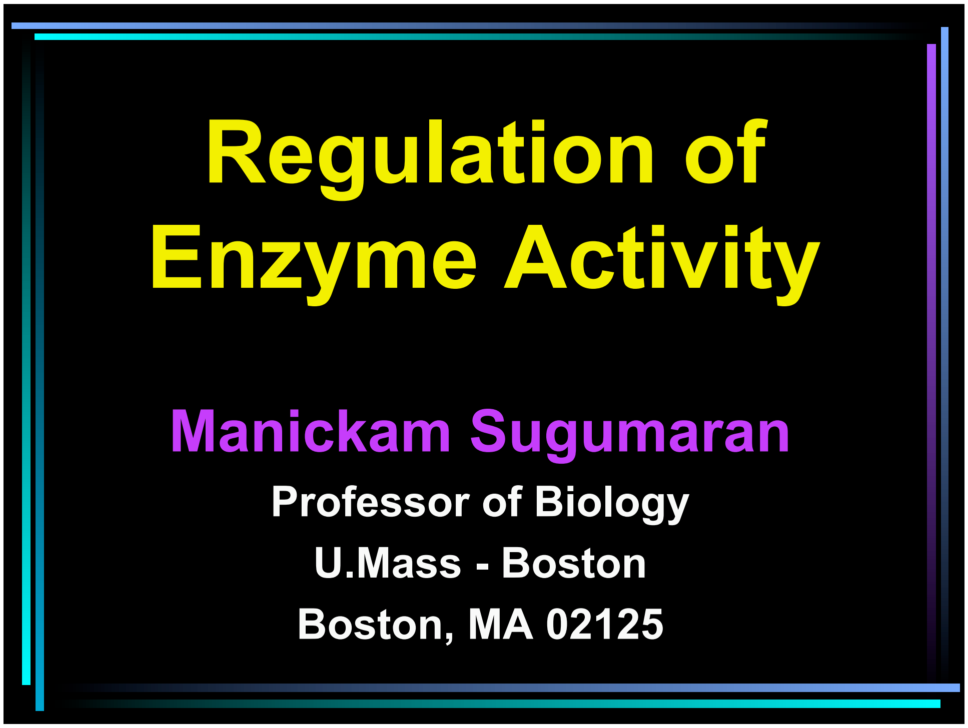

Regulation of Enzyme Activity

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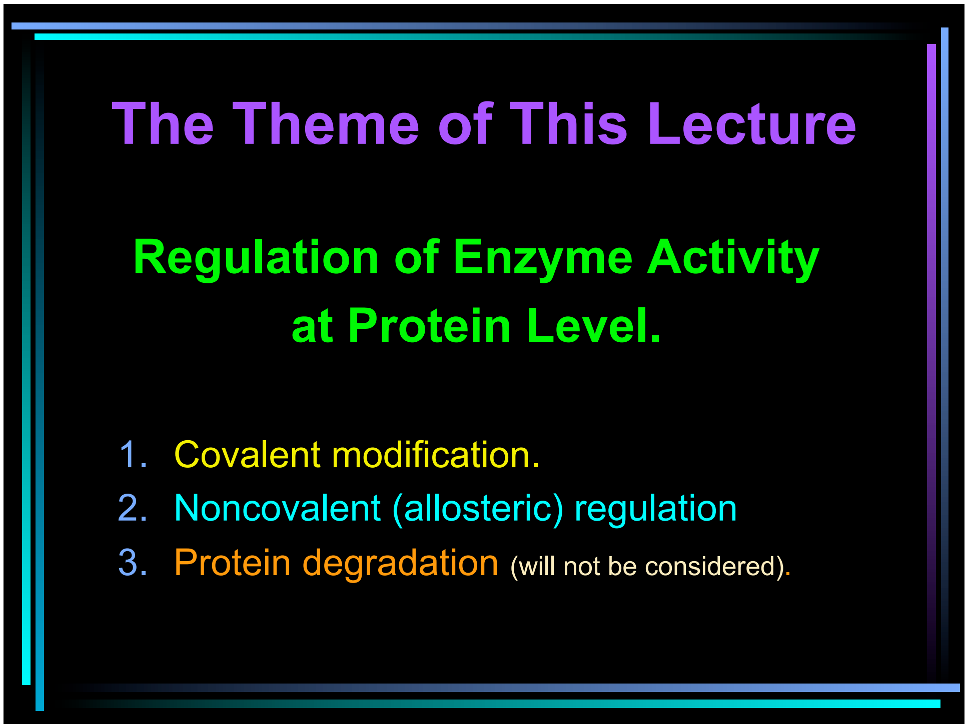
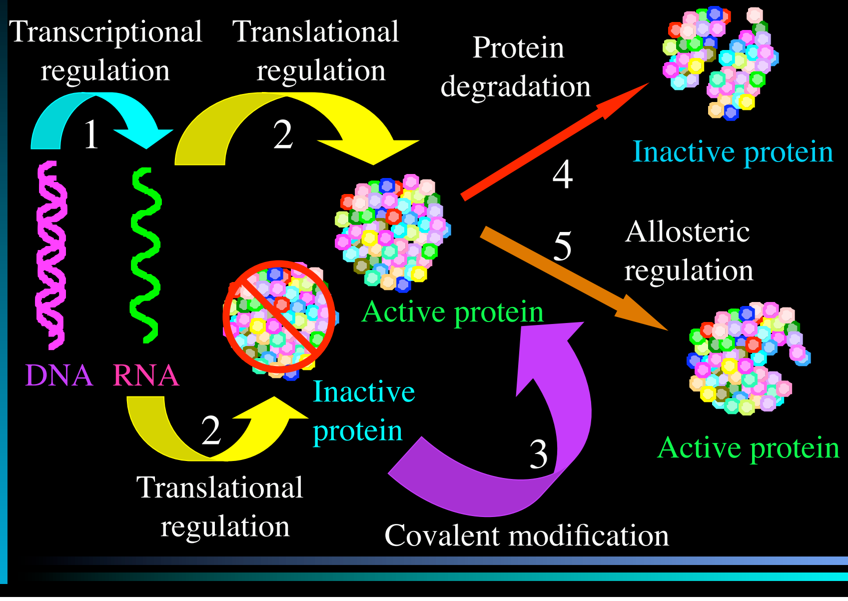


The Theme of This Lecture

Regulation of Enzyme Activity at Protein Level.

1. Covalent modification.
2. Noncovalent (allosteric) regulation
3. Protein degradation (will not be considered).

Regulation at Different Levels



Transcriptional regulation

1

Translational regulation

2

Protein degradation

4

Inactive protein

DNA

RNA

Active protein

5

Allosteric regulation

Inactive protein

2

Translational regulation

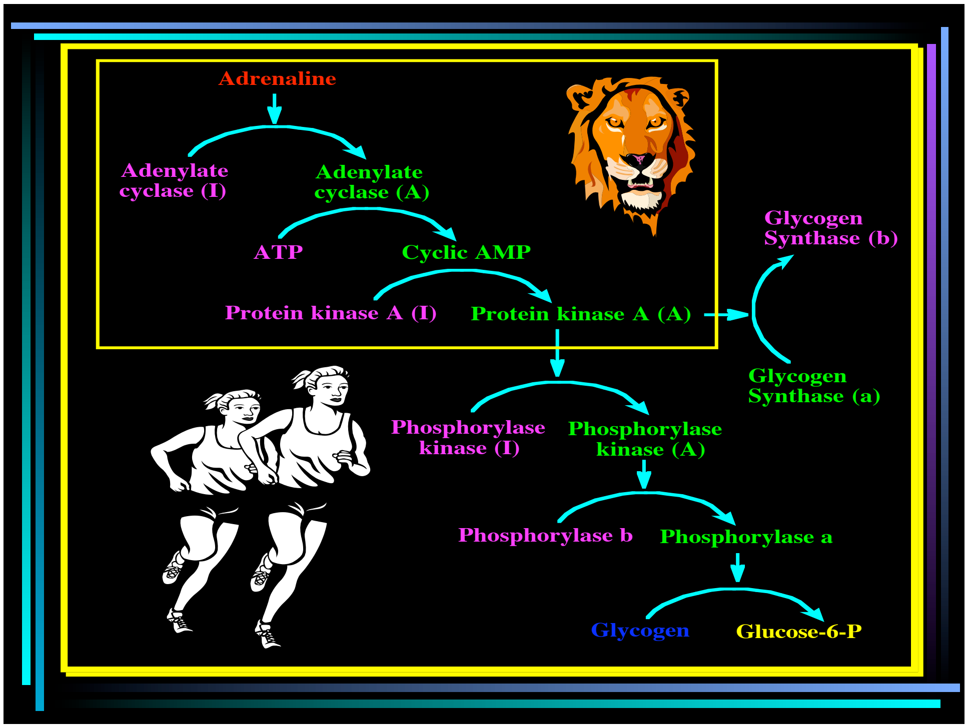
3

Active protein

Covalent modification

Why regulate?

- Emergency situation - Response should be rapid.
- Resource availability - should adjust to availability of resources from both internal and external sources.



Adrenaline

Adenylate cyclase (I) → Adenylate cyclase (A)

ATP → Cyclic AMP

Protein kinase A (I) → Protein kinase A (A)

Glycogen Synthase (a) → Glycogen Synthase (b)

Phosphorylase kinase (I) → Phosphorylase kinase (A)

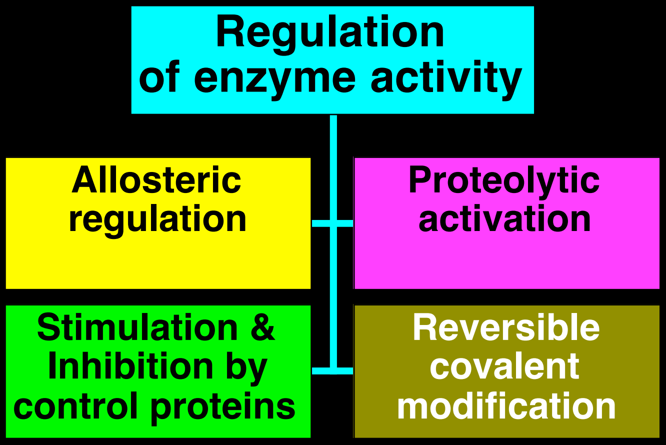
Phosphorylase b → Phosphorylase a

Glycogen → Glucose-6-P

Amplification of enzyme activity

- Approximately 0.1 nmoles of adrenaline per gram of muscle will trigger the synthesis of 25 μmoles of glucose -1-phosphate per minute per gram of muscle.
- **This represents an amplification of 250,000 fold.**

Four kinds of regulation



Regulation of enzyme activity

- Allosteric regulation
- Proteolytic activation
- Stimulation & Inhibition by control proteins
- Reversible covalent modification

Proteolytic activation

This kind of activation is irreversible.

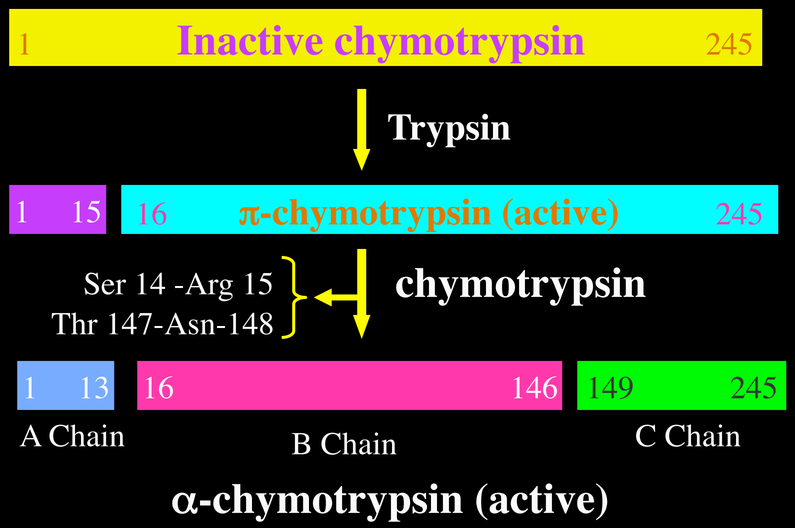
Once the protein is activated,
the process cannot be reversed.

Active protein can only be controlled
by other kinds of regulation.

Examples of Proteolytic Activation

- Zymogen activation.
- Blood clotting.
- Complement activation.
- Prophenoloxidase activation.
- Inactive hormones to active hormones.

Activation mechanism of Chymotrypsin



1 **Inactive chymotrypsin** 245

Trypsin

1 15 | 16 **π-chymotrypsin (active)** 245

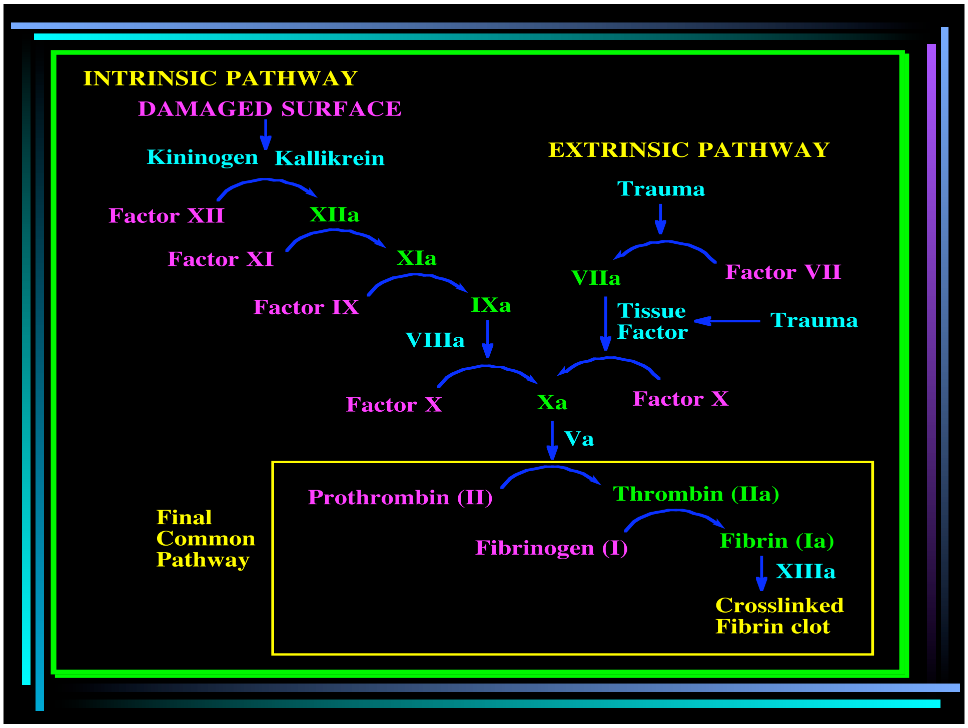
Ser 14 -Arg 15
Thr 147-Asn-148

chymotrypsin

1 13 | 16 146 | 149 245

A Chain | B Chain | C Chain

α-chymotrypsin (active)



INTRINSIC PATHWAY

DAMAGED SURFACE

Kininogen Kallikrein

Factor XII XIIa

Factor XI XIa

Factor IX IXa

VIIIa

Factor X Xa

Va

EXTRINSIC PATHWAY

Trauma

VIIa Factor VII

Tissue Factor

Trauma

Factor X

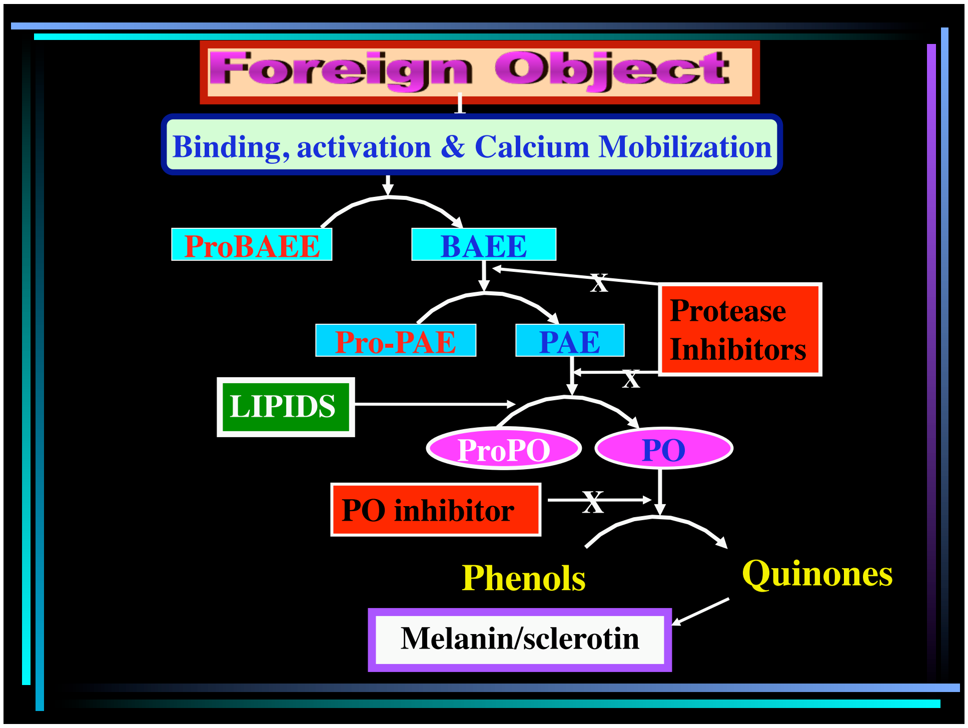
Final Common Pathway

Prothrombin (II) Thrombin (IIa)

Fibrinogen (I) Fibrin (Ia)

XIIIa

Crosslinked Fibrin clot



Foreign Object

Binding, activation & Calcium Mobilization

ProBAEE BAEE

Protease Inhibitors

X

Pro-PAE PAE

X

LIPIDS

ProPO PO

PO inhibitor

X

Phenols Quinones

Melanin/sclerotin

Reversible Covalent Modification

A single trigger rapidly switches
a whole pathway on or off

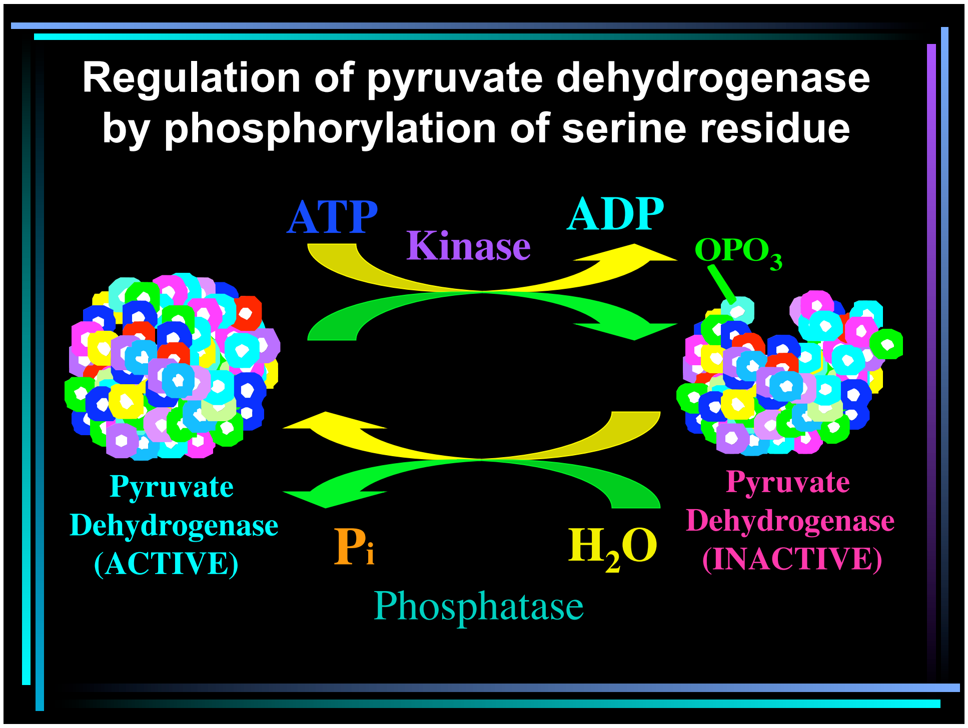
Examples of reversible covalent modifications

- Phosphorylation - dephosphorylation
- Adenylation - deadenylation.
- Uridinylation -deuridinylation.
- Thiol disulfide exchange.
- Methylation - demethylation.
- Acetylation -deacetylation.

Reversible covalent modification - Phosphorylation

While running, glycogen phosphorylase activity is enhanced by phosphorylation.

At the same time glycogen synthase activity is shut off by phosphorylation.



Regulation of pyruvate dehydrogenase by phosphorylation of serine residue

ATP

Kinase

ADP

OPO_3

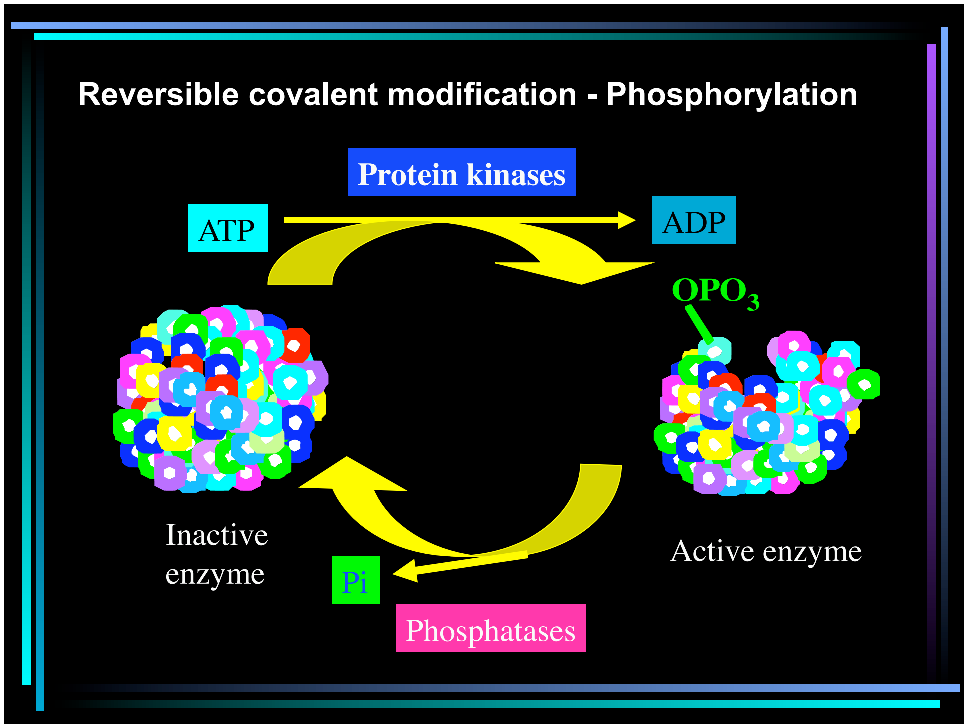
Pyruvate Dehydrogenase (ACTIVE)

P_i

H_2O

Pyruvate Dehydrogenase (INACTIVE)

Phosphatase



Reversible covalent modification - Phosphorylation

Protein kinases

ATP

ADP

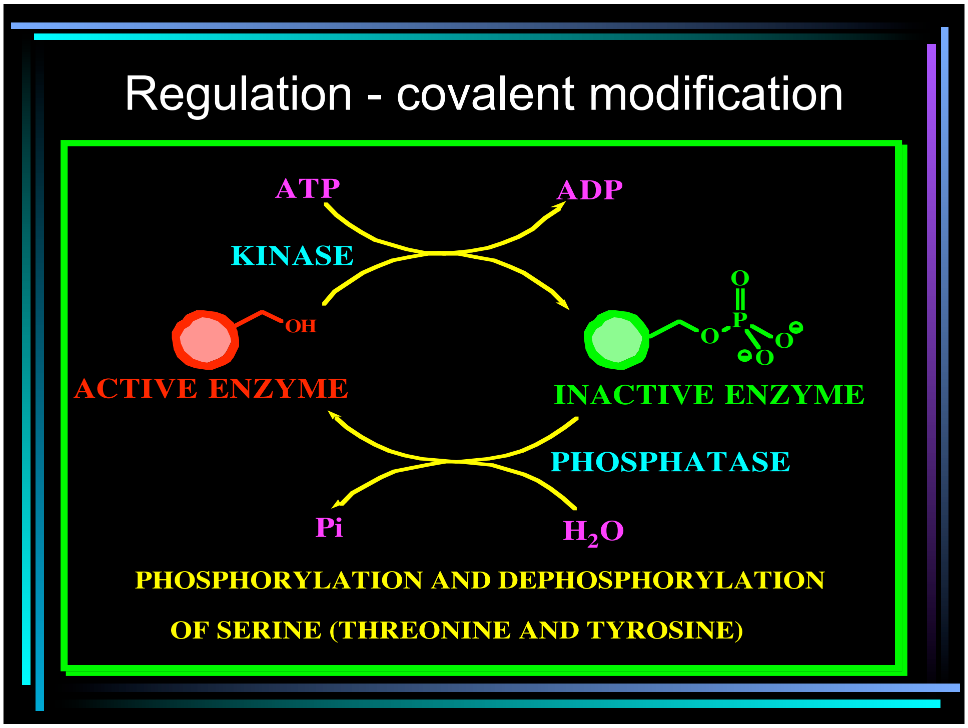
OPO_3

Inactive enzyme

Active enzyme

Pi

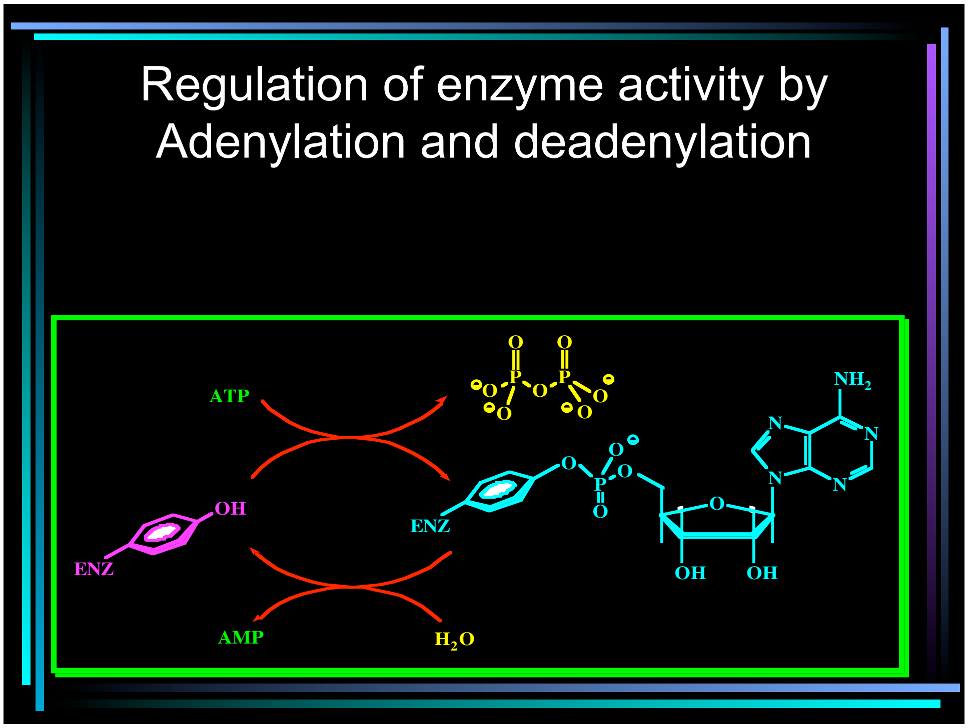
Phosphatases



Regulation - covalent modification

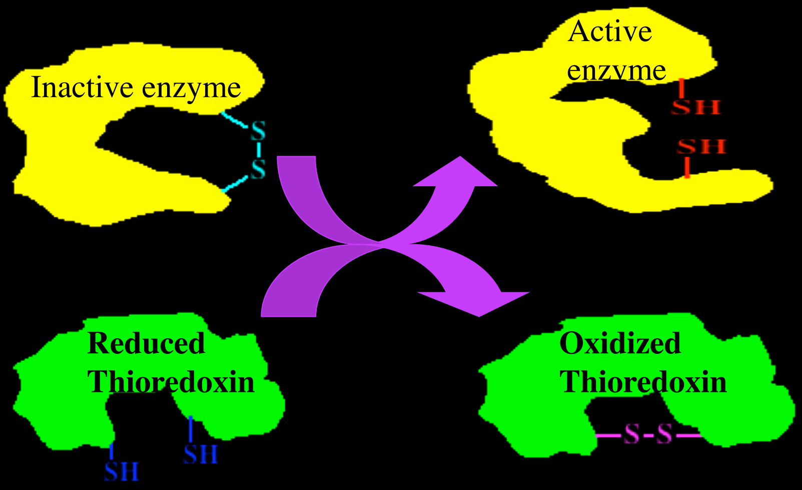
PHOSPHORYLATION AND DEPHOSPHORYLATION

OF SERINE (THREONINE AND TYROSINE)



Regulation of enzyme activity by Adenylation and deadenylation

Thiol-disulfide exchange as a regulatory mechanism



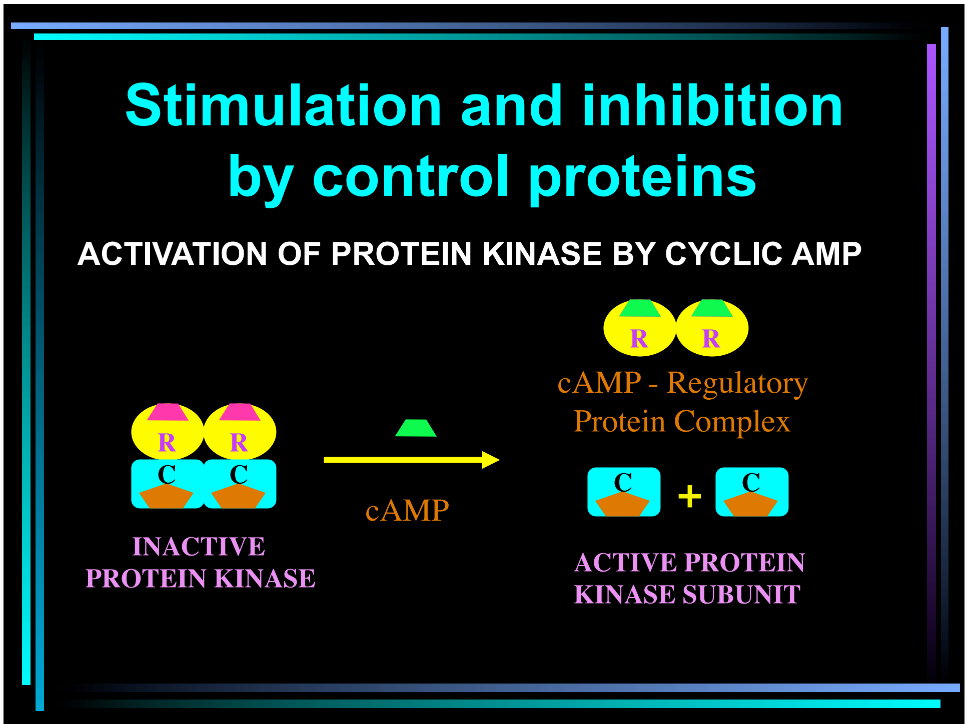
Enzymes regulated by reduction (in plants)

Activation by disulfide reduction

- Fructose-1,2-bisphosphatase
- NADP-Malate dehydrogenase
- Thylakoid ATP-synthase

Inhibition by disulfide reduction

- Phosphofructokinase



Stimulation and inhibition by control proteins

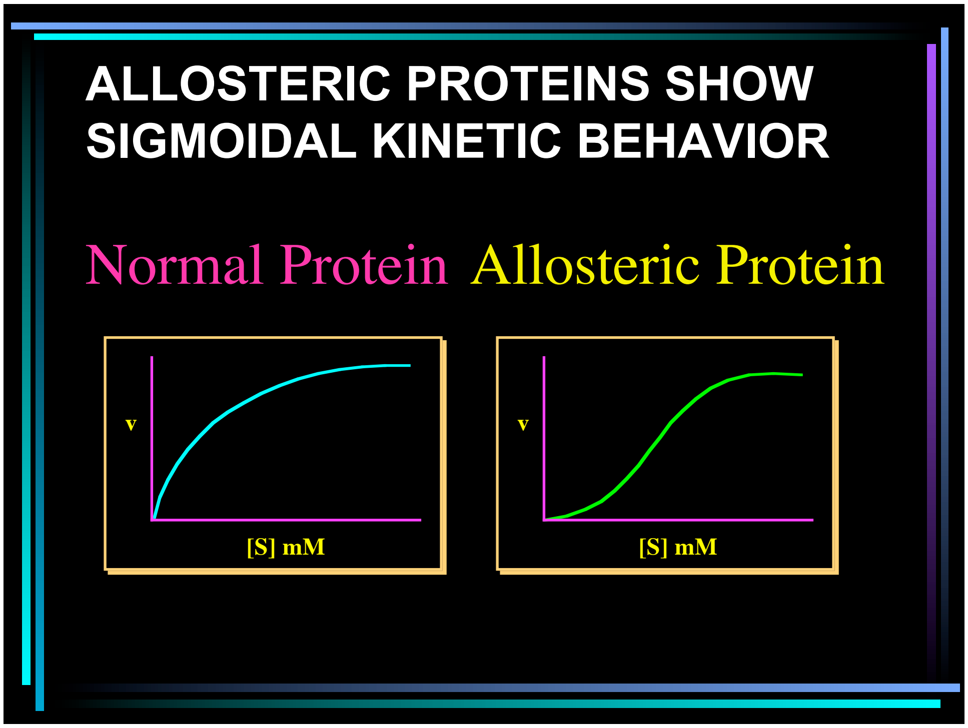
ACTIVATION OF PROTEIN KINASE BY CYCLIC AMP

INACTIVE PROTEIN KINASE

cAMP

cAMP - Regulatory Protein Complex

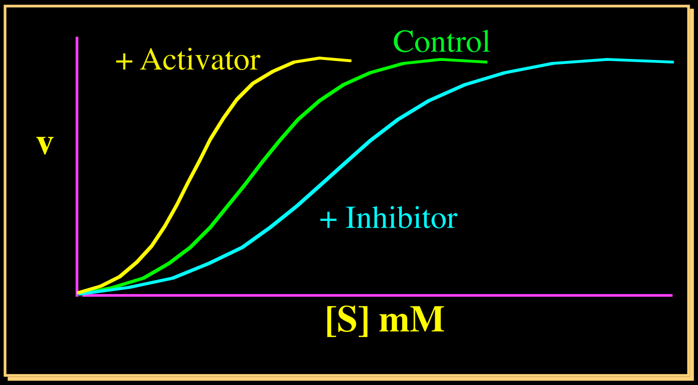
ACTIVE PROTEIN KINASE SUBUNIT



ALLOSTERIC PROTEINS SHOW SIGMOIDAL KINETIC BEHAVIOR

Normal Protein Allosteric Protein

Effect of activator and Inhibitor



Activator decreases sigmoidity; Inhibitor increases sigmoidity

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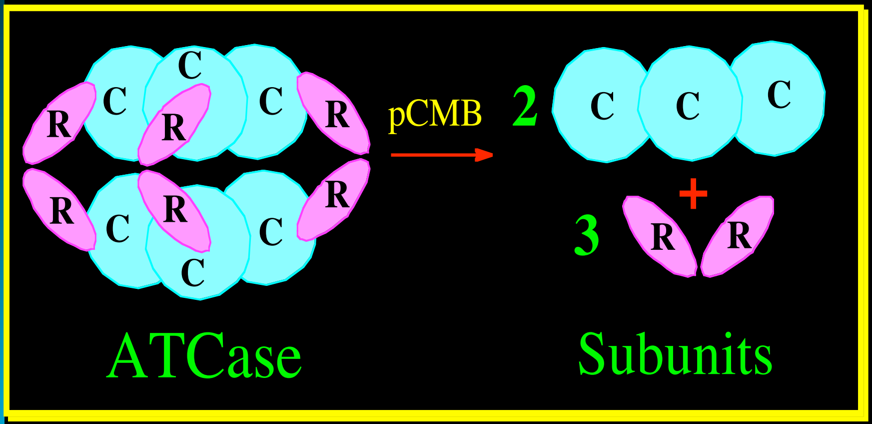
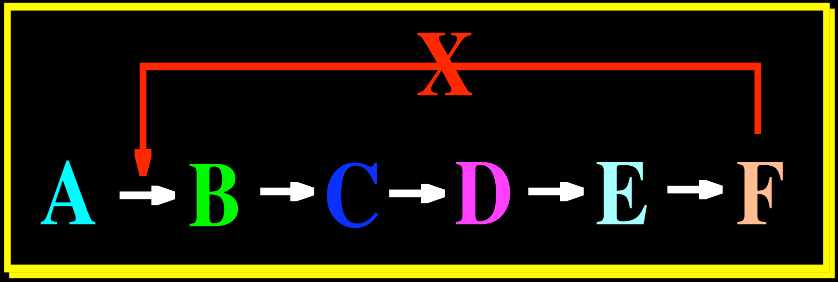
ATCase treated with mercurials do not exhibit allosteric kinetics. This desensitization is caused by the separation of catalytic and regulatory subunits by the reaction with mercurials.



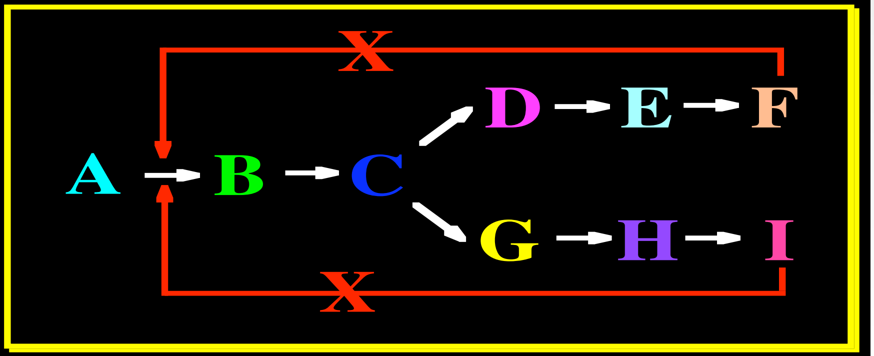
End Product Inhibition

If F is available by some chance,
the pathway should be shut off.
End product inhibition provides this regulation



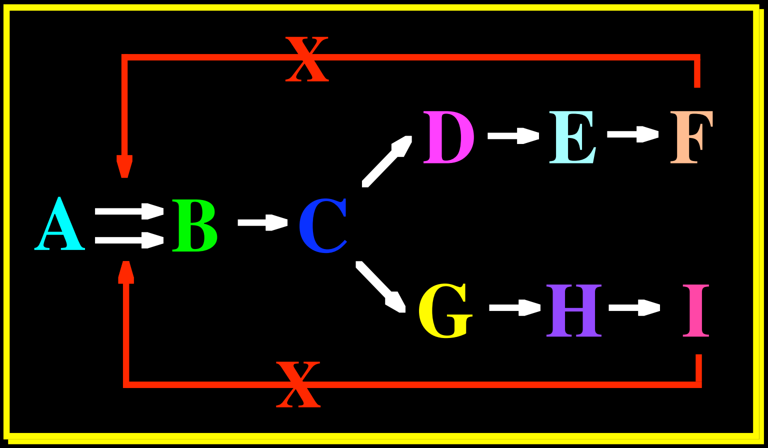
Regulation of Branch Pathways

In a branched pathway, both end products (F and I)
should inhibit the pathway at the starting point.
But when one is in excess, obviously the synthesis of
the other will also be inhibited by this mechanism.



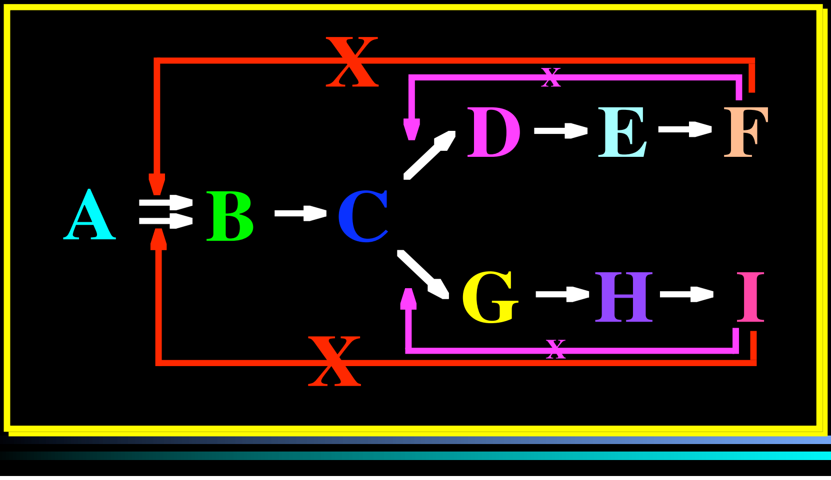
Regulation of Branch pathways

Branched pathways are therefore regulated by Isoenzymes.



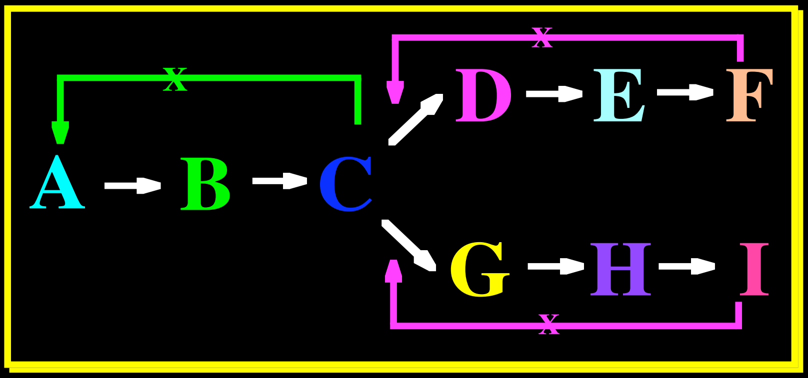
Branch Pathway Inhibitions

Branch pathways are additionally regulated
at the branch sites by the end products



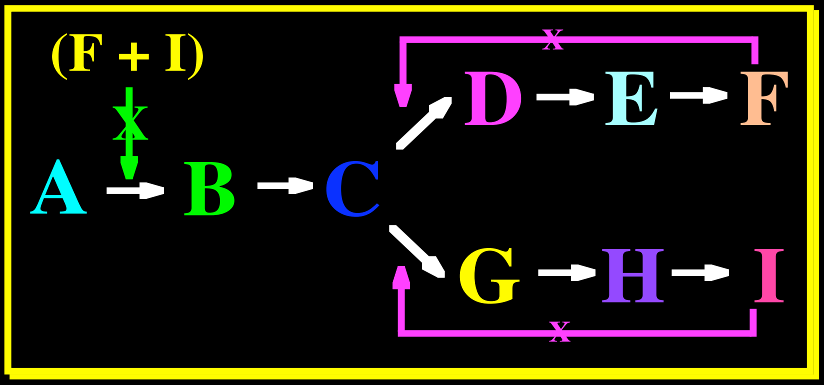
Sequential Feedback Inhibition

F inhibits C to D conversion; I inhibit C to G conversion and C inhibits A to B conversion.

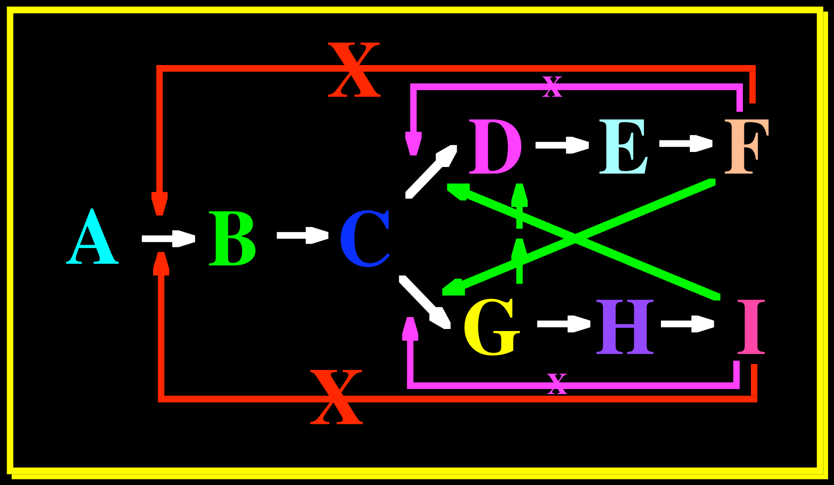


Concerted Feedback Inhibition

Only the high levels of F and I together can inhibit A to B conversion. They will individually exhibit control only at the branching sites.



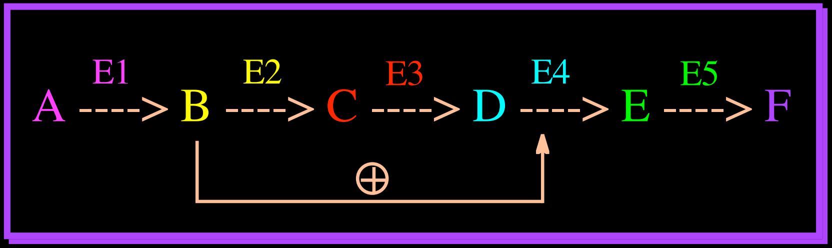
Inhibition and Activation in a Branch Pathway



Cumulative Feedback Inhibition

The first enzyme is partly controlled by each end product.

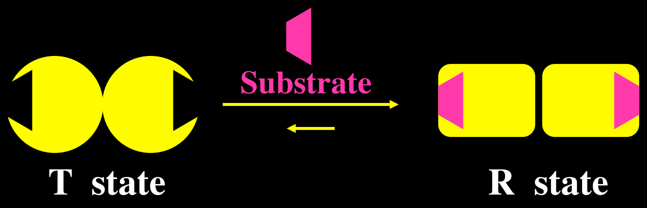
Feed forward activation



A --E1--> B --E2--> C --E3--> D --E4--> E --E5--> F

Excess B activates E4 so that the metabolic pathway goes forward.

Concerted model or symmetry model



The enzyme exists only in two states

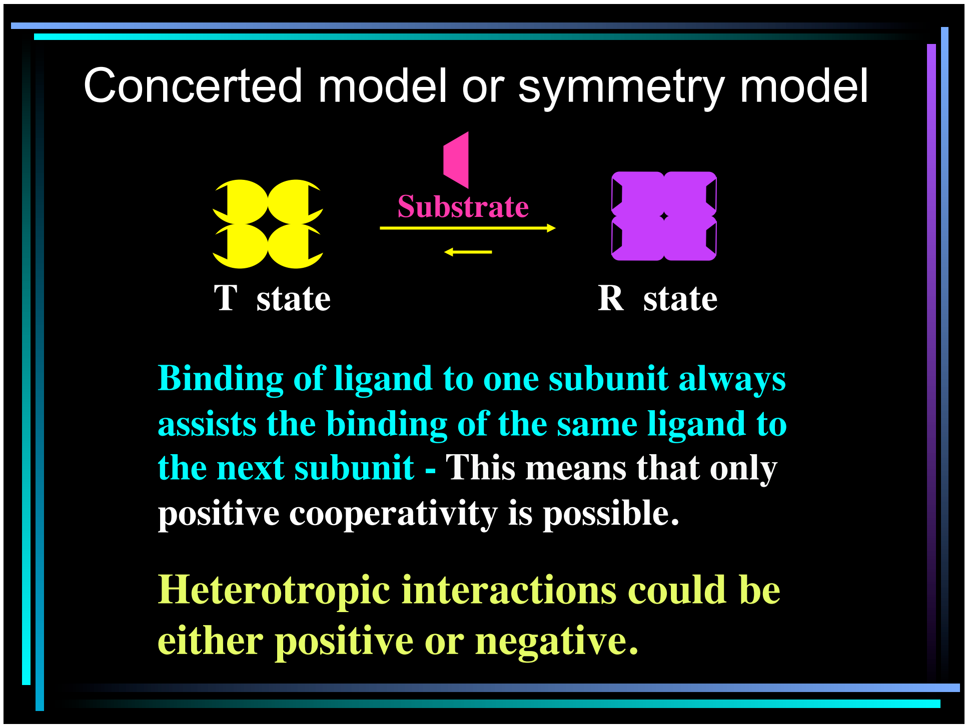
The two states are T (taut or tensed) and R (relaxed)

Substrates and activators have great affinity for R state

Inhibitors have higher affinity for T state

Ligands affect the equilibrium between T and R states

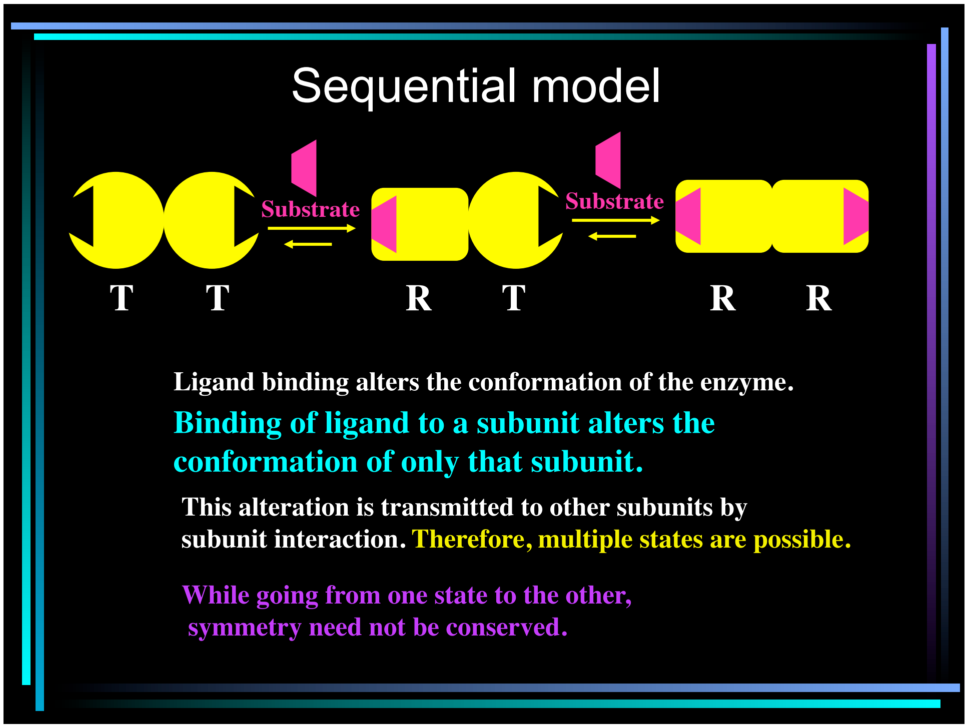
While going from one state to the other symmetry must be conserved.



Concerted model or symmetry model

Binding of ligand to one subunit always assists the binding of the same ligand to the next subunit - This means that only positive cooperativity is possible.

Heterotropic interactions could be either positive or negative.



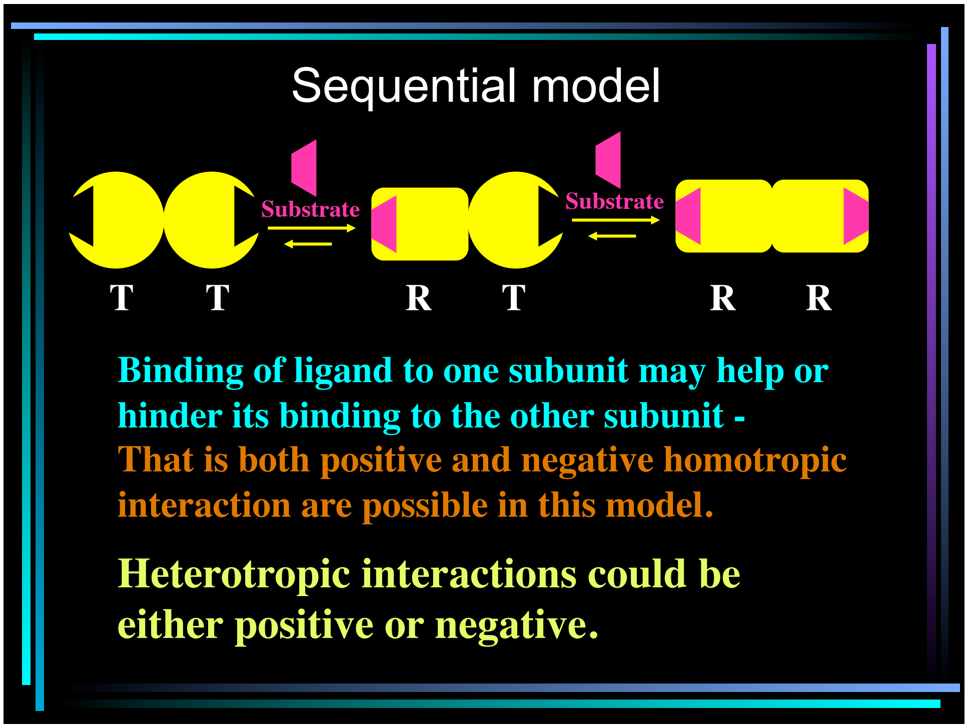
Sequential model

Ligand binding alters the conformation of the enzyme.

Binding of ligand to a subunit alters the conformation of only that subunit.

This alteration is transmitted to other subunits by subunit interaction. Therefore, multiple states are possible.

While going from one state to the other, symmetry need not be conserved.

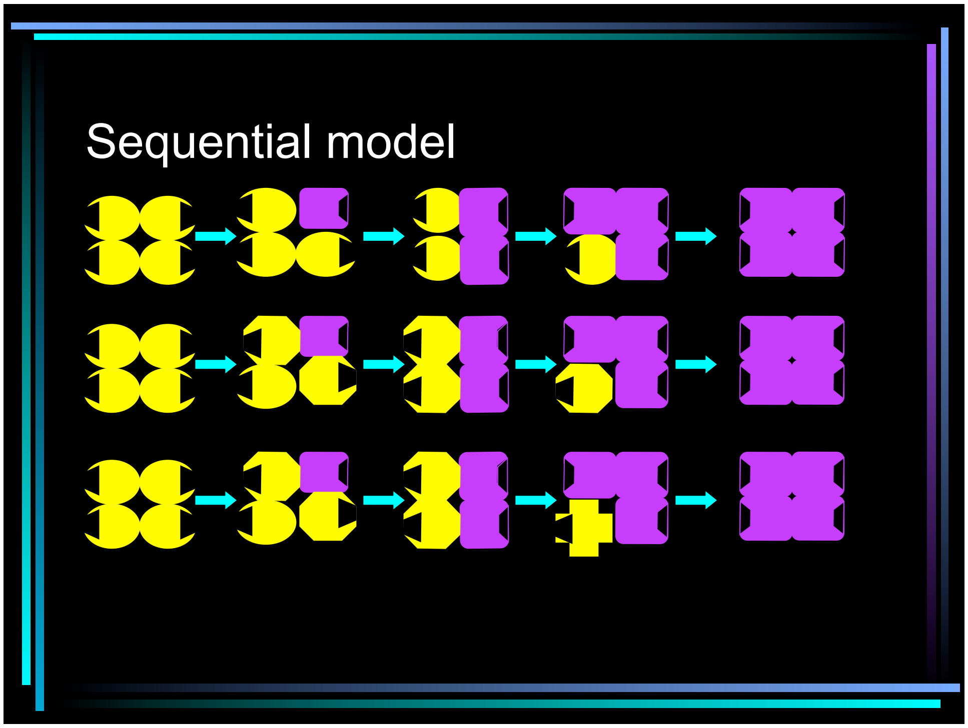


Sequential model

Binding of ligand to one subunit may help or hinder its binding to the other subunit -

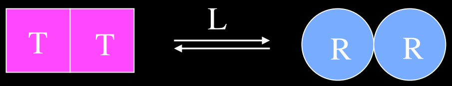
That is both positive and negative homotropic interaction are possible in this model.

Heterotropic interactions could be either positive or negative.



Sequential model

$T_o \rightleftharpoons R_o$



Allosteric constant L $= T_o/R_o$

T = Taut or tight; Stable form binds to S weakly (or no binding)
R = Relaxed form, unstable; binds to substrate tightly

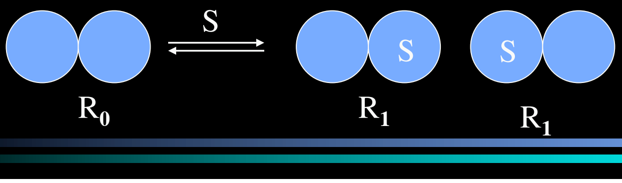
For simplicity of deriving the equation, let us consider a two subunit model, where only R binds to S.

$R_o + S \rightleftharpoons R_1$

Equilibrium constant K_R for this process is

$$K_R = 2\,[R_o]\,[S]/[R_1]$$

2 is the statistical factor because S can bind to either of the two empty subunits.



Allosteric models

Monod Wyman and Changeux model
(MWC model)

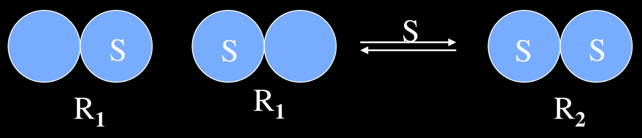
Note:
K_R is the microscopic dissociation constant.

By reasons of symmetry, and because of the binding of Of any one ligand is assumed to be intrinsically Independent of the binding of any other, the K_R values Are the same for all homologous sites.

$$R_1 + S \rightleftharpoons R_2$$

Equilibrium constant for the second binding, $K_R = [R_1]\,[S]/\,2\,[R_2]$

Statistical factor 2 appears in the denominator,
as S can come out of either of the two bound subunits.



Since, $K_R = 2\,[R_o]\,[S]/[R_1]$

$[R_1] = 2\,[R_o]\,[S]/\,K_R = 2\alpha\,[R_o]$ where $\alpha = [S]/\,K_R$

Similarly, $[R_2] = [R_1]\,[S]/2\,[K_R]$ Or $[R_2] = [R_1]\,\alpha\,/\,2$

Substituting for $[R_1]$, we get, $[R_2] = \alpha^2\,[R_o]$

Y = Fractional sites occupied by the ligand is defined as

$$Y = \frac{\text{Occupied sites}}{\text{Total sites}} = \frac{[R_1] + 2\,[R_2]}{2\,([T_o] + [R_o] + [R_1] + [R_2])}$$

$$Y = \frac{2\,\alpha\,[R_o] + 2\,\alpha^2\,[R_o]}{2\,([T_o] + [R_o] + 2\,\alpha\,[R_o] + \alpha^2\,[R_o])}$$

Dividing Nr and Dr by 2 $[R_o]$, we get

$$Y = \frac{\alpha\,(1 + \alpha)}{([T_o]\,/[R_o]) + 1 + 2\,\alpha + \alpha^2}$$

Since, $L = [To] / [Ro]$ and $(1 + 2\,\alpha + \alpha^2)$ is $(1 + \alpha)^2$

The above equation simplifies to $Y = \dfrac{\alpha\,(1 + \alpha)}{L + (1 + \alpha)^2}$

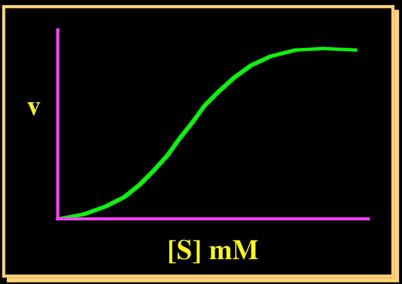
Y is related to velocity of an enzyme reaction.
Since v is dependent on the fractional sites occupied by the substrate, $v = Y\,V_{max}$ or $Y = v/V_{max}$

$$Y = \frac{\alpha\,(1 + \alpha)}{L + (1 + \alpha)^2}$$

This equation was derived with the assumption, that the enzyme has two subunits. If it has n subunits, The equation is

$$Y = \frac{\alpha\,(1 + \alpha)^{n-1}}{L + (1 + \alpha)^n}$$

This equation defines a sigmoidal curve. A plot of Y (or v) versus α (or [S]) gives a sigmoidal graph.



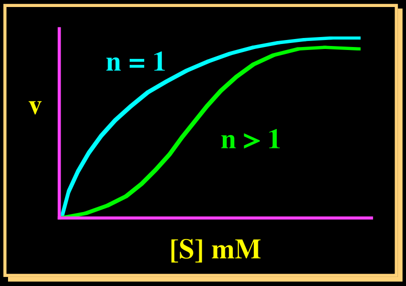
The sigmoidity depends on the values of n, and L.

$$Y = \frac{\alpha\,(1 + \alpha)^{n-1}}{L + (1 + \alpha)^{n}}$$

If n = 1 (there is only one subunit per enzyme molecule), the equation reduces to Michaelis - Menten type.

$$Y = \frac{\alpha\,(1 + \alpha)^{0}}{L + (1 + \alpha)^{1}} = \frac{\alpha}{L' + \alpha}$$

Hence, for sigmoidal curve, you need at least 2 subunits. Higher the value of n, higher will be sigmoidity.



Value of L $T_o \rightleftarrows R_o$ $L = T_o / R_o$

$$Y = \frac{\alpha\,(1 + \alpha)^{n-1}}{L + (1 + \alpha)^{n}}$$

Let us assume $L = 10^4$,
For every one of R_o, there will be 10^4 of T_o
The addition of S causes removal of R_o from equilibrium
So more and more T_o gets converted to R_o for S binding.

Since T--> R causes concerted changes in the structure of both subunits, the proportion of enzyme in R form increases progressively as more and more S are added. Thus binding of S is said to be Cooperative.

If an activator is present, say L is reduced to 10^3 (this means for every 10^3 T_o there is one R_o), we need less S to shift the equilibrium to R state. Therefore, sigmoidity decreases if L is small. In the same way, if an inhibitor is present, say $L = 10^5$, sigmoidity increases.

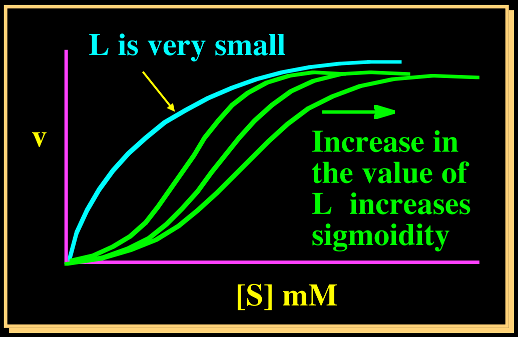
Therefore, higher the value of allosteric constant L, higher will be the sigmoidity and lower the value of L, lower will be sigmoidity.

If the value of L becomes very low, it can be ignored from denominator. So the equation again reduces to M. M. type:

In $Y = \dfrac{\alpha\,(1+\alpha)^{n-1}}{L + (1+\alpha)^{n}}$

If L is omitted, the equation is

$$Y = \frac{\alpha\,(1+\alpha)^{n-1}}{(1+\alpha)^{n}} = \frac{\alpha}{1+\alpha}$$



Effect of Activator and Inhibitor

If we add the effect of inhibitors and activators to this equation, We get:

$$Y = \frac{\alpha\,(1+\alpha)^{n-1}}{L + (1+\alpha)^{n}}$$

$$Y = \frac{\alpha\,(1+\alpha)^{n-1}}{L\left\{\dfrac{(1+\beta)^{n}}{(1+\tau)^{n}}\right\} + (1+\alpha)^{n}}$$

Where

$\beta = K_I\,[I]$ $\tau = K_A\,[A]$

If $\beta = 0$, and $\tau = 0$ you get a normal sigmoidal graph.
When an inhibitor is present, it stabilizes T state.
β is increased and you get an increase in sigmoidity.
When an activator is present, it stabilizes the R state,
τ is increased and sigmoidity is decreased.

So far, we dealt with a special case where S binds to only R.
If S also binds to T, but less effectively than it does to R,
The equation for allosteric interaction becomes:

$$Y = \frac{\alpha\,(1+\alpha)^{n-1} + LC\,\alpha\,(1+C\alpha)^{n-1}}{(1+\alpha)^{n} + L\,(1+C\alpha)^{n}} \quad \text{Where } C = K_T / K_R$$

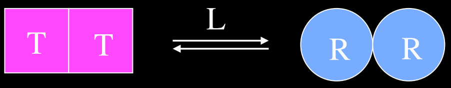
K_T is the intrinsic binding constant for S to bind to T

K_R is the intrinsic binding constant for S to bind to R

Lower the value of C (that is binding occurs only to R state
As we originally considered) higher will be the sigmoidity.
Higher the value of C, lower will be the sigmoidity.

Derivation of equation - Binding to both sites.

$T_o \rightleftharpoons R_o$



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T = Taut or tight; Stable form binds to S weakly
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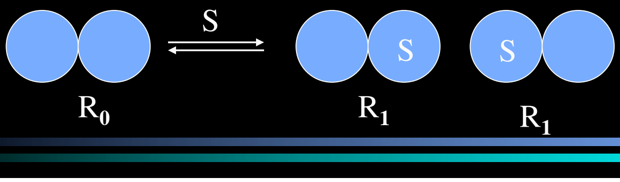
$R_o + S \rightleftharpoons R_1$

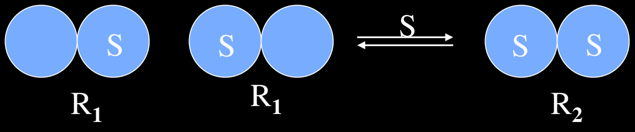
Equilibrium constant K_R for this process is
$K_R = 2\,[R_o]\,[S]/[R_1]$
2 is the statistical factor because S can bind to either of the two empty subunits.



$$R_1 + S \rightleftharpoons R_2$$

Equilibrium constant for the second binding, $K_R = [R_1]\,[S]/\,2\,[R_2]$

Statistical factor 2 appears in the denominator,
as S can come out of either of the two bound subunits.



Since, $K_R = 2\,[R_o]\,[S]/[R_1]$

$[R_1] = 2\,[R_o]\,[S]/\,K_R = 2\alpha\,[R_o]$ where $\alpha = [S]/\,K_R$

Similarly, $[R_2] = [R_1]\,[S]/2\,[K_R]$ Or $[R_2] = [R_1]\,\alpha\,/\,2$

Substituting for $[R_1]$, we get, $[R_2] = \alpha^2\,[R_o]$

For T state: $T_o + S \rightleftharpoons T_1$ $K_T = 2\,[T_o]\,[S]/[T_1]$

$[T_1] = 2\,[T_o]\,[S]/\,K_T$

Let us assume $c = K_R/K_T$

Therefore, $K_T = K_R/c$, the above equation becomes,

$[T_1] = 2\,[T_o]\,c\,[S]/\,K_R$

Since, $\alpha = [S]/\,K_R$

We can reduce this further to $[T_1] = 2\,[T_o]\,c\,\alpha$

On the same reasoning, $[T_2] = 1/2\,\{[T_1]\,[S]/\,K_T\}$

$$[T_2] = \frac{2\,[T_o]\,c\,\alpha\,[S]}{2\quad K_T} = \frac{[T_o]\,c\,\alpha\,[S]\,c}{K_R} = [T_o]\,(c\,\alpha)^2$$

Y = Fractional sites occupied by the ligand $Y = \frac{\text{Occupied sites}}{\text{Total sites}}$

$$Y = \frac{([R_1] + 2\,[R_2]) + ([T_1] + 2\,[T_2])}{2\,\{([R_o] + [R_1] + [R_2]) + ([T_o] + [T_1] + [T_2])\}}$$

Substituting $R_1 = 2\alpha\,[R_o]$; $R_2 = \alpha^2\,[R_o]$; $T_1 = 2c\alpha\,[T_o]$; and $T_2 = (c\alpha)^2\,[T_o]$;

$$Y = \frac{2\alpha\,[R_o] + 2\,\alpha^2\,[R_o] + 2c\alpha\,[T_o];\, + 2\,(c\alpha)^2\,[T_o]}{2\,\{([R_o] + 2\alpha\,[R_o] + \alpha^2\,[R_o]) + ([T_o] + 2c\alpha\,[T_o] + (c\alpha)^2\,[T_o])\}}$$

$$Y = \frac{2\alpha\,[R_o]\,(1+\alpha) + 2c\alpha\,[T_o]\,(1+c\alpha)}{2\,\{[R_o]\,(1+\alpha)^2 + [T_o]\,(1+c\alpha)^2\}}$$

Canceling 2 and dividing by $[R_o]$

$$Y = \frac{\alpha\,(1+\alpha) + c\alpha\,[T_o]/[R_o]\,(1+c\alpha)}{(1+\alpha)^2 + [T_o]/[R_o]\,(1+c\alpha)^2}$$

Since $L = [T_o]/[R_o]$

$$Y = \frac{\alpha\,(1+\alpha) + L\,c\alpha\,(1+c\alpha)}{(1+\alpha)^2 + L\,(1+c\alpha)^2}$$

General equation

$$Y = \frac{\alpha\,(1+\alpha) + L\,c\alpha\,(1+c\alpha)}{(1+\alpha)^2 + L\,(1+c\alpha)^2}$$

This equation is for 2 subunit case. If we have n subunits, the equation can be modified as

$$Y = \frac{\alpha\,(1+\alpha)^{n-1} + L\,c\alpha\,(1+c\alpha)^{n-1}}{(1+\alpha)^n + L\,(1+c\alpha)^n}$$

If c = 1 that is if S binds to both T and R states equally well the equation reduces to

$$Y = \frac{\alpha\,(1+\alpha)^{n-1} + L\,\alpha\,(1+c\alpha)^{n-1}}{(1+\alpha)^n + L\,(1+c\alpha)^n} = \frac{(1+L)\,\alpha\,(1+\alpha)^{n-1}}{(1+L)\,(1+\alpha)^n}$$

Y becomes = $\alpha/(1+\alpha)$ or Michaelis Menten equation

$$Y = \frac{\alpha\ (1+\alpha)^{n-1} + L\,c\alpha\,(1+c\alpha)^{n-1}}{(1+\alpha)^{n} + L\,(1+c\alpha)^{n}}$$

If L is approaching 0 (that is the equilibrium is largely in favor of R state), we can omit the terms containing L as they will be very small and the equation reduces to Michaelis Menten equation again.

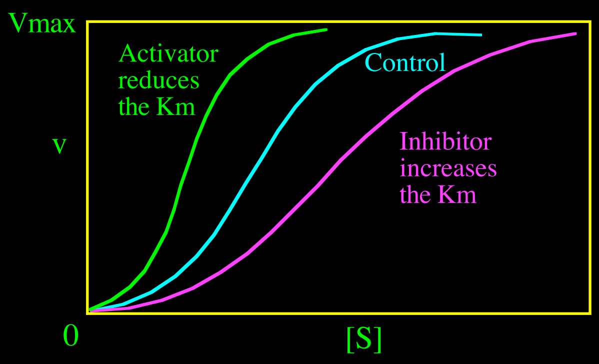
$$Y = \frac{\alpha\ (1+\alpha)^{n-1}}{(1+\alpha)^{n}} = \alpha / (1+\alpha)$$

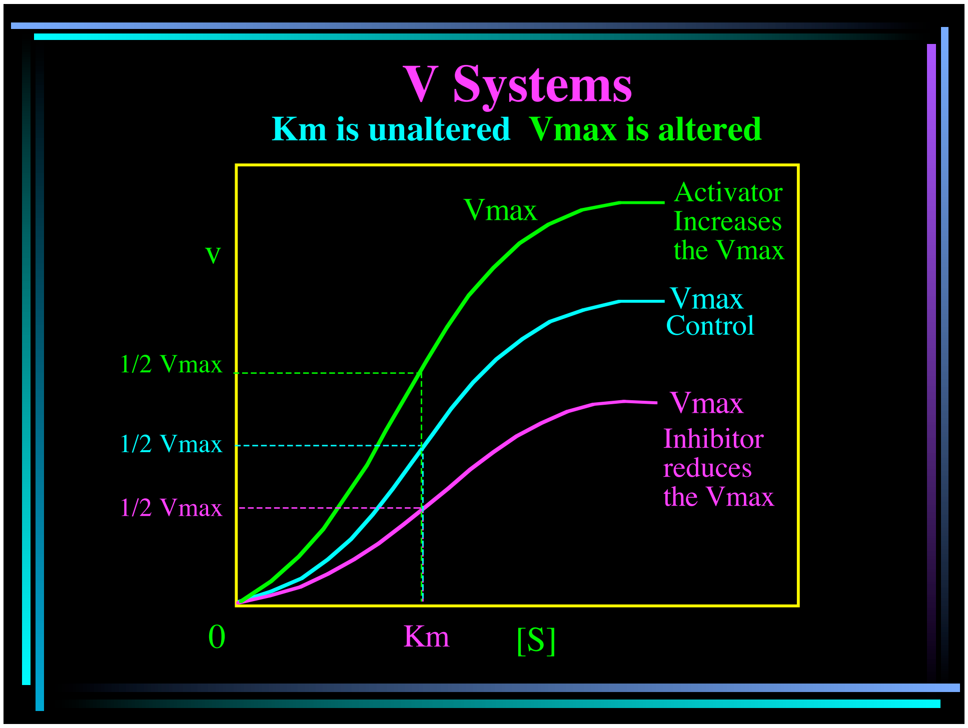
Thus larger the value of L, large will be sigmoidity
Lower the value of L, lower will be the sigmoidity.

Two types of effects - K system and V system

K Systems

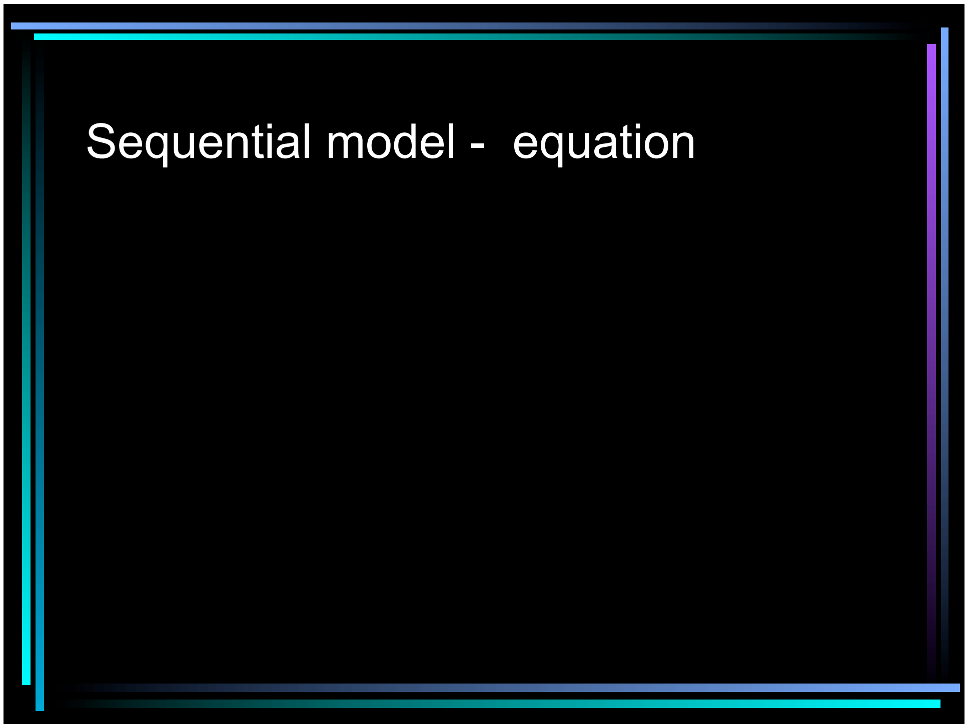
Km is altered
Vmax is unaltered



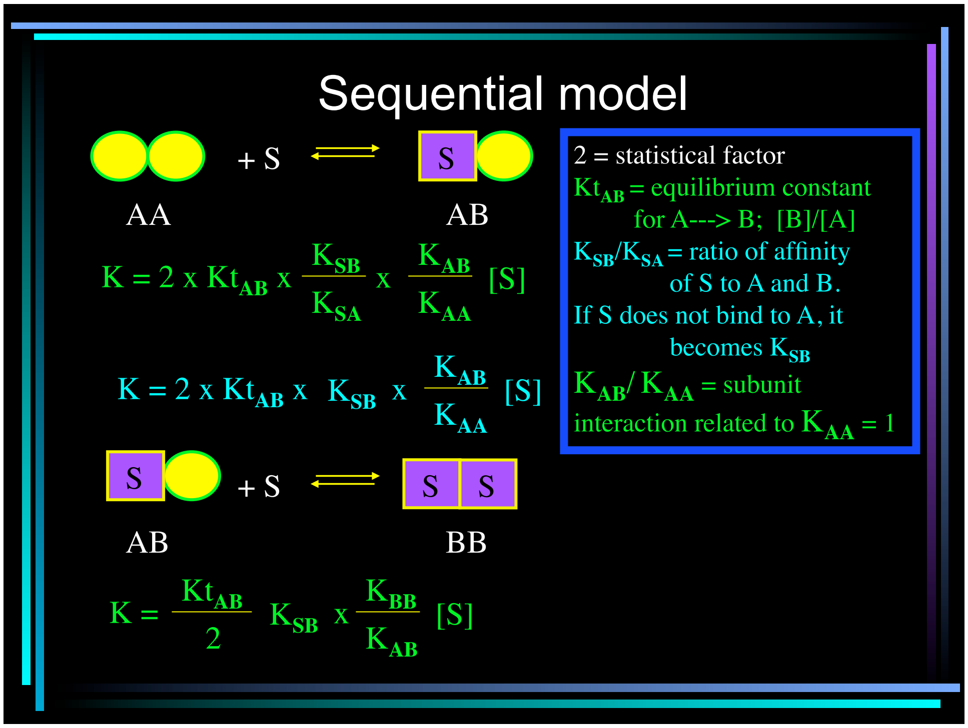


V Systems

Km is unaltered Vmax is altered



Sequential model - equation



Sequential model

AA + S ⇌ AB

$$K = 2 \times Kt_{AB} \times \frac{K_{SB}}{K_{SA}} \times \frac{K_{AB}}{K_{AA}} [S]$$

$$K = 2 \times Kt_{AB} \times K_{SB} \times \frac{K_{AB}}{K_{AA}} [S]$$

AB + S ⇌ BB

$$K = \frac{Kt_{AB}}{2} K_{SB} \times \frac{K_{BB}}{K_{AB}} [S]$$

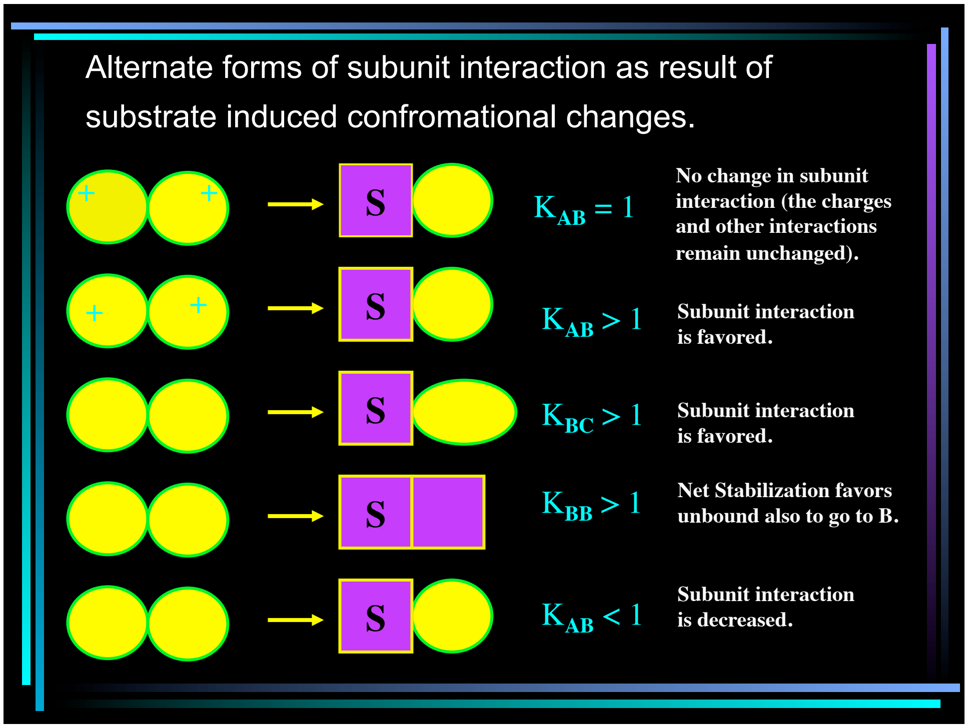
2 = statistical factor

Kt_{AB} = equilibrium constant for A---> B; [B]/[A]

K_{SB}/K_{SA} = ratio of affinity of S to A and B.

If S does not bind to A, it becomes K_{SB}

K_{AB}/K_{AA} = subunit interaction related to $K_{AA} = 1$



Alternate forms of subunit interaction as result of substrate induced confromational changes.

$K_{AB} = 1$ **No change in subunit interaction (the charges and other interactions remain unchanged).**

$K_{AB} > 1$ **Subunit interaction is favored.**

$K_{BC} > 1$ **Subunit interaction is favored.**

$K_{BB} > 1$ **Net Stabilization favors unbound also to go to B.**

$K_{AB} < 1$ **Subunit interaction is decreased.**

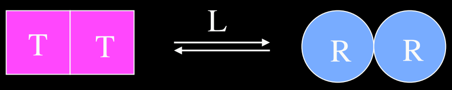
If K_{BB}/K_{AB} is >> K_{AB}/K_{AA}, binding of S is increased to the second in comparison to the first
Hence it is positive cooperativity.
(There is net stabilization, $K_{AB} = 1$ and $K_{BB} > 1$)

If K_{BB}/K_{AB} is << K_{AB}/K_{AA}, binding of S is decreased to the second in comparison to the first
Hence it is negative cooperativity.
(BB shows repulsion $K_{BB} < K_{AB} = 1$)

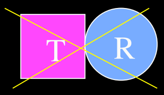
If K_{BB}/K_{AB} is = K_{AB}/K_{AA}, binding of S is same to the second as that of the first
Hence no cooperativity.
$K_{AB} = K_{BB} = 1$) Michaelis Menten type kinetics

Differences between models

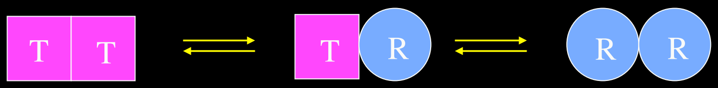
In the symmetry model, there is pre-existing equilibrium. Only two states are possible. Ligands stabilizes different states. The two states have to remain in the same form to conserve the symmetry.



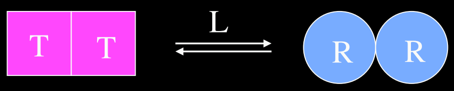
That is if one subunit changes from T to R, the other T subunit also automatically changes to R.
No mixed state is allowed here.



In Sequential model, there is no equilibrium . Ligand induces conformational changes that stabilize different states.
More states than two states are possible. It is possible to have mixed states. Symmetry need not be conserved here.

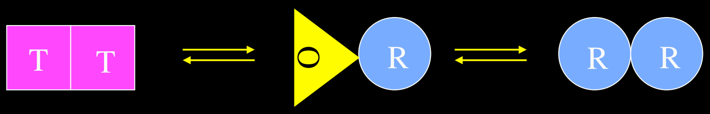


In the symmetry model, only two states are allowed for the enzyme say R form and the T form.



These two states are in pre-existing equilibrium.
Ligand binding affects this equilibirum.

In Sequential model, it is possible to have more than two states for the enzymes.



Thess states are not in preexisting equilibrium.
Ligand induced conformational changes cause the Changes in the subunit structure.

In the Symmetry model only positive homotropic interaction is allowed. [S binding always helps another S binding]

Heterotropic interactions could be positive or negative. [S and I binding is negative heterotropic interaction and S and A binding is positive heterotropic interaction].

In the Sequential model, both homotropic and heterotropic interactions could be positive or negative.

[That is even S binding to one subunit could inhibit The S binding to the next subunit (negative homotropic interaction is possible here).

Difference between two models

Concerted model (MWC model)	**Sequential model (KNF model)**
Based on Pre-existing equilibrium Ligands stabilizes them	Based on induced fit theory. Ligand induces conformational changes
Symmetry should be conserved	No symmetry. Mixed states allowed
Only two states are allowed. All transitions occur simultaneously	Many states are possible. Different ligands stabilize differently
Predicts M. M. kinetics if the subunits are in the active form	M. M. kinetics only when there is no change in subunit interaction
Simple. Restricted application	Complex. General application.
Same states could stabilized by different ligands (A & S → R)	Different states are stabilized by different ligands slightly.
Homotropic interaction are only positive	Homotropic interactions could be both positive and negative.

Hill’s equation

Oxygen binding to hemoglobin (Hg)

$Hg(O_2)_4 \rightleftharpoons Hg + 4O_2$

A general equation is:

$ES_n \rightleftharpoons E + nS$

Saturation $Y = \frac{[S]^n}{K^n + [S]^n}$ ....Equation 1

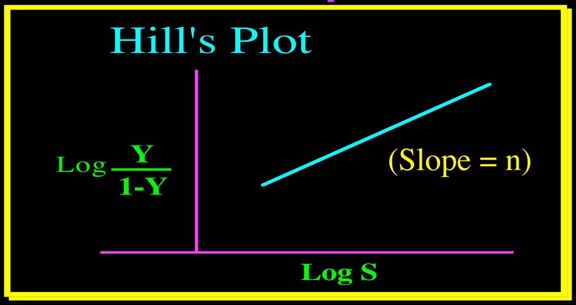
$1 - Y = 1 - \frac{[S]^n}{K^n + [S]^n}$ or $= \frac{K^n}{K^n + [S]^n}$ ....Equation 2

Divide equation 1 by 2 $\frac{Y}{1 - Y} = \frac{[S]^n}{K^n}$ ....Equation 3

log of equation 3 yeilds the Hill's equation:

$$\log \frac{Y}{1 - Y} = n \log [S] - n \log K$$

Hill’s plot



n = 1 - For Michaelis Menten type

n < 1 - Negative cooperativity

n > 1 - Positive cooperativity

(Myoglobin n = 1; Hemoglobin n = 2.8)

Scatchard plot

$E + S \rightleftharpoons ES$ $\quad Ka = \frac{[ES]}{[E]_o\,[S]}$ equation 1

Concentration of bound total E is
where n is total number of sites $\quad = n[E]_t$ equation 2

According to law of Mass action,
(unbound = total - bound) $\quad [E]_o = n[E]_t - [ES]$ equation 3

Substitution equation 3 in 1, $\quad Ka = \frac{[ES]}{(n[E]_t - [ES])[S]}$

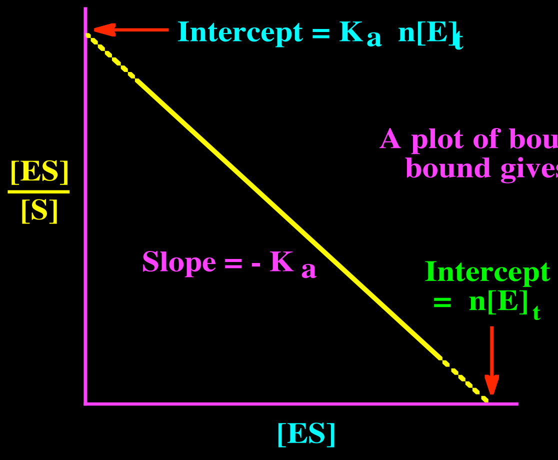
or $\quad Ka\,(n[E]_t - [ES]) = \frac{[ES]}{[S]}$

SCATCHARD PLOT:

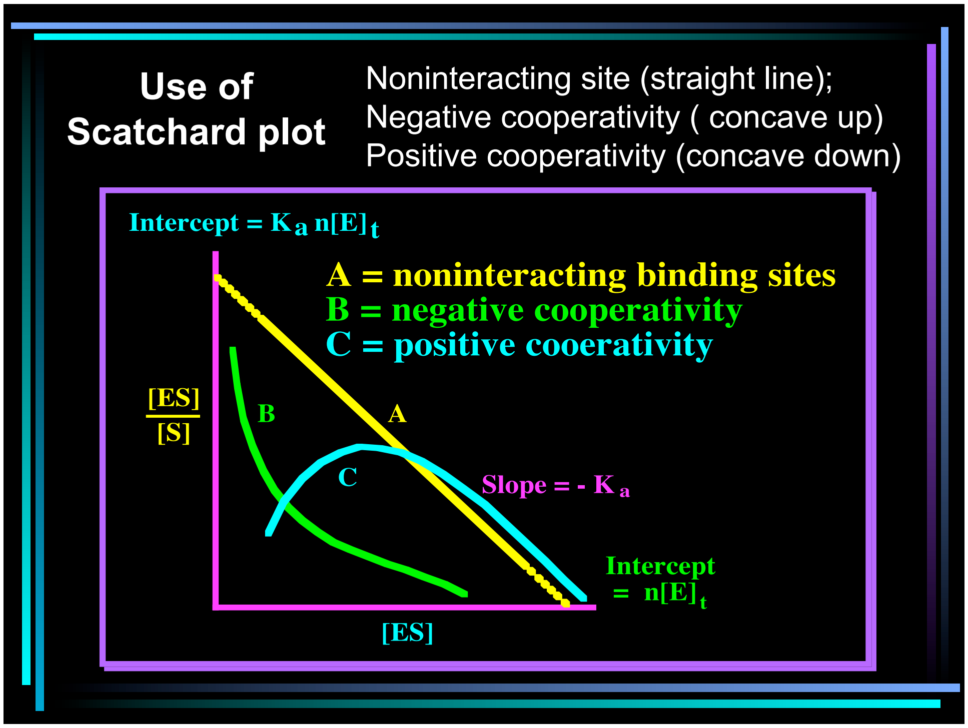
A plot of bound by free
versus bound gives a
straight line.

$$\frac{[ES]}{[S]} = Ka\,n[E]_t - Ka\,[ES]$$

Scatchard plot



A plot of bound by free versus
bound gives a straight line

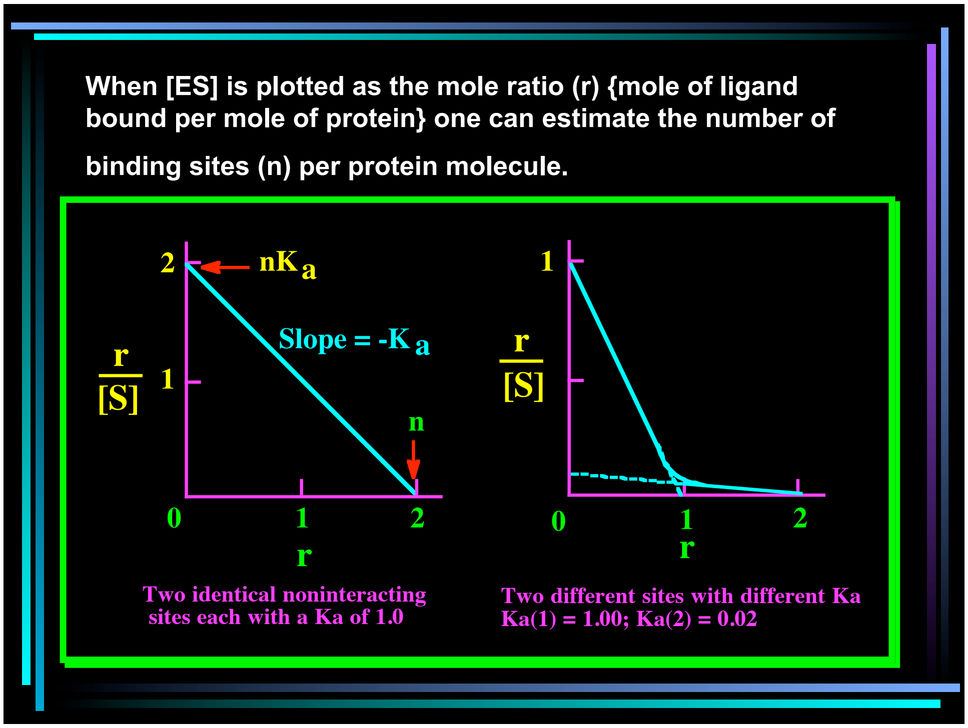


Use of Scatchard plot

Noninteracting site (straight line);
Negative cooperativity (concave up)
Positive cooperativity (concave down)

Intercept = $K_a\, n[E]_t$

A = noninteracting binding sites
B = negative cooperativity
C = positive cooerativity



When [ES] is plotted as the mole ratio (r) {mole of ligand bound per mole of protein} one can estimate the number of binding sites (n) per protein molecule.

Two identical noninteracting sites each with a Ka of 1.0

Two different sites with different Ka
Ka(1) = 1.00; Ka(2) = 0.02

$$R_S = \frac{\text{Substrate concentration at 0.9 saturation}}{\text{Substrate concentration at 0.1 saturation}}$$

Since $Y = [S]/K_m + [S]$, the value at 90% and 10% saturation are

$$0.9 = [S_{0.9}] / K_m + [S_{0.9}] \quad \text{and} \quad 0.1 = [S_{0.1}] / K_m + [S_{0.1}]$$

Therefore, $0.9\ K_m + 0.9\ [S_{0.9}] = [S_{0.9}]$ or
$0.9\ K_m = 0.1\ [S_{0.9}]$ or $[S_{0.9}] = 9\ K_m$

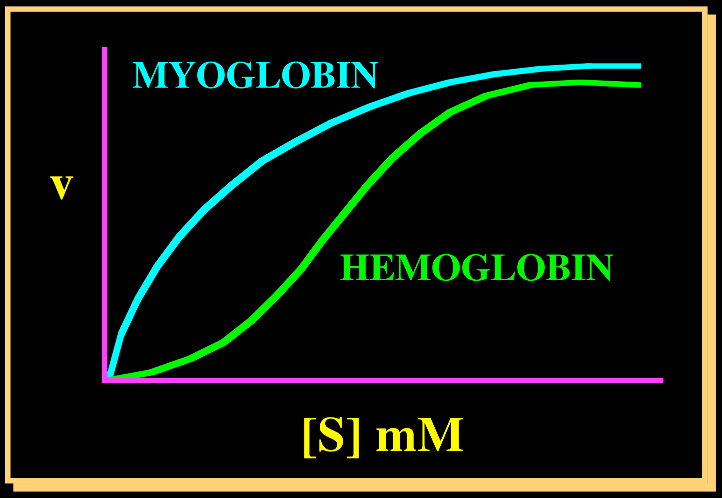
Similarly, $0.1\ K_m + 0.1\ [S_{0.1}] = [S_{0.1}]$ or
$0.1\ K_m = 0.9\ [S_{0.1}]$ or $[S_{0.1}] = 1/9\ K_m$

Hence, $R_S = \dfrac{9\ K_m}{1/9\ K_m}$ = 81 for Michaelis Menten type.

$R_s > 81$ for negative cooperativity
Rs < 81 for positive cooperativity.

Example of allosteric enzyme

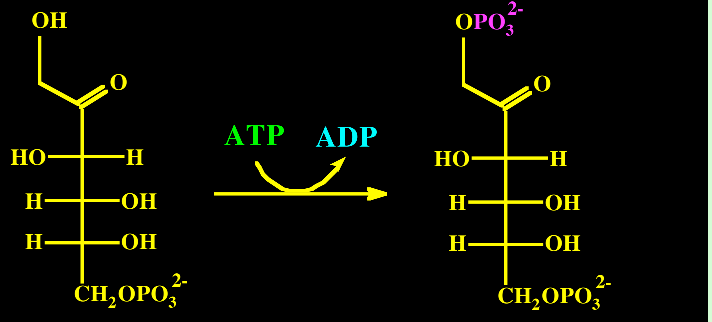
MYOGLOBIN - MICHAELIS-MENTEN KINETICS.
HEMOGLOBIN - SIGMOIDAL KINETICS



Myoglobin	Hemoglobin
Monomeric in nature.	Tetrameric in nature
	Two kind of subunits. $\alpha_2\beta_2$.
M.M. Kinetics.	Sigmoidal kinetics.
pH has no drastic effect.	pH inhibits O_2 binding.
CO_2 - No effect.	CO_2 inhibits O_2 binding.
Diphosphoglycerate - No effect	Diphosphoglycerate inhibits O_2 binding.
Only one form.	Exists in two forms - oxy form is different from deoxy form.

Phosphofructokinase -1

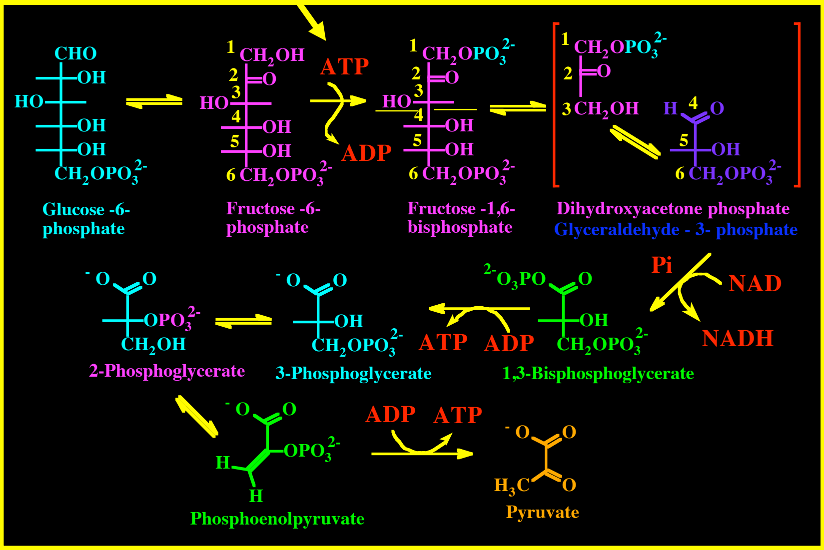
Reaction catalyzed by Phosphofructokinase -1



Fructose 6 - phosphate

Fructose 1,6 - bisphosphate

Phosphofructokinase is an important enzyme in glycolysis



Glucose -6- phosphate

Fructose -6- phosphate

Fructose -1,6- bisphosphate

Dihydroxyacetone phosphate

Glyceraldehyde - 3- phosphate

2-Phosphoglycerate

3-Phosphoglycerate

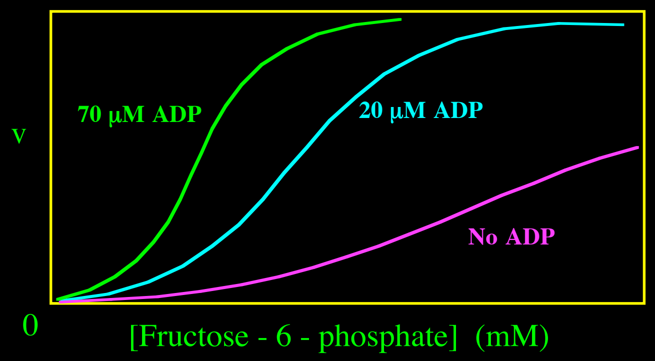
1,3-Bisphosphoglycerate

Phosphoenolpyruvate

Pyruvate

Phosphofructokinase of E. coli Activated by ADP and inhibited by Phosphoenolpyruvate.

Blangy, D., Buc, H and Monod, J.   J. Mol. Biol. 31, 13-35 (1968).



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