Polymerization of Cytoskeletal Filaments

The most striking feature of cytoskeletal filaments is their length. Actin filaments range in length from ~35 nm in the cortex (just underneath the plasma membrane) of erythrocytes and other cells (Byers and Branton, 1985), to 10 to 100 μm in the stereocilia of hair cells, the sensory receptors of the vertebrate inner ear (Tilney and Tilney, 1988). Microtubules range in length from 1 μm or less in the mitotic spindle of the yeast S. pombe (Ding et al., 1993), through 100 μm in the axons of rat neurons (Bray and Bunge, 1981), to greater than 1 mm in insect sperm (Ashburner, 1989) and the comb plates of ctenophores (Tamam, 1973). The lengths of intermediate filaments are also in the micron range (Quirlan et al., 1995). Thus the cytoskeletal filaments span the scale from molecular to cellular. Because the individual proteins that make up these filaments are only a few to a few tens of nanometers long, it follows that cytoskeletal filaments contain tens to tens of thousands of protein subunits. One of the major points made in this chapter is that the existence of such long filaments requires a special structural adaptation: Filaments must be multistranded because single-stranded filaments are generally very short due to their tendency to break in the middle.

A second striking feature of actin filaments and microtubules is that their assembly and disassembly occur primarily by the addition and subtraction of subunits at one or both ends. This is clearly demonstrated in vitro where polymerization onto stable seeds can be visualized for actin by electron microscopy (Pollard, 1986), and for tubulin by video-enhanced light microscopy (Walker et al., 1988). Microtubules in vivo also grow by monomer addition onto their ends, as can be demonstrated by injection of fluorescently labeled tubulin into cells (Soltys and Borisy, 1985). In the case of actin, there are many observations that are consistent with polymerization of...
of visualizing individual actin filaments under the light microscope. Taken together, these in vitro and in vivo results demonstrate that end-polymerization is the major growth and shrinkage mechanism for actin filaments and microtubules. By contrast, the spontaneous breakage and annealing of actin filaments and microtubules in vitro is very slow, though there are proteins that sever actin filaments (such as gelsolin; Yin and Stossel, 1979) and others that sever microtubules (such as katanin; McNally and Vale, 1993). We will see in this chapter that end-polymerization is another consequence of actin and microtubules being multistranded—because of the contacts between the strands, it is energetically less favorable to break the filament in the middle than to remove a subunit from the end. The polymerization of intermediate filaments is not well understood and will not be discussed.

**Passive Polymerization: The Equilibrium Polymer**

As a first step to understanding polymerization, we will consider some very simple models. These models ignore the important fact that both actin and tubulin are nucleotide triphosphatases. The free energy derived from this hydrolysis reaction endows cytoskeletal polymerization with unusual and unexpected properties not found in crystallization or other forms of “passive” self-assembly. Some of these properties will be discussed in Chapter 11. But in order to appreciate the added richness provided by nucleotide hydrolysis, it is necessary to understand first the physical constraints that exist when a free energy source is not tapped during polymerization. For this reason we consider the passive polymerization of the so-called equilibrium polymer.

The simplest equilibrium polymer is the single-stranded filament shown in Figure 9.1. This is also known as the Einstein polymer (Hill, 1987). Whereas the single-stranded model has the advantage that its properties can be derived quite easily, it predicts that the average polymer is only a few subunits long. This fundamental weakness will be corrected later by considering the more realistic two-stranded model of Figure 9.2.

To solve the single-stranded model, we assume that all the individual subunit-addition reactions have the same dissociation constant, $K$. That is, we write

$$A_n + A_1 \overset{k_{\text{on}}}{\underset{k_{\text{off}}}{\underset{\text{addition}}{\longrightarrow}}} A_{n+1} \quad \frac{[A_n] \cdot [A_1]}{[A_{n+1}]} = K = \frac{k_{\text{off}}}{k_{\text{on}}} \quad n \geq 1 \quad (9.1)$$

where $A_1$ denotes the monomer and $A_n$ denotes the $n$-mer. The dissociation constant has the same unit as concentration, namely M, and is the reciprocal of the equilibrium constant (Chapter 5). It is more convenient to use the dissociation constant than the equilibrium constant because the latter has the awkward unit M$^{-1}$. Another reason to use the dissociation constant is that it is equal to the critical concentration, an important parameter for describing polymerization (see below). Because we are going to discuss polymerization of both actin filaments and microtubules, we need to adopt a potentially ambiguous terminology. We will refer to the building block of the filament as a subunit, which can be in either a monomeric or polymeric form. This definition is
unambiguous in the case of actin because the building block is the 45 kDa actin monomer. However, in the case of tubulin, there is a potential ambiguity because tubulin is a dimer of $\alpha$- and $\beta$-tubulin subunits. But because the dimer cannot be split into its two constituent subunits without irreversibly denaturing the protein, the dimer really is the building block and, for the purposes of polymerization, we will refer to it as a "subunit" that can even be in a "monomeric" form. This ought not create confusion. The dissociation constant is defined in terms of the ratios of concentrations. Even though it is often useful to write it as a ratio of the dissociation rate constant ($k_{off}$) and the second-order association rate constant ($k_{on}$) as shown in Equation 9.1, it is important to realize that the equilibrium concentration of the $n$-mers does not depend on the individual rates or the details of the mechanism by which the subunits come off and go on. Instead, the equilibrium concentration depends only on the equilibrium constant, which in turn depends only on the differences in energy of the subunits when they are in their various states of aggregation. This is a consequence of Boltzmann’s law (Chapter 5).

The Einstein polymer is a crude model. It is a useful starting point for understanding polymerization because it is simple, but it is important to realize that the model is inexact. For example, the dissociation constants for each of the addition reactions shown in Figure 9.1 are assumed to be equal. However, in reality, the equilibrium constants are expected to depend on the length of the polymer, though the differences are fairly small. The reason for this length dependence can be appreciated by considering the physical meaning of the dissociation constant. The dissociation constant is associated with a "standard free energy change," $\Delta G^0$, via

$$K = \exp\left(\frac{\Delta G^0}{RT}\right) \text{ moles/liter}$$

(Chapter 5). $\Delta G^0$ is the free energy change when the reaction occurs under standard conditions. In the molecular spirit of this book, we consider $\Delta G^0$ as an energy per molecule rather than per mole and so use $kT$, rather than $RT$, as the denominator in the exponential. The standard state is 1 M concentration, by chemical convention. We can interpret $\Delta G^0$ as the sum of a potential energy, usually negative, associated with formation of the bond, and an entropic
1 M concentration to being bound to other subunits in a polymer. This interpretation allows us to see why the dissociation constants are not all the same: When a monomer binds to a long polymer it loses more translational and rotational entropy than when it binds to another monomer, because a dimer moves and rotates more freely than a long polymer. Thus the association between two monomers will be stronger than the association between a monomer and a long polymer, and so the dissociation constants will be smaller for the former reaction. Using quantum mechanical calculations for objects the size of proteins and assuming that the polymers are rigid cylinders, Hill (1987) estimated that the dissociation constant is about ten times smaller for the monomer–monomer reaction compared to the monomer–long polymer reaction. These calculations also show that once a polymer is more than about 10 subunits long it can be considered to be a “long polymer” in the sense that the dissociation constant for binding to a 10-mer is within a factor of 2 of that for an infinitely long polymer. Thus we can think of this entropic effect as influencing nucleation but not growth. Despite this length-dependence of the dissociation constants, the conclusion I am going to draw regarding the equilibrium lengths of polymers is not qualitatively affected. Indeed, because the length dependence implies that smaller polymers are even more favorable, the conclusion that single-stranded filaments are short is reinforced.

**Single-Stranded Filaments Are Short**

The crucial question is: How does the average length of the polymers depend on the total concentration of subunits available for polymerization?

It is relatively easy to show that at equilibrium the lengths of the polymers are exponentially distributed

\[ [A_n] = K \exp \left( -\frac{n}{n_0} \right) \]  \hspace{1cm} (9.3)

(Appendix 9.1). In other words, there are always fewer \((n+1)\)-mers than \(n\)-mers, and the ratio of \((n+1)\)-mers to \(n\)-mers is a constant. As the total concentration of subunits, \([A_1]\), is increased, the average length of the polymers increases. However, even when \([A_1]\) is much greater than the dissociation constant \(K\), the average length of a single-stranded filament is still modest:

\[ n_{av} = \sqrt{\frac{[A_1]}{K}} \]  \hspace{1cm} (9.4)

This equation illustrates the fundamental shortcoming of the single-stranded model if it is to be applied to cytoskeletal filaments. It predicts that even when the total subunit concentration is one hundred times greater than the dissociation constant, the average polymer would contain only about 10 subunits. Yet cytoskeletal filaments must be designed to contain thousands of subunits. For a single-stranded polymer this would require total concentrations of subunits that are millions of times the dissociation constant. But for actin the total concentration (~200 μM; Bray, 1992) is only ~1000 times the dissociation constant.
The discrepancy is even greater for tubulin, where the total concentration (~20 μM) is only about twice the monomer concentration (~10 μM) (Zhai and Borisy, 1994).

The reason why the single-stranded model predicts short filaments is that the ends of the polymers are not very unfavorable from an energetic point of view—a monomer can decrease the free energy of the system equally well by associating with another monomer as it can by associating with a long polymer.

**Multistranded Filaments Are Long**

It could be argued that the reason why real filaments are much longer than predicted by the single-stranded model is that they are not at equilibrium, and that, if one waited long enough, the filaments would eventually shorten to the predicted lengths. Alternatively, it is possible that the free energy derived from nucleotide hydrolysis could somehow be used to overcome the shortcomings of the single-stranded model. However, these possibilities are not consistent with the observation that long filaments can be grown with ADP-actin, which has a relatively high dissociation constant (Pollard, 1986) and for which there is no source of free energy.

Instead, there is a much simpler explanation: Actin filaments and microtubules are multistranded, and multistranded filaments are inherently longer. The two-stranded model (Figure 9.2) differs from the one-stranded model because it has two different classes of bonds, one within the strands and one

\[ K_1 = \frac{[A_1][A_1]}{[A_2]} \]

\[ K_2 = \frac{[A_1][A_1]}{[A_2^*]} \]

\[ K = \frac{[A_1][A_2]}{[A_3]} \]

\[ K = \frac{[A_1][A_n]}{[A_{n+1}]} \]
between the strands. As a result, there are two different nuclei, $A_2^+$ and $A_2^-$, and three different dissociation constants, $K$, $K_1$, and $K_2$. The model is solved in Appendix 9.2. Like the single-stranded model, it predicts that the lengths of the polymers are exponentially distributed, but the average length is much greater

$$n_{av} \equiv \sqrt{\frac{K_1}{K}} \sqrt{\frac{[A_1]}{K}}$$

(9.5)

(assuming $[A_1] > K$). For example, the dissociation constants for actin are $K \sim 1 \mu M$ (see Table 11.1), and $K_1 \equiv K_2 \equiv 0.1 M$ (see below). If the total subunit concentration, $[A_1]$, is $10 \mu M$, then $n_{av} \equiv 1000$, corresponding to a $2.75 \mu m$-long actin filament. Thus the two-stranded model predicts filament lengths that are consistent with the polymer lengths found in cells. The reason why the two-stranded filaments are much longer than single-stranded filaments is that the ends of a two-stranded filament are energetically unfavorable, so there will be only a low concentration of them at equilibrium.

**Multistranded Filaments Grow and Shrink at Their Ends**

A second crucial difference between single-stranded and multistranded polymerization is that the lengthening and shortening of multistranded filaments occurs almost exclusively by monomer addition and subtraction at the ends. This is expected to hold even when the polymer is not at equilibrium, provided that the monomer concentration is not vanishingly small (in which case only annealing could take place). That growth and shrinkage of multistranded polymers occur at the ends is the key to the regulation of polymerization of actin filaments and microtubules. By providing stable nuclei it is possible to control the location of filament polymerization. Furthermore, it is possible to stabilize a filament by simply capping it, and it is not necessary to bind stabilizing proteins all along its length. These issues of nucleation and capping will be considered in more detail in Chapter 11.

The rate of elongation of multistranded filaments, in subunits per second, is given by

$$\frac{dn}{dt} = k_{on}[A_1] - k_{off}$$

(9.6)

where $k_{on}$ and $k_{off}$ are the on- and off-rates and $[A_1]$ is the monomer concentration. In general, the on- and off-rates will differ at the two ends (Chapter 11), but for the moment we will consider only one end and assume that the other end is capped.

The proof that growth and shrinkage occur exclusively by monomer addition and subtraction is quite subtle, but worth going through because it illustrates a number of important points about the kinetic properties of large molecules. Consider first the annealing reactions that lead to an increase in length of an $n$-mer:

$$\frac{dn}{dt} = k_{on}[A_1] + 2k_{on,2}[A_2] + \ldots + mk_{on,m}[A_m] + \ldots$$
where the first term on the right-hand side corresponds to addition of monomers, the second term corresponds to addition of dimers, and so on. If all the second-order annealing rate constants \( k_{\text{on,}m} \) were equal to the second-order monomer association rate constant \( k_{\text{on}} \), then the total growth rate would be

\[
\frac{dn}{dt} = k_{\text{on}} \sum_{n=1}^{\infty} m[A_n] = k_{\text{on}} [A_1]
\]

where \([A_1]\) is the total concentration of subunits. This shows that elongation is not necessarily an end property because, in general, \([A_1]\) > \([A_n]\), and in some cases \([A_1]\) >> \([A_n]\). However, when \( m \) is large, the translational and rotational diffusion of \( n \)-mers become very slow. The diffusion coefficients scale as \( \ln(m)/m \) for translation, \( 1/m \) for axial rotation, and \( \ln(m)/m^3 \) for rotation about an axis perpendicular to the filament axis (Chapter 6). As a result, the second-order annealing rates will become diffusion limited for large \( m \) and will be much slower than the monomer association rate. Because the second order on-rate constant for the monomer, \( k_{\text{on}} \approx 10^6 \text{M}^{-1}\text{s}^{-1} \) (see Tables 11.1 and 11.2), is already close to the diffusion-limited rate (Berg and von Hippel, 1985; Northrup and Erickson, 1992), it is likely that the annealing rate of even 10mers will be significantly less than \( k_{\text{on}} \). The strong length dependence of the perpendicular rotation term (\( \ln(m)/m^3 \)) means that we can consider the annealing of all but the shortest polymers to be “frozen out,” so that only monomer and short polymers will contribute significantly to the growth rate. But the concentration of short polymers is much less than that of the monomers (by a ratio \( [A_n]/[A_1] \approx K/K_1 \approx 10^{-5} \), see Appendix 9.2). As a consequence, the total contribution of annealing to growth will be minor. In other words, growth is by monomer addition, with rate \( k_{\text{on}} [A_1] \).

Now consider breakage. The change in length due to breakage is

\[
\frac{dn}{dt} = -k_{\text{off},1} - 2k_{\text{off},2} - \ldots - mk_{\text{off},m} - \ldots
\]

where \( k_{\text{off},m} \) is the rate constant for the breaking off of an \( m \)-mer. Because the dissociation constant for annealing/breakage of an \( m \)-mer is independent of \( m \) (i.e., independent of the length of the fragment), the freezing out of the annealing of long fragments as discussed in the previous paragraph means that the breakage into long fragments must also be frozen out. This unexpected result is an example of the Principle of Microscopic Reversibility: At the molecular level, the breakage rate is frozen out because long polymer fragments diffuse away from each other so slowly that they have a high chance of re-annealing before they escape. Thus only the dissociation of monomers and the breaking off of smaller oligomers will contribute significantly to shortening. However, the total contribution from small oligomers is small because breaking a two-stranded filament requires severing three bonds, whereas removing a subunit from the end requires severing only two bonds (Figure 9.3A). As a consequence, \( k_{\text{off},m}/k_{\text{off}} \approx K/K_1 \approx 10^{-5} \) for \( m > 1 \) (Appendix 9.3). This means that
Figure 9.3  Breaking a two-stranded filament is difficult
Dissociation of the terminal subunit from a two-stranded filament (A) is more likely to occur than breakage in the middle because it involves breaking only two bonds rather than three. By contrast, breakage of and dissociation from a one-stranded filament involves breaking only one bond (B).

shorten primarily by breakage because the breakage and dissociation both require only one bond to be severed (Figure 9.3B).

Other Properties of Multistranded Filaments

Multistranded filaments have a number of other interesting and important properties. Though our discussion has centered on 2-stranded filaments, qualitatively similar results hold for 3-stranded, 4-stranded (thin filaments comprising two actin filaments and two tropomyosin filaments), and even 13-stranded filaments (microtubules).

1. There exists a critical concentration, $K_c$, such that when the total subunit concentration ([A]), is less than $K_c$, there are hardly any polymers, whereas when the total subunit concentration exceeds $K_c$, almost all the excess subunits go into filaments. When the monomer concentration is above the critical concentration, the polymers will grow, whereas below the critical concentration the polymers will shrink. Thus the critical concentration is the monomer concentration at which the elongation rate is zero; comparison of Equations 9.6 with Equation 9.1 therefore shows that

$$K_c = \frac{k_{off}}{k_{on}}$$

(9.7)

These properties are shown graphically in Figure 9.4A,B. Note that when the total subunit concentration is greatly in excess of the critical concentration, the monomer concentration approaches the critical concentration.

2. The concentrations of the nuclei, $A_2^*$ and $A_2^{**}$ are very small, equal to $-K^*/K_1$ and $K^*/K_2$, respectively. In our numerical example, $K = 1 \text{ mM}$, $K_1 = K_2 = 0.1 \text{ M}$. Thus the concentrations of the nuclei are $0.1 \text{ nM}$. If the cell can provide more nuclei than this, then little growth from spontaneously formed nuclei will occur. The Arp2/3 complex (Machesky et al., 1994, Welch et al., 1997) nucleates actin polymerization and is one mechanism that permits cells to regulate their rate of actin polymerization in response to extracellular or intracellular signals (Machesky et al., 1999).
Figure 9.4  Polymerization of a two-stranded filament

(A) Growth rate for a multistranded filament according to Equation 9.6. (B) The concentration of subunits in their monomeric and polymeric forms as a function of the total subunit concentration. Note the sharp transition in polymer concentration about the critical concentration. (C) Mean length as a function of the total subunit concentration. In these examples, the off-rate constant is 1 s⁻¹, the on-rate constant is 1 μM⁻¹s⁻¹, and the dissociation constant (equal to the critical concentration) is 1 μM.

3. The ends of filaments will be predominantly blunt rather than having long protofilaments growing from them. This follows from the extra stability afforded by a subunit filling the "snug" site (see Figure 9.2).

4. The mean lengths of filaments increase very steeply about the critical concentration (see Figure 9.4C). Thus slight changes in the free monomer concentration, mediated by proteins that bind to monomers and sequester them (e.g., thymosin-β4, which binds to actin; statulin, which binds to tubulin; Safer et al., 1991; Gigant et al., 2000), are expected to have a profound effect on the polymer length.

**Binding Energies and the Loss of Entropy**

I need to elaborate on the binding constants used in our example. For the two-stranded filaments there are two bonds, one between protofilaments, with equilibrium constant $K_1$, and one within protofilaments, with dissociation constant $K_2$ (see Figure 9.2). Thus we might think that when both bonds are formed, the "binding energy" is simply the sum of the two separate contributions, and that the dissociation constant $K$ is simply the product of $K_1$ and $K_2$. However, this is not the case. The reason is that, as first mentioned in the discussion of the
 generally negative, and an entropic energy, $\Delta G_\text{s}$, which is generally positive due to the loss of translational and rotational entropy as a result of bond formation (Jencks, 1981). That is, $\Delta G^0_i = \Delta G_1 + \Delta G_2 + \Delta G_5 + \Delta G_7 + \Delta G_9 + \Delta G_{12}$ (i = 1, 2). Now, when a monomer binds to a snug site to form two bonds, the entropic cost is taken only once because the translational and rotational entropy is lost only once when the first bond is formed. According to this model, $\Delta G^0 = \Delta G_1 + \Delta G_2 + \Delta G_5 + \Delta G_7 + \Delta G_9 + \Delta G_{12}$. However, there is also another term: The formation of both bonds at the same time may require strain in the monomer or the polymer; furthermore, some of the entropy loss will be compensated for by vibrational entropy of the newly formed bonds. Thus a positive “interaction energy,” $\Delta G_{12}$, must also be included. Thus we write the standard free energy for formation of the two bonds together as

$$\Delta G^0 = \Delta G_1 + \Delta G_2 + \Delta G_5 + \Delta G_{12} = \Delta G^0_1 + \Delta G^0_2 - \Delta G_5 + \Delta G_{12} \tag{9.8}$$

In practice it is difficult to separate the entropic term ($\Delta G_5$) from the interaction-energy term ($\Delta G_{12}$), so the reader may wonder why this is a useful description of a protein–protein association. However, this approach seems preferable to the usual $\Delta H^0 - T\Delta S^0$ formulation because the standard enthalpy ($\Delta H^0$) and entropy ($-T\Delta S^0$) are very difficult to interpret for complex structures such as proteins interacting in water. In contrast, our mechanical picture of a protein–protein bond provides many qualitative insights into the mechanical interactions between proteins and is especially useful when interpreting how changes in protein conformation could alter binding strengths by inducing strain in the subunits and therefore increasing the interaction energy (Chapters 5 and 11).

The dissociation constant associated with Equation 9.8 is

$$K = \exp\left(\frac{\Delta G^0}{kT}\right) = K_1 K_2 \exp\left(\frac{-\Delta G_5 + \Delta G_{12}}{kT}\right) \tag{9.9}$$

where $K_i = \exp[(\Delta G_1 + \Delta G_2)/kT]$. The magnitude of the entropy-interaction term ($\Delta G_5 - \Delta G_{12}$) is uncertain. In many texts the term is simply ignored (e.g., Israelachvili, 1992). This is the same as setting it equal to zero and interpreting $\Delta G^0_i$ as binding energies alone. On the other hand, quantum mechanical calculations that assume that the bonds between subunits are completely rigid predict an entropic term equal to $-40 kT$ (Chothia and Janin, 1975). The best experimental estimate for the entropy-interaction term is $-10 kT$, based on the polymerization of tubulin (Erickson and Pantaloni, 1981), actin (Erickson, 1989), and sickle hemoglobin (Cao and Ferrone, 1997); on the binding of dehydrogenase to avidin (Jencks, 1981); and on the modeling of protein–protein association constants in terms of solvation energies (Horton and Lewis, 1992).

The entropy-interaction term for actin is likely to be about $10 kT$ in order to account for filament lengths in the micron range. Let us first consider an extreme case where $\Delta G_5 - \Delta G_{12} = 0$, and the entropy is ignored. Then, for a dissociation constant $K \sim 1 \mu M$, we need $K_1 = K_2 \sim 1 \mu M$, which gives a mean length of only $\sim 100$ (Equation 13.6 with $A_i/K = 31$). This is significantly shorter than the 1000 that is typical in cells and in vitro. On the other hand, if we take the other extreme of a very large entropic term, $\Delta G_5 - \Delta G_{12} = \Delta G_5 = 40 kT$, then
$K_1 = K_2 \sim 10^6 M (\ell)$ and two extremely weak bonds give a strong bond. In this case, the mean length is $\sim 3 \times 10^6$, which is too long. If we take an intermediate value of $\Delta G_5 - \Delta G_{12} = kT \ln(10,000) \equiv 9 kT$, then $K = 1 \mu M$, $K_1 = K_2 = 0.1 M$, and if the total subunit concentration is 10 times the critical concentration, then the mean length is 1000. Erickson (1989) also argues that the entropy-interaction term must be about $10 kT$ in order to account for the low frequency of breakage and annealing of actin filaments.

There is a simple mechanical explanation for why the entropic term should be only $\sim 10 kT$. Since the Debye length at physiological ionic strength is $\sim 1$ nm, it is likely that within this distance electrostatic forces will bring proteins into precise alignment (Schurr, 1970a,b; Chapter 5). Thus most of the entropy loss has occurred by the time the protein’s position and orientation have been restricted to within 0.5 to 1.0 nm (at which point intermolecular forces take over), rather than within the 0.05 to 0.1 nm characteristic of the stereospecific alignment that results when the final bond is formed. Thus the translational entropy lost going from 1 M concentration (corresponding to 1 molecule per (1.2 nm)$^3$ to the bound state is approximately equal to that lost as a result of being concentrated $\sim 2$ fold in each of the three directions, a total of perhaps $(3\ln2)\k \equiv 2 \k$. The loss of rotational entropy will be greater because a 50 kDa protein has a circumference of $\sim 16$ nm: Alignment within 1 nm in each of three dimensions is associated with a total entropy loss of $8 \k (\equiv (3\ln2)\k)$. Thus a total entropy loss of $10 \k$ is reasonable. If concentration by another factor of ten in each of six dimensions were necessary, the entropy loss would increase by an additional $14 \k (\equiv 6\ln10\k)$, to $24 \k$, nearer to that predicted by the quantum mechanical calculations.

**Structure and Dimensionality**

A key feature of linear polymers is that, at equilibrium, the lengths of the filaments are exponentially distributed. In other words, the polymers are polydisperse: They have a wide range of sizes. This is quite different from polymerization in two and three dimensions, where, at equilibrium, all subunits in excess of the critical concentration have aggregated into a single oligomer (Israelachvili, 1991). In two dimensions the aggregate is a “raft”; in three it is a clump or a crystal. In both cases, we expect that at equilibrium there will be just one raft or one clump. The reason why there is polydispersion in one dimension but precipitation in higher dimensions can be understood by considering the “surfaces” of the various polymers. In one dimension the surface corresponds to the two ends of a filament. In two dimensions it is the perimeter of a raft. And in three it is the surface of a clump. We can think of polymerization as being driven by the minimization of the surface areas. Now in the case of filaments, the fusion of two filaments reduces the number of ends by two, but this reduction is independent of the initial lengths of the filaments.
clumps. In other words, if there is a driving force for aggregation, then in two and three dimensions this driving force gets larger as the clumps get larger and this drives the system to precipitate.

**Summary**

Irrespective of the details of polymerization, two things are clear: Multistranded filaments are much longer than single-stranded filaments, and multistranded filaments polymerize and depolymerize by subunit addition and subtraction at their ends, whereas single-stranded filaments change length by annealing and breaking. These functional differences between single-stranded and multistranded filaments highlight the importance of the intrastrand contacts in actin filaments. If there were no intrastrand contacts (i.e., \( K_2 = \infty \) in Figure 9.2), then the actin filament would be effectively single-stranded and would not have the observed functional properties (Erickson, 1989).

**Problems**

9.1 **Average length** Show that the average length of the polymers, \( n_{av} \) (i.e., not counting the monomers and nuclei) is approximately equal to the characteristic length \( n_0 \).

9.2** Three-stranded model Find the critical concentration and the mean polymer length for a three-stranded filament. (Difficult!)

9.3* Transition about the critical concentration Show that for a two-stranded filament the mean filament length, \( n_{av} \), depends very sensitively on the total subunit concentration near the critical concentration (i.e., when \( [A_4] = K \)):

\[
\frac{dn_{av}}{d([A_4]/K)} = \sqrt[3]{\frac{K_1}{K}}
\]

Show that the slope \( \sim 1000 \) in the numerical example used in the text (\( K_1 = 0.1 \) M and \( K = 1 \) \( \mu \)M).

*Asterisks denote more advanced problems.