

# CHAPTER 14

## Allostery

Protein molecules that play critical cellular roles are usually switchable—that is, their function can be turned on or off. This modulation of activity can occur through covalent modification, such as phosphorylation, or through the binding of ligand molecules. In either case, the active site of the protein is altered in such a way that the activity of the protein changes. In the most general sense, such communication between two sites in a protein (the active site and the site of modification or binding) is referred to as **allostery**. Allosteric regulation involves the stabilization of one conformation of the protein with respect to alternative conformations, thereby altering the activity of the protein.

Allosteric proteins are critical for the responsiveness of cells to external signals. Allostery is also important for the regulation of metabolic pathways. For example, as the levels of a metabolite molecule build up in the cell, the molecule binds to enzymes involved in its synthesis and turns them off allosterically. Such systems are discussed in Chapter 16. Allostery was originally discovered in the oxygen transport protein hemoglobin, where it increases the efficiency of oxygen uptake and release. We shall discuss hemoglobin in detail in this chapter.

Before beginning a discussion of allostery in hemoglobin, we introduce the concept of ultrasensitivity, which refers to situations in which the response of a protein to an input is sharper than expected from the hyperbolic response that results from a simple binding equilibrium. Ultrasensitivity in molecular systems can arise from many different mechanisms, but allosteric proteins are usually at the heart of the mechanism.

We first illustrate the concept of ultrasensitivity by discussing how bacteria switch from directed movement to tumbling movements in response to the presence of chemical attractants or repellants. In another example, also drawn from signal transduction, we describe how sets of coupled protein kinases give rise to ultrasensitive responses in signaling systems. We then turn to the major focus of the chapter, which is to illustrate the basic principles of allostery using oxygen transport as an example. We shall see that hemoglobin is allosteric, and that its structure changes in response to the binding of oxygen. This structural change makes hemoglobin ultrasensitive to oxygen concentration and a much more efficient oxygen transporter than it would be otherwise.

### A. ULTRASENSITIVITY OF MOLECULAR RESPONSES

#### 14.1 Molecular outputs that depend on independent binding events switch from on to off over a 100-fold range in input strength

Cells continually make decisions that affect how they respond to their environment and grow. The decision-making circuits in cells typically consist of proteins (or, in some cases, RNAs) that process “inputs” and generate “outputs,” as

illustrated in Figure 14.1. At the heart of each such processing unit is an interaction between a protein (or RNA) and another molecule that binds to it (a ligand). The output generated by such a unit depends on the strength of the interaction between the protein and the ligand. Hence, an important aspect of understanding how such processing units work is the thermodynamics of binding interactions, which was the focus of Chapters 12 and 13.

There are many examples of proteins that are components of decision-making circuits (see Figure 14.1). Every enzyme in the cell can be thought of in this way, because an enzyme binds to a substrate (the “input”) and generates a product (the “output”). Cell-surface receptors, such as the epidermal growth factor receptor (see Section 12.13) are activated when they bind to hormones, and the outputs of the activated receptor cause changes in cellular behavior. Transcription factors that bind to DNA alter the rates at which genes are transcribed, depending on how strongly they are bound to their target DNA elements.

In the simplest case, the output generated by a protein is proportional to the amount of complex formed by the input ligand molecule and the protein. The rate of an enzyme-catalyzed reaction, for example, is proportional to the concentration of the enzyme-substrate complex (see Chapter 16). If the binding of one ligand molecule does not affect the binding of the others (that is, if the binding events are independent), then the formation of the complex as a function of ligand concentration is described by a hyperbolic binding isotherm (see Figure 12.4). In such a situation, the output as a function of input strength is also hyperbolic (Figure 14.2A).

Recall from Section 12.10 that the fractional saturation,  $f$ , of a protein increases from 0.1 to 0.9 as the ligand concentration is increased ~100-fold, from  $\sim 0.1K_D$  to  $10K_D$ , where  $K_D$  is the dissociation constant for the interaction. This is illustrated in Figure 14.2B, in which the input strength (for example, concentration of the ligand) is shown on a logarithmic scale. As made clear in Figure 14.2B, if the binding events are independent, then the protein responds gradually to increases in the concentration of the ligand, switching from off (unbound) to on (bound) over two log units of concentration. Such a response is referred to as a **graded response**. Graded responses are also known as **linear responses** because the output is a linear function of the input when the input strength is low, as you can see by considering Equation 12.11.

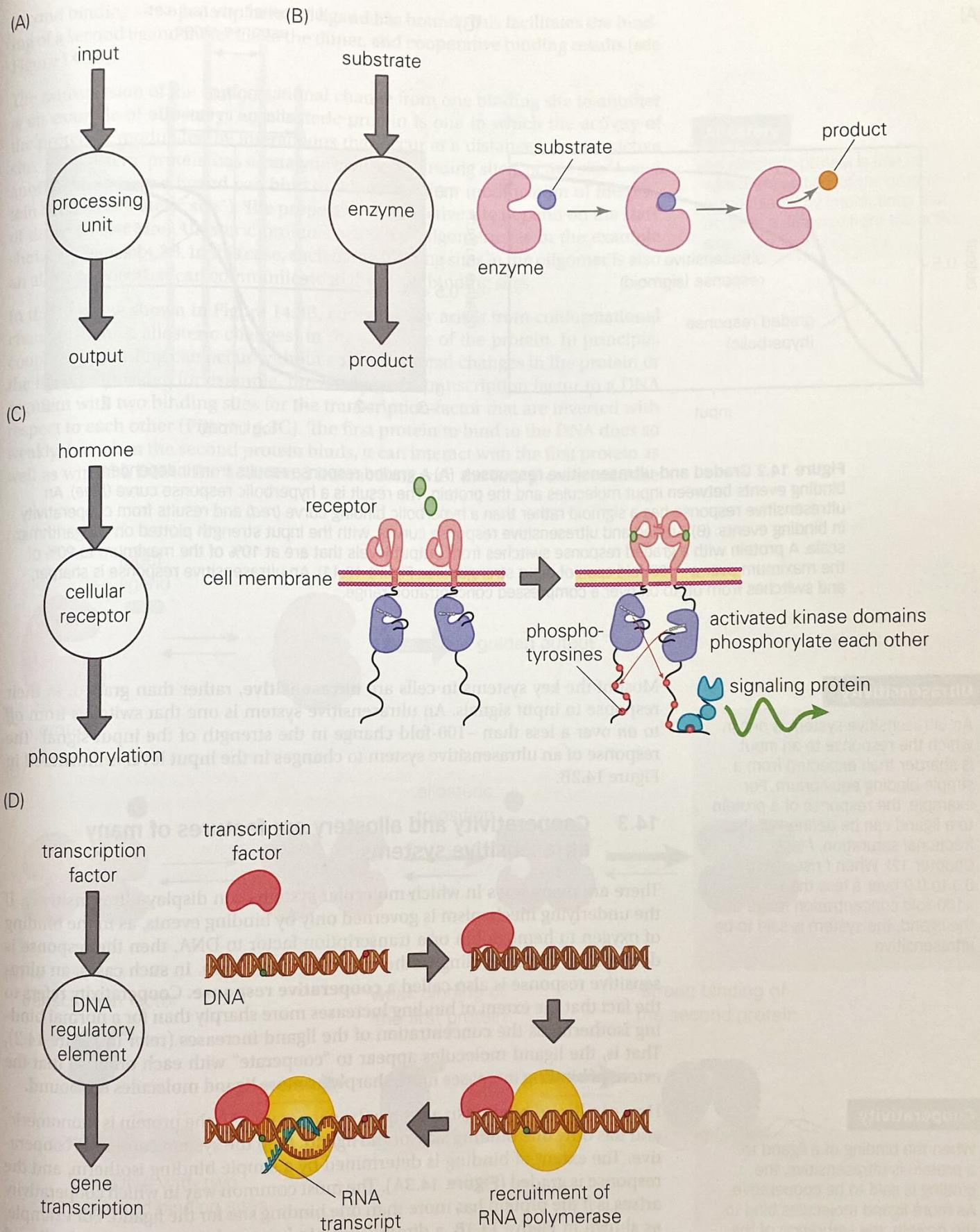
### Graded response

An output function that depends on the input in a hyperbolic fashion, as in a simple binding equilibrium, is known as a graded, or linear, response. In such a response, the output switches from ~10% to ~90% of the maximum response over a 100-fold change in input strength.

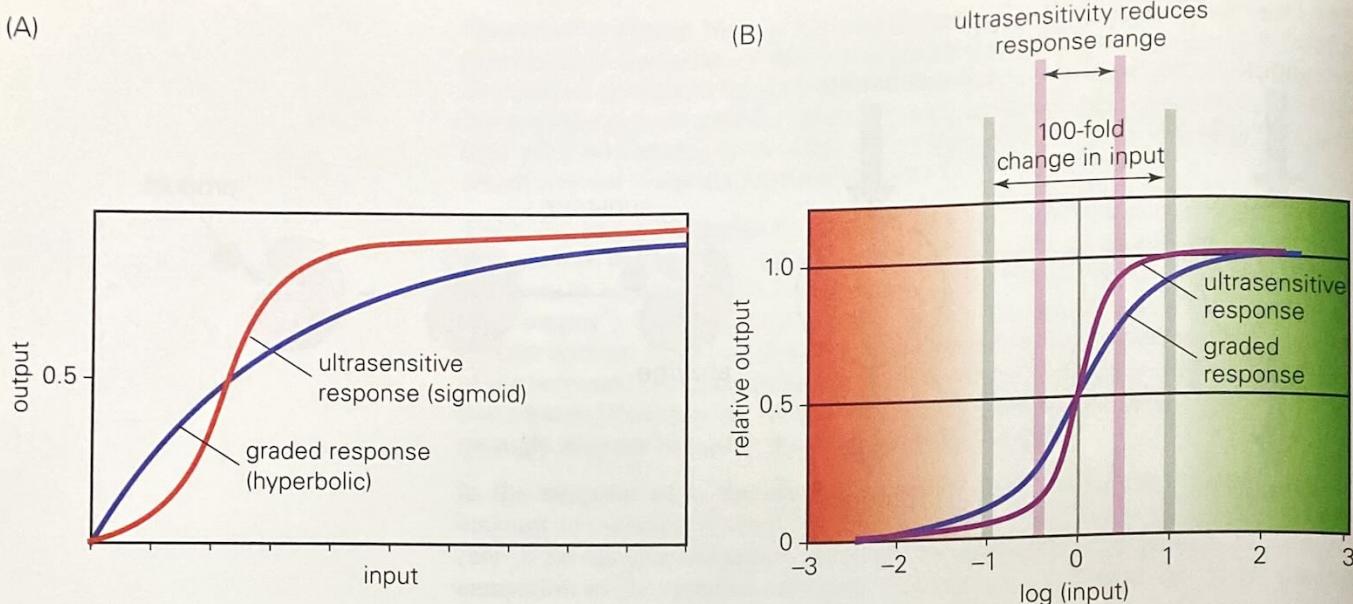
## 14.2 The response of many biological systems is ultrasensitive, with the switch from off to on occurring over a less than 100-fold range in concentration

A graded response is much too sluggish to be useful for many biological systems. The ~100-fold increase in the input signal strength (for example, concentration of the ligand) that is required to switch the system from on to off has several undesirable features. First, in living systems, the concentrations of molecules do not usually change by 100-fold as processes occur. One example, which we study in more detail in part B of this chapter, is the function of hemoglobin in transporting oxygen. There is only a three-fold difference in dissolved oxygen concentration between the lungs and the tissues, and if the response of hemoglobin to oxygen concentration were graded, it would be a very inefficient oxygen transporter.

Second, cells have to be able to respond decisively to small changes in the concentrations of the molecules that they detect. A graded response is not very sensitive to small changes in concentration, and it does not produce a switch-like response to an input signal. The need for a sharp, switch-like response can be understood by imagining that we were in a house that was catching fire. Our noses are able to detect trace amounts of the molecules that are in smoke and our nervous system galvanizes us to make a rapid response as soon as smoke is detected. A switch-like response is essential for both the rapid response and for preventing too many false alarms. A graded response in our noses would lead to unfortunate outcomes.



**Figure 14.1 Proteins as input processing units.** (A) Schematic representation of the conversion of inputs into outputs. (B) The substrates of enzymes can be thought of as inputs, with their products as the outputs. (C) Cell signaling systems respond to inputs, such as hormones, and generate responses, such as phosphorylation (refer to Figure 12.15 for a more detailed discussion of the receptor shown here). (D) Transcription factors bind to regulatory elements in DNA and help recruit RNA polymerase. This initiates transcription, as explained in Chapter 1. (The concepts illustrated here are based on a discussion in D. Bray, *Nature* 376: 307–312, 1995.)



**Figure 14.2 Graded and ultrasensitive responses.** (A) A graded response results from independent binding events between input molecules and the protein. The result is a hyperbolic response curve (blue). An ultrasensitive response has a sigmoid rather than a hyperbolic binding curve (red) and results from cooperativity in binding events. (B) Graded and ultrasensitive response curves, with the input strength plotted on a logarithmic scale. A protein with a graded response switches from output levels that are at 10% of the maximum to 90% of the maximum over a ~100-fold span of input strength (see Figure 12.11). An ultrasensitive response is sharper, and switches from off to on over a compressed concentration range.

### Ultrasensitivity

An ultrasensitive system is one in which the response to an input is sharper than expected from a simple binding equilibrium. For example, the response of a protein to a ligand can be defined as the fractional saturation,  $f$  (see Chapter 12). When  $f$  rises from 0.1 to 0.9 over a less than ~100-fold concentration range of the ligand, the system is said to be ultrasensitive.

Most of the key systems in cells are **ultrasensitive**, rather than graded, in their response to input signals. An ultrasensitive system is one that switches from *off* to *on* over a less than ~100-fold change in the strength of the input signal. The response of an ultrasensitive system to changes in the input level is illustrated in Figure 14.2B.

### 14.3 Cooperativity and allostery are features of many ultrasensitive systems

There are many ways in which molecular systems can display ultrasensitivity. If the underlying mechanism is governed only by binding events, as in the binding of oxygen to hemoglobin or a transcription factor to DNA, then the response is determined by the binding isotherm for the interaction. In such cases, an ultrasensitive response is also called a **cooperative response**. Cooperativity refers to the fact that the extent of binding increases more sharply than for a normal binding isotherm, as the concentration of the ligand increases (refer to Figure 14.2). That is, the ligand molecules appear to “cooperate” with each other so that the extent of binding increases more sharply as more ligand molecules are bound.

### Cooperativity

When the binding of a ligand to a protein is ultrasensitive, the binding is said to be cooperative. As more ligand molecules bind to the protein, the saturation of the protein increases more sharply than would be expected for a normal binding event, as if the ligand molecules “cooperate” with each other.

How can ligand binding to a protein be cooperative? If the protein is monomeric, and has only one binding site for the ligand, then the system cannot be cooperative. The extent of binding is determined by a simple binding isotherm, and the response is graded (Figure 14.3A). The most common way in which cooperativity arises is if the protein has more than one binding site for the ligand. For example, as shown in Figure 14.3B, a dimeric protein has two binding sites for the ligand per dimeric unit. Now imagine that in the absence of ligand both binding sites are closed, so that it is difficult for ligand molecules to bind. The protein changes conformation when the ligand binds to it, thus opening up one binding site. If the dimer is so constructed that the conformational change that occurs when ligand binds to one subunit of the dimer is transmitted to the other subunit, then the

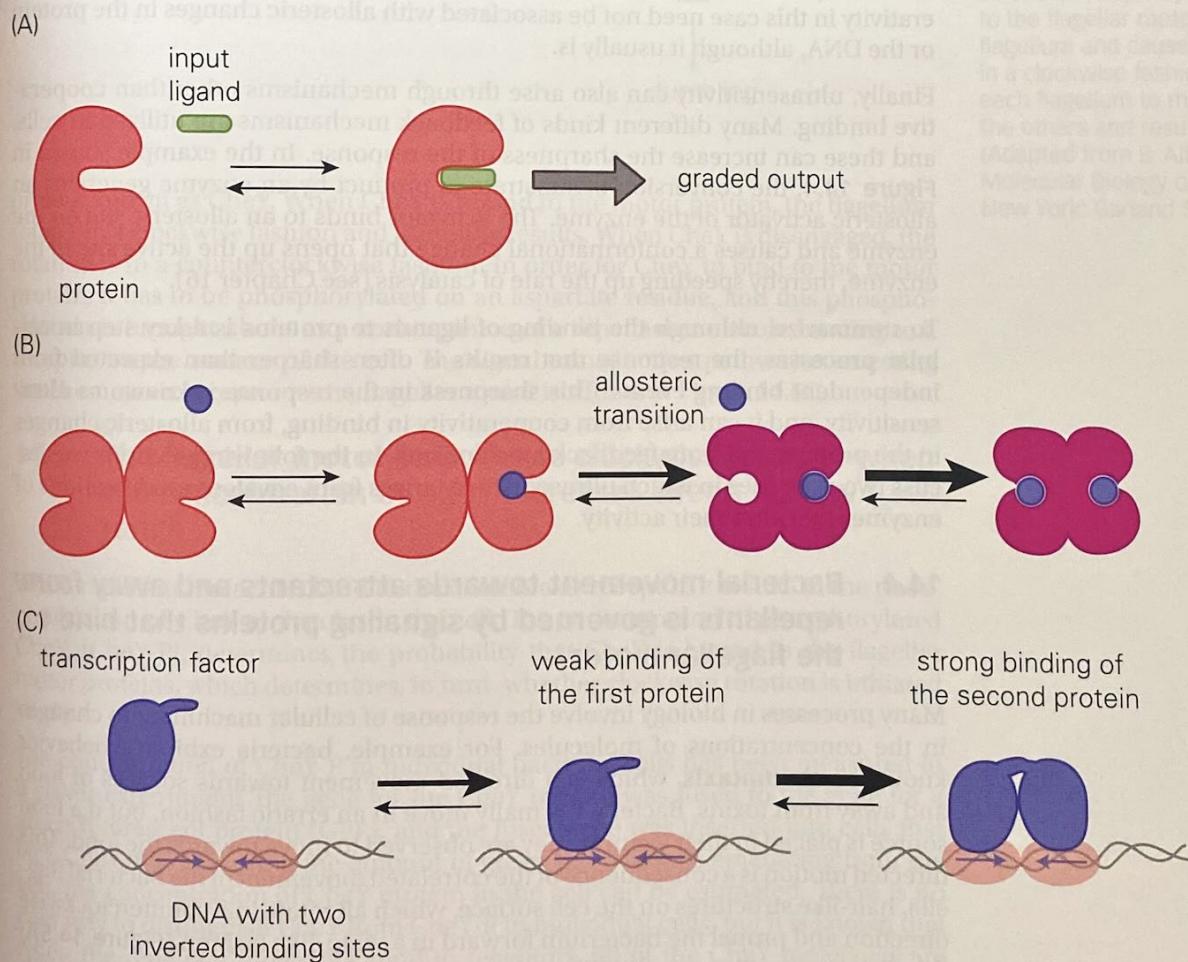
second binding site opens up before a ligand has bound. This facilitates the binding of a second ligand molecule to the dimer, and cooperative binding results (see Figure 14.3B).

The transmission of the conformational change from one binding site to another is an example of **allostery**. An allosteric protein is one in which the activity of the protein is modulated by interactions that occur at a distance from the active site. An allosteric protein has a catalytic center or binding site ("active site"), and another site where a ligand can bind or where covalent modification of the protein occurs ("allosteric site"). The properties of the active site depend on the state of the allosteric site. Allosteric proteins are often oligomeric, as in the example shown in Figure 14.3B. In this case, each of the binding sites in the oligomer is also an allosteric site that can communicate to the other binding sites.

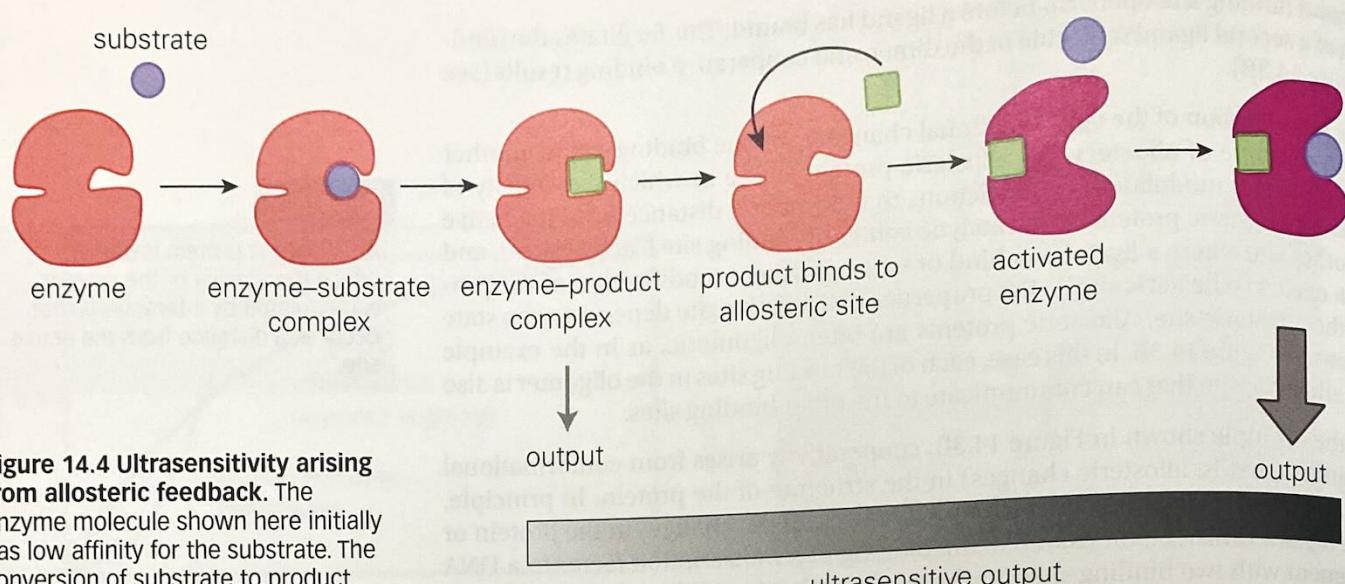
In the example shown in Figure 14.3B, cooperativity arises from conformational changes (that is, allosteric changes) in the structure of the protein. In principle, cooperative binding can occur without conformational changes in the protein or the ligand. Consider, for example, the binding of a transcription factor to a DNA segment with two binding sites for the transcription factor that are inverted with respect to each other (Figure 14.3C). The first protein to bind to the DNA does so weakly, but when the second protein binds, it can interact with the first protein as well as with the DNA. The additional interactions with the first protein increase

### Allostery

An allosteric protein is one in which the activity of the protein is modulated by interactions that occur at a distance from the active site.



**Figure 14.3 Cooperativity and allostery.** (A) A monomeric protein with a single binding site cannot display ultrasensitivity (the output is graded). (B) A dimeric protein with two binding sites can display ultrasensitivity if the conformations of the two binding sites are coupled. Ligand binding to one site causes an allosteric change in the other, thereby increasing affinity for the ligand. Ligand binding to such a system is cooperative. (C) Cooperative binding can occur without allostery. In this example, a transcription factor has two closely spaced and inverted binding sites on DNA. If two molecules of the transcription factor interact with each other when bound to DNA, then cooperative binding results without necessitating a conformational change.



**Figure 14.4 Ultrasensitivity arising from allosteric feedback.** The enzyme molecule shown here initially has low affinity for the substrate. The conversion of substrate to product activates the enzyme because the product molecule can bind to an allosteric site on the enzyme, causing a conformational change that opens up the active site.

the affinity of the second protein for DNA, leading to cooperative binding. Cooperativity in this case need not be associated with allosteric changes in the protein or the DNA, although it usually is.

Finally, ultrasensitivity can also arise through mechanisms other than cooperative binding. Many different kinds of feedback mechanisms are utilized in cells, and these can increase the sharpness of the response. In the example shown in **Figure 14.4**, the conversion of substrate to product by an enzyme generates an activator that binds to an allosteric site on the enzyme and causes a conformational change that opens up the active site of the enzyme, thereby speeding up the rate of catalysis (see Chapter 16).

To summarize, although the binding of ligands to proteins is a key step in cellular processes, the response that results is often sharper than expected from independent binding events. This sharpness in the response is known as ultrasensitivity, and it can arise from cooperativity in binding, from allosteric changes in the protein, and from feedback mechanisms. In the following sections we discuss two examples in which ultrasensitivity arises from covalent modifications of enzymes that alter their activity.

#### 14.4 Bacterial movement towards attractants and away from repellants is governed by signaling proteins that bind to the flagellar motor

Many processes in biology involve the response of cellular machinery to changes in the concentrations of molecules. For example, bacteria exhibit a behavior known as **chemotaxis**, which is a directed movement towards sources of food and away from toxins. Bacteria normally move in an erratic fashion, but if a food source is placed in their vicinity, they are observed to move towards the food. This directed motion is a consequence of the correlated movement of the bacterial flagella, hair-like structures on the cell surface, which all rotate in a counterclockwise direction and propel the bacterium forward in a whip-like fashion (**Figure 14.5A**; flagella are also illustrated in Figure 1). If there is a chemical repellent present, instead, then the flagella start to rotate in a clockwise fashion and disengage from each other. The uncorrelated rotations of the disengaged flagella cause the bacteria to tumble and, eventually, to move away from the repellent (**Figure 14.5B**).

Each bacterial flagellum is driven by a motor protein that is embedded in the cell wall (**Figure 14.5C**). The direction of rotation of the flagellum is controlled by a

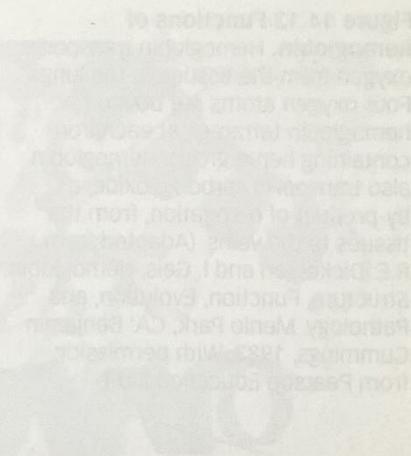
switch. At the heart of this process is the control of the activity of the MAP kinases by phosphorylation. Just how the sequential coupling of the activation of the kinases results in ultrasensitivity is well understood, but complicated. We shall not discuss this further, but turn instead to allosteric regulation in hemoglobin, which is much simpler to analyze in detail.

## B. ALLOSTERY IN HEMOGLOBIN

### 14.10 Allosteric proteins exhibit positive or negative cooperativity

In Chapter 12, where we studied the thermodynamics of ligand binding, the discussion was centered on binding interactions in which the binding events are independent of each other. That is, the binding of one ligand molecule to a protein is assumed to have no influence on the strength of the interaction between other ligand molecules and their binding sites. Many molecular recognition events in biology, in contrast, are cooperative, with the binding of one ligand molecule to a protein resulting in alterations in the affinity of the protein for other ligand molecules. Such systems are by nature allosteric.

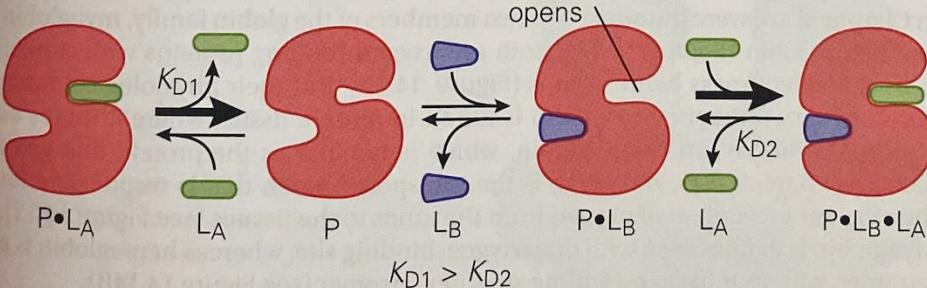
In the most general case, consider a protein, P, with two distinct binding sites (**Figure 14.12**). One site is specific for one kind of ligand, L<sub>A</sub>, and the other site is specific for a different ligand, L<sub>B</sub>. If P is allosteric, then the binding of L<sub>A</sub> to P affects the strength of the interaction with L<sub>B</sub> and vice versa. For example, as illustrated in Figure 14.12, the binding of L<sub>A</sub> in the absence of L<sub>B</sub> is characterized by a dissociation constant K<sub>D1</sub>. When L<sub>B</sub> binds to P, it induces a conformational change such that the structure of the binding site for L<sub>A</sub> is altered. If this structural change results in tighter binding, then the dissociation constant for L<sub>A</sub> in the presence of L<sub>B</sub>, K<sub>D2</sub>, is smaller than K<sub>D1</sub>. This phenomenon is known as **positive cooperativity**, because the two ligands cooperate to increase the strengths of the interactions with the protein. Alternatively, if the binding of L<sub>B</sub> to P decreases the strength of the interaction of L<sub>A</sub> with P, then the phenomenon is known as **negative cooperativity**. Positive cooperativity is much more common in nature than negative cooperativity.



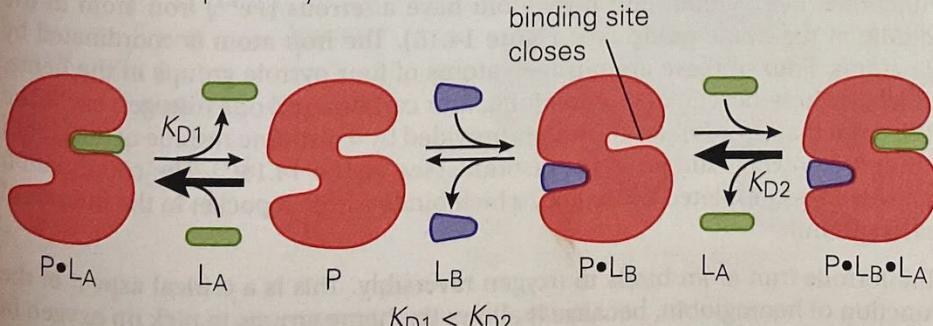
#### Positive and negative cooperativity

When two or more ligands bind to a protein in such a way that they mutually reinforce each of their binding affinities, the phenomenon is called positive cooperativity. If the ligands make it more difficult for each other to bind, the phenomenon is called negative cooperativity.

(A) positive cooperativity

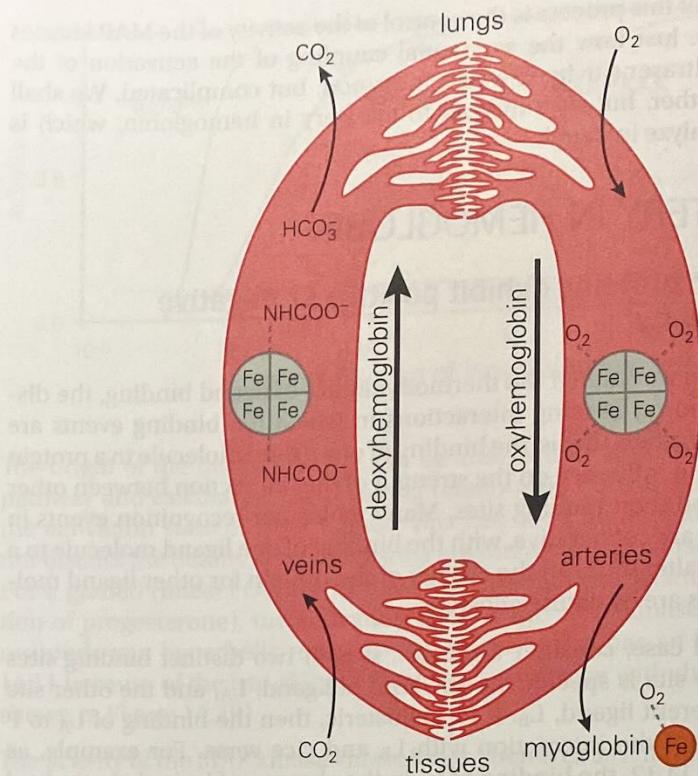


(B) negative cooperativity



**Figure 14.12 Positive and negative cooperativity.** (A) Positive cooperativity occurs when two ligands, L<sub>A</sub> and L<sub>B</sub>, mutually reinforce their affinities with a protein, P. In the binding scheme shown here, L<sub>B</sub> increases the affinity of L<sub>A</sub> for P. The reverse is also true, although not shown explicitly. (B) Negative cooperativity occurs when each of the ligands decreases the binding affinity of the other.

**Figure 14.13 Functions of hemoglobin.** Hemoglobin transports oxygen from the tissues to the lungs. Four oxygen atoms are bound per hemoglobin tetramer, at each iron-containing heme group. Hemoglobin also transports carbon dioxide, a by-product of respiration, from the tissues to the veins. (Adapted from R.E. Dickerson and I. Geis, *Hemoglobin: Structure, Function, Evolution, and Pathology*. Menlo Park, CA: Benjamin-Cummings, 1983. With permission from Pearson Education Ltd.)



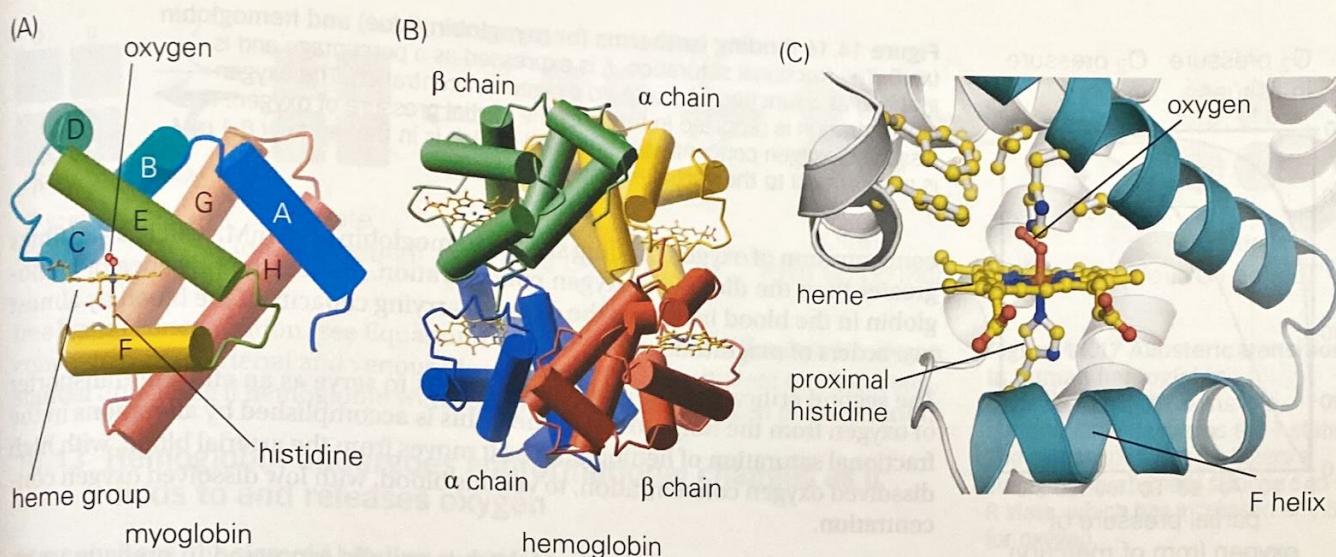
As discussed in Section 14.3 (see Figure 14.3), a very common kind of allostery occurs when a protein or a protein assembly has more than one binding site for the same ligand. This occurs naturally if the protein is an oligomer (for example, a dimer, trimer, or tetramer). If such a multimeric protein is allosteric, then when the ligand binds to the empty protein a conformational change in the protein is induced such that the structures of the other binding sites are altered, and the dissociation constants for the next ligands to bind are different from the first one. The oxygen-transport protein hemoglobin (Figure 14.13) is an example of such a multimeric and allosteric system, and we analyze its mechanism in detail in the following sections.

### 14.11 The heme group in hemoglobin binds oxygen reversibly

In Chapter 5, we were introduced to two members of the globin family, myoglobin and hemoglobin (Figure 14.14). Both are oxygen-binding proteins with similar folds that encompass heme groups (Figure 14.15), but their physiological functions are very different. Myoglobin is found in muscle tissue, where it serves as a reservoir for oxygen. Hemoglobin, which is familiar as the protein that gives blood its characteristic red color, is the transport protein that is responsible for the efficient movement of oxygen from the lungs to the tissues (see Figure 14.13). Myoglobin is a monomer, with one oxygen-binding site, whereas hemoglobin is a tetramer, with four oxygen-binding sites per tetramer (see Figure 14.14B).

Functional hemoglobin and myoglobin have a ferrous ( $\text{Fe}^{2+}$ ) iron atom in the middle of the heme group (see Figure 14.15). The iron atom is coordinated by six atoms. Four of these are nitrogen atoms of four pyrrole groups in the heme, which are heterocyclic rings containing four carbons and one nitrogen each. The fifth atom that coordinates the iron is provided by a histidine residue of the globin subunit, known as the proximal histidine (see Figure 14.14C). The coordination of the iron is completed by oxygen, which binds within a pocket in the interior of each subunit.

The ferrous iron atom binds to oxygen reversibly. This is a critical aspect of the function of hemoglobin, because it allows the heme groups to pick up oxygen in

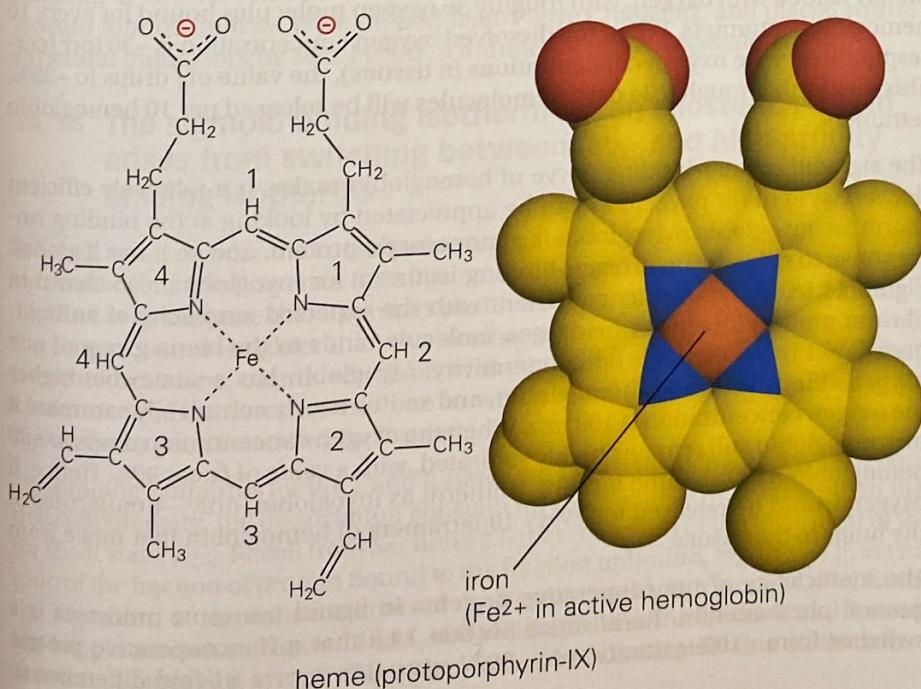


the lungs but also to let it go in the tissues. The heme iron is readily oxidized to  $\text{Fe}^{3+}$  (ferric), and part of the function of the protein is to protect the heme group and to make the iron atom difficult to oxidize. Once oxidized, the iron atom no longer binds oxygen reversibly.

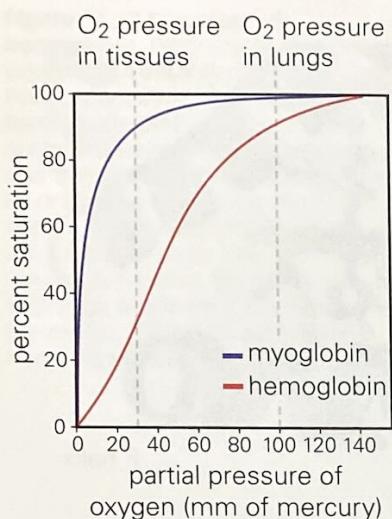
### 14.12 Hemoglobin increases the solubility of oxygen in blood and makes its transport to the tissues more efficient

Hemoglobin has at least two critical roles to play in our respiratory systems. First, it greatly increases the oxygen carrying capacity of the blood. Oxygen is not particularly soluble, and the dissolved oxygen concentration in blood is  $\sim 0.1 \text{ mM}$  ( $10^{-4} \text{ M}$ ). As we shall see, the dissolved oxygen concentration changes by a factor of three between arterial and venous blood, but  $0.1 \text{ mM}$  is a good reference value. The hemoglobin concentration in human blood is about  $15 \text{ g deciliter}^{-1}$ , using a common clinical unit. Since the molecular weight of a hemoglobin tetramer is  $\sim 70,000$ , this means that the concentration of hemoglobin in the blood is  $\sim 2 \text{ mM}$  ( $2 \times 10^{-3} \text{ M}$ ). Because each tetramer has four oxygen binding sites, the effective

**Figure 14.14 Globin and heme structures.** (A) Structure of myoglobin. (B) Structure of human hemoglobin. (C) Close up of one of the four heme binding sites in hemoglobin, with oxygen bound to the iron atom. (PDB codes: A, 1MBC; B, 1A00.)



**Figure 14.15 Structure of the heme group, also known as protoporphyrin-IX.**



**Figure 14.16 Binding isotherms for myoglobin (blue) and hemoglobin (red).** The fractional saturation,  $f$ , is expressed as a percentage and is graphed as a function of dissolved oxygen concentration. The oxygen concentration is reported in terms of the partial pressure of oxygen. The dissolved oxygen concentration in blood, which is in the range of 0.1 mM, is proportional to the partial pressure of oxygen.

concentration of oxygen binding sites in hemoglobin is  $\sim 8$  mM, which is  $\sim 80$  times greater than the dissolved oxygen concentration. Hence, the presence of hemoglobin in the blood increases the oxygen carrying capacity of the blood by almost two orders of magnitude.

The second critical function of hemoglobin is to serve as an efficient transporter of oxygen from the lungs to the tissues. This is accomplished by alterations in the fractional saturation of hemoglobin as it moves from the arterial blood, with high dissolved oxygen concentration, to venous blood, with low dissolved oxygen concentration.

The oxygen concentration in blood,  $[O_2]$ , is usually expressed in pressure units, because the partial pressure of oxygen gas in equilibrium with dissolved oxygen is measured readily. The commonly used unit of partial pressure is the torr, with 1 torr corresponding to 1 mm of Hg at  $20^\circ C$  ( $760$  torr = 1 atm of pressure). These nuances need not concern us here, because the concentration of oxygen in molar units is proportional to the partial pressure. The oxygen concentration of venous blood is  $\sim 30$  torr, whereas it is  $\sim 100$  torr in arterial blood.

The rather small difference (about three-fold) in dissolved oxygen concentration between venous and arterial blood means that hemoglobin would be a very inefficient transporter of oxygen if it did not have an ultrasensitive response to oxygen. To appreciate this, let us first look at the binding isotherm for hemoglobin, shown in Figure 14.16. The binding isotherm is clearly not hyperbolic. Instead, it switches between a concave and a convex shape, resembling a distorted form of the letter "S." Such a binding isotherm is referred to as a **sigmoid binding curve**. The sigmoid shape of the hemoglobin oxygen binding isotherm is characteristic of an allosteric system with positive cooperativity and is a feature that is essential for the proper delivery of oxygen to the tissues from the lungs.

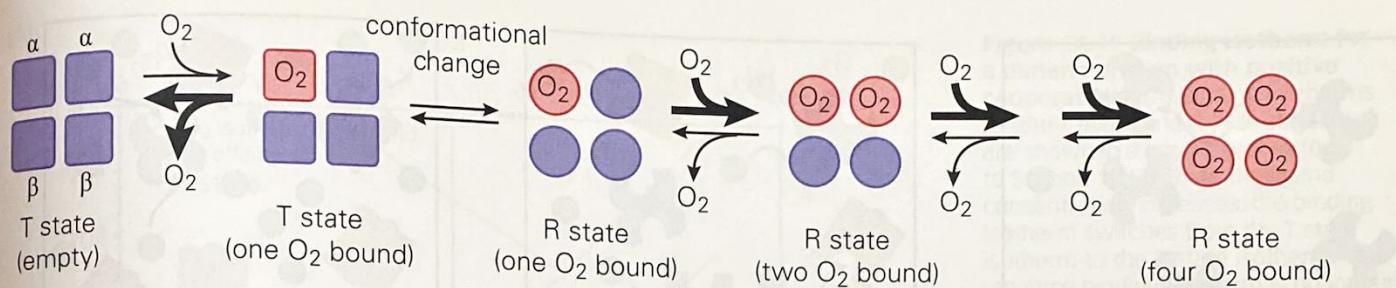
When the oxygen concentration is 100 torr, the fractional saturation,  $f$ , is  $\sim 90\%$ . That is, as the hemoglobin is carried through the lungs, it becomes nearly completely loaded with oxygen, with roughly 36 oxygen molecules bound for every 10 hemoglobin tetramers. When the dissolved oxygen concentration is  $\sim 30$  torr (corresponding to the oxygen concentrations in tissues), the value of  $f$  drops to  $\sim 30\%$ . This means that roughly 24 oxygen molecules will be released per 10 hemoglobin tetramers.

The sigmoid oxygen-binding curve of hemoglobin makes it a relatively efficient delivery system for oxygen, as can be appreciated by looking at the binding isotherm for myoglobin. Myoglobin is a monomeric protein, and so it has a graded response to oxygen. The oxygen-binding isotherm for myoglobin, also shown in Figure 14.16, is hyperbolic, consistent with the expected simplicity of an equilibrium process in which one oxygen molecule binds to the heme group of one myoglobin molecule with no cooperativity. Myoglobin has a somewhat higher oxygen affinity than does hemoglobin, and so it is nearly completely saturated if the oxygen concentration is 100 torr. When the oxygen concentration drops to  $\sim 30$  torr, myoglobin still remains highly saturated, with a value of  $f$  near 90%. Hence, if hemoglobin had the same binding isotherm as myoglobin, only  $\sim 4$  molecules of oxygen would be released for every 10 tetramers of hemoglobin that move from the lungs to the tissues.

The inefficiency of noncooperative proteins in ligand transport processes is a general phenomenon. Recall from Section 14.9 that a noncooperative protein switches from  $\sim 10\%$  saturation to  $\sim 90\%$  saturation over a 81-fold difference in

### Sigmoid binding curve

When the binding of ligands to a protein is cooperative, the shape of the binding curve (fractional saturation versus ligand concentration) is no longer hyperbolic. Instead, it resembles an "S" shape and is called sigmoid (for sigma, the Greek letter S).



free ligand concentration (see Equation 14.3). The three-fold difference in oxygen concentration in arterial and venous blood is therefore insufficient to trigger substantial unloading if hemoglobin were not ultrasensitive to oxygen concentration.

### 14.13 Hemoglobin undergoes conformational changes as it binds to and releases oxygen

As we shall see, the sigmoid binding curve for hemoglobin arises from conformational changes that are brought about by oxygen binding. Empty hemoglobin has four oxygen-binding sites that all have low affinity for oxygen (Figure 14.17). This conformation of hemoglobin is referred to as the “tense” or T state of the system. The first oxygen to bind to such a hemoglobin tetramer does so with difficulty, but this initial binding event triggers a conformational change in the assembly such that the three remaining binding sites are switched to a conformation that has much higher affinity for oxygen. This conformation is called the “relaxed” or R state of the system. The last oxygen molecule to bind to R-state hemoglobin binds ~1000 times more tightly than does the first oxygen molecule to bind to T-state hemoglobin. It is this switch in binding affinity that allows hemoglobin to pick up oxygen with high affinity in the lungs, but then release it with alacrity in the tissues.

We shall study the origins of the sigmoid binding curve of hemoglobin in two stages. First, by analyzing a simpler system with just two binding sites, we shall understand why allosteric systems have sigmoid binding curves (Sections 14.14 and 14.15). Next, we shall look at the structural mechanisms that underlie the ability of hemoglobin to alter the structure of its oxygen-binding sites in response to oxygen binding at a distant site (Sections 14.16 and 14.17). Our current understanding of allostery has its roots in concepts first developed for hemoglobin by Jacques Monod, Jeffries Wyman, and Jean-Pierre Changeux, and placed within a structural framework by Max Perutz, a founder of protein crystallography.

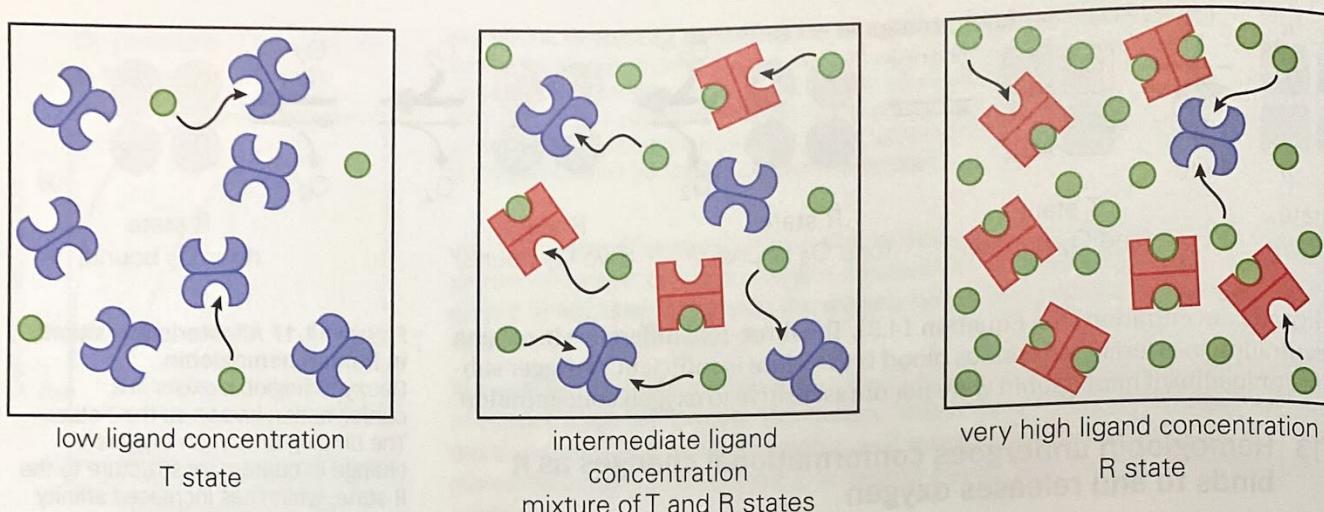
### 14.14 The sigmoid binding isotherm for an allosteric protein arises from switching between low- and high-affinity binding isotherms

We shall now consider the consequences of allostery for a dimeric protein, P, with two equivalent binding sites for a ligand, L, that can bind to either site (Figure 14.18). Let us suppose that P exhibits positive cooperativity, so that the binding of the second ligand is tighter than the binding of the first one. P switches between two conformations, one in which all of the binding sites are in the low-affinity conformation (the T state), and one in which all of the binding sites are in the high-affinity conformation (the R state).

The binding isotherms for the T and R states are shown in Figure 14.19A. For positive cooperativity, the dissociation constant for the T state,  $K_{D1}$ , is larger than that for the R state,  $K_{D2}$ . Recall from Section 12.5 that the graph of the logarithm of the ratio of the fraction of protein bound to the fraction unbound,  $\log[f/(1-f)]$ , versus the logarithm of the concentration of the ligand,  $\log[L]$ , is a straight line. In the log-log plot shown in Figure 14.19A, the binding isotherms for the T and R states are straight lines that are parallel to each other, with the R-state line on the left.

**Figure 14.17 Allosteric transitions in human hemoglobin.**

Deoxyhemoglobin exists in a conformation known as the T state. The binding of oxygen triggers a change in quaternary structure to the R state, which has increased affinity for oxygen.



**Figure 14.18 A dimeric allosteric protein.** The protein switches between two conformations, denoted T (low affinity, blue) and R (high affinity, red). When the ligand concentration is low, all of the protein is in the T state, and ligand binds with difficulty. As the ligand concentration increases, the protein starts to switch to the R state as ligand binds, and ligand affinity increases. This is an example of positive cooperativity.

We assume that the structure of P is always symmetrical—that is, conformations in which the two subunits have different conformations are disallowed. A very common form of allostery in biological systems (including hemoglobin) involves symmetrical multisubunit molecules, and for such systems the condition of symmetry is a reasonable one to impose. Notice that the dimeric molecules depicted in Figure 14.18 are all symmetrical, with both subunits in the R-state conformation, or both in the T-state conformation.

First, consider what happens when the ligand concentration is very low. At this point, most of the protein molecules are empty, and all of the available binding sites correspond to the low-affinity binding site (T state, dissociation constant  $K_{D1}$ ). At very low ligand concentrations, the binding reaction will be governed by the isotherm corresponding to the low-affinity T state, as shown in Figure 14.19B. As the ligand concentration increases, more and more of the protein molecules have one ligand bound and have switched to the R-state conformation, which has higher affinity for the ligand (dissociation constant  $K_{D2}$ ). The population of proteins is now mixed, with some in the T state and some in the R state. Ligand molecules now have a choice of binding sites and the thermodynamics of binding cannot be understood without a detailed calculation, which we shall examine in Section 14.15. Finally, when the ligand concentration is very high, all of the protein molecules have at least one site occupied, and so essentially all of the available sites are in the high-affinity R state. The binding of the ligand is now governed by the binding isotherm corresponding to the high-affinity site (see Figure 14.19B).

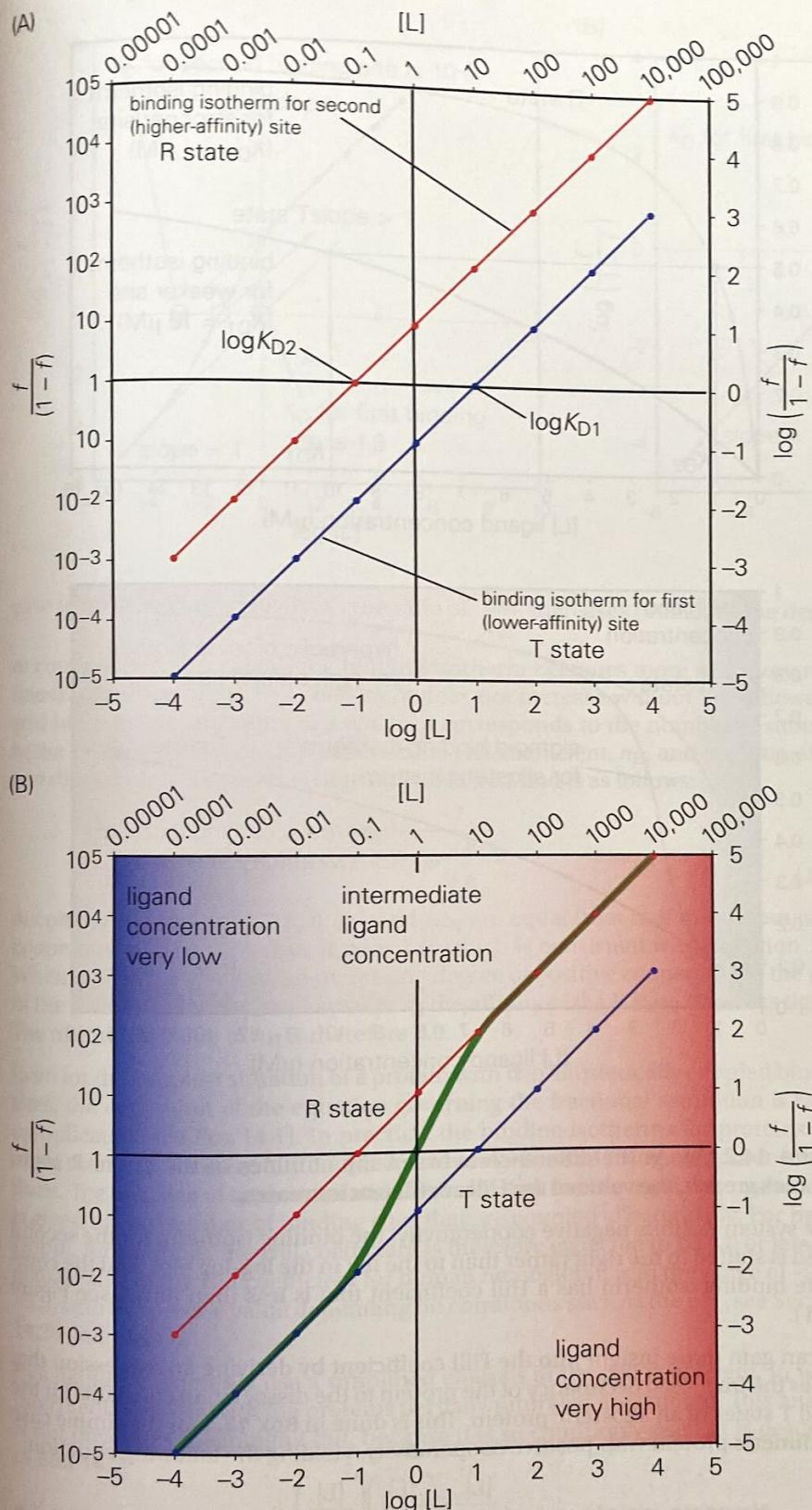
Figure 14.20 shows a graph of the data using normal (that is, not logarithmic) axes. The binding isotherm switches from one hyperbolic binding curve at low concentration to another one at high concentration, resulting in the sigmoidal shape that is characteristic of positive cooperativity.

### 14.15 The degree of cooperativity between binding sites in an allosteric protein is characterized by the Hill coefficient

The log-log plot for the binding isotherm of a noncooperative protein is governed by the following equation (see Equation 12.18 in Section 12.5):

$$\log\left(\frac{f}{1-f}\right) = \log[L] - \log K_D \quad (14.4)$$

According to Equation 14.4, the slope of the binding isotherm is 1.0. For an allosteric system with positive cooperativity, the slope of the binding isotherm is also ~1.0 at the two extremes of ligand concentration. At intermediate values of ligand



**Figure 14.19 Binding isotherm for a dimeric protein with positive cooperativity.** (A) Binding isotherms for the T (blue) and R (red) states are shown in a log-log plot (refer to Section 12.5). (B) As the ligand concentration increases, the binding isotherm switches from the T state isotherm to the R state isotherm. The resulting binding isotherm is no longer linear (green). The ligand concentration is expressed in micromolar units.

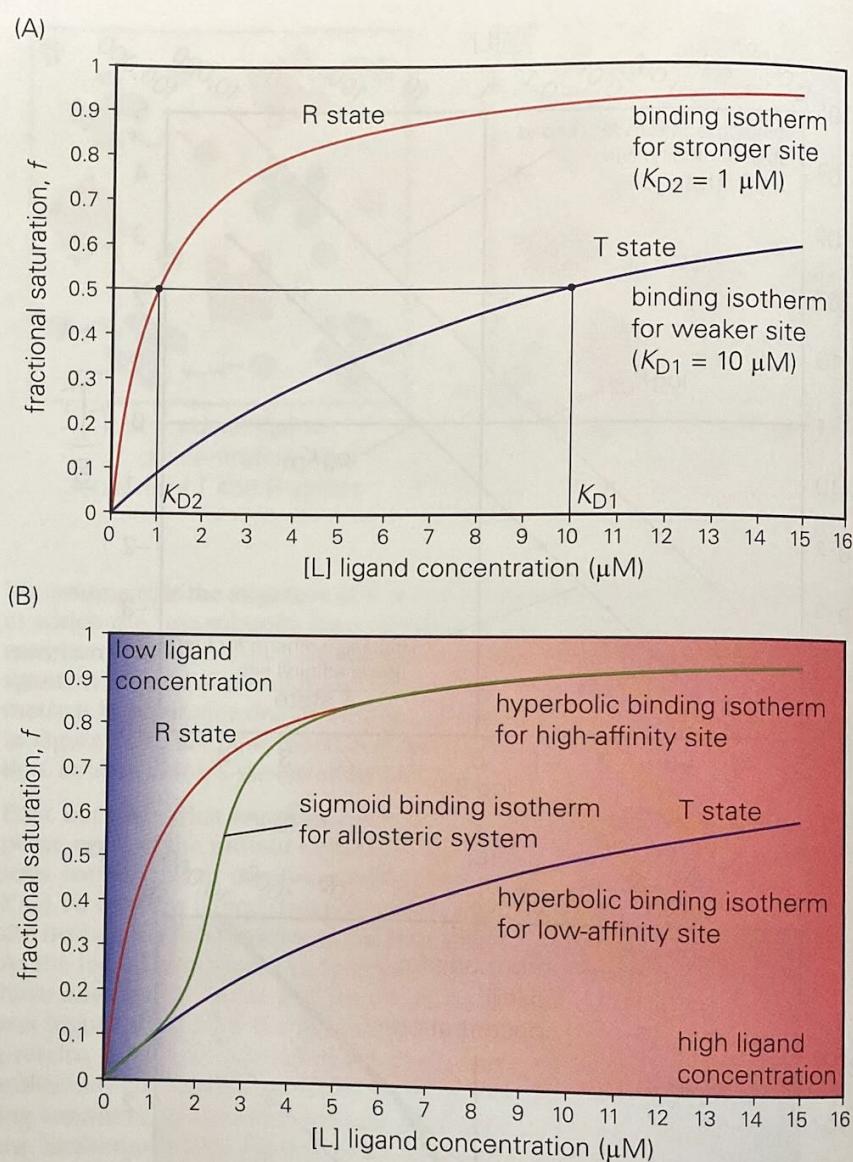
concentration, the binding isotherm curves up and has a slope that is greater than unity. The slope of the binding isotherm at the point where the protein is half saturated [that is,  $\log \left( \frac{f}{1-f} \right)$  is zero] is known as the **Hill coefficient**,  $n_H$ .

Figure 14.21 Hill coefficients for positive and negative cooperativity. (A) The binding isotherm for a dimeric protein is shown. The equation is given as described in Box 14.1. The plot shows positive cooperativity, with the values for the T and R states being 1.5 and 10<sup>2</sup>/M, respectively. At the point of half saturation, when  $\log \left( \frac{f}{1-f} \right) = 0$ , the slope of the binding curve is greater than 1.0. The value at this point is defined as the Hill coefficient, reported in values from Equation 14.1. (B) The binding isotherm for a dimeric protein with negative cooperativity. The T and R states have Hill values of 1.0 and 10<sup>2</sup>/M, respectively. In this case, the binding isotherm is nearly linear for intermediate concentrations of ligand, and the slope becomes steeper as the ligand concentration is increased.

### Hill coefficient

This parameter, named after the physiologist Archibald Hill, reflects the steepness of the log-log binding isotherm at the point when the protein is half saturated with ligand. A noncooperative system has a Hill coefficient of unity. Systems exhibiting positive and negative cooperativity have Hill coefficients that are greater than and less than unity, respectively.

**Figure 14.20 Binding isotherm graphed with normal axes for a dimeric protein with positive cooperativity.** (A) Binding isotherms for the T state (blue) and R state (red). (B) The net binding isotherm (green) is a combination of the T and R-state isotherms.



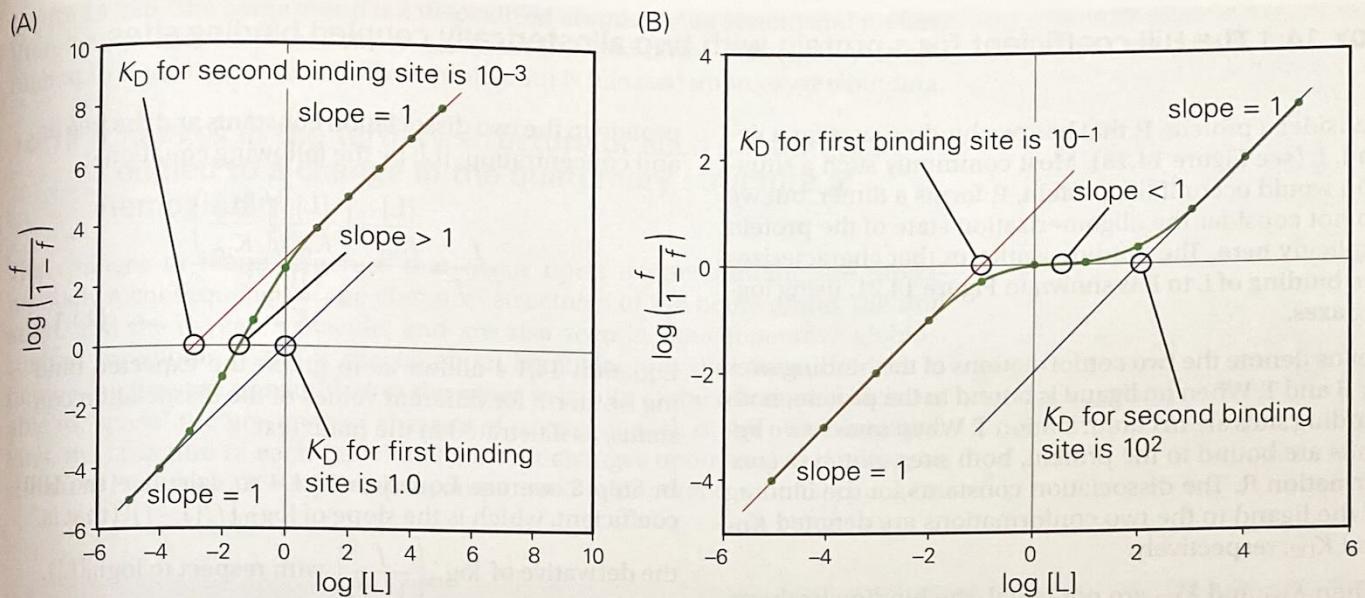
(Figure 14.21A). As the difference between the affinities of the T and R states becomes greater, the value of the Hill coefficient increases.

If the system exhibits negative cooperativity, the binding isotherm for the second ligand is shifted to the right rather than to the left in the log-log plot, and the composite binding isotherm has a Hill coefficient that is less than unity (see Figure 14.21).

We can gain more insight into the Hill coefficient by deriving an expression that relates the fractional occupancy of the protein to the dissociation constants for the R and T states of an allosteric protein. This is done in **Box 14.1** for the simple case of a dimeric protein with positive cooperativity, yielding the following equation:

$$\frac{f}{1-f} = \frac{\frac{[L]}{K_{D1}} + \left( \frac{[L]}{K_{D1}} \right) \left( \frac{[L]}{K_{D2}} \right)}{1 + \frac{[L]}{K_{D1}}} \quad (14.5)$$

Equation 14.5 allows us to plot the binding isotherms as a function of different values of  $K_{D1}$  and  $K_{D2}$  (the graphs in Figure 14.21 are based on Equation 14.5). In the



case of positive cooperativity, as the ratio of  $\frac{K_{D1}}{K_{D2}}$  increases (that is, as the degree

of cooperativity increases), the binding isotherm becomes more and more nonlinear. The slope of the Hill coefficient does not increase without limit, however, and has a maximum value of 2.0, which corresponds to the number of subunits in the protein. A relationship between the Hill coefficient,  $n_H$ , and the ratio of the two dissociation constants is derived in Box 14.1 and is as follows:

$$n_H = \frac{2}{1 + \sqrt{\frac{K_{D2}}{K_{D1}}}} \quad (14.6)$$

According to Equation 14.6, if  $K_{D1}$  and  $K_{D2}$  are equal (that is, if the system is not cooperative), then the value of  $n_H$  is 1.0, which is consistent with Equation 14.4. When the system exhibits an increasing degree of positive cooperativity, the ratio in the denominator becomes smaller as the affinity of the R state becomes tighter. The maximum value of  $n_H$  is therefore 2.0.

Even for the simplest situation of a protein with two allosterically coupled binding sites, the derivation of the equation governing the fractional saturation is rather complicated (see Box 14.1). In practice, the binding isotherms for proteins with more than two allosterically coupled sites are generated using computer simulations. The analysis of such simulations reveals that the maximum value of  $n_H$  is given by the number of binding sites that are coupled allosterically. For hemoglobin, for example, the Hill coefficient is therefore expected to be 4.0 or less. The actual value of the Hill coefficient for human hemoglobin is in the range of ~2.5 to ~3.5, with the precise value depending on conditions such as the pH (see Sections 14.20 and 14.21).

Biochemists commonly use a simplified analysis of the Hill coefficient, in which intermediate states with the proteins partially saturated are ignored. This analysis is not physically meaningful but, because it is so commonly used, it is described in Box 14.2.

#### 14.16 The tertiary structure of each hemoglobin subunit changes upon oxygen binding

Crystal structures of ligand-bound and ligand-free hemoglobin, first determined by Max Perutz, have led to the elucidation of the structural basis for the cooperativity in oxygen binding. The key feature of the mechanism is the change in the

**Figure 14.21** Hill coefficients for positive and negative cooperativity.

(A) The binding isotherm for a dimeric protein is shown, calculated using Equation 14.5, as described in Box 14.1. The protein exhibits positive cooperativity, with the values of  $K_D$  for the T and R states being 1.0 M and  $10^{-3}$  M, respectively. At the point

of half saturation, when  $\log \left( \frac{f}{1-f} \right)$

is zero, the slope of the binding curve is greater than 1.0. The slope at this point is defined as the Hill coefficient,  $n_H$ , and its value is 1.94 (see Equation 14.6). (B) The binding isotherm for a dimeric protein with negative cooperativity. The T and R states have  $K_D$  values of  $10^{-1}$  M and  $10^2$  M, respectively. In this case, the binding isotherm has a slope less than unity for intermediate concentrations of ligand. The value of  $n_H$  is 0.061, according to Equation 14.6. The ligand concentration is expressed in micromolar units.

### Box 14.1 The Hill coefficient for a protein with two allosterically coupled binding sites

Consider a protein, P, that has two binding sites for a ligand, L (see Figure 14.18). Most commonly such a situation would occur if the protein, P, forms a dimer, but we do not consider the oligomerization state of the protein explicitly here. The binding isotherm that characterizes the binding of L to P is shown in Figure 14.21, using log-log axes.

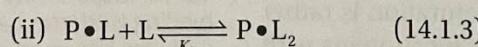
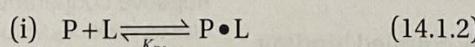
Let us denote the two conformations of the binding sites by R and T. When no ligand is bound to the protein, both binding sites are in conformation T. When one or two ligands are bound to the protein, both sites switch to conformation R. The dissociation constants for the binding of the ligand to the two conformations are denoted  $K_{D1}$  and  $K_{D2}$ , respectively.

When  $K_{D1}$  and  $K_{D2}$  are not equal, the binding isotherm is no longer linear. The Hill coefficient,  $n_H$ , characterizes the steepness of the curvature of the binding isotherm. The slope of the binding curve is not uniform and, in order to be precise, we define the Hill coefficient as the slope of the binding isotherm at the point when the protein is 50% saturated with ligand—that is, when the value of  $f$  is 0.5 and  $\log[f/(1-f)]$  is zero (see Figure 14.21).

The value of the Hill coefficient,  $n_H$ , for a protein, P, with two allosterically coupled binding sites is given by:

$$n_H = \frac{2}{1 + \sqrt{\frac{K_{D2}}{K_{D1}}}} \quad (14.1.1)$$

Equation 14.1.1 is obtained by treating the binding of the ligand to the protein as if it were described by the following two sequential binding equilibria:



In order to derive Equation 14.1.1, we proceed in two steps. In step 1, we relate the ratio of bound and unbound

protein to the two dissociation constants and the free ligand concentration, [L], by the following equation:

$$\frac{f}{1-f} = \frac{\frac{[L]}{K_{D1}} + \left( \frac{[L]}{K_{D1}} \right) \left( \frac{[L]}{K_{D2}} \right)}{1 + \frac{[L]}{K_{D1}}} \quad (14.1.4)$$

Equation 14.1.4 allows us to graph the expected binding isotherm for different values of the dissociation constants, as illustrated in the main text.

In Step 2, we use Equation 14.1.4 to calculate the Hill coefficient, which is the slope of  $\log_{10}[f/(1-f)]$  (that is, the derivative of  $\log_{10}\left(\frac{f}{1-f}\right)$  with respect to  $\log_{10}[L]$ ), evaluated at the ligand concentration when  $f=0.5$ :

$$\text{Hill coefficient, } n_H = \frac{d \log_{10}\left(\frac{f}{1-f}\right)}{d \log_{10}[L]} \quad \boxed{\text{evaluated at } f=0.5} \quad (14.1.5)$$

Evaluation of this derivative gives us the desired definition of the Hill coefficient,  $n_H$  (Equation 14.1.1). Steps 1 and 2 are straightforward, but they involve rather tedious algebra and repeated application of the chain rule of differentiation. You can work out these relationships yourself by using the definitions of the dissociation constants and the fractional saturation. In practice, allosteric binding interactions are usually estimated empirically by numerical analysis of actual binding data, or by analyzing binding equilibria using the principles of statistical thermodynamics. You may, if you are interested, refer to the paper by Attila Szabo and Martin Karplus on a statistical thermodynamic model for hemoglobin, in the reading list at the end of this chapter.

state of electron spins of the iron atom of the heme group upon coordination by oxygen. In the absence of oxygen, the iron atom is coordinated by five nitrogen atoms, four provided by the heme group and one provided by the proximal histidine (Figure 14.14C). The five-coordinate iron atom has high electron spin (that is, the d-electrons are in different orbitals and are not paired) and, consequently, an ionic radius that is too large to be accommodated within the central cavity of the heme group.

The heme group is therefore buckled in the deoxy form, and the iron atom is popped out of the plane of the four nitrogen atoms of the heme group (Figure 14.22A). Oxygen binding converts the iron atom from the five-coordinate state with high electron spin to a six-coordinate state with low spin (pairing electrons in orbitals), which results in a reduction of the effective size of the iron atom. The iron atom can now be accommodated within a planar heme group, as seen in