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

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

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 ($K_a$)

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

Units?

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 ($\theta$) is proportional to the ligand concentration

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

Substitute in $[PL] = K_a [L][P]$,

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

When $[L] \gg [PL] + [P]$, $[L]$ is constant (usually true for small ligands in cells)
The fraction of occupied ligand-binding sites $\theta$ depends on $[L]$ and the binding affinity $K_d = [L]$:

$$\theta = \frac{[L]}{[L] + K_d} = \frac{[L]}{2[L]} = 0.5$$
A protein with higher affinity for a ligand has a higher binding curve and lower $K_d$. 
Protein-ligand dissociation constants ($K_d$’s) vary over several orders of magnitude.

### Table 5–1: Some Protein Dissociation Constants

<table>
<thead>
<tr>
<th>Protein</th>
<th>Ligand</th>
<th>$K_d$ (M)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Avidin (egg white)†</td>
<td>Biotin</td>
<td>$1 \times 10^{-15}$</td>
</tr>
<tr>
<td>Insulin receptor (human)</td>
<td>Insulin</td>
<td>$1 \times 10^{-10}$</td>
</tr>
<tr>
<td>Anti-HIV immunoglobulin (human)‡</td>
<td>gp41 (HIV-1 surface protein)</td>
<td>$4 \times 10^{-10}$</td>
</tr>
<tr>
<td>Nickel-binding protein (E. coli)</td>
<td>Ni$^{2+}$</td>
<td>$1 \times 10^{-7}$</td>
</tr>
<tr>
<td>Calmodulin (rat)§</td>
<td>Ca$^{2+}$</td>
<td>$3 \times 10^{-6}$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$2 \times 10^{-5}$</td>
</tr>
</tbody>
</table>

Table 5-1
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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 2$^{nd}$ site (where $L_2$ binds).

Modulator ($L_1$) is an ‘inhibitor’ if it decreases affinity at 2$^{nd}$ 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.