Mathematical models for mechanically induced morphogenetic pattern formation

Nesenberend, D.N.

Chapter 1

Introduction

Have you ever looked up to the sky to see a flock of birds glide through the sky? Or have you turned on a nature documentary to see a school of fish in the water? Did you see them moving together, forming shapes and evading predators? In both these cases, we have individual units, birds or fish, that make their own decisions based on their environment. By signaling and responding to each other, these individual units can collectively be something larger, which has many benefits for these species.

Essentially, your body is somewhat comparable to this flock of birds or school of fish; it consists of tens of billions of cells, all working together seamlessly to help you work, dance, eat and right now, read this thesis. These cells can be viewed as individual units; they can even continue to live when they are isolated in the lab. Similarly to birds and fish, cells can respond to their environment and give signals to other cells.

I still remember how intrigued I was when I first learned about cells and their communication methods. However, there was a question that was never really answered: **How is it possible that such a large amount of individual building blocks can work together to form one functioning, cohesive body?** What I soon realized was that much is actually unknown about the mechanisms behind the collective behavior of cells and that we are far from understanding the human body as a whole. It is very challenging to find these exact mechanisms, especially when solely using an experimental approach.

Mathematical modeling can be a useful tool in unraveling the mechanisms behind collective cell behavior, particularly when used in conjunction with experimental research. When a hypothesis is implemented on the cellular or even molecular level using mathematical formulation, we can predict what the results are on a larger scale or collective level. This thesis studies the mathematics of *morphogenesis*, the emergence of biological growth and form. Before discussing the details of the mathematics that we have used to study morphogenesis, we will briefly explain the basics of the underlying biology.

1.1 Morphogenesis

The body forms from what was once a single fertilized egg cell, over time forming complex shapes and structures needed for the body to function. To form a multicellular structure, the single cell needs to grow and divide many times. In this progression, cells must become the right type through a process known as *cell differentiation*. As part of cell differentiation, cells adopt a certain shape to function optimally. For example, intestinal cells exhibit a highly deformed surface morphology that increases their surface area, allowing for more efficient nutrient absorption compared to a flat cell surface [20].

The formation of properly functioning organs and tissues depends on the collective organization of many cells. The formation of these complex structures relies on cells sending and receiving signals, which help to determine, for example, their differentiation fate and migration patterns [17]. Analogously to the cellular scale, organs and tissues need to have a certain shape to function optimally. For example, the bones surrounding your lungs (ribs) are more flat and have a cage-shaped structure to protect many organs it surrounds, while the bones of the legs are long and strong to allow walking and dancing [19]. The process of cells, tissues, and organs forming into particular shapes and structures is called *morphogenesis* [40]. Morphogenesis does not occur exclusively during embryonic development. For example, morphogenetic processes must occur during wound healing [149]. Furthermore, (deregulation of) morphogenetic processes can play an important role in diseases such as cancer. An interesting example is angiogenesis, the formation of new blood vessels from an existing one, a natural process that a tumor can hijack to be supplied with extra nutrients and oxygen.

In this section, we dive deeper into the building blocks and signaling regulators needed for morphogenesis. We discuss these two things separately, but there is much overlap. The goal is not to give a complete overview, but rather to highlight some of the most important players in morphogenesis in the context of this thesis.

1.1.1 Building blocks: cells and the extracellular matrix

There are many different types of cells in the body, all tuned to a specific task. Here we focus on some general cellular features that are important for (cellular) morphogenesis. The cell surface is called the cell membrane and is made up of a lipid bilayer that contains numerous proteins. The cell membrane has an important function of forming a barrier between the intracellular space and the extracellular environment, regulating the exchange of substances [32]. The cell membrane also plays a role in the structural integrity of the cell, particularly in dynamic processes such as cell division [75].

The shape of the cell membrane is strongly influenced by the cytoskeleton. The cytoskeleton consists of different types of filamentous protein structures that form a network-like architecture throughout the cytoplasm, connecting to the cell membrane and inducing mechanical stability [35]. Since the cytoskeleton is constantly changing and adapting, it plays a role in dynamically shaping the cell and movement [35]. One type of cytoskeletal protein of interest is the family of septins, recognized for their

essential role in cell division [9] and its ability to bind to the cell membrane [130]. In the next section, we will explain the function of septin in more detail.

Surrounding the cells, there is a fibrous, network-like structure referred to as the extracellular matrix (ECM). The ECM consists of many different types of fibers [52], providing strength to tissues and organs. Cells can bind to the ECM with protein clusters that are embedded in the cytoplasm, called focal adhesions [124]. Cells actively regulate the ECM by breaking it down and producing it accordingly. The quantity and composition of the ECM varies throughout the body [66].

1.1.2 Signaling regulators: morphogens and mechanics

Crucial to morphogenesis is the ability for cells to communicate with one another and to sense and respond to their environment. One specific cue that cells can respond to and use for communication is through morphogens, a generic name introduced for all types of molecules that regulate morphogenesis [135]. An example of a type of morphogen is the Wnt growth factor that can form a gradient throughout tissues, regulating cell fate, for example, in central nervous system development [113]. Another interesting type of morphogens are the vascular endothelial growth factors (VEGFs), that, for example, can be secreted by tumors to activate blood vessel cells nearby to grow new blood vessels towards the tumor [24]. Many morphogenetic processes are guided by morphogens that *activate* and morphogens that *inhibit* a certain cell type and/or protein expression to make spatial patterning possible, for example, in embryonic finger development [107]. We will further discuss the mathematics behind these activator-inhibitor processes in Section 1.2.1.

A protein type that influences morphology on the cellular level is septin. This family of cytoskeletal proteins is known to influence cell shape by inducing local curvature of the cell membrane, but interestingly enough, septin is also responding to the local curvature. In addition to septins, a wide range of other morphogens can modulate or be modulated by curvature of the cell membrane [63, 43] or tissue [155, 53], indicating that curvature is an important morphogenetic cue.

Next to curvature, mechanical cues in the sense of stiffness, stress and strain are also important in regulating morphogenetic processes. For example, hydrostatic pressure in the lungs leads to a strain in cells, inducing cell differentiation [153]. The ECM plays an important role in giving and transmitting mechanical cues [82]. In vitro experiments of cells on ECM-like material have shown that cells spread more on stiffer material [13, 104] and can follow a stiffness gradient, in a process called *durotaxis* [61]. Furthermore, cells can respond to an external stretch by orienting parallel to the strain [119]. Cells have been proposed to sense the mechanical properties of ECM through their focal adhesions, where the strength of this connection depends on the build-up force [111]. We explain the concepts of stiffness, stress, and strain in more detail in Section 1.2.5.

1.2 Modeling techniques

In the above we have discussed a number of key building blocks and signaling regulators that play a role in a certain morphogenetic process; however, it is hard to understand how these factors work together to build to a particular morphology. Mathematical modeling is a suitable tool to use when trying to understand key mechanisms at play in a particular morphogenetic process. Modeling can help explain the pattern-forming processes observed in nature or in experiments, but can also function to test the sufficiency of a particular hypothesis (e.g., do these factors and their interactions suffice to yield a particular spatial structure?) or discriminate between main issues and side issues.

One way of modeling is cell-based modeling [92]. Cell-based models formulate single cell behavior in mathematical rules and simulate the system with many cells to understand collective cell behavior over time. This technique is used in the Cellular Potts Model, an energy based, discrete approach, that we elaborate on in Section 1.2.4. Another method would be to describe the dynamic behavior of cells and morphogens as a continuum, using partial differential equations (PDEs), which offer the advantage that they can be analyzed. In Section 1.2.1 we explain the theory behind reaction diffusion equations, a type of PDEs commonly used in morphogenesis. In Section 1.2.3 we explain how to derive evolution equations from the energy of the system. We discuss two theoretical frameworks that can be used to include mechanics in morphogenetic processes; continuum mechanics to address stiffness, stress and strains (Section 1.2.5) and differential geometry that can be used to handle (tissue-/ cell-) surface shape and concept such as curvature (Section 1.2.2).

1.2.1 Reaction-diffusion equations

Morphogens are integral to many morphogenetic processes. Morphogens can work as regulators by activating an inhibiting cellular processes. An important method of transport for morphogens is by *diffusion*. Diffusion is the passive spreading of molecules through a liquid; for example, when you put a tea bag in a mug with hot water, you see the tea slowly releasing from the bag and moving through the mug until a uniform concentration is obtained. The interactions between morphogens and their spreading can be modeled using *reaction-diffusion equations*, with general formulation:

$$\frac{\partial u}{\partial t} = D\Delta u + f(u). \tag{1.1}$$

In equation (1.1), $u \in \mathbb{R}^n$ is a vector where each component represents, for example, a morphogen or cell concentration distribution over space and time. The notation $\frac{\partial u}{\partial t}$ represents the speed of change of u. The diffusion is described by the Laplace operator Δ that reduces to $\Delta u = \frac{\partial^2 u}{\partial x^2}$ when one spatial dimension is modeled. The diffusion constant D is a diagonal matrix that describes the speed of diffusion per component. Furthermore, f is a function that describes the interactions between the components of u.

Alan Turing was in 1952 the first to show that a slow diffusing (i.e. short-range) activator and a quick diffusing (i.e. long-range) inhibitor can form patterns, in the sense of spatially inhomogeneous concentration quantities [135]. This somewhat counterintuitive finding, in the sense that diffusion would be expected to lead to homogeneous concentration distributions, has had a great impact on research in morphogenesis. Since this seminal paper, many different reaction-diffusion based pattern forming models have been developed [122, 38, 45], which have been used to better understand numerous morphogenetic processes [2, 87, 89]. Reaction diffusion equations are part of a larger class of *evolution equations*, i.e. ordinary differential equations (ODEs) or partial differential equations (PDEs) that describe the temporal evolution of some state. In Sections 1.3.1 and 1.3.2 we discuss analytical methods that can be used to study evolution equations, which are most relevant in the context of this thesis.

1.2.2 Differential Geometry of curves and surfaces

In many morphogenetic processes, tissue and cell shape play an important role, not only because morphogenesis is by definition a shape-forming process, but also since geometric cues can be key regulators in this process. Differential geometry can be used to mathematically describe the shape of a biological surface in a continuous way.

We can reduce the complexity of the model by studying a curve instead of a surface, which can already provide useful insights into the morphogenetic process. Generally, a *parametrized smooth plane curve*, is a smooth map $\gamma : I \to \mathbb{R}^2$ for an open interval $I \subset \mathbb{R}$ [120]. However, to simplify analysis, we focus throughout this thesis on curves that can be written as a *graph*, $\gamma(x) = (x, h(x))$, where $h : I \to \mathbb{R}$ smooth enough. An important concept for a curve in the context of morphogenesis is *curvature*, that describes the rate at which the curve turns. For the graph representation, we can derive the curvature $K(x)$ to be

$$K(x) = \frac{h_{xx}}{(1 + h_x^2)^{3/2}} \qquad [120].$$

Analogously, we can define a parametrized smooth surface as the smooth map $X : U \to \mathbb{R}^3$, where $U \subset \mathbb{R}^2$ is an open set [120]. Extending the definition for curvature to a surface is not straightforward. First, we need to explain some other important geometric concepts when working with surfaces. In every point P on the surface we can define two vectors,

$$\mathbf{e_i} \coloneqq \partial_i \mathbf{X}, \quad i \in \{1, 2\},$$

which are both tangent to the surface that span the *tangent plane* [27]. Together with the *unit normal vector*,

$$\mathbf{n} = \frac{\mathbf{e_1} \times \mathbf{e_2}}{\|\mathbf{e_1} \times \mathbf{e_2}\|}$$

the tangent vectors provide a local coordinate system [27]. Figure 1.1 shows how to obtain the *normal curvature* in a certain direction. Here, we take the normal curvature in point P in the direction of vector t, that is tangent to the surface. Vector t and unit normal vector $\mathbf{n}$ uniquely select a plane that "cuts" through the surface in a curve. The normal curvature in the direction of $\mathbf{t}$ can now be computed by taking the curvature of that curve in point P. The two extrema of the normal curvature over all directions are called the *principal curvatures*. The product and half the sum of the principal curvatures are called the *Gaussian curvature* and the *mean curvature*, respectively.

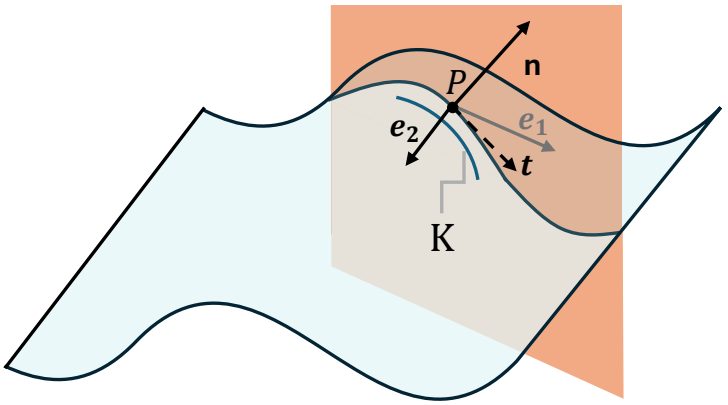

Figure 1.1: Explanation of normal curvature in point P in direction of vector t.

1.2.3 Energy functionals and calculus of variations

In some morphogenetic processes, the dynamic behavior of, for example, a morphogen concentration or a tissue surface is unclear from conceptual understanding or empirical data; however, it can be apparent which states are more energetically favorable than others. In such cases we can use an *energy functional* to assign a number (energy value) to a function or a curve [37]. An example of an energy functional is the Helfrich energy,

$$J = \int_{\Gamma} \left(\kappa_c(2H - C_0)^2 + \bar{\kappa}_c K\right) dA, \tag{1.2}$$

that takes a surface Γ (which can represent, for example, a cell membrane) and assigns the energy based on its geometry [51]. The functional (1.2) uses the deviation of the local mean curvature H from a spontaneous curvature C_0 that is a constant, the Gaussian curvature K and constants κ_c and $\bar{\kappa}_c$.

It is expected that a system goes to a minimum of the energy functional to obtain the most energetically favorable state, however how and at what speed an energy is minimized cannot be inferred from the functional itself. One possible method to derive

an evolution equation from an energy functional is through an L^2-gradient flow,

$$\frac{\partial y}{\partial t} = -\frac{\delta J}{\delta y}, \tag{1.3}$$

for a general functional $J[y]$ of function y. In equation (1.3), $\frac{\delta J}{\delta y}$ represents the *variational derivative*, i.e. "a derivative of a functional". We can compute the variational derivative using

$$\left\langle \frac{\delta J}{\delta y}, p \right\rangle = \lim_{\epsilon \to 0} \frac{J[y + \epsilon p] - J[y]}{\epsilon},$$

where $p(x)$ is a *test function* and $\langle , \rangle$ the L^2 inner product [91]. Next to this continuous approach, we can also use discrete energy-based models, for example the Cellular Potts model, discussed in the next section.

1.2.4 Cellular Potts Model

The *Cellular Potts model* (CPM) is an energy-based on-grid model, often used to describe cell shape and collective cell behavior. For the two-dimensional CPM, we work on a square lattice $\Lambda \subset \mathbb{Z}^2$, where to every lattice site $\vec{x} \in \Lambda$ is assigned a number $\sigma(\vec{x})$ (see Figure 1.2) [44]. One cell is described by a usually connected patch of lattice sites, that are all assigned the same number; $C(p) = \{\vec{x} : \sigma(\vec{x}) = p, \vec{x} \in \Lambda\}$, for some value $p \in \{1, 2...N_c\}$, where N_c is the number of cells. The medium consists of all lattice sites $\vec{x}$ that are assigned number $\sigma(\vec{x}) = 0$. Different type of cells can be introduced by function τ that assigns a cell type to a cell number. To a certain CPM state, a Hamiltonian energy (H) is attributed,

$$H = \sum_{(\vec{x}, \vec{x}')} J\big(\tau(\sigma(\vec{x})), \tau(\sigma(\vec{x}))\big)(1 - \delta(\sigma(x), \sigma(x')) + \lambda \sum_{\sigma \in \{1,..N_c\}} (a(\sigma) - A(\tau))^2. \tag{1.4}$$

The first part of equation (1.4) represents the energetic favorability of cells in contact with each other (or with the medium). Here we sum over all pairs of neighboring lattice sites $\vec{x}$ and $\vec{x}'$. Furthermore, J is the contact energy and δ the Kronecker delta function to only include lattice sites from different cells. The second part of equation (1.4) keeps the cells from growing or shrinking extensively by penalizing deviations in surface area ($a(\sigma)$) from a resting area $A(\tau)$, with Lagrange multiplier λ. The dynamic evolution of the CPM states are now done as followed. We randomly choose a lattice site $\vec{x}'$ and a random neighbor $\vec{x}$ and we try to copy state $\sigma(\vec{x})$ into $\vec{x}'$. Then we compute ΔH, which is the difference in Hamiltonian H between before and after the *copy attempt*. If the copy attempt is energetically favorable, i.e. the energy difference is negative $\Delta H < 0$, we accept the copy attempt. If it is positive $\Delta H \geq 0$, we accept the copy attempt with the Boltzmann probability $P = e^{-\Delta H/T}$, where constant T is the *cellular temperature*. One CPM time step is called a *Monte Carlo step* and consists of $|\Lambda|$ copy attempts.

By tuning the contact energy, the CPM with Hamiltonian (1.4) can show the effect of cell sorting over time [44]. By extending this Hamiltonian and adding other biological components, the model can be used to elucidate numerous other morphogenetic processes, e.g. angiogenesis and vasculogenesis [98, 24]. A possible extension is adding a "work term" to the computed value of ΔH. This term can represent cellular activity and can be used to model processes such as durotaxis.

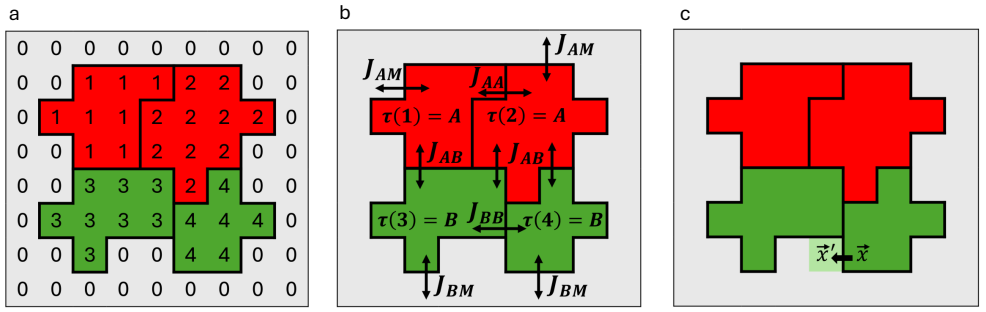

Figure 1.2: Graphical explanation of the Cellular Potts Model. **a.** Every lattice site is assigned a number ($\sigma(\vec{x})$), referring to the cell (or medium) that it belongs to. Here we have four different cells. **b.** Cells can belong to a particular set of cell types (τ). Here we have cell type A (red) and B (green), the gray area displays the medium. Contact between cells or cell and medium contributes to contact energy, that can differ depending on the cell type or medium involved (J_{AA}, J_{AB}, J_{AM} etc.). **c.** For the evolution of the cell states, we choose a lattice site $\vec{x}'$ and neighbor x and attempt to copy the number of $\vec{x}$ into $\vec{x}'$.

1.2.5 Continuum mechanics

Cells can sense and respond to mechanical cues such as stresses and strains in their environment, crucial for morphogenesis. Therefore, it can be relevant to take into account the mechanics of the ECM or the cell when trying to model a morphogenetic process. One way of doing that is by the theory of continuum mechanics. In this theoretical approach, individual molecules and particles are neglected and the material is treated as a continuum [108]. The main question is, how much will a certain material deform (*strain*), when a certain set of forces (*stress*) is working on it?

Here we discuss two types of deformation, normal and shear. Figure 1.3a shows a graphical description of a material under normal stress σ, that is defined as

$$\sigma := \frac{F}{A_0},$$

where F is the force and A_0 the initial area on which the force is exerted. The strain that is a result of normal stress is defined as

$$\epsilon := \frac{L - L_0}{L_0},$$

where L_0 is the initial length and L is the length after stress is exerted. Shear stress, τ comes from a force exerted parallel to a surface, resulting in shear strain γ, see Figure 1.3b. Shear stress and shear strain (using engineering notation) are defined as

$$\tau := \frac{F}{A_0} \quad \text{and} \quad \gamma := \frac{\Delta x}{L_0},$$

respectively, where F is the force, A_0 the initial area on which the force is exerted, L_0 the initial length and Δx the deformation. Note that when we do computations on materials with stresses and strains, these can vary point-wise, and the definitions need to change accordingly; taking derivatives instead of fractions and using stress and strain tensors, combining the different types of stress and strain in one formulation. For a more complete overview, we refer to [108].

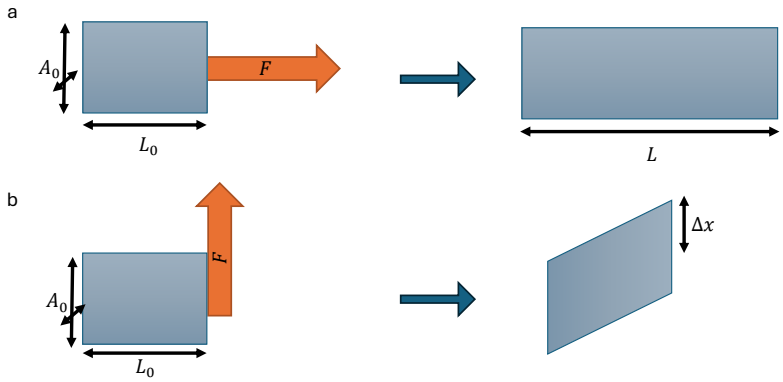

Figure 1.3: Graphical explanation of stress and strain. **a.** Deformation when normal stress is applied **b.** Deformation when shear stress is applied.

The amount of strain that is observed under a certain amount of stress differs per material. In Figure 1.4a we plot the stress-strain curve for a linear material. The slope of the stress-strain curve is defined as the *stiffness* of the material, which is called *Young's modulus* E when normal strain is used and *Shear modulus* G when shear strain is used. Interestingly, these two moduli are related with formula $E = 2G(1+\nu)$ when the material is isotropic. Here, ν is *Poisson's ratio*, a ratio that quantifies how much a material shrinks in one direction when it is extended in the perpendicular direction. Some materials, such as the ECM, do not have a linear relationship between stress and strain, but show *strain stiffening* i.e. an increase in stiffness when the material is more strained, see Figure 1.4b. The Finite Element Method (FEM) can be used to numerically solve the PDE that describes the relationship between stress and strain [64].

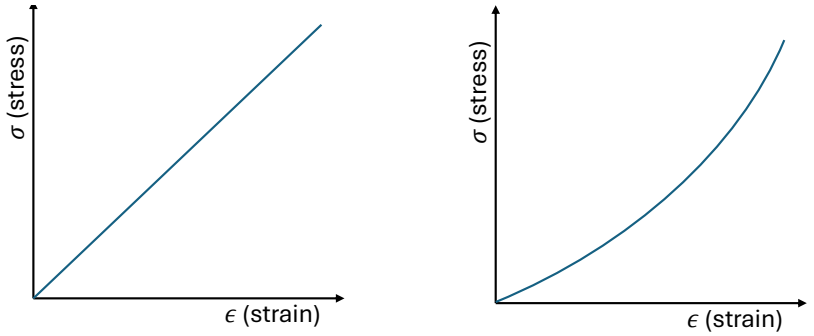

Figure 1.4: Stress strain curve. **a.** Linear relation **b.** Strain stiffening.

Continuous mechanics is a commonly used tool when modeling morphogenetic processes. For example, Ahmed et al. have simulated collective cell motility induced by mechanical stimuli in the context of wound healing [1]. Verhees et al. have described the deformation of the cell itself, connecting it to intercellular signaling processing [138]. Furthermore, van Oers et al. have combined numerical solution of a discretised continuum mechanics model (FEM) with the CPM to understand cellular network formation in in vitro experiments [98].

1.3 Model analysis methods

By analyzing the models presented in Section 1.2 we can determine the temporal (over time) and spatial (over space) behavior. Here we discuss some of the analysis methods used in this thesis, which are a combination of analytical (pen-and-paper) and numerical (computer-implemented) methods.

1.3.1 Asymptotic expansions

In Section 1.2.1 and 1.2.3 we showed continuous dynamic model approaches relevant in the context of morphogenesis. The set of PDEs used in such models can be difficult to study analytically, especially when nonlinear terms and higher-order derivatives are present. A viable strategy to study the system analytically is to employ the intrinsic scales of the processes. Some processes might be much faster than others, for example, the diffusion of a small morphogen versus a large morphogen or the propagation of a mechanical cue versus the diffusion of a morphogen. By appointing a small parameter (after rescaling), we can use analytical methods to study a system of differential equations.

Conventionally, ε is used to denote a small parameter. We often use notation $0 < \varepsilon \ll 1$, which means "for sufficiently small ε". When such a small parameter can be appointed, we can try to find the solution to the differential equation as an expansion in ε;

$$u = u_0 + \varepsilon u_1 + \varepsilon^2 u_2 + \mathcal{O}(\varepsilon^3). \tag{1.5}$$

The big O ($\mathcal{O}$) notation in equation (1.5) is defined as follows. For some functions f and ϕ we have that $f(\varepsilon) = \mathcal{O}(\phi(\epsilon))$ as $\varepsilon \downarrow 0$ if there exists constants $\hat{\epsilon}$ and k such that $|f(\varepsilon_0)| \leq |\phi(\varepsilon_0)|$ for all $\varepsilon_0 \in (0, \hat{\varepsilon})$ [55]. Note that when u is dependent on x and t expansion (1.5) might not be correct for all x and t, e.g. when $u_1 = t$, then the second term becomes $\mathcal{O}(1)$ for $t = \mathcal{O}(1/\epsilon)$.

In equation (1.5), we write solution u in terms of components that have the highest contribution to the solution, u_0, u_1 and u_2, but we neglect *higher order terms*, since the contribution of the $O(\varepsilon^3)$ terms are getting smaller and smaller when ε becomes smaller. By plugging in the expansion in the differential equation, we can sometimes find solutions to the lowest order terms, obtaining an approximate solution for u. Notably, the solutions for u_0, u_1, ... are typically not unique or consistent with the boundary conditions or initial conditions of the differential equation. Then it might be necessary to "glue" the solutions together using boundary layers or interior layers. These boundary and interior layers are typical examples of where the solution expansion is no longer correct.

1.3.2 Phase space analysis and Geometric Singular Perturbation Theory

It is not always necessary to find exact or approximate solutions to a system of differential equations when studying its behavior, as is the case when using asymptotic expansions. When dealing with a system of ordinary differential equations (ODEs), *phase space analysis* can be a useful alternative [88]. In this analysis method, every variable is represented by one of the axes, and the orbits in this space represent the solutions to the ODE. To explain this method, we use the following example of a system of ODEs

$$\begin{aligned} \frac{du}{dx} &= f_1(u, v) = v, \\ \frac{dv}{dx} &= f_2(u, v) = \alpha(u^3 - u), \end{aligned} \tag{1.6}$$

for variables u and v and parameter α. We will denote orbits (solutions to system (1.6)) by $\gamma(x; (u_0, v_0)) = (u(x), v(x))$, where (u_0, v_0) represents the initial value. First, we study orbits that consist of a single point, the *steady states* of the system, i.e. the variable values for which $\frac{du}{dx} = \frac{dv}{dx} = 0$. System (1.6) has steady states $P^0 = (0, 0)$, $P^+ = (1, 0)$ and $P^- = (-1, 0)$. Especially in the context of modeling a biological system, it is important to determine the *stability* of a steady state. When a steady state is stable, small disturbances to the state do not grow, instead the state will stay close to the steady state. The character of steady states can be determined using the *Jacobian matrix*,

$$Df = \begin{pmatrix} \frac{\partial f_1}{\partial u} & \frac{\partial f_1}{\partial v} \\ \frac{\partial f_2}{\partial u} & \frac{\partial f_2}{\partial v} \end{pmatrix} = \begin{pmatrix} 0 & 1 \\ \alpha(3u^2 - 1) & 0 \end{pmatrix},$$

by evaluating it in a steady state and computing its eigenvalues. When the real part of all eigenvalues is negative, the steady state is stable, but when the real part of at

least one eigenvalue is positive, the steady state is unstable. For $\alpha < 0$, the Jacobian evaluated in P^0 provides one positive and one negative eigenvalue, corresponding to a *stable manifold*,

$$W^s(P^0) := \{(u_0, v_0) : \lim_{x\to\infty} \gamma(x; (u_0, v_0)) = P_0\}, \tag{1.7}$$

and *unstable manifold* $W^u(P^0)$ (see definition (1.7) for $x \to -\infty$). When the real part of the eigenvalues of the Jacobian (e.g. P_0 for $\alpha < 0$) are unequal to zero, a steady state is called *hyperbolic*.

System (1.6) contains a conserved quantity, the *Hamiltonian*,

$$H(u, v) = \frac{1}{2}v^2 - \frac{\alpha}{2}\left(\frac{1}{2}u^4 - u^2\right),$$

that has property $\frac{dH}{dx} = \frac{\partial H}{\partial u}\frac{du}{dx} + \frac{\partial H}{\partial v}\frac{dv}{dx} = -f_2 f_1 + f_1 f_2 = 0$. From the Hamiltonian that describes the level curves of solutions in phase space and the other collected information we can derive a visual description of the phase space, see Figure 1.5. Figure 1.5 shows a range of types of orbits; γ_{hom} is a *homoclinic orbit*;

$$\lim_{x\to-\infty} \gamma_{hom}(x; (u_0, v_0)) = \lim_{x\to\infty} \gamma_{hom}(x; (u_0, v_0)) = P^0,$$

γ_{het} is a *heteroclinic orbit*;

$$\lim_{x\to-\infty} \gamma_{het}(x; (u_0, v_0)) = P^- \quad \text{and} \quad \lim_{x\to\infty} \gamma_{het}(x; (u_0, v_0)) = P^+,$$

and γ_{per} is a *periodic orbit*; there is a T, such that for all x

$$\gamma_{per}(x; (u_0, v_0)) = \gamma_{per}(x + T; (u_0, v_0)).$$

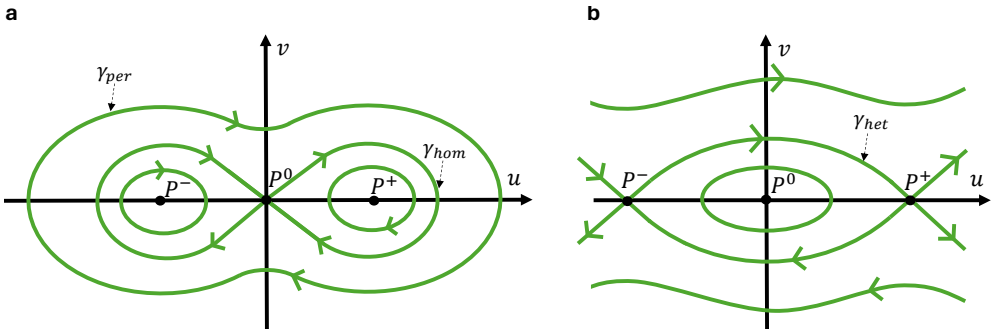

Figure 1.5: Sketch of the phase plane of system (1.6), **a.** for $\alpha < 0$; **b.** for $\alpha > 0$.

Interestingly, system (1.6) has an important link to reaction-diffusion equation (1.1) for one spatial dimension, one variable (u), $f = -f_2$ and for $D = 1$. That is, system (1.6) describes the steady state phase space of the reaction-diffusion equation, derived by setting $\frac{\partial u}{\partial t} = 0$ and $v := \frac{du}{dx}$. Using this approach, we can analyze the *existence* of steady states to a partial differential equation. In particular, existence does not mean that solutions are also *stable* with respect to time evolution, which is necessary for the solutions to be *observable*. To prove observability, more extensive analysis is necessary.

Unfortunately, when modeling morphogenetic processes, we often have to deal with more than one equation. If we for example have to analyze a system of two reaction-diffusion equations,

$$\begin{pmatrix} u_t \\ z_t \end{pmatrix} = \begin{pmatrix} d & 0 \\ 0 & 1 \end{pmatrix} \begin{pmatrix} u_{xx} \\ z_{xx} \end{pmatrix} - \begin{pmatrix} f(u,z) \\ g(u,z) \end{pmatrix}, \tag{1.8}$$

where $u_{xx} := \frac{\partial^2 u}{\partial x^2}$ and $u_t := \frac{\partial u}{\partial x}$, we obtain a steady state phase space of four dimensions, which is much harder to analyze than two. However, if we know from empirical observations that the diffusion of one of the variables is very small compared to the other parameters, we can utilize Geometric Singular Perturbation Theory (GSPT) to analyze the steady state phase space.

To briefly explain GSPT, we consider system (1.8), for $u_t = z_t = 0$ and $d = \varepsilon^2$. Setting $u_1 = u$, $u_2 = \varepsilon u_x$, $z_1 = z$ and $z_2 = z_x$, we obtain the following system of ODEs,

$$\begin{aligned} \varepsilon \frac{du}{d\xi} &= \hat{f}(u,z), \\ \frac{dz}{d\xi} &= \hat{g}(u,z). \end{aligned} \tag{1.9}$$

for small parameter $0 < \varepsilon \ll 1$, for $u, z \in \mathbb{R}^2$ and with smooth functions $\hat{f}$ and $\hat{g}$. By rescaling space, $\xi := \frac{x}{\varepsilon}$, we can reformulate system (1.9) as

$$\begin{aligned} \frac{du}{dx} &= \hat{f}(u,z), \\ \frac{dz}{dx} &= \varepsilon \hat{g}(u,z). \end{aligned} \tag{1.10}$$

System (1.9) and (1.10) are equivalent for $\varepsilon > 0$ and are called the *slow* and *fast* system respectively. However, since ε is very small, we can learn a lot about the dynamics of the system by setting $\varepsilon = 0$ and analyzing the *reduced* systems. If we set $\varepsilon = 0$ for the fast dynamics, system (1.10), we can deduce the collection of steady states given a constant z, to obtain the *critical manifold*, $\mathcal{M}_0 \subset \{\hat{f}(u,z) = 0\}$. Furthermore, we can obtain a collection of unstable and stable manifolds, see definition (1.7), that together define the stable and unstable manifolds of the critical manifold; $W^s(\mathcal{M}_0)$ and $W^u(\mathcal{M}_0)$.

To understand what happens when $0 < \varepsilon \ll 1$, we can utilize *Fenichels theorems* [33, 50]. These theorems precisely state when a critical manifold and its stable and unstable manifolds persist for $0 < \varepsilon \ll 1$ and, when they persist, that they stay close to their $\varepsilon = 0$ counterparts. Furthermore, we can use Fenichel's theorems to concatenate a slow piece of orbit (on the critical manifold) to a fast piece of orbit. The theory can be used to prove the existence of particular orbits for $0 < \varepsilon \ll 1$, for example a heteroclinic orbit that has a fast jump from one critical manifold to another.

1.4 Experiments and Collaboration

An extensive range of experimental approaches can be applied to study morphogenesis. Generally there are two types of experiments; *in vivo* meaning inside a living organism and *in vitro* meaning outside a living organism. The advantage of in vitro experiments is that certain interactions or processes can be studied in isolation from the rest of the body. It can be very useful to combine experimental research with mathematical modeling in an interdisciplinary project [73].

For this type of research project, where experiments and modeling are performed alongside each other, interdisciplinary collaboration is often necessary, which can present some challenges. Most research groups contain scientists that have the same field of expertise and most scientific workshops and conferences are attuned for scientists of a particular discipline, making it hard to meet possible experimental collaborators. Once a possible collaboration partner is selected, it might take some work to convince them that a mathematical modeling approach can be useful in the context of the experiments that are being performed. There is often a steep learning curve for both collaborative partners; "you need to learn how to speak each other's language" [25]. Since both types of research can have their own challenges and timelines, the project needs to be adjusted accordingly [116]. For example, not every physical quantity can be easily measured in an experimental setup and data can be noisy. On the flip side, mathematical models are limited in the sense that when all biological behavior is put in, the model is prone to overfitting and there are less possibilities for analysis e.g. more differential equations make phase space analysis increasingly more involved. As a mathematician, you need to be flexible to attune your modeling approach to what is the expected physical mechanism, something that can change when more experimental data is acquired.

Collaborative interdisciplinary projects, where mathematical modeling is used to further understand and develop experimental research do have many advantages. When designing a mathematical model for an explicit application, you are forced to think about the problem in a mechanistic way, which can already increase understanding of the physical problem. Then, the model can be used to test if a certain hypothesis can be enough to explain observed behavior. The model can also be used to predict outcomes for yet untested parameter regimes [148]. These outcomes can be used to design new experiments, that can test the predicted outcome of the model.

1.5 Thesis outline

In this thesis, we use a variety of different modeling and analysis techniques to further understand morphogenesis. We focus on morphogenetic processes where mechanical signals, curvature, stresses and strains play an important role. In the first part, we analytically study a generic model, to push the boundaries of the theoretical knowledge. Then, in Chapter 3 and 4 we present two interdisciplinary collaborative projects where models are explicitly designed to further understand a specific in vitro experiment. Since the work presented in these chapters is the result of interdisciplinary projects, chapters 3 and 4 are written for a broader audience. For example, in Chapter 4 we focus on biologically relevant outcomes in the result section and have chosen to present the analytical work in the supplementary section. The final chapter integrates elements from the two preceding chapters. Here we use a combination of numerical and analytical tools to better understand the in vitro observations. Where alignment is an emergent phenomenon in the Chapter 3, it is an input for the model in Chapter 4. In this section, we give an overview of the different chapters and explain which model techniques and methods are used.

In **Chapter 2**, we study a generic model for morphogenesis with curvature and a morphogen as the main signaling regulators. We describe the surface of a tissue or cell by a curve, using differential geometry in the graph representation (Section 1.2.2). From the Helfrich energy we derive an evolution equation for the curve, assuming an L^2-gradient flow (Section 1.2.3). The morphogen dynamics is assumed to be a reaction diffusion equation (Section 1.2.1), depending on the local curvature of the curve. The resulting system of differential equations, a mechanochemical model, describes a mechanism of an evolving curve with a morphogen diffusing on it, where the local morphogen concentration induces curvature and vise versa. We assume a global arclength constraint (i.e. the curve has a fixed length) and periodic boundary conditions that together induce a non-local inhibiting effect of the curvature; positive curvature in one place induces negative curvature in another place.

We use phase space analysis and GSPT to study the temporal steady states of the system of evolution equations (Section 1.3.2). Here we use that mechanical changes happen much quicker than diffusion. Depending on the strength of interplay between the morphogen and the curvature, we obtain a qualitatively different solution. For a weak interplay, we obtain a family of periodic orbits that lie exclusively on the critical manifold. For a strong interplay, we obtain a family of periodic orbits that jump between two critical manifolds, exhibiting a solution that has pieces of orbit that move slowly in space and that move quickly in space. Due to the graph assumption and periodic boundary conditions, the solution must adhere to a set of constraints to be observable. We show that the solutions do not adhere to these constraints when the small parameter is too small. Lastly, we use numerical simulation to study the observability and temporal stability of the steady state solutions further, observing the analyzed steady state orbits. This chapter introduces the first application of these analytical tools within the context of mechanochemical models.

Chapter 3 presents a collaborative project that employs a mathematical model to analyze a specific in vitro experiment. In this experimental approach, a synthetic hydrogel is synthesized that could serve as a representation of the ECM. Different from most synthetic hydrogels, such as the widely used polyacrylamides,, this hydrogel has strain-stiffening behavior; when the strain exceeds the critical strain, the material becomes stiffere with strain (with a certain strain-stiffening slope). If cells are cultured in the hydrogel, they align with their neighbors in a radial symmetric pattern, starting on the outside of the domain. Using the CPM (Section 1.2.4) coupled to a FEM that describes the stresses and strains of the hydrogel (Section 1.2.5), we model the behavior of the cells in the hydrogel. We assume that the hydrogel is an isotropic, linear elastic material, and we include strain stiffening by changing the response of the cells to stiffness through a function called 'perceived stiffness'. Furthermore, cells can interact with the hydrogel by actively exerting forces on the gel based on the shape of CPM and by responding to the perceived stiffness through a durotaxis term in the Hamiltonian.

The model indicates that there might be a radially symmetric prestrain on the hydrogel that the cells use to align radially. Furthermore, the model shows a qualitatively similar result as the experiments when testing different hydrogel compositions, namely, when decreasing the (normalized) strain-stiffening slope or increasing the stiffness, the cells show less radial alignment in both model and experiments. The model predicts that for lower cell concentrations, the radial alignment effect is less strong. To test this hypothesis, experiments were performed with lower cell concentrations, showing a qualitatively similar response as the model. These findings suggest that the radial alignment is a collective cell response; cells need each other to form this pattern.

Chapter 4 reports on a different collaborative project, where we study the cytoskeletal protein family septin. In vitro experiments with septins on lipid membrane surfaces with a fixed wavy geometry have shown that septins respond to curvature by reorienting themselves in the direction of positive curvature. Furthermore, when septins are incubated with deformable lipid membrane vesicles, they deform the surface, forming both stripe and spot patterns. We propose an energy-based, continuous mathematical model to describe the orientation of septin given the geometry of the surface. The energy is based on two assumptions. First, septins prefer to align themselves parallel to their neighbors. Second, septins respond to the geometry of the surface by trying to minimize the difference between the normal curvature in the direction of the septin (see Section 1.2.2) and the preferred local curvature, that is a constant. We use the L^2-gradient flow to derive the evolution equation of the septin orientation profile on a fixed geometry (Section 1.2.3). Numerical analysis of the model shows qualitatively similar results to the experimental results on fixed wavy surfaces when the strength of alignment with the neighbors is small and the preferred local curvature is positive. Since we can appoint a small parameter, we use asymptotic expansions (see Section 1.3.1) to study the steady state solution, increasing the understanding of the simulation results. To further study the response of the surface geometry to septin, we run simulations for surfaces with a wave and bump geometries, representing the stripe and spot

patterns respectively, varying in size and period. These simulations provide energy diagrams for both these pattern types, predicting the size and period of the bumps and waves and that the bumps prefer to go inwards. The numerical simulation on the bump geometry predicts septin orientation profiles on these experimentally untested surfaces.

1.6 Outlook

This thesis presents a variety of approaches that use mathematical modeling to study morphogenesis, both in a theoretical framework and in conjunction with experimental research. There are many possible avenues for continuing this research.

Guiding future experiments The models that we use for the projects executed in collaboration with experimental researchers show qualitative behavior that has not been observed experimentally. By extending the experimental approach, these model predictions can be tested, either strengthening the support for the validity of the model or possibly showing when the model fails, which can increase the understanding of the underlying biology. For example, in Chapter 3, the model predicts that for a very high strain stiffening slope, cells align perpendicular to the substrate boundary. By changing hydrogel composition, it might be possible to increase strain-stiffening slope and test this parameter regime. Moreover, in Chapter 4, experiments of septin orientation profiles have been done on one type of fixed surface geometry, namely the wave geometry. Currently, our collaborators are working on extending this research to alternative geometries. The model predicts different septin orientation profile depending on the size and period of the bump geometry, that can be tested experimentally.

Quantitative comparison and data fitting Throughout this research, every comparison between model and experimental research is qualitative; we focus on trends and general behavior; however, we do not fit the model to the data. To make the comparison stronger, it would be very good to perform parameter fitting and quantitatively compare the model with the data. For both projects, more quantitative data have to be obtained before such data fitting is possible. Parameters that are fitted to the data can provide better model predictions for yet unexplored experimental conditions.

Model extensions: new processes and new analysis When modeling a specific experiment, assumptions for the underlying processes have to be made. Some aspects of these processes can be described quite accurately by the model; in addition, the model can often be extended in order to study and increase the understanding of additional mechanisms that may underlie the observed biological behavior. For example, in Chapter 3 we model strain stiffening by adding perceived strain stiffening

to the cellular response. Cellular behavior can possibly be predicted more accurately when a strain-stiffening material is modeled directly. Furthermore, in Chapter 4, we do not directly model the deformations of the lipid membrane surface, when comparing to experiments where that is appropriate. The deformation of the surface can be added to the model in a similar approach as in Chapter 2. The resulting model can possibly be analyzed using GSPT, possibly proving the existence of the steady state solutions. The theoretical framework of Chapter 2 could also be extended further, by investigating the temporal stability of the solutions analytically.

Overall, the work presented in this thesis applies a variety of tools to address research questions in the field of morphogenesis. We show that analytical tools such as GSPT can be very useful when studying mechanochemical models. Furthermore, we demonstrate in the context of two specific experimental approaches how mathematical modeling in close collaboration with experimentalists can be very useful in increasing the understanding of the underlying mechanisms. Hopefully, this work can be an inspiration for studying a wide variety of problems in morphogenesis.