Anisotropy in cell mechanics

Schakenraad, K.K.

Chapter 1

Introduction

Physics and biology have traditionally been completely separated fields within the natural sciences. This started to change in the twentieth century, when state-of-the-art optical and electronic imaging technologies were first used to obtain a better understanding of biology at the scale of single molecules. The most well-known example is probably the discovery of the double-helix structure of DNA, using X-ray radiation, by Watson and Crick in 1953 [1] (see Figure 1.1a). Other examples include fluorescence microscopy [2], optical tweezers [3], magnetic tweezers [4], atomic force microscopy [5], and combinations of these methods [6]. Around the same time, physics and biology also started to get entangled at a theoretical level due to the introduction of mathematics in biology. Whereas the language of mathematics has always played a crucial role in physics since Sir Isaac Newton published his *Principia* in the seventeenth century, biology has traditionally been a purely experimental science. In the twentieth century this changed with the emergence of *mathematical biology*, a field that theoretically studies biological systems using mathematical tools. Applications of mathematical biology include pattern formation [7], population dynamics [8], and physical models of cells and tissues [9, 10].

More recently, physics and (mathematical) biology got further entangled when people started to realize that both fields could profit from closer collaborations. On the one hand, the enormous complexity of biological systems serves as an inspiration for both engineering and fundamental physics. From an engineering perspective, the ingenuity of biological materials serves as an inspiration for designing new man-made materials, with applications in robotics [11] and tissue engineering [12]. In fundamental physics, new theories are required to describe biological systems. Living entities, such as cells or entire organisms, actively consume energy to move, exert forces on their environment, and perform various other tasks. The challenge of understanding the physics of these living systems inspired the emergence of new areas of physics and mathematics, such as non-equilibrium statistical mechanics [13], pattern formation [7], and active matter [14].

On the other hand, insights from physics have greatly helped in getting a better understanding of experimental and mathematical biology at various scales. On the smallest length scales, the mechanical properties of DNA molecules have been elucidated both

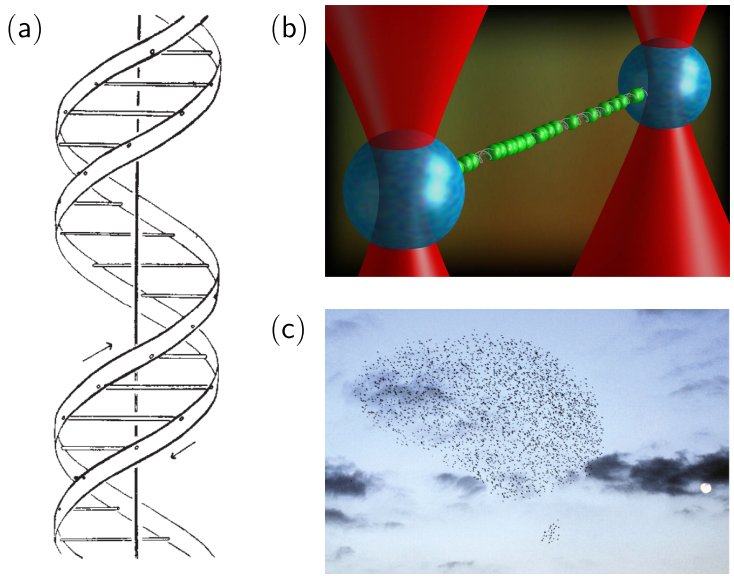

Figure 1.1. Examples of the interplay between physics and biology. (a) The double-helix structure of DNA was discovered by Watson and Crick using X-ray radiation. (b) Physical techniques and theories have helped to eludicate the mechanical propertiees of DNA. In this illustration, a DNA molecule is attached to two beads (blue) and stretched by moving two optical tweezers (red) apart. (c) Groups of living entities, such as a flock of birds, have inspired the field of active matter, a field in physics that studies matter that can actively consume energy to move and exert forces. Figure (a) was reprinted from Ref. [1] with permission from Springer Nature, copyright 1953. Figure (b) was printed with permission from Iddo Heller. Figure (c) was adapted from Ref. [15] with permission from Annual Reviews, copyright 2014.

experimentally, using stretching and twisting experiments [16–18] (see Figure 1.1b), and theoretically, using statistical mechanics [19]. On much larger length scales, mathematical models have shown that mechanical interactions are crucial in, for instance, the formation of new blood vessels [20, 21] and embryonic development [22–24]. On even larger length scales, insights from active matter have helped to understand the collective behaviour of animal groups, such as schools of fish or flocks of birds [25–27] (Figure 1.1c).

At intermediate biological scales, between those of single molecules and those of entire organisms, we find cells, the basic biological units that make up all life. It is at this intermediate length scale of a single cell that the importance of physics in biology has become most apparent in the past decades. A good example is given by the seminal experiment by Engler *et al.* [28]. In this experiment the authors studied differentiation of

stem cells, a process in which stem cells specialize by becoming, for instance, nerve cells, muscle cells, or bone cells. Although stem cell differentiation is traditionally believed to be triggered chemically by the detection of signaling molecules [29], Engler *et al.* showed that this process is also affected by the mechanical properties of the cell's environment. In particular, they showed that cells lying on a soft surface (mimicking the brain) have a large probability of differentiating into nerve cells, whereas those on top of surfaces of intermediate stiffness (mimicking muscles) differentiate most likely into muscle cells and those on stiff surfaces (mimicking bone) differentiate into bone cells. Other research has shown that the influence of physics on stem cell differentiation is not limited to the rigidity of the underlying surface, as stem cell differentiation is also affected by, for instance, the internal structure of the underlying surface [30], the spreading area [31] and the shape [32, 33] of the cell itself, and the geometry of and the mechanical tension in the cell's internal cytoskeleton [32, 33]. These results by no means disprove the importance of biochemistry in cell biology, but they clearly demonstrate the need to understand cell biology from the perspective of physics as well.

The interplay between physics, mathematics, and biology in the emerging field of *cell mechanics* ranges much further than stem cell differentiation alone. For instance, the shape of cells plays a role in cell division, growth, death, nuclear deformation, and gene expression [34–37], and the migration of cells strongly depends on the mechanical properties of their environment [38]. From a biomedical perspective, mechanical interactions between cells and their environment play an important role in processes such as wound healing [39] and in diseases such as asthma [40] and cancer [41, 42]. In particular, several studies have shown that the mechanical properties of cancer cells change when they become metastatic [43–45], demonstrating that a fundamental understanding of cell mechanics is required for successful future cancer treatments and other biomedical applications. In this thesis we take a step back from these biological and biomedical applications, and focus on expanding the fundamental understanding of cell mechanics.

1.1 The cytoskeleton

In **Part I** of this thesis we study how the cell's *cytoskeleton* affects, and is affected by, the cell shape and how it influences the forces that cells exert on their surroundings. The cytoskeleton is a complex network of filaments and proteins inside the cell with many different functionalities [29]. It gives the cell structural integrity, determines the positions of organelles inside the cell, directs intracellular transport, splits a dividing cell into two, and allows the cell to regulate its shape and motion. Loosely speaking, the cytoskeleton is for a cell what the combination of muscles and bones is for the human body. The cytoskeleton of most animal cells consists of three different types of filaments. *Microtubules* are hollow cylindrical structures made from the polymer tubulin, making them the most rigid filaments. They are responsible for the internal structure of the cell, determine the positions of several organelles, organize intracellular transport of materials, and form the mitotic spindle that accurately divides the genetic material between

two daughter cells during cell division. *Intermediate filaments* are a family of filaments that bend easily, making them much less rigid than microtubules. Networks of cross-linked intermediate filaments provide the cell with mechanical stability [29]. The third type of cytoskeletal filaments are *actin filaments*, also called microfilaments, which play an important role in the generation of cellular forces and in cell migration. In this thesis we focus on the role of actin in cell mechanics.

Actin filaments consist of two strands of polymers of the protein actin which are helically twisted around each other. Actin filaments have a diameter of 5-9 nm, and their bending rigidity is between those of microtubules and intermediate filaments, with a persistence length of about 10 μm. Actin filaments, in collaboration with many cross-linking proteins, self-organize into many different structures inside the cell. In the *cell cortex*, the layer just beneath the cell membrane, they support the membrane and regulate the shape and movement of the cell boundary. In cells under tension, this *cortical actin* can be highly contractile, minimizing the length of the cell boundary. During cell division, cortical actin forms a contractile ring that splits the cell into two. Actin in the cell cortex is also responsible for *filopodia* and *lamellipodia*, thin and wide protrusions of the cell membrane, respectively, that are crucial during cell migration [29]. In the cell interior, away from the edge, actin filaments form gel-like branched networks as well as linear bundles called *stress fibers* [46, 47]. A crucial property of actin filaments is that they are polar, meaning that their head (called *barbed end*) is different from their tail (called *pointed end*). This polarization allows motor proteins called *myosin* to move along actin filaments, always toward the barbed end, by consuming energy using ATP hydrolysis. In bundles of oppositely aligned filaments present in stress fibers and the actin cortex, this property allows myosin motors, that bind simultaneously to two opposite filaments, to exert forces in opposite directions on these filaments, thereby contracting the actin bundle.

1.1.1 Adherent cells

In **Part I** of this thesis we investigate individual cells that adhere to an adhesive surface, which we refer to as the substrate. These adhesive surfaces lack the realism of the complex three-dimensional environment of cells *in vivo*, but they are an excellent platform for exploring the key mechanisms in cell mechanics in a controlled and reproducible setting [48]. For instance, the response of cells to the mechanical properties of their environment can be studied by varying the substrate stiffness. This stiffness does not only affect stem cell differentiation, as we discussed above, but it also has prominent consequences for the shape and actin cytoskeleton of the cell. On soft substrates, cells have a round shape, a small spreading area, and an isotropic actin cytoskeleton. On stiffer substrates, the cell's aspect ratio [49] and spreading area [49–52] increase, and stress fibers are formed [51, 53]. Consequently, cells adhering to stiff surfaces obtain an essentially flat, two-dimensional shape. They adhere to the substrate using adhesion receptors called *focal adhesions* [54], large protein complexes in the cell membrane that mechanically connect intracellular actin bundles with the outside world, in this case the

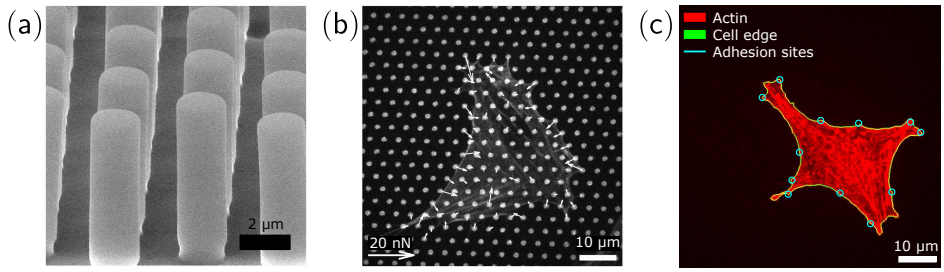

Figure 1.2. Cells on top of an array of stiff microfabricated pillars. (a) Scanning electron microscopy image of micropillars. The micropillars have a 2 µm diameter and a height of 6.9 µm. Scalebar corresponds to 2 µm. (b) A cell (3T3 fibroblast) on top of a two-dimensional array of micropillars. The arrows indicate the orientations and magnitudes of the forces that the cell exerts on the micropillars. Scalebar is 10 µm and the arrow in the bottom left corresponds to a force of 20 nN. (c) A cell (epithelioid GEβ3) adhering to a micropillar array assumes a concave (i.e., curved inwards) shape. The cell boundary (green) consists of cellular arcs that connect two sites of strong adhesion to the substrate (cyan circles). The actin in the cell is visualized in red using tetramethyl isothiocyanate rhodamine phalloidin. Scalebar is 10 µm. Figures (a) and (b) were adapted from Ref. [55]. Copyright (2014) American Chemical Society.

substrate. Because these focal adhesions keep them in place, cells on stiff substrates are under considerable mechanical tension and the cytoskeleton generates mainly contractile forces due to contraction of actin bundles [48].

The adhesive surfaces that are used in experimental studies on adherent cells can be divided into two main types. One of these types is a *microfabricated elastomeric pillar array* [55–57], shown in Figure 1.2a. This substrate consists of a lattice of pillars with a diameter in the µm range, whose tops are coated with fibronectin, a protein which is also present in the natural environment of cells and to which focal adhesions can bind. Cells deposited on a stiff micropillar array lie on top of a bed of micropillars and adhere to a limited number of them, mainly distributed along the cell boundary. At these *adhesion sites*, the cell exerts forces on the substrate, often referred to as *traction forces*, which can be measured by observing the deflections of the micropillars [55–57] (see Figure 1.2b). Due to the contractility of the actin cytoskeleton, adherent cells on stiff micropillar arrays assume a typical concave (i.e., curved inwards) shape, with the cell boundary consisting of *cellular arcs* connecting two consecutive adhesion sites (see, for example, the cell in Figure 1.2c). *Micropatterned substrates* are an often used alternative to micropillar arrays. These are flat surfaces coated with a specific pattern of fibronectin [58]. Although less straightforward than on micropillar arrays, traction forces can be measured on micropatterned substrates using a technique called traction force microscopy [59–61]. The big advantage of micropatterned substrates, with respect to micropillar arrays, is that the well-defined shape of the adhesive part of the surface ensures predictable and reproducible cell shapes, facilitating an easier comparison between experimental findings and

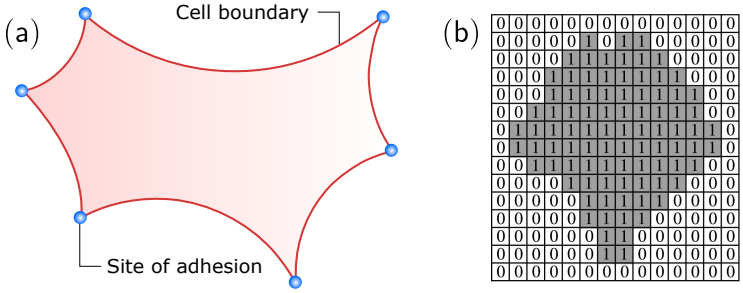

Figure 1.3. Models for cell shape used in this thesis. (a) In a contour model, the shape of a cell is modelled by describing the location of the one-dimensional cell boundary. The cell boundary adheres to the underlying substrate at sites of adhesion, indicated by the blue circles. Contour models are used in Chapters 2 and 3 of this thesis. (b) In the Cellular Potts Model, space is represented by a discrete lattice of pixels and the cell is represented by the collection of pixels that are labelled with the number 1. The Cellular Potts Model is used in Chapter 4 of this thesis. Figure (b) was reprinted from Ref. [69] with permission from Elsevier.

the predictions of mathematical models.

Mathematical models complement experimental approaches because they can help to interpret experimental findings and often raise questions that inspire new experiments. Several types of these mathematical models have been proposed to study the shape of adherent cells and the traction forces they exert on the substrate [48]. The simplest type of model is a two-dimensional *contour model* [62–67], in which the shape of a cell is fully described by the location of the one-dimensional cell boundary (see Figure 1.3a). Each contour model predicts this location based on a particular choice of intracellular forces. For cells adhering to a small number of discrete adhesion sites, such as cells on micropillar arrays, the cell boundary is a collection of cellular arcs that connect two adjacent adhesion sites. The simplest contour model is the Simple Tension Model (STM), first proposed by Bar-Ziv *et al.* [62] and later expanded by Bischofs *et al.* [63, 64]. This model assumes that the locations of the adhesion sites are fixed and known, and calculates the resulting shape of a cellular arc by considering the competition between contractile forces in the cell bulk, which model the contractility of the internal actin cytoskeleton, and contractile forces in the cell contour, which model the contractility of the actin cortex. The STM predicts that cellular arcs are curved inwards and have a circular shape, and succesfully describes cellular shape and traction forces observed in experiments of several cell types on adhesive surfaces [63, 64]. The STM was extended in more advanced contour models by inclusion of other intracellular forces, such as bending elasticity of the cell membrane [65, 68] or an elastic contribution to the contractility of the actin cortex [63, 64].

An alternative to contour models is given by *whole-cell models* [48], which explicitly

define the two-dimensional shape of the cell by modeling the cell interior. An example is given by cable network models [70, 71], which describe the actin cytoskeleton as a network of cables that pull on the cell edge. When these cables are contracting due to myosin activity [72], this model reproduces the circular arcs predicted by contour models [62–64]. Many whole-cell models are rooted in *continuum mechanics* and describe the cytoskeleton as a continuous medium rather than by explicitly modeling its discrete constituents. In these models, the cytoskeleton can be represented as an elastic [68, 73] or viscoelastic medium [74], or by more sophisticated models that include several biomechanical and biochemical aspects [75–79]. The *Cellular Potts Model* (CPM), on the other hand, is a computational model that discretizes space and represents a cell as a collection of lattice sites on an often two-dimensional lattice (see Figure 1.3b). During a CPM simulation, lattice sites can be added to or subtracted from the cell, allowing it to grow, shrink, change shape, and move. This dynamics is governed by a *Hamiltonian*, an energy functional which describes the various intracellular and intercellular forces in the model. The Cellular Potts Model was developed in 1992 by Glazier and Graner to describe the demixing of two types of cells [80, 81]. Later, the CPM has been extended with many biomechanical and biochemical aspects to describe a wide range of multicellular processes [9], such as embryonic development [22, 23], tumor growth [82], and blood vessel formation [20, 21]. More recently, the Cellular Potts Model has been employed to model the shape of single cells. For instance, Vianay *et al.* used the CPM to successfully predict a variety of shapes for cells on a dotlike micropattern [83], whereas Albert and Schwarz developed a CPM based on the Simple Tension Model to describe the shape and traction forces of cells adhering to continuous micropatterns of arbitrary shape [69, 84].

1.1.2 Liquid crystals

Most mathematical models on shape and traction forces of adherent cells, including many models discussed above, are *isotropic*, meaning that the cytoskeleton in those models contracts in all directions equally. However, many cells on stiff adhesive substrates develop actin stress fibers [51, 53], which are often oriented parallel to other stress fibers in their vicinity (see Figure 1.4a). Therefore, the cytoskeleton of these cells is *anisotropic* and experimental studies have demonstrated that both cell shape [85–88] and traction forces [89, 90] are strongly affected by this cytoskeletal anisotropy. In **Part I** of this thesis, we develop anisotropic models for the actin cytoskeleton employing the theory of *nematic liquid crystals* [91]. Liquid crystals are materials whose smallest constituents are anisotropic particles, giving them mechanical and optical properties intermediate between those of simple liquids and those of solid crystals. Liquid crystals have many applications [92], but are best known for the technological development of the Liquid Crystal Display (LCD) [93]. *Nematic* liquid crystals consist of elongated particles that, like in liquids, have no long-range positional order and can freely flow. However, due to their anisotropy, the particles align with one another, giving them long-range directional order as is the case in solid crystals [94], see Figure 1.4b. An extensive continuum mechanics framework has been developed to describe the physics of nematic liquid crys-

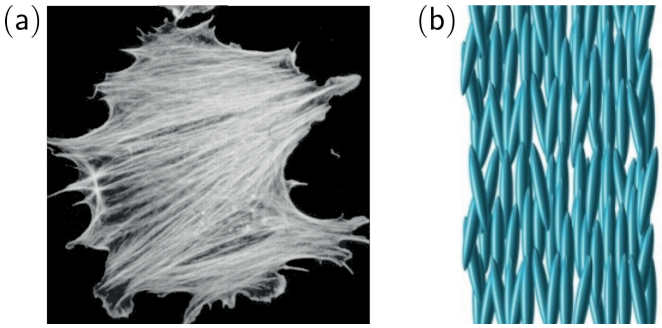

Figure 1.4. The anisotropy of the actin cytoskeleton can be modeled by using the theory of nematic liquid crystals. (a) An adherent cell (3T3 fibroblast) with visualized actin stress fibers. The stress fibers are oriented parallel to other stress fibers in their vicinity, making the actin cytoskeleton anisotropic. (b) Nematic liquid crystals consist of elongated particles that have long-range directional order but no long-range positional order. Their anisotropic nature makes them perfectly suitable for modeling the actin cytoskeleton. Figure (a) is reprinted from Ref. [112] with permission from AAAS, and Figure (b) was adapted from Ref. [113].

tals [91], including the interactions with confining boundaries [95] and the emergence of *topological defects* [96], locations where the particle orientation is ill-defined. This theoretical framework has been applied to a variety of systems, ranging from polymer solutions [97] and droplets of elongated colloidal particles [98] to rod-like viruses [99] and the mitotic spindle [100]. More recently, liquid crystal theory has been extended to describe *active* nematic liquid crystals [101], which consist of anisotropic particles that actively exert forces by consuming energy. These active constituents collectively give rise to an active bulk stress [102, 103], and lead to new phenomena such as active turbulence [104, 105] and complex dynamics of topological defects [106]. The active nature of this theory makes it a natural framework to describe various biological systems such as collections of swimming microorganisms [102], mixtures of cytoskeletal filaments and molecular motor proteins [107, 108], growing bacterial colonies [109], and confluent cell layers [110, 111].

1.2 Cell migration

In **Part II** of this thesis we shift our focus from the shape of non-motile cells to the process of cell migration, which is essential for many biological processes. For instance, *neutrophils*, a type of white blood cell, move to locations of infections where they attack hostile invaders such as bacteria. *Fibroblasts* migrate toward damaged tissue where they play a crucial role in wound healing. Cell migration also plays a crucial role in cancer metastasis, a process in which cancer cells leave a primary tumor and migrate to a distant organ to form a secondary tumor. Some animal cells, such as sperm cells,

achieve migration by swimming through a fluid using a *flagellum*, a tail that is used for propulsion. However, most animal cells migrate by crawling over a surface rather than by swimming. Although the detailed mechanisms of the crawling process are different for each cell type, the general idea behind the mechanism is the same for most of them. First, the leading edge of the cell moves forward because actin polymerization in the cell cortex, in the form of filopodia, lamellipodia, or pseudopodia, pushes the cell membrane forward. Then, the cell forms adhesions to the surface at this leading edge and, finally, contractile forces in the cytoskeleton pull the rest of the cell along [29]. Various mathematical models have been proposed to describe this process [114, 115]. These models vary both in numerical techniques and in the biological phenomena they describe. A relatively simple approach uses Langevin equations to model the stochastic dynamics of the cell position based on experimental data [116, 117]. More complex models explicitly model individual focal adhesions and stress fibers [118, 119], or describe cell shape and the dynamics of the actin cytoskeleton using phase-field models [120, 121], hydrodynamic models [122, 123], or Cellular Potts Models [124, 125].

Both *in vivo* and in experiments on surfaces, the direction of cellular migration can be biased by so-called *directional cues*, asymmetries in the surroundings of the cell that stimulate the cell to move in a specific direction. The most well-known directional cue is *chemotaxis*, the ability of cells to sense and respond to local gradients in the concentration of certain chemicals. Both prokaryotic cells (such as bacteria) [126] and eukaryotic cells (such as animal cells) [127] can perform chemotaxis, which can be positive (i.e., motion toward large concentrations) or negative (i.e., motion toward small concentrations). Chemotaxis has been extensively studied, both experimentally [128] and theoretically [129, 130], and plays a crucial role in many processes in the human body, such as in the above-mentioned migration of neutrophils toward sites of infection [29]. However, similar to what we discussed earlier for stem cell differentiation, it has become increasingly clear in the last decades that cell migration is not solely dictated by biochemistry. Instead, many mechanical cues have been found that play an important role in dictating the direction of cell migration. The most well-known examples of these are *haptotaxis*, the migration of cells from small to large densities of adhesion sites, and *durotaxis*, the migration of cells from soft to rigid mechanical environments [131–136].

In **Chapter 5** we study a recently discovered mechanical cue called *topotaxis*, the directed migration of cells due to asymmetries in the local topographical properties of the environment. Several kinds of topographically anisotropic substrates have been shown to direct cell migration, including surfaces with asymmetric adhesive patterns [137–139], substrates with spatial gradients in the density of micropillars [140, 141], and substrates with tilted micropillars [137, 142]. In all of these examples, cell motion is biased due to topographical cues at sub-cellular length scales. These small-scale directional cues cause an anisotropic response of the cytoskeleton, thereby biasing the direction of migration. Wondergem and coworkers demonstrated, however, that topotaxis can also be achieved by topographical cues at length scales larger than the cell itself [143]. They studied highly motile cells, moving on a substrate that contains cell-sized obstacles that

force the cells to move around them, and showed that cells migrate from regions of large obstacle densities to regions of low obstacle densities. In **Chapter 5** we zoom out from the cell's internal structure and cytoskeleton, which we studied in Part I. Inspired by the experiments on cells by Wondergem *et al.* [143], we study topotaxis of active Brownian particles (ABPs), a simple model for structureless self-propelled particles that is extensively studied in the field of active matter [144, 145]. Despite their simplicity, directed motion of ABPs has been demonstrated using asymmetric periodic potentials [146–148], arrays of asymmetric obstacles [149, 150], and asymmetric channels [151–154]. ABPs have even been demonstrated to perform chemotaxis [155, 156], durotaxis [157], and phototaxis [158], making them an excellent model system for identifying the basic physical principles behind directed cell migration.

1.3 Outline of the thesis

In this thesis we investigate the role of anisotropy in cell mechanics. The thesis is organized as follows. In **Part I** we combine analytical calculations, computer simulations and *in vitro* experiments to study cells adhering to stiff adhesive substrates. We investigate the mechanical interplay between the shape of these cells, the orientation of their actin stress fibers, and the traction forces that they exert on the underlying substrate. In **Chapter 2** we develop a theory for the shape of cells adhering to adhesive micropillar arrays. We extend previous isotropic contour models of cellular adhesion by explicitly introducing the directed contractile forces generated by actin stress fibers. Given the orientations of stress fibers in adherent cells, we predict cell shape as well as the directions of cellular traction forces, and we compare these predictions to experimental observations on epithelioid and fibroblastoid cells. We demonstrate that the arcs of cells with an anisotropic cytoskeleton are well described by segments of an ellipse. The aspect ratio of this ellipse is dictated by the degree of anisotropy of the internal cell stresses, and the orientation of the ellipse is dictated by the orientations of the stress fibers. Our work shows that cells can control the anisotropy of their shape by regulating the anisotropy of their cytoskeleton.

In **Chapter 3** we reverse this question, and ask how the shape of a cell influences the orientations of its stress fibers. We study the interplay between cell shape and stress fiber orientation by combining the model for cell shape, developed in Chapter 2, with a model for the cytoskeleton based on liquid crystal theory. We perform numerical simulations that predict both cell shape and the orientations of stress fibers, and again compare our results to experimental observations on epithelioid and fibroblastoid cells adhering to a micropillar array. We find that stress fiber orientation is determined by a competition between alignment with the cell edge and alignment with one another in the bulk of the cell. Our work highlights the importance of the boundary conditions, imposed by cell shape, in understanding the internal structure of the actin cytoskeleton.

In **Chapter 4** we study the interplay between cell shape, stress fiber orientation and traction forces on stiff micropatterned surfaces. To be able to model cells with continu-

ous adhesion with the substrate, rather than through a limited number of adhesion sites on top of micropillars, we implement the concepts developed in Chapters 2 and 3 into the Cellular Potts Model (CPM). As was the case for pre-existing contour models, previous CPM implementations model the contractility of the cytoskeleton using isotropic forces. In **Chapter 4**, we introduce the anisotropic contractility of the cytoskeleton in the Cellular Potts Model, and validate our model by comparing our numerical results on stress fiber distributions and traction forces to previously reported experimental data. Our numerical results show that traction forces are strongly biased by the local stress fiber orientation, and reproduce previously reported anisotropic traction force distributions. Our findings demonstrate that an anisotropic model for the actin cytoskeleton is required for accurately predicting cellular traction forces.

In **Part II** of this thesis we study cell migration on a substrate that contains cell-sized obstacles. We zoom out from the internal structure of the cell, which we studied in Part I, and investigate the motion of active Brownian particles (ABPs) in **Chapter 5**. Using a combination of numerical simulations and analytical arguments, we study the motion of ABPs in obstacle lattices of both constant and non-constant densities, and demonstrate the emergence of topotaxis of active Brownian particles. This finding demonstrates that persistent migration of cells is sufficient to obtain topotaxis, even in the absence of any more complex mechanical or biochemical mechanisms.

Finally, in **Chapter 6** we summarize the most important findings of the research presented in this thesis. We discuss the implications of our results on the field of cell mechanics and suggest the most promising directions for future research.