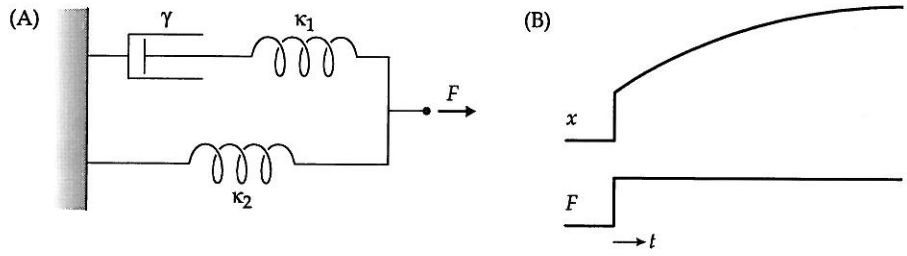
2.9 A Voigt element consists of a spring and a dashpot in series. When one end is held fixed and a constant force is abruptly applied to the other at time zero, how does the system move?

2.10 Show that the motion of the Maxwell element (Figure A, below), in response to a force F, is

$$x(t) = x_0 - (x_0 - y_0)\exp\left(-\frac{t}{\tau}\right) \qquad x_0 = \frac{F}{\kappa_2} \qquad y_0 = \frac{F}{\kappa_1 + \kappa_2} \qquad \tau = \gamma\left(\frac{1}{\kappa_1} + \frac{1}{\kappa_2}\right)$$

as plotted in Figure B.



CHAPTER

3

Mass, Stiffness, and Damping of Proteins

The purpose of this chapter is to get a feeling for what proteins are like as mechanical devices. How rigid are they? How quickly do they move and change shape? And what is the quality of their motion: When a protein is struck by a force, does it ring like a tuning fork (underdamped motion), or does it creep monotonically into a new shape (overdamped motion)? To answer these questions, I begin this chapter with a discussion of the material properties of proteins—their density, their elasticity, and the frictional forces that damp their motion. Proteins have similar densities and rigidities to hard plastics and Plexiglas. However, owing to their small size, the viscous forces from the surrounding fluid are large compared to the inertial forces. Consequently, the global motions of proteins are overdamped, meaning that proteins relax monotonically into new conformations. Thus a protein, as a mechanical device, is like a little plastic toy. But if we were to scale it up by a factor of 10^7 so that a 5-nm-diameter protein becomes a 50-mm-diameter device that would fit into the palm of one's hand, then we would have to increase the viscosity by the same amount (i.e., bathe it in treacle) in order to damp out any tendency for oscillation.

Mass

Mass equals density, ρ, times volume, V:

$$m = \rho V \tag{3.1}$$

The densities of various amino acids, proteins, organelles, and cells are given in Table 3.1. Proteins are composed of relatively light elements—carbon, oxygen, nitrogen, and hydrogen—and are about 40% denser than water, with different proteins having slightly different densities. We take the average density of proteins to be 1.38×10^3 kg/m^3.

Table 3.1 Densities of molecules, proteins, organelles, and cells relative to water

Substance	Density (relative to water[a])
Water	1.00
Glycerol	1.26
Glycine	1.16 (solid)
Alanine	1.40 (solid)
Glutamic acid	1.46 (solid)
Hemoglobin	1.33 (in solution)
Trypsin	1.38 (in solution)
Lysosyme	1.42 (in solution)
Chromosome	1.36
Virus	1.15
Mitochondrion	1.18
Synaptic vesicle	1.05
Erythrocyte	1.10
Fibroblast	1.05

Source: Rickwood, 1984; Kaye and Laby, 1986; Creighton, 1993.
[a]The density of water at 20°C is 998 kg/m^3.

The SI unit of mass is the kilogram (kg). However, in biochemistry the mass of proteins and other biomolecules is usually expressed as **molecular mass**, defined as the mass in grams of a mole of the molecules. The unit is the **dalton** (Da). According to this definition, a hydrogen atom has a molecular mass of 1 Da, corresponding to an actual mass of 1.66×10^{-24} g ($1 \text{ g} \div N$, where N is the Avogadro constant given in the table on the endpapers) or 1.66×10^{-27} kg. A protein of molecular mass 100,000 Da, or 100 kDa, has a mass of 166×10^{-24} kg.

The density of proteins is such that each kDa of protein occupies a volume of about 1.2 nm^3. A 100 kDa protein therefore has a volume of 120 nm^3; if it were spherical its diameter would be 6 nm. Because the average molecular mass of an amino acid is 119.4 Da (weighted according to amino acid frequency in globular proteins; Creighton, 1993), there are ~7 amino acids per nm^3.

Elasticity

A solid is **homogenous** if its mechanical properties are identical throughout, and it is **isotropic** if these properties do not depend on direction. If a small tensile force F is applied to a homogenous, isotropic solid of uniform cross-sectional area A (Figure 3.1), it is found experimentally that the **strain**, the relative length change $\Delta L/L$, is proportional to the **pressure**, the force per unit area:

$$\frac{F}{A} = E\frac{\Delta L}{L} \tag{3.2}$$

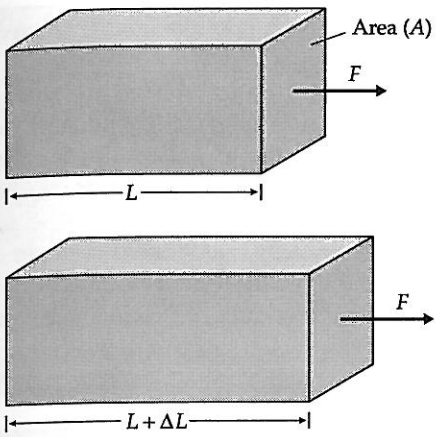


Figure 3.1 A solid strained by a tensile force

Because the extension (ΔL) is proportional to the force, this is an example of Hooke's law. The constant of proportionality in Equation 3.2, E, is known as the **Young's modulus**, or the **elastic modulus**. Because strain is the ratio of lengths and is therefore dimensionless, the Young's modulus has the same units as pressure, namely newtons per square meter (N/m^2) or pascals (Pa). The Young's moduli of various materials and proteins are given in Table 3.2. Note

Table 3.2 Young's moduli and tensile strength of materials

Material	Young's modulus, E (GPa)	Tensile strength (GPa)[a]
Carbon nanotube	1300	14
Diamond	1200	—
Steel (stainless)	211	1.1 (wire)
Glass (quartz)	73	1 (fiber)
Wood (fir, along grain)	16	0.06
Plexiglas	3	0.05
Plastic (polypropylene)	2.4	0.035
Teflon (PTFE)	0.34	0.022
Rubber (polyisoprene)	0.02	0.017
Silk (*Bombyx mori*)	5–10	0.3–0.6
Keratin (hair)	2.4	0.2
Actin	2.3	0.03
Collagen	2	0.1
Tubulin	1.9	—
Elastin	0.002	0.002

Source: Data for nonproteins from Tennent, 1971; Kaye and Laby, 1986; Wong et al., 1997. Data on proteins from Table 8.5 (from Wainwright et al., 1976; Kaye and Laby, 1986) and from Fraser and Macrae, 1980; Tsuda et al., 1996.

[a]Note that drawing a material out into a wire or fiber increases its tensile strength (Gordon, 1984).

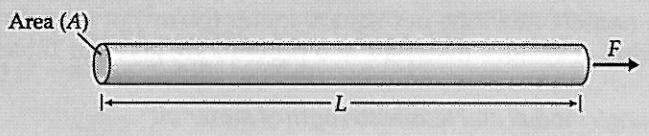
that the most rigid proteins have similar Young's moduli to Plexiglas (Perspex) and hard plastics!

For many materials—for example, metals, plastics, and structural proteins—Hooke's law applies only for forces that cause strains up to 0.1 to 1 percent. At higher forces the material yields; the yield pressure is called the **tensile strength**. By contrast, some resilient materials such as rubber and proteins like elastin and titin can be strained up to 100% or more.

The Young's modulus is a material property, meaning that it does not depend on the object's size or shape. On the other hand, the stiffness of an object does depend on its size and shape (as well as its Young's modulus). This is illustrated in the following examples.

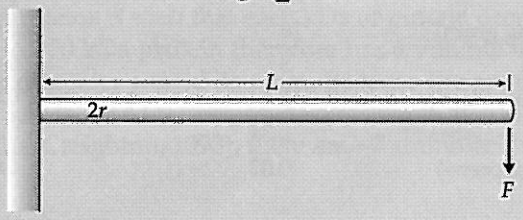
Example 3.1 Stiffness of a rod under tension The longitudinal spring constant of the rod shown in the figure is:

$$\kappa = \frac{F}{\Delta L} = \frac{EA}{L}$$



Example 3.2 The cantilever spring A rod subject to a bending force (as shown in the figure below) has a stiffness (see Equation 6.5 and Figure 6.2)

$$\kappa = \frac{4\pi}{3}\frac{Er^4}{L^3}$$

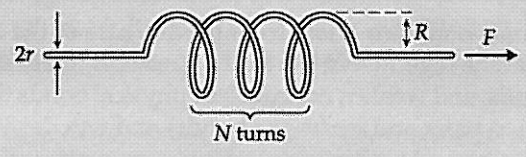


In general, proteins are neither homogenous nor isotropic due to their complex atomic structures. For this reason, care must be taken when considering their mechanical properties. For a nonhomogenous, nonisotropic solid, there are as many as 21 elastic parameters for *every* point in the material (Kittel, 1996): An exact description of the elasticity of a material could therefore be as complex as the full atomic description! By contrast, a homogenous and isotropic material has just two elastic parameters, the Young's modulus and Poisson's

Example 3.3 The coiled spring The coil in the figure has a stiffness

$$\kappa \cong \frac{1}{4N}\frac{Gr^4}{R^3}$$

where the shear modulus, G, is defined in Problem 3.6.



ratio, the latter being a measure of how much the cross-sectional area changes as the material is stretched (Problem 3.6). Thus the description of the elastic properties of a material is greatly simplified if the material is homogenous and isotropic.

Is it valid to think of proteins as having material properties, or must we always think in terms of their atomic structures? This question is related to the domain concept of structural biology (Creighton, 1993) in which proteins are thought of as comprising fairly rigid domains joined by more flimsy connecting regions (Yguerabide et al., 1970; Mendelson et al., 1973; Gerstein et al., 1994). In this picture the hinging or twisting of domains is attributed to the less substantial thickness of the connections, in the same way that a rubber dumbbell bends about its linking rod not because the rod is composed of a weaker material, but because it has a reduced cross-section. Thus the domain concept encompasses the idea that proteins have material properties.

Are there experiments to support the notion that proteins can be thought of as mechanically isotropic, at least to a first approximation? In the case of the globular proteins actin and tubulin, which polymerize to form cytoskeletal filaments, the Young's moduli are found to be approximately independent of the direction of the applied force, indicating that there is no drastic departure from isotropy (Chapter 8). In addition, the Young's moduli of several filamentous proteins are similar, despite their quite different atomic structures (see Tables 3.2 and 8.5); this suggests the existence of a material property that is independent of the atomic details. On the other hand, wet hair has significant mechanical anisotropy: The Young's modulus measured using longitudinal forces is an order of magnitude greater than that measured using transverse forces, due to the orientation of the constituent coiled coils. Nevertheless, even in this case, the anisotropy may be described quite simply.

In summary, the concept that proteins have material properties derives support from both structural and mechanical studies. The simplicity of the material description over the atomic one makes it a useful conceptual tool for understanding protein mechanics. Furthermore, the material description can be readily tested and refined by mechanical experiments on proteins; by contrast,

the tools necessary for relating mechanical measurements to atomic descriptions of proteins via molecular dynamics simulations are only now being developed (Krammer et al., 1999; Marszalek et al., 1999).

The Molecular Basis of Elasticity

The rigidity of most materials arises from the stiffness of the bonds that hold the constituent atoms together. In the case of proteins, there are strong, covalent chemical bonds, and weaker, noncovalent physical bonds that include electrostatic bonds (ion pairs and hydrogen bonds) and van der Waals bonds.

The energy of a bond holding together two atoms depends on the separation, r. At equilibrium, when there is no net force acting on the atom, the energy is a minimum. The separation for which the energy is a minimum is the bond length, r_0. The energy profile, $U(r)$, forms a well as shown in Figure 3.2, and at the bottom of the well the profile is approximately parabolic: $U(r) \cong U_0 + \frac{1}{2}\kappa(r - r_0)^2$, where U_0 is the bond energy and κ is the stiffness of the bond. To

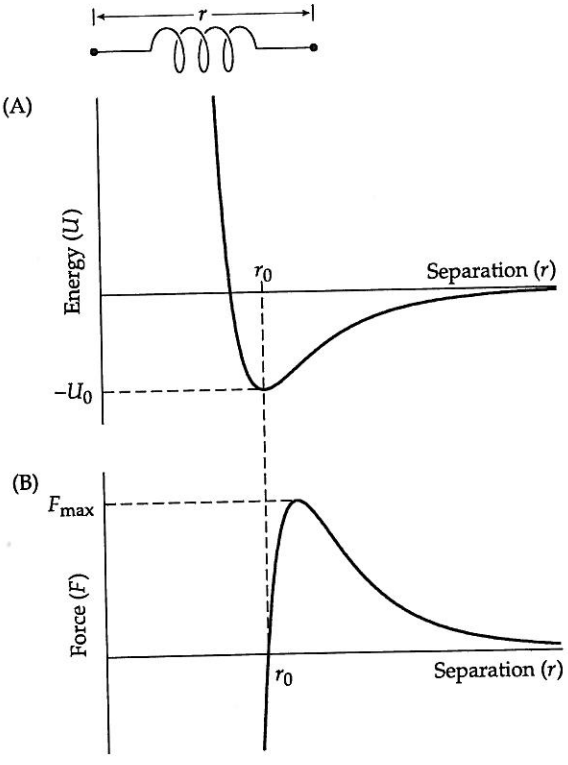


Figure 3.2 Energy and force of a van der Waals bond between two atoms
(A) The bond energy (U) plotted against the center-to-center spacing (r). (B) The force ($F = dU/dr$) required to stretch the bond. The energy function used is the **Lennard–Jones potential,**

$$U(r) = -U_0\left[2\left(\frac{r_0}{r}\right)^6 - \left(\frac{r_0}{r}\right)^{12}\right]$$

The first term on the right-hand side corresponds to the attractive component, which falls off with the sixth power of the distance according to the van der Waals interaction. The second term is repulsive and corresponds to a "steric" force (Israelachvili, 1991). The potential is a minimum when the force is equal to zero, and this occurs when $r = r_0$. The force is a maximum when $r \cong 1.11r_0$; when stretched beyond this distance, the bond breaks. The asymmetry of the energy profile means that the stiffness is not a constant, though for small strains (<1%) the stiffness changes by less than 10%. The asymmetry also means that the bond expands when heated, because, as the temperature increases, the bond vibrates more, and the atoms spend less time at the bottom of the well (at separation r_0), and more time at higher energy levels that are associated with a larger average bond length due to the asymmetry.

stretch the bond a small distance $(r - r_0)$ requires an applied force $F(r) = dU/dr \cong \kappa(r - r_0)$. In other words, for small forces, the extension of the bond, $r - r_0$, is proportional to the force: Hooke's law holds. For larger tensile forces, Hooke's law breaks down and the bond becomes softer. Eventually a maximum force is reached; beyond this force the bond breaks (see Figure 3.2), and the material yields.

If we knew the spring constant of every bond in a material, then we could calculate its Young's modulus (and other elastic parameters). This approach is taken in molecular dynamics, in which the stiffness and length of each covalent, ionic, and van der Waals bond is specified, as well as the bending and torsional stiffnesses of the covalent bonds (Levitt, 1974; Tung et al., 1984; McCammon and Harvey, 1987). To illustrate how the stiffness of the constituent bonds determines the rigidity of a material, consider a hypothetical material composed of a cubic lattice of identical atoms, shown in Figure 3.3, connected by bonds of stiffness κ and length r_0. Suppose that a force is applied perpendicularly to one face of the lattice such that each bond experiences a tensile force F. Each bond will be stretched a distance Δr according to Hooke's law: $F = \kappa \Delta r$. Dividing through by r_0^2, we have $F/r_0^2 = (\kappa/r_0)(\Delta r/r_0)$. Now F/r_0^2 is the force

Example 3.4 The Young's modulus of a covalent solid The stiffness of the C–C single bond is ~550 N/m and the bond length is 0.14 nm (Tung et al., 1984). If carbon formed a cubic lattice, its Young's modulus would be ~4×10^{12} Pa, or 4000 GPa, along the [100] axis. This overestimates the Young's modulus of diamond by a factor of three (see Table 3.2). The overestimation occurs because carbon is tetravalent rather than hexavalent, and the tetrahedral coordination of each atom with its nearest neighbors (Moore, 1972) means that bond bending contributes additional compliance.

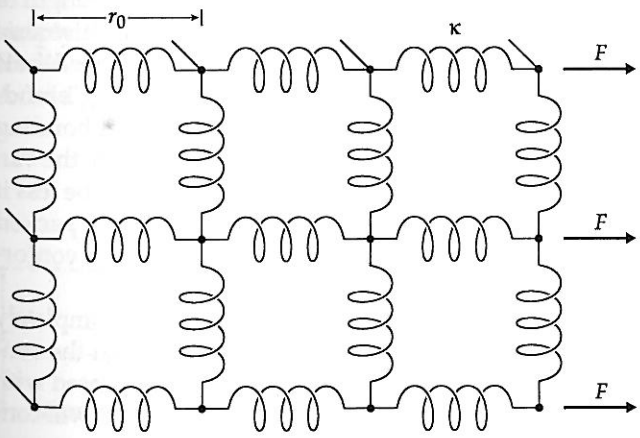


Figure 3.3 A cubic lattice of springs

per unit area and $\Delta r/r_0$ is the strain in each bond (and thus the strain in the whole material). Comparing this with the definition of Young's modulus (Equation 3.2), we see that for this hypothetical material, $E = \kappa/r_0$. Note that κ/r_0 has the same units as Young's modulus.

Table 3.2 shows that the Young's moduli of proteins are much less than that of solids that are held together by covalent bonds (e.g., diamond), metallic bonds, or electrostatic bonds (e.g., glass). This is because the compliance of proteins (the reciprocal of the stiffness) is dominated by the softer van der Waals bonds between the uncharged amino acids: The van der Waals bonds are the weak links in the structure. Evidence for this statement comes from the following "back of the envelope" calculation. The van der Waals potential energy between two solids whose planar surfaces are separated by a distance D is

$$U(D) = -U_0\left[\tfrac{1}{3}\left(\frac{D_0}{D}\right)^2 - \tfrac{1}{3}\left(\frac{D_0}{D}\right)^8\right] \text{ energy per unit area} \qquad (3.3)$$

(Israelachvili, 1991; and see Heinz and Hoh, 1999 for several other force laws used to interpret AFM measurements). D_0 is the resting (equilibrium) separation, which we take to be twice the van der Waals radius (Creighton, 1993): $D_0 \cong 0.3$ nm. U_0 is equal to twice the surface energy (also called the surface tension): $U_0 \cong 40$ mJ/m^2 = 10 kT/nm^2 = 40 pN/nm for molecules like oils and hydrocarbons that are composed of hydrogen, carbon, oxygen, and nitrogen (Israelachvili, 1991). The first term in the square brackets in Equation 3.3 corresponds to the attractive dipole–dipole interactions, whereas the second term arises from steric repulsion between adjacent atoms. In Appendix 3.1 it is shown that a solid composed of uncharged amino acids of diameter ~0.6 nm held together by van der Waals bonds is expected to have a Young's modulus of ~4 GPa. The experimental finding that the Young's moduli of filamentous proteins are in the range 1 to 5 GPa (see Table 3.2 and Chapter 8), close to this theoretical value, therefore supports the notion that the rigidity of proteins is primarily limited by the rigidity of the van der Waals bonds. A further corollary of the van der Waals model is that the maximum tensile strength of a protein ought to be 0.1 to 0.2 GPa (Appendix 3.1). These values are also similar to those of proteins (see Table 3.2), again supporting the van der Waals model for protein rigidity. A possible exception is silk: Its high Young's modulus and tensile strength are probably due to the strong hydrogen bonding along the β-sheet backbone of the silk molecules. In general, though, the van der Waals rigidity is expected to be an upper limit. The rigidity will be less if the protein is not well folded (i.e., the amino acids are not well packed together) or if the protein fluctuates between a number of different conformational states (Chapter 5).

Rubber and flexible proteins such as titin resist deformation for a completely different reason than do rigid materials. Deformations tend to align the constituent polymeric chains (Figure 3.4), and the loss of entropy associated with such alignment makes the deformation energetically unfavorable. We will con-

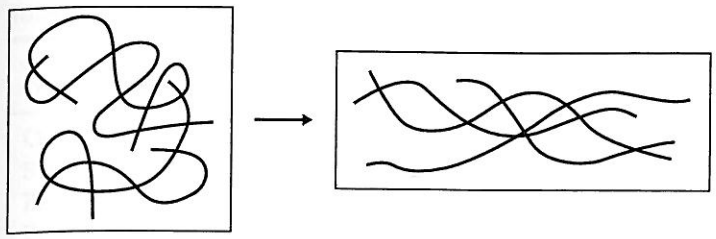


Figure 3.4 Deformation of a rubber-like material

sider the statistical physics of rubber-like elasticity in more detail in Chapter 6, and show that for small deformations, the stiffness is approximately constant, so Hooke's law again applies. At higher strains, the chains become taut, and the stiffness increases, in contrast to the behavior of rigid materials, which get softer before eventually yielding.

Viscous Damping

As a protein changes shape, it is subject to two types of damping forces that slow its motion. The first arises from the viscosity of the surrounding fluid. We call this solvent friction. The second arises from transitory interactions between amino acids that slide with respect to one another as the protein changes shape. We call this **protein friction**. The two types of damping have a similar molecular origin, as is shown below.

Viscosity is defined as follows. When two surfaces submerged in a fluid are moved slowly with respect to each other as shown in Figure 3.5, it is found experimentally that the force per unit area required to produce the shear, F/A, is proportional to the velocity gradient, dv/dx in the fluid between them:

$$\frac{F}{A} = \eta \frac{dv}{dx} \tag{3.4}$$

The constant of proportionality, η, is called the coefficient of viscosity, or simply the viscosity. Because the unit of pressure is the pascal and the unit for velocity gradient (also called shear rate) is s^{-1}, the unit of viscosity is Pa·s. A fluid for which the viscosity is independent of the velocity gradient is called

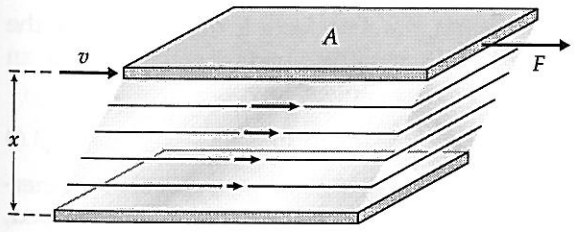


Figure 3.5 Velocity gradient in a viscous fluid between two surfaces

Table 3.3 Viscosities of various liquids

Liquid	Viscosity at 20°C (mPa·s)
Acetone	0.32
Water	1.00
N-hexadecane	3.34
Phenol	12.7
Motor oil (S.A.E. 30)	30
Olive oil	84
Ficoll 400 (50% w/v)	600
Glycerol	1408

Note: Data from Tennent, 1971; Kaye and Laby, 1986; and Resnick et al., 1992, except for Ficoll 400 (Pharmacia), a highly branched polysaccharide of molecular mass 400 kDa.

a **Newtonian fluid**. Most common liquids are Newtonian. The most common deviation from Newtonian behavior is **shear thinning**, the tendency for the viscosity to decrease at high velocity gradients. The viscosity of several fluids is given in Table 3.3.

An object moving through a viscous fluid will experience a drag force that opposes its motion. The magnitude of the drag force depends on the pattern of fluid flow around the object. This, in turn, depends on the **Reynolds number** defined by

$$Re = \frac{\rho L v}{\eta} \tag{3.5}$$

where ρ is the density of the liquid, L is the characteristic length of the object (in the direction of the flow), v is the speed of the object, and η is the viscosity. Note that the Reynolds number is dimensionless, and its physical meaning is that it is the ratio of the inertial and the viscous forces. The Reynolds number tells you what opposes the acceleration of an ocean liner or a bacterium (see Table 3.4). For the ocean liner ($Re >> 1$), it is the mass; for the bacterium ($Re << 1$), it is the drag, and the mass does not matter. If the Reynolds number is less than 1, the flow is laminar and nonturbulent and is referred to as **creeping flow**. The Reynolds number scales with the size of the object, L: The smaller the object, the smaller the ratio of inertial to viscous forces. As illustrated in Table 3.4, microscopic objects like cells and proteins have a Reynolds number less than 1.

At low Reynolds number (i.e., $Re < 1$), the drag force is proportional to the speed, and for a sphere of radius r moving at constant velocity v in an unbounded fluid, the drag force is given by **Stokes' law**:

$$F_d = -\gamma v = -6\pi\eta r v \quad \text{or} \quad \gamma = 6\pi\eta r \tag{3.6}$$

Stokes' law also holds when the velocity is not constant, provided that the inertial forces are less than the viscous forces (i.e., the motion is overdamped, Appendix 3.2). A nearby surface significantly increases the drag coefficient

Table 3.4 Reynolds numbers

Object	Size	Speed	Density of the fluid (kg/m3)	Viscosity of the fluid (Pa·s)	Reynolds number
Ocean liner	100 m	30 m/s	1000	10^{-3}	3×10^{9}
Swimmer	2 m	1 m/s	1000	10^{-3}	2×10^{6}
Bee	10 mm	0.14 m/s	1.3	18×10^{-6}	100
Protein	6 nm	8 m/s	1000	10^{-3}	0.05
Bacterium	2 μm	25 μm/s	1000	10^{-3}	5×10^{-5}

(Happel and Brenner, 1983): For example, if the surface of a sphere is within a radius of a plane surface, then the drag is increased ~40%.

Example 3.5 Jar of honey The viscosity of honey is ~100 Pa·s. According to Stokes' law, to pull a honey spoon, a sphere of diameter 20 mm, out of a jar of honey at a rate of 1 m/s requires a force of about 18 N. We can check that the Reynolds number is ~0.2, so Stokes' law does apply. This force corresponds to the weight of a 1.8 kg mass, so it is not surprising that we can actually pick up the jar with the viscous force.

The Molecular Basis of Viscosity

Although the molecular basis for the viscosity of gases is well understood, that of liquids is not. In an ideal gas, the force needed to shear two adjacent planes in the gas arises from the transfer of momentum due to the diffusion of the gas molecules from regions of high speed to regions of low speed. Because the rate of diffusion (and therefore the change in momentum) increases with temperature, the viscosity of gases actually increases with temperature! By contrast, the viscosity of liquids decreases with temperature; this suggests that the viscosity of liquids is due to intermolecular bonds that break more rapidly at higher temperatures.

I now derive a simple theory of viscosity based on molecular friction between two surfaces (Figure 3.6). Suppose that the molecules on one surface

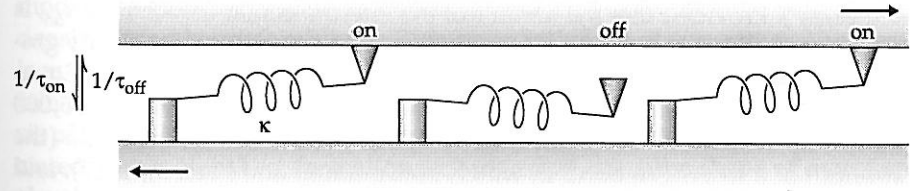


Figure 3.6 Protein friction due to transient crosslinks between two surfaces

make transitory crosslinks with the molecules on an adjacent surface, and that the surfaces slide past one another at a speed that is slow compared to the breaking of the crosslinks—that is, while the crosslink is attached, the surfaces move through a distance that is small compared to the molecular size. Let the rate of detachment be $1/\tau_{on}$ (the reciprocal of the time, τ_{on}, that they spend attached), and suppose the speed of movement is v. Then each molecule will be stretched by an average amount $\tau_{on}v$ during each attachment. If the stiffness of each molecular bond is κ, then the average force opposing the shear is $-\kappa\tau_{on}v$. If each molecule spends a fraction p of its time attached as a crosslink, then the average force per molecule is $-p\kappa\tau_{on}v$. Because this force is proportional to the speed, we can think of it as being a drag force. The associated drag coefficient (per molecule) is

$$\gamma = p\kappa\tau_{on} \tag{3.7}$$

This drag coefficient is independent of speed provided that $p\tau_{on}$ is independent of speed, which occurs if both the attachment and detachment rates are independent of the speed. For low speeds this is likely to be the case. A similar expression has been derived by Schoenberg (1985), Tawada and Sekimoto (1991), and Leibler and Huse (1993).

Equation 3.7 accounts for many features of viscous damping. As expected, the damping increases with the number of molecular bonds between the surfaces. The drag increases as the attached time increases because the crosslinks get more stretched and produce a greater force. The drag also increases with the stiffness of the crosslinks, again because a greater opposing force is generated. Because the attached time is expected to decrease with temperature, so too will the viscosity (p is expected to change little with temperature). Furthermore, for large speeds, corresponding to large shear rates, we expect the attached time to decrease as the shear disrupts the bonds. Thus the model predicts that the viscosity should eventually decrease as the velocity gradient is increased, giving rise to shear thinning.

We can develop this approach a little further to derive an approximate expression for the viscosity of a liquid in terms of molecular parameters. The number of molecules per unit area is $\sim 1/\delta^2$, where δ is the dimension of the molecule. The separation of the layers is also $\sim\delta$. We can therefore write

$$\frac{\text{Force}}{\text{Area}} = \frac{p\kappa\tau_{on}v}{\delta^2} = \left(p\kappa\delta^2\right)\left(\frac{1}{\delta^3}\right)\left(\tau_{on}\right)\left(\frac{v}{\delta}\right) \cong wc\tau_{on}\frac{dv}{dx} \tag{3.8}$$

where w is the intermolecular bond energy and c is the concentration (molecules/m^3). Provided that the lifetime of the intermolecular association, τ_{on}, is a constant (see below), then the force will be proportional to the velocity gradient, dv/dx, as expected for the viscous force in a Newtonian fluid (see Equation 3.4). The corresponding viscosity is $\eta \cong wc\tau_{on}$. For water, c = 55,000 moles/m^3 = 33×10^{27} molecules/m^3, $w = 8\ kT \cong 32 \times 10^{-21}$ J/molecule (the strength of a hydrogen bond in water; McCammon and Harvey, 1987), and $\tau_{on} \cong 10$ ps (Eisenberg and Kauzmann, 1969). The predicted viscosity is there-

fore ~10 mPa·s, which is close to (though a little higher than) the viscosity of water at room temperature, ~1 mPa·s. This analysis assumes that the distances through which the crosslinks are deformed are much less than the size of the bonds. This relationship is satisfied provided that the velocity gradient is much smaller than the detachment rate, $1/\tau_{on}$. In the case of water, the analysis should be valid for velocity gradients up to ~ 10^9 s^{-1}, which corresponds to a very high shear rate.

Because the sliding of protein domains past each other also entails the breaking and unbreaking of numerous weak bonds, we expect that protein movements will be slowed down by internal viscosity. Internal viscosity, or protein friction, has been measured in relaxed muscle fibers where the myosin heads make transitory crossbridges to the actin filaments (Brenner et al., 1982). Molecular dynamics modeling suggests that the interior of proteins is liquid-like and hence should possess viscosity (McCammon et al., 1977). Protein friction has been measured in myoglobin where it is found to contribute a damping force that is four times greater than that from the solvent (Ansari et al., 1992). The magnitude of the internal friction for other proteins is not known. However, because the viscosities of aromatics and light oils are in the range of 1 to 30 times that of water, we expect protein friction to be at least as important as solvent friction. If more long-lived crosslinks must be broken, then protein friction could be much larger. A challenge for experimentalists is to measure the internal viscosity, preferably at the single-protein level, to determine the extent to which protein friction limits the speed of conformational changes.

The Global Motions of Proteins Are Overdamped

In Chapter 2 I established criteria for whether the motion of an object in response to a mechanical force is oscillatory (underdamped) or monotonic (overdamped). It was shown that the behavior depends on the relative magnitudes of the inertial and viscous forces. These in turn depend on the material properties of the object—its mass, stiffness, and damping. In this chapter I have described the material properties of proteins. We are therefore now in a position to determine whether the global motions of proteins are underdamped or overdamped. The answer is arrived at through a scaling argument: As the dimension of an object gets smaller, the viscous forces increase relative to the inertial forces, and as a result, *the global motions of small, comparatively soft objects such as proteins in aqueous solution are expected to be overdamped.*

To develop the scaling argument, we consider first a crude mechanical model of a globular protein as a homogenous and isotropic cube with side L, density ρ, and Young's modulus E, damped by a fluid of viscosity η. The mass is $m = \rho V = \rho L^3$. The stiffness is $\kappa = EL$ (Example 3.1), assuming that the protein is globular rather than elongated. (See the end of this section for an analysis of elongated proteins such as cytoskeletal filaments.) The drag force associated with a global conformational change that alters the shape of a protein should be roughly given by Stokes' law, for which the drag coefficient is $\gamma \cong$

$3\pi\eta L$ (Appendix 3.3). In Chapter 2 I showed that the motion is overdamped if the ratio $4m\kappa/\gamma^2$ is less than 1. In the present case

$$\frac{4m\kappa}{\gamma^2} = \frac{4 \cdot \rho L^3 \cdot EL}{(3\pi\eta L)^2} = \left(\frac{2}{3\pi}\right)^2 \frac{\rho E}{\eta^2} L^2 \tag{3.9}$$

The important feature of this equation is that it shows how the ratio scales with dimension, L: The smaller the object, the smaller the ratio, and the less is the tendency for oscillation. The reason for this scaling behavior is that while the damping and the stiffness decrease in proportion to the length, the mass decreases much faster (to the third power), so the inertial forces decrease more quickly than the viscous forces. While the numerical term on the right-hand side of Equation 3.9 depends on the particular model, the scaling behavior does not.

How small must a protein be to ensure that its motion is overdamped and that it does not oscillate when subject to an external force? The most rigid proteins have Young's moduli, E, on the order of 1 GPa (see Table 3.2). The density, ρ, is on the order of 10^3 kg/m^3, and the viscosity of water, η, is on the order of 1 mPa·s (see Table 3.3). Thus for a rigid protein in water, $\eta^2/\rho E \cong 1$ nm^2, and according to Equation 3.9, the motions of globular proteins or protein domains of diameter less than a characteristic length $L_c \cong (3\pi/2)\sqrt{\eta^2/\rho E} \cong 5$nm will be overdamped. This length corresponds to a medium-sized globular protein of ~1000 amino acids. Thus the model predicts that global motions of rigid globular proteins or protein domains of molecular weight less than 100 kDa should be overdamped. This conclusion is confirmed by the more accurate analysis presented in Appendix 3.2. The analysis in the Appendix also justifies the use of Stokes' law for the drag coefficient, which strictly applies only if the motion is overdamped. Although the quality of motion of proteins is difficult to measure experimentally, molecular dynamics modeling studies lend support to these arguments (McCammon et al., 1976).

There are several additional arguments for why the motions of even large proteins (L>5 nm) ought to be overdamped:

1. The rigidity of allosteric, energy-transducing proteins such as motor proteins and the ribosome (Example 3.6) is likely to be much less than that of rigid proteins like those of the cytoskeleton. Consider a protein that undergoes a fairly modest conformational change of 1 nm, corresponding approximately to the size of a nucleotide or an amino acid. Many proteins and protein complexes such as motors (Chapter 12), G-proteins, and ribosomes (Frank, 1998) undergo substantially larger conformational changes (for review, see Gerstein et al., 1994). Suppose that the conformational change is associated with a large amount of mechanical work, say 100×10^{-21} J (= 25 kT) equal to the free energy of hydrolysis of the gamma phosphate bond of one molecule of ATP (Chapter 14). Because the mechanical work done on the proteins is equal to $\frac{1}{2}\kappa x^2$ the stiffness is 0.2 N/m, only ~1% of the stiff-

Example 3.6 Ribosome If a large protein were to oscillate, how fast and how large might these oscillations be? Consider the ribosome, a globular protein–RNA enzyme complex of diameter ~30 nm (Ban et al., 1999, Clemons et al., 1999). The ribosome is the molecular machine that synthesizes proteins. If the ribosome were very rigid ($E = 1$ GPa), and the only damping came from the surrounding fluid, then it would oscillate at a frequency of $\sim(\kappa/m)^{0.5}/2\pi$ Hz $= (E/\rho)^{0.5}/2\pi L \sim 5$ GHz, corresponding to a period of 200 ps. The oscillation would decay quickly, with a time constant of $2m/\gamma \sim (2/3\pi)\rho L^2/\eta \sim 200$ ps (Equation A2.1 in the Appendix). In other words, the oscillations would die out after only a few cycles. The magnitude of the oscillations would depend on the size of the force. Suppose that the force did work on the protein equal to 100×10^{-21} J ($= 25\ kT$), the free energy associated with the hydrolysis of one molecule of ATP (Chapter 14). If we think of this chemical energy as being converted into mechanical potential energy within the protein during the protein synthesis reaction, then the amplitude, x, of the deformation would be only ~0.8 Å (energy $= \frac{1}{2}\kappa x^2$, and we assume that ribosome is as rigid as a cytoskeletal protein with $\kappa = EL = 30$ N/m). The oscillations, if they occurred, would be very small indeed. Considering that the lifetimes of different chemical states are in the order of microseconds to milliseconds, it is unlikely that such small oscillations, even it they were to occur, would play important roles in the chemistry of protein synthesis.

ness of a rigid protein of length 10 nm and Young's modulus 2 GPa. This low value of stiffness leads to a much greater characteristic length of 50 nm, implying that even the motion of a ribosome, one of the largest protein machines, would be overdamped. Because this calculation used a small value for the conformational change and a large value for the work, even this low stiffness is likely to be an overestimate; indeed, the stiffness of motor proteins is on the order of only ~1mN/m. This argues strongly that protein motions are overdamped.

2. We expect that protein friction due to the fluid-like nature of the interior of proteins (McCammon and Harvey, 1987) will further dampen out any tendency to oscillate. There is little data on the magnitude of this effect, though experimental data of Ansari et al. (1992) indicate that the internal damping of myoglobin is four times greater than the external damping from the fluid.
3. Elongated proteins are more highly damped than globular proteins of the same molecular weight (Appendix 3.4). This is because as the aspect ratio increases, the damping increases while the stiffness decreases. For example, if the aspect ratio is 10, then the characteristic length for stretching motions is ~100 nm, while that for bending motions is ~4000 nm. Thus the motions of proteins with large axial ratios will always be overdamped.

4. For a protein filament, the damping ratio actually *increases* as the length increases: The longer the filament the more highly damped (Appendix 3.4), a scaling behavior opposite to that of globular proteins. *This leads to the important conclusion that the motion of the cytoskeleton is overdamped.*

I have belabored this discussion of mechanical damping because the quality of motion of a protein is very important for understanding how it works. Overdamping rules out many wacky ideas about high-frequency resonances and long-distance information transfer and processing in proteins (e.g., Penrose, 1994). Instead, we have a relatively simple view of proteins as mechanical devices that move monotonically into new structural states in response to applied (and internally generated) forces.

To get a feeling for how proteins move, imagine that the size of a protein were increased by a factor of 10^7, so that a 5-nm-diameter protein became a mechanical device of diameter 50 mm, fitting nicely in the palm of one's hand. Let's keep the density and Young's modulus the same so our device could as well be built of plastic or Plexiglas. Now, if the viscosity of the fluid bathing the device were increased by the same factor by putting it in treacle, then the ratio of the inertial to the viscous forces will the same for both protein and device (Equation 3.9). The Reynolds number will be unchanged and the pattern of fluid flow will be preserved (just scaled in size). However, to deform the plastic device to the same relative extent will require a much larger force because the device has a much greater cross-sectional area: Whereas a force of only 1 pN might be needed to induce a protein conformational change of 1 nm (corresponding to a strain of 20%), a force of 100 N, corresponding to a weight of 10 kg, would be required to produce the same strain in the plastic device. In response to the respective forces, the protein and the mechanical device will move at the same initial speed, but because the protein conformational change is so much smaller, the relaxation of the protein will be complete in much less time: A relaxation that took an almost imperceptible 100 ns for the protein will take a leisurely 1 s for the device.

The Motions of the Cytoskeleton and Cells Are Also Overdamped

One might expect, based on the scaling argument for globular proteins, that cells, whose linear dimensions are some 1000 times larger than those of proteins, might undergo underdamped, oscillatory motions. However, experimental measurements show that this is not the case: The motions of cells are very highly damped. For example, the cytoplasm of macrophages that have ingested 1-μm-diameter magnetic particles can be perturbed using a weak external magnetic field. The particles reorient extremely slowly, with time constants of minutes. The apparent intracellular viscosity is very high, ~1000 Pa·s (a million times the viscosity of water) for velocity gradients of 0.01 s^{-1} (Valberg and Feldman, 1987). The motion is highly overdamped. Because actin gels crosslinked with the actin binding protein ABP have similar viscoelastic properties to cells (Zaner and Valberg, 1989; see Problem 3.6 for the definition of viscoelasticity), it is likely that the

viscoelasticity of cells arises from the stiffness of and damping on the cytoskeletal filaments. And because the long cytoskeletal filaments are highly damped, as argued in the previous section, so too are cells.

The high apparent viscosity of cytoplasm measured in the Valberg and Feldman experiment is still consistent with the cytoplasm being an aqueous environment. Indeed, small fluorescent probes of diameter ~1 nm are highly mobile inside cells, having rotational and translational diffusion coefficients similar to those in aqueous solution (Fushimi and Verkman, 1991). Even particles of diameter 6 nm, corresponding to that of a 100 kDa protein, diffuse quickly inside cells, as though the viscosity were about three times that of water (Luby-Phelps et al., 1987; Seksek et al., 1997). But larger particles are not nearly so mobile: 50 to 500-nm-diameter particles diffuse very slowly inside cells, indicating an apparent viscosity 30 to 300 times that of water (Luby-Phelps et al., 1987; Alexander and Rieder, 1991). Thus it appears that the cytoskeletal filaments form a gel with a mesh size of ~50 nm. Small solutes and proteins can readily diffuse through the pores, but the motion of larger particles, such as ribosomes and organelles, is severely restricted.

Even in cases where the cytoskeletal filaments are highly aligned and tightly crosslinked for maximum rigidity, the viscous forces dominate the inertial ones; this is true for cilia and flagella, which are composed of microtubules, as well as for the stereocilia of hair cells, which are composed of tightly crosslinked actin filaments (Chapter 8). Thus, even though it is conceivable that the rigidity of the whole cell could be large enough to result in underdamped motion, the cytoskeletal filaments are too sparsely crosslinked to make the network sufficiently rigid. It is only at the organismal level, where large multicellular structures are in contact with air, that oscillations—of the belly of an elephant, for example—are possible.

Summary

The rigidity of cytoskeletal proteins such as actin, tubulin, and keratin, which serve structural roles in cells, is similar to that of hard plastics like polypropylene, but substantially less than that of materials such as metal, glass, and wood. This is because proteins are held together by relatively weak van der Waals bonds. The rigidity of protein machines, proteins that are designed to undergo large conformational changes as they transduce chemical energy into mechanical work, is expected to be substantially less than that of structural proteins. As proteins move and change shape, they experience damping forces from the surrounding fluid as well as from internal friction. These viscous forces arise from the rapid making and breaking of bonds. Owing to the small size of proteins, the viscous forces on proteins are generally much greater than the inertial forces. Consequently, the global motions of proteins, especially less rigid ones, are highly overdamped: They creep rather than oscillate when subject to applied forces. The motions of the long, thin cytoskeletal filaments are also overdamped, due to their large aspect ratios. This, in turn, causes the motion of cells to be overdamped.

Problems

3.1 The Young's modulus of an active muscle (in the longitudinal direction) is ~40 MPa (Bagshaw, 1993). What is the spring constant of a muscle of length 100 mm and cross-sectional area 1000 mm^2?

3.2 By how much would such a muscle be extended by the weight of a mass of 10 kg? What is the fractional extension?

3.3 Using the Young's modulus for actin in Table 3.2 and a cross-sectional area of 20 nm^2, calculate the stiffness of a 1-μm-long actin filament.

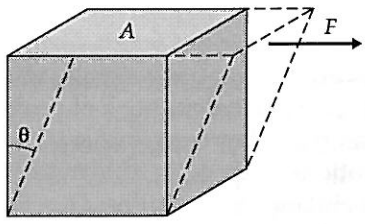
3.4 Given that there are ~10^{15} actin filaments per square meter of cross-sectional area of a muscle and that the cross-sectional area of an actin filament is 20 nm^2, calculate the fraction of the cross-section occupied by actin. If a muscle were composed solely of continuous actin filaments (stretching from one end of the muscle to the other) occupying this volume fraction, what would be its Young's modulus? How does this compare with the value stated in Problem 3.1? What conclusions can you draw about the molecular basis for the rigidity of muscle? (Note that the actin filaments are not continuous but instead alternate with the myosin-containing thick filaments [see Figure 1.1]. The compliance contributed by actin is similar to that contributed by the crosslinks [the myosin heads] between the thin and thick filaments [Chapter 8].)

3.5 Show that the relaxation time of a rigid globular protein whose motion is highly damped by the solvent is ~10 ps, independent of the size of the protein.

[Answer: $\tau = \frac{\gamma}{\kappa} \sim \frac{3\pi\eta L}{EA/L} = 3\pi\frac{L^2}{A}\frac{\eta}{E} \cong 3\pi\frac{\eta}{E} \sim 10\,\text{ps}$ using $\eta = 1$ mPa·s/m and $E = 1$ GPa.]

3.6 Viscoelasticity Another way to deform a material is to place it under shear (see the figure below). In this case:

$$\frac{F}{A} = G\theta$$



where G is the **shear modulus**. For a homogenous isotropic material, the shear modulus is related to the Young's modulus by $G = E/2(1 + \sigma)$. The parameter σ is Poisson's ratio, the relative amount of sideways contraction ($\Delta w/w$) of the material compared to the lengthwise strain ($\Delta L/L$): $\Delta w/w = \sigma(\Delta L/L)$. **Poisson's ratio** lies between –1 and +0.5; the lower value applies to a material that has constant shape, and the upper value applies to a material that has constant volume (i.e., incompressible). For an incompressible solid, $G = E/3$. For most materials, $0.2 \leq \sigma \leq 0.5$ (Kaye and Laby, 1986).

If the deformation of an elastic material requires the breaking and remaking of internal molecular bonds, then it will not respond instantaneously to an applied force, but instead it will relax more slowly to its new shape. We say that the material is **viscoelastic**. With the aid of Figure 3.5 and the figure opposite in Problem 3.6, we can write the equation of motion in response to a shearing force:

$$\frac{F}{A} = \eta \frac{d\theta}{dt} + G\theta$$

This is formally the same as the equation of motion of a damped spring—i.e., a spring in series with a dashpot (see Figure 2.3B). The material will deform with a time constant equal to η/G. Note that the relaxation time constant is also a material property because it does not depend on size.

If a protein has an internal viscosity (protein friction) 4 times that of water, a Young's modulus of 1 GPa, and a Poisson ratio of 0.25, calculate the relaxation time constant. Compare this to Problem 3.5.