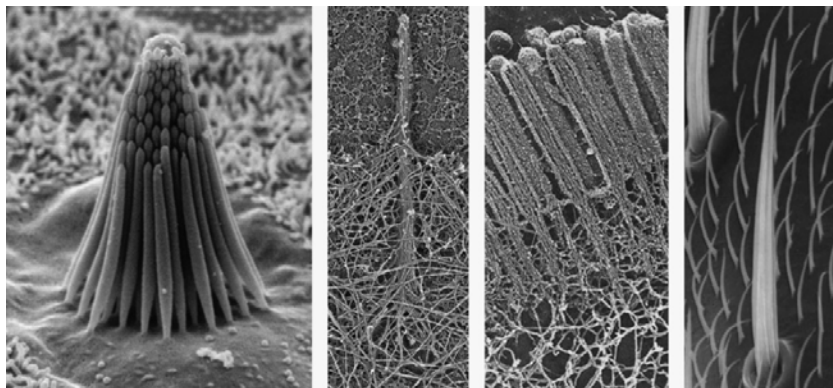


4. the cytoskeleton - fiber bundle models



bathe, heussinger, claessens, bausch, frey [2008]

me239 mechanics of the cell

1

day	date	topic	notes	material
tue	apr 03	introduction I - cell biology		s01 s01
thu	apr 05	introduction II - cytoskeletal biology, stem cells	n02	s02 l02
tue	apr 10	introduction III - structural mechanics	n03	s03
thu	apr 12	biopolymers I - energy, tension, bending	n04	s04
thu	apr 12	homework I - biopolymers, directed stem cell differentiation	h01	m04
tue	apr 17	biopolymers II - entropy, FJC and WLC model	n05	s05
thu	apr 19	biopolymers III - polymerization kinetics in amoeba	n06	s06 m06
tue	apr 24	cytoskeletal mechanics I - fiber bundle model for filopodia	n07	s07 m07
thu	apr 26	cytoskeletal mechanics II - network model for red blood cells	n08	s08
thu	apr 26	homework II - cytoskeleton, cell mechanics challenges	h02	m10
tue	may 01	cytoskeletal mechanics III - tensegrity model for generic eukaryotic cells	n09	s09 m09
thu	may 03	biomembranes I - micropipette aspiration in white blood cells and cartilage cells	n10	s10
tue	may 08	biomembranes II - lipid bilayer, soap bubble, cell membrane	n11	s11
thu	may 10	biomembranes III - energy, tension, shear, bending	n12	s12
tue	may 15	mechanotransduction I - inter- and intracellular signaling, bone cells	n13	s13
tue	may 15	homework III - micropipette aspiration, final project	h03	m12
thu	may 17	summary and midterm preparation	n14	s14
tue	may 22	midterm		
thu	may 24	mechanotransduction II - electrophysiology in nerve cells	n16	s16
tue	may 29	mechanotransduction III - excitation contraction in skeletal muscle and heart cells	n17	s17
thu	may 31	mechanics of the cell - the inner life	n18	l01 l02
tue	jun 05	final projects - oral presentations	p02	
thu	jun 07	no class		
fri	jun 08	final projects - written projects due	p01	

me239 mechanics of the cell

2

biopolymers

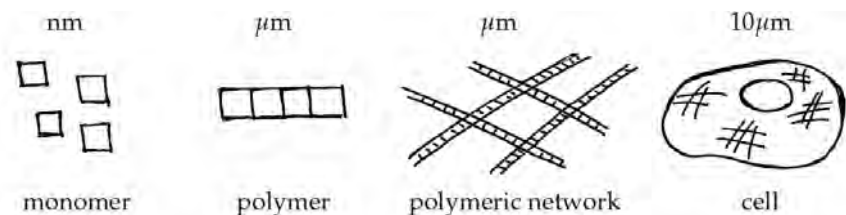
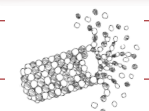


Figure 3.1. Biopolymers. Characteristic length scales on the cellular and subcellular level.

3.1 biopolymers - motivation

3

polymerization of actin and tubulin



ON RATES AND OFF RATES

A linear polymer of protein molecules, such as an actin filament or a microtubule, assembles (polymerizes) and disassembles (depolymerizes) by the addition and removal of subunits at the ends of the polymer. The rate of addition of these subunits (called monomers) is given by the rate constant k_{on} , which has units of $M^{-1} sec^{-1}$. The rate of loss is given by k_{off} (units of sec^{-1}).

polymer (with n subunits) + subunit

polymer (with $n + 1$ subunits)

THE CRITICAL CONCENTRATION

The number of monomers that add to the polymer (actin filament or microtubule) per second will be proportional to the concentration of the free subunit ($k_{on}C$), but the subunits will leave the polymer end at a constant rate (k_{off}) that does not depend on C . As the polymer grows, subunits are used up, and C is observed to drop until it reaches a constant value, called the **critical concentration** (C_c). At this concentration the rate of subunit addition equals the rate of subunit loss. At this equilibrium,

$$k_{on} C = k_{off}$$

so that

$$C_c = \frac{k_{off}}{k_{on}} = \frac{1}{K}$$

(where K is the equilibrium constant for subunit addition; see Figure 3-44).

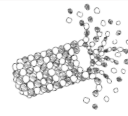
alberts, johnson, lewis, raff, roberts, walter [2002]



3.2 biopolymers - polymerization

4

polymerization of actin and tubulin



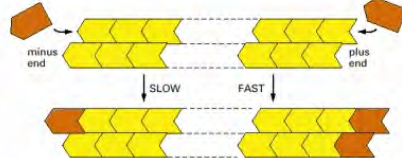
PLUS AND MINUS ENDS

The two ends of an actin filament or microtubule polymerize at different rates. The fast-growing end is called the **plus end**, whereas the slow-growing end is called the **minus end**. The difference in the rates of growth at the two ends is made possible by changes in the conformation of each subunit as it enters the polymer.



This conformational change affects the rates at which subunits add to the two ends.

Even though k_{on} and k_{off} will have different values for the plus and minus ends of the polymer, their ratio k_{on}^+/k_{on}^- —and hence C_{crit} —must be the same at both ends for a simple polymerization reaction (no ATP or GTP hydrolysis). This is because exactly the same subunit interactions are broken when a subunit is lost at either end, and the final state of



the subunit after dissociation is identical. Therefore, the ΔG for subunit loss, which determines the equilibrium constant for its association with the end, is identical at both ends: if the plus end grows four times faster than the minus end, it must also shrink four times faster. Thus, for $C > C_c$, both ends grow; for $C < C_c$, both ends shrink. The nucleoside triphosphate hydrolysis that accompanies actin and tubulin polymerization removes this constraint.

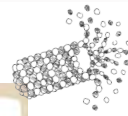
alberts, johnson, lewis, raff, roberts, walter [2002]

THE CELL

3.2 biopolymers - polymerization

5

polymerization of actin and tubulin

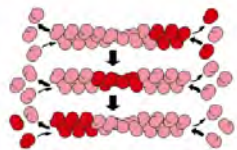


TREADMILLING

One consequence of the nucleotide hydrolysis that accompanies polymer formation is to change the critical concentration at the two ends of the polymer. Since k_{on}^+ and k_{on}^- refer to different reactions, their ratio k_{on}^+/k_{on}^- need not be the same at both ends, so that:

$$C_c \text{ (minus end)} > C_c \text{ (plus end)}$$

Thus, if both ends of a polymer are exposed, polymerization proceeds until the concentration of free monomer reaches a value that is above C_c for the plus end but below C_c for the minus end. At this steady state, subunits undergo a net assembly at the plus end and a net disassembly at the minus end at an identical rate. The polymer maintains a constant length, even though there is a net flux of subunits through the polymer, known as **treadmilling**.



DYNAMIC INSTABILITY

Microtubules depolymerize about 100 times faster from an end containing GDP tubulin than from one containing GTP tubulin. A GTP cap favors growth, but if it is lost, then depolymerization ensues.



Individual microtubules can therefore alternate between a period of slow growth and a period of rapid disassembly, a phenomenon called **dynamic instability**.

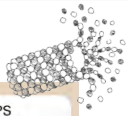
alberts, johnson, lewis, raff, roberts, walter [2002]

THE CELL

3.2 biopolymers - polymerization

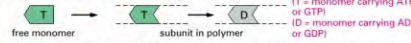
7

polymerization of actin and tubulin



NUCLEOTIDE HYDROLYSIS

Each actin molecule carries a tightly bound ATP molecule that is hydrolyzed to a tightly bound ADP molecule soon after its assembly into polymer. Similarly, each tubulin molecule carries a tightly bound GTP that is converted to a tightly bound GDP molecule soon after the molecule assembles into the polymer.



Hydrolysis of the bound nucleotide reduces the binding affinity of the subunit for neighboring subunits and makes it more likely to dissociate from each end of the filament (see Figure 16-11 for a possible mechanism). It is usually the **D** form that adds to the filament and the **T** form that leaves.

Considering events at the plus end only:



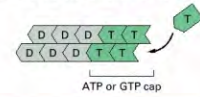
As before, the polymer will grow until $C = C_c$. For illustrative purposes, we can ignore k_{on}^+ and k_{off}^+ since they are usually very small, so that polymer growth ceases when

$$k_{on}^+ C = k_{off}^+ \quad \text{or} \quad C_c = \frac{k_{off}^+}{k_{on}^+}$$

This is a steady state and not a true equilibrium, because the ATP or GTP that is hydrolyzed must be replenished by a nucleotide exchange reaction of the free subunit ($D \rightarrow T$).

ATP CAPS AND GTP CAPS

The rate of addition of subunits to a growing actin filament or microtubule can be faster than the rate at which their bound nucleotide is hydrolyzed. Under such conditions, the end has a "cap" of subunits containing the nucleoside triphosphate—an ATP cap on an actin filament or a GTP cap on a microtubule.



DYNAMIC INSTABILITY and **TREADMILLING** are two behaviors observed in cytoskeletal polymers. Both are associated with nucleoside triphosphate hydrolysis. Dynamic instability is believed to predominate in microtubules, whereas treadmilling may predominate in actin filaments.

alberts, johnson, lewis, raff, roberts, walter [2002]

THE CELL

3.2 biopolymers - polymerization

6

polymerization of actin and tubulin

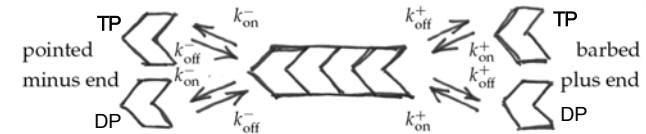
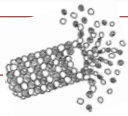


Figure 3.4: Eight rate constants for polymerization capture and release in non-symmetric actin and microtubules filaments.

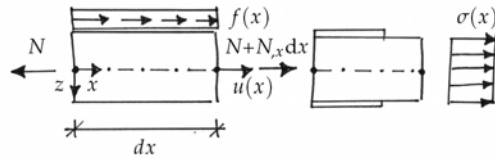
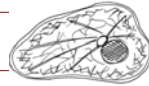
	k_{on}^+ [1/(μ M s)]	k_{off}^+ [1/s]	k_{on}^- [1/(μ M s)]	k_{off}^- [1/s]	C_{crit}^+ μ M	C_{crit}^- μ M
ATP-actin	11.60	1.40	1.30	0.80	0.12	0.62
ADP-actin	3.80	7.20	0.16	0.27	1.90	1.70
GTP-tubulin	8.90	44.00	4.30	23.00	4.90	5.30
GDP-tubulin	0	733	0	915	n/a	n/a

Table 3.1: Measured rate constants of actin filaments and microtubules

3.2 biopolymers - polymerization

8

axial deformation - tension



$$EA u_{,xx} + f = 0 \quad \text{with} \quad EA \dots \text{axial stiffness}$$

cross section area $A = \pi r^2$

	r	A	E	EA
microtubule	12.5 nm	491 nm ²	1.9 · 10 ⁹ N/m ²	93 · 10 ⁻⁸ N
intermediate filament	5.0 nm	79 nm ²	2.0 · 10 ⁹ N/m ²	15 · 10 ⁻⁸ N
actin filament	3.5 nm	39 nm ²	1.9 · 10 ⁹ N/m ²	7 · 10 ⁻⁸ N

Table 3.1: Axial stiffness EA of major constituents of cytoskeleton: microtubules, intermediate filaments and actin filaments

3.3 biopolymers - energy

9

concept of persistence length



- stiffer filaments are straighter \propto bending stiffness EI
- cooler filaments are straighter \propto inverse temperature kT

$$A = \frac{EI}{kT} \quad \dots \quad \text{persistence length}$$

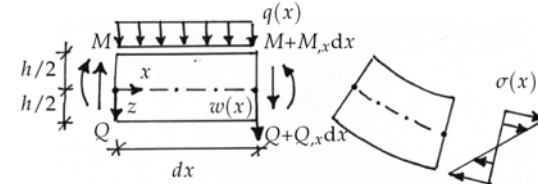
	r	E	EI	$A = [EI]/[kT]$
microtubule	12.5 nm	1.9 · 10 ⁹ N/m ²	364 · 10 ⁻²⁵ Nm ²	8.800 mm
intermediate filament	5.0 nm	2 · 10 ⁹ N/m ²	10 · 10 ⁻²⁵ Nm ²	0.240 mm
actin filament	3.5 nm	1.9 · 10 ⁹ N/m ²	2 · 10 ⁻²⁵ Nm ²	0.048 mm

Table 3.6: Persistence lengths of major constituents of cytoskeleton at room temperature: microtubules, intermediate filaments and actin filaments

3.3 biopolymers - entropy

11

transverse deformation - bending



$$q = EI w_{,xxxx} \quad \text{with} \quad EI \dots \text{bending stiffness}$$

for circular cross sections $I = \pi r^4 / 4$

	r	I	E	EI
microtubule	12.5 nm	19,175 nm ⁴	1.9 · 10 ⁹ N/m ²	364 · 10 ⁻²⁵ Nm ²
intermediate filament	5.0 nm	491 nm ⁴	2 · 10 ⁹ N/m ²	10 · 10 ⁻²⁵ Nm ²
actin filament	3.5 nm	118 nm ⁴	1.9 · 10 ⁹ N/m ²	2 · 10 ⁻²⁵ Nm ²

Table 3.2: Bending stiffness of major constituents of cytoskeleton: microtubules, intermediate filaments and actin filaments

3.3 biopolymers - energy

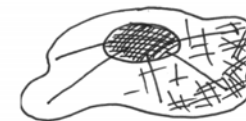
10

from molecular level to cellular level



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?

- could we then predict the **stiffness of the overall assembly**?
- how does the filament microstructure affect **cytoskeletal properties**?
- how can we calculate the **macroscopic network properties** from the individual microscopic filament properties?



elements of the cytoskeleton
microtubules
intermediate filaments
actin filaments

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.

4.1 mechanics of the cytoskeleton

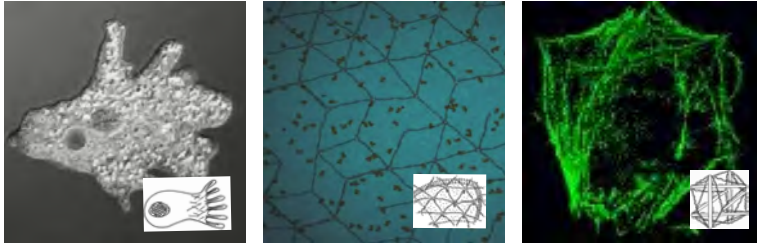
12

mechanics of the cytoskeleton



three examples

- **fiber bundle model** for filopodia
- **network model** for red blood cell membranes
- **tensegrity model** for generic cell structures



4.1 mechanics of the cytoskeleton

13

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. **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 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.

4.2 fiber bundle model for filopodia

15

microstructural arrangement of actin

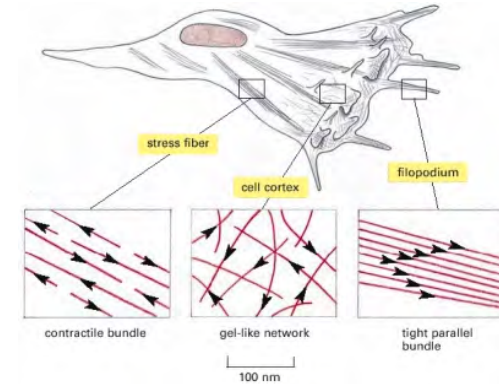


Figure 4.2.1. A crawling cell, drawn to scale, is shown with three areas enlarged to show the arrangement of actin filaments. The actin filaments are shown in red, with arrowheads pointing toward the plus end. Stress fibers are contractile and exert tension. The cell cortex underlies the plasma membrane. Filopodia are spike-like projections of the plasma membrane that allow a cell to explore its environments.

alberts, johnson, lewis, raff, roberts, walter [2002]

4.2 fiber bundle model for filopodia

14

filopodia and lamellipodia

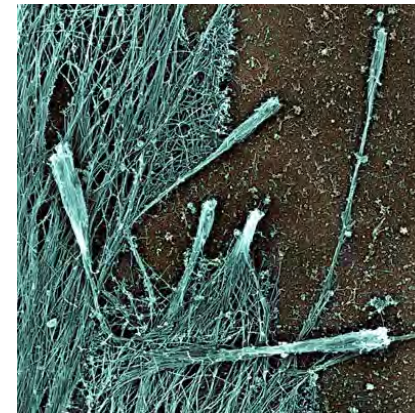


Figure 4.2.2. Filopodia and lamellipodia are actin-rich protrusions important for cell motility. This electron micrograph shows exaggerated filopodia with club-like shape. Filopodia are filled with bundled actin filaments which were born in and converged from the lamellipodial network.

czech, svitkina, yang [2007]

4.2 fiber bundle model for filopodia

16

filopodia and other fiber bundles of F-actin

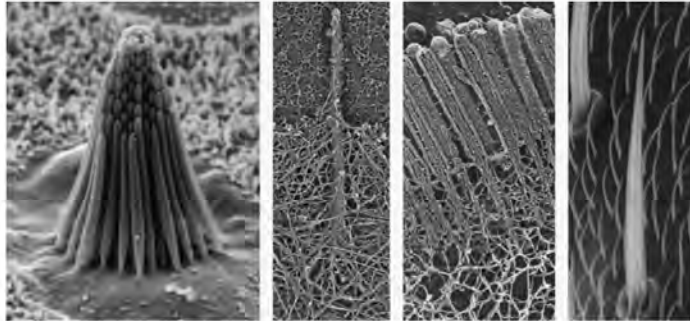


Figure 4.2: Fiber bundles of F-actin. Ciliary bundle from the sensory epithelium of a bullfrog sacculus consisting of stereocilia, filopodium protruding from the lamellipodium of a mouse melanoma cell, epithelial microvilli, and *Drosophila* neurosensory micro- and macrochaete bristles.

bathe, heussinger, claessens, bausch, frey [2008]

4.2 fiber bundle model for filopodia

17

assembly of crosslinked actin filaments

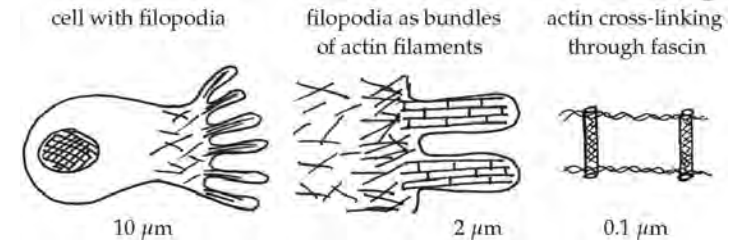
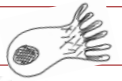


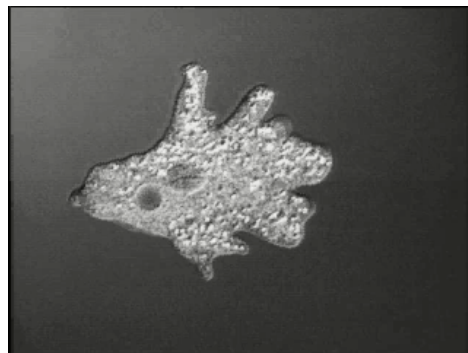
Figure 4.3: Bundles of actin filaments tightly crosslinked through fascin are known as filopodia. The mechanical properties of filopodia play an essential role in various different physiological processes including hearing, cell migration, and growth.

filopodia are very thin structures approximately 0.2 μm in diameter. they can easily extend up to 2.0 μm . 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.

4.2 fiber bundle model for filopodia

18

pushing the envelope



this single-celled amoeba crawls around by using actin polymerization to push out pseudopods, or false feet, to explore new territory. at the same time, organelles move in complex patterns within the cell.

alberts, johnson, lewis, raff, roberts, walter [2002]

4.2 fiber bundle model for filopodia

19

pushing the envelope



simplified model for cell locomotion

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

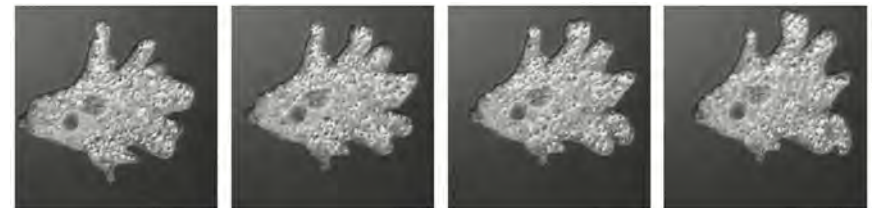


Figure 4.4: Single-celled amoeba crawling around by using actin polymerization to push out pseudopods to explore new territory. Organelles move in complex patterns within the cell.

alberts, johnson, lewis, raff, roberts, walter [2002]

4.2 fiber bundle model for filopodia

20

pushing the envelope

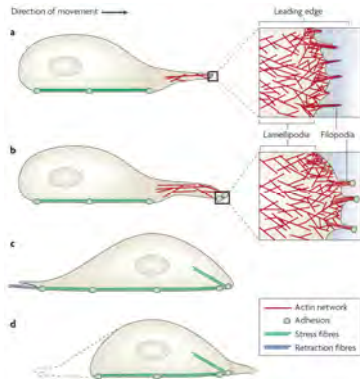


Figure 4.2.3. Cell migration is dependent on different actin filament structures. Motility is initiated by an actin-dependent protrusion of the leading edge, which is composed of lamellipodia and filopodia. **a)** These protrusive structures contain actin filaments, with elongating barbed ends orientated towards the plasma membrane. **b)** During cellular extension, new adhesions with the substratum are formed under the leading edge. **c)** Next, the nucleus and the cell body are translocated forward through actomyosin based contraction forces that might be mediated by focal adhesion-linked stress fibers, which also mediate the attachment to the substratum. **d)** Then, retraction fibers pull the rear of the cell forward, adhesions at the rear of the cell disassemble and the trailing edge retracts.

mattila & lappalainen [2008]

4.2 fiber bundle model for filopodia

21

pushing the envelope - membrane force

$$F_{\text{fil}} \doteq F_{\text{mem}}$$

- membrane forces acting on a cylinder of radius r_{fil}

$$F_{\text{mem}} \approx 5 r_{\text{fil}} \frac{\text{pN}}{\text{nm}}$$

- radius r_{fil} of the filopodium

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

- force exerted by the cell membrane on the filopodium cylinder

$$F_{\text{mem}} \approx 5 \sqrt{n} r_{\text{act}} \text{ pN/nm}$$

4.2 fiber bundle model for filopodia

23

pushing the envelope - filament force

$$F_{\text{fil}} \doteq F_{\text{mem}} \quad \text{critical force} \quad F_{\text{crit}} = \frac{\pi^2 EI}{L_{\text{crit}}^2} \quad \text{thus} \quad F_{\text{fil}} = \frac{\pi^2 EI}{[2L]^2} = \frac{\pi^2 EI}{4L}$$

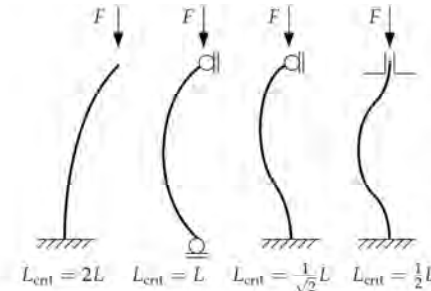


Figure 4.5: The four Euler buckling modes. As the buckling length L_{crit} decreases from $L_{\text{crit}} = 2L$ to $L_{\text{crit}} = 1/2L$ from left to right, the critical buckling force $F_{\text{crit}} = \pi^2 EI / L_{\text{crit}}^2$, or rather the resistance to buckling, increases.

4.2 fiber bundle model for filopodia

22

pushing the envelope - critical length

Newton's third law: actio = reactio

$$F_{\text{fil}} \doteq F_{\text{mem}}$$

$$F_{\text{fil}} = \frac{\pi^2 EI}{[2L]^2} = \frac{\pi^2 EI}{4L}$$

$$F_{\text{mem}} \approx 5 \sqrt{n} r_{\text{act}} \text{ pN/nm}$$

$$\frac{\pi^2 EI}{4L_{\text{crit}}^2} = 5 \sqrt{n} r_{\text{act}} \frac{\text{pN}}{\text{nm}} \quad \text{thus} \quad L_{\text{crit}} = \frac{\pi}{2} \sqrt{\frac{EI}{5 \sqrt{n} r_{\text{act}} \text{ pN/nm}}}$$

$$E = 1.9 \cdot 10^9 \text{ N/m}^2 = 1.9 \text{ GPa} \quad r_{\text{act}} = 3.5 \text{ nm} \quad \text{moment of inertia } I$$

- loose assembly
- tightly crosslinked

4.2 fiber bundle model for filopodia

24

case I - loosely assembled actin filaments



$$L_{crit} = \frac{\pi}{2} \sqrt{\frac{EI}{5\sqrt{n} r_{act} pN/nm}}$$

moment of inertia I

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

$$E = 1.9 \cdot 10^9 \text{ N/m}^2 = 1.9 \text{ GPa} \quad r_{act} = 3.5 \text{ nm}$$

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

$$n = 30 \text{ filaments} \quad L_{crit} = 0.416 \mu\text{m}$$

much too low - disagrees with observations of 2um

4.2 fiber bundle model for filopodia

25

case II - tightly crosslinked actin filaments



$$L_{crit} = \frac{\pi}{2} \sqrt{\frac{EI}{5\sqrt{n} r_{act} pN/nm}}$$

moment of inertia I

$$I = \frac{\pi r_{fil}^4}{4} = n^2 \frac{\pi r_{act}^4}{4} \quad \text{with} \quad r_{fil} = \sqrt{n} r_{act}$$

$$E = 1.9 \cdot 10^9 \text{ N/m}^2 = 1.9 \text{ GPa} \quad r_{act} = 3.5 \text{ nm}$$

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

$$n = 30 \text{ filaments} \quad L_{crit} = 2.278 \mu\text{m}$$

better model - agrees with observations of 2um

4.2 fiber bundle model for filopodia

26

assembly of crosslinked actin filaments

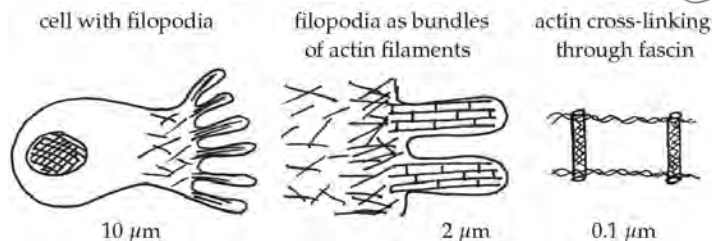


Figure 4.3. Bundles of actin filaments tightly crosslinked through fascin are known as filopodia. The mechanical properties of filopodia play an essential role in various different physiological processes including hearing, cell migration, and growth.

filopodia are very thin structures approximately 0.2 um in diameter. they can easily extend up to 2.0um. they typically polymerize and depolymerize at rates of approximately 10 um/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.

4.2 fiber bundle model for filopodia

27

pushing the envelope

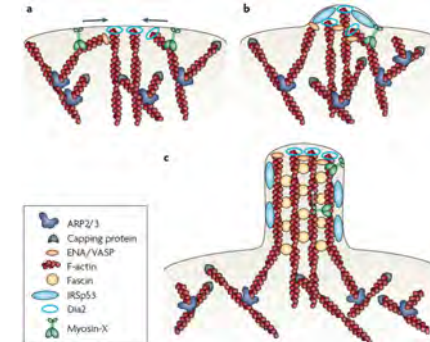
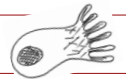


Figure 4.2.4. A working model for filopodia formation. The model describes functions of key proteins at different stages during filopodia formation. a) A subset of uncapped actin filaments are targeted for continued elongation. The barbed ends of these elongating actin filaments are converged together through the motor activity of myosin-X, leading to the initiation of a filopodium. b) When the primary filopodium begins to push the plasma membrane, IRSp53 might further facilitate plasma membrane protrusion by directly deforming or tubulating the membrane. c) The incorporation of the actin crosslinking protein fascin in the shaft of the filopodium generates a stiff actin filament bundle. At this stage, myosin-X might localize adhesion molecules to the filopodium tip by barbed directed movement and attach the elongating actin filament barbed ends to the plasma membrane.

mattila & lappalainen [2008]

4.2 fiber bundle model for filopodia

28

pushing the envelope

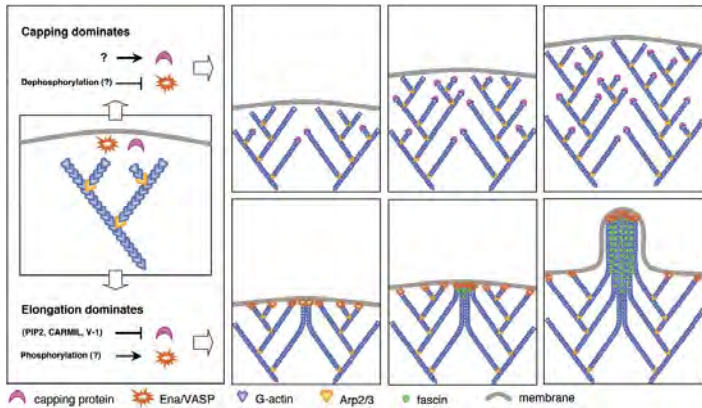


Figure 4.2.5. Model for mechanisms of regulation of lamellipodial versus filopodial protrusion by filament capping.
mejillano, kojima, applewhite, gertler, svitkina, borsy [2004]

4.2 fiber bundle model for filopodia

29

visualization of cell locomotion

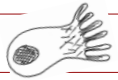


Figure 4.2.6. Fibroblasts cultured on a very thin sheet of silicon rubber. Attachment of the cells, followed by contraction of their cytoskeleton, has caused the rubber substratum to wrinkle.
alberts, johnson, lewis, raff, roberts, walter [2002]

4.2 fiber bundle model for filopodia

30