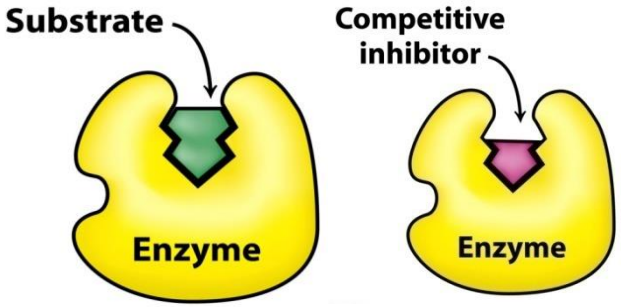
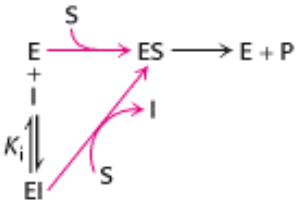
2.2 Reversible Inhibitors

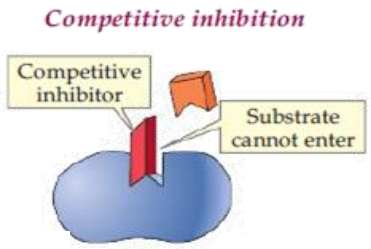
- **Characterized by a rapid dissociation of the enzyme-inhibitor complex**
- **Usually these inhibitors bind through non-covalent interactions & inhibitor maintains a reversible equilibrium with the enzyme**
- **Reversible inhibitors can be divided into two classes: competitive & noncompetitive**
- **The double-reciprocal plots are highly useful for distinguishing among these inhibitors**

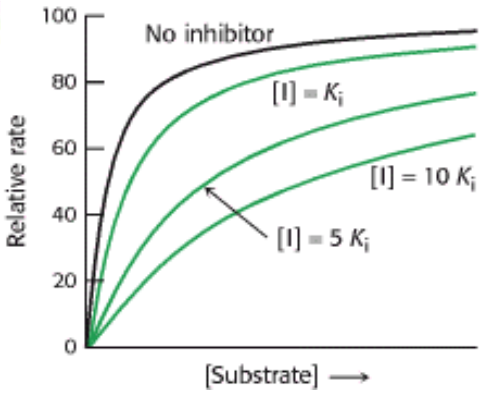
2.2.A. Competitive inhibition



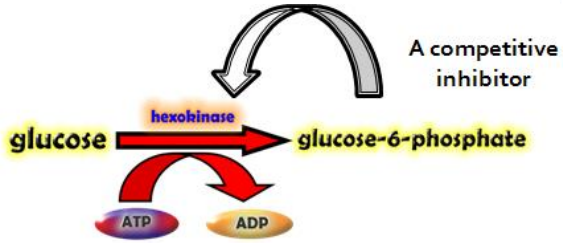


- **The inhibitor competes with substrate**

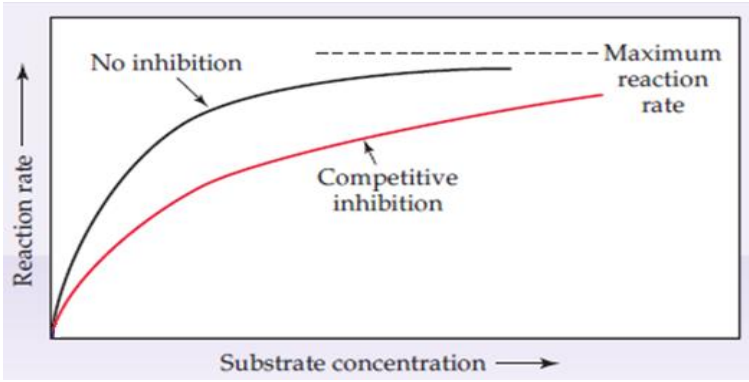




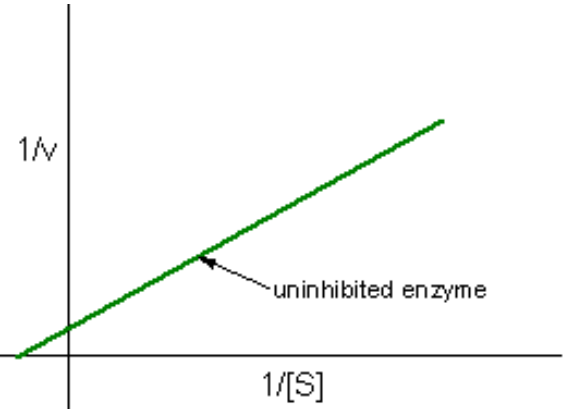
- **Increasing [S] can overcome the inhibition (V_{max})**

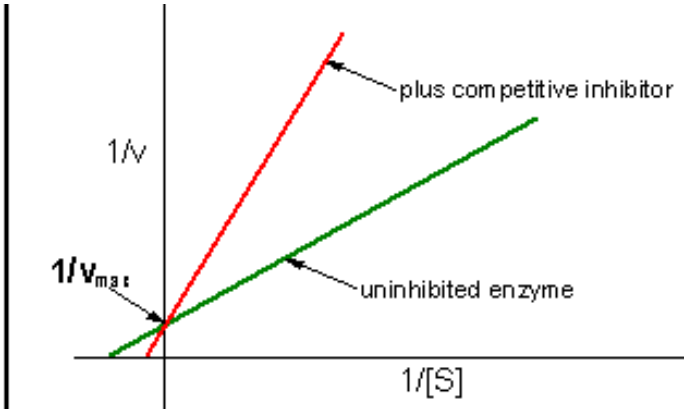


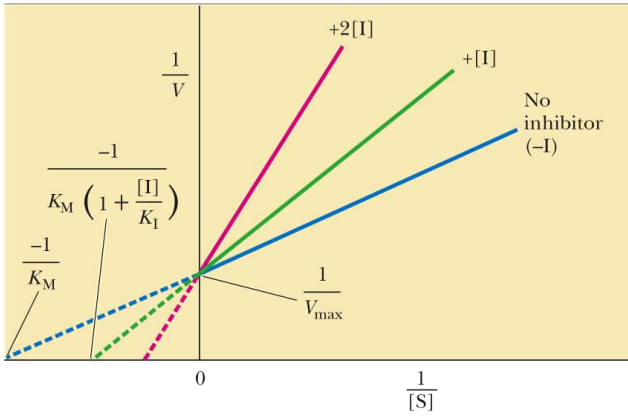
- **Does K_M change?**



- **Significance (ex. Hexokinase)**

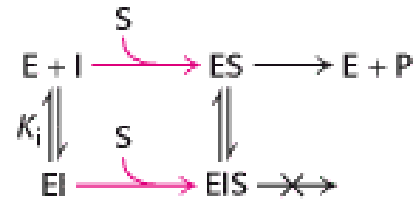


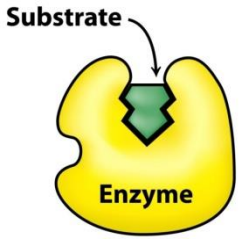


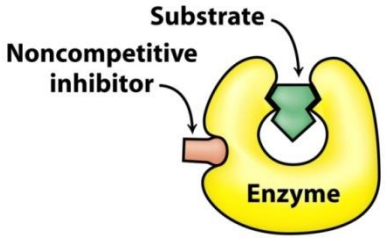


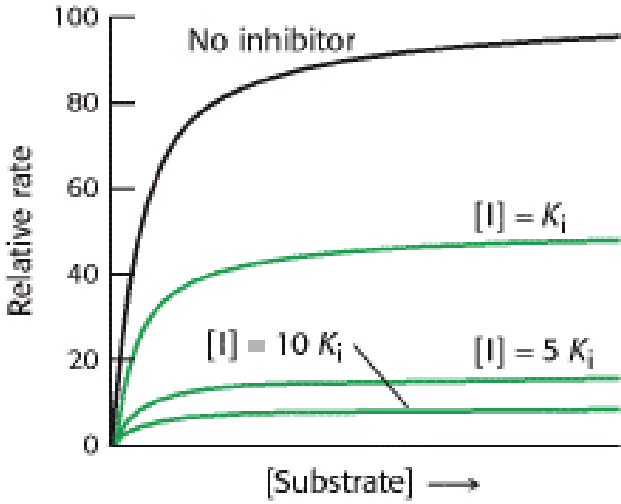
2.2.B. Noncompetitive inhibition

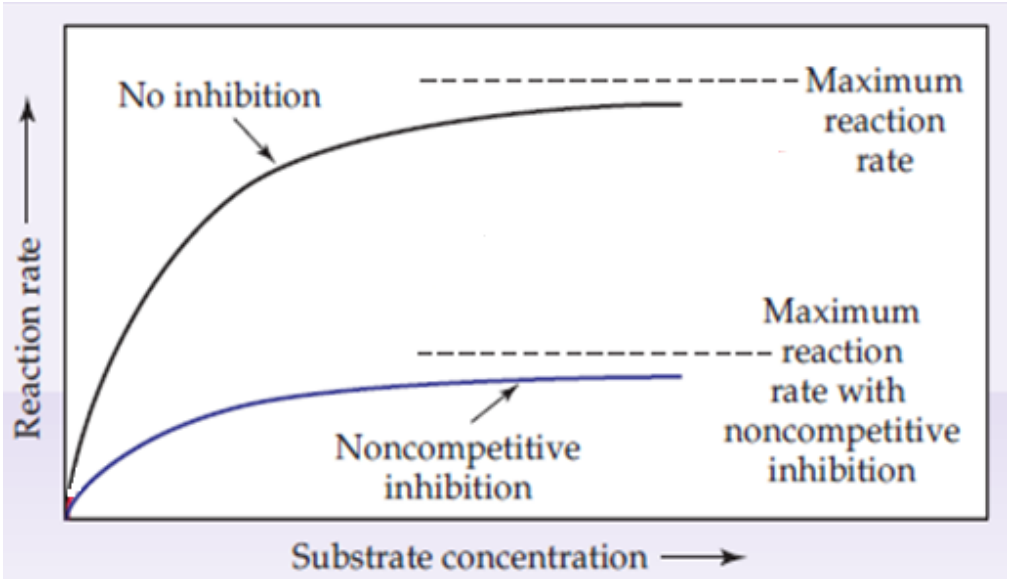
- **The inhibitor binds at a site other than the active site**
- **The complex does not proceed to form product or has a lower efficiency**
- **V_{max} vs. K_M**
- **Can we reach V_{max}?**

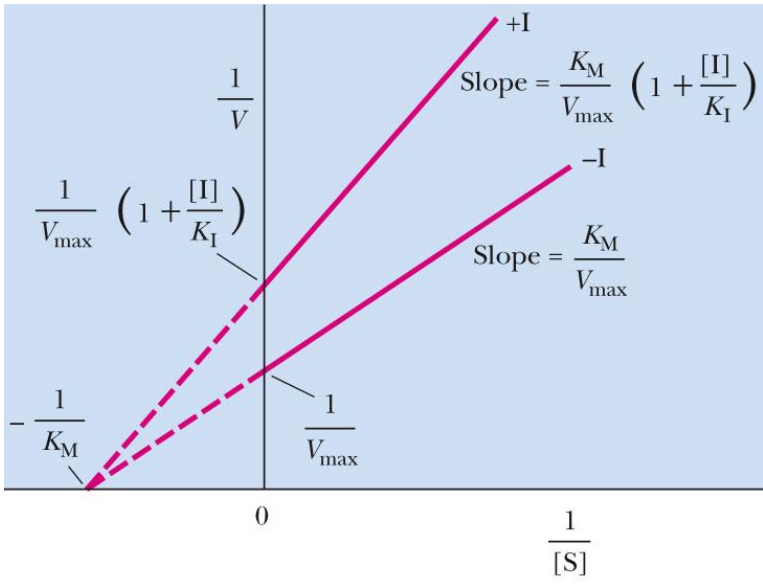












3. REGULATION THROUGH CONFORMATIONAL CHANGES

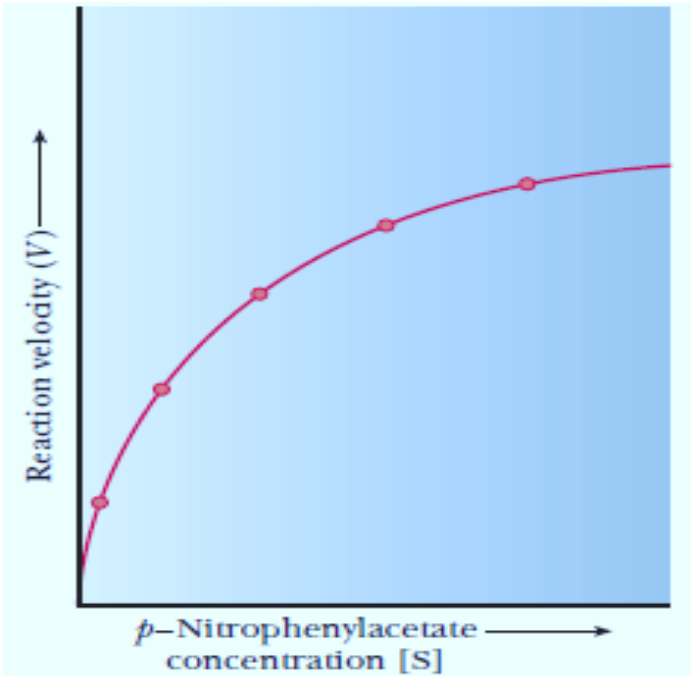
- **These regulatory mechanisms include**
  - **A. Allosteric activation and inhibition;**
  - **B. Phosphorylation or other covalent modification;**
  - **C. Protein-protein interactions between regulatory & catalytic subunits or between two proteins;**
  - **D. Proteolytic cleavage**

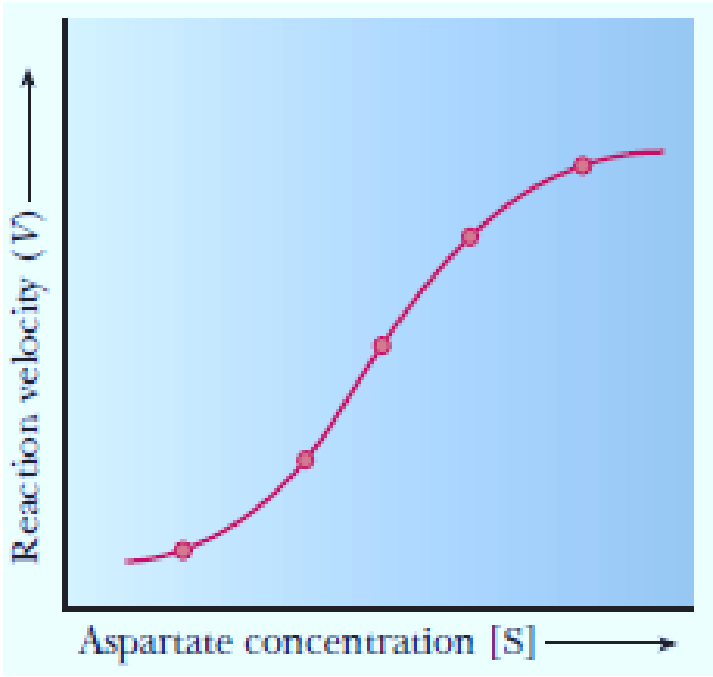
- **These types of regulation can rapidly change an enzyme from an inactive form to a fully active conformation**

3.A. Not all enzymes follow Michaelis-Menten equation; Chymotrypsin vs. ATCase

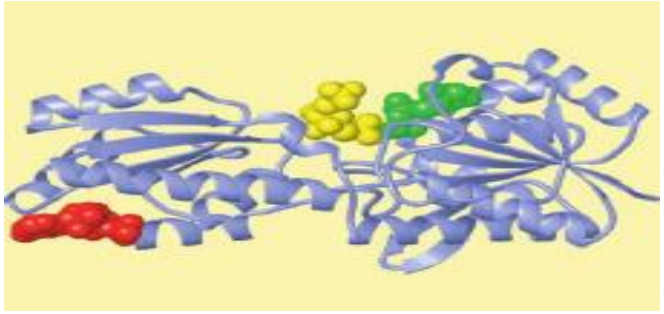
- **Chymotrypsin: Specificity for aromatic residues mainly. Also, hydrolysis of ester bonds**
- **Aspartate transcarbamoylase (ATCase): synthesis of CTP & UTP for RNA and DNA synthesis**

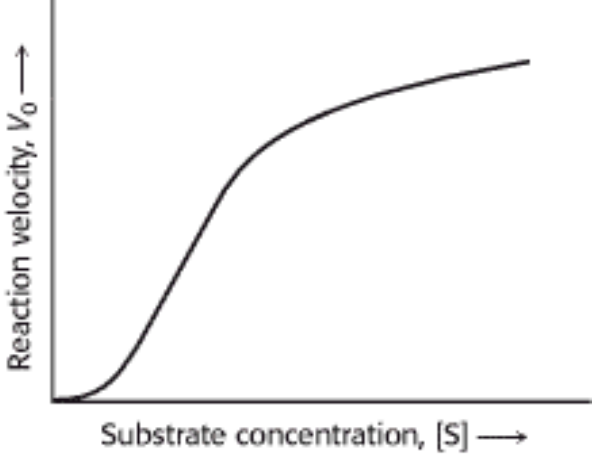
$$\text{Carbamoyl phosphate} + \text{Aspartate} \rightarrow \text{Carbamoyl aspartate} + HPO_4^{2-}$$



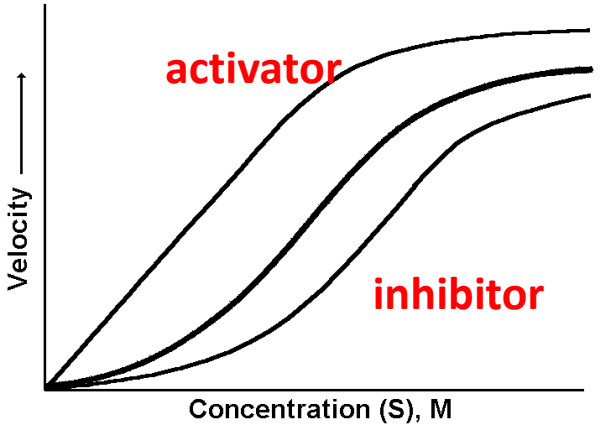


Allosteric regulation





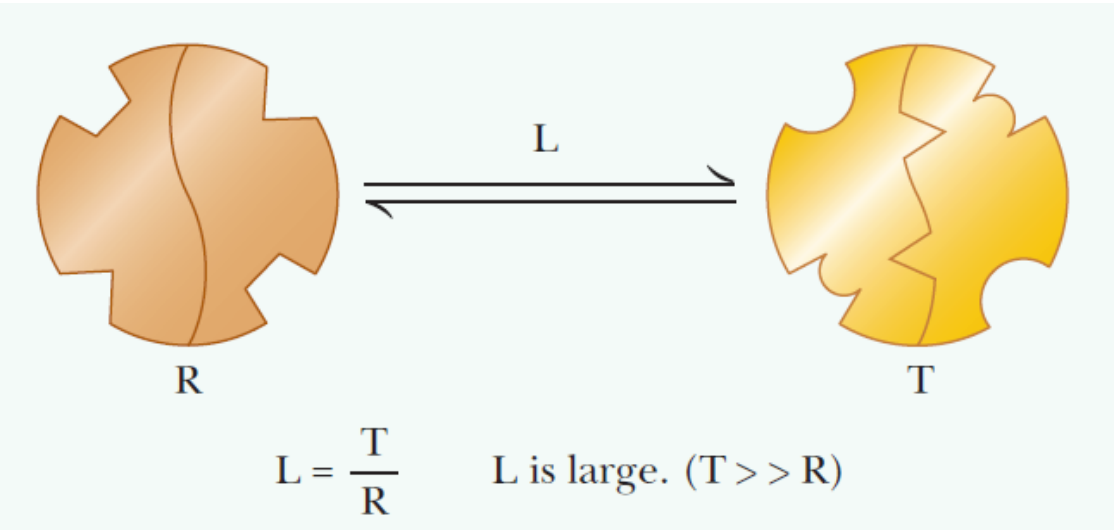
- **What are allosteric enzymes? A multi-subunit enzyme with catalytic subunit(s) and regulatory subunit(s)**
- **Binding triggers a conformational change in the active site**
- **The Michaelis-Menten model can't explain the kinetic properties**
- **The effect of the modulators (allosteric modifiers)**
- **Homotropic vs. heterotropic**
- **The substrate concentration at half of the V_{max} is called ($K_{0.5}$)**
- **Allosteric inhibitors have a much stronger effect on enzyme velocity**

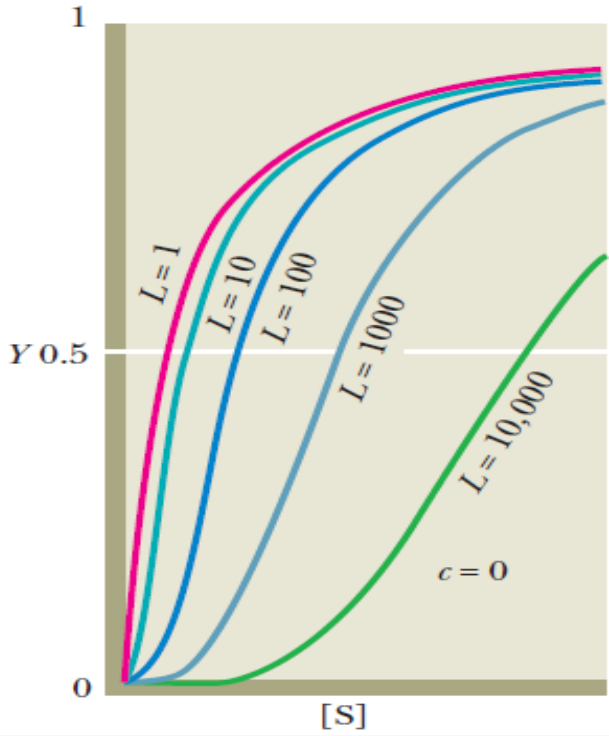


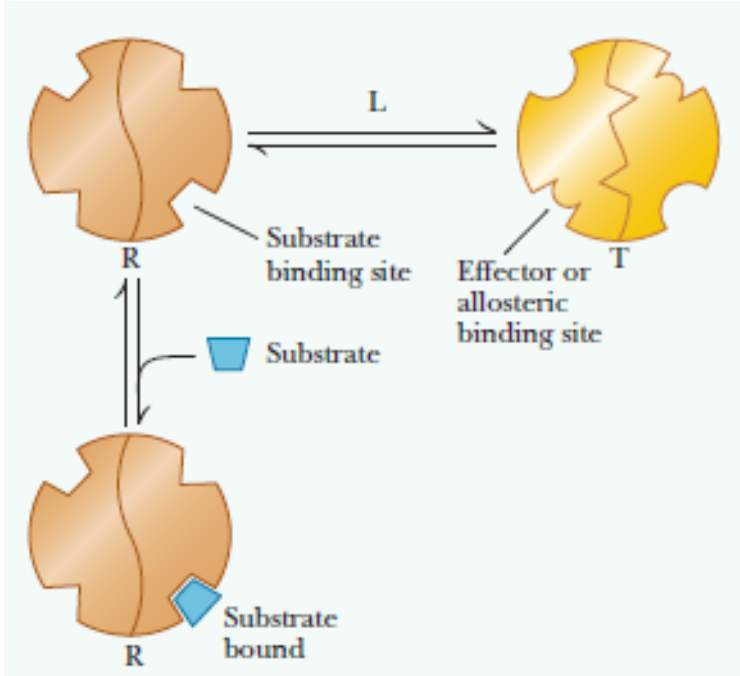
The effect of modifiers on V_{max} & $K_{0.5}$

How do allosteric enzymes work?

- Two conformations: more active (R) & less active or inactive (T),
- The equilibrium ratio (T/R) is called L and assumed to be high
- As L (T/R) increases, the shape becomes more sigmoidal

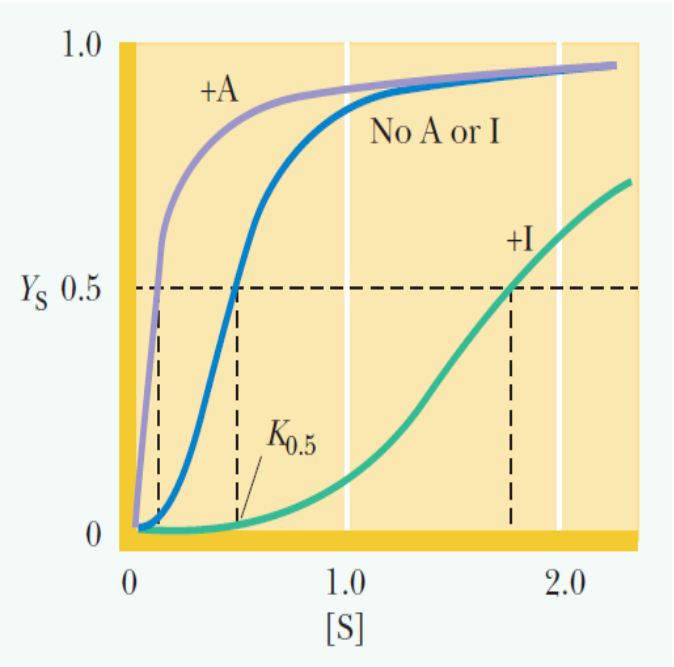


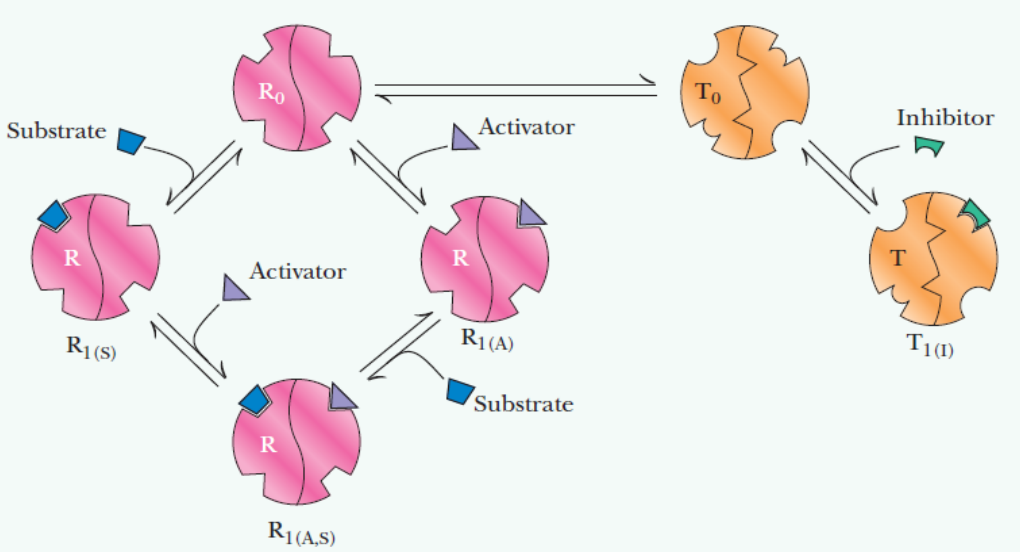


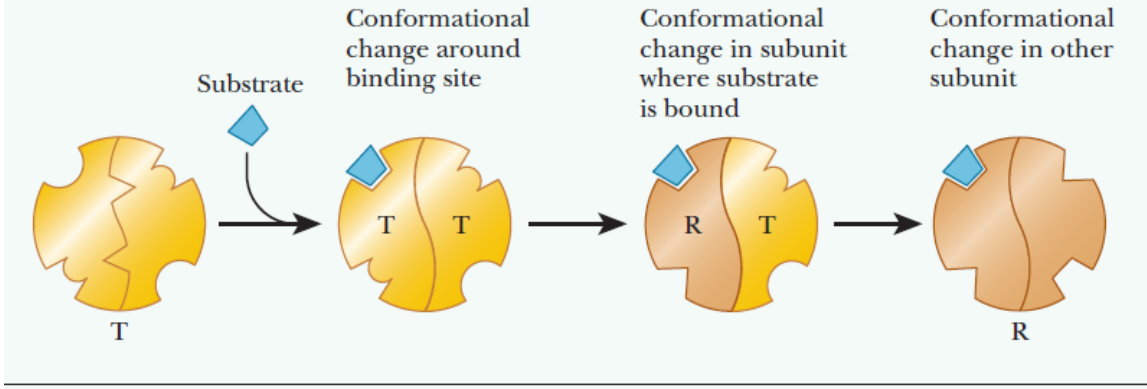


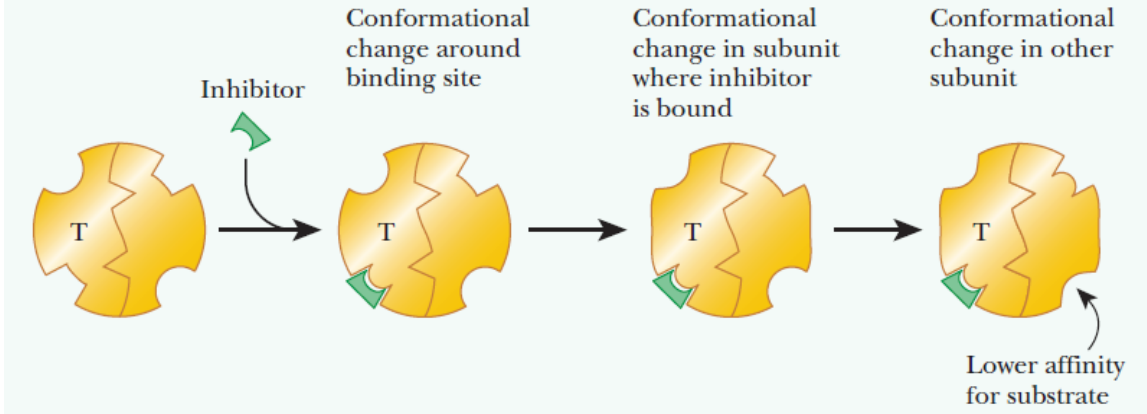
Concerted or sequential?

- **Either substrate or activator must be increased to overcome the effects of the allosteric inhibitor**
- **Conformational change**

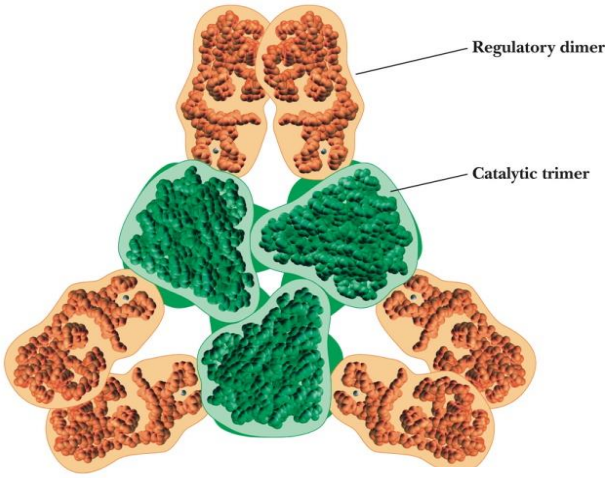








Allosteric regulation – ATCase "synthesis of pyrimidine nucleotides"



- **ATCase and Hb are allosteric proteins (cooperative behavior)**
- **Catalytic can be separated from regulatory (hyperbolic)**
- **Cooperativity in relation to substrate**
- **CTP is an inhibitor of ATCase (feedback inhibition), ATP is an activator**