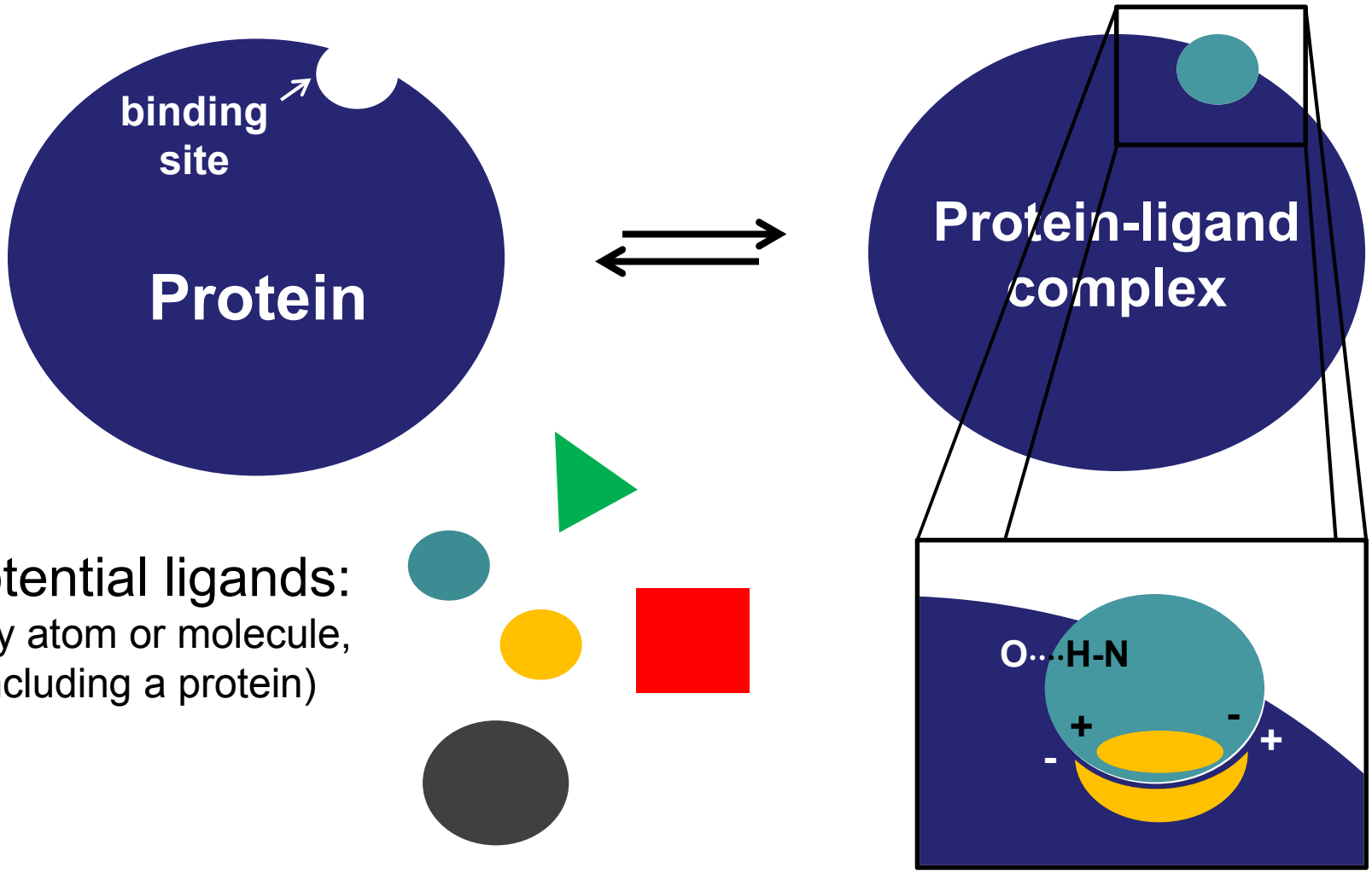
A protein binds a ligand through a specific, reversible interaction

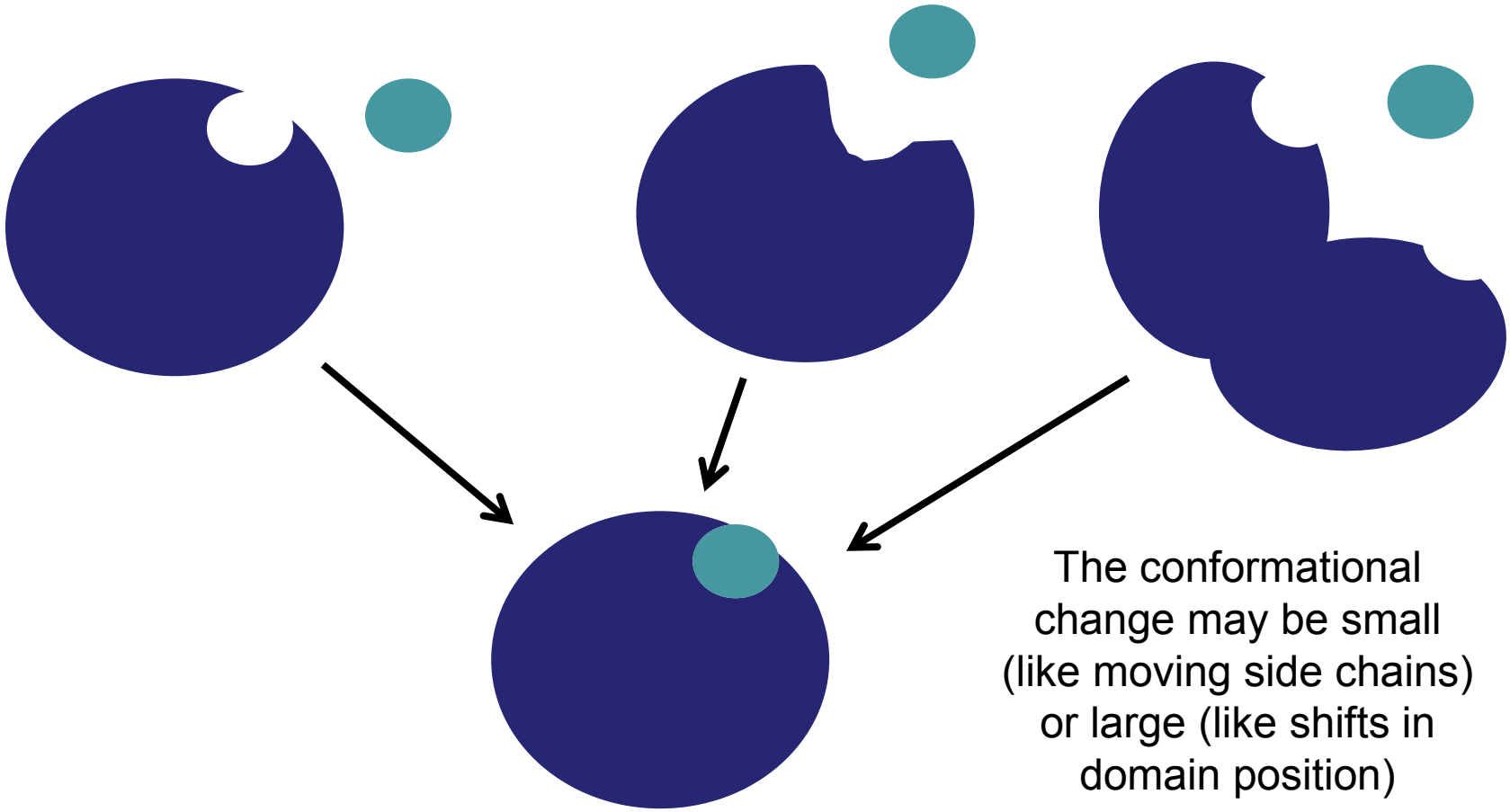


Potential ligands:
(any atom or molecule, including a protein)

Ligand binding may cause a conformational change in the protein that enhances binding

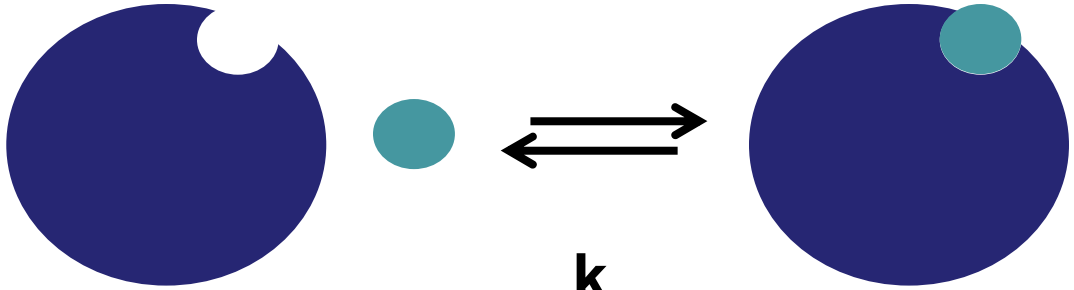
Lock and key model

Induced-fit model



The conformational change may be small (like moving side chains) or large (like shifts in domain position)

The association constant (K_a) provides a measure of affinity between protein & ligand



$$P + L \underset{k_d}{\overset{k_a}{\rightleftharpoons}} PL$$

equilibrium constant (uppercase K)

Units?

$$K_a = \frac{[PL]}{[P][L]} = \frac{k_a}{k_d}$$

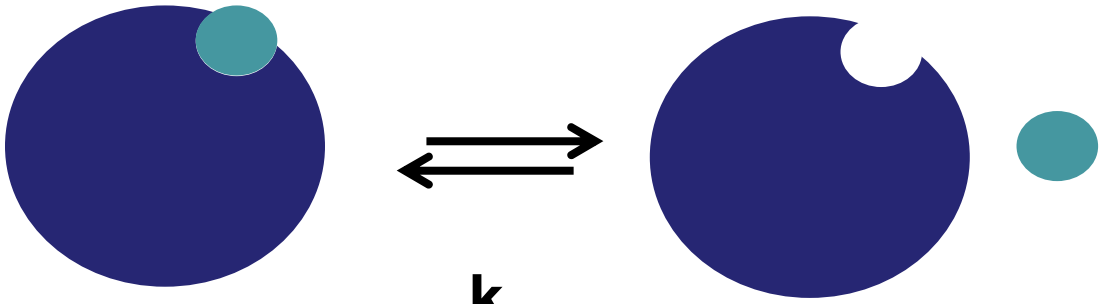
rate constants (lowercase k)

Units?

Rate constants are proportionality constants, describing the fraction of the pool that reacts in a given amount of time

Ex: if $k_d = 0.03\ s^{-1}$, then 3% of PL dissociates per second

The dissociation constant (K_d) is analogous to the association constant (K_a)



$$PL \underset{k_a}{\overset{k_d}{\rightleftharpoons}} P + L$$

Units?

$$K_d = \frac{[P][L]}{[PL]} = \frac{k_d}{k_a} = \frac{1}{K_a}$$

Note: K_a, K_d, k_a, & k_d are constant under set conditions; they can change with changes in temperature, pH, [salt], …

The fraction of occupied binding sites (θ) is proportional to the ligand concentration

$$\mathbf{P + L \rightleftharpoons PL}$$

When $[L] \gg [PL] + [P]$, $[L]$ is constant (usually true for small ligands in cells)

$$\theta = \frac{binding\ sites\ occupied}{total\ binding\ sites} = \frac{[PL]}{[PL] + [P]}$$

Substitue in $[PL] = K_a[L][P]$

$$\theta = \frac{K_a[L][P]}{K_a[L][P]+[P]} = \frac{K_a[L]}{K_a[L]+1} = \frac{[L]}{[L]+\frac{1}{K_a}} = \frac{[L]}{[L]+K_d}$$

The fraction of occupied ligand-binding sites θ depends on [L] and the binding affinity

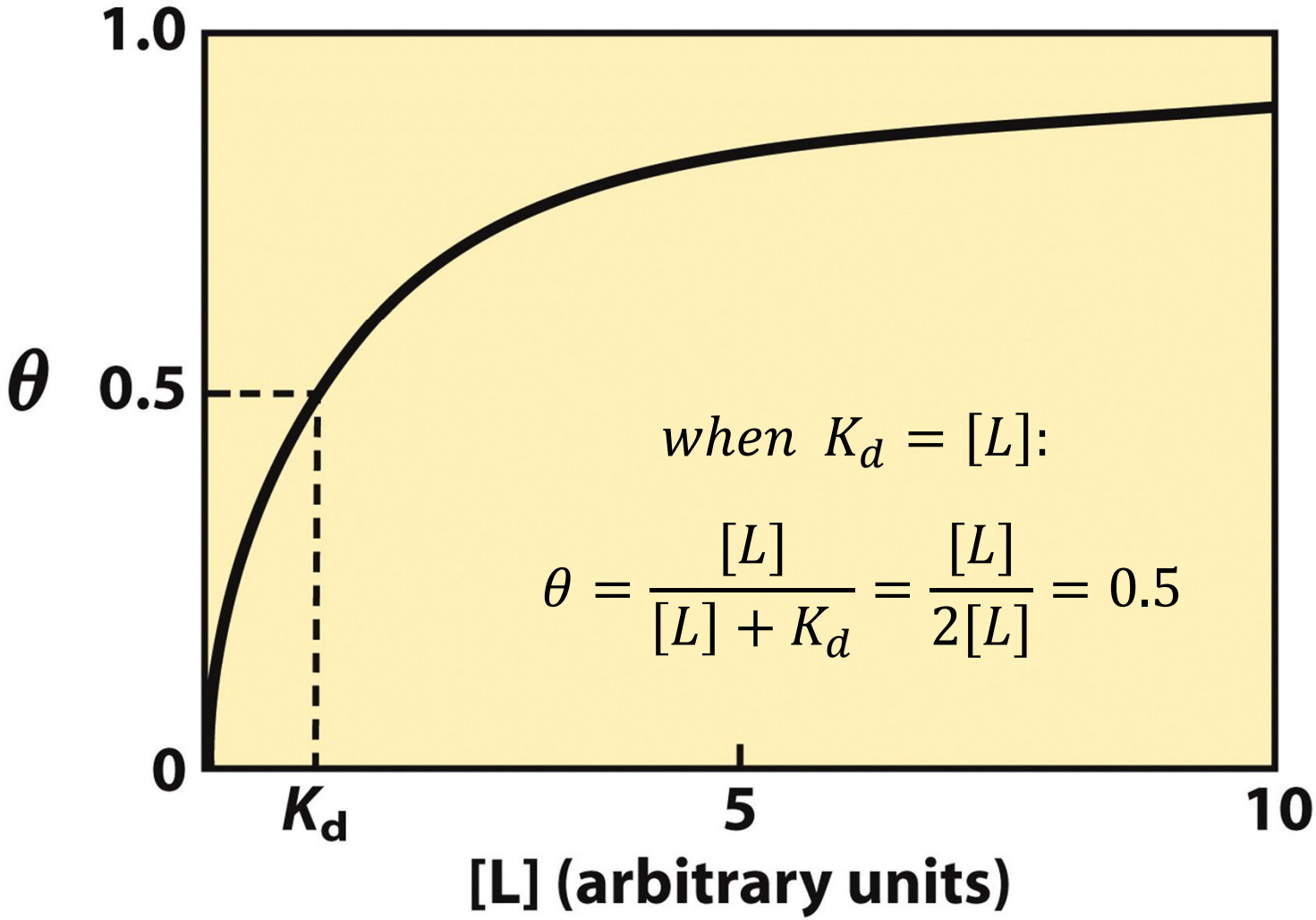
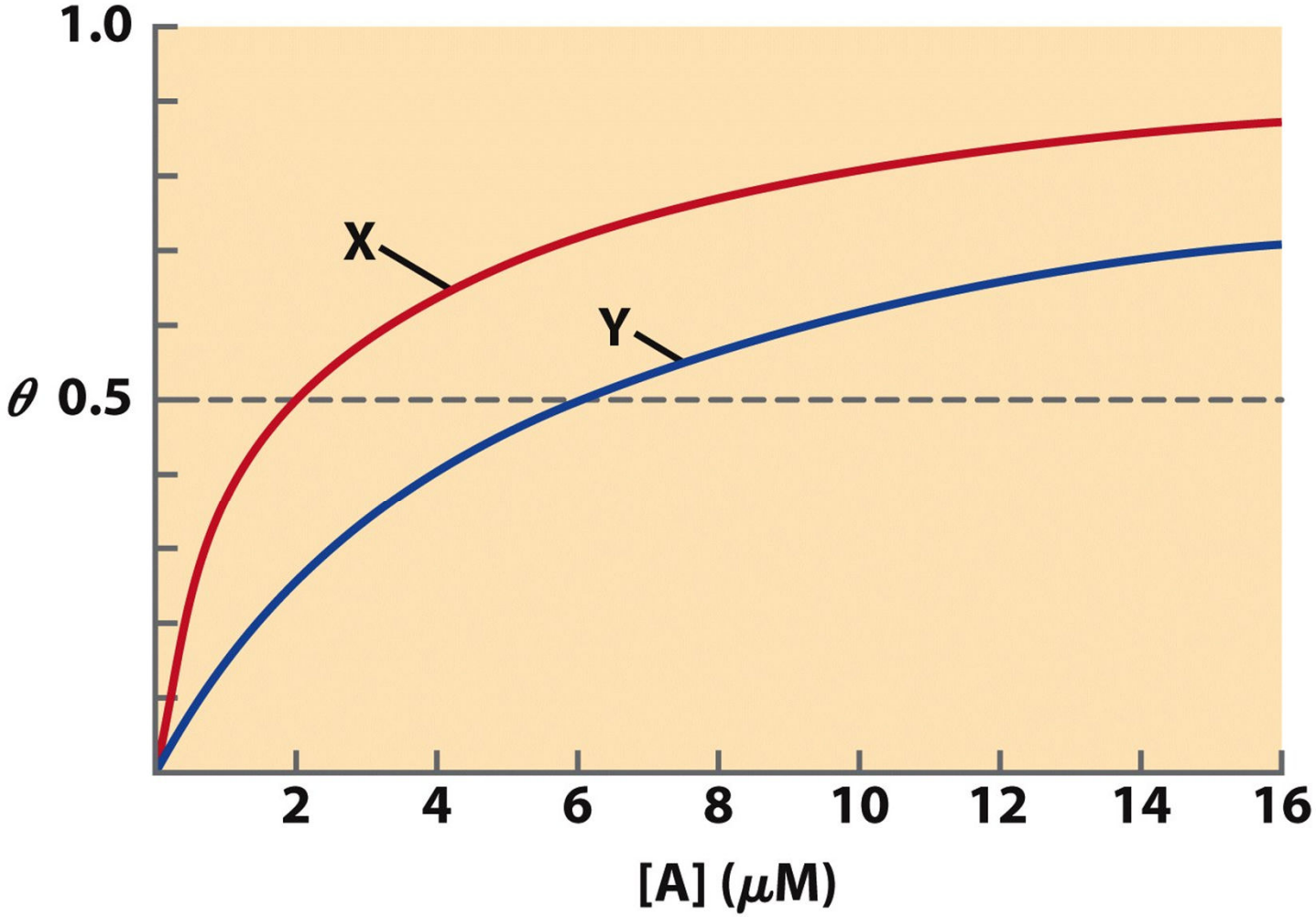


Figure 5-4a
Lehninger Principles of Biochemistry, Fifth Edition

A protein with higher affinity for a ligand has a higher binding curve and lower K_d



Unnumbered 5 p157
Lehninger Principles of Biochemistry, Fifth Edition

Protein-ligand dissociation constants (K_d's) vary over several orders of magnitude

TABLE 5–1 Some Protein Dissociation Constants

Protein	Ligand	K_d (M)*
Avidin (egg white)†	Biotin	1×10^{-15}
Insulin receptor (human)	Insulin	1×10^{-10}
Anti-HIV immunoglobulin (human)‡	gp41 (HIV-1 surface protein)	4×10^{-10}
Nickel-binding protein (*E. coli*)	Ni^{2+}	1×10^{-7}
Calmodulin (rat)§	Ca^{2+}	3×10^{-6}
		2×10^{-5}

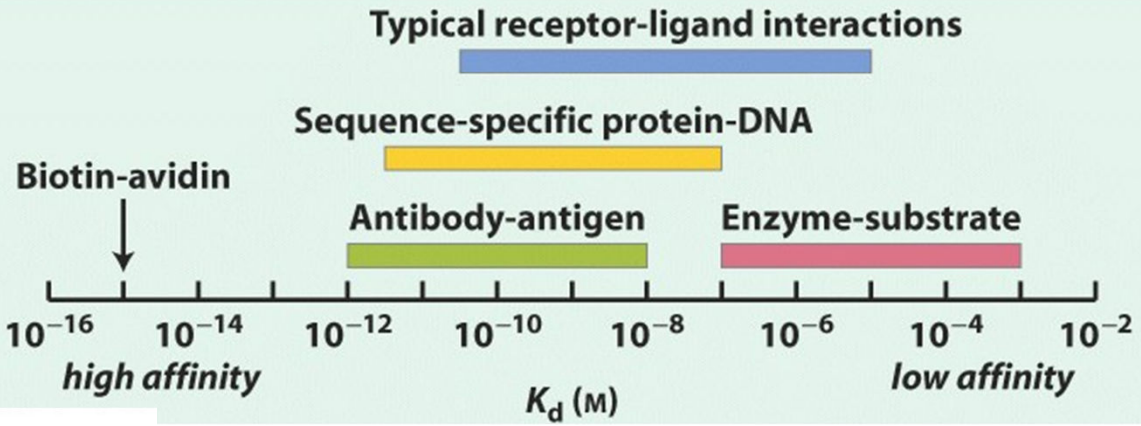
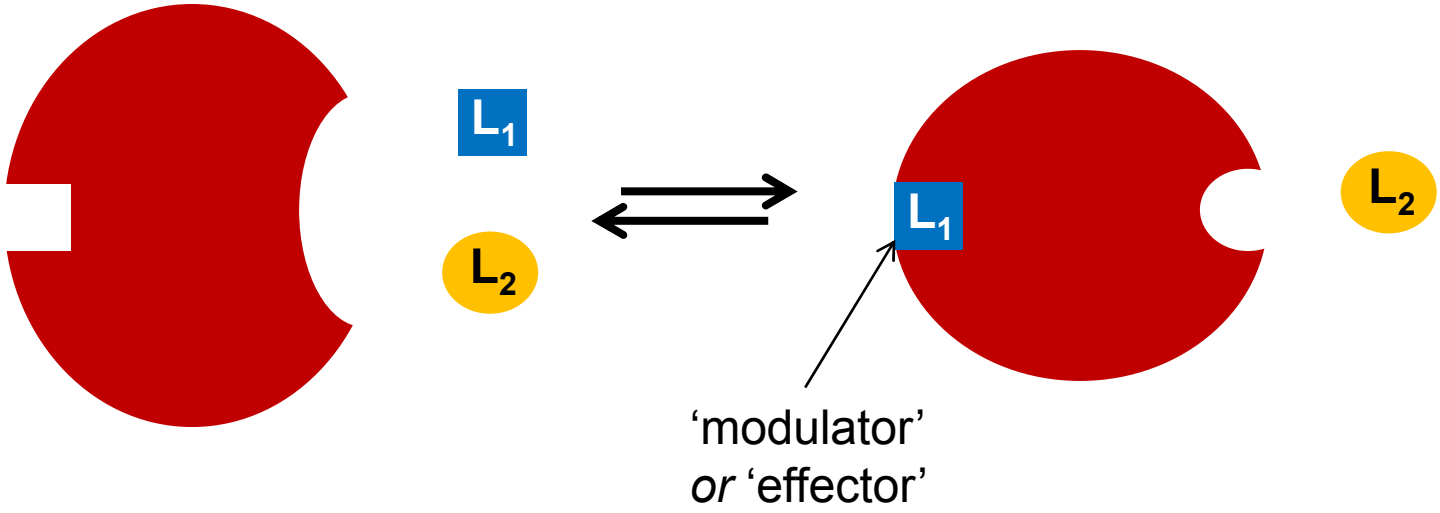


Table 5-1
Lehninger Principles of Biochemistry, Fifth Edition

Some proteins have varying affinity for a ligand, depending on their conformation

Allosteric protein

Binding of a ligand (L_1) to one site affects binding properties of another site (via a conformational change in the protein)

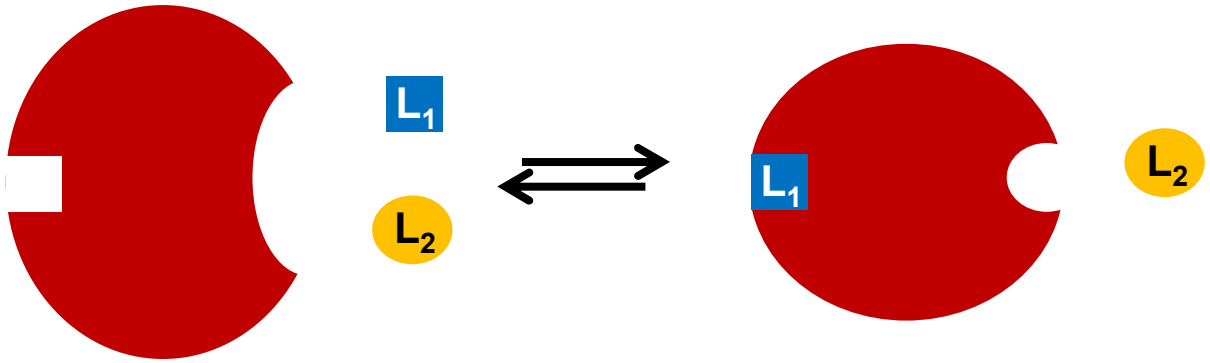


Modulator (L_1) is an 'activator' if it increases affinity at 2nd site (where L_2 binds)
Modulator (L_1) is an 'inhibitor' if it decreases affinity at 2nd site (where L_2 binds)

Allostery may involve different ligands, the same ligands, or both

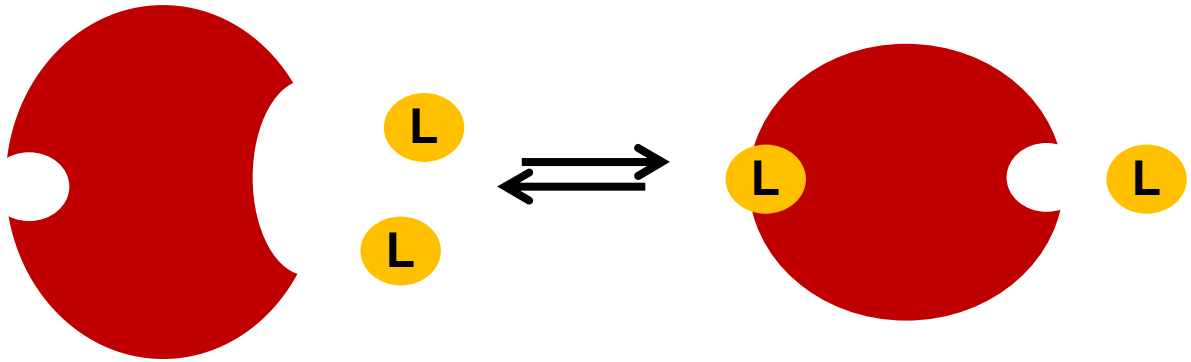
Heterotropic interaction

Modulator and other ligand are different

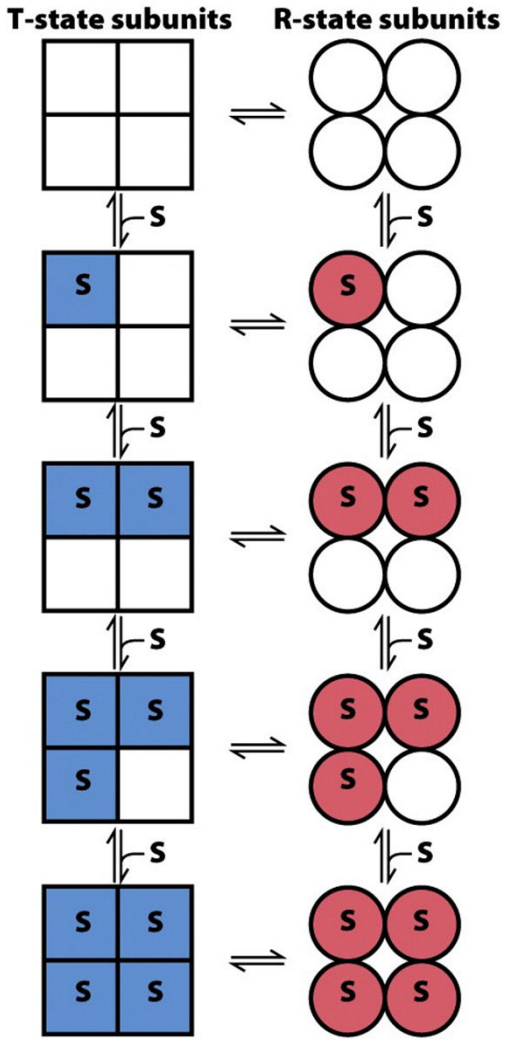


Homotropic interaction (cooperativity)

Modulator and other ligand are the same

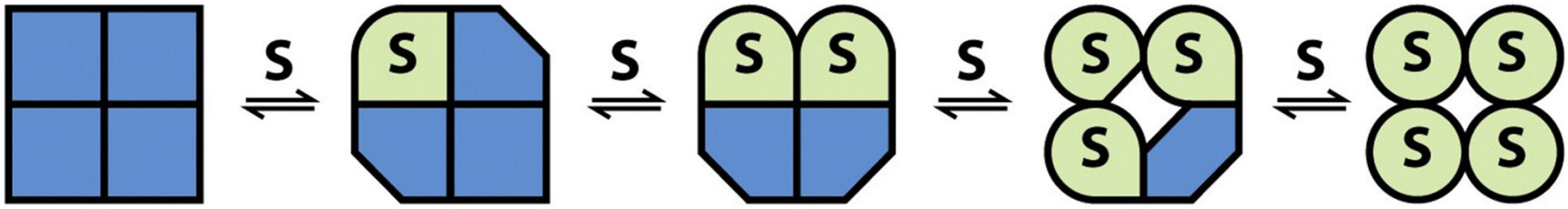


The symmetry (concerted) model of cooperativity requires symmetry of the allosteric protein



- Subunits can adopt one of two possible conformations: T or R
- All subunits *must* adopt the same conformation (protein is always symmetric)
- Ligand (S) can bind to:
  - T-state with low affinity
  - R-state with high affinity
- Equilibrium between T and R states is influenced by ligand binding
- Switching between T and R is *concerted*; all subunits transition simultaneously

The sequential model of cooperativity allows multiple conformations for each subunit



- Subunits can adopt multiple conformations
- Binding of ligand (S) induces conformational changes in the bound subunit and in neighboring subunits
- Different subunits may have different conformations, each with different ligand affinities
- Bound conformations may have higher or lower affinity for ligand than the free protein

Binding curves for allosteric proteins vary depending on the presence of modulators