4. the cytoskeleton - fiber bundle models

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

3.1 biopolymers - motivation

3.2 biopolymers - polymerization

alberts, johnson, lewis, raff, roberts, walter [2002]
3.2 biopolymers - polymerization

**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 upon its assembly into polymer. Similarly, each tubulin subunit carries a tightly bound GTP that is hydrolyzed to a tightly bound GDP molecule upon the assembly into 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 16–17 for a possible mechanism). It is usually the form that adds to the filament and the form that leaves. Considering events at the plus end only:

$$[A]_n = [GDP]_n$$

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

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 ($[\text{ATP}]$ or $[\text{GTP}]$).

**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 nucleotides is hydrolyzed. Under such conditions, the end has a “cap” of subunits containing the nucleotide 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 nucleotide triphosphate hydrolysis. Dynamic instability is believed to predominate in microtubules, whereas treadmilling may predominate in actin filaments.

**Table 3.1: Measured rate constants of actin filaments and microtubules**

<table>
<thead>
<tr>
<th></th>
<th>$k_{on}^{-1}$ [1/µM]</th>
<th>$k_{off}^{-1}$ [1/s]</th>
<th>$k_{on}$ [1/µM]</th>
<th>$k_{off}$ [1/s]</th>
<th>$C_{crit}^{+}$ µM</th>
<th>$C_{crit}^{-}$ µM</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP-actin</td>
<td>11.60</td>
<td>1.40</td>
<td>1.30</td>
<td>0.80</td>
<td>0.12</td>
<td>0.62</td>
</tr>
<tr>
<td>ADP-actin</td>
<td>3.80</td>
<td>7.20</td>
<td>0.16</td>
<td>0.27</td>
<td>1.90</td>
<td>1.70</td>
</tr>
<tr>
<td>GTP-tubulin</td>
<td>8.90</td>
<td>44.00</td>
<td>4.30</td>
<td>23.00</td>
<td>4.90</td>
<td>5.30</td>
</tr>
<tr>
<td>GDP-tubulin</td>
<td>0</td>
<td>733</td>
<td>0.27</td>
<td>915</td>
<td>n/a</td>
<td>n/a</td>
</tr>
</tbody>
</table>

**3.3 biopolymers - energy**

**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 $k_{off}$ refer to different reactions, their ratio $C_{crit}^{-}$/$C_{crit}^{+}$ need not be the same at both ends of the polymer, so that $C_{crit}^{-}$ (minus end) > $C_{crit}^{+}$ (plus end).

Thus, if both ends of a polymer are exposed to equal polymerization proceeds until the concentration of free monomer reaches a value that is above $C_{crit}$ for the plus end but below $C_{crit}$ for the minus end, an steady state, actin subunits undergoes net assembly at the plus end and a net disassembly at the minus end and 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**

Microtubule depolymerization about 100 times faster than an actin filament containing GDP subunits faster than an actin filament containing GDP subunits. A GTP cap favors growth, but if it is lost, then depolymerization.

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

**Axial Deformation - Tension**

$$EA \frac{d^2x}{dx^2} + f = 0$$

with

$$EA \frac{d^2u}{dx^2} = 0$$

**Cross section area** $A = \pi r^2$

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

<table>
<thead>
<tr>
<th></th>
<th>$r$</th>
<th>$A$</th>
<th>$E$</th>
<th>$EA$</th>
</tr>
</thead>
<tbody>
<tr>
<td>microtubule</td>
<td>12.5 nm</td>
<td>491 nm²</td>
<td>1.9⋅10⁹ N/m²</td>
<td>93⋅10⁻⁸ N</td>
</tr>
<tr>
<td>intermediate filament</td>
<td>5.0 nm</td>
<td>79 nm²</td>
<td>2.0⋅10⁹ N/m²</td>
<td>15⋅10⁻⁸ N</td>
</tr>
<tr>
<td>actin filament</td>
<td>3.5 nm</td>
<td>39 nm²</td>
<td>1.9⋅10⁹ N/m²</td>
<td>7⋅10⁻⁸ N</td>
</tr>
</tbody>
</table>
transverse deformation - bending

\[ q = EI \, w_{xxxx} \quad \text{with} \quad EI \quad \ldots \text{bending stiffness} \]

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

<table>
<thead>
<tr>
<th></th>
<th>( r )</th>
<th>( I )</th>
<th>( E )</th>
<th>( EI )</th>
</tr>
</thead>
<tbody>
<tr>
<td>microtubule</td>
<td>12.5 nm</td>
<td>19,175 nm(^4)</td>
<td>1.9 \times 10^9 N/m(^2)</td>
<td>364 \times 10^{-25} Nm(^2)</td>
</tr>
<tr>
<td>intermediate filament</td>
<td>5.0 nm</td>
<td>491 nm(^4)</td>
<td>2.1 \times 10^9 N/m(^2)</td>
<td>10 \times 10^{-25} Nm(^2)</td>
</tr>
<tr>
<td>actin filament</td>
<td>3.5 nm</td>
<td>118 nm(^4)</td>
<td>1.9 \times 10^9 N/m(^2)</td>
<td>2 \times 10^{-25} Nm(^2)</td>
</tr>
</tbody>
</table>

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

concept of persistence length

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

\[ A = \frac{EI}{kT} \quad \ldots \text{persistence length} \]

<table>
<thead>
<tr>
<th></th>
<th>( r )</th>
<th>( E )</th>
<th>( EI )</th>
<th>( A = [EI]/[kT] )</th>
</tr>
</thead>
<tbody>
<tr>
<td>microtubule</td>
<td>12.5 nm</td>
<td>1.9 \times 10^9 N/m(^2)</td>
<td>364 \times 10^{-25} Nm(^2)</td>
<td>8.800 mm</td>
</tr>
<tr>
<td>intermediate filament</td>
<td>5.0 nm</td>
<td>2 \times 10^9 N/m(^2)</td>
<td>10 \times 10^{-25} Nm(^2)</td>
<td>0.240 mm</td>
</tr>
<tr>
<td>actin filament</td>
<td>3.5 nm</td>
<td>1.9 \times 10^9 N/m(^2)</td>
<td>2 \times 10^{-25} Nm(^2)</td>
<td>0.048 mm</td>
</tr>
</tbody>
</table>

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

3.3 biopolymers - energy

3.3 biopolymers - entropy

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?

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

three examples

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

elements of the cytoskeleton

microtubules
intermediate filaments
actin filaments

4.1 mechanics of the cytoskeleton
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 amoeba 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 utilize 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.

**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]

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.

Čech, Svitkova, Yang [2007]

Filopodia and other fiber bundles of F-actin

**Figure 4.2.3.** Fiber bundles of F-actin. Ciliary bundle from the sensory epithelium of a bullfrog saccule consisting of stereocilia, filopodium protruding from the lamellipodium of a mouse melanoma cell, epithelial microvilli, and drosophila neurosensory micro- and macrochaete bristles.

Bethe, Haussinger, Claesson, Bausch, Fray [2008]
Filopodia are very thin structures approximately 0.2 μm in diameter. They can easily extend up to 1.5 μm. They typically polymerize and depolymerize at rates of approximately 10 μm/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**

- **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 4.2.3.** 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 oriented 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.

**Figure 4.2.4.** Single-celled amoeba crawling around by using actin polymerization to push out pseudopods, or false feet, 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

pushing the envelope - filament force

\[ F_{\text{fil}} \doteq F_{\text{mem}} \]

Critical force

\[ F_{\text{crit}} = \frac{\pi^2 EI}{L_{\text{crit}}^2} \]

Thus

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

\[ L_{\text{crit}} = \frac{2L}{\sqrt{n}} \]

\[ L_{\text{crit}} = L \]

\[ L_{\text{crit}} = \frac{L}{\sqrt{n}} \]

\[ L_{\text{crit}} = \frac{L}{n} \]

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

\[ E = 1.9 \cdot 10^9 \text{N/m}^2 = 1.9 \text{ GPa} \]

\[ r_{\text{act}} = 2.5 \]

moment of inertia

\[ I = n I_{\text{act}} \]

with

\[ I_{\text{act}} = \frac{\pi r_{\text{act}}^4}{4} \]

\[ E = 1.9 \cdot 10^9 \text{N/m}^2 \cdot n \pi/4 [3.5 \cdot 10^{-9}]^4 \text{m}^4 \approx 0.17769 \mu\text{m} n^{1/4} \]

4.2 fiber bundle model for filopodia

pushing the envelope - membrane force

\[ F_{\text{fil}} \doteq F_{\text{mem}} \]

- Membrane forces acting on a cylinder of radius \( r_{\text{fil}} \)

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

- Radius \( r_{\text{fil}} \) of the filopodium

\[ A_{\text{fil}} = \pi r_{\text{fil}}^2 \]

\[ A_{\text{act}} = n \pi r_{\text{act}}^2 \]

\[ A_{\text{fil}} = A_{\text{act}} \]

thus

\[ r_{\text{fil}} = \sqrt{n} r_{\text{act}} \]

- Force exerted by the cell membrane on the filopodium cylinder

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

4.2 fiber bundle model for filopodia

Pushing the envelope - critical length

Newton’s third law: actio = reactio

\[ F_{\text{fil}} \doteq F_{\text{mem}} \]

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

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

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

Thus

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

\[ I = n I_{\text{act}} \]

with

\[ I_{\text{act}} = \frac{\pi r_{\text{act}}^4}{4} \]

\[ E = 1.9 \cdot 10^9 \text{N/m}^2 \cdot n \pi/4 [3.5 \cdot 10^{-9}]^4 \text{m}^4 \approx 0.17769 \mu\text{m} n^{1/4} \]

\[ n = 30 \text{ filaments} \]

\[ L_{\text{crit}} = 0.416 \mu\text{m} \]

Much too low - disagrees with observations of 2um
### 4.2 Fiber Bundle Model for Filopodia

**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. 
- When the primary filopodium begins to push the plasma membrane, INPP51 might further facilitate plasma membrane protrusion by directly deforming or tubulating the membrane. 
- 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-end directed movement and attach the elongating actin filament barbed ends to the plasma membrane.

**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.

---

- **Case II - Tightly Crosslinked Actin Filaments**

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

- Moment of Inertia \( I = \frac{\pi}{4} r_{\text{fil}}^4 = n^2 \frac{\pi}{4} r_{\text{act}}^4 \) with \( r_{\text{fil}} = \sqrt{n} r_{\text{act}} \)

\[ E = 1.9 \cdot 10^9 \text{ N/m}^2, r_{\text{act}} = 2.5 \]

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

Better model - agrees with observations of 2um