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Insights from modeling metabolism and amoeboid cell motility in the immune system

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Chapter 1

Introduction

Multicellular organisms, including plants and animals, continuously struggle with infections by bacteria, fungi and viruses. In animals, infections are cleared by the innate and acquired immune system. Despite the successes of biomedical research in fighting infectious diseases, many disease processes are still little understood. In this thesis, we will focus on two processes involved in fighting infections: metabolism, and immune cell motility and navigation. Specifically, we use mathematical and computational models to address questions surrounding these processes. In this chapter, we first explain the link between metabolism and immune cell motility, before we introduce both the main questions and modeling methods for these subjects.

1.1 Cell migration and metabolism: linked by infection

During infection, metabolism shifts from maintenance of the body to fighting the infection. This happens at a single cell scale as well as on tissue or organism scale. Examples of common global changes are the production of heat in inflamed tissues or fever on the scale of the entire body [1]. Some infectious diseases, for example tuberculosis, are associated with wasting, a rapid and involuntary loss of muscle and fat tissue and a still little understood metabolic change. As these tissues can serve as energy storage, the breakdown of these tissues is paired with the release of a large amount of energy. It is yet unclear which processes would require such an amount of energy and which changes in metabolism allow for this. A potential process that is under debate is whether immune cells in tuberculosis lesion exhibit the Warburg effect, which is the rapid conversion of glucose into lactate, despite the availability of oxygen to fully oxidize the glucose [2]. Furthermore, it is unclear how wasting is induced: by the pathogen/infection or by the host [3]?

Local changes in metabolism can be seen in many types of immune cells and occur at different stages of infection clearance; immune cells, such as leukocytes or cytotoxic T lymphocytes, first have to locate pathogens and then neutralize them. A number of different metabolic demands are the production of ATP to fuel cell migration, the production of reactive oxygen species and other compounds that destroy the pathogen, and cytokine production to recruit more cells to infected areas [4, 5].

However, some metabolic changes in immune cells will not occur unless the immune system finds the pathogen within the body. Detection of pathogens is thought to happen by random encounters of immune cell and pathogen. Navigation through a tissue is greatly influenced by the type of motility immune cells display and determines the occurrence of such random encounters [6, 7]. How immune cells such as lymphocytes and leukocytes navigate through a tissue is a topic of ongoing research. The extracellular matrix (ECM), the network surrounding cells within tissues, is thought to play an important role [8]. As a framework around cells in tissues, it can function as a scaffold for cells to push off from, as well as a physical barrier blocking cell movement. Besides that, the ECM also plays a role in signaling, both by binding signaling molecules and by its composition and stiffness. By modeling immune cell motility, we can untangle the roles of the ECM and study the effect of the ECM as a substrate, an obstacle or its signaling function separately.

1.2 Metabolism

In studying metabolism, there are two main questions: which metabolites are present and at what rate are they being converted into one another. Considering the changes in metabolism during infection, and the wide range of involved processes, we should not be limited to a small set of metabolites. Metabolomics can study a wide array of molecules using techniques such as NMR and mass-spectrometry, and thus answer the first question. Combining metabolomics data with other data and methods can be used to study reactions rates, so called systems metabolomics [10]. A rough indication of the present enzymes and, by proxy, fluxes, can be obtained through gene expression and proteomic data [11]. Detailed flux information can be derived from metabolomics combined with isotope labeling. However, this requires good prior knowledge of the metabolic network [12]; by extensive calculation of fluxes and the corresponding isotope distributions along the network, one can match fluxes to the measured

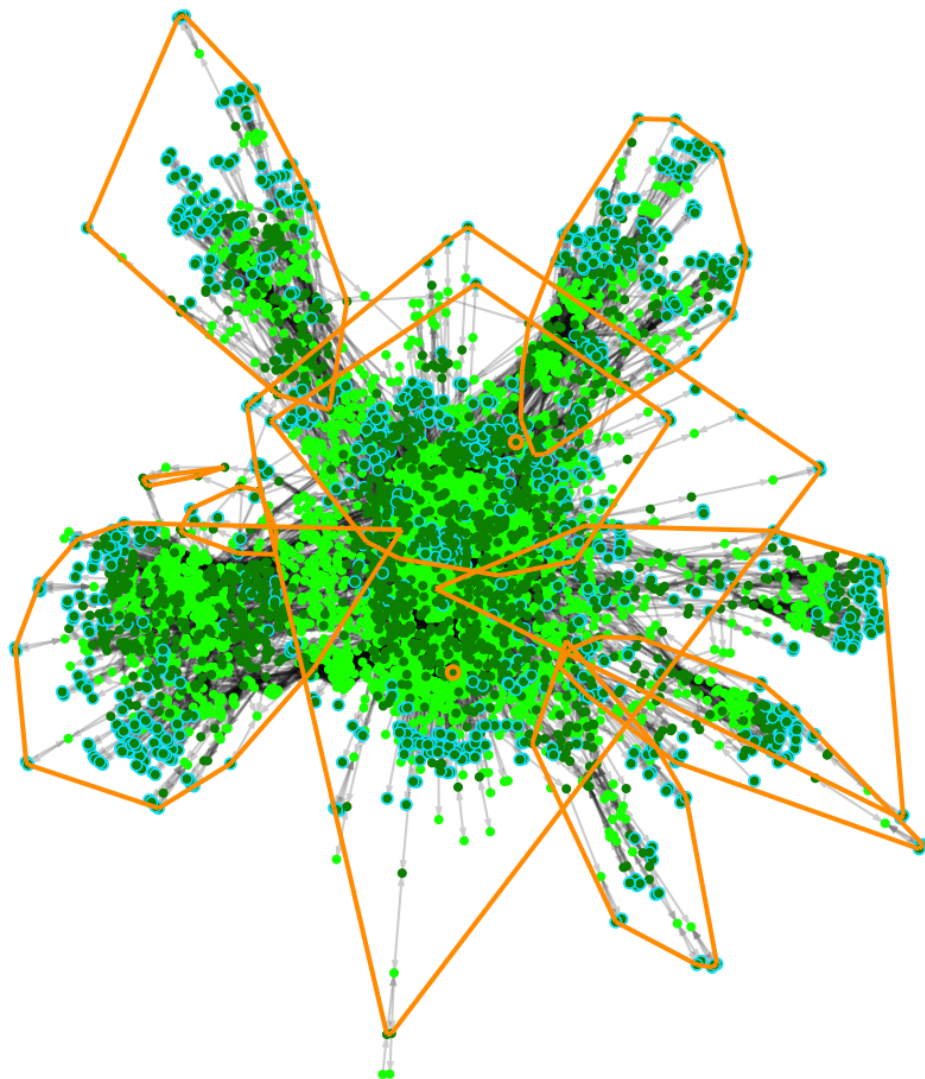


FIGURE 1.1: **Visualization of the metabolic network from ZebraGEM 2.0.** Light green nodes: reactions. Dark green nodes: metabolites (with cyan lining: end point metabolites). Orange boxes: cellular compartments. Visualization made using ModelExplorer [9].

isotope labeled metabolomics data [13]. This prior knowledge, collected into a metabolic model, can be used for other modeling efforts as well.

Aside from being a computational tool in isotope labeling, metabolic models can be used as a predictive tool of how internal and external changes alter an organism's metabolism. Current models of metabolism exist varying in the level of details. Kinetic models are systems of coupled ordinary differential equations that describe the change of metabolites over time. Often, they are limited to single pathways for which they can give detailed time-dependent predictions of metabolite concentrations and metabolite flows [14]. They come, however, at the cost of large numbers of parameters. These parameters can be obtained through experiments, and a kinetic *S. cerevisiae* metabolism model [15] and, more recently, a kinetic model of *E. coli* core metabolism [16] have been established. Nonetheless, this requires extensive studies, as kinetics are influenced by substrate concentration, temperature, pH, and many other factors. Another angle is taken in constraint-based metabolic models, or stoichiometric models. As the stoichiometry of many metabolic pathways is known, the metabolic network can be represented by a stoichiometric matrix. Constraint-based metabolic models explore the properties of the network, and how the network itself and physical and chemical properties constrain fluxes through that network [17]. They can predict the most efficient metabolic pathway to create certain metabolites or alternative pathways in case of disruption by blocking or limiting certain reactions.

The most commonly used method with constraint-based modeling is Flux Balance Analysis (FBA) [18]. The main assumption of this method is a steady state of internal metabolites; hence, flux balance. As the concentration of metabolites, c , is governed by $\frac{dc}{dt} = S \cdot f$, with S the stoichiometric matrix corresponding to the metabolic network and f the fluxes through that network, this assumption can be written as $\frac{dc}{dt} = S \cdot f = 0$. This gives a system of linear equations. However, usually this system of equations is underdetermined, resulting in a multidimensional solution space with an infinite number of solutions (Figure 1.2). This brings us to another important assumption: the organism optimizes metabolism for a certain metabolic objective. This metabolic objective depends on the research question, but frequent objectives are maximization of biomass production (i.e. growth), maximization of energy production (i.e. ATP production), or maximal production of a specific desirable compound like a drug compound. In addition, metabolites flowing in and out of the system are limited, resulting in additional constraints. With these two assumptions and the constraints, the model then gives the following linear programming problem (LP) that can be solved computationally:

Optimize

$$f_{obj}$$

such that:

$$\begin{aligned} S \cdot f &= 0, \\ a_i &\leq f_i \leq b_i \end{aligned}$$

The solution of that LP problem is a set of reaction fluxes that ascertain steady state and optimize the objective.

The main limitation of FBA is that it predicts fluxes in equilibrium with available nutrients. However, in most natural systems, nutrient availability varies over time. Extending the FBA with a simple system of ordinary differential equations, one can do dynamic FBA [19], and account for the fluctuations of nutrients and growing organisms, described by the following equations:

$$\frac{dc}{dt} = S \cdot fB, \quad \frac{dB}{dt} = f_B B, \quad (1.1)$$

where B denotes the biomass of the organism and f_B is the growth rate per unit biomass obtained from computing the fluxes. One example of where dynamic FBA is applied, is the modeling of small populations of bacteria. Although dynamic FBA is a dynamic model, the time scale is coarser than for kinetic metabolic models, hence allowing for the use of FBA as an intermediary step. Another limitation of FBA is that it gives only a single solution from the usually large solution space. The outlines of this solution space can be explored by using flux variability analysis (FVA, Figure 1.2). Here, aside from optimizing the objective, each single reaction in the network is minimized and maximized [20], which draws the contours of the solution space.

Lastly, for many organisms, and especially multicellular ones, data on nutrient availability is hard to obtain or even define, and finding the right constraints is impossible. However, other data can be collected much easier, such as gene expression data, or proteomics and metabolomics data. These data can be integrated into the constraints-based modeling framework as additional constraints. Multiple methods on how to incorporate experimental data in constraints-based modeling have been developed, depending on the specific type of data; gene expression profiles [21, 22], proteomics data [23, 24] and metabolomics data [23, 25, 22, 26].

Essential to the integration of gene expression data is that the genes are represented in the model. Most of these constraint-based models, especially

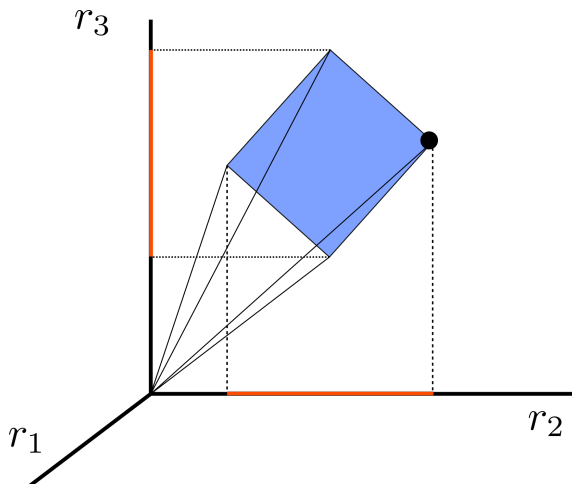


FIGURE 1.2: Solution space of constraints based models. Constraints on the fluxes ($r_{1,2,3}$) result in a convex subspace that represents feasible flux distributions. Optimizing a certain objective, as in FBA, gives an optimal solution space (blue face) of which LP solvers give a single point (black point). By individually minimizing and maximizing each flux within the optimal solution space (FVA), one can draw the contours (red projections on r_2 and r_3 axis) of the optimal solution space.

in recent years, are so called whole-genome scale metabolic reconstructions and, hence, contain all metabolic genes, or at least all suspected metabolic genes, of an organism. The corresponding metabolic reactions then form the metabolic network. By including all metabolic reactions, these model encompass an organism's metabolic network. The relation between reactions and genes is described by gene-protein-reaction associations (GPR) [27]. Many organisms are already captured in such whole-genome scale metabolic reconstructions. Most of those organisms are unicellular organisms, such as a multitude of *E. coli* strains [28] and *S. cerevisiae* [29]. Multicellular organisms, and of special interest for studying immune response, vertebrates, are less well represented. To current date, whole-genome scale metabolic reconstruction of human [30], mouse [31], Chinese hamster [32], rat [33], zebrafish [34, 35], and cod [36] have been assembled. Although the metabolic diversity among vertebrates is limited in comparison to the zoo of reconstructions of unicellular organisms, organism specific models are still preferable. Essential nutrients differ among organisms, think of vitamin C that is essential for humans but not for most other vertebrates, and inositol, which zebrafish are unable to produce themselves [37]. Furthermore, genetic structure between vertebrates is sufficiently different to

justify separate reconstructions, as GPRs become dramatically different.

1.3 Cell migration

The metabolic demands of immune cells are diverse and change during the infection. Powering cell migration is an important demand from the beginning of infection. Immune cells have to be able to find the pathogen. Both how they manage to propel themselves and how they navigate through tissues play a role in their ability of finding the pathogen. Here, we shortly discuss how immune cells move, how to analyze cell motility and distinguish different types of motilities, and finally, how cell motility is modeled.

1.3.1 Amoeboid motion

Most eukaryotic cells move by reorganizing their internal structure, the cytoskeleton, and hence their shape. This type of motion is called amoeboid motion. Amoeboid cell motility is driven by actin polymerization (Figure 1.3). Actin filaments are polarized and polymerization occurs mainly at one end. Due to thermal fluctuations of the cell membrane, actin subunits can be added in the space between an existing filament and the membrane. When an actin subunit is added to the actin filament, its attached ATP is hydrolyzed. This releases energy and combined with the elongation of the actin filament, this results in a force that can be used to push the cell membrane forward [38].

The polymerizing actin is organized in a network, an actin front. The size and shape of this network can be seen in the deformation of the cell. Actin fronts pushing out the cell membrane form a flat structure called a lamellipodium. Lamellipodia vary in size and stability. Very broad and stable lamellipodia are seen in keratocytes and these cells often cover very straight trajectories [39]. Other cells, for example the cellular slime mold *Dictyostelium*, have multiple, forking lamellipodia which they sometimes retract, and hence, change direction more often than keratocytes [40].

With so many regulating compounds, cells can also swiftly react to external signaling. The best known example of this is chemotaxis, where cells migrate towards or away from a compound such as food, oxygen or signaling molecules. Membrane located receptors influence in migration direction by relaying their signals to the actin polymerization regulating factors [41] such as the Arp2/3 complex which influences branching of actin filaments [42], and the antagonistic small GTPases Rac1 and RhoA which determine cell polarization [43]. Another cue that relays to the actin machinery is durotaxis, where the cell responds to the stiffness of the

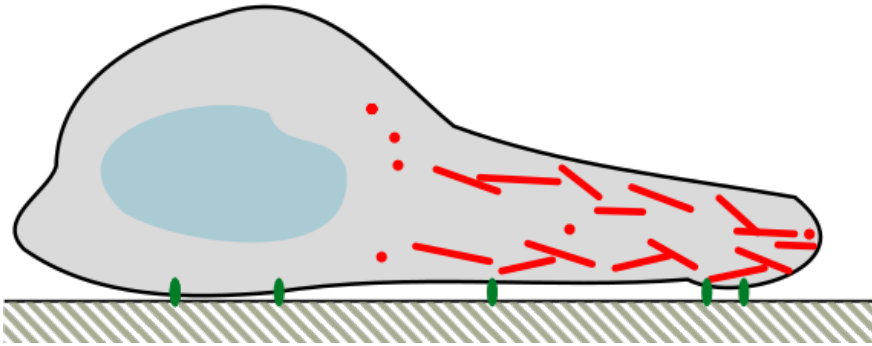


FIGURE 1.3: **Schematic view of actin polymerization within a lamellipodium** Red: actin filaments and actin monomers. Blue: cell nucleus. Green: cell-matrix adhesion complexes. Shaded gray: substrate with extracellular matrix. Actin filaments are polymerized at the front (right) of the cell. This generates a force that can push the cell membrane outwards. Actin filaments attached to the ECM can transfer the force more efficiently to outwards motion.

extracellular matrix through focal adhesions, protein complexes connecting the cytoskeleton to the ECM [44]. In this thesis, we argue that topotaxis, guidance by topographical cues in the environment, could also work by changing the actin polymerization, simply by putting obstacles in the way of cells.

1.3.2 Analysing immune cell motility

There are many determining characteristics in cell motility. Hence, describing cell motility by just a single characteristic is insufficient to fully capture the behavior of a motile cell. The most simple characteristic of cell motion is speed. Defining an instantaneous speed requires some considerations. Using a short time frame to define instantaneous speed can measure fluctuations of the cell membrane which do not contribute to actual cell displacement, and overestimates cell speed. On the other hand, measuring instantaneous speed with too large time steps disregards actual short excursions of the cell, and underestimates cell speed. Hence, choosing a time frame for computing the instantaneous velocity requires some consideration. For experimental data, there are additional constraints in the feasible spatial and time resolution.

The trajectory of cells can be described as a random walk: they regularly stop and move into a new direction. Random walk theory has brought forth

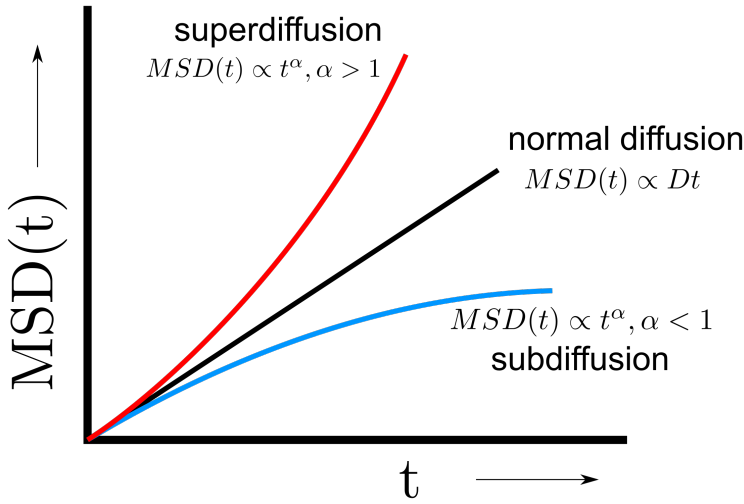


FIGURE 1.4: Mean squared displacement curve of diffusive, superdiffusive and subdiffusive processes

a number of characteristics and methods that we can apply to study cell motility. The first measure we discuss is the mean squared displacement (MSD). It is a measure of the deviation of the position of a random walker, particle or cell from a reference position over time, defined by:

$$MSD(t) = \langle |x(t) - x_0|^2 \rangle, \quad (1.2)$$

where the mean is taken either over a number of cells or over multiple time points. The MSD can be interpreted as the area explored by a population of cells in a given time and is closely related to the concept of diffusivity.

By computing the MSD of a large number of cell trajectories, information can be extracted from the curve of the MSD. If the MSD increases linearly over time, the observed cells are performing a Brownian random walk. Einstein and Sutherland derived independently that the MSD of Brownian walkers is given by

$$MSD(t) = 2nDt, \quad (1.3)$$

with n the dimension in which the walk takes place, and D the diffusion constant of the walker [45, 46]. Hence, from a linear MSD, we can straightforwardly compute the diffusivity of the cells.

Brownian walkers do not show any correlation in their step directions. However, some cells are able to maintain their direction for some amount

of time. This is called persistence. One can recognize persistent random walkers when the MSD curve shows a more than linear increase with respect to time, i.e. $MSD(t) \propto t^\alpha$ with $\alpha > 1$ (figure 1.4). This is called superdiffusion. The time scale at which $\alpha > 1$ is an interesting matter. For persistent random walkers, $\alpha > 1$ on short time scales, but on longer timescales the walker still perform a Brownian walk and $\alpha = 1$. The diffusivity is then no longer related to the slope of the MSD curve only. Instead, for persistent random walkers a different expression gives us the MSD, the so called Fürth's equation [47]:

$$MSD(t) = 2v_0^2\tau t - 2(v_0\tau)^2(1 - e^{-t/\tau}), \quad (1.4)$$

with v_0 an intrinsic cell velocity and τ the cell persistence time, i.e. the time frame in which the cell keeps moving in the same direction. The effective diffusivity of these walkers in two dimensions is given by $\frac{1}{2}v_0^2\tau$. So, the cell can explore more area by increasing its intrinsic velocity, or by increasing its persistence time. Random walks with $\alpha > 1$ on all time scales are called superdiffusive. A good example are Lévy walkers [48], which have fractal-like property.

A less than linear increase in MSD with time ($\alpha < 1$) is also possible and is called subdiffusion (Figure 1.4). Usually, subdiffusion is caused by some physical constraint on the cell, like limited space in which the cells can move around or forces keeping the cell in place.

Cell persistence can also be measured by the meandering index. This index is defined as the net displacement divided by the total displacement of a cell:

$$\frac{\|x_T - x_0\|}{\sum_{i=1}^T \|x_i - x_{i-1}\|},$$

with T the duration in time steps of the cell trajectory. A high index corresponds to straighter trajectories, and a low index to more convoluted trajectories. An important factor is the time resolution, similar to the computation of cell speed. Too low resolution will overestimate the index, and higher resolutions will most likely always result in a lower index.

Finally, we can define the 'directional bias' as the population mean velocity in a direction of interest. If the mean is equal to zero, there is no bias in that direction, and if the mean is significantly different from zero, there is. Directional bias can arise by factors such as chemotaxis and other forms of taxis.

1.3.3 Modeling amoeboid motion

In analysis of cell tracking data, a common question is the characterization of the type of cell motility observed in the data: which random walk recapitulates the same statistical properties as the cell tracking data [49, 50, 51, 52]? These types of analyses show that T cells display a Brownian walk, persistent random walk, Lévy walk or subdiffusive walk, depending on the tissue they reside in [53, 54, 55, 56, 57], but they give no insight in what mechanisms underlie those different types of motility.

Mechanistic models have focused on how the cellular components such as actin, myosin, Arp2/3 and other actin network regulating factors, focal adhesion complexes, and feedback from Rac and Rho signaling and phosphoinositide contribute to cell polarization and cell locomotion [58, 59, 60, 61, 62]. We cannot possibly describe all the work, and hence direct the reader to some reviews on this topic [63, 64].

In this thesis, we are interested in studying the effect of cell-ECM interactions on the type of cell motility using mathematical models. We have a number of requirements for our model: 1) we want a model that includes cell-ECM interactions on a subcellular scale, 2) the effects of cell-ECM interaction remain localized within the cell and hence we want our model to integrate intracellular processes and cell and cytoskeleton plasticity, and 3) the model should be coarse-grained enough that we can simulate sufficiently many cell tracks for statistical analysis. The combination of these three requirements further requires us to restrict to cell motility models that take the shape of the cell into account. These types of models can be found in the modeling framework of phase field models [65, 66, 67] and the Cellular Potts model [68]. Phase field models are a good tool in understanding the role of different components in cell motility such as adhesion, contractility and actin polymerization, yet, they are computationally quite expensive which makes them less suitable for the generation of cell trajectories. In this thesis, we use the Cellular Potts modeling framework, described in detail in Chapters 3 and 4, and shortly introduce it here.

1.3.4 Cellular Potts model

The Cellular Potts model, also known as the Glazier-Graner-Hogeweg model, is a lattice based model of deformable cells [68]. A single cell consists of a connected set of lattice sites having spin, or cell ID, denoted by σ . Cells move by both copying their σ into neighboring lattice sites or other σ 's being copied into their lattice sites (Figure 1.5). Copy attempts are done iteratively by a Metropolis algorithm. The algorithm selects a random lattice site u and one of its neighboring lattice sites v . If $\sigma(u) \neq \sigma(v)$, $\sigma(v)$

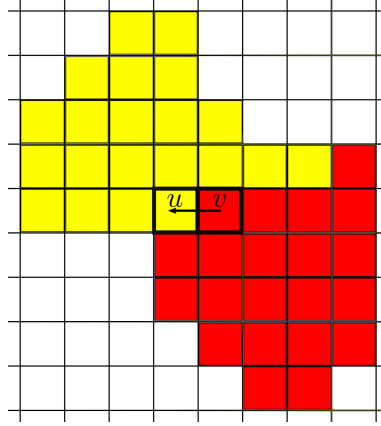


FIGURE 1.5: **Copy attempt in the Cellular Potts model** A configuration of the CPM with a yellow and red cell. A random lattice site u and one of its neighbors v are chosen. As $\sigma(u) \neq \sigma(v)$, lattice site u can obtain $\sigma(v)$ causing the red cell to expand and the yellow cell to retract. Whether this copy attempt is actually successful depends on the Hamiltonian $\mathcal{H}$.

can be copied into u . Whether this copy attempt is accepted depends on the balance of forces described in the Hamiltonian $\mathcal{H}$. If a copy attempt decreases $\mathcal{H}$, it is always accepted. For copy attempts which increase $\mathcal{H}$, the Boltzmann probability is used, which allows for stochasticity in cell movement. In total, the probability of a successful copy attempt is given by:

$$P(\Delta\mathcal{H}_{v \rightarrow u}) = \begin{cases} 1 & \text{if } \Delta\mathcal{H} < 0 \\ e^{-(\Delta\mathcal{H}/T)} & \text{if } \Delta\mathcal{H} \geq 0 \end{cases}, \quad (1.5)$$

where T denotes a temperature of the system. For the Metropolis algorithm, a number of consecutive copy attempts equal to the total number of lattice sites is called a Monte Carlo step (MCS) and is used as a time measure. Within one MCS, each lattice site is expected to have been updated exactly once. Rejection-free versions of the Metropolis algorithm, which exclude sampling over neighbors with identical σ , have modified definitions of the Monte Carlo step [69], but are more efficient in computation time.

The Hamiltonian describes the balance of forces in the model. Terms included in the Hamiltonian can be varied to include cellular mechanisms of interest, such as adhesion energies between cells and between cell and medium, area constraints to ensure cell area, or perimeter constraints to influence cell shape. An example Hamiltonian including these terms could

look like this [70]:

$$\mathcal{H} = \sum_{x,y} J_{\sigma_x \sigma_y} (1 - \delta_{\sigma_x, \sigma_y}) + \lambda_{\text{area}} \sum_{\sigma} (a_{\sigma} - A_{\sigma})^2 + \lambda_{\text{perimeter}} \sum_{\sigma} (p_{\sigma} - P_{\sigma})^2. \quad (1.6)$$

Some dissipative processes are not represented in the Hamiltonian directly, but are accounted for in $\Delta\mathcal{H}$ as additional work terms. One example is chemotaxis. A very simple chemotaxis algorithm for the CPM is to compare concentrations C at u and v such that $\Delta\mathcal{H}_{\text{chem}} = \chi(C(v) - C(u))$, where χ denotes the strength of chemotaxis [71]. This influences the probability of a successful copy attempt by replacing $\Delta\mathcal{H}$ in Eq. 1.5 by the sum $\Delta\mathcal{H}_{\text{total}} = \Delta\mathcal{H} + \Delta\mathcal{H}_{\text{chem}}$.

Cell motility in the CPM with the Hamiltonian described by Eq. 1.6 is limited to random passive fluctuations of the membrane and has low diffusivity. Multiple extensions of the CPM have been developed to simulate actively moving cells [58, 72, 59, 73]. In this thesis, we mainly use the Act model to model actively moving immune cells [73]. This extension models actin dynamics in a phenomenological way: the branching and polymerizing actin network pushes the membrane outward. This is represented in the Act model through so called Act values: aside from keeping track of the σ of each lattice site, each lattice site u now also has an Act value $Act(u)$. These values indicate how recently that lattice site was added to the cell and can be viewed as a measure of actin activity. More recently active sites can be viewed as containing polymerizing actin network pushing against the membrane, and cell extension from lattice sites with a local neighborhood of high Act values is favored. Similarly, retraction at such lattice sites is suppressed, according to:

$$\Delta\mathcal{H}_{Act}(u \rightarrow v) = \frac{\lambda_{Act}}{\max_{Act}} \left(\sqrt{\prod_{y \in \text{NB}(u)} Act(y)} - \sqrt{\prod_{y \in \text{NB}(v)} Act(y)} \right). \quad (1.7)$$

Here, λ_{Act} is a parameter determining the strength of this process in relation to the other terms in the Hamiltonian. Once a lattice site is added to a cell, that site will obtain the maximum Act value $\max_{Act}$. This initiates a feedback loop where further extension at the same edge of cell remains favored, resulting in cell polarization. The use of the geometric means in Eq. 1.7 ensures that neighborhoods where all Act values are high are favored and neighborhoods with ‘holes’ are nullified. A biological interpretation for this is that actin subunits contribute to growth of the actin network by attaching to the already existing network. By using the geometric mean, attachment of actin subunits to the existing network is enforced.

By tuning the parameters $\max_{Act}$ and λ_{Act} , the stability and strength of the cell front can be tuned. This results in persistent random walks with a variety in persistence times, and different motility behaviors can be observed, such as *Dictyostelium*-like motion and keratocyte-like motion.

1.4 Thesis overview

This thesis is organized as follows. In Chapter 2, we present ZebraGEM 2.0, an improved whole-genome scale metabolic reconstruction for zebrafish. The improvements include the addition of GPRs and the oxidative phosphorylation pathway, and make it possible to use the model for knock-out studies, simulating respiration experiments and predicting changes in metabolism based on gene expression data. We specifically study zebrafish metabolism upon infection with *Mycobacterium marinum* integrating gene expression data from control and infected zebrafish larvae.

The following chapters will focus on cell motility in response to the environment and revolves around the question how environmental input shapes and guides cell motility. Chapter 3 explores the different types of lymphocyte motility that can arise by the interactions between cell and extracellular matrix. We introduce an extension of the Act model with adhesion dynamics that can both show cell motion on a short time scale as well as derive statistical properties of cell motility. We observe a range of motility modes: 1) Brownian walks, 2) stick-and-slip walks where cells alternate between sufficient adhesion and too little adhesion to gain traction, 3) highly persistent walks, and 4) short-term persistent long-term subdiffusive walks.

In Chapter 4, we focus on a different role of the environment, namely how obstacles in the environment can guide cell movement. Immune cells have to navigate within tissues around the other cells in the tissue as well as the extracellular matrix. A model system for the role of obstacles in cell motility has previously been set up to study *Dictyostelium discoideum* cells in a gradient in density of cell sized pillars [74]. The *Dictyostelium discoideum* cells display actin-driven amoeboid motility, very similar to immune cells, and are shown to be guided by this gradient in pillar density from areas with high pillar density to area with low pillar densities. This process is called topotaxis: cell movement guided by topographical cues. Previous work on Active Brownian particles, which perform a persistent random walk, similar to the *Dictyostelium* cells, has shown that part of the topotaxis effect can be explained by altered cell persistence in the pillar grid [75]. However, the extent to which Active Brownian particles perform topotaxis

is lower than measured in *Dictyostelium* cells. We hypothesize that the amoeboid motility of *Dictyostelium discoideum* is better captured by the Act model and show that both deformable cell shape and active reorientation upon collision make cells more efficient at topotaxis.

The final chapter on cell motility is Chapter 5, but this also marks our return to zebrafish. Here, leukocyte trajectories from a tail-wounding assay in zebrafish larvae are analyzed. Two mutants in the TLR-signalling pathway are studied and we show that leukocyte migration towards the wounded area is significantly lowered in the mutant compared to wild type.

Finally, we conclude this thesis in Chapter 6, where we discuss the results of this thesis and propose ideas for future work.

