Bioinformatics: Network Analysis

Enzyme Kinetics

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Luay Nakhleh, Rice University

- A catalyst accelerates a chemical reaction without itself being consumed and without changing the equilibrium constant (K_{eq}) of the reaction.

- Catalysts only speed up the approach to equilibrium.

- The most important catalysts in living systems are enzymes.

- Enzymes are protein molecules which fold into a specific three dimensional structure to allow interaction with a substrate at a location on the enzyme called the active site.

Enzyme Kinetics

- Enzyme kinetics is a branch of science that deals with the many factors that can affect the rate of an enzyme-catalyzed reaction.

- The most important factors include the concentration of enzyme, reactants, products, and the concentration of any modifiers such as specific activators, inhibitors, ...

Kinetic Modeling of Enzymatic Reactions

- In 1902, Brown proposed an enzymatic mechanism for invertase, catalyzing the cleavage of saccharose to glucose and fructose.

- This mechanism holds in general for all one-substrate reactions without backward reaction and effectors, such as

$$\mathrm{E} + \mathrm{S} \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} \mathrm{ES} \xrightarrow{k_2} \mathrm{E} + \mathrm{P}$$

reversible formation of enzyme-substrate complex ES from free enzyme E and substrate S

irreversible release of the product P

Kinetic Modeling of Enzymatic Reactions

- The previous model is often used when carrying out *in vitro* kinetic assays because under these conditions it is assumed that the product has a negligible concentration and therefore the reverse rate is zero.

- Unlike in vitro conditions, most reactions in show some degree of reversibility *in vivo*, which leas to the more general model:

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} E + P$$

Kinetic Modeling of Enzymatic Reactions

$$\mathrm{E} + \mathrm{S} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{ES} \xrightarrow{k_2} \mathrm{E} + \mathrm{P}$$

- The ODE system for the dynamics of this reaction reads

$$\frac{\mathrm{d}S}{\mathrm{d}t} = -k_1 E \cdot S + k_{-1} ES,$$

$$\frac{\mathrm{d}ES}{\mathrm{d}t} = k_1 E \cdot S - (k_{-1} + k_2) ES,$$

$$\frac{\mathrm{d}E}{\mathrm{d}t} = -k_1 E \cdot S + (k_{-1} + k_2) ES,$$

$$\frac{\mathrm{d}P}{\mathrm{d}t} = k_2 ES.$$

Kinetic Modeling of Enzymatic Reactions

- The reaction rate is equal to the negative decay rate of the substrate as well as to the rate of product formation:

$$v = -\frac{\mathrm{d}S}{\mathrm{d}t} = \frac{\mathrm{d}P}{\mathrm{d}t}.$$

Michaelis-Menten Kinetics

- This ODE system cannot be solved analytically.

- Different assumptions have been made to simplify this system.

- Michaelis and Menten considered a quasi-equilibrium between the free enzyme E and the enzyme substrate complex ES:

$$k_1, k_{-1} \gg k_2.$$

Michaelis-Menten Kinetics

- Briggs and Haldane assumed that during the course of reaction, a state is reached where the concentration of the ES complex remains constant, the so-called quasi-steady state. That is,

$$\frac{\mathrm{d}ES}{\mathrm{d}t} = 0.$$

- This assumption is justified only if the initial substrate concentration is much larger than the enzyme concentration, S(t=0)>>E, otherwise such a state will never be reached.

Michaelis-Menten Kinetics

$$\frac{dS}{dt} = -k_1 E \cdot S + k_{-1} ES,$$

$$\frac{dES}{dt} = k_1 E \cdot S - (k_{-1} + k_2) ES,$$

$$\frac{dE}{dt} = -k_1 E \cdot S + (k_{-1} + k_2) ES,$$

$$\frac{dP}{dt} = k_2 ES.$$

$$\frac{dES}{dt} + \frac{dE}{dt} = 0 \quad \text{or} \quad E_{\text{total}} = E + ES = \text{constant}.$$

The enzyme is neither produced nor consumed in this reaction.

Michaelis-Menten Kinetics

$$\frac{\mathrm{d}ES}{\mathrm{d}t} = k_1 E \cdot S - (k_{-1} + k_2) ES$$

$$+ \quad \frac{\mathrm{d}ES}{\mathrm{d}t} + \frac{\mathrm{d}E}{\mathrm{d}t} = 0 \quad \text{or} \quad E_{\text{total}} = E + ES = \text{constant.}$$

$$+ \quad \frac{\mathrm{d}ES}{\mathrm{d}t} = 0.$$

$$ES = \frac{k_1 E_{\text{total}} S}{k_1 S + k_{-1} + k_2} = \frac{E_{\text{total}} S}{S + (k_{-1} + k_2)/k_1}$$

Michaelis-Menten Kinetics

$$v = -\frac{dS}{dt} = \frac{dP}{dt}.$$

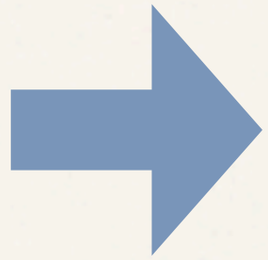
$$+ \quad \frac{dP}{dt} = k_2 ES$$

$$+ \quad ES = \frac{k_1 E_{total} S}{k_1 S + k_{-1} + k_2} = \frac{E_{total} S}{S + (k_{-1} + k_2)/k_1}$$

$$v = \frac{k_2 E_{total} S}{S + ((k_{-1} + k_2)/k_1)}$$

Michaelis-Menten Kinetics

$$v = \frac{k_2 E_{\text{total}} S}{S + ((k_{-1} + k_2)/k_1)}$$



$$v = \frac{V_{\max} S}{S + K_m}$$

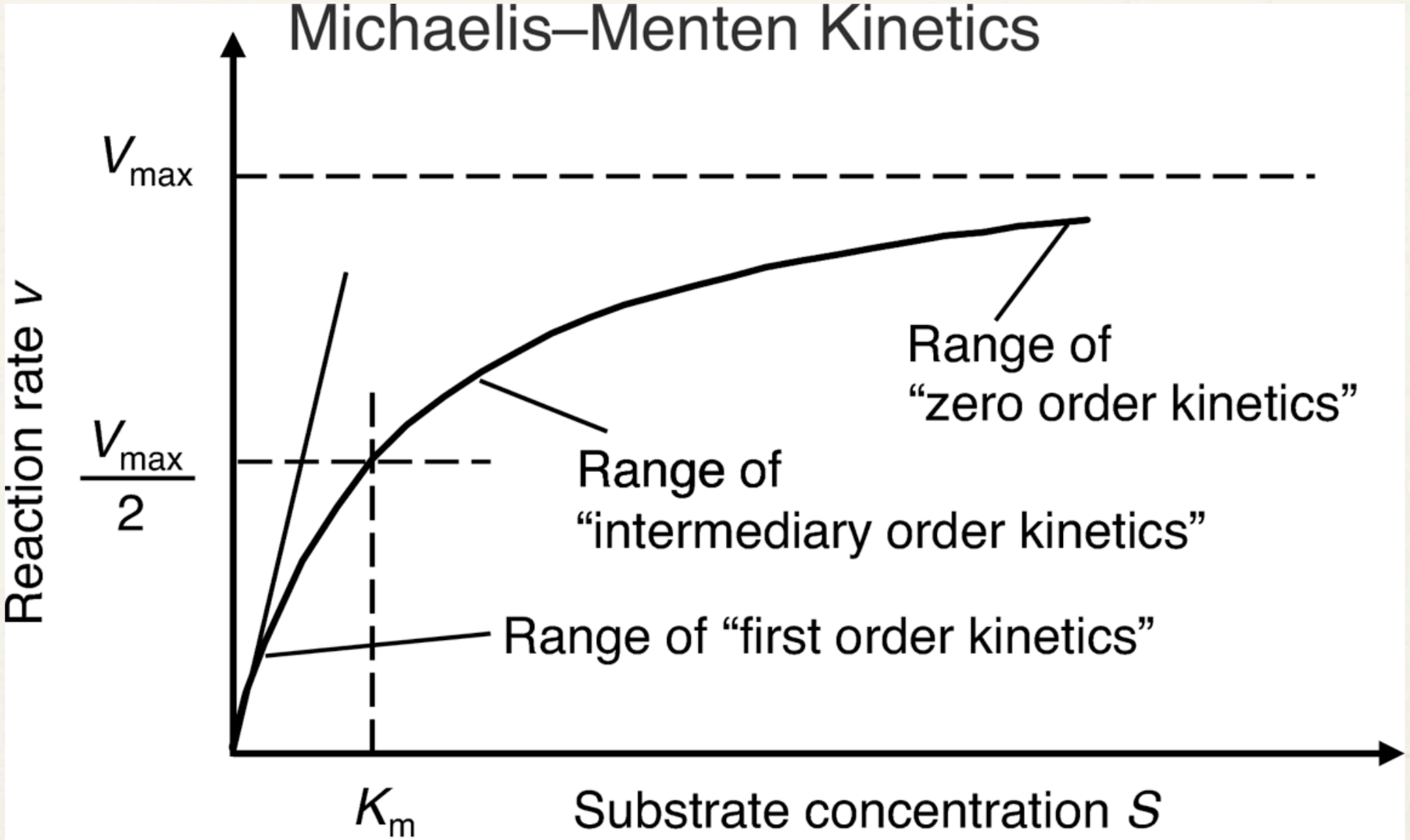
$$V_{\max} = k_2 E_{\text{total}}$$

The maximal velocity
(the maximal rate that can be attained
when the enzyme is completely saturated
with substrate)

$$K_m = \frac{k_{-1} + k_2}{k_1}$$

The Michaelis constant
(the substrate concentration that yields
the half-maximal reaction rate)

Michaelis-Menten Kinetics



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Michaelis-Menten Kinetics: How to derive a rate equation

1. Draw a wiring diagram of all steps to consider. It contains all substrates and products (S and P), and *n* free or bound enzyme species (E and ES).

2. The right sides of the ODEs for the concentrations changes sum up the rates of all steps leading to or away from a certain substance. The rates follow mass action kinetics.

3. The sum of all enzyme-containing species is equal to the total enzyme concentration E_{total}. This constitutes one equation.

4. The assumption of quasi-steady state for n-1 enzyme species together with (3) result in n algebraic equations for the concentrations of the *n* enzyme species.

5. The reaction rate is equal to the rate of product formation. Insert the respective concentrations of enzyme species resulting from (4).

Parameter Estimation and Linearization of the Michaelis-Menten Equation

- To assess the values of the parameters V_{max} and K_m for an isolated enzyme, one measures the initial rate for different concentrations of the substrate.

- Since the rate is a nonlinear function of the substrate concentration, one has to determine the parameters by nonlinear regression.

- Another way is to transform the equation

$$v = \frac{V_{max} S}{S + K_m}$$

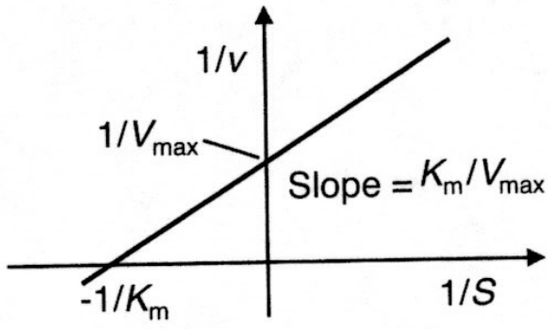
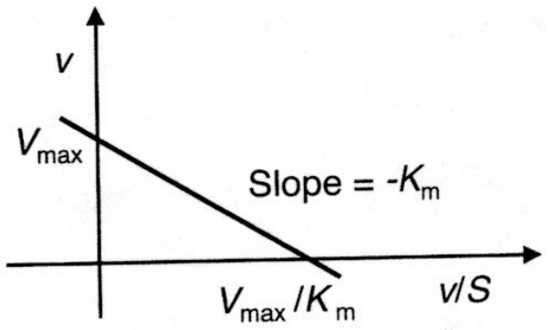
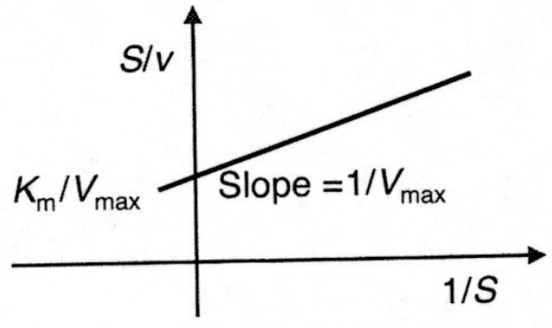
to a linear relation between variables and then apply linear regression.

Parameter Estimation and Linearization of the Michaelis-Menten Equation

- The advantage of the transformed equations is that one may read the parameter value more or less directly from the graph obtained by linear regression of the measurement data.

Parameter Estimation and Linearization of the Michaelis-Menten Equation

Table 2.2 Different approaches for the linearization of Michaelis–Menten enzyme kinetics.

	Lineweaver–Burk	**Eadie–Hofstee**	**Hanes–Woolf**
Transformed equation	$\frac{1}{v} = \frac{K_m}{V_{max}}\frac{1}{S} + \frac{1}{V_{max}}$	$v = V_{max} - K_m\frac{v}{S}$	$\frac{S}{v} = \frac{S}{V_{max}} + \frac{K_m}{V_{max}}$
New variables	$\frac{1}{v}, \frac{1}{S}$	$v, \frac{v}{S}$	$\frac{S}{v}, S$
Graphical representation			

Michaelis-Menten for Reversible Reactions

- Consider the following reaction:

$$\mathrm{E} + \mathrm{S} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{ES} \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} \mathrm{E} + \mathrm{P}$$

- The product formation is given by

$$\frac{\mathrm{d}P}{\mathrm{d}t} = k_2 ES - k_{-2} E \cdot P = v.$$

- The respective rate equation reads

$$\begin{aligned} v &= E_{\text{total}} \frac{Sq - P}{Sk_1/(k_{-1}k_{-2}) + 1/k_{-2} + k_2/(k_{-1}k_{-2}) + P/k_{-1}} \\ &= \frac{(V_{\text{max}}^{\text{for}}/K_{\text{m}S})S - (V_{\text{max}}^{\text{back}}/K_{\text{m}P})P}{1 + S/K_{\text{m}S} + P/K_{\text{m}P}}. \end{aligned}$$

Michaelis-Menten for Reversible Reactions

$$v = E_{\text{total}} \frac{Sq - P}{Sk_1/(k_{-1}k_{-2}) + 1/k_{-2} + k_2/(k_{-1}k_{-2}) + P/k_{-1}}$$
$$= \frac{(V^{\text{for}}_{\text{max}}/K_{\text{m}S})S - (V^{\text{back}}_{\text{max}}/K_{\text{m}P})P}{1 + S/K_{\text{m}S} + P/K_{\text{m}P}}.$$

- The parameters $V^{\text{for}}_{\text{max}}$ and $V^{\text{back}}_{\text{max}}$ denote the maximal velocity in forward and backward direction, respectively, under zero product or substrate concentration, and the parameters $K_{\text{m}S}$ and $K_{\text{m}P}$ denote the substrate or product concentration causing half maximal forward or backward rate.

- They are related by:

$$K_{\text{eq}} = \frac{V^{\text{for}}_{\text{max}} K_{\text{m}P}}{V^{\text{back}}_{\text{max}} K_{\text{m}S}}.$$

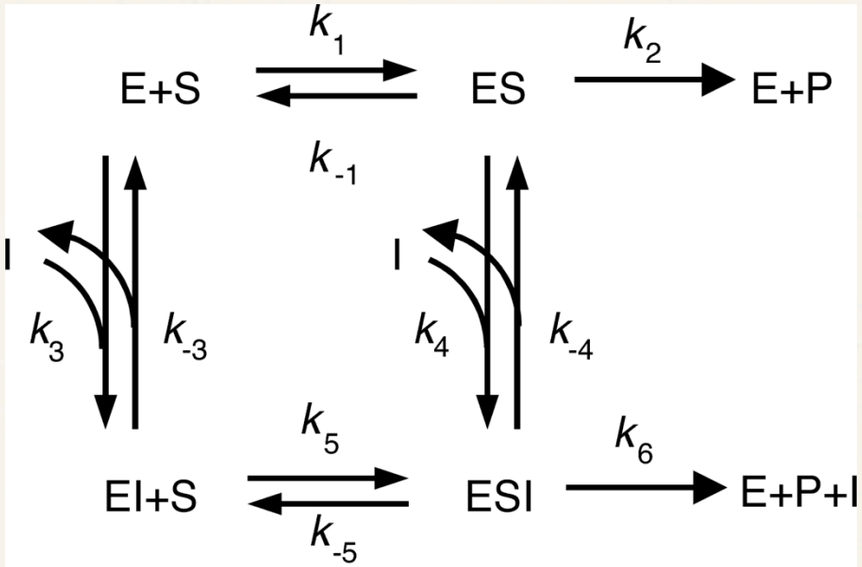
Regulation of Enzyme Activity by Effectors

- Enzymes may be targets of effectors, both inhibitors and activators.

- Effectors are small molecules, or proteins, or other compounds that influence the performance of the enzymatic reaction.

- Basic types of inhibition are distinguished by the state in which the enzyme may bind the effector (i.e., the free enzyme E, the enzyme-substrate complex ES, or both) and by the ability of different complexes to release the product.

Regulation of Enzyme Activity by Effectors



standard Michalis-Menten kinetics	reactions 1 and 2
competitive inhibition	reactions 1, 2, and 3 (and not 4, 5, and 6)
uncompetitive inhibition	reactions 1, 2, and 4
noncompetitive inhibition	reactions 1, 2, 3, 4, and 5
partial inhibition	occurrence of reaction 6

Regulation of Enzyme Activity by Effectors

- The rate equations are derived according to the following scheme:

1. Consider binding equilibriums between compounds and their complexes:

$$K_m \cong \frac{k_{-1}}{k_1} = \frac{E \cdot S}{ES},\ K_{I,3} = \frac{k_{-3}}{k_3} = \frac{E \cdot I}{EI},\ K_{I,4} = \frac{k_{-4}}{k_4} = \frac{ES \cdot I}{ESI},\ K_{I,5} = \frac{k_{-5}}{k_5} = \frac{EI \cdot S}{ESI}. \tag{2.29}$$

Note that, if all reactions may occur, the Wegscheider condition [12] holds in the form

$$\frac{k_1 k_4}{k_{-1} k_{-4}} = \frac{k_3 k_5}{k_{-3} k_{-5}}, \tag{2.30}$$

which means that the difference in the free energies between two compounds (e.g., E and ESI) is independent of the choice of the reaction path (here via ES or via EI).

2. Take into account the moiety conservation for the total enzyme (include only those complexes, which occur in the course of reaction):

$$E_{total} = E + ES + EI + ESI. \tag{2.31}$$

3. The reaction rate is equal to the rate of product formation

$$v = \frac{dP}{dt} = k_2 ES + k_6 ESI. \tag{2.32}$$

Equations (2.29)–(2.31) constitute four independent equations for the four unknown concentrations of E, ES, EI, and ESI. Their solution can be inserted into Eq. (2.32).

Table 2.3 Types of inhibition for irreversible and reversible Michaelis–Menten kinetics[a].

Name	Implementation	Equation – irreversible case	Equation – reversible case	Characteristics
Competitive inhibition	I binds only to free E; P-release only from ES complex $k_{\pm4} = k_{\pm5} = k_6 = 0$	$v = \frac{V_{max} S}{K_m \cdot i_3 + S}$	$v = \frac{V^f_{max}(S/K_{mS}) - V^r_{max}(P/K_{mP})}{(S/K_{mS}) + (P/K_{mP}) + i_3}$	K_m changes, V_{max} remains same. S and I compete for the binding place; high S may out compete I.
Uncompetitive inhibition	I binds only to the ES complex; P-release only from ES complex $k_{\pm3} = k_{\pm5} = k_6 = 0$	$v = \frac{V_{max} S}{K_m + S \cdot i_4}$	$v = \frac{V^f_{max}(S/K_{mS}) - V^r_{max}(P/K_{mP})}{1 + ((S/K_{mS}) + (P/K_{mP}))i_4}$	K_m and V_{max} change, but their ratio remains same. S may not out compete I
Noncompetitive inhibition	I binds to E and ES; P-release only from ES $K_{I,3} = K_{I,4}$, $k_6 = 0$	$v = \frac{V_{max} S}{(K_m + S)i_3}$	$v = \frac{V^f_{max}(S/K_{mS}) - V^r_{max}(P/K_{mP})}{(1 + (S/K_{mS}) + (P/K_{mP}))i_4}$	K_m remains, V_{max} changes. S may not out compete I
Mixed inhibition	I binds to E and ES; P-release only from ES $K_{I,3} \neq K_{I,4}$, $k_6 = 0$	$v = \frac{V_{max} S}{K_m \cdot i_4 + S \cdot i_3}$		K_m and V_{max} change. $K_{I,3} > K_{I,4}$: competitive–noncompetitive inhibition $K_{I,3} < K_{I,4}$: noncompetitive–uncompetitive inhibition
Partial Inhibition	I may bind to E and ES; P-release from ES and ESI $K_{I,3} \neq K_{I,4}$, $K_6 \neq 0$	$v = \frac{V_{max} S[1 + \{(k_6 I)/(k_2 K_{I,3})\}]}{K_m i_4 + S i_3}$		K_m and V_{max} change. if $k_6 > k_2$: activation instead of inhibition.

[a]These abbreviations are used: $K_{I,3} = \frac{k_{-3}}{k_3}$, $K_{I,4} = \frac{k_{-4}}{k_4}$, $i_3 = 1 + \frac{I}{K_{I,3}}$, $i_4 = 1 + \frac{I}{K_{I,4}}$.

Substrate Inhibition

- In some cases, a further substrate binds to the enzyme-substrate complex, yielding the complex ESS that cannot form a product.

- This form of inhibition is reversible if the second substrate can be released.

- The rate equation can be derived using the scheme of uncompetitive inhibition by replacing the inhibitor by another substrate. It reads

$$v = k_2 ES = \frac{V_{max} S}{K_m + S(1 + (S/K_I))}.$$

Binding of Ligands to Proteins

- Consider binding of one ligand (S) to a protein (E) with only one binding site:

$$E + S \rightleftharpoons ES$$

- The binding constant K_B is given by

$$K_B = \left(\frac{ES}{E \cdot S} \right)_{eq}$$

$dES/dt = k_1 * E * S - k_{-1} * ES = 0$
$\Rightarrow k_1 / k_{-1} = ES / (E * S)$

- The reciprocal of K_B is the dissociation constant K_D.
- The fractional saturation Y of the protein is determined by the number of subunits that have bound ligands, divided by the total number of subunits.

$$Y = \frac{ES}{E_{total}} = \frac{ES}{ES + E} = \frac{K_B \cdot S}{K_B \cdot S + 1}$$

Binding of Ligands to Proteins

- At a process where the binding of S to E is the first step followed by product release and where the initial concentration of S is much higher that the initial concentration of E, the rate is proportional to the concentration of ES and it holds

$$\frac{v}{V_{max}} = \frac{ES}{E_{total}} = Y$$

Positive Homotropic Cooperativity and the Hill Equation

- If the protein has several binding sites, then interactions may occur between these sites, i.e., the affinity to further ligands may change after binding of one or more ligands.

- This phenomenon is called *cooperativity*.

- *Positive* or *negative cooperativity* denotes increase or decrease in the affinity of the protein to a further ligand, respectively.

- *Homotropic* or *heterotropic cooperativity* denotes that the binding to a certain ligand influences the affinity of the protein to a further ligand of the same or another type, respectively.

Positive Homotropic Cooperativity and the Hill Equation

- Consider a dimeric protein ($\mathbf{E_2}$) with two identical binding sites.

- The binding to the first ligand (**S**) facilitates the binding to the second ligand:

$$E_2 + S \xrightarrow{\text{slow}} E_2S$$

$$E_2S + S \xrightarrow{\text{fast}} E_2S_2$$

- The fractional saturation is given by

$$Y = \frac{E_2S + 2 \cdot E_2S_2}{2 \cdot E_{2,\text{total}}} = \frac{E_2S + 2 \cdot E_2S_2}{2 \cdot E_2 + 2 \cdot E_2S + 2 \cdot E_2S_2}.$$

- If the affinity to the second ligand is strongly increased by binding to the first ligand, then E_2S will react with S as soon as it is formed and the concentration of E_2S can be neglected.

Positive Homotropic Cooperativity and the Hill Equation

- In the case of complete cooperativity, i.e., every protein is either empty or fully bound, the previous equation reduces to

$$E_2 + 2S \rightarrow E_2S_2$$

$dE_2S_2/dt = k_1 * E_2 * S^2 - k_{-1} * E_2S_2 = 0$
$\Rightarrow k_1/k_{-1} = E_2S_2/(E_2 * S^2)$
$\Rightarrow k_1/k_{-1} = E_2S_2/((E_{total} - E_2S_2) * S^2)$
$\Rightarrow K_B = E_2S_2/((E_{total} - E_2S_2) * S^2)$
$\Rightarrow E_2S_2 * (1 + K_B * S^2) = K_B * S^2 * E_{total}$
$\Rightarrow E_2S_2/E_{total} = (K_B * S^2)/(1 + K_B * S^2) = S^2/(K_D + S^2)$

- The binding constant reads

$$K_B = \frac{E_2S_2}{E_2 \cdot S^2},$$

and the fractional saturation is

$$Y = \frac{2 \cdot E_2S_2}{2 \cdot E_{2,\text{total}}} = \frac{E_2S_2}{E_2 + E_2S_2} = \frac{K_B \cdot S^2}{1 + K_B \cdot S^2}.$$

Positive Homotropic Cooperativity and the Hill Equation

- Generally, for a protein with n subunits, it holds:

$$v = V_{max} \cdot Y = \frac{V_{max} \cdot K_B \cdot S^n}{1 + K_B \cdot S^n}.$$

- This is the general form of the *Hill equation*.

- The quantity *n* is termed the *Hill coefficient*.

Acknowledgments

- *"Systems Biology: A Textbook,"* by E. Klipp et al., 2009.