ENZYMES

A. Binding
B. Catalysis
C. Nomenclature
D. Catalysis
   1. Transition State Theory
   2. Catalytic strategies (What)
   3. Mechanistic strategies (How)

E. Quantifying the Catalytic Power: Kinetics
   1. Review
   2. Enzyme Kinetics
   3. Initial rate (v_0) vs. [S] for enzymic reaction;
   4. ES complex
   5. Rate expression; Michaelis-Menten Kinetics
   6. Meaning of rate expression (M-M eqn)
   7. Collection and manipulation of data
      a. Lineweaver-Burk; double reciprocal; $1/v_0$ vs. $1/[S]$
   8. Inhibition
      a. Irreversible: protein modification
      b. Reversible: Competitive, Uncomp., Noncomp.

F. Active-site identification
   1. Determine mechanism-ordered *versus* random
   2. pH studies;
   3. Protein modification; Irreversible
   4. X-ray crystallography structure; cleft

G. Energetics of Catalysis
   1. The $\Delta\Delta G^‡$ is negative
   2. The $\Delta\Delta G^‡ = \Delta\Delta H^‡ - T\Delta\Delta S^‡$; bonding & proximity effects
   3. Rate dependent on $(\mathbf{k}T/h)EXP(-\Delta G^‡/RT)$
   4. Examples; Proline Racemase, HIV protease

H. Enzyme Mechanisms
   1. Proteases
      a. Introduction; roles and types
         i. Roles: Serine:
         ii. Types of mechanisms; Serine, Thiol, Acid, Metallo
   2. Serine Proteases

NEXT
- Reading: Ch5; 157-160, Ch6; 213-220
- Homework #18

NEXT
- Reading: Ch5; 148-149, 152-157,160-164
- Homework #19

Lecture 18 (10/25/24)

a. Reaction & specificity
b. Activation (zymogens); Ile α-amino pK_a impt.
c. Active Site Determination
   i. esterase activity
   ii. burst kinetics => two-steps (Ping-pong bi bi)
   iii. protein modification
   iv. pH studies
   v. X-ray crystallography
d. Proposed mechanism
   i. Catalytic triad (Ser-His-Asp) – highly conserved
   ii. Mechanism; tetrahedral intermediates and stabilize t.s. with oxy-anion hole
   iii. Old; charge relay (but Ser-195 does not have the correct pK_a)
   iv. Low-barrier Hydrogen bonds - Role for Asp
e. Specificity
   i. Chymotrypsin *versus* elastase

1. Other protease mechanisms

I. Enzyme Regulation
   1. Introduction
   2. Strategies
      a. Gene Regulation
      b. Covalent Modification
      c. Allosteric Control
   3. Covalent Modification
      a. Proteolysis, Protein modifications
         i. Phosphorylation
            a) Kinases
            b) Phosphatase
            c) Example-PKA
   4. Allosteric Control
      a. Regulation nomenclature
         i. On/off switch
         ii. Example: ATCase
      b. Review; binding curves; why sigmoidal
      c. Hill Equation
      d. Physical models; 3° and 4° conf. changes
         i. T_o vs. R_o
         ii. Sequential; KNF
         iii. Concerted (symmetry); MWC
         iv. Examples of measuring these parameters

H. Hemoglobin
   1. Roles of Hb
      a. Oxygen transport
      b. CO_2 binding
      c. Blood buffer: Bohr effect

Enzyme Regulation

Control by Covalent Modification:

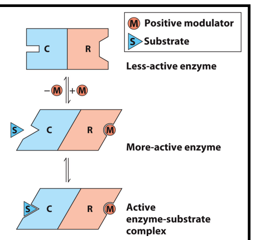
EXAMPLE: Protein Kinase A

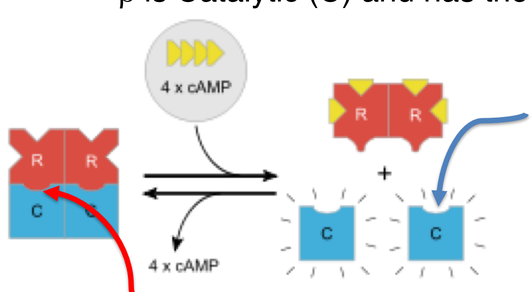
AKA: cAMP-dependent Protein Kinase

$\alpha_2\beta_2$ (90 kDa & 40 kDa)

α is Regulatory (R)

β is Catalytic (C) and has the kinase activity





The active site recognizes a particular site on substrate proteins
Target sites have the consensus sequence:
$R^{R}_{K}XSB$

small — Large hydrophobic

Why is PKA inactivated when bound to R_2?

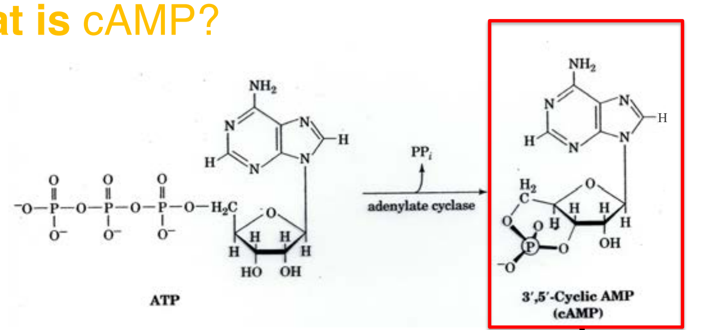
R has a sequence: RRGAI that fits into the C active site!

How is this equilibrium shifted? Cyclic-AMP (cAMP)

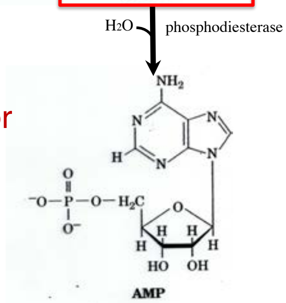
Enzyme Regulation

Control by Allostery:

What is cAMP?



For PKA, cAMP is known as a
positive heterotropic allosteric effector



Enzyme Regulation

Control by Allostery:

What is Allostery or Allosteric Control?

(meaning: ‘allo’ = another, ‘steric’ = place, site, space)

Control by binding to another site; NOT the active site

Nomenclature for **allosteric effector** molecules:

- molecules that are the substrate = homotropic
- molecules that are the not the substrate = heterotropic

- molecules that activate the enzyme = positive
- molecules that inhibit the enzyme = negative

Hence, cAMP is a positive heterotropic allosteric effector for PKA

Enzyme Regulation

Control by Allostery:

Recall binding curves:

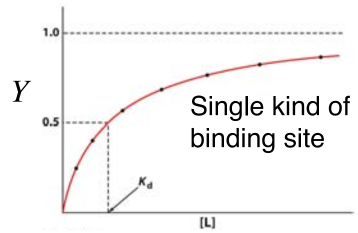
$$R + L \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} R{\cdot}L$$

$$K_D = \frac{[R][L]}{[R \bullet L]}$$

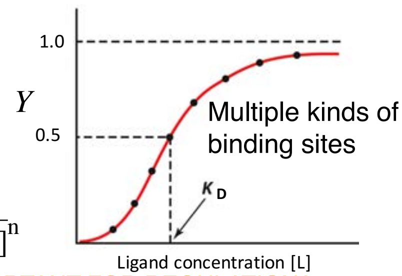
Fraction bound (Y) =

$$\frac{[R \bullet L]}{[R]_T}$$

$$Y = \frac{[L]}{K_D + [L]}$$



$$Y = \frac{[L]^n}{K_D' + [L]^n}$$



WHY IS THIS SIGMOIDAL BEHAVIOR IMPORTANT FOR REGULATION?

IF AFFORDS A WAY TO MAKE AN ON/OFF SWITCH

Enzyme Regulation

Control by Allostery:

Example of famous binding proteins:

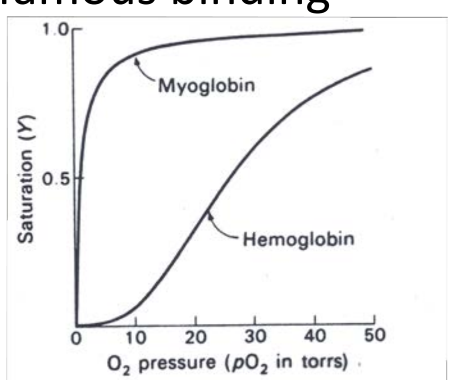
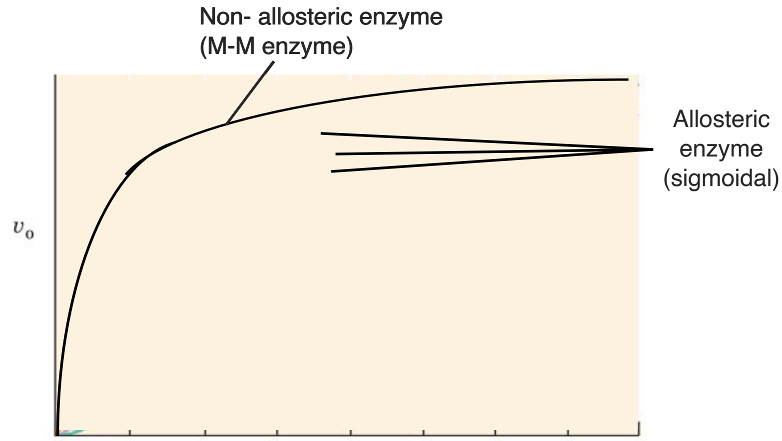


Figure 7-20
Oxygen dissociation curves of myoglobin and hemoglobin. Saturation of the oxygen-binding sites is plotted as a function of the partial pressure of oxygen surrounding the solution.

Enzyme Regulation

Control by Allostery:

Allosteric Effectors can change binding/kinetic behavior

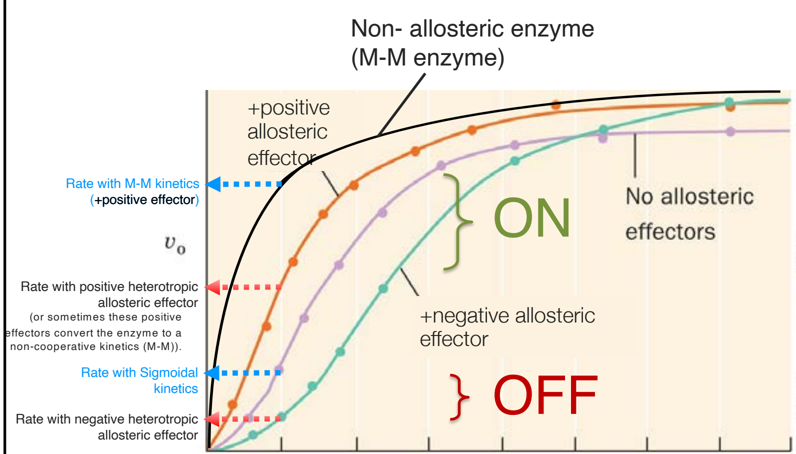


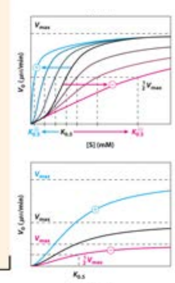
If this is physiological concentration of substrate, we have an ON/OFF switch!

Enzyme Regulation

Control by Allostery:

Allosteric Effectors can change binding/kinetic behavior





If this is physiological concentration of substrate, we have an ON/OFF switch!

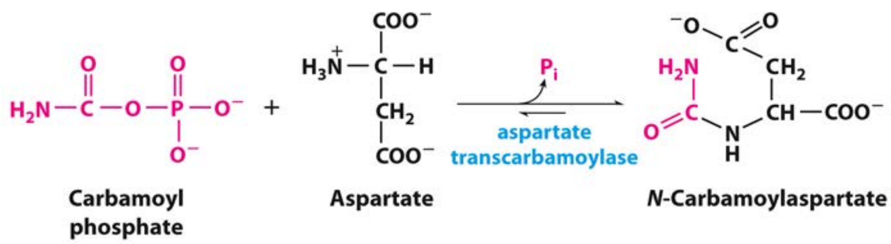
From book: At increasing conc., positive effectors (blue) or negative effectors (red) can affect the K_m or the V_{max} to achieve the same switch.

Enzyme Regulation

Control by Allostery:

Allosteric Effectors can change binding/kinetic behavior

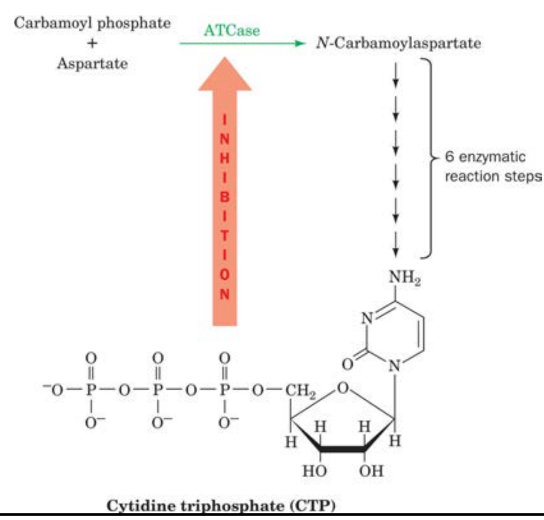
EXAMPLE: Aspartate Transcarbamoylase (ATCase)



Enzyme Regulation

Control by Allostery:

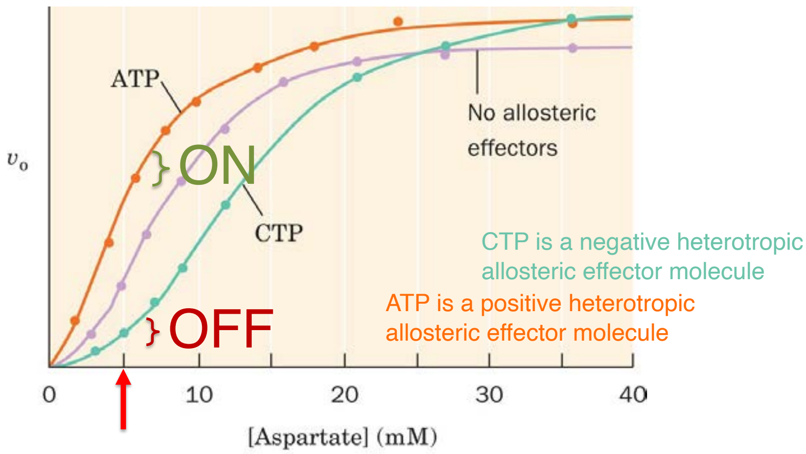
Pyrimidine Biosynthesis: ATCase Controlled by Classic Feedback Inhibition



Control by Allostery:

Enzyme Regulation

Allosteric Effectors: ATCase Reaction



After Kantrowitz, E.R., Pastra-Landis, S.C., and Lipscomb, W.N., *Trends Biochem. Sci.* 5, 125 (1980).

Control by Allostery:

Enzyme Regulation

Allosteric Effectors can change binding/kinetic behavior

<u>Other examples:</u>

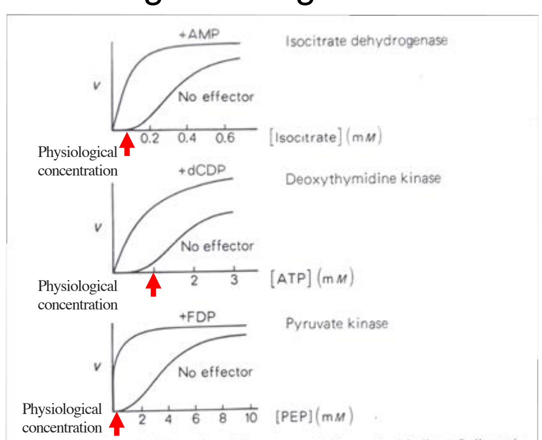


FIGURE 10·4. The abolition of positive cooperativity on the binding of allosteric effectors to some enzymes. Note the dramatic increases in activity at low substrate concentrations on the addition of adenosine monophosphate to isocitrate dehydrogenase, of deoxycytosine diphosphate to deoxythymidine kinase, and of fructose 1,6-diphosphate to pyruvate kinase; this shows how the activity may be "switched on" by an allosteric effector (PEP = phosphoenolpyruvate). [From J. A. Hathaway and D. E. Atkinson, *J. Biol. Chem.* **238**, 2875 (1963); R. Okazaki and A. Kornberg, *J. Biol. Chem.* **239**, 275 (1964); R. Haeckel, B. Hess, W. Lauterhorn, and K.-H. Würster, *Hoppe-Seyler's Z. Physiol. Chem.* **349**, 699 (1968).]

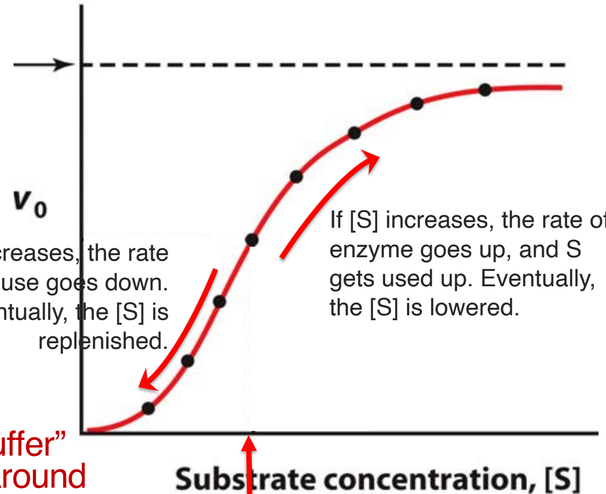
Enzyme Regulation

Control by Allostery:

Why has sigmoidal kinetics evolved?

$A \rightarrow S \rightarrow P \rightarrow Q$

- Not just to create ON/OFF switches
- Maintain homeostasis



This acts to "buffer" the activity at around ½V_{max}, often right at the homeostatic [S].

Physiological concentration of substrate.

Enzyme Regulation

Control by Allostery:

What is the degree of cooperativity?

Michaelis-Menton Equation

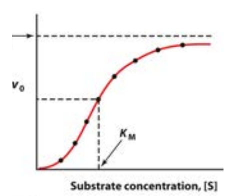
$$v_0 = \frac{V_{max}[S]}{K_m + [S]}$$

Like binding, we can also express as fraction of maximum rate

$$\frac{v_0}{V_{max}} = \frac{[S]}{K_m + [S]}$$

Recall for cooperative binding, there is an exponent term on the [S].

$$\frac{v_0}{V_{max}} = \frac{[S]^{n'}}{K'_m + [S]^{n'}}$$



What is K'_m term?

The K'_m term is a mixture of values for both poor- (at low [S]) and high-affinity (at higher [S]) binding sites.

What is the value of this n' term?

The n' term is related to the degree of cooperativity.

Enzyme Regulation

Control by Allostery:

In 1913, Archibald Hill derived an equation to measure both the K'_m term, and the n' term: The Hill Equation

$$\frac{\text{Fraction of } [E]_T \text{ as } [ES] \text{ (Bound)}}{\text{Fraction of } [E]_T \text{ as } [E] \text{ (Free)}} = \frac{Y}{1-Y} = \frac{\dfrac{[S]^{n'}}{K'_m + [S]^{n'}}}{1 - \dfrac{[S]^{n'}}{K'_m + [S]^{n'}}} \times \frac{(K'_m + [S]^{n'})}{(K'_m + [S]^{n'})}$$

Related to $\frac{v_0}{V_{max}}$

$$= \frac{[S]^{n'}}{K'_m}$$

Take log of both sides

Sometimes you will see:
n' = h, the Hill coefficient

$$\boxed{\log\left(\frac{Y}{1-Y}\right) = n' \log [S] - \log K'_m}$$

(Eqn for line → y = ax + b)

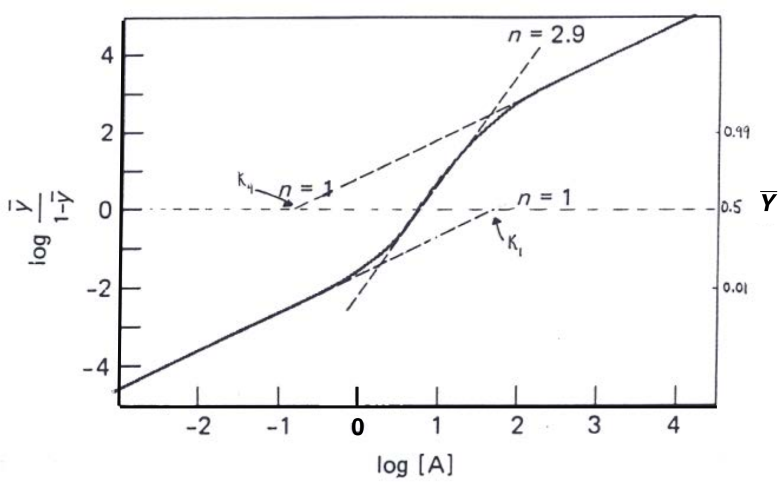
This has the form of a line with n' as the slope and $-\log K'_m$ as the x-intercept in a plot of log Y/(1-Y) *versus* log [S]. This is a "Hill Plot."

Enzyme Regulation

Control by Allostery:

The Hill Plot

$$\log\left(\frac{Y}{1-Y}\right) = n' \log [S] - \log K'_m$$



Theoretical maximum cooperativity = # of binding sites

Positive cooperativity: n > 1

Non-cooperative: n = 1 at low and high [S]

Negative cooperativity: n < 1

Control by Allostery:

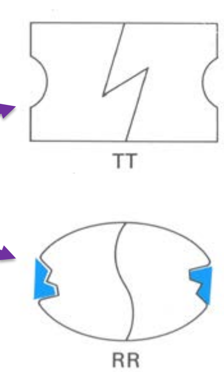
Enzyme Regulation

Physically, how does this cooperativity work?

In other words, how is this cooperativity accomplished at the molecular level?

Recall that sigmoidal behavior requires more than one subunit. Therefore, subunits must "communicate," or binding of one subunit changes the subunit-subunit interface, and this changes how the non-bound subunits will bind.

EXAMPLE:
- Dimer
- Two conformations
  - unbound enzyme = T (tense) state
  - bound enzyme = R (relaxed) state
- Conformation at interface differs
- Binding site differs with binding easier (tighter) in the R-state



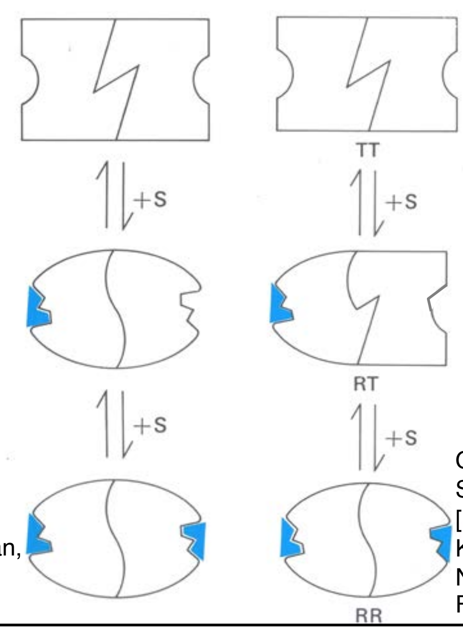
Control by Allostery:

Enzyme Regulation

Two ways have been proposed to explain the transition for T-state to R-state binding.

This type of cooperativity would be homotropic (caused by the substrate itself)

All at once = CONCERTED [Proposed by Monod, Wyman, & Changeux (MWC)]

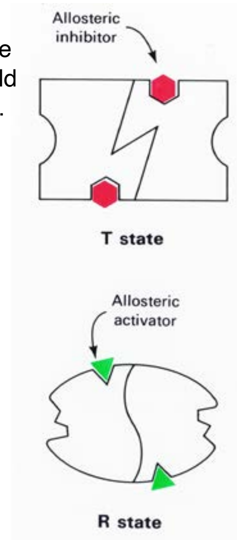


One at a time = SEQUENTIAL [Proposed by Koshland, Nemethy, & Filmore (KNF)]

Enzyme Regulation

Control by Allostery:

If it binds to the T-state, it would be an inhibitor. (negative heterotropic allosteric effector)



If it binds to the R-state, it would be an activator. (positive heterotropic allosteric effector)

Heterotropic cooperativity can be achieved by binding at a different allosteric site.

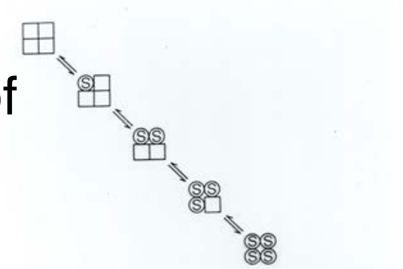
Enzyme Regulation

Control by Allostery:

How about for a tetramer?

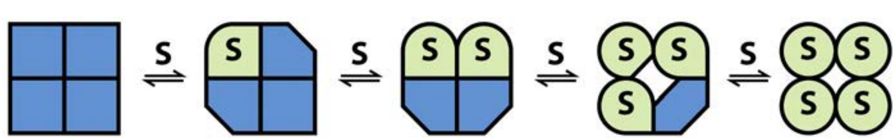
Sequential Model of cooperative regulation

The Koshland-Némethy-Filmer (KNF) sequential model



The KNF model for the binding of ligands to a tetrameric protein.

- S does not bind to the T-state.
- Binding of ligand to one subunit changes its conformation.
- That conformational change influences neighboring subunits through changes at the subunit-subunit interface.
- The binding to the neighboring subunits is easier (K_d decreases).
- Model has many different binding and dissociation constants (very complicated rate equations).



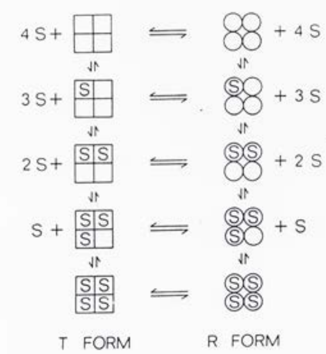
Control by Allostery:

Enzyme Regulation

Concerted Model of cooperative regulation

- Looks more complicated, but its simpler in concept and mechanism
- An equilibrium exists between the T-state and the R-state
- Binding of substrate is easier to the R-state
- Binding of substrate to one subunit shifts the equilibrium.
- That shift brings neighboring subunits, which are also high affinity; more "good" sites.

The Monod-Wyman-Changeux (MWC) concerted mechanism

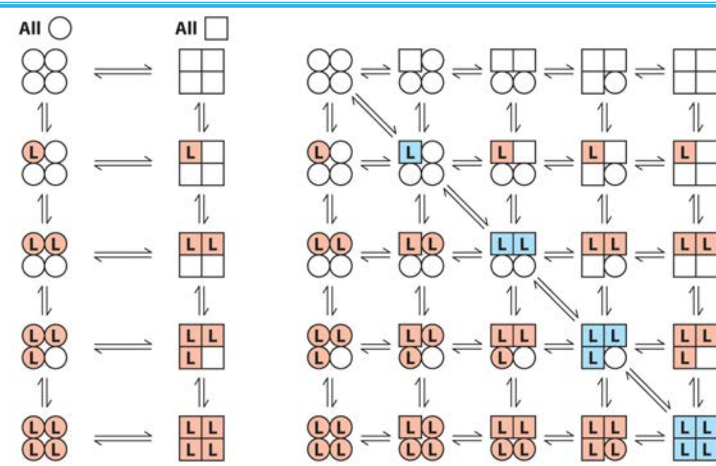


The MWC model for the binding of ligands to a tetrameric protein.

Control by Allostery:

Enzyme Regulation

Two Models of Cooperativity: Concerted vs. Sequential



All at once = CONCERTED [MWC]

One at a time = SEQUENTIAL [KNF]

Enzyme Regulation

Control by Allostery:

We can write the rate equation for the Concerted Model of cooperative regulation:

The equilibrium between the T-state and the R-state = $[T_0]/[R_0] = L$

Binding of substrate is easier to the R-state, which is reflected in a ratio of dissociation constants = c = K_R / K_T (less than 1.0).

A rate equation can be derived using these values: For a dimer…..

$$\frac{v_0}{V_{max}} = \frac{k_{cat}[ES]}{k_{cat}[E]_T} = \frac{[ES]}{[E]_T} = \frac{\text{Bound S}}{\text{Total sites}} = Y = \frac{\frac{[S]}{K_R}\left(1+\frac{[S]}{K_R}\right)^{(n-1)}}{L+\left(1+\frac{[S]}{K_R}\right)^{(n)}}$$

For dimer, exponent in denominator

If L=0 (no T-state) …….

$$Y = \frac{\frac{[S]}{K_R}}{\left(1+\frac{[S]}{K_R}\right)} = \frac{[S]}{K_R + [S]}$$

Recall eqn for coop:

$$\frac{v_0}{V_{max}} = \frac{[S]^{n'}}{K'_m + [S]^{n'}}$$

Enzyme Regulation

Control by Allostery:

$L = [T_0]/[R_0]$

$c = K_R / K_T$

($K_R << K_T$)

Allosteric constants for some proteins

Protein	Ligand	Number of binding sites (n)	Hill constant (h)	L	c	Ref.
Hemoglobin	O_2	4	2.8	3×10^5	0.01	1
Pyruvate kinase (yeast)	Phosphoenol-pyruvate	4	2.8	9×10^3 [a]	0.01[a]	2
Glyceraldehyde 3-pnosphate dehydrogenase (yeast)	NAD^+	4	2.3	60	0.04	3

[a] Estimated by the author.

1 S. J. Edelstein, *Nature, Lond.* **230**, 224 (1971).

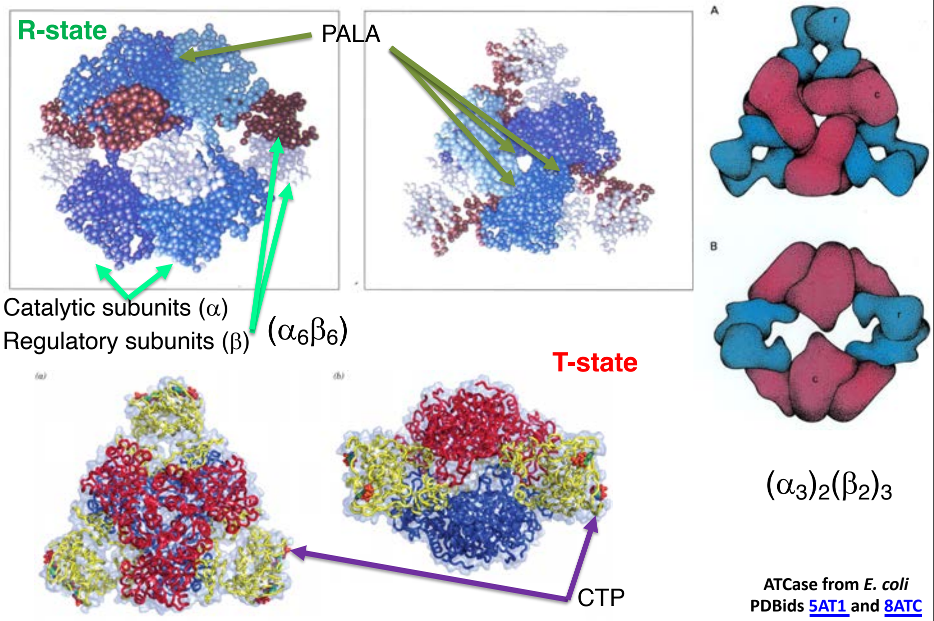
2 R. Haeckel, B. Hess, W. Lauterhorn, and K.-H. Würster, *Hoppe-Seyler's Z. Physiol. Chem.* **349**, 699 (1968); H. Bischofberger, B. Hess, and P. Röschlau, *Hoppe-Seyler's Z. Physiol. Chem.* **352**, 1139 (1971).

3 K. Kirschner, E. Gallego, I. Schuster, and D. Goodall, *J. Molec. Biol.* **58**, 29 (1971).

Is there structural evidence for these T- and R-states?

Enzyme Regulation

Control by Allostery: **Aspartate Transcarboxylase (ATCase)**



ATCase from *E. coli*
PDBids 5AT1 and 8ATC

Enzyme Regulation

Control by Allostery:

ATCase: Conformational Changes: **T-State vs. R-State**

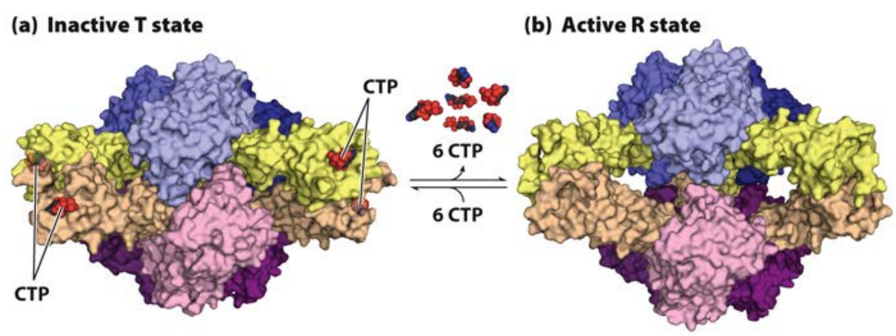
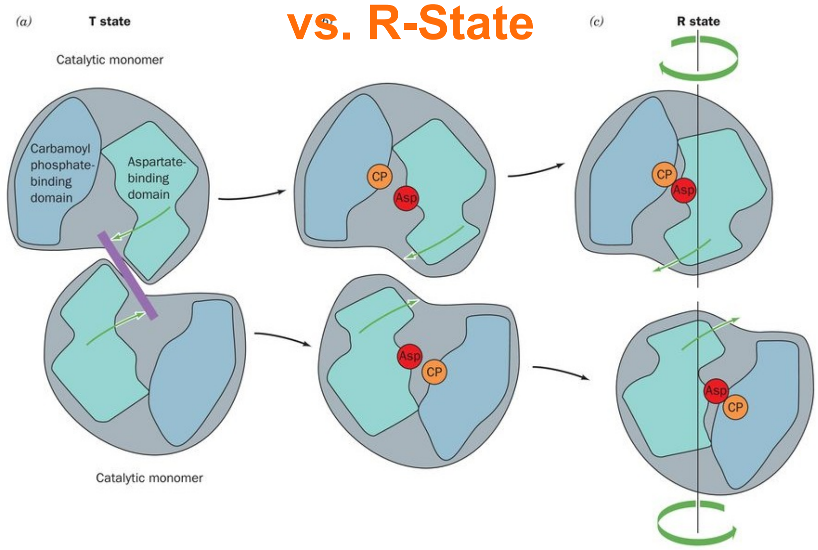


Figure 6-34

Enzyme Regulation

Control by Allostery:

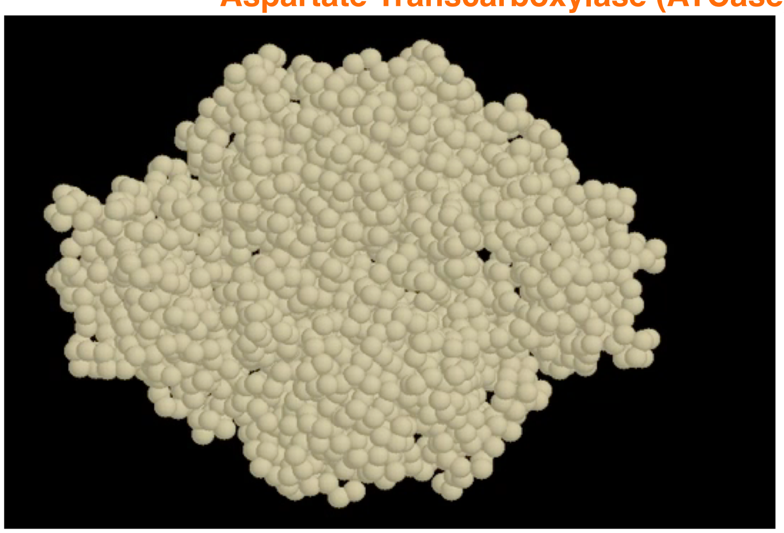
ATCase: Conformational Changes: **T-State vs. R-State**



Enzyme Regulation

Control by Allostery:

Aspartate Transcarboxylase (ATCase)



http://garlandscience.com/garlandscience_resources/resource_detail.jsf?landing=student&resource_id=9780815344469_CH03_QTM02

Enzyme Regulation

Key Concepts for Control of Enzyme Activity

•Allosteric effectors bind to multisubunit enzymes, such as aspartate transcarbamoylase, thereby inducing cooperative conformational changes that alter the enzyme's catalytic activity (T and R states).

•Phosphorylation and dephosphorylation of an enzyme such as glycogen phosphorylase by protein kinases can control its activity by shifting the equilibrium between more active and less active conformations (T and R states).

You should be able to:

• Compare and contrast the actions of an allosteric effector, a competitive enzyme inhibitor, and a noncompetitive inhibitor.
• Explain the structural basis for cooperative substrate binding and allosteric control in ATCase.
• Why are such allosteric enzymes composed of more than one catalytic subunit?
• Distinguish between KNF and MWC models for cooperativity and how homotropic and heterotropic effectors act
• Understand what can be derived from a Hill Plot