Bio-Systems Modeling and Control

Lecture 21

Enzymes

Cooperation in Enzymes

Enzymes with Multiple Binding Sites

- Many enzymes have more than one binding site for substrate molecules.
- Example: Hemoglobin (Hb), the oxygen-carrying protein in red blood cells, has 4 binding sites for O_2 molecules.
- In such cases (as will be shown) reaction velocity may no longer be of the simple Michaelis-Menten hyperbolic curve type.
- It may have a sigmoid shape.

Types of Enzyme-Substrates Interactive Behavior

- Basic cooperation: Binding of substrate molecules to some of the active sites affect the binding of subsequent substrates molecules.
- Allosteric effect: Binding one substrate (or other molecule) at one site can affect the binding of different type of substrate, at a different site of the same enzyme.
- If a molecule enhances the binding of the other substrates, it is called activator (or cofactor).
- If it decreases the subsequent activity, it is called an inhibitor.

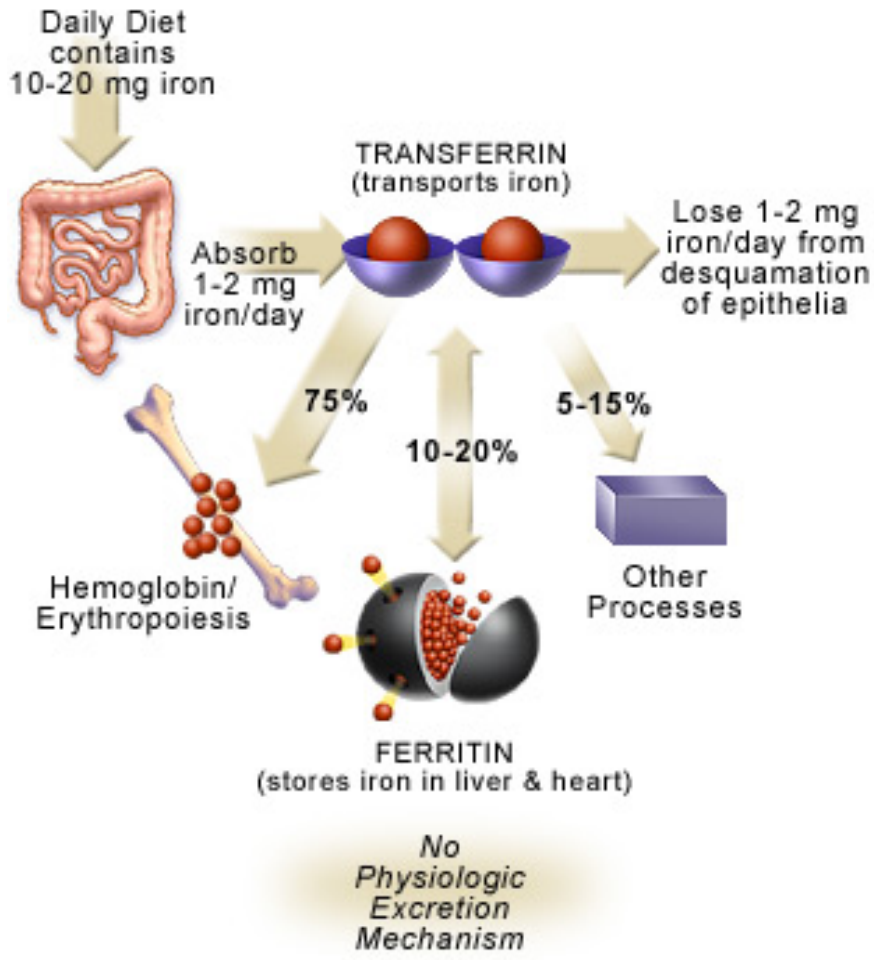
Why and How is Enzyme Activity Controlled?

- Without any regulation of reaction rates, cellular metabolism would be uncoordinated and undirected, proceeding at rates varying from explosive to almost-nonexistent.
- Control can be effected to both – enzyme synthesis, as well as enzyme activity. In this lecture we focus on the latter.

A few words about the Control of Enzyme Synthesis

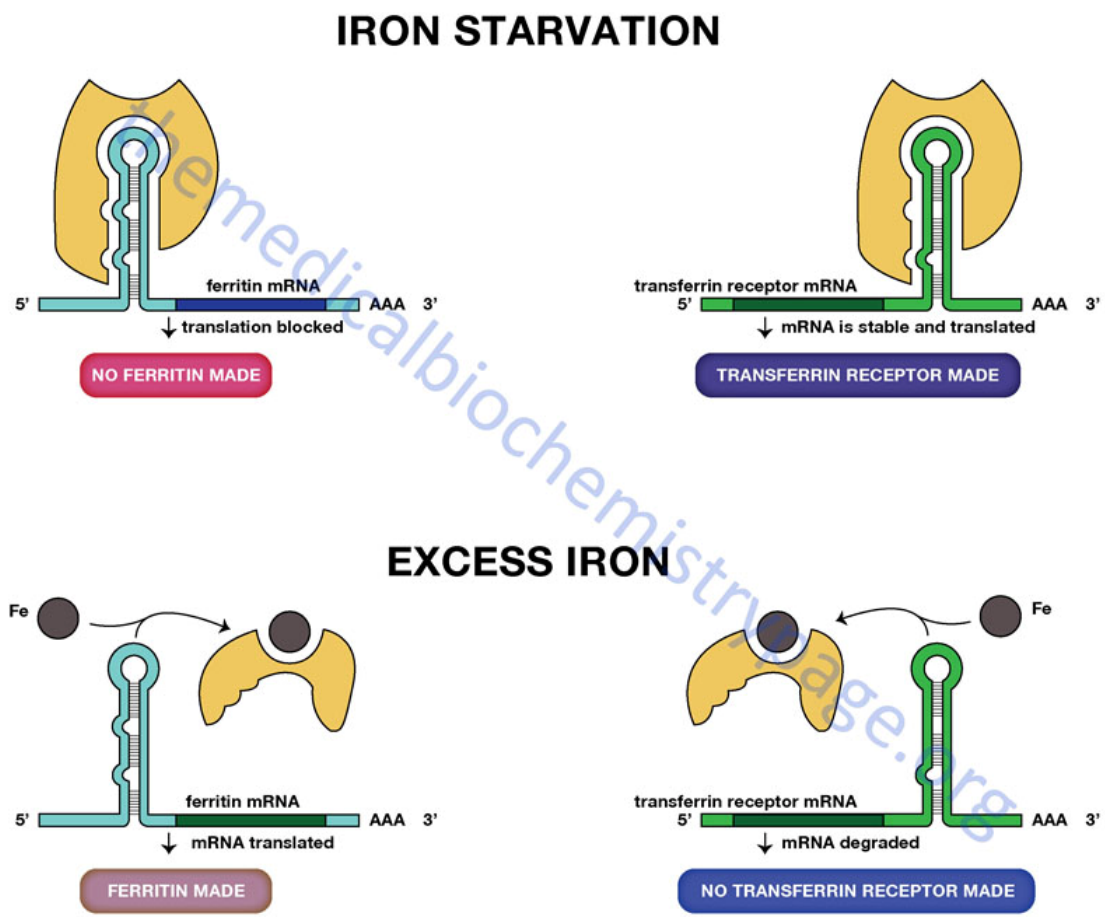
- The quantity of an enzyme in a cell depends on the balance between enzyme rate of destruction (due to temperature effect denaturing, or due to breaking down by other enzymes called proteolytic enzymes), and rate of synthesis.
- Normally, rate of enzyme synthesis is regulated by the rate of transcription of the gene encoding the creation of such an enzyme protein.
- Often, enzyme is created only after an initial quantity of the substrate is sensed.

Example: Regulation of Iron in the Blood



- It is important to regulate the amount of iron (Fe) in the blood, which is transported in the blood by the protein "transferrin".
- Excess iron is stored in certain cells. It is bound to another protein "ferritin".

Example: Regulation of Iron in the Blood



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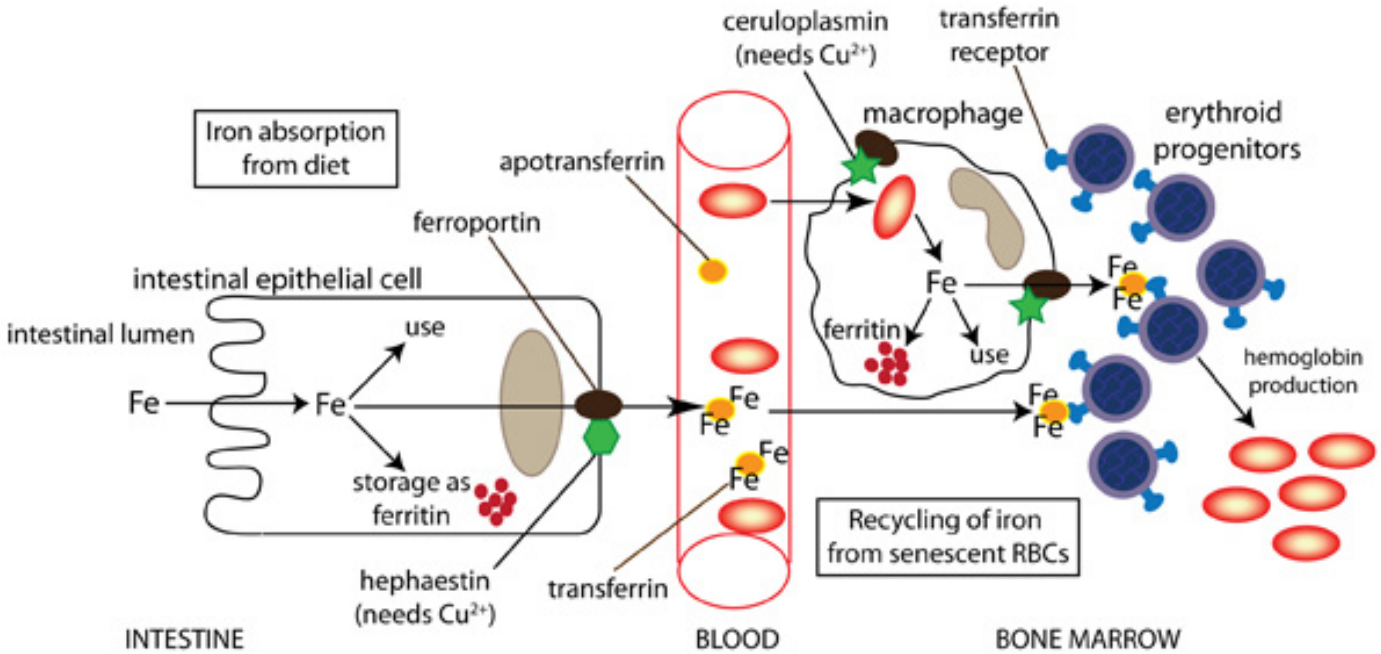
- The more transferrin the more iron is there in the blood.
- The more ferritin (in certain cells) the less iron is there in the blood.

Example: (Control of Enzyme Synthesis) Regulation of Iron in the Blood

- The regulation of "transferrin vs. ferritin" occurs via an "Iron Regulatory Protein (IRP)". How?



Blood Iron Regulation – IRP and IRE

- IRP can either bind to iron (Fe) or to an "Iron Response Element (IRE)", which is part of the RNA strands, for production of either ferritin or transferrin receptor.
- Case 1: Iron deficiency (low iron level in the blood) → IRP has an increased affinity for binding to IRE's ←→ {Whenever IRP binds to IRE on the transferrin receptor RNA, this stabilizes that RNA strand and causes increased synthesis of the transferrin receptor}

Blood Iron Regulation – Case 1: Iron Deficiency

Low iron level in the blood → IRP has an increased affinity for binding to IRE's ←→{Whenever IRP binds to IRE on the transferrin receptor RNA, this stabilizes that RNA strand and causes increased synthesis of the transferrin receptor} and {Whenever IRP binds to the IRE on the ferritin RNA, this blocks the translation of that RNA strand into protein → decreased synthesis of ferritin} → Overall: Low iron → increased production of transferrin receptor, decreased production of ferritin.

Blood Iron Regulation – Case 2: Iron Excess

High iron level in the blood → IRP has a decreased affinity for binding to IRE's ←→{Whenever less IRP binds to IRE on the transferrin receptor RNA, there is a decrease in the synthesis of the transferrin receptor} and {Whenever less IRP binds to the IRE on the ferritin RNA, there is an overall increased synthesis of ferritin} → Overall: Low iron → decreased production of transferrin receptor, increased ferritin production.

Going back to Control of Enzyme Activity

Enzyme with Two Active Sites

$$S + E \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} C_1 \xrightarrow{k_2} E + P$$

$$S + C_1 \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} C_2 \xrightarrow{k_4} C_1 + P$$

- Enzyme can exist in three states: Free molecule E, a complex with one occupied site C_1 and a complex with two occupied sites C_2.

Mass-Action Reaction Equations

$$S + E \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} C_1 \xrightarrow{k_2} E + P$$

$$S + C_1 \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} C_2 \xrightarrow{k_4} C_1 + P$$

$$\frac{dc_1}{dt} = k_1 es - (k_{-1} + k_2 + k_3 s)c_1 + (k_{-3} + k_4)c_2$$

$$\frac{dc_2}{dt} = k_3 s c_1 - (k_{-3} + k_4)c_2$$

$$\frac{ds}{dt} = -k_1 es + (k_{-1} - k_3 s)c_1 + k_{-3}c_2$$

$$\frac{de}{dt} = -k_1 es + (k_{-1} + k_2)c_1$$

$$\frac{dp}{dt} = k_2 c_1 + k_4 c_2$$

Initial conditions:
$s(0)=s_0$,
$e(0)=e_0$,
$c_1(0)=c_2(0)=$
$=p(0)=0$

Similarly to previous derivations we obtain $e+c_1+c_2=e_0$ and:

$$S + E \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} C_1 \xrightarrow{k_2} E + P$$

$$S + C_1 \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} C_2 \xrightarrow{k_4} C_1 + P$$

$$\frac{dc_1}{dt} = k_1 e_0 s - (k_{-1} + k_2 + k_1 s + k_3 s)c_1 + (k_{-3} + k_4 - k_1 s)c_2$$

$$\frac{dc_2}{dt} = k_3 s c_1 - (k_{-3} + k_4)c_2$$

$$\frac{ds}{dt} = -k_1 e_0 s + (k_{-1} + k_1 s - k_3 s)c_1 + (k_1 s + k_{-3})c_2$$

ICs: $s(0)=s_0$, $c_1(0)=c_2(0)=0$

Applying quasi-steady-state assumption $dc_1/dt \approx dc_2/dt \approx 0$, yields:

$$c_1 \approx \frac{K_2 e_0 s}{K_1 K_2 + K_2 s + s^2}$$

$$c_2 \approx \frac{e_0 s^2}{K_1 K_2 + K_2 s + s^2}$$

$$K_1 = \frac{k_{-1} + k_2}{k_1} \qquad K_2 = \frac{k_{-3} + k_4}{k_3}$$

We can now find the speed of reaction V, from the $dp/dt = k_2 c_1 + k_4 c_2$ equation.

Quasi-Steady-State for c_1, c_2: Reaction Velocity

$$c_1 \approx \frac{K_2 e_0 s}{K_1 K_2 + K_2 s + s^2}$$

$$c_2 \approx \frac{e_0 s^2}{K_1 K_2 + K_2 s + s^2}$$

$$K_1 = \frac{k_{-1} + k_2}{k_1} \qquad K_2 = \frac{k_{-3} + k_4}{k_3}$$

$$V = \frac{dp}{dt} = k_2 c_1 + k_4 c_2 = \frac{(k_2 K_2 + k_4 s) e_0 s}{K_1 K_2 + K_2 s + s^2}$$

Reaction Velocity – Extreme Case 1

$$V = \frac{(k_2 K_2 + k_4 s) e_0 s}{K_1 K_2 + K_2 s + s^2}$$

- If active sites act independently and identically:
- Then $k_1 = 2k_3 = 2k_+$, $2k_{-1} = k_{-3} = 2k_-$ and $2k_2 = k_4$
- The above factor of 2 occurs because two identical binding sites are involved in the reaction, doubling the amount of the reactant.

Reaction Velocity – No Cooperativity

$$V = \frac{(k_2K_2 + k_4s)e_0s}{K_1K_2 + K_2s + s^2} = \frac{2k_2e_0(K+s)s}{K^2 + 2Ks + s^2} = 2\frac{k_2e_0s}{K+s}$$

- If active sites act independently and identically:
- Then $k_1=2k_3=2k_+$, $2k_{-1}=k_{-3}=2k_-$ and $2k_2=k_4$
- Let $K=k_-/k_+$ is the equilibrium constant for the individual binding site.
- Reaction rate simply doubles.

Reaction Velocity – Extreme Case 2 (Positive Cooperativity)

$$V = \frac{(k_2K_2 + k_4 s)e_0 s}{K_1K_2 + K_2 s + s^2} \rightarrow \frac{k_4 e_0 s^2}{K_m{}^2 + s^2} = \frac{V_{\max} s^2}{K_m{}^2 + s^2}$$

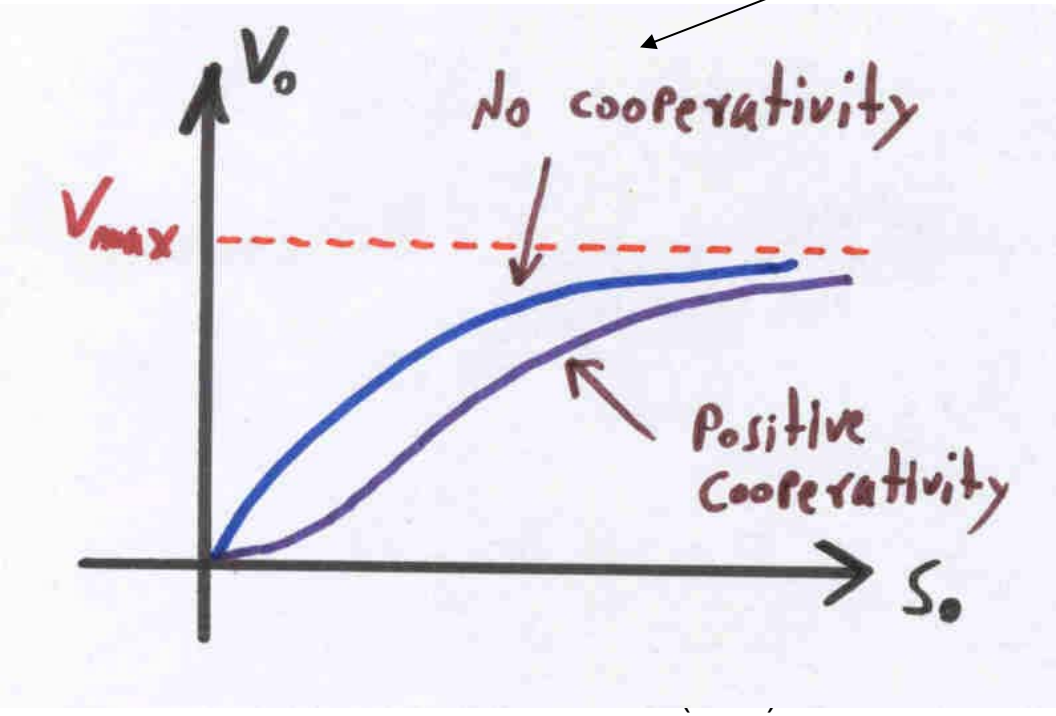
- If binding of the first substrate is slow, but that with one site bound, binding of the second is fast – this is called large positive cooperativity:
- Then $k_1 \rightarrow 0$ (that is "very small") , $k_3 \rightarrow \infty$ (that is "very large"), while the product k_1k_3 stays constant. (other constants – fixed)
- Then $K_2 \rightarrow 0$ and $K_1 \rightarrow \infty$ while $K_1K_2 = K_m{}^2$ is constant.

Reaction Velocity Extreme Cases

Positive Cooperativity

$$V = \frac{V_{\max} s^2}{K_m^{\ 2} + s^2}$$

$$V = 2\frac{k_2 e_0 s}{K + s}$$

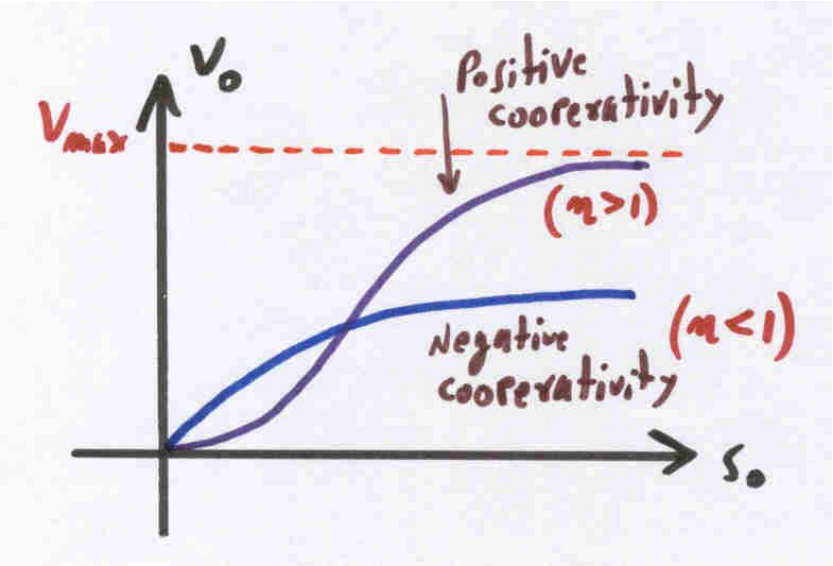


In between the two exterme cases:

Hill Function

Curve Fitted Hill Equation

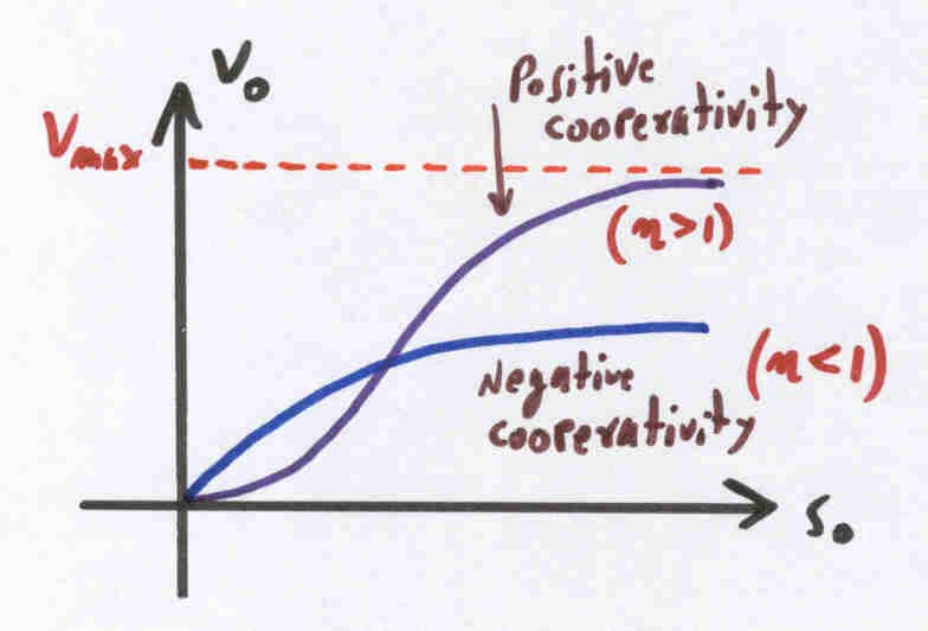
$$V = \frac{V_{\max} s^n}{K_m^{\ n} + s^n}$$



- $K_m^{n'} = K_1K_2\ldots K_{n'}$ (n' equilibrium constants for an n'-site enzyme)
- Positive cooperativity is if n>1, No cooperativity if n=0, Negative cooperativity is if n<1.

Negative Cooperativity

- Binding of the first substrate molecule decreases the rate of subsequent binding.
- In such a case we usually have a small value of k_3.
- Main effect: A decrease in the maximum speed.



Hill Plot (from Hill's equation)

$$n\ln(s) = n\ln(K_m) + \ln\left(\frac{V}{V_{\max} - V}\right)$$

- Vary s_0 and measure V_0.
- Plot $\ln(V_0/(V_{max}-V_0))$ vs. $\ln(s_0)$ is expected to be a straight line of slope n.
- The unknowns n, V_{max} and K_m are found by curve fitting.

Hill Equation Applications

- Even though, theoretically, n should be an integer, in practice, after doing the curve fitting, the resulting n is often non-integer.
- Hill equation is often used in reactions whose detailed intermediate steps are not known, but for which cooperative behavior is suspected.

Bottom Line Approximated Model for Cooperative Enzyme Reactions

$$\frac{dp}{dt} \approx \frac{V_{\max} s^n}{K_m^{\ n} + s^n}$$

- The above approximation is for cases where there are multiple active sites for identical substrates.
- Scientists often use such a model to represent a chain of enzyme reactions.