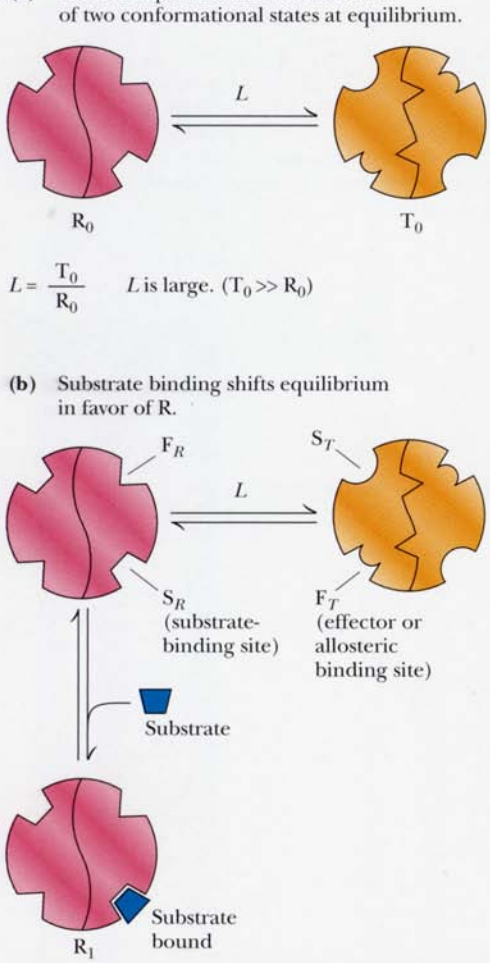


FIGURE 15.8 Monod–Wyman–Changeux (MWC) model for allosteric transitions. Consider a dimeric protein that can exist in either of two conformational states, R or T. Each subunit in the dimer has a binding site for substrate S and an allosteric effector site, F. The promoters are symmetrically related to one another in the protein, and symmetry is conserved regardless of the conformational state of the protein. The different states of the protein, with or without bound ligand, are linked to one another through the various equilibria. Thus, the relative population of protein molecules in the R or T state is a function of these equilibria and the concentration of the various ligands, substrate (S), and effectors (which bind at F_R or F_T). As [S] is increased, the T/R equilibrium shifts in favor of an increased proportion of R conformers in the total population (that is, more protein molecules in the R conformational state).

15.3 Can a Simple Equilibrium Model Explain Allosteric Kinetics?

Monod, Wyman, and Changeux Proposed the Symmetry Model for Allosteric Regulation

In 1965, Jacques Monod, Jeffries Wyman, and Jean-Pierre Changeux proposed a theoretical model of allosteric transitions based on the observation that allosteric proteins are oligomers. They suggested that allosteric proteins can exist in (at least) two conformational states, designated **R,** signifying "relaxed," and **T,** or "taut," and that, in each protein molecule, all of the subunits have the same conformation (either R or T). That is, molecular symmetry is conserved. Molecules of mixed conformation (having subunits of both R and T states) are not allowed by this model.

In the absence of ligand, the two states of the allosteric protein are in equilibrium:

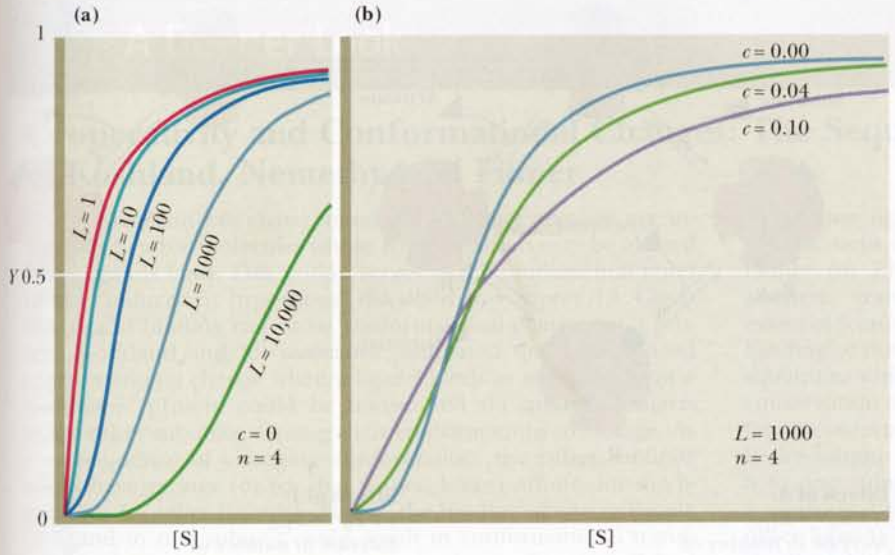
$$R_0 \rightleftharpoons T_0$$

(Note that the subscript "0" signifies "in the absence of ligand.") The equilibrium constant is termed *L*: $L = T_0/R_0$. *L* is assumed to be large; that is, the amount of the protein in the T conformational state is much greater than the amount in the R conformation. Let us suppose that $L = 10^4$.

The affinities of the two states for substrate, S, are characterized by the respective dissociation constants, K_R and K_T. The model supposes that $K_T \gg K_R$. That is, the affinity of R_0 for S is much greater than the affinity of T_0 for S. Let us choose the extreme where $K_R/K_T = 0$ (that is, K_T is infinitely greater than K_R). In effect, we are picking conditions in which S binds only to R. (If K_T is infinite, T does not bind S.)

Given these parameters, consider what happens when S is added to a solution of the allosteric protein at conformational equilibrium (Figure 15.8). Although the relative $[R_0]$ concentration is small, S will bind "only" to R_0, forming R_1. This depletes the concentration of R_0, perturbing the T_0/R_0 equilibrium. To restore equilibrium, molecules in the T_0 conformation undergo a transition to R_0. This shift renders more R_0 available to bind S, yielding R_1, diminishing $[R_0]$, perturbing the T_0/R_0 equilibrium, and so on. Thus, these linked equilibria (Figure 15.8) are such that S-binding by the R_0 state of the allosteric protein perturbs the T_0/R_0 equilibrium with the result that S-binding drives the conformational transition, $T_0 \longrightarrow R_0$.

In just this simple system, *cooperativity* is achieved because each subunit has a binding site for S, and thus, *each protein molecule has more than one binding site for S.* Therefore, the increase in the population of R conformers gives a progressive increase in the number of sites available for S. The extent of cooperativity depends on the relative T_0/R_0 ratio and the relative affinities of R and T for S. If *L* is large (that is, the equilibrium lies strongly in favor of T_0) and if $K_T \gg K_R$, as in the example we have chosen, cooperativity is great (Figure 15.9). Ligands



Biochemistry Now™ ANIMATED FIGURE 15.9
The Monod–Wyman–Changeux model. Graphs of allosteric effects for a tetramer ($n = 4$) in terms of Y, the saturation function, versus [S]. Y is defined as [ligand-binding sites that are occupied by ligand]/[total ligand-binding sites]. **(a)** A plot of Y as a function of [S], at various L values. **(b)** Y as a function of [S], at different c, where $c = K_R/K_T$. (When $c = 0$, K_T is infinite.) *(Adapted from Monod, J., Wyman, J., and Changeux, J-P., 1965. On the nature of allosteric transitions: A plausible model.* Journal of Molecular Biology *12:92.)* **See this figure animated at http://chemistry.brookscole.com/ggb3**

such as S here that bind in a cooperative manner, so that binding of one equivalent enhances the binding of additional equivalents of S to the same protein molecule, are termed **positive homotropic effectors.** (The prefix *homo* indicates that the ligand influences the binding of like molecules.)

Heterotropic Effectors Influence the Binding of Other Ligands

This simple system also provides an explanation for the more complex substrate-binding responses to positive and negative effectors. Effectors that influence the binding of something other than themselves are termed **heterotropic effectors.** For example, effectors that promote S binding are termed **positive heterotropic effectors** or **allosteric activators.** Effectors that diminish S binding are **negative heterotropic effectors** or **allosteric inhibitors.** Feedback inhibitors fit this class. Consider a protein composed of two subunits, each of which has two binding sites: one for the substrate, S, and one to which allosteric effectors bind, the *allosteric site.* Assume that S binds preferentially ("only") to the R conformer; further assume that the *positive heterotropic effector,* A, binds to the allosteric site only when the protein is in the R conformation and the *negative allosteric effector,* I, binds at the allosteric site only if the protein is in the T conformation. Thus, with respect to binding at the allosteric site, A and I are competitive with each other.

Positive Effectors Increase the Number of Binding Sites for a Ligand

If A binds to R_0, forming the new species $R_{1(A)}$, the relative concentration of R_0 is decreased and the T_0/R_0 equilibrium is perturbed (Figure 15.10). As a consequence, a relative $T_0 \rightarrow R_0$ shift occurs in order to restore equilibrium. The net effect is an increase in the number of R conformers in the presence of A, meaning that more binding sites for S are available. For this reason, A leads to a decrease in the cooperativity of the substrate saturation curve, as seen by a shift of this curve to the left (Figure 15.10). Effectively, the presence of A lowers the apparent value of L.

Negative Effectors Decrease the Number of Binding Sites Available to a Ligand

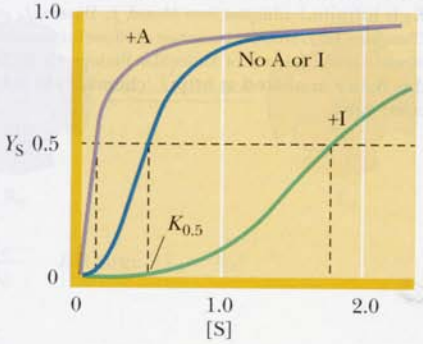
The converse situation applies in the presence of I, which binds "only" to T. I binding will lead to an increase in the population of T conformers, at the expense of R_0 (Figure 15.10). The decline in $[R_0]$ means that it is less likely for S (or A) to bind. Consequently, the presence of I increases the cooperativity

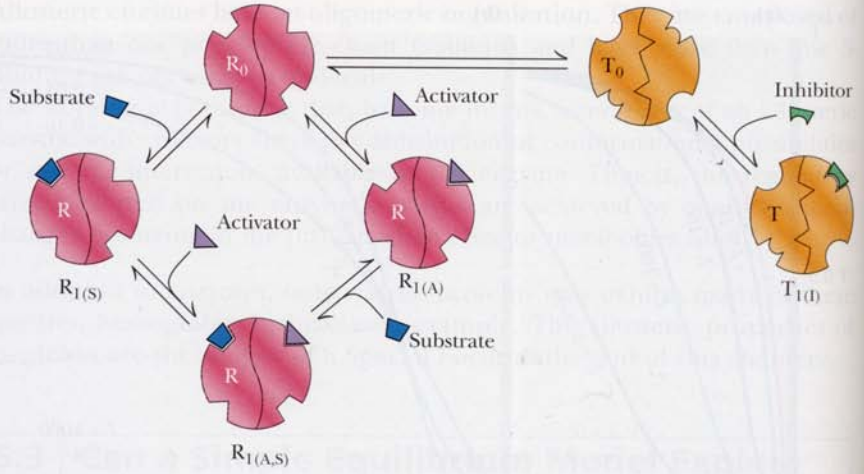
A dimeric protein that can exist in either of two states: R_0 or T_0.
This protein can bind three ligands:

1) Substrate (S) : A positive homotropic effector that binds only to R at site S

2) Activator (A) : A positive heterotropic effector that binds only to R at site F

3) Inhibitor (I) : A negative heterotropic effector that binds only to T at site F





Effects of A:
$A + R_0 \rightarrow R_{I(A)}$
Increase in number of R-conformers shifts $R_0 \rightleftharpoons T_0$ so that $T_0 \rightarrow R_0$

(1) More binding sites for S made available.

(2) Decrease in cooperativity of substrate saturation curve. Effector A lowers the apparent value of L.

Effects of I:
$I + T_0 \rightarrow T_{I(I)}$
Increase in number of T-conformers (decrease in R_0 as $R_0 \rightarrow T_0$ to restore equilibrium)

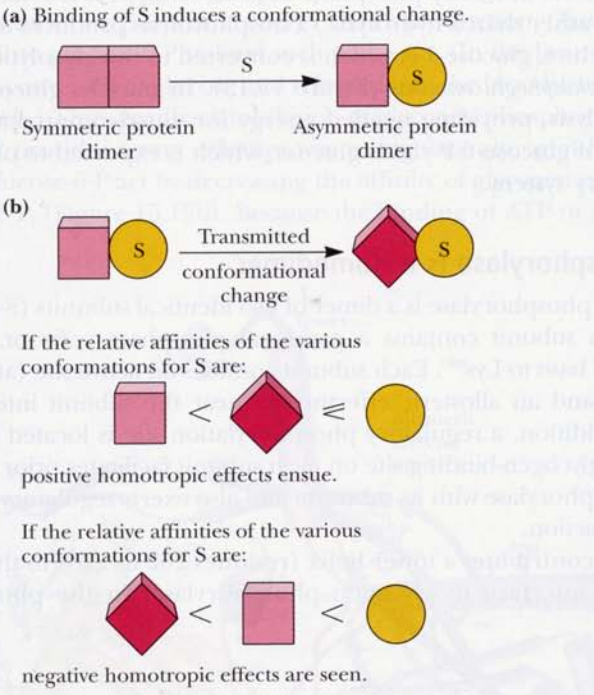
Thus, I inhibits association of S and A with R by lowering R_0 level. I increases cooperativity of substrate saturation curve. I raises the apparent value of L.

Biochemistry Now™ ACTIVE FIGURE 15.10 Heterotropic allosteric effects: A and I binding to R and T, respectively. The linked equilibria lead to changes in the relative amounts of R and T and, therefore, shifts in the substrate saturation curve. This behavior, depicted by the graph, defines an allosteric "K system." The parameters of such a system are that (1) S and A (or I) have different affinities for R and T and (2) A (or I) modifies the apparent $K_{0.5}$ for S by shifting the relative R versus T population. **Test yourself on the concepts in this figure at http://chemistry.brookscole.com/ggb3**

(that is, the sigmoidicity) of the substrate saturation curve, as evidenced by the shift of this curve to the right (Figure 15.10). The presence of I raises the apparent value of L.

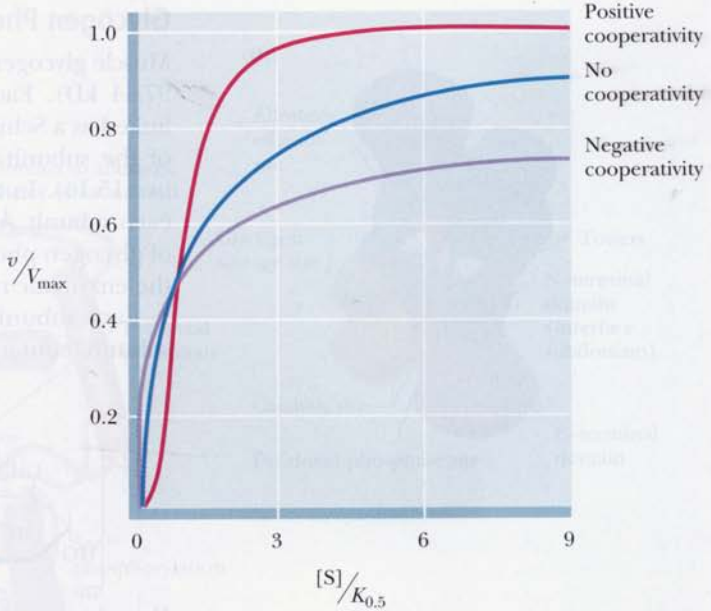
Cooperativity and Conformational Changes: The Sequential Allosteric Model of Koshland, Nemethy, and Filmer

Daniel Koshland has championed the idea that proteins are inherently flexible molecules whose conformations may be altered when ligands bind. This notion serves as the fundamental tenet of the "induced-fit hypothesis" discussed in Chapter 13. Given that ligand binding can cause conformational changes in a protein, Koshland and his associates postulated that the induced conformational change when a ligand binds to one subunit of a multimeric protein could be transmitted via subunit contacts to the other subunits, causing their conformations to change. As a consequence of changing conformation, the other subunits might have greater (or for that matter, lesser) affinity for the ligand (or for other ligands). That is, the binding of one molecule of ligand to one subunit could result in conformational transitions in the protein that make it easier or harder for other ligand molecules to bind to the other subunits. Depending on the nature of such coupled conformational changes, virtually any sort of allosteric interaction is possible.



▲ The Koshland–Nemethy–Filmer sequential model for allosteric behavior. **(a)** S binding can, by induced fit, cause a conformational change in the subunit to which it binds. **(b)** If subunit interactions are tightly coupled, binding of S to one subunit may cause the other subunit to assume a conformation having a greater (positive homotropic) or lesser (negative homotropic) affinity for S. That is, the ligand-induced conformational change in one subunit can affect the adjoining subunit. Such effects could be transmitted between neighboring peptide domains by changing alignments of nonbonded amino acid residues.

Because ligand binding and conformational transitions are distinct steps in a sequential pathway, the Koshland, Nemethy, Filmer (or KNF) model is dubbed the **sequential model** for allosteric transitions. The accompanying figure depicts the essential features of this model in a hypothetical dimeric protein. Binding of the ligand S induces a conformational change in the subunit to which it binds. Note that there is no requirement for conservation of symmetry here; the two subunits can assume different conformations (represented here as a square and a circle). If the subunit interactions are tightly coupled, then binding of S to one subunit could cause the other subunit(s) to assume a conformation having more, or less, affinity for S (or some other ligand). The underlying mechanism rests on the fact that the ligand-induced conformational change in one subunit can transmit its effects to neighboring subunits by changing the interactions and alignments of amino acid residues at the interface between the subunits. Depending on the relative ligand affinity of the conformation adopted by the neighboring subunit, the overall effect on further ligand binding may be positive, negative, or neutral (see accompanying graph). Note that in negative cooperativity, the response (binding) at $[S] > K_{0.5}$ is less than that seen for the "no cooperativity" (or Michaelis–Menten) situation. Negative cooperativity is not possible in the MWC model. Thus, the KNF model is more general than the MWC model in covering all allosteric possibilities—positive, negative, or no cooperativity. Approximately half of all known allosteric enzymes display negative cooperativity.



▲ Theoretical curves for the binding of a ligand to a protein having four identical subunits, each with one binding site for the ligand. The fraction of maximal binding is plotted as a function of $[S]/K_{0.5}$.