

4 Mechanics of the cytoskeleton

4.1 Motivation

In the previous section, we have seen how biopolymers dynamically assemble and disassemble during polymerization. We have discussed the individual mechanical properties such as Young's modulus E , the axial stiffness EA , the bending stiffness EI , and the persistence length A for individual filaments. In particular, have talked about actin filaments, intermediate filaments, and microtubules. Now, assuming we know the mechanical properties of the individual filaments, what does that actually tell us about the assembly of filaments that we find in the cell? Or, to put it differently, if we knew

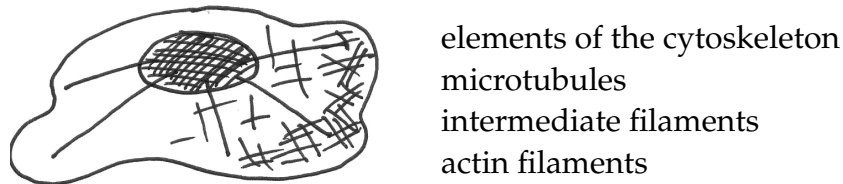


Figure 4.1: The cytoskeleton provides structural stability and is responsible for forces during cell locomotion. Microtubules are thick hollow cylinders reaching out from the nucleus to the membrane, intermediate filaments can be found anywhere in the cytosol, and actin filaments are usually concentrated close to the cell membrane.

the structural arrangement of filaments, could we then predict the stiffness of the overall assembly? How does the filament microstructure affect cytoskeletal properties? Or, more precisely, how can we calculate the macroscopic network properties from the individual microscopic filament properties? In mechanics, the derivation of macroscopic parameters based on microscopic considerations is referred to as homogenization. In this chapter, we illustrate the homogenization by means of three different examples, the fiber bundle model for filopodia, the network model for red blood cell membranes, and the tensegrity model for generic cell structures.

4.2 Fiber bundle model for filopodia

Filopodia are thin dynamic cytoplasmic projections composed of tight bundles of long actin filaments extending from the leading edge of migrating cells. Sometimes, the name filopodia is used to describe all different kinds of cytoskeletal protrusions including thick filopodia, cell feet, and amoebae pseudopods, [3, 29–31, 38]. Filopodia contain actin filaments cross-linked into bundles by actin-binding proteins such as fimbrin. Many types of motile cell such as fibroblasts or keratinocytes use filopodia for cell

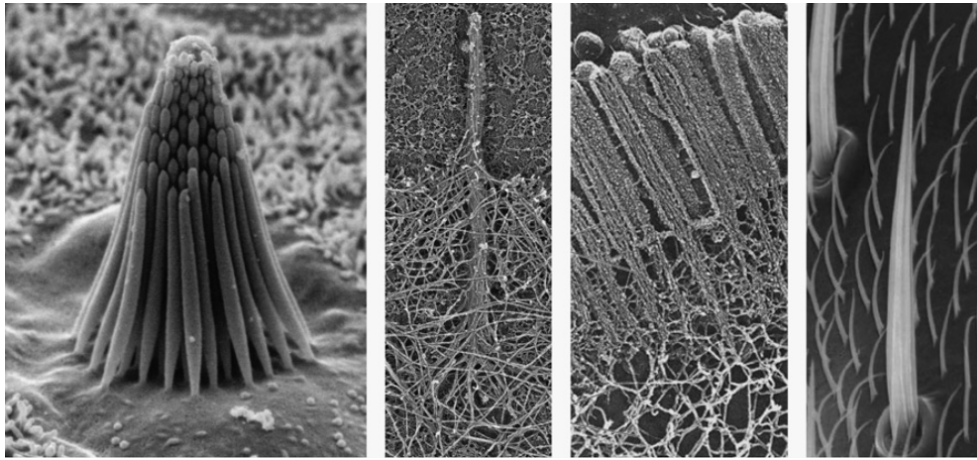


Figure 4.2: Fiber bundles of F-actin. Ciliary bundle from the sensory epithelium of a bullfrog sacculus consisting of stereocilia, filopodium protruding from the lamellipodium of a mouse melanoma cell, epithelial microvilli, and drosophila neurosensory micro- and macrochaete bristles, adopted from [3]

locomotion. Filopodia at the leading edge of a migrating cell seem to explore the extracellular matrix and surfaces of other cells. Once they have identified appropriate targets, they form focal adhesions linking the cell surface to the substratum further down the migratory pathway. The contraction of stress fibers then retracts the rear of the cell and the cell crawls forwards.

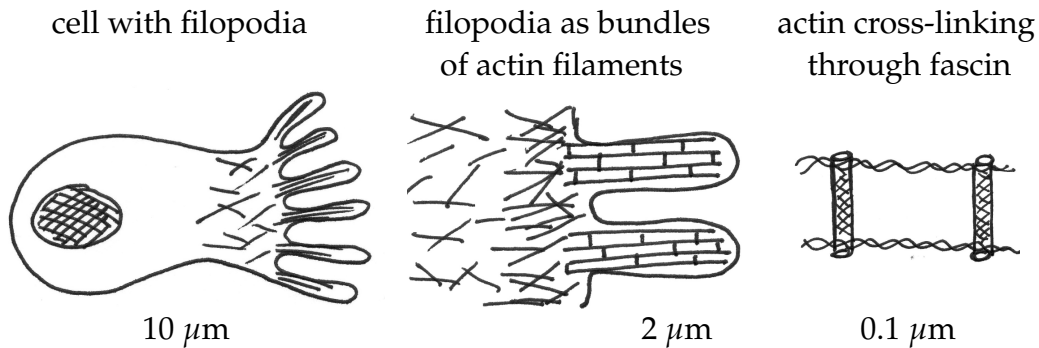


Figure 4.3: Filopodia as bundles of actin filaments tightly cross-linked through fascin

Filopodia are very thin structures approximately $0.2 \mu\text{m}$ in diameter. They can easily extend $1.5 \mu\text{m}$, occasionally they can be more than $20 \mu\text{m}$ long, see figure 4.3. They typically polymerize and depolymerize at rates of approximately $10 \mu\text{m}/\text{min}$. The mechanical properties of filopodia play an essential role in various different physiological processes, including hearing, cell migration, and growth. Despite their importance to cell function, the structural architecture responsible for their overall mechanical behavior remains largely unknown. In this chapter, we explore the mechanics of cytoskeletal fiber bundles using a simple Euler buckling model that accounts for the discrete nature of constituent actin filaments and their distinct cross-linking proteins [31].

Pushing the envelope

The simplified model for cell locomotion essentially consists of three steps

- (i) protrusion ... polymerization at the leading edge of the cell
- (ii) attachment ... formation of focal adhesions to link the cell to the surface
- (iii) retraction ... contraction of stress fibers to retract the rear of the cell

There are a lot of open questions related to the phenomenon of protrusion that might be partly explained by looking at the mechanics of filament bundles: Why are filopodia $\approx 1\mu\text{m}$ long? Is their length related to mechanics? What if filopodia were longer? How does polymerization push the cell membrane?

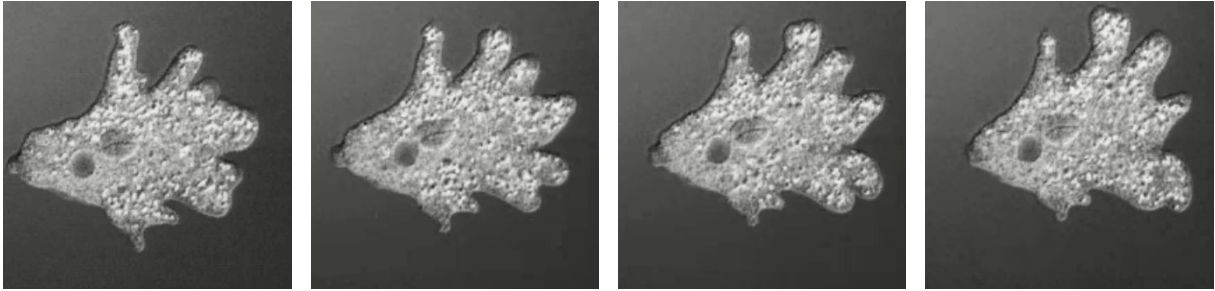


Figure 4.4: Single-celled amoeba crawling around by using actin polymerization to push out pseudopods to explore new territory. Organelles move in complex patterns within the cell, adopted from [1].

Filament buckling

First, we draw a free body diagram with the filopodium approximated as a cylinder of radius r_{fil} . This cylinder pushes against the cell membrane with a maximum force F_{fil} . Due to surface tension, the cell membrane exerts a membrane force of F_{mem} on the cylinder.

$$F_{\text{fil}} \doteq F_{\text{mem}} \quad (4.2.1)$$

This is actually Newton's third law: actio = reactio. For every action, there is an equal and opposite reaction. We are asking ourselves the question what is the maximum critical force F_{crit} a filopodium can bear? Obviously, this force is limited by filopodium buckling. You might remember the four Euler modes of buckling displayed in Figure 4.5. For all four Euler buckling modes, the critical buckling force is given as follows.

$$F_{\text{fil}} = F_{\text{crit}} = \frac{\pi^2 EI}{2 L_{\text{crit}}^2}$$

So, in Figure 4.5, which one is the most likely to buckle? The structure on the left is the least supported and thus the most flexible structure with the longest buckling length and thus the highest critical buckling force. As the buckling length L_{crit} decreases from $L_{\text{crit}} = 2L$ to $L_{\text{crit}} = 1/2L$ from left to right, the critical buckling force F_{crit} , or rather the

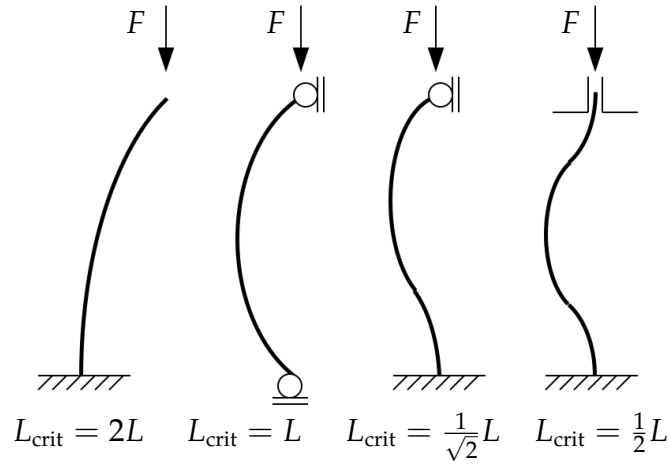


Figure 4.5: The four Euler buckling modes. As the buckling length L_{crit} decreases from $L_{\text{crit}} = 2L$ to $L_{\text{crit}} = 1/2L$ from left to right, the critical buckling force $F_{\text{crit}} = \pi^2 EI / L_{\text{crit}}^2$, or rather the resistance to buckling, increases.

resistance to buckling, increases. For us, the relevant buckling load is the most critical one from case (i) with $L_{\text{crit}} = 2L$, where $L = L_{\text{fil}}$ is actually the filopodia length we are looking for.

$$F_{\text{fil}} = \frac{\pi^2 EI}{[2L_{\text{fil}}]^2} = \frac{\pi^2 EI}{4L_{\text{fil}}^2} \quad (4.2.2)$$

So now, what is the force F_{mem} acting on the filopodia bundle as it pushes against the cell membrane? The membrane forces acting on a cylinder of radius r_{fil} can be approximated analytically [37].

$$F_{\text{mem}} \approx 5 r_{\text{fil}} \frac{\text{pN}}{\text{nm}} \quad (4.2.3)$$

What is the radius r_{fil} of the filopodium? We can assume that the area of the the filopodium A_{fil} is equivalent to the cross section area A_{act} of a cylinder composed of n actin fibers. The radius of this cross section can then be computed as follows.

$$\begin{aligned} A_{\text{fil}} &= \pi r_{\text{fil}}^2 & A_{\text{fil}} &= A_{\text{act}} & \text{thus} & & r_{\text{fil}} &= \sqrt{n} r_{\text{act}} \\ A_{\text{act}} &= n \pi r_{\text{act}}^2 \end{aligned} \quad (4.2.4)$$

This tells us that the force exerted by the cell membrane on the filopodium cylinder scales with the square root of the number of actin filaments

$$F_{\text{mem}} \approx 5 \sqrt{n} r_{\text{act}} \frac{\text{pN}}{\text{nm}} \quad (4.2.5)$$

From Newton's third law (4.2.1) together with equations (4.2.2) and (4.2.5),

$$F_{\text{fil}} \doteq F_{\text{mem}} \quad \text{thus} \quad \frac{\pi^2 EI}{4L_{\text{fil}}^2} = 5 \sqrt{n} r_{\text{act}} \frac{\text{pN}}{\text{nm}} \quad (4.2.6)$$

we can derive the critical filopodia length L_{crit} beyond which they would start to buckle.

$$L_{\text{fil}} = \frac{\pi}{2} \sqrt{\frac{EI}{5\sqrt{n} r_{\text{act}} \text{ pN/nm}}} \quad (4.2.7)$$

From table 3.2 we know the Young's modulus $E = 1.9 \cdot 10^9 \text{ N/m}^2 = 1.9 \text{ GPa}$ and the radius $r_{\text{act}} = 2.5 \text{ nm}$ of individual actin filaments. But what is the moment of inertia I of a bundle of actin fibers? Of course, the moment of inertia of a complex microstructure of crosslinked filaments is difficult to predict, but we can consider two limit case: (i) a loose assembly of actin filaments and (ii) tightly crosslinked filaments. These might tell us something about the real microstructure of filopodia.

4.2.1 Loosely assembled filaments of actin

First, let us assume the actin filaments in filopodia are loosely assembled without any further crosslinking. In that case, the overall moment of inertia I

$$I = n I_{\text{act}} \quad \text{with} \quad I_{\text{act}} = \frac{\pi r_{\text{act}}^4}{4} \quad (4.2.8)$$

would simply scale linearly with the number of actin filaments n within the fiber bundle. This would give us a critical filopodia length of

$$L_{\text{fil}} = \frac{\pi}{2} \sqrt{\frac{1.9 \cdot 10^9 \text{ N/m}^2 \cdot n \pi/4 [3.5 \cdot 10^{-9}]^4 \text{ m}^4}{5\sqrt{n} 3.5 \cdot 10^{-12} \text{ N}}} \approx 0.17769 \mu\text{m} n^{1/4} \quad (4.2.9)$$

According to this model, the critical length of filopodia would scale with the fourth root of the number of actin filaments $n^{1/4}$ within the fiber bundle. Let us assume there are about $n = 30$ filaments in one filopodium. Then the critical buckling length

$$L_{\text{fil}} = 0.416 \mu\text{m} \quad (4.2.10)$$

would be about half a micron. Now, the observed length of filopodia is something in the order of $2\mu\text{m}$ or even up to $20\mu\text{m}$. This is much larger than the critical length $L_{\text{fil}} = 0.416 \mu\text{m}$ we have calculated with our model! There must be a mechanism that increases the buckling length!

4.2.2 Tight crosslinked filaments of actin

Let us now consider the second limit case, for which the actin filaments are tightly crosslinked. Accordingly, the overall moment of inertia I would be a function of the overall radius of the filament bundle r_{fil} .

$$I = \frac{\pi r_{\text{fil}}^4}{4} = n^2 \frac{\pi r_{\text{act}}^4}{4} \quad (4.2.11)$$

By using equation (4.2.4) with $r_{\text{fil}} = \sqrt{n} r_{\text{act}}$ we see that the moment of inertia scales quadratically with the number of actin filaments. This give us a critical filopodia length of

$$L_{\text{fil}} = \frac{\pi}{2} \sqrt{\frac{1.9 \cdot 10^9 \text{ N/m}^2 \cdot n^2 \pi/4 [3.5 \cdot 10^{-9}]^4 \text{m}^4}{5 \sqrt{n} 3.5 \cdot 10^{-12} \text{ N}}} \approx 0.17769 \mu\text{m} n^{3/4} \quad (4.2.12)$$

which scales with $n^{3/4}$. For $n = 30$ filaments in one filopodium, the critical buckling length before buckling would be

$$L_{\text{fil}} = 2.278 \mu\text{m}. \quad (4.2.13)$$

This value is of the order of the observed filopodium length. The model of tightly crosslinked actin filaments thus seems more realistic than the model of loosely attached filaments!