BINDING EQUILIBRIA

Formation of a protein-ligand complex (PL):

\[ P + L \rightleftharpoons PL \]  (1)

Reverse reaction, dissociation of PL:

\[ PL \rightarrow P + L \]  (2)

Equilibrium constant for reaction 2 is \( K_d \) (dissociation constant)

\[ K_d = \frac{[P][L]}{[PL]} \]  (3)

A large value for \( K_d \) means that the equilibrium for reaction 2 tends to the right, and that the ligand is loosely bound.

A small value for \( K_d \) means that the equilibrium for reaction 2 tends to the left, and that the ligand is tightly bound.

Degree of saturation and its dependence on ligand concentration:

At very high [L], the equilibrium of reaction (1) shifts to the right, and nearly all of the protein is in the form PL. The protein is said to be saturated with ligand, because all the available binding sites are occupied.

Let \([P_T] = \) the total protein concentration = [P] + [PL]

The degree of saturation, \( Y \) or \( \theta \), is defined as the fraction of protein which has ligand bound.

Degree of saturation \( (\theta) = \frac{[PL]}{[P_T]} = \frac{[PL]}{[P] + [PL]} \)  (4)

Substituting \([P] = \frac{K_d[PL]}{[L]} \), eqn. (4) becomes

\[ \frac{[PL]}{[P_T]} = \frac{[PL]}{[L]} + [PL] = \frac{1}{K_s + [L]} \]

Rewriting this, \( \theta = \frac{[PL]}{[P_T]} = \frac{[L]}{K_s + [L]} \)  (5)

Plotting \( \frac{[PL]}{[P_T]} \) vs. \([L]\) according to equation (4) gives a hyperbolic saturation curve.

**Note 1:** As \([L]\) approaches infinity (when \([L]\) is very large), \([L]\) is much greater than \(K_d\), so \(K_d + [S] \rightarrow [S]\).

\[ \therefore \text{As } [L] \rightarrow \infty, \quad \theta = \frac{[PL]}{[P_T]} = \frac{[L]}{K_s + [L]} \rightarrow \frac{[L]}{[L]} = 1 \]

Meaning: At very high [L], all the protein is in the form PL and none in the form P. The protein is saturated.

**Note 2:** \( K_d \) has units of mM (molar), i.e. \( K_d \) is a concentration. What is the significance of this concentration?

When \([L] = K_d\), \( \theta = \frac{[L]}{K_s + [L]} = \frac{[L]}{[L] + [L]} = \frac{1}{2} \)

\( \therefore \) \( K_d \) is the concentration of L at which \( \frac{[PL]}{[P_T]} = \frac{1}{2}, \) i.e. the conc. of L at which the protein is half-saturated, sometimes given the symbol \( L_{50} \). A low \( K_d \) means that it takes a low conc. of L to half-satiate the protein, and a high \( K_d \) means that half-saturation requires a higher conc. of L.

Hence, Equation (4) can also be written in the form \( \theta = \frac{[L]}{L_{50} + [L]} \)  (6).

**Note 3:** When the molecule which binds to the protein is a gas, then the equations contain the partial pressure of the gas rather than its molar concentration. Thus, for \( O_2 \) binding to myoglobin, equations (5) and (6) become \( \theta = \frac{pO_2}{K_d + pO_2} \) and \( \theta = \frac{pO_2}{P_{O_2} + pO_2} \).
ENZYME KINETICS -- THE LIMITING CASE OF RAPID BINDING EQUILIBRIUM

An enzymatic reaction in which a substrate $S$ is converted to a product $P$ can be represented by the following two-step reaction scheme:

$$E + S \stackrel{k_1}{\rightarrow} ES \stackrel{k_2}{\rightarrow} E + P \quad (P = \text{product}) \quad (1)$$

where $k_1$ and $k_2$ are the rate constants for the binding (or association) and release (or dissociation) of substrate from the active site of the enzyme, and $k_2$ (also called $k_{cat}$) is the rate constant for the conversion of bound substrate to product.

In some cases the binding and release of substrate are very rapid compared to the rate at which the actual chemical conversion occurs. Then the first step of the reaction is approximately at equilibrium, i.e. the rates of association and dissociation are equal, and there is no net change in the amount of substrate bound. Mathematically,

rate of association = rate of dissociation

$$k_1 [E][S] = k_{-1} [ES]$$

$$\frac{[E][S]}{[ES]} = \frac{k_{-1}}{k_1} = K_d,$$ the substrate dissociation constant

The velocity of the reaction is simply the rate of formation of product,

$$v = \frac{d[P]}{dt} = k_2 [ES]$$

But we know from equation 2, in the handout on binding equilibria, that

$$[ES] = [E][S] \frac{K_d + [S]}{[S]}$$

Therefore

$$v = k_2 [E][S] \frac{[S]}{K_d + [S]} \frac{K_d + [S]}{[S]} = V_{max} \frac{[S]}{K_d + [S]}$$

Plotting $v$ vs. $[S]$ gives a hyperbolic curve as shown. $v$ approaches a value of $V_{max}$ at high substrate concentrations, and $K_d$ is the value of $[S]$ at which $v = 1/2 \ V_{max}$.
1. A system containing an enzyme actively catalyzing a reaction is not at equilibrium, because a net chemical change is occurring, namely, the conversion of substrate to product. The steps in this conversion and the corresponding rate constants can be represented by the following scheme:

\[ E + S \overset{k_1}{\underset{k_{-1}}{\rightleftharpoons}} ES \overset{k_2}{\longrightarrow} E + P \quad (P = \text{product}) \quad (1) \]

This system does reach a steady state, in which the concentration of the intermediate ES remains constant. This happens because ES is being broken down at exactly the same rate as it is being formed. Analogly, a sink with a leaky stopper. If water flows into the sink at the same rate at which it drips out, the water level in the sink remains constant.

2. Why does a steady state occur?

Rate of formation of ES from E and S:

\[ \frac{d[ES]}{dt} = k_1[E][S] \quad (2) \]

(Note: If we are working under conditions where we can measure initial velocity, we can ignore the reverse reaction) E + P → ES, since [P] is initially zero.)

Rate of breakdown of ES to E + P or E + S: \[ \frac{d[ES]}{dt} = k_2[ES] + k_{-1}[ES] = [ES](k_{-1} + k_2) \quad (3) \]

Initially, [ES] = 0, so rate of breakdown of ES is zero. As time proceeds, [ES] increases, so the rate of ES breakdown gradually increases (eqn. 3). At the same time [E] decreases, so the rate of ES formation gradually decreases (eqn. 2). Eventually, the rate of breakdown of ES = rate of formation of ES. At that point, for every E that combines with S to give ES, a molecule of ES breaks down to form P (or S) and regenerate E. Therefore, [ES] remains constant. This is called steady state.

3. Definition of the Michaelis constant, \( K_m \):

At equilibrium, the equilibrium constant for the dissociation reaction ES ⇌ E + S is given by

\[ K_d = \frac{[E][S]}{[ES]} \quad (1) \]

or, since any equilibrium constant can be expressed as the ratio of two rate constants, \( K_d = \frac{k_{-1}}{k_1} \quad (4) \).

At steady state, the fraction \( \frac{[E][S]}{[ES]} \) is once again constant. This constant is assigned the symbol \( K_m \), the Michaelis constant, or the apparent dissociation constant.

i.e. \( K_m = \frac{[E][S]}{[ES]} \) for steady state \((5)\)


4. What is the value of $K_m$?

At steady state, the rate of formation equals the rate of breakdown:

$$k_1[E][S] = [ES](k_{-1} + k_2)$$

$$\therefore \frac{[E][S]}{[ES]} = \frac{k_1 + k_2}{k_1} \quad \text{or} \quad K_m = \frac{k_1 + k_2}{k_1} \quad (6)$$

If we compare equations 4 and 6, we see that $K_m$ is greater than $K_d$, because there is an extra term, $k_2$, in the numerator. This makes perfect sense. Remember, the dissociation constant is the concentration of $S$ needed to keep the enzyme half-saturated. If we are constantly removing ES by forming product, we will need a higher concentration of $S$ to keep the enzyme half-saturated than if no product were being formed. Hence the apparent steady state dissociation constant is greater than the equilibrium dissociation constant. The greater the value of $k_2$ (the faster product is formed), the greater $K_m$ will be.

In many cases, $k_2$ is much smaller than $k_{-1}$, i.e., the rates of formation and dissociation of the ES complex are rapid compared to the rate of catalysis. In this case, the enzyme and its substrate are always at equilibrium. Then the term $k_2$ is negligible, and $K_m$ is equal to $K_S$ (the apparent constant equals the true constant). Although this is not always true, $K_m$ is usually taken to be a measure of the affinity of the enzyme for the substrate, with a low $K_m$ indicating tight substrate binding and a high $K_m$ weak substrate binding.

4. Expressing [ES] as a function of [S]:

At equilibrium, $\frac{[E][S]}{[ES]} = K_d$.

Algebraic derivation on equilibrium binding handout gave $[ES] = \frac{[E][S]}{K_d + [S]}$.

At steady state, $\frac{[E][S]}{[ES]} = K_m$. A similar derivation gives $[ES] = \frac{[S]}{K_m + [S]}$ \quad (7).

5. Initial velocity ($V$) as a function of [S]:

$$E + S \xrightarrow{k_1} ES \xrightarrow{k_{-1}} E + P$$

Initial velocity $V = \frac{d[P]}{dt} = k_2 [ES]$ \quad (8)

Substituting Equation (7),

$$V = k_2 \frac{[S]}{K_m + [S]}$$

or, defining $V_{max} = k_2 [E]$,

$$V = V_{max} \frac{[S]}{K_m + [S]}$$ \quad (9)

Eqn. 9 is the Michaelis-Menten equation. A plot of $V$ vs. [S] according to equation 9 gives a hyperbolic saturation curve.
6. Meaning of $V_{\text{max}}$:

$$V = k_2[\text{ES}] \quad \text{and} \quad V_{\text{max}} = k_2 [\text{E}]$$

$V_{\text{max}}$ is the initial velocity when $[\text{ES}] = [\text{E}]$

$V_{\text{max}}$ is the value that $V$ approaches when $[S]$ is so large that all $E$ has been converted to ES, i.e., when the enzyme is completely saturated with substrate.

7. Meaning of $K_m$:

Note: $K_m$ has units M (molar)

When $[S] = K_m$,

$$V = V_{\text{max}} \frac{[S]}{[S] + [S]} = \frac{1}{2} V_{\text{max}}$$

$K_m$ is the substrate concentration needed to attain an initial velocity of $\frac{1}{2} V_{\text{max}}$.

A high $K_m$ means you need a high substrate concentration to make the reaction proceed.

Remember --

$K_m$ has units of concentration,
and $V_{\text{max}}$ has units of velocity, so

**Km DOES NOT EQUAL 1/2 Vmax!!!**
**Enzyme Kinetics**

\[ E + S \xrightleftharpoons[K_1]{} ES \xrightarrow[K_2]{k} E + P \]

**Rapid Bimolecular Equilibrium**

- The enzyme-substrate complex is in rapid equilibrium with the free enzyme and substrate.
- At equilibrium, \[ K_1 \text{ and } K_{-1} >> K_2 \]

**Steady State**

- \( k_2 \) is not negligible (vs. \( k_{-1} \)).
- At steady state, the rate of forming \( ES \) = rate of breaking down \( ES \).

\[ \frac{[ES]}{[E+S]} = \frac{k_1}{k_{-1}} \]

\[ \frac{[ES]}{[E+S]} = \frac{k_2}{k_{-1}} \]

\[ \Theta = \frac{[ES]}{[E+S]} = \frac{k_1}{k_{-1}} \]

\[ V = \frac{d[E]}{dt} = k_2 [ES] \]

\[ = k_2 \frac{k_1 [E]}{K_m + [S]} \]

\[ = \frac{V_{max} K_m}{K_m + [S]} \]  

\( V_{max} \cdot \Theta \)

**Summary**

- \( K_m \) is the Michaelis constant.
- \( V_{max} \) is the maximum reaction rate.
- \( [S] \) is the substrate concentration.