3 Biopolymers

3.1 Motivation

3.2 Polymerization

The structural stability of the cell is provided by the cytoskeleton. Assembling and disassembling dynamically, the cytoskeleton enables cell movement through a highly controlled synthesis of filaments in one direction of the cell with the front denoted as the leading edge. But how do these filaments move through the cell? Filaments are able to move through the synthesis of subunits, see figure 3.1. Although both sides of

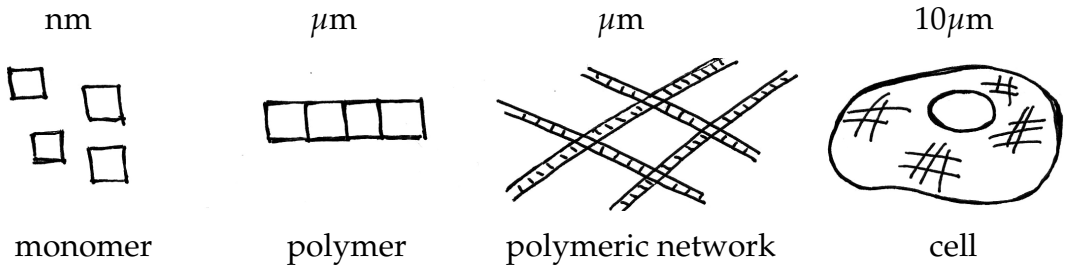

Figure 3.1: Length scales on the cellular and subcellular level.

a filament are able to grow, one end of the filament denoted as the plus end or barbed end is usually more dynamic and will grow faster than the minus end or pointed end. Filamental subunits are polarized and must be added onto the filament in the correct orientation for synthesis to occur. Subunit removal is mediated by the same process,

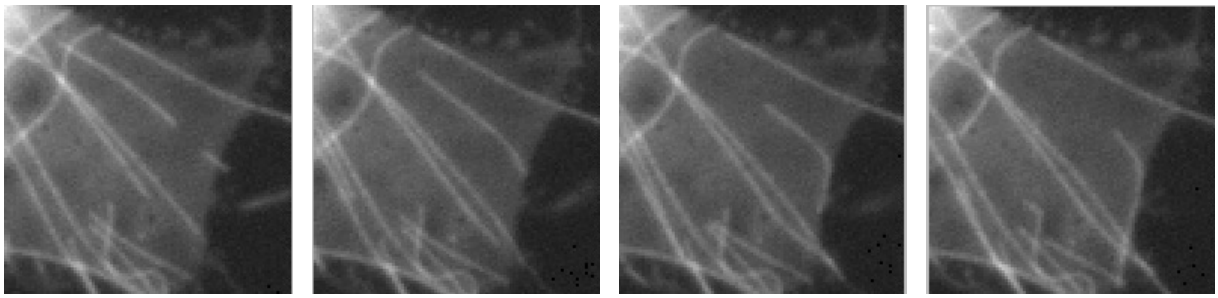

Figure 3.2: In vivo time sequence of fluorescently tagged cytoplasmic microtubules. Microtubules are nucleated and anchored by their minus ends at the centrosome and turn over by depolymerization and repolymerization at their plus ends. Free microtubules move toward the periphery by treadmilling growing at the plus end while shrinking at the minus end [37]

again with the plus end being able shrink faster than the minus end. Figure 3.2 displays the polymerization and depolarization of fluorescently tagged microtubules. In this section, we will study by which mechanisms filaments grow and shrink through polymerization. The figure illustrates how free microtubules move around driven by a phenomenon called treadmilling that we will analyze in the following section.

3.2.1 Polymerization of an idealized polymer

The evolution of number of monomers n in a single filament is controlled by monomer capture and release. Experiments have shown that, to a first approximation, monomer capture depends linearly on the free monomer concentration C in the solution.

$$\frac{\mathrm{d}n}{\mathrm{d}t} = +k_{\mathrm{on}}\, C \qquad \text{... monomer capture} \tag{3.2.1}$$

Here, $\mathrm{d}n$ / $\mathrm{d}t$ denotes the evolution of the number of monomers n within a single polymer, k_{on} is the capture rate with units $[k_{\mathrm{on}}]$=[1/(concentration $\cdot$ time)] and C is the free

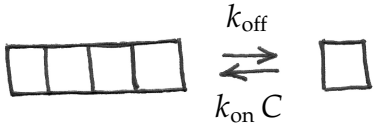

Figure 3.3: Model of idealized polymerization with addition and removal of subunits.

monomer concentration with $[C]$=[concentration]. The concentration, the number of free monomers per volume, is typically given in M, where 1 M= 1 mol / liter, with 1 mol being equivalent to 6.02 $\times 10^{23}$ monomers. In contrast to monomer capture, monomer release independent of the concentration.

$$\frac{\mathrm{d}n}{\mathrm{d}t} = -k_{\mathrm{off}} \qquad \text{... monomer release} \tag{3.2.2}$$

Here, k_{off} is the release rate with units $[k_{\mathrm{on}}]$=[1/time]. Summarizing both monomer capture and release, we obtain the simplest form of polymerization kinetics.

$$\frac{\mathrm{d}n}{\mathrm{d}t} = +k_{\mathrm{on}}\, C - k_{\mathrm{off}} \tag{3.2.3}$$

We can analyze this equation further by asking ourselves What is the condition for the polymer to remain at constant length?. At constant length, the number of monomers has to be constant, i.e., n =const.

$$\frac{\mathrm{d}n}{\mathrm{d}t} = +k_{\mathrm{on}}\, C_{\mathrm{crit}} - k_{\mathrm{off}} \doteq 0 \qquad \text{thus} \qquad C_{\mathrm{crit}} = \frac{k_{\mathrm{off}}}{k_{\mathrm{on}}} \tag{3.2.4}$$

At the critical free monomer concentration C_{crit}, i.e., the ratio between release rate k_{off} and capture rate k_{on}, a polymer releases as many monomers as it captures, i.e., it remains at constant length. In this idealized model, two rate constants, k_{off} and k_{on}, govern polymerization dynamics.

3.2.2 Polymerization of actin and tubulin

In reality, polymerization is somewhat more complex for two reasons. For actin and tubulin, the subunit of microtubules, (i) the subunits are not symmetric, i.e., they add to the two ends of the filament with preferred orientation giving rise to an oriented filament and (ii) the free subunits carry triphosphate nucleotide (ATP for actin and GTP for tubulin) which is hydrolized to diphosphate (ADP for actin and GDP for tubulin) after polymerization. As a natural consequence, the ends of the polymers grow at

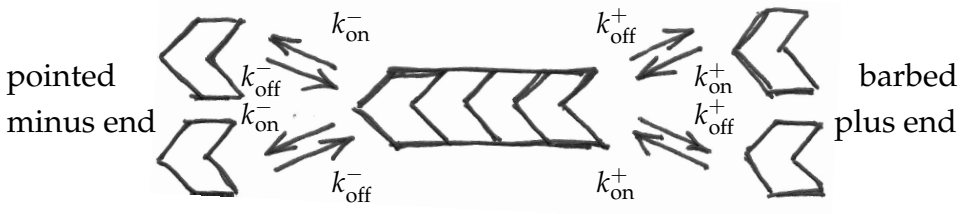

Figure 3.4: Eight rate constants for polymerization capture and release in non-symmetric actin and microtubules filaments.

different rates. The faster growing end is often referred to as the + end or barbed end, the slower growing end as the - end or pointed end. This implies, that $2^3 = 8$ rate constants are needed to describe actin or tubulin polymerization. Table displays the

	k_{on}^+ [1/(μMs)]	k_{off}^+ [1/s]	k_{on}^- [1/(μMs)]	k_{off}^- [1/s]	C_{crit}^+ μM	C_{crit}^- μM
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

experimentally measured rate constants that characterize the polymerization of actin filaments and microtubules. Experimental measurements reveal that (i) capture and release rates k_{on}, k_{off} are larger at the plus than at the minus end, and (ii) caputure rates of triphosphate are larger than those of diphosphate at both ends.

Polymerization of microtubules

For microtubules, the critical concentration of the faster growing plus end grows at a rate of $\mathrm{d}n^+ / \mathrm{d}t$ with rate constants k_{on}^+ and k_{off}^+. The critical concentration for the plus end C_{crit}^+ follows from the ratio of the two growth constants.

$$C_{crit}^+ = \frac{k_{off}^+}{k_{on}^+} = \frac{44}{8.9}\,\mu\mathrm{M} = 4.94\,\mu\mathrm{M},$$

The slower growing end grows at a different rate of $\mathrm{d}n^- / \mathrm{d}t$ with rate constants k_{on}^- and k_{off}^-. Accordingly, it grows at a different critical concentration C_{crit}^- than the plus end.

$$C_{\mathrm{crit}}^- = \frac{k_{\mathrm{off}}^-}{k_{\mathrm{on}}^-} = \frac{23}{4.3}\,\mu\mathrm{M} = 5.35\,\mu\mathrm{M}.$$

In essence, for this particular example, the critical concentration for both ends is thus almost equivalent, $C_{\mathrm{crit}}^+ = k_{\mathrm{off}}^+ / k_{\mathrm{on}}^+ \approx k_{\mathrm{off}}^- / k_{\mathrm{on}}^- = C_{\mathrm{crit}}^-$ although the rate constants at both ends differ by a factor two. This implies that at a given concentration either both ends shrink or grow.

$$\begin{aligned} C &< C_{\mathrm{crit}} \quad \ldots \text{ shrinkage at plus and minus end} \\ C_{\mathrm{crit}} &< C \quad \ldots \text{ growth at plus and minus end} \end{aligned}$$

This does not imply, however, that shrinkage and growth rates at both ends are identical. For microtubules, the process of shrinkage is approximately two times faster than growth.

Polymerization of microtubules Determine the time that it takes for a plus end of a microtubule to grow 5μm from the centrosome to the cell membrane. Assume a monomer concentration of $C = 10\mu$M and a tubulin dimer length of 8nm. Use the growth equation $\mathrm{d}n / \mathrm{d}n = k_{\mathrm{on}} C - k_{\mathrm{off}}$ with the parameters of GTP-tubulin, i.e., $k_{\mathrm{on}}^+ = 8.9/(\mu\mathrm{Ms})$ and $k_{\mathrm{off}}^+ = 44/\mathrm{s}$ $\mathrm{d}n / \mathrm{d}t = [\,8.9 \cdot 10 - 44\,]$ /s=45/s. The required number of tubulin dimers is $n = 5\mu$m / 8nm=5000/8=625. The required time follows from $\mathrm{d}n / \mathrm{d}t = 45/s = t/n$, thus $t = n / [\mathrm{d}n / \mathrm{d}t] = 625 / 45$ s = 13.89 s. It takes 13.89 seconds for the microtubule to grow from the centrosome to the cell membrane. But how long would it take for the polymer to shrink to zero length? Let's assume microtubule shrinks only from the membrane end. Then, $\mathrm{d}n / \mathrm{d}n = k_{\mathrm{on}} C - k_{\mathrm{off}}$ with the parameters of GDP-tubulin, i.e., $k_{\mathrm{on}}^+ = 0/(\mu\mathrm{Ms})$ and $k_{\mathrm{off}}^+ = 733/\mathrm{s}$. Then $\mathrm{d}n / \mathrm{d}t = [\,0 - 733\,]$ /s=-733/s. With $n = 625$, the required time is $t = n / [\mathrm{d}n / \mathrm{d}t] = -625 / -733$ s = 0.85 s. Polymer shrinkage takes 0.85 s. In this example, polymer shrinkage is approximately 16 times faster than polymer growth.

Polymerization of actin

The faster growing plus end of actin filaments grows at a rate of $\mathrm{d}n^+/\mathrm{d}t$ with rate constants k_{on}^+ and k_{off}^+. The critical concentration for the fast growing plus end is C_{crit}^+.

$$C_{\mathrm{crit}}^+ = \frac{k_{\mathrm{off}}^+}{k_{\mathrm{on}}^+} = \frac{1.4}{11.6}\,\mu\mathrm{M} = 0.12\,\mu\mathrm{M},$$

The slower growing minus end grows at a different rate $\mathrm{d}n^-/\mathrm{d}t$ defined through the rate constants k_{on}^- and k_{off}^- defining its critical concentration C_{crit}^-.

$$C_{\mathrm{crit}}^- = \frac{k_{\mathrm{off}}^-}{k_{\mathrm{on}}^-} = \frac{0.8}{1.3}\,\mu\mathrm{M} = 0.62\,\mu\mathrm{M}.$$

In addition to the growth and shrinkage states at both ends, there is now an additional intermediate interval at which the plus and grows whereas the minus end shrinks.

$$\begin{aligned} C < C^{+}_{\text{crit}} \quad &\ldots \quad \text{shrinkage at plus and minus end} \\ C^{+}_{\text{crit}} < C < C^{-}_{\text{crit}} \quad &\ldots \quad \text{growth at plus end and shrinkage at minus end} \\ C^{-}_{\text{crit}} < C \quad &\ldots \quad \text{growth at plus and minus end} \end{aligned}$$

It is interesting to explore the steady state related to this intermediate interval at which the overall polymer neither shrinks nor growths. The condition for this steady state implies that the overall growth rates is zero.

$$\frac{\mathrm{d}n^{+}}{\mathrm{d}t} + \frac{\mathrm{d}n^{-}}{\mathrm{d}t} = k^{+}_{\text{on}}\, C_{\text{std}} - k^{+}_{\text{off}} + k^{-}_{\text{on}}\, C_{\text{std}} - k^{-}_{\text{off}} \doteq 0 \quad \text{thus} \quad C_{\text{std}} = \frac{k^{+}_{\text{off}} + k^{-}_{\text{off}}}{k^{+}_{\text{on}} + k^{-}_{\text{on}}} \tag{3.2.5}$$

For actin, the steady state concentration at which the polymer remains at constant length while the plus and growth and the minus end shrinks is at

$$C_{\text{std}} = \frac{k^{+}_{\text{off}} + k^{-}_{\text{off}}}{k^{+}_{\text{on}} + k^{-}_{\text{on}}} = \frac{1.4 + 0.8}{11.6 + 1.3}\,\mu\text{M} = 0.17\,\mu\text{M}$$

which is right between the two critical concentrations.

$$C^{+}_{\text{crit}} = 0.12\,\mu\text{M} \leq C_{\text{std}} = 0.17\,\mu\text{M} \leq C^{-}_{\text{crit}} = 0.62\,\mu\text{M}$$

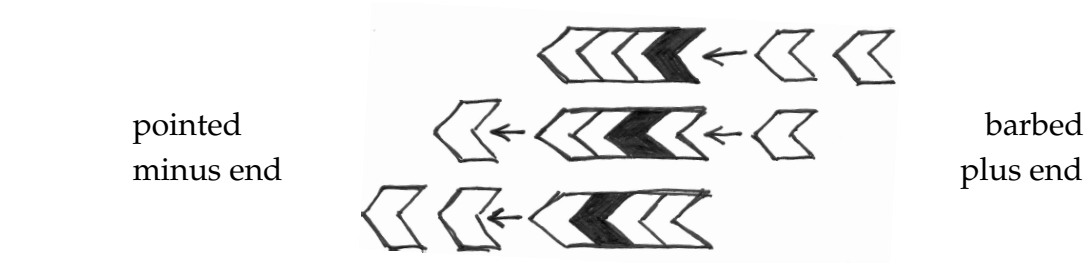

Figure 3.5: During treadmilling, monomers move along the filament from the plus end to the minus end while the overall filament might remain at constant length.

Treadmilling The special situation when one end of a filament polymerizes while the other end depolymerizes is referred to as treatmilling. Treadmilling can be observed in many cellular cytoskeletal filaments, especially in actin filaments and microtubules. The filament grows at the plus or barbed end while, at the same, it shrinks at the minus or pointed end. From the outside, it seems as if segments of the filament move across the cytosol [1]. In general, treadmilling may occur at different rates at both ends. At a particular concentration at which the speed of growth at the plus end is equal to the rate of growth at the minus end the net length of the treadmilling filament remains unchanged. This is state is called steady-state treadmilling.