Anisotropy in cell mechanics

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Chapter 6

Final conclusions and outlook

In this thesis we studied the effects of anisotropy on two fundamental aspects of cell mechanics: the role of the actin cytoskeleton in determining cell shape and the generation of traction forces (Part I), and cell migration in an asymmetric crowded environment (Part II). In Part I we focused on the role of actin stress fibers, bundles of actin filaments that can contract under the influence of the motor protein myosin. By contracting, stress fibers can adjust the cell shape and exert forces on the environment of the cell. The structure and function of stress fibers is relatively well understood [46, 47] and several experimental studies have shown their importance in the anisotropy of the cytoskeleton and the anisotropy of traction forces [89, 90], but most theoretical models for cell contractility describe cells as isotropic objects [63, 64, 68, 73, 196, 197, 232]. In Part I we combined analytical calculations, computer simulations and *in vitro* experiments to study the geometrical and mechanical properties of cells with a highly anisotropic cytoskeleton adhering to adhesive substrates. Our findings highlight the crucial mechanical interplay between the actin cytoskeleton, which dictates the shape and traction forces of cells, and the cell shape, which, in turn, determines the structure of the actin cytoskeleton.

In Chapter 2 we studied how the orientation of the stress fibers affects the shape of the cell. We extended a previous isotropic contour model for cell contractility called the Simple Tension Model [63, 64]. When cells adhere to the substrate at a small number of discrete adhesion sites, the Simple Tension Model predicts that the cell has a concave shape and that each part of the cell edge between two adhesion sites, called a cellular arc, can be approximated by a segment of a circle. We extended this contour model by introducing the effects of anisotropic contractility due to actin stress fibers, and predict that cellular arcs of anisotropic cells are better approximated by segments of an ellipse. The aspect ratio of this ellipse is determined by the degree of anisotropy of the internal cell stresses, and the orientation of the long axis is parallel to the local orientation of stress fibers along that cellular arc. We validated our model predictions by studying epithelioid and fibroblastoid cells [173] on microfabricated elastomeric pillar arrays [55–

57]. We demonstrated that the arcs of cells with an anisotropic cytoskeleton are well approximated by a unique ellipse, which is for each cellular arc oriented parallel to the local stress fiber orientation along that arc. Additionally, we demonstrated that the traction forces that the cell exerts on the micropillar array are affected significantly by the anisotropy of the cytoskeleton. Our work shows that cells can control the anisotropy of their shape and traction forces by regulating the anisotropy of their cytoskeleton.

In Chapter 3 we reversed the question from Chapter 2, and asked how the shape of the cell affects the orientation of the stress fibers. We presented a phenomenological model for stress fiber orientation based on the continuum theory of nematic liquid crystals [91]. This model for the cytoskeleton was coupled with the model for cell shape, developed in Chapter 2, to study the mechanical interplay between cell shape and the organization of the actin cytoskeleton. Our model predicts that the orientation of the stress fibers is governed by an interplay between alignment of stress fibers with one another in the bulk of the cell and alignment of stress fibers with the cell edge. We compared our model predictions with experimental data on epithelioid and fibroblastoid cells [173] on microfabricated elastomeric pillar arrays [55–57], and demonstrated good qualitative agreement. Because our phenomenological model for the cytoskeleton does not explicitly take into account a number of biochemical pathways that are important in the generation of cytoskeletal anisotropy and traction forces [56, 176, 196, 198, 199, 206, 207, 210, 211], our theoretical predictions do not perfectly agree with the experimental data. Importantly, however, our work demonstrates that the formation and organization of the actin cytoskeleton cannot be understood from processes at the sub-cellular scale alone, but that it is crucial to take into account the boundary conditions imposed by the shape of the cell.

In Chapter 4 we build on the work in Chapters 2 and 3 and implement our model for cell shape in the framework of the Cellular Potts Model (CPM). Combining this Cellular Potts Model with the liquid crystal model for the cytoskeleton developed in Chapter 3, we study cells adhering to adhesive micropatterns that ensure reproducible cell shapes [58]. Our model predictions qualitatively reproduce experimentally observed stress fiber distributions of several cell types on differently shaped micropatterns. Additionally, this approach allowed us to calculate traction forces on micropatterned substrates. Our numerical predictions show that the traction forces are strongly biased by the local stress fiber orientation, consistent with experimental observations [89, 90] but different from many earlier models [69, 196, 234, 235]. Comparing the predictions to previously published experimental data of several cell types on different pattern shapes [43, 89, 196], we reproduce prominent anisotropic features in traction force patterns that were not captured in earlier isotropic models [69, 196]. These findings demonstrate the importance of carefully considering the configuration of the actin cytoskeleton in the study of cellular traction forces.

In Part II of this thesis we shifted our focus to cells migrating in a crowded environment, and studied large-scale topotaxis. This process was first observed by Wondergem *et al.* [143] in experiments of highly motile cells moving on a substrate in between

cell-sized obstacles. In the presence of a gradient in the density of these obstacles, the cells migrate, on average, in the direction of lower obstacle densities. Inspired by these observations, in Chapter 5 we studied large-scale topotaxis of active Brownian particles (ABPs), which represent a simple model system for self-propelled particles. This allowed us to zoom out from the internal structure of the cell that we studied in Part I, and to study the role of persistent cell migration in large-scale topotaxis. We demonstrated numerically that ABPs perform topotaxis and that topotaxis is stronger for particles with larger persistence lengths and for lattices with steeper density gradients. Using a combination of numerical simulations and analytical arguments, we studied ABPs in regular obstacle lattices and showed that the origin of ABP topotaxis lies in an effective persistence length that depends on the local obstacle density. Our work demonstrates that persistent migration is on itself sufficient to drive large-scale topotaxis, even in the absence of any more complex biochemical regulatory mechanisms.

6.1 Outlook

Our work has revealed a number of promising directions for future work. In Part I, we identified several possibilities to extend our models with additional biochemical and biomechanical mechanisms to further improve the agreement with experimental data. These potential model extensions include spatial variations in actin densities [43, 176, 196, 210, 211, 240], the distinction between different stress fiber subtypes [242], interactions of stress fibers with the substrate in the cell interior [56, 118, 198, 199], the increase of cytoskeletal tension as a function of substrate area [195–197] or substrate stiffness [198], and the evaluation of traction forces in the cell interior [69, 136].

The most promising direction for future work is, arguably, extending our model to study the role of cytoskeletal anisotropy in cell spreading and migration. This could be achieved by taking into account, for instance, actin filament turnover and the viscoelasticity of stress fibers [206, 207], the dynamics of focal adhesions [136, 189], or cellular protrusions and retractions [216]. A natural platform for achieving this goal is the Cellular Potts Model, in which the model for stress fiber contractility we developed in Chapter 4 could be combined with previously published CPM implementations of cell migration based on the formation of a lamellipodium at the front of the cell [124, 125]. As cell migration crucially depends on pulling forces at the back of the cell [243, 244], integrating our cytoskeleton model in these existing Cellular Potts Models would be an important step forward in realistically modeling cell migration.

In Part II we were inspired by cell migration in crowded environments and demonstrated that active Brownian particles (ABPs) perform topotaxis. In the future, it is important to understand how sensitive topotaxis of active particles is with respect to the details of the model, such as the details of particle-obstacle interactions [149, 271, 286–288], the shape of the obstacles [149, 150, 260], or the type of active motion [260, 263, 273, 283–285]. From a biophysical perspective, however, the most important question is what our findings imply for cellular large-scale topotaxis. As we commented in the

discussion of Chapter 5, the efficiency of ABPs to perform topotaxis is about a factor 5 lower than that of highly motile cells [143]. Investigating the origin of this large discrepancy is an important next step toward a better understanding of cellular large-scale topotaxis. Here, we speculate about a number of possible explanations for the origin of this discrepancy, and show preliminary data that presents a first step toward identifying this origin. Possible explanations include, but are not limited to:

1. ABPs in Chapter 5 are modelled as hard, non-deformable objects. Cells, on the other hand, are highly flexible and can take on many different shapes, as we have seen in Part I of this thesis. This property allows cells to squeeze themselves through narrow spaces between the obstacles [143], whereas ABPs either move through spaces effortlessly (if they fit) or not at all (if they do not fit).

2. Cells can adhere to obstacles using various physical or chemical interactions. This might guide them through an obstacle lattice in a way that is not possible for ABPs.

3. Cells respond differently to collisions with obstacles than ABPs do. As we explained in Chapter 5, obstacles in our model slow down ABPs and change the direction in which they move, but they do not change the particle orientation. In other words, obstacles affect the direction in which particles move, but they do not affect the direction in which the partcicles *try* to move. This particle-obstacle interaction is realistic for active colloids [260], but cells crawling on a substrate are likely to show a more complicated response upon encountering an obstacle.

A first step toward identifying which of the explanations above might contribute to the suprising efficiency of experimentally observed large-scale topotaxis of cells [143], is currently being undertaken by Van Steijn *et al.* [293]. They numerically study large-scale topotaxis by employing more biologically-realistic models of cell migration based on the Cellular Potts Model (CPM). In particular, they adapt the standard CPM, which we discussed in Chapters 1 and 4, in two different ways to include persistent cell motion. The first method is based on the persistent motion described in Chapter 5 of this thesis: each cell is assigned an orientation vector $\boldsymbol{p}$, which evolves in time according to Eq. (5.1). Consistent with ABPs in Chapter 5, the orientation vector is not affected by encounters with obstacles. Then, the Hamiltonian H is adapted such that cell motion in the direction of $\boldsymbol{p}$ becomes more likely, whereas motion in the opposite direction becomes less likely. We call this method for cellular persistence in the CPM "vector based persistence".

The second method of cellular persistence is based on a phenomenological description of actin polymerization at the leading edge of the cell developed by Niculescu *et al.* [125]. In this model, called the "Act model", each lattice site in the cell is assigned an "activity value" that keeps track of the time that the lattice site was included in the cell. A term is then added to the Hamiltonian which favors the cell to expand at lattice sites that were recently added to the cell. In this way, the cell is likely to keep forming protrusions at the same side, which leads to persistent migration. However, unlike CPM cells with vector based persistence, these cells do not stubbornly keep trying to move in

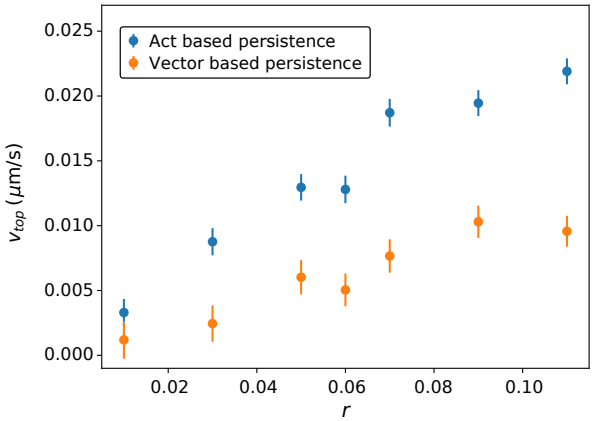

Figure 6.1. Topotactic velocity v_{top} (see Chapter 5) as a function of the dimensionless obstacle density gradient r (Chapter 5) for two different models of highly motile and persistently migrating cells based on the Cellular Potts Model [293]. The model with "vector based persistence" implements persistent cell motion based on the vector $\boldsymbol{p}$ in Chapter 5, whereas cells with "Act based persistence" move persistently due to a phenomenological model of actin protrusion dynamics at the cell's leading edge [125]. This Figure was printed with permission from Leonie van Steijn.

the same direction when they encounter an obstacle. Instead, they quickly "forget" the direction in which they were previously migrating, and start moving in a new direction.

By comparing the topotactic abilities of these two types of persistently migrating cells in the Cellular Potts Model, Van Steijn *et al.* [293] investigate to what extent explanation 3 might cause the difference in topotactic efficiency between ABPs and highly motile cells. Figure 6.1 shows the topotactic velocity v_{top} as a function of the dimensionless obstacle density gradient r (see Chapter 5) for highly motile CPM cells with vector based persistence and highly motile CPM cells with persistence based on the Act model [293]. The cells with Act based persistence have about twice the topotactic velocity of cells with vector based persistence for all values of r. This result demonstrates that the "smart" way in which Act based persistent cells adapt their direction of motion upon encountering an obstacle allows them to perform topotaxis more efficiently than cells that stubbornly keep trying to move in the same direction. Returning to the results of Chapter 5, this suggests that the limited topotactic efficiency of ABPs with respect to highly motile cells can in part be explained by the fact that their orientation is not affected by interactions with obstacles. However, experimentally observed large-scale topotaxis is about five times as efficient as that of ABPs, suggesting that other explanations, such as those listed above, might also contribute to the discrepancy. In the future, it is worth investigating this in greater detail. On the theoretical side, for example, the topotactic efficiency of ABPs could be compared to that of CPM cells with vector based persistence to test explanation 1, and the adhesion affinity of cells with the obstacles

can be varied in the Cellular Potts Model to test explanation 2. On the experimental side, the large-scale topotactic efficiency could be compared for different types of persistently migrating cells, such as leukocytes [292], amoeba [289], or invasive (amoeboid) cancer cells [290, 291]. Additionally, the actin dynamics within the cell could be studied during cell-obstacle interactions to shed more light on the reorientation dynamics and to inspire more realistic models of cell migration. Together, these efforts will contribute to a better biological and biophysical understanding of cell migration in crowded environments, which can potentially inspire biomedical applications in, for instance, malaria [294, 295] or cancer [251] treatments.