beta = 1$, $r = 1$, $M = 1$, $R = 10$.

Figure A.13. Numerical plots of $F(iy)$ (antiphase) as $y$ changes from $R$ to $0$ for different values of the delay $\tau$. (a) $\tau = 2.5 < \tau_{hp}$, $\Delta_{\Gamma^+} ArgF = -\pi/4$. $Re(F) > 0$. (b) $\tau = \tau_{hp} = 3.15$. (c) $\tau = 3.2 > \tau_{hp}$, $\Delta_{\Gamma^+} ArgF = 7\pi/4$. Other parameters: $L = 1$, $D = 1$, $\beta = 1$, $r = 1$, $M = 1$, $R = 10$.

In the particular case $D = \beta = r = 1$, the critical time delay (in-phase) is $\tau_{hp} \approx 2.436$. For $\tau = 2.3 < \tau_{hp}$ and $\tau = 2.5 > \tau_{hp}$, we plot $ReF(iy)$ and $ImF(iy)$ in Figure A.12. It follows from these plots that

$$N_0 = \frac{1}{4} + \frac{1}{\pi} \Delta_{\Gamma^+} ArgF(\lambda) = \begin{cases} \frac{1}{2} - \frac{1}{4} = 0, & \text{if } \tau = 2.3 < \tau_{hp}, \\ \frac{1}{3} + \frac{7}{4} = 2, & \text{if } \tau = 2.5 > \tau_{hp}. \end{cases}$$

(A.31)

Therefore, there are two eigenvalues associated with the in-phase oscillation in $Re\lambda > 0$ when $\tau > \tau_{hp}$. This holds until $\tau$ is further increased to the continuation of the antiphase oscillation.
Figure A.14. Contour plots of $\max(\Re(\lambda),0)$ in the $(D, \tau)$ plane with $D \in [0.1,1]$ and $\tau \in [0,5]$. (a) Eigenvalues of in-phase mode. (b) Eigenvalues of antiphase mode. The numerical results indicate that for a diffusion coefficient $D \geq 0.3$ and sufficiently large time delay, the eigenvalue corresponding to the synchronous mode has a larger positive real part. Parameters: $L = 1$, $\beta = 1$, $r = 1$, $M = 1$.

Hopf curve; see Figure A.1(a). A similar result for the eigenvalues associated with the antiphase oscillation is shown in Figure A.13. The critical time delay (antiphase) is $\tau_{hp} \approx 3.15$. Finally, in Figure A.14, we plot the real parts of the eigenvalues associated with the in-phase and antiphase mode for different $D$ and $\tau$. We see that for a diffusion coefficient $D \geq 0.3$ and sufficiently large time delay, the eigenvalue corresponding to the synchronous mode has a larger positive real part, indicating that it is the dominant mode. This indicates that for an arbitrary perturbation near the steady state, we would expect to see in-phase oscillations for most initial conditions.

A.3.2 Winding number for $\beta < r$.  

Following from Lemma 1, for any $\beta > 0$ and $r \in (r_k(\beta), r_{k+1}(\beta)]$, fixed, there are $k$ solutions in $\Re \lambda > 0, \Im \lambda > 0$. Here $\{r_k(\beta)\}_k$ are solutions to equation (A.29); see Figure A.15. Since the conjugate $\bar{\lambda}$ is also an eigenvalue, it follows that

$$N_\infty = 2k, \quad \text{for } r \in (r_k(\beta), r_{k+1}(\beta)], \quad k = 0, 1, 2, \cdots.$$  

(A.32)

For simplicity, we will focus on the parameter regime with $r \leq r_2$, for which the number of poles is

$$N_\infty = \begin{cases} 0, & \text{if } r \in (0, r_1(\beta)], \\ 2, & \text{if } r \in (r_1(\beta), r_2(\beta)]. \end{cases}$$  

(A.33)
**Figure A.15.** Intersection points of $\cos(\tau \sqrt{r^2 - \beta^2})$ and $-\beta/r$. (a) Plot of $\cos(\tau \sqrt{r^2 - \beta^2})$ (blue) and $-\beta/r$ (red) as a function of $r$. As $r$ increased from $\beta$ to $\infty$, these two functions intersect many times at $r = r_i(\beta), i \in \mathbb{N}$. (b) the smallest positive solution $r_1(\beta)$ as a function of $\beta$. For the parameter $(\beta, r)$ lies below the the curve, there are no zeros of $H(\lambda) = \lambda + \beta + re^{-\lambda \tau}$ on the right-half plane. Baseline parameters $\beta = 0.6$, $\tau = 3$.

**Figure A.16.** $Re F(\lambda)$ and $Im F(\lambda)$ as $\lambda$ travels along $\Gamma_{I^+} = \{iy\}$ with $y$ changes from 100 to 0.01. (a) $r = 0.75 < r_{hp} = 0.81$. Since $Im F > 0$, we have $\Delta_{\Gamma_{I^+}} = -\pi/4$. (b) $r = 0.9 \in (r_{hp}, r_1), \Delta_{\Gamma_{I^+}} = 7\pi/4$. (c) $r = 1 \in (r_1, r_2), \Delta_{\Gamma_{I^+}} = -\pi/4$. Other parameters: $\beta = 0.6$, $\tau = 3$, $D = 1$, $L = 1$, $R = 100$.

For the sake of illustration, suppose that $\beta = 0.6, \tau = 3$. We find that there are two types of critical values of $r$:

1. the critical value at a Hopf point of $F$ occurs at $r_{hp} \approx 0.81$. If $r < r_{hp}$, then the eigenvalue has negative real part and the steady state is linearly stable.

2. the first two positive solutions of equation (A.29): $r_1 \approx 0.96$ and $r_2 \approx 1.56$. If $0 < r < r_1$, there are no poles inside the contour $\Gamma$, i.e., $N_\infty = 0$; whereas if $r_1 < r < r_2$, there are two poles in conjugate pairs, i.e., $N_\infty = 2$. 
Figure A.17. Hopf bifurcation in \((\beta, r)\) plane. Parameters \(D = 1, \tau = 3, L = 1\).

For \(0 < r < r_{hp}, r_{hp} < r < r_1\) and \(r_1 < r < r_2\), we plot the real and imaginary part of \(F\) in Figure A.16. The numerical result suggests

\[
\Delta r_j \text{Arg}F(\lambda) = \begin{cases} 
-\frac{\pi}{4}, & \text{if } r \in (0, r_{hp}), \\
\frac{7\pi}{4}, & \text{if } r \in (r_{hp}, r_1), \\
-\frac{\pi}{4}, & \text{if } r \in (r_1, r_2).
\end{cases}
\]

Hence the formula of the Argument Principle (A.25) gives

\[
N_0 = N_\infty + \frac{1}{4} + \frac{1}{\pi} \Delta r_j \text{Arg}F(\lambda) = \begin{cases} 
0 + \frac{1}{4} - \frac{1}{4} = 0, & \text{if } r \in (0, r_{hp}), \\
0 + \frac{1}{4} + \frac{7}{4} = 2, & \text{if } r \in (r_{hp}, r_1), \\
2 + \frac{1}{4} - \frac{1}{4} = 2, & \text{if } r \in (r_1, r_2).
\end{cases} \quad (A.34)
\]

The numerical result suggests that for the parameter \(r\) above the Hopf curve in \((\beta, r)\) plane (see Figure A.17), and \(r \in [r_{hp}, r_2]\), there are two eigenvalues with positive real parts. For \(r = 0.9, 1\), the eigenvalues are \(\lambda = 0.058 \pm 0.6i, 0.029 \pm 0.588i\), respectively.

### A.4 Extensions of the analysis

So far we have focused on one example of a DDE, the delayed logistic equation, and assumed that the coupling between each compartment and the bulk is the same (symmetric coupling). In this section, we briefly explore extensions of our analysis to (a) asymmetric coupling and (b) a PDE–DDE model based on the Mackey–Glass equation [100]. In the latter case, we show that most of our results still hold, but that the dominant mode for large \(D\) is now the antiphase solution rather than the in-phase solution.
**A.4.1 Asymmetric coupling**

Let $\beta_1$ and $\beta_2$ denote the diffusive coupling between the two compartments and the bulk. In previous sections, we took $\beta_1 = \beta_2 = \beta$ (symmetric coupling). Here we briefly explore what happens in the case of asymmetric coupling, $\beta_1 \neq \beta_2$. Equations (A.1) and (A.2) become

\[
\frac{\partial C}{\partial t}(x, t) = D \frac{\partial^2 C}{\partial x^2}, \quad 0 < x < L, \ t > 0,
\]

\[
D \frac{\partial}{\partial x} C(0, t) = \beta_1 (C(0, t) - X_1(t)),
\]

\[
-D \frac{\partial}{\partial x} C(L, t) = \beta_2 (C(L, t) - X_2(t)), \quad (A.35)
\]

and

\[
\frac{dX_1}{dt}(t) = \beta_1 (C(0, t) - X_1(t)) + f(X_1(t), X_1(t - \tau)), \quad (A.36a)
\]

\[
\frac{dX_2}{dt}(t) = \beta_2 (C(L, t) - X_2(t)) + f(X_2(t), X_2(t - \tau)). \quad (A.36b)
\]

It can be checked that the nonnegative steady-state solutions are $X_1 = X_2 = C(x) = 0$ and $X_1 = X_2 = C(x) = 1$. Although the steady-state solution is the same as the case when $\beta_1 = \beta_2$, the amplitude and phase of the oscillations at the two end compartments are sensitive to the coupling parameters $\beta_1$ and $\beta_2$; see Figure A.18. For asymmetric coupling, the two oscillators are less likely to be synchronized. Firstly, the phases of the solutions can be different. Secondly, the weakly coupled oscillator has a relatively larger amplitude; see Figure A.18 (a, b, d). The difference of the amplitudes decreases when the asymmetry of the two coupling strengths gets smaller; see Figure A.18 (a, b).

To explore the effect of asymmetric coupling on the Hopf bifurcation, we take $\beta_2 = 1$ and plot the Hopf bifurcation curves in the $(\beta_1, \tau)$ plane for different values of $D$ in Figure A.19. For $D = 0.2$ and $D = 0.3$, there are two Hopf branches, which have different limiting values of time delay as $\beta_1$ goes to 0; see Figure A.19 (a). As $\beta_1 \to 0$, the Hopf bifurcation curve (blue) has the critical time delay $\tau \to \pi/2$ which is the critical time delay of the delayed logistic equation. This suggests that diffusion has a weak effect on the time delay of the weakly coupled compartment $X_1$. As $\beta_1 \to 0$, the time delay on the second Hopf branch (dashed green line) is the same as the critical time delay of the model given by equations (A.35) and (A.36) with $\beta_1 = 0$. The two Hopf branches separate the $(\beta_1, \tau)$ plane into three different regions; see Figure A.19 (a). In region 1, below the Hopf curve, the
Figure A.18. Change of the phase and amplitude of the oscillations with different values of the coupling parameter $\beta_1$. The other coupling parameter $\beta_2$ is fixed to be 1. (a,b) $\beta_1 = 0.1$ and $\beta_1 = 0.5$. The oscillator with a weaker coupling has a larger amplitude. The difference between the amplitudes becomes smaller as $\beta_1$ increased from 0.1 to 0.5. (c) Symmetric coupling $\beta_1 = \beta_2$. The two oscillators have the same amplitude and phase. (d) Strongly coupled oscillators with $\beta_1 = 2$. The difference of the amplitude or phase is small when the coupling strength is strong at both ends. Initial conditions: $X_1(t) = X_2(t) = 1.1, C(x,t) = 1$. Parameters: $D = 0.2, \tau = 2.5, \beta_2 = 1$. Other parameters are the same as in Figure A.1.

steady-state solution is stable. In region 2, above the Hopf curve, the steady-state solution is unstable and there are periodic solutions of $X_{1,2}$ with different phases and amplitudes. For the sake of illustration, we take $\beta_1 = 0.1$, and plot the numerical solution with time delay below or above the critical time delay in Figure A.19 (c,d). In region 3, there exist an in-phase oscillation and oscillations with different phases; see Figure A.18 (b,c). In particular, the in-phase oscillation occurs when $\beta_1 = \beta_2$.

In Figure A.20, we compare the numerical solutions for $\beta_1 < \beta_2$ and $\beta_1 = \beta_2$. For $\beta_1 = \beta_2$, if $\tau$ is below the second Hopf branch, then the numerical solution with initial condition $X_1 = X_2$ converges to the steady state; if $\tau$ is above the second Hopf branch, then the solution changes to periodic solutions with different phases; see Figure A.20 (a,b).
For $\beta_1 < \beta_2$, the numerical solution of $X_{1,2}$ starts with the same phase and amplitude but ends up with different phases and amplitudes; see Figure A.20 (c,d).

### A.4.2 PDE coupled with the Mackey–Glass equation

In this section, we replace the delayed Logistic equation (A.3) by another classical delay differential equation, namely, the Mackey–Glass equation [100]. Let

$$f(x(t), x(t - \tau)) = a_1 \frac{x(t - \tau)}{1 + x^n(t - \tau)} - a_2 x(t).$$  \hfill (A.37)

If $a_2/a_1 > 1$, then $f$ has a positive solution at

$$x = \sqrt[n]{a_1/a_2 - 1}.$$

Suppose that we set $a_2 = 1$. Sample Hopf bifurcation curves of the Mackey–Glass equation

$$\frac{dX}{dt} = a_1 \frac{X(t - \tau)}{1 + X^n(t - \tau)} - a_2 X(t)$$

are shown in Figure A.21 (a). For both $n = 10$ and $n = 15$, the critical time delay decreases as $a_1$ increases. For $a_1 = 2$ and $n = 10, 15$, the critical time delay is $\tau \approx 0.47, 0.27$, respectively.
Figure A.20. Numerical solution with different $\tau$ and $\beta_1$ close to $\beta_2$. (a, b) $\beta_1 = \beta_2$ and $\tau = 2.2, 2.5$, respectively. The solution converges to the steady state for a smaller time delay $\tau = 2.2$ while it converges to a periodic solution (in-phase) for $\tau = 2.5$. The time delay $\tau = 2.2$ is below the time delay on the second Hopf branch; see Figure A.19 (a). (c, d) $\beta_1 = 0.9 < \beta_2$ and $\tau = 2.2, 2.5$, respectively. The numerical solution of $X_{1,2}$ starts with the same phase and amplitude but ends up with different phases and amplitudes. Initial condition: $X_1(t) = X_2(t) = 1.2$ and $C(x, t) = 1$. Other parameters: $\beta_2 = 1$, $D = 0.2$.

We then take $a_2 = 2$ and consider the full PDE–Mackey–Glass model (A.1-A.2) with $f$ given by the equation (A.37). The Hopf bifurcation curves are plotted in Figure A.21. The critical time delay increases as the diffusion coefficient increases, which is similar to our result of the PDE–Logistic model; see Figure A.1. On the other hand, in contrast to the PDE–Logistic equation, the mode of the oscillation emerging from a Hopf bifurcation is antiphase rather than in-phase for a wide range of diffusion coefficients. This result suggests that the oscillation mode depends on the explicit form of the delay differential equations. For $D = 0.5$ and $n = 10$, the numerical solution with different time delays near the critical time delay $\tau_{hp} \approx 0.55$ is shown in Figure A.21 (d-e). The existence of oscillation for $\tau = 0.57 > \tau_{hp}$ suggests that the Hopf bifurcation is supercritical. The oscillation modes are both antiphase for $\tau = 0.57$ and $\tau = 0.6$. Although an in-phase oscillation is also observed for $\tau = 0.6$, we find that it is unstable with respect to perturbations near the initial condition $X_1 = X_2 = 1$. 
A.5 Conclusions

In this appendix, we analyzed a one-dimensional PDE–DDE model consisting of a pair of delayed logistic equations or Mackey–Glass equations coupled by one-dimensional bulk diffusion. We used linear stability analysis to derive the associated characteristic equation and then solved this equation numerically to plot the Hopf curves as a function of various model parameters. In the parameter regions above the Hopf bifurcation curves, our numerical results suggest that there are two different oscillation modes (reflecting the exchange symmetry of the system): in-phase and antiphase. The selection of these modes is sensitive to the diffusion coefficient, the time delay, and the explicit form of delay feedback.
APPENDIX B

NUMERICS

In this appendix, we briefly present the numerical simulation methods used in our model for growth cone membrane polarization via microtubules. Firstly, we describe the numerical scheme used to solve the 2D active transport model given by equations (3.1a) and (3.1b), and how we handle a discontinuous velocity field. Secondly, we describe the numerical scheme used to solve the 2D reaction diffusion model of stathmin given by equations (3.10) and (3.11). The average MT length is calculated by evaluating the integrals (3.46) and (3.17) using the Trapezoidal Rule.

B.1 Simulation of the active transport model

For simplicity, we consider the square domain \( \Omega = [0, L] \times [0, L] \). The method can easily be generalized to a rectangular domain \([0, L] \times [0, R]\). \( \Omega \) is divided into \( N^2 \) cells \( \{ \Omega_{ij} \}_{i,j=1..N} \) with (see Figure B.1)

\[
\Omega_{ij} = [x_{i-\frac{1}{2}}, x_{i+\frac{1}{2}}] \times [z_{j-\frac{1}{2}}, z_{j+\frac{1}{2}}].
\]

Each cell is centered at \((x_i, z_j) = ((i - \frac{1}{2})h, (j - \frac{1}{2})h)\) where \( h \) is the space step. The solution of \( c(x, z, t) \) and \( u(x, t) \) at each cell at time \( t = t_n \) is approximated by a cell average

\[
U^n_{ij} = \frac{1}{h} \int_{x_{i-\frac{1}{2}}}^{x_{i+\frac{1}{2}}} u(x, t_n) dx,
\]

\[
C^n_{ij} = \frac{1}{h^2} \int_{\Omega_i} c(x, z, t_n) dx dz.
\]

Let \( k \) be the time step, a classical finite volume scheme is given as

\[
C^n_{ij} + 1 = C^n_{ij} - \frac{k}{h} (F_{i+\frac{1}{2}j} - F_{i-\frac{1}{2}j} + F_{i,j+\frac{1}{2}} - F_{i,j-\frac{1}{2}}),
\]

where the numerical flux \( F \) is defined by

\[
F_{i,j-\frac{1}{2}} = v_{ij-1} C^n_{ij} - \frac{D}{h} (C^n_{ij} - C^n_{ij-1}), \quad F_{i-\frac{1}{2}j} = -\frac{D}{h} (C^n_{ij} - C^n_{i-1,j}),
\]

\[
F_{i,j+\frac{1}{2}} = v_{ij} C^n_{ij+1} - \frac{D}{h} (C^n_{ij+1} - C^n_{ij}), \quad F_{i+\frac{1}{2}j} = -\frac{D}{h} (C^n_{i+1,j} - C^n_{ij}).
\]
Here we use the upwind scheme for advection flux. The advection term is treated explicitly for computational cost while the diffusion term is treated implicitly for stability.

The no flux boundary condition at \( x = 0, L \) and \( z = 0 \) gives

\[
F_{2,j} = F_{N+2,j} = F_{i,1} = 0, \quad \text{for } i, j = 1, \cdots, N. \tag{B.4}
\]

The flux conservation condition at \( z = R \) requires that

\[
F_{i,N+1/2} = k_+ C^n_i - k_- U^n_i, \quad \text{for } i = 1, \cdots, N. \tag{B.5}
\]

The reaction term is treated explicitly for simplicity.

The difficulty of solving the equations numerically is to deal with the jump discontinuity of velocity function \( \nu(x, z) = \nu_0 H(\phi(x) - z) \) at the interface \( \{z = \phi(x)\} \). One way to solve the interface problem is to use the smoothing method. The idea is to approximate the discontinuous function \( \nu(x, z) \) by a sequence of smooth functions \( \nu_\epsilon(x, z) \). One choice for smoothing the Heaviside function is

\[
H_\epsilon(x) = \frac{1}{1 + e^{-2x/\epsilon}}.
\]

The smoothing function of the velocity function \( \nu(x, z) \) is then constructed as

\[
\nu_\epsilon(x, z) = \nu_0 H_\epsilon(\phi(x) - z). \tag{B.6}
\]
B.2 Simulation of the stathmin model

We solve the reaction diffusion system of the stathmin model given by equations (3.10) and (3.11) in the two space dimensions by the alternating direction implicit method. It consists of two steps of solving the 2D equations explicitly in one spatial direction and implicitly in the other spatial direction. For the first step, we discretize the diffusion term implicitly in the x-direction and explicitly in the z-direction and solve it for time \( t_n + 1/2 \). For the second step, we discretize the diffusion term implicitly in the z-direction and explicitly in the z-direction and solve it for time \( t_{n+1} \). The scheme is presented below.

For \( 1 \leq i \leq N \),

\[
\frac{S_{on}^{n+1/2}(i,j) - S_{on}^n(i,j)}{dt/2} = D_s \frac{S_{on}^{n+1/2}(i+1,j) - 2S_{on}^{n+1/2}(i,j) + S_{on}^{n+1/2}(i-1,j)}{dx^2} \\
+ D_s \frac{S_{on}^n(i,j+1) - 2S_{on}^n(i,j) + S_{on}^n(i,j-1)}{dz^2} \\
- k_{on} \frac{S_{on}^n(i,j) + S_{on}^{n+1/2}(i,j)}{2}.
\]

For \( 1 \leq j \leq N \),

\[
\frac{S_{off}^{n+1/2}(i,j) - S_{off}^n(i,j)}{dt/2} = D_s \frac{S_{off}^{n+1/2}(i+1,j) - 2S_{off}^{n+1/2}(i,j) + S_{off}^{n+1/2}(i-1,j)}{dx^2} \\
+ D_s \frac{S_{off}^n(i,j+1) - 2S_{off}^n(i,j) + S_{off}^n(i,j-1)}{dz^2} \\
- k_{off} \frac{S_{off}^n(i,j) + S_{off}^{n+1/2}(i,j)}{2}.
\]

\[
\frac{S_{on}^{n+1}(i,j) - S_{on}^{n+1/2}(i,j)}{dt/2} = D_s \frac{S_{on}^{n+1}(i+1,j) - 2S_{on}^{n+1/2}(i,j) + S_{on}^{n+1/2}(i-1,j)}{dx^2} \\
+ D_s \frac{S_{on}^n(i,j+1) - 2S_{on}^n(i,j) + S_{on}^n(i,j-1)}{dz^2} \\
- k_{on} \frac{S_{on}^n(i,j) + S_{on}^{n+1}(i,j)}{2}.
\]

\[
\frac{S_{off}^{n+1}(i,j) - S_{off}^{n+1/2}(i,j)}{dt/2} = D_s \frac{S_{off}^{n+1}(i+1,j) - 2S_{off}^{n+1/2}(i,j) + S_{off}^{n+1/2}(i-1,j)}{dx^2} \\
+ D_s \frac{S_{off}^n(i,j+1) - 2S_{off}^n(i,j) + S_{off}^n(i,j-1)}{dz^2} \\
- k_{off} \frac{S_{off}^n(i,j) + S_{off}^{n+1}(i,j)}{2}.
\]
The reflecting boundary conditions at $x = 0, L$ and $z = 0$ are discretized as

$$S^n_{\text{off}}(0, j) = S^n_{\text{off}}(1, j), \quad S^n_{\text{off}}(N + 1, j) = S^n_{\text{off}}(N, j), \quad S^n_{\text{off}}(i, 1) = S^n_{\text{off}}(i, 0),$$

$$S^n_{\text{on}}(0, j) = S^n_{\text{on}}(1, j), \quad S^n_{\text{on}}(N + 1, j) = S^n_{\text{on}}(N, j), \quad S^n_{\text{on}}(i, 1) = S^n_{\text{on}}(i, 0).$$

The boundary condition at $z = R$ is discretized using a first-order accurate approximation to the derivative with respect to $z$ and an explicit method for the reaction term. That is, at the second step,

$$- \frac{D_s}{\delta} S^{n+1}_{\text{off}}(i, N + 1) - S^{n+1}_{\text{off}}(i, N) = k_{\text{on}} S^{n+1/2}_{\text{off}}(i, N) - r_{\text{on}}(i) k_{\text{off}} S^{n+1/2}_{\text{on}}(i, N),$$

$$- \frac{D_s}{\delta} S^{n+1}_{\text{on}}(i, N + 1) - S^{n+1}_{\text{on}}(i, N) = -k_{\text{on}} S^{n+1/2}_{\text{off}}(i, N) + r_{\text{on}}(i) k_{\text{off}} S^{n+1/2}_{\text{on}}(i, N).$$
REFERENCES


