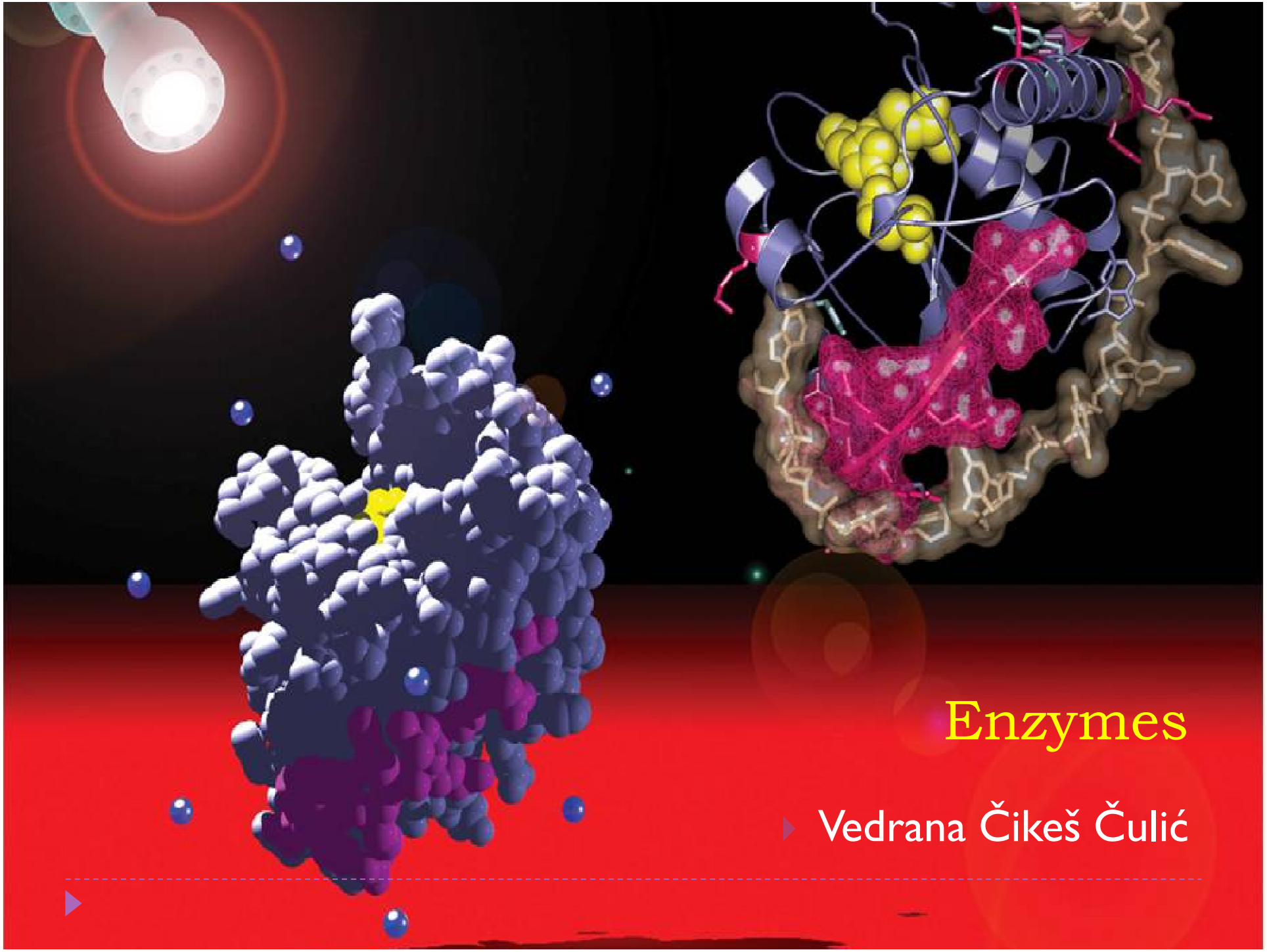
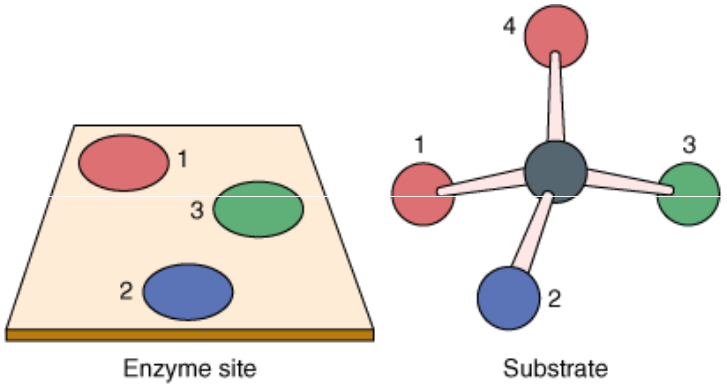


Enzymes

Vedrana Čikeš Čulić

Enzymes = catalysts

- PROTEINS
- SUBSTRATE(S) to PRODUCT(S)
- High catalytic efficiency – enhance the rate of reaction to 10^6 – $1o^9$ times
- EXTREMELY SPECIFIC:
  - catalyze only one or a small set of closely related reactions
  - absolute stereospecificity



- no side-products
- activity can be regulated

Classification of enzymes

- Classified depending on the TYPE OF REACTION they catalyzed
- SIX classes
- NAME OF ENZYME: type of substrate + -ase

- ATPase – degradation of ATP
- ATP synthase – synthesis of ATP

Main enzyme classes

- Oxidoreductases — oxidations and reductions
- Transferases —transfer of moieties
- Hydrolases —*hydrolytic* cleavage of C—C, C—O, C—N and other covalent bonds
- Lyases —cleavage of C—C, C—O, C—N and other covalent bonds by *atom elimination,* generating double bonds
- Isomerases —geometric or structural changes *within* a molecule
- Ligases —joining together (ligation) of two molecules in reactions coupled to the hydrolysis of ATP

IUB classification

- International Union of Biochemists (IUB), Enzyme Commission (EC)
- EC 4 digit mark

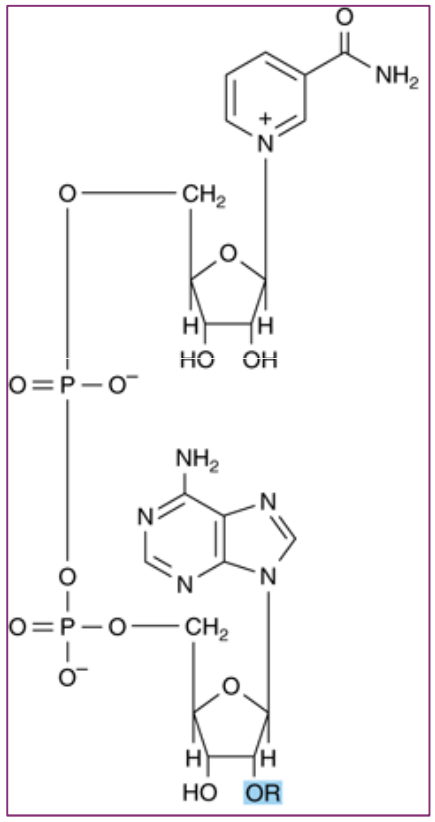
- Example: nucleoside-monophosphate-kinase EC 2.7.4.4.
  Catalyzes:

$$ATP + NMP \rightarrow ADP + NDP$$

1. number: class (transferase)
2. number: subclass (transferase which transfers phosphate group)
3. number: subsubclass (phosphate group as acceptor)
4. number: precisely describes acceptor (nukleoside monophosphate)

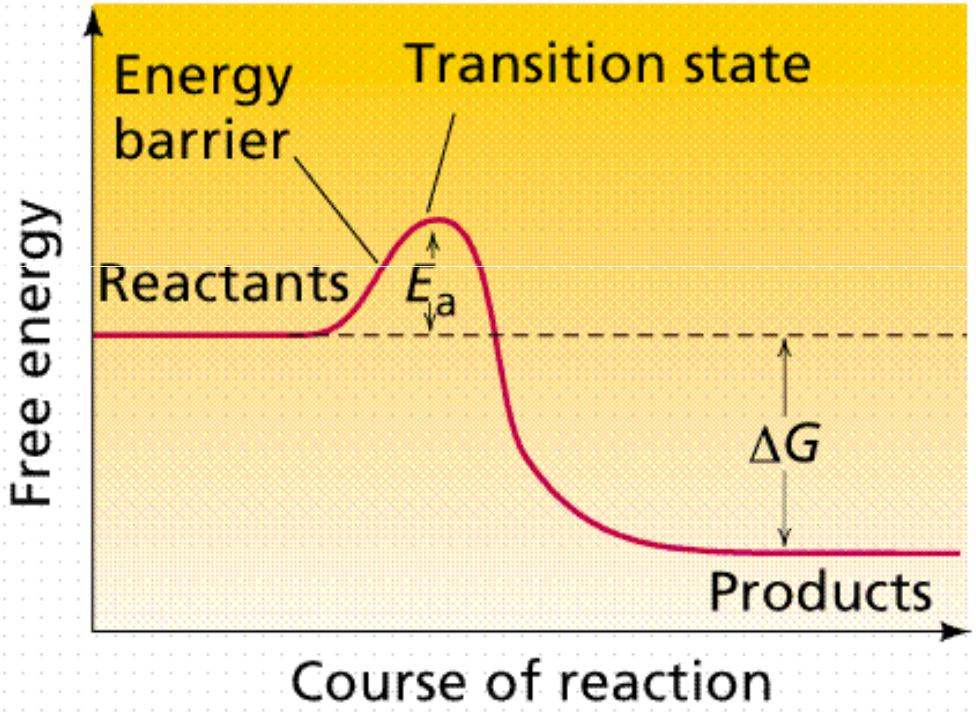
Prosthetic Groups, cofactors & coenzymes

- PROSTETIC GROUPS – tightly integrated into the enzyme structure (e.g. metals in metalloenzymes)
- COFACTORS – bind in dissociable manner to enzyme or substrate (e.g. metals in metal-activated enzymes)
- COENZYMES – recyclable shuttles; stabilize reactive species and facilitate binding of substrate to enzyme



NAD(P)$^+$

Reaction kinetics



ACTIVATION ENERGY $\Delta G_F = E_a$

$\Delta G_F = E_a = G_{transitional\ state} - G_{substrate}$

- energy required for formation of products from reactants
- determines the reaction rate

FREE REACTION ENERGY ΔG

$\Delta G = \Delta G_{products} - \Delta G_{reactants}$

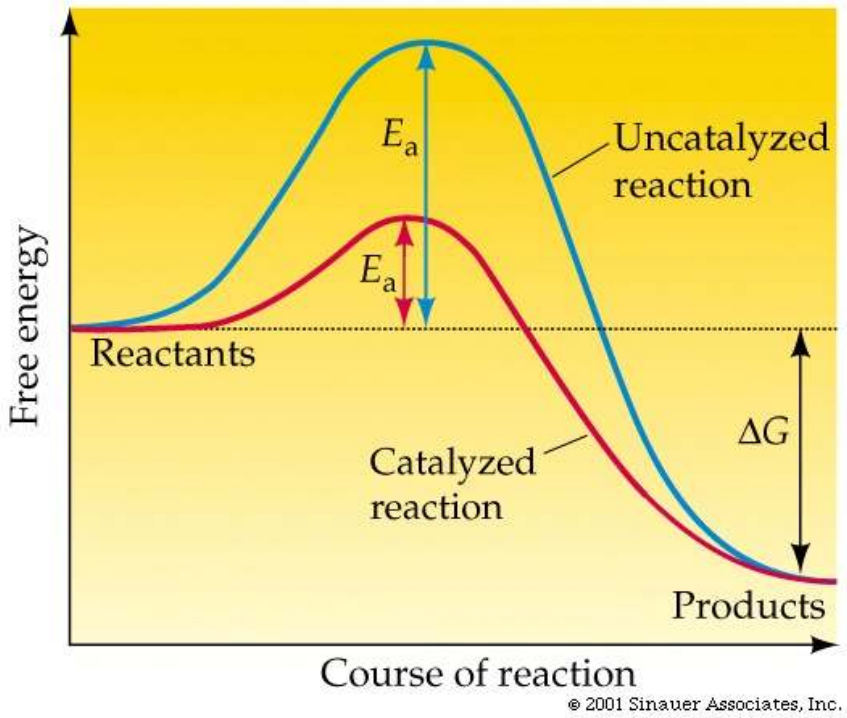
- is independent of the mechanism of the reaction
- provides no information concerning *rates* of reactions
- describes *spontaneity* and the *direction* in which a chemical reaction will tend to proceed

Advantage of enzymatic catalysis comparing to chemical catalysis

- CHEMICAL catalysis often requires:
  - high temperature
  - pressure
  - extreme pH values

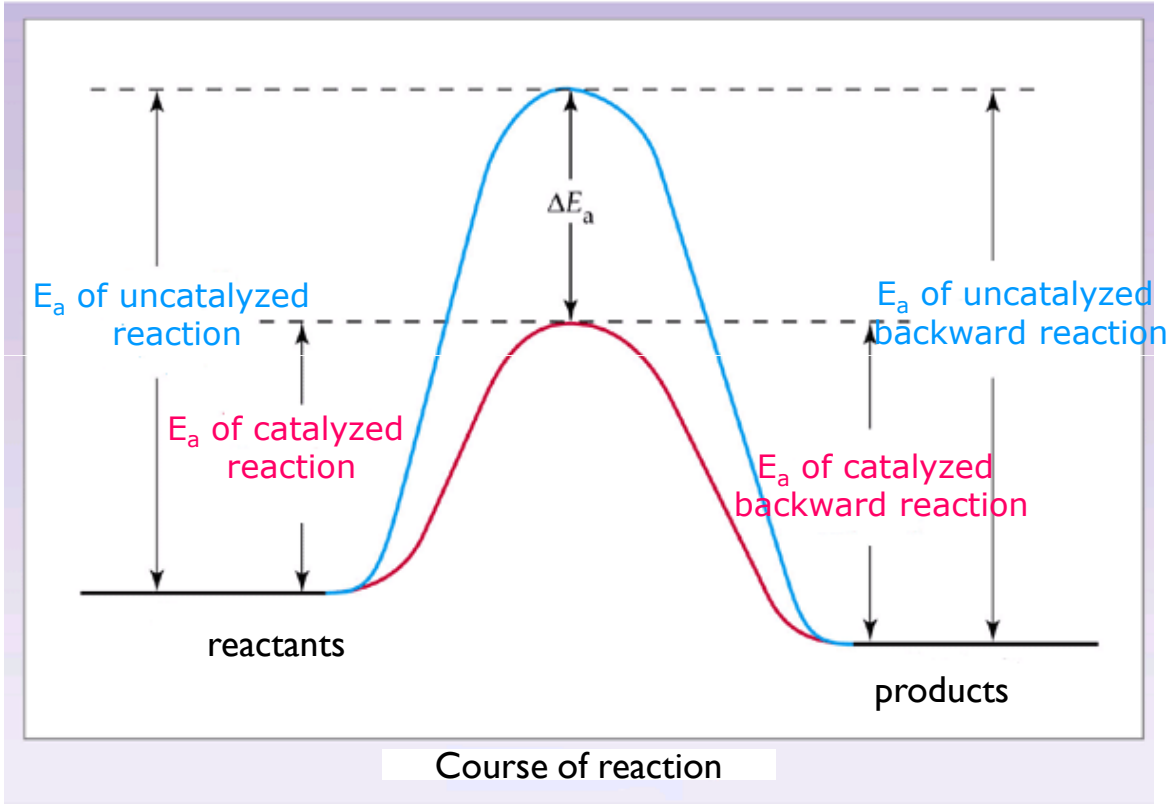
- ENZYMATICALLY catalyzed reactions proceed in milder reaction conditions
  - enzymatic catalysis is effective in physiological conditions (pH=7, T=37 °C)

Enzymes lower the activation energy barrier for a reaction



- Enzymes facilitate the formation of transition states
- Substrates: reactants in enzymatically catalyzed reactions

Basic characteristics of enzymatic catalysis



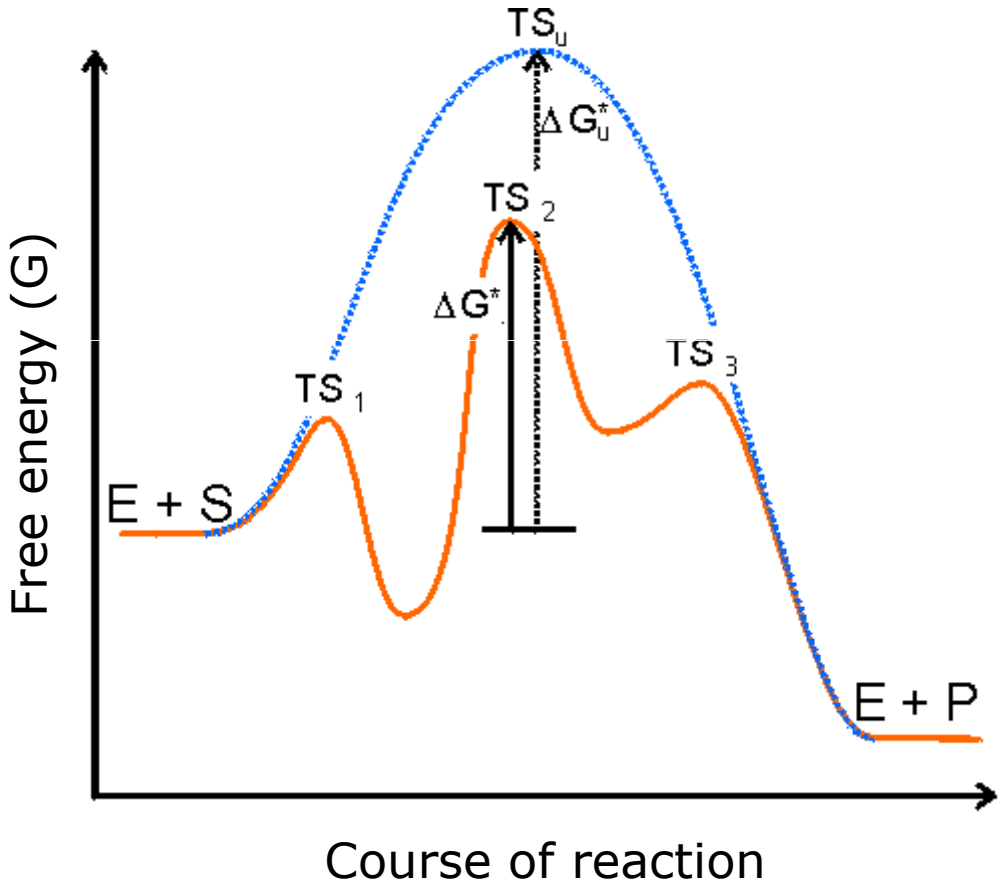
k_1, k_{-1} = constants of chemical reaction rates

$$A \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} B \qquad K = \frac{k_{-1}}{k_1}$$

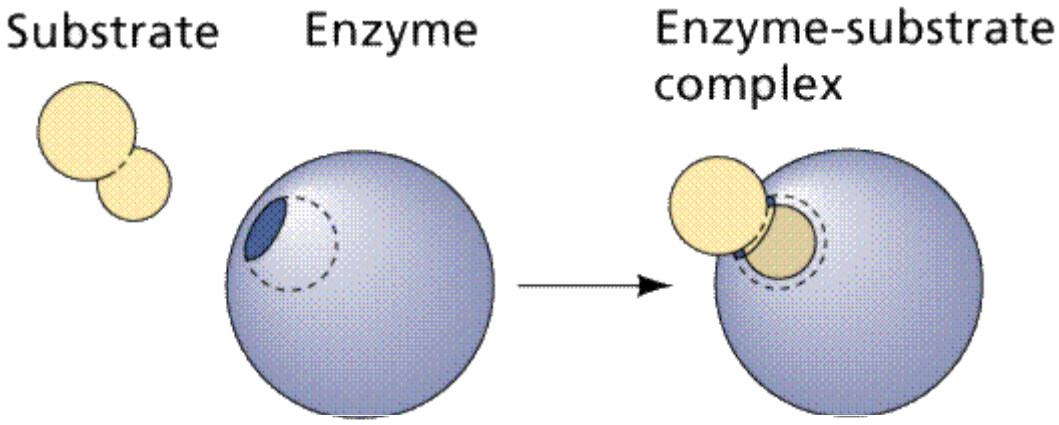
- Enzymes enhance the rates of both way reactions equally
- They accelerate the achievement of equilibrium, but do not alter the position of equilibrium
- Enzymes accelerate only thermodynamically favorable reaction ($\Delta G < 0$)

Enzymatically catalyzed reactions often involve more reaction stages

- Catalyzed reaction involves few transition stages (TS_1, TS_2, TS_3)
- Total reaction rate is determined by the slowest stage – stage with the highest activation energy

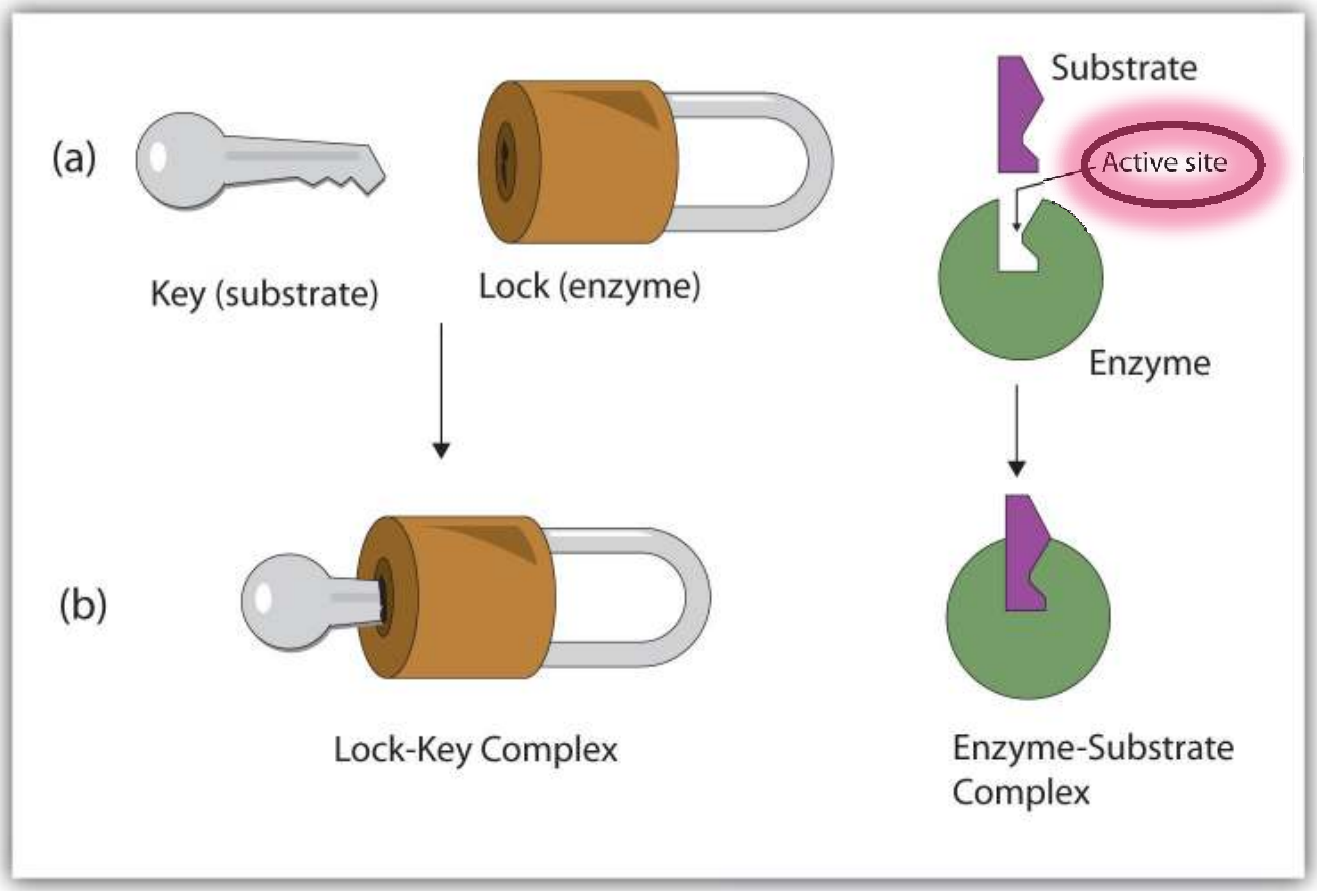


Formation of enzyme-substrate complex is the first step in the enzymatic catalysis



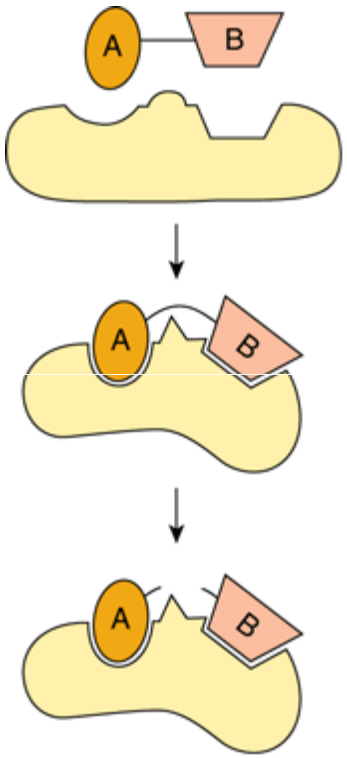
- Reaction of enzyme and substrate is very specific
- Substrate binds to specific site on enzyme: ACTIVE SITE
- ES complex approaches substrates and directs them favorably for reaction
- Interaction of enzyme and substrate in active site induces a formation of transition state

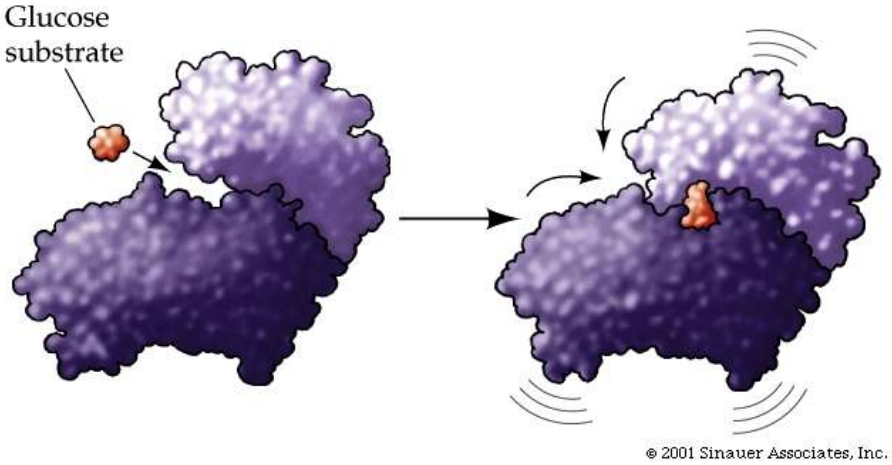
Key – Lock model



Active site of enzyme has the shape complementary to the shape of substrate

Induced fit model

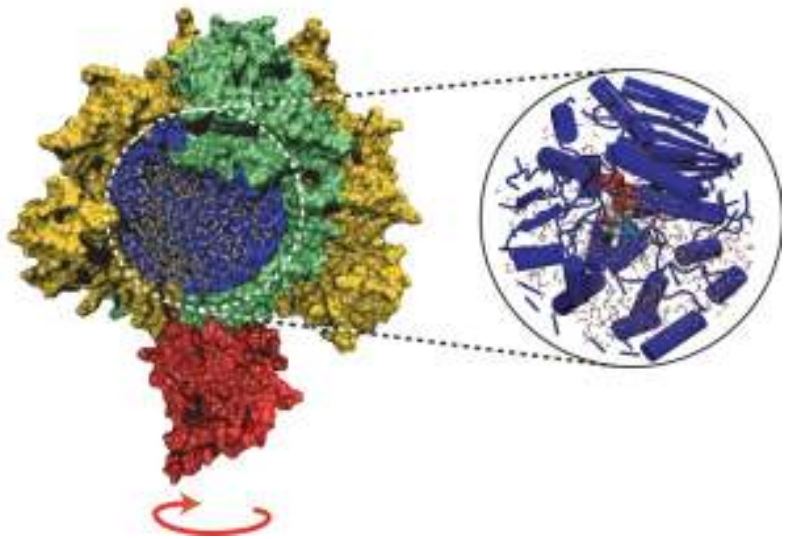




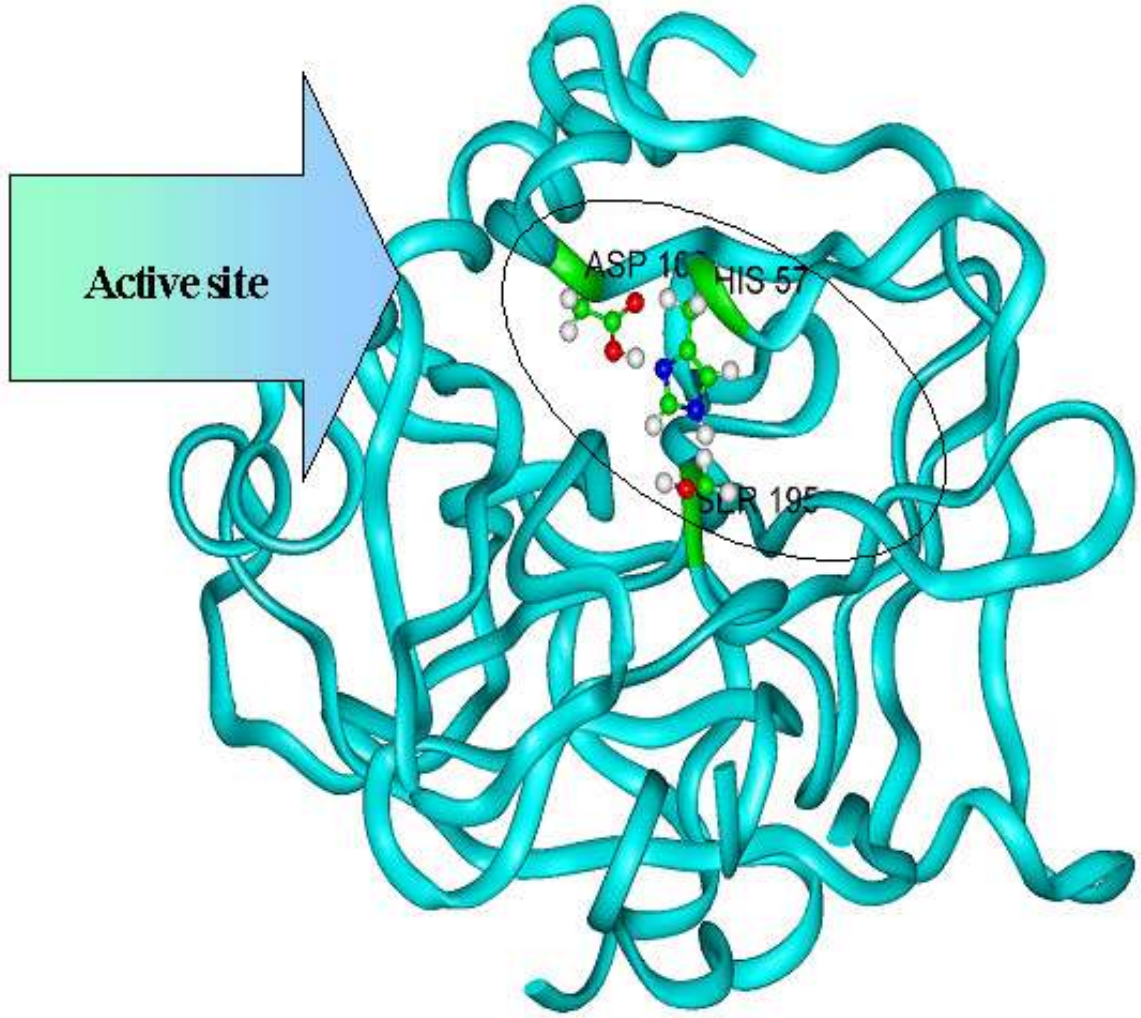
Complementary shape of active site is formed after binding of substrate to enzyme

Active site

- Takes small part of the enzyme
- Three dimensional gaps in the structure of the enzyme
- Contains binding amino acids which bind substrates and cofactors with weak forces
- Contains catalytically important amino acids involved in formation and cleavage of chemical bonds
- Specificity of binding depends on precisely determined rearrangement of atoms in active site

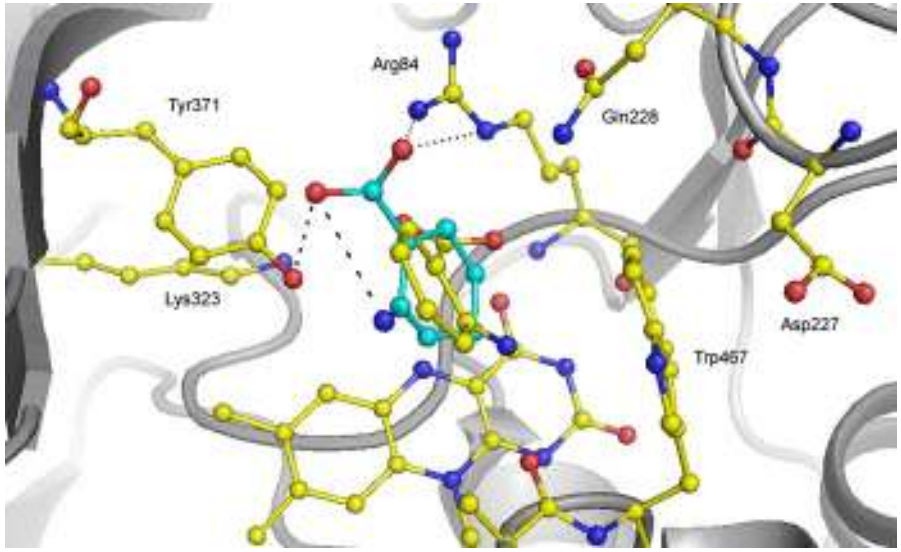


Active site includes amino acids which are distant in primary structure



Substrate binds to enzyme with weak forces

- ES complex is formed of weak non-covalent interactions between linking amino acid residues in enzyme active site

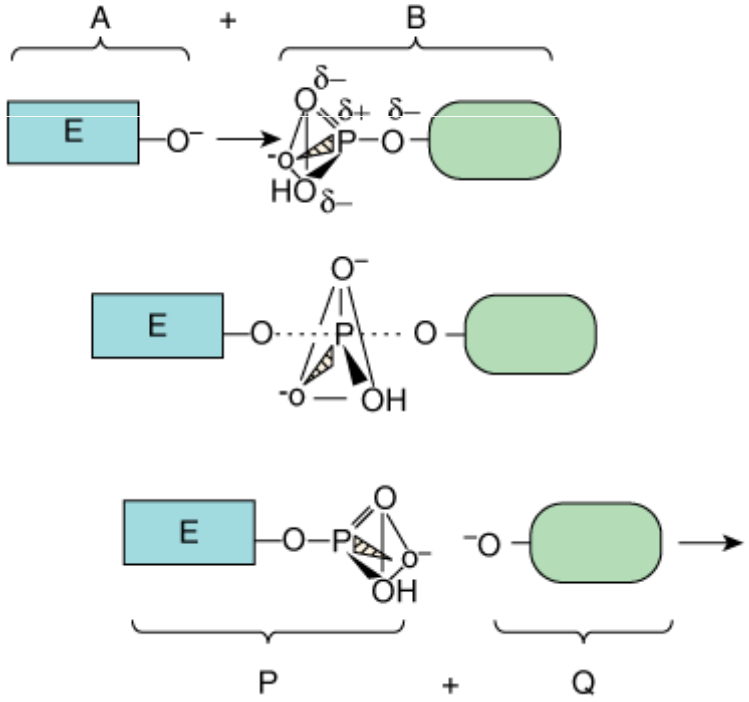


Reversible interactions in ES complex

- Electrostatic interactions (6 kJ/mol) depend on ionization groups electric charge of enzyme and substrate
- H-bonds between proton donor and proton acceptor
- Van der Waals interactions (2-4 kJ/mol) as a consequence of asymmetric arrangement of electronic cloud
- Hydrophobic interactions: association of non-polar molecules increases water entropy

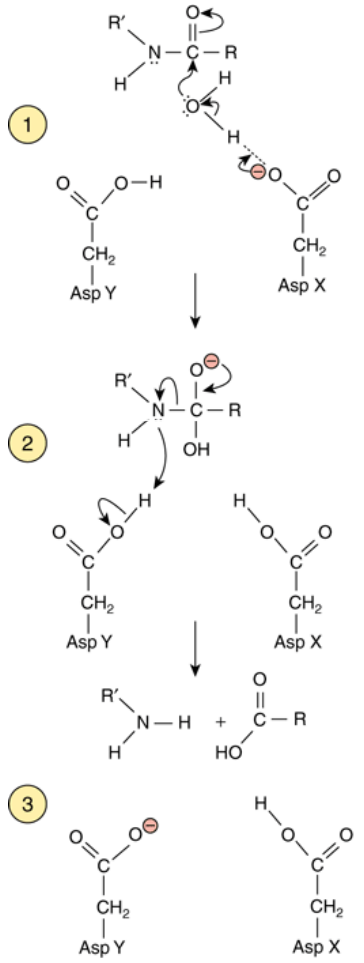
Catalytic amino acid residues

- Participate in CLEAVAGE and FORMING of covalent bonds
- Stabilize transition state



Enzymes employ multiple mechanisms to facilitate catalysis

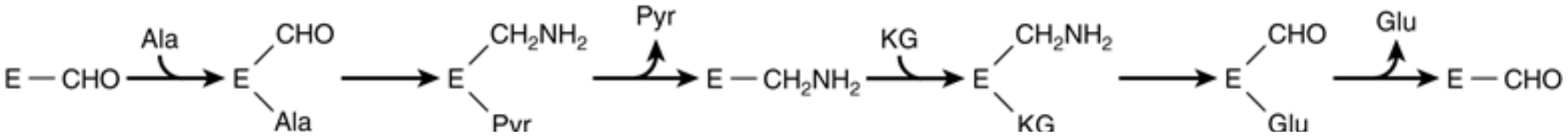
- Acid–Base Catalysis
  - proton transfer from the donor, or to proton acceptor, reduces the energy of the transition state



Mechanism for catalysis by an aspartic protease such as HIV protease

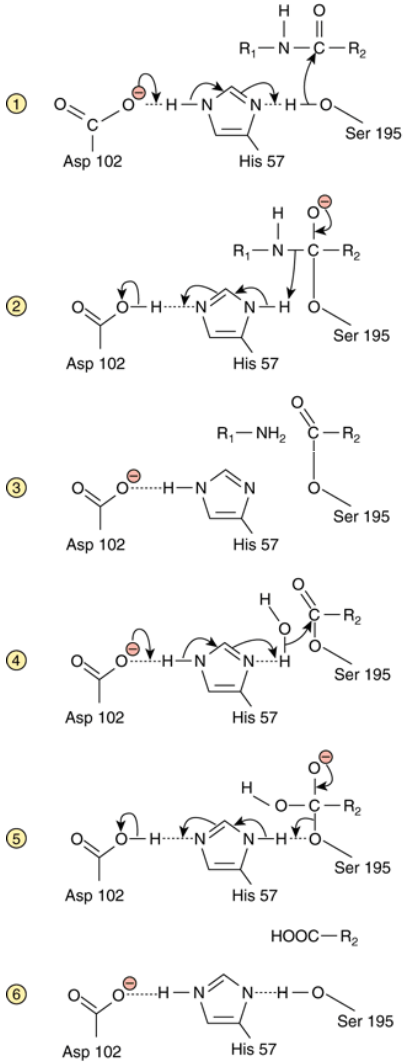
Covalent catalysis

- during catalysis specific reactive groups in the active site of the enzyme is temporarily covalently modified

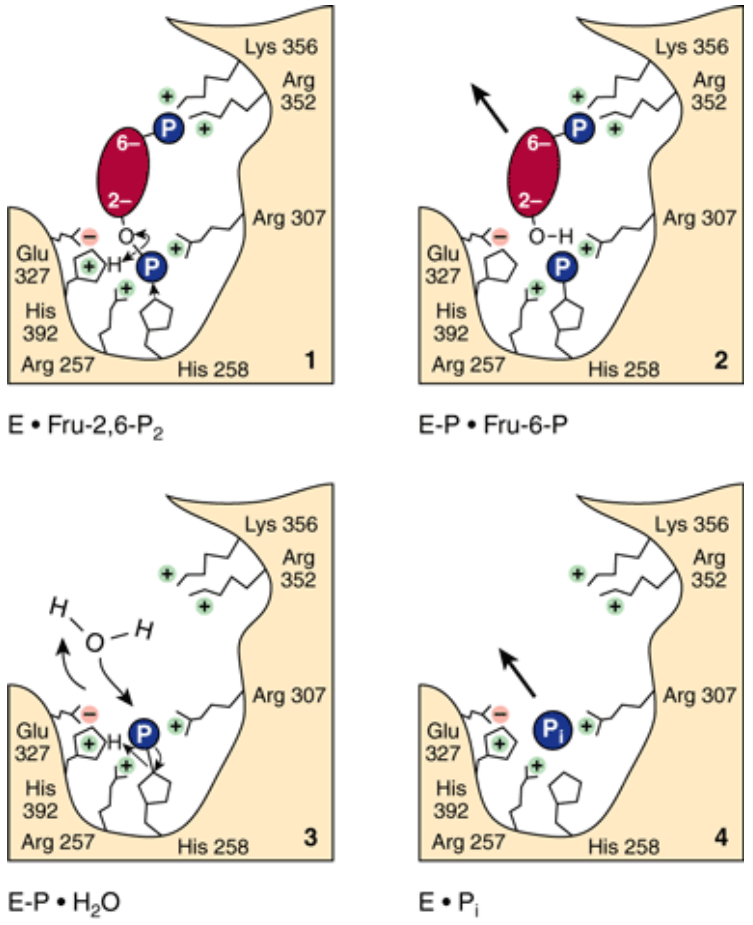


"Ping-pong" mechanism for transamination

Covalent catalysis



Catalysis by chymotrypsin



Catalysis by fructose-2,6-bisphosphatase

Amino Acid Sequences in the Neighborhood of the Catalytic Sites of Several Bovine Proteases

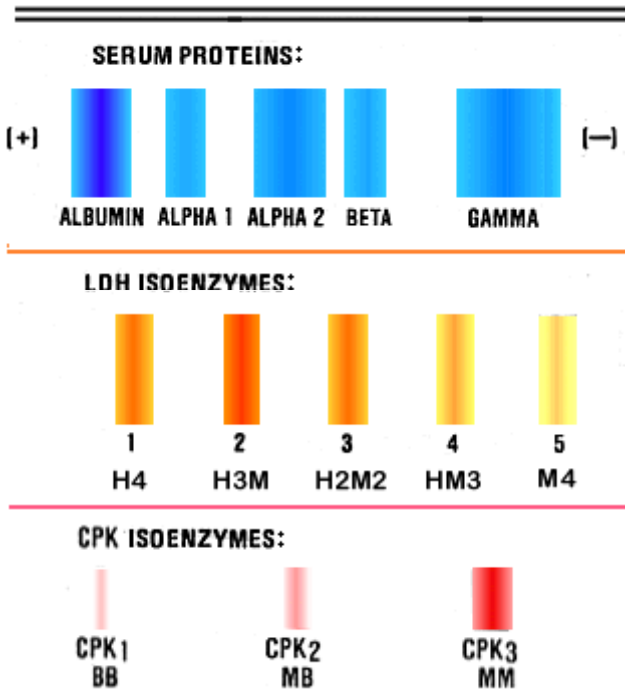
Enzyme	Sequence Around Serine	Sequence Around Histidine
Trypsin	D S C Q D G G G PVV C S G	K V V S A A C Y K S G
Chymotrypsin A	S S C M G D G G P L V C K K	N V V T A A G G V T T
Chymotrypsin B	S S C M G D G G P L V C Q K	N V V T A A C G V T T
Thrombin	D A C E G D G G P F V M K S	P V L T A A C L L Y P

Catalytic residues are highly conserved

- Catalysis with metal ion
  - electrophilic catalyst that stabilizes the negative charge
- Catalysis by Proximity
  - reactants approaching accelerates the chemical reaction

Isozymes

- Distinct enzyme forms that catalyze the same reaction



Enzyme kinetics

- Gives information of enzymatically catalyzed reaction RATE
- Measures enzyme AFINITY to substrate or inhibitor
- Provides insight to reaction MECHANISM

Enzymatic reaction RATE

Reaction $A + B \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} C + D$

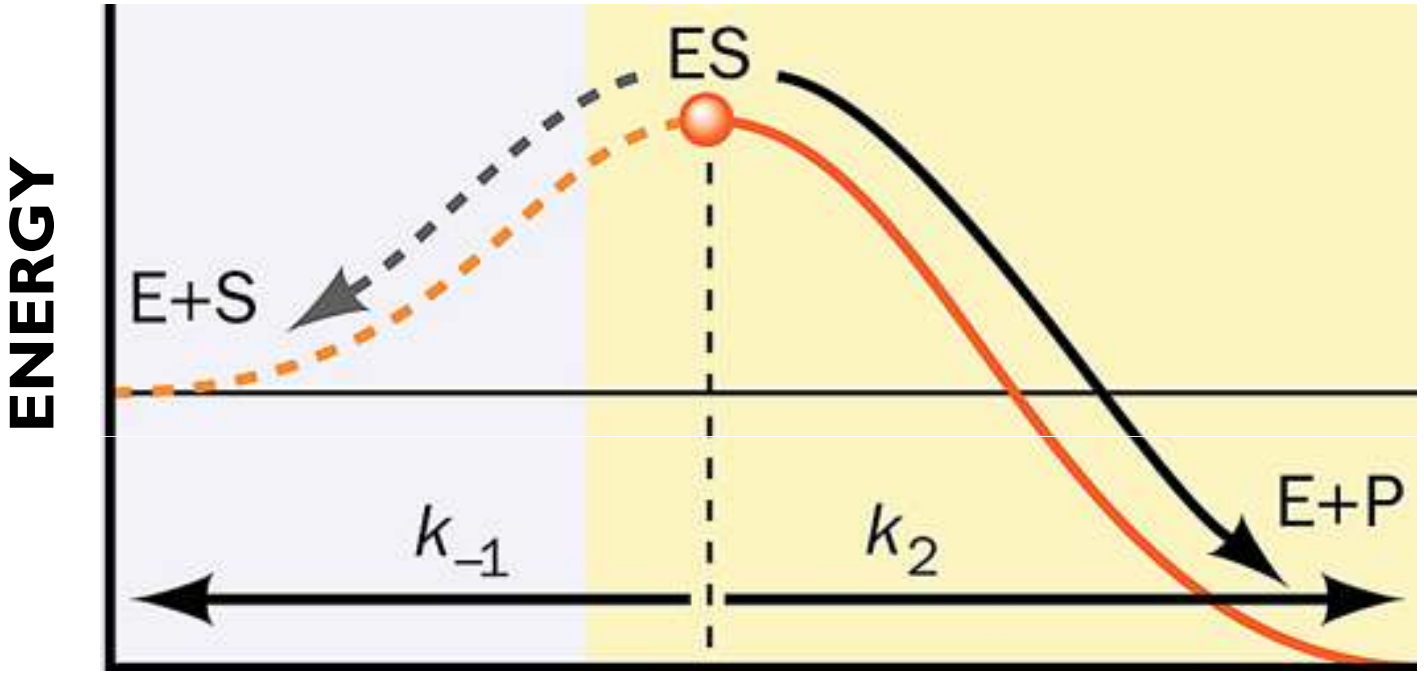
Reaction rate $= k_1[A][B]$

k_1 is reaction rate constant

Rate of reverse reaction $= k_{-1}[C][D]$

k_{-1} is reverse reaction rate constant

Simple model of enzymatically catalyzed reaction



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

$$V = k_2 [ES]$$

STEADY STATE

Concentration of intermediates isn't changing, while concentration of initial substances and product is changing

rate of ES formation = rate of ES dissociation

rate of ES formation

$$\frac{\Delta[ES]}{\Delta t} = k_1[E][S]$$

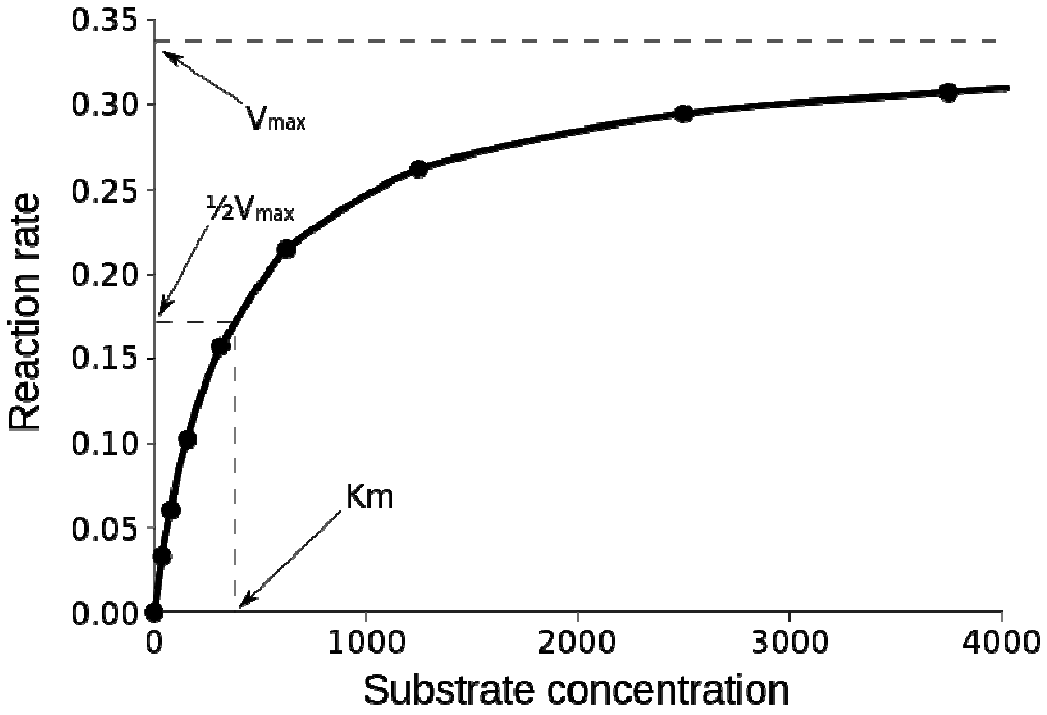
rate of ES dissociation

$$-\frac{\Delta[ES]}{\Delta t} = k_{-1}[ES] + k_2[ES]$$

$$k_1[E][S] = k_{-1}[ES] + k_2[ES]$$

$$[ES] = \frac{[E][S]}{k_{-1} + k_2/k_1}$$

The Michaelis–Menten equation



Reaction kinetics shows saturation – reaction reaches MAXIMUM RATE

Michaelis-Menten equation

$$V_{init} = \frac{V_{max}[S]}{K_M + [S]}$$

Michaelis constant

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

Maximum reaction velocity

$$V = k_2 [ES]$$

Enzyme-substrate complex

Total enzyme

$$[ES] = [E]_o$$

$$V_{max} = k_2[E]_o$$

Maximum velocity V_{max} is reached when all enzyme sites are saturated with substrate

Limiting case: value of k_2 is negligibly small

- k_2 determines total reaction rate

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

When is $k_2 <<< k_{-1}$

$$K_M = \frac{k_{-1}}{k_1} = K_d$$ (dissociation constant)

K_M is equal to constant of dissociation of complex ES if k_2 is much less then k_{-1}

K_M – measure of enzyme **afinity** to substrate

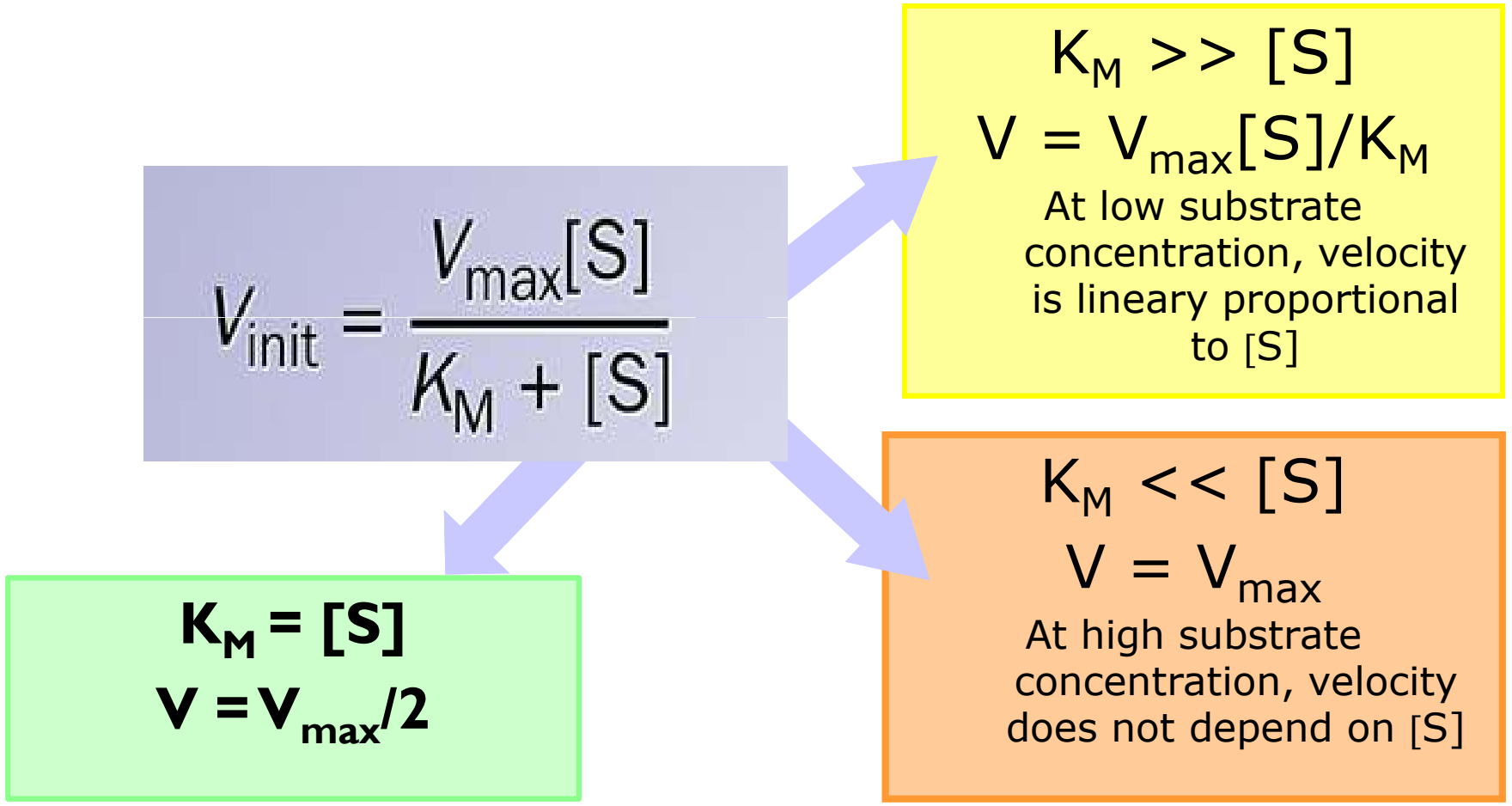
$k_2 <<< k_{-1}$

$$\frac{k_{-1}}{k_1} = K_M = K_d$$

K_d = dissociation constant

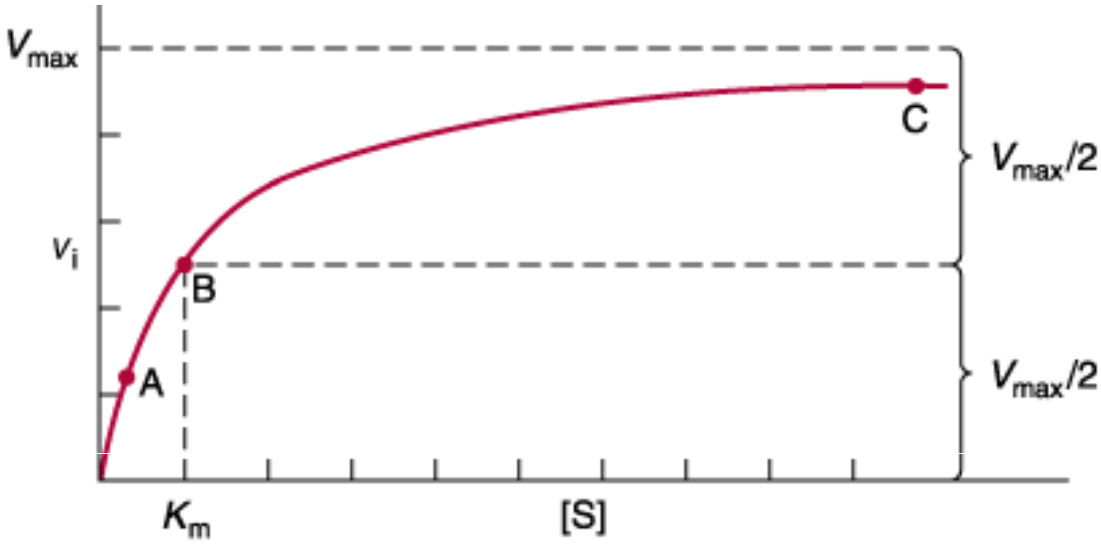
- **high K_M = weak enzyme afinity to substrate**
- **low K_M = strong enzyme afinity to substrate**

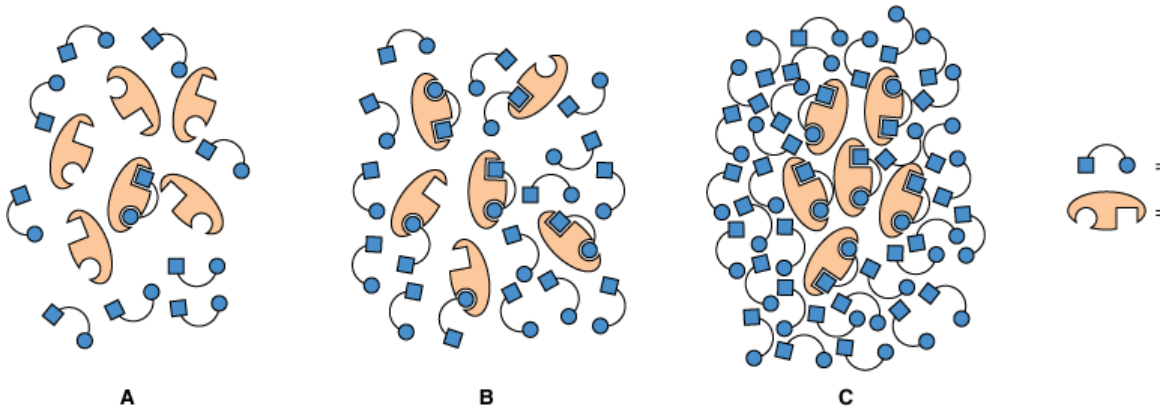
Change in velocity of enzymatically catalyzed reaction depending on substrate concentration



K_M value

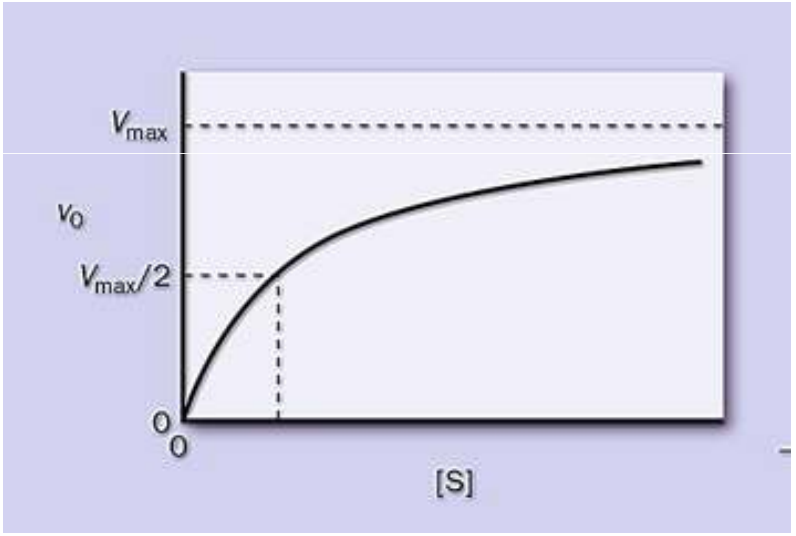
The Michaelis constant K_m is the substrate concentration at which v_i is half the maximal velocity ($V_{max}/2$) attainable at a particular concentration of the enzyme





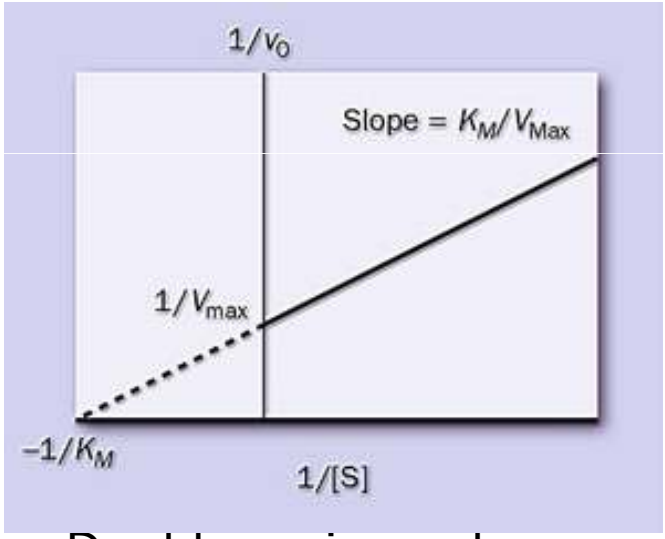
Determination of catalytic constants (V_{max} and K_M) of enzymatically catalyzed reaction

$$V_{init} = \frac{V_{max}[S]}{K_M + [S]}$$



Michaelis-Menten graph
hyperbolic curve
v *vs* [S]

$$\frac{1}{V} = \frac{K_M}{V_{max}}\frac{1}{[S]} + \frac{1}{V_{max}}$$



Double reciprocal
Lineweaver-Burk graph
linear form
1/v *vs* 1/[S]

Enzyme turnover number

$$k_{cat} = V_{max}/[E_o]$$

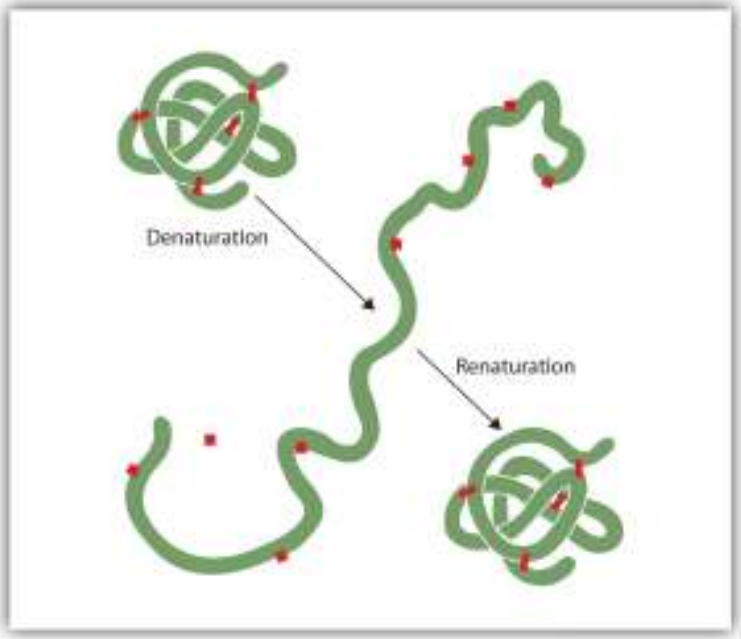
unit: $mol\ s^{-1}/mol = s^{-1}$

Moles of substrate turned to product in time unit (second) for mole of enzyme, when enzyme is completely saturated with substrate

Enzymes are active when they are in native conformation

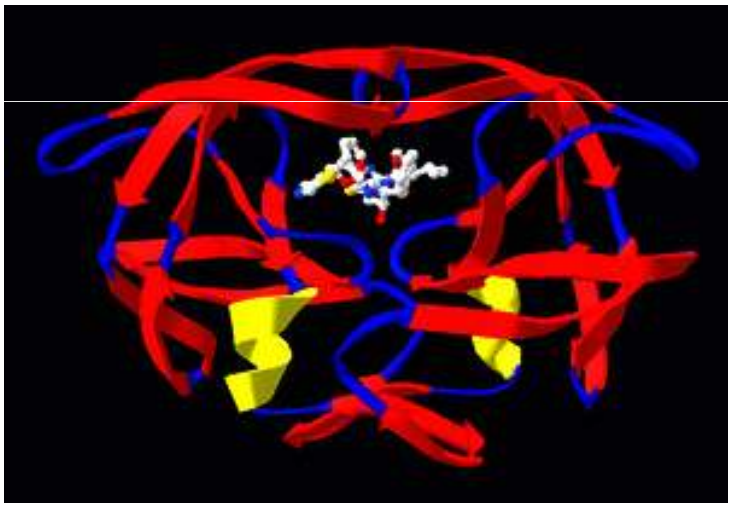
- Enzymes are sensitive to reaction conditions

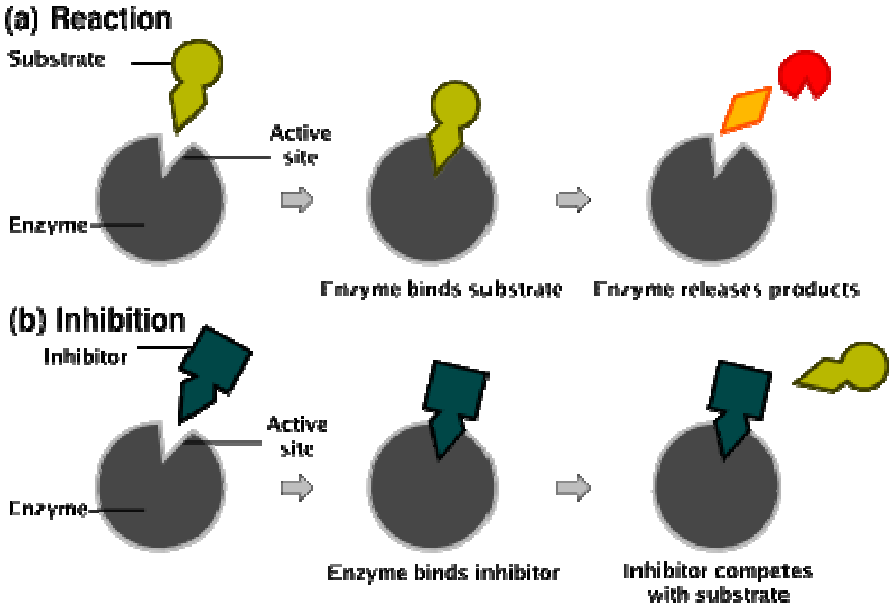
- Enzymes loose activity in conditions of extreme temperature, pH, ionic strength, presence of detergent and other matters that denaturate proteins



Inhibition of emzymatic activity

Enzyme activity can be inhibited by specific small molecules and iones

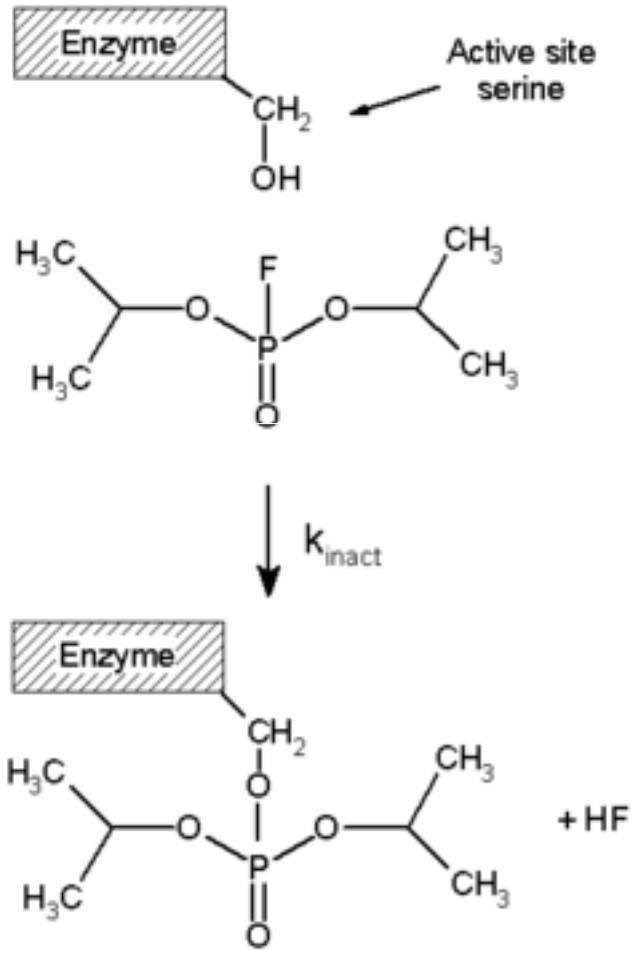




Irreversible inhibition is covalent modification of enzyme with inhibitor or tightly inhibitor binding in active site of enzyme

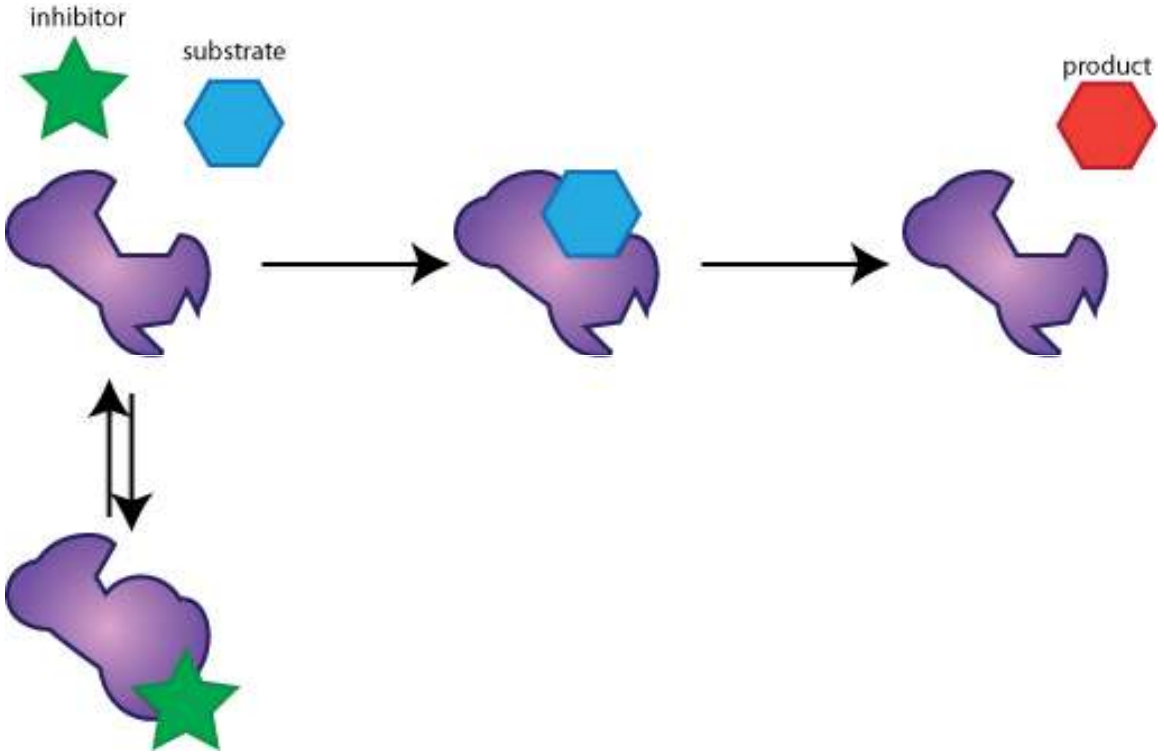
- Group-specific reagents
  - chemically modify amino acid residues in active site
- Substrate analogs
  - structurally similar to substrate
  - covalently modify amino acid residues in active site
- Suicide inhibitors
  - modified substrates
  - enzyme transforms them to reactive intermediate which inactivates enzyme by covalent modification

Irreversible inhibitor

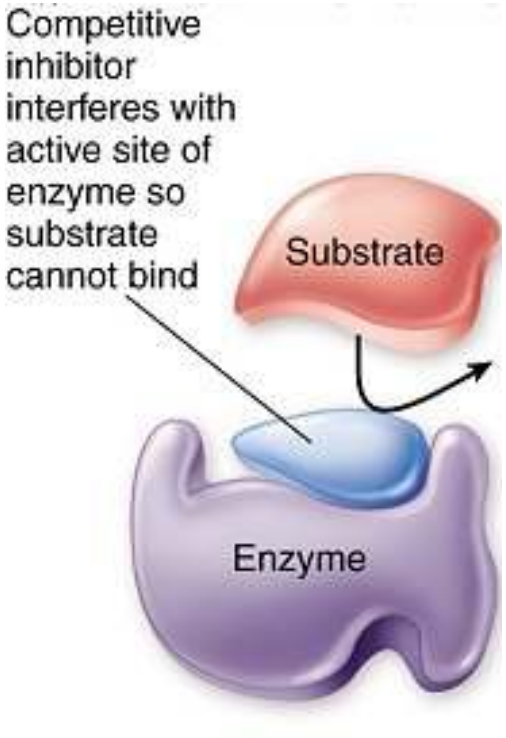


Reaction of the irreversible inhibitor diisopropylfluorophosphate (DFP) with a serine protease) (chemical warfare)

Reversible inhibition is fast dissociation of enzyme:inhibitor complex



Competitive inhibition

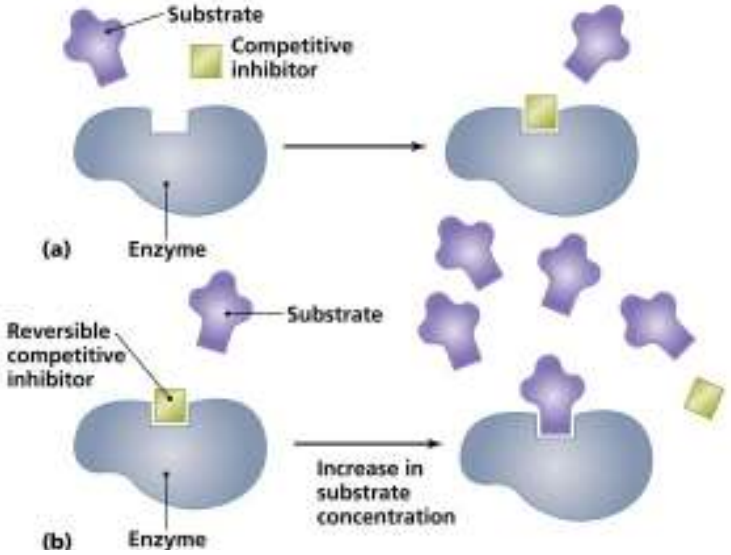


$$E + S \underset{}{\overset{K_m}{\rightleftharpoons}} ES \longrightarrow E + P$$

$$+ \; I \quad \Big\Vert \; K_I$$

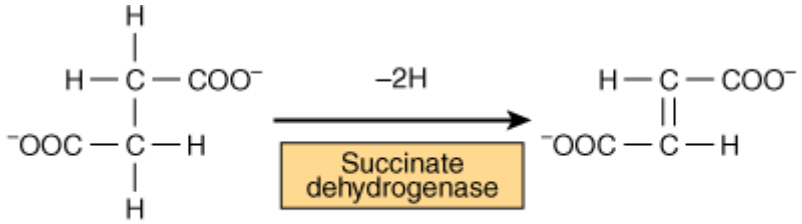
$$EI$$

Enzyme binds substrate (ES) or inhibitor (EI), but not both (EIS)

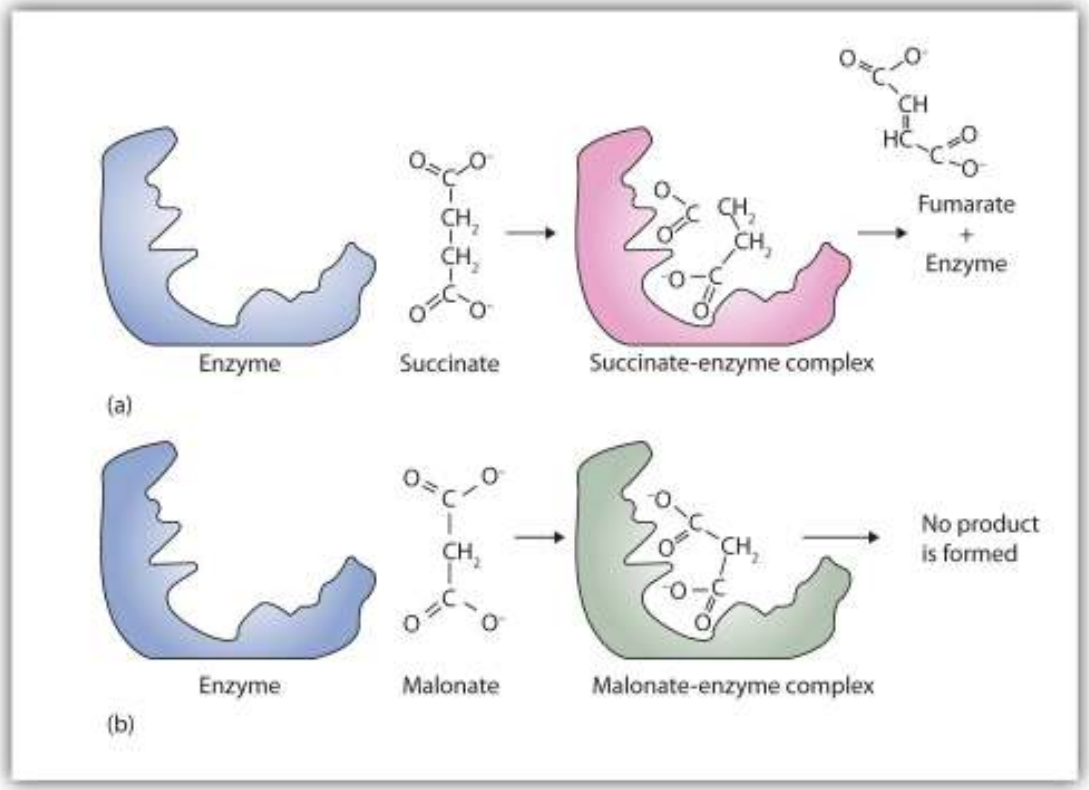


- Competitive inhibitor slows catalysis by decreasing the number of free enzyme molecules available to bind substrate
- Can be overcome with high substrate concentration

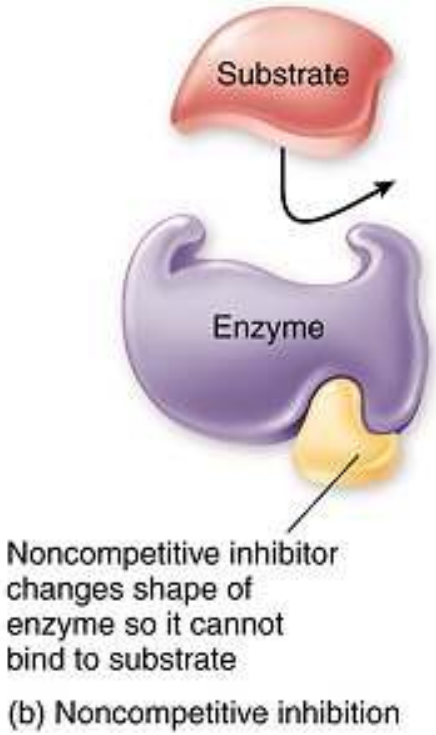
Example of competitive inhibition

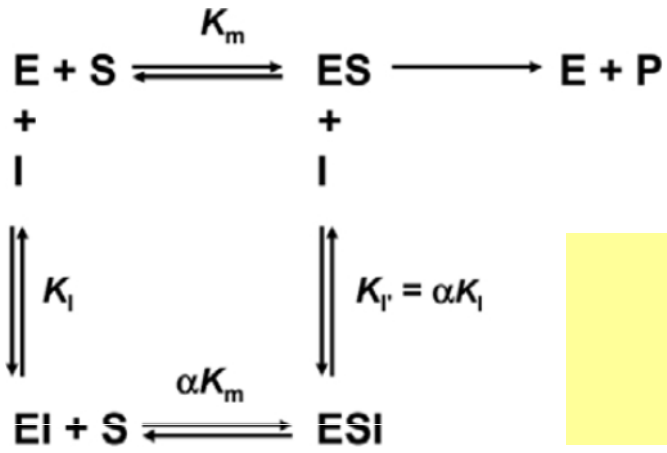


Succinate **Fumarate**



Noncompetitive inhibition



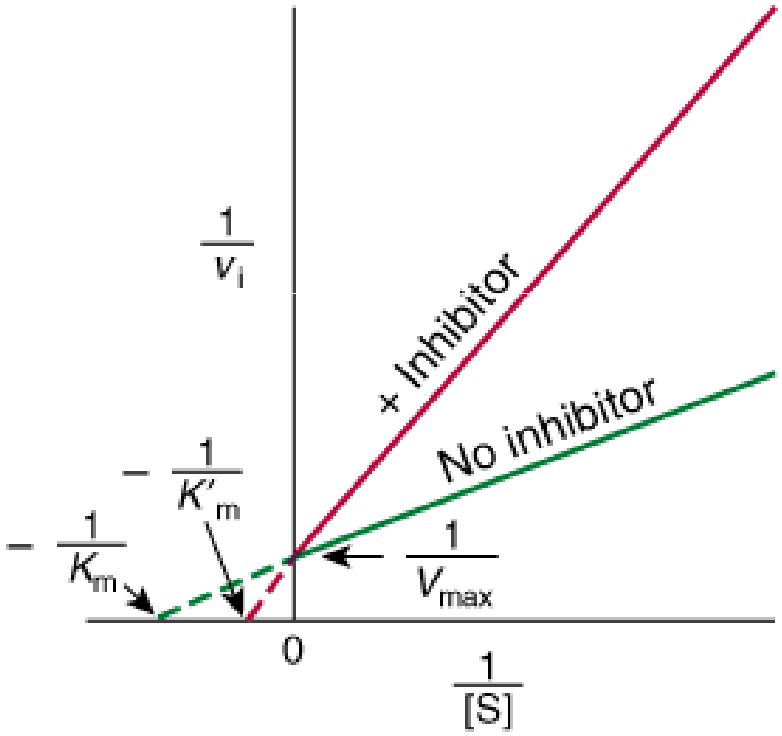


Enzyme binds both substrate and inhibitor

- Noncompetitive inhibitor decreases enzyme turnover number
- Can not be overcome by increasing the substrate concentration

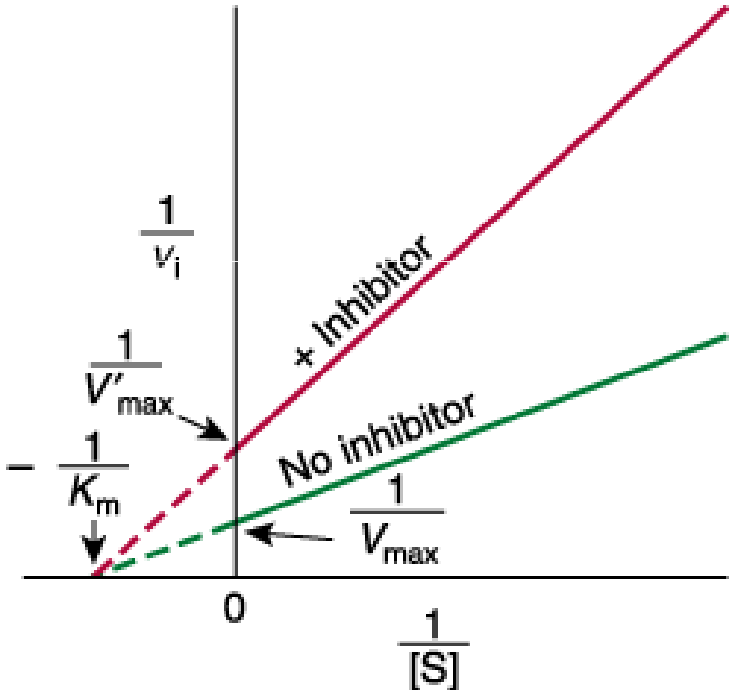
Competitive and noncompetitive inhibition can be distinguished by measuring the reaction velocity under different concentrations of substrate and inhibitor

competitive inhibitor



- does not affect V_{max}
- raises apparent K_M for the substrate

noncompetitive inhibitor



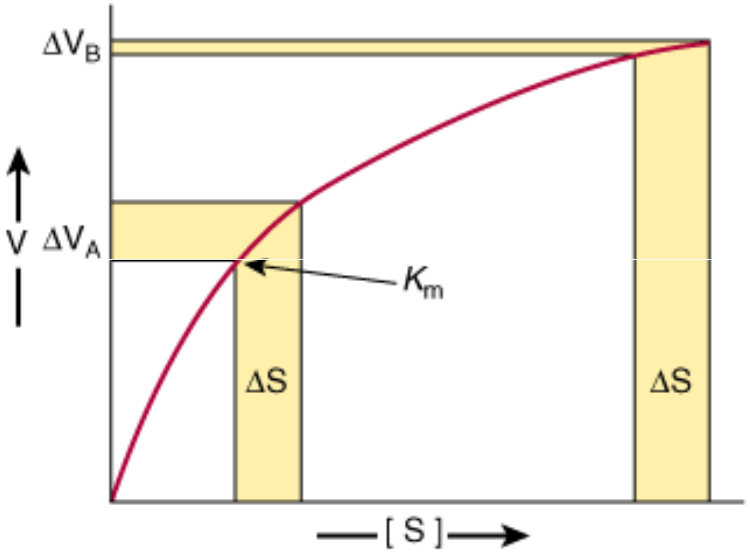
- does not affect K_M
- decreases V_{max}

Enzymes

Regulation of activity

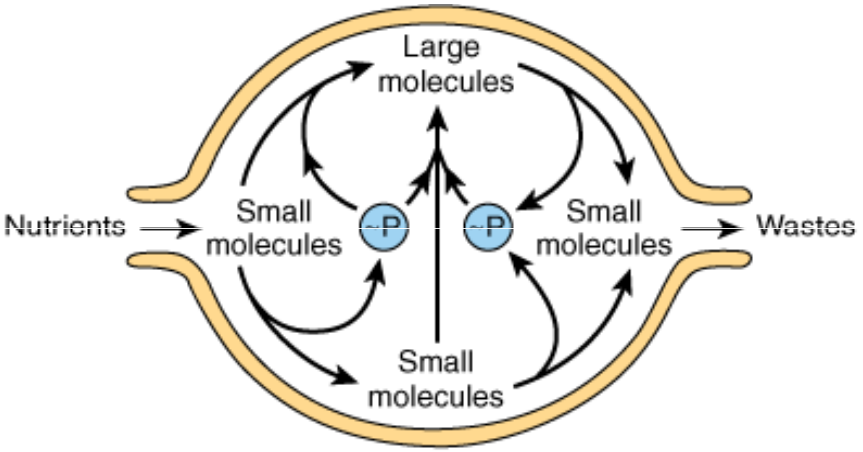
Regulation of metabolite flow can be active or passive

PASSIVE



maximal enzyme rate responds to changes in substrate level

ACTIVE



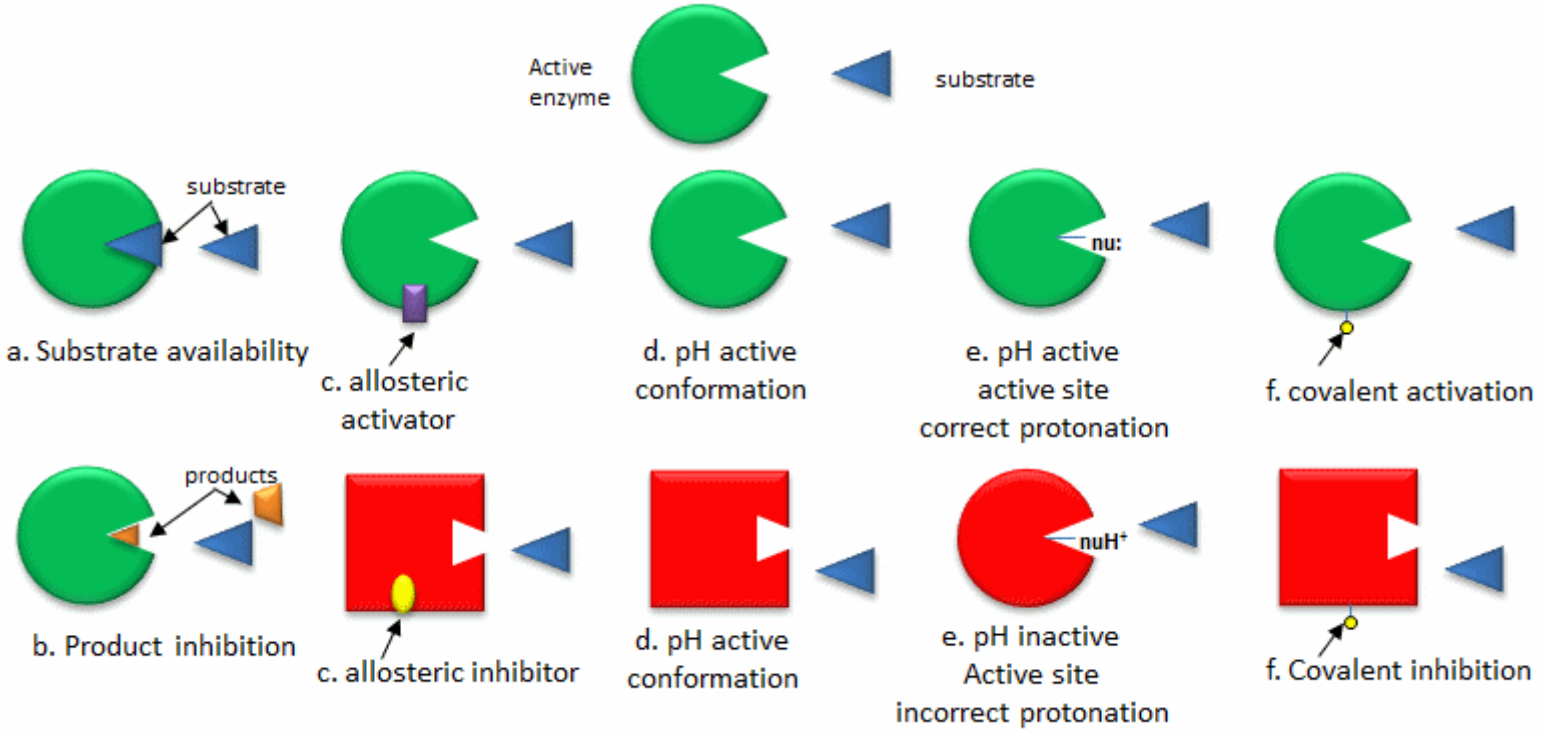
enzyme efficiency responds to internal and external signals

Regulations of enzyme activity

- *Change in the number of enzyme molecules*
  - regulation of synthesis rate (regulation of protein concentration by regulation of gene expression)
  - regulation of degradation rate
- *Change in the enzyme activity*
  - activation of inactive precursor by proteolysis
  - reversible covalent modification: groups binding
  - inhibition by feedback (inhibition by end product)
  - allosteric regulation: change in enzyme conformation by binding the regulatory molecules on site which is different of substrate binding site (activators, inhibitors)

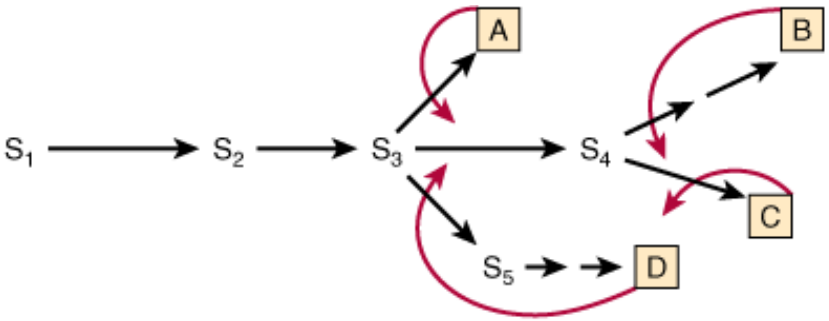
Regulating catalytic activity

- Allosteric regulation - binding of dissociable ligands
- Covalent modification

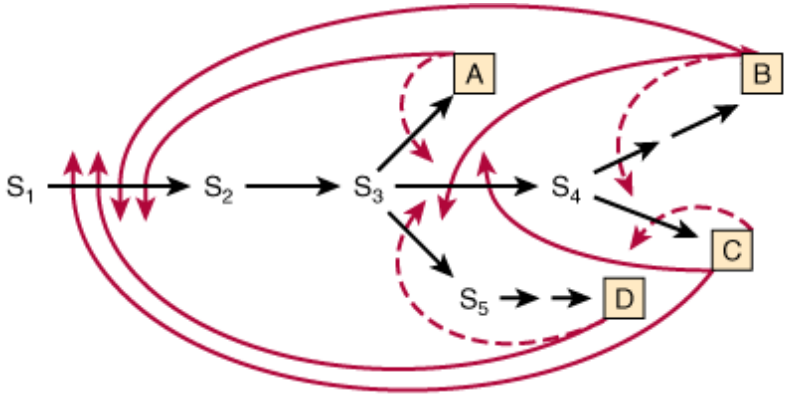


Feedback inhibition or inhibition by end product

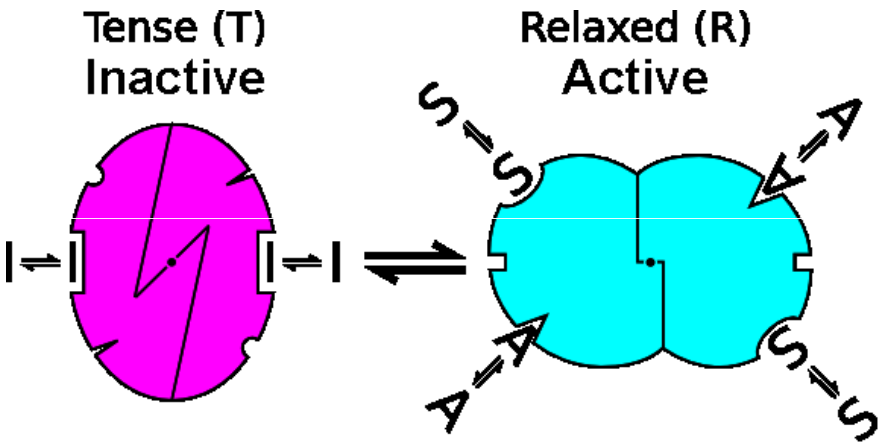
Sites of feedback inhibition in a branched biosynthetic pathway. S_1–S_5 are intermediates in the biosynthesis of end products A–D. Straight arrows represent enzymes catalyzing the indicated conversions. Curved red arrows represent feedback loops and indicate sites of feedback inhibition by specific end products.



Multiple feedback inhibition in a branched biosynthetic pathway. Superimposed on simple feedback loops (dashed red arrows) are multiple feedback loops (solid red arrows) that regulate enzymes common to biosynthesis of several end products.

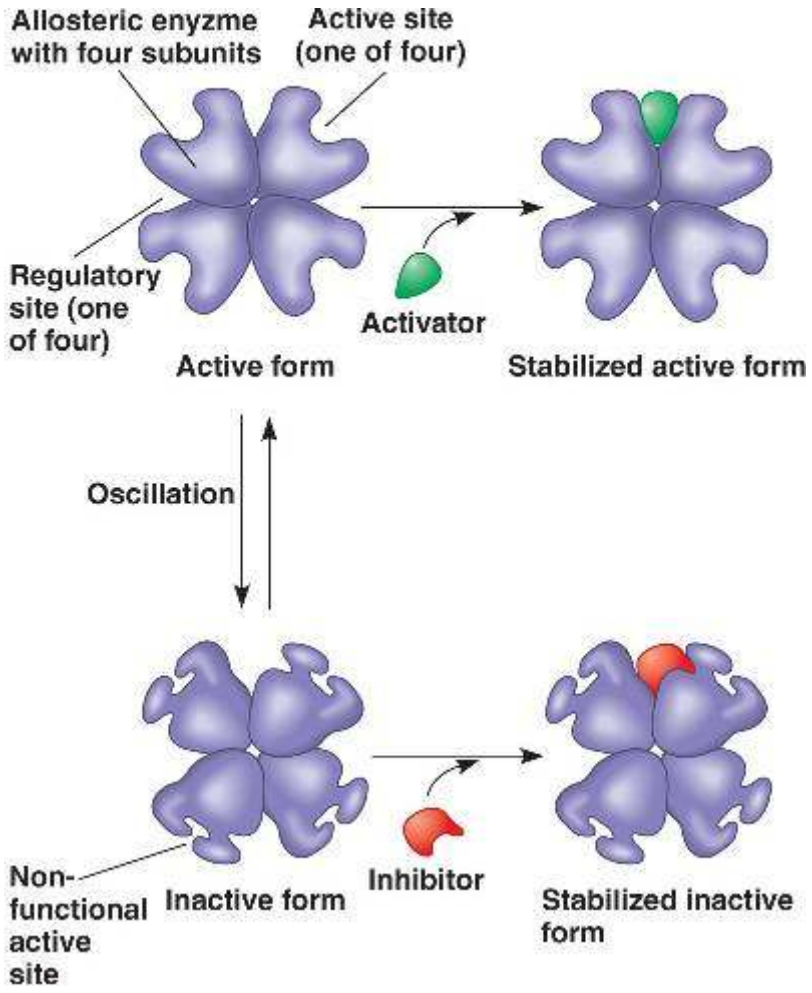


Allosteric enzymes



- Greek *allos* (other) *stereos* (shape)
- Regulatory enzymes – change the shape or conformation by binding the effectors
- Do not obey Michaelis-Menten kinetics
- Composed of few subunits
- Can exist in two states
  - R – relaxed state
  - T – tense state

Allosteric activation/inhibition

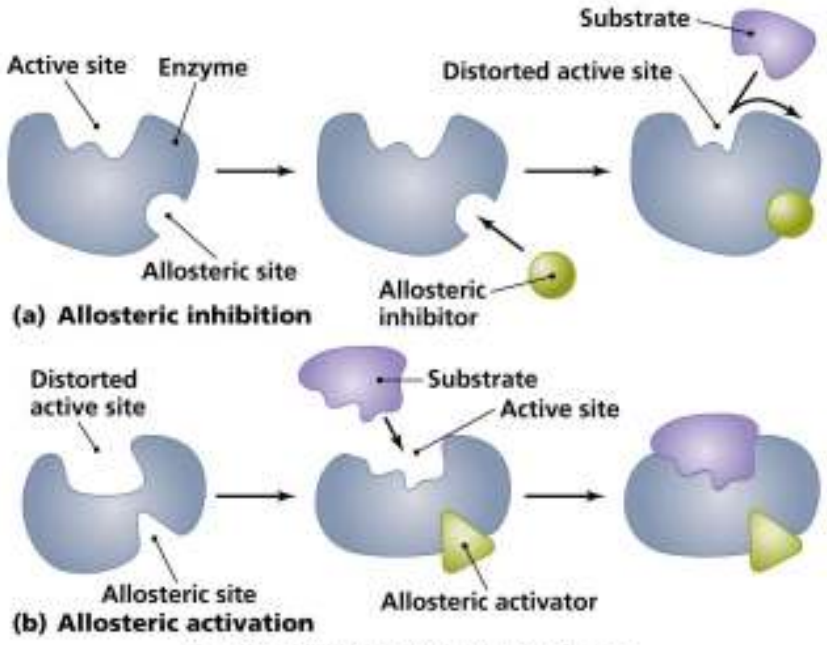


- Allosteric activator: shifts the conformational equilibrium towards the R (active) form

- Allosteric inhibitor: shifts the conformational equilibrium towards the T (inactive) form

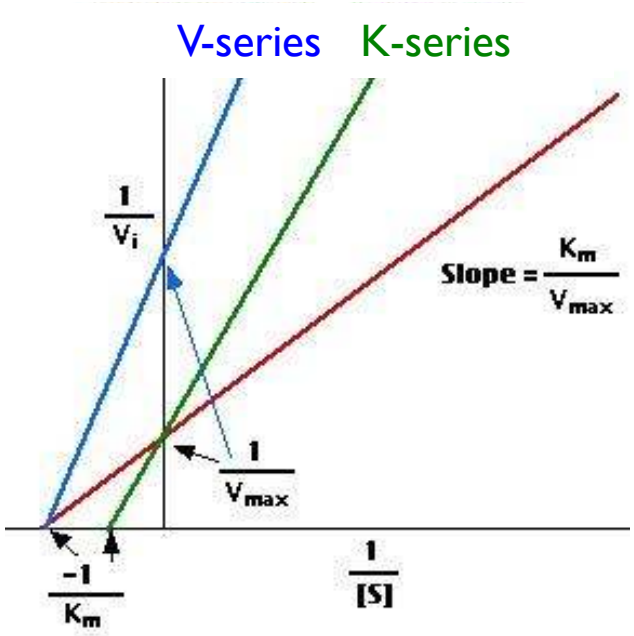
Allosteric enzymes

Allosteric & catalytic sites are spatially distinct

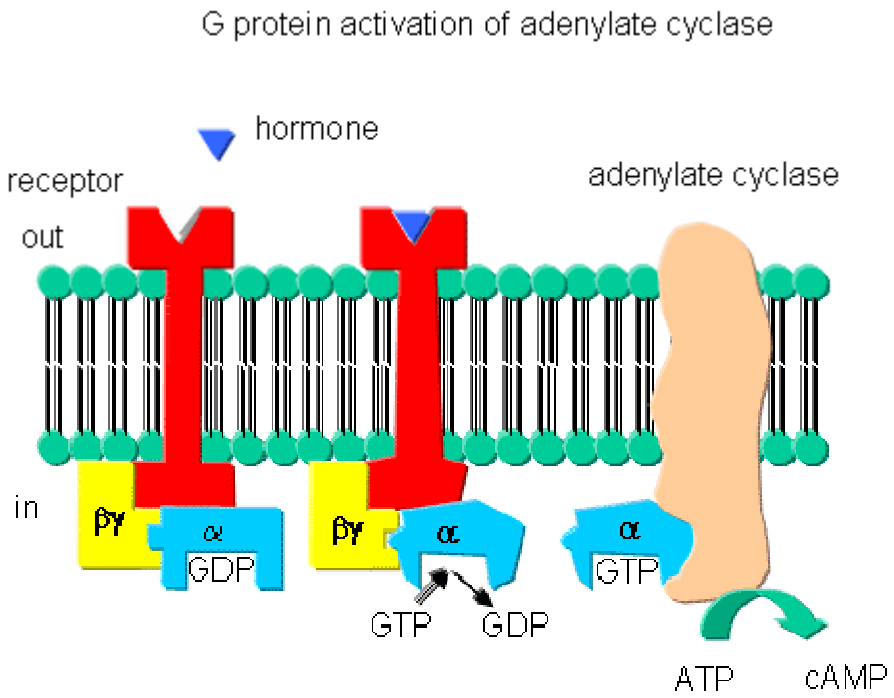


Allosteric effects may be on K_m or on V_{max}

- K-series – increases K_m
- V-series – decreases V_{max}

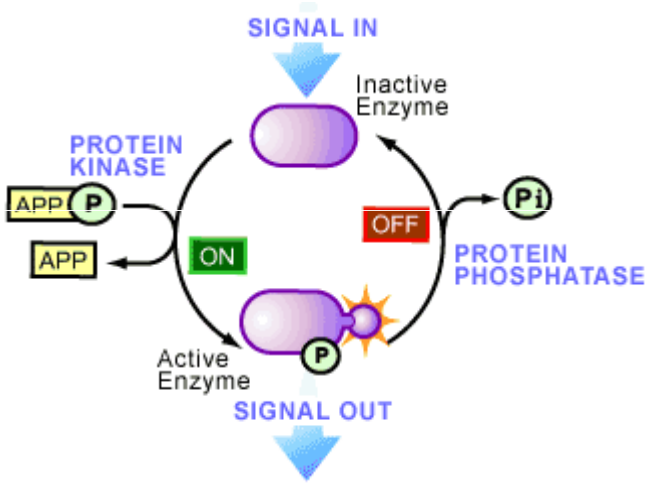


Allosteric second messengers



Phosphorylation is very efficient way of enzyme activity regulation

- The most common form of the reversible regulation by covalent modification
- Protein kinases phosphorylate proteins and thus change their activity
- Usually phosphorylated are specific Ser, Tyr or Thr amino acid residue of target protein
- Protein phosphatases dephosphorylate proteins

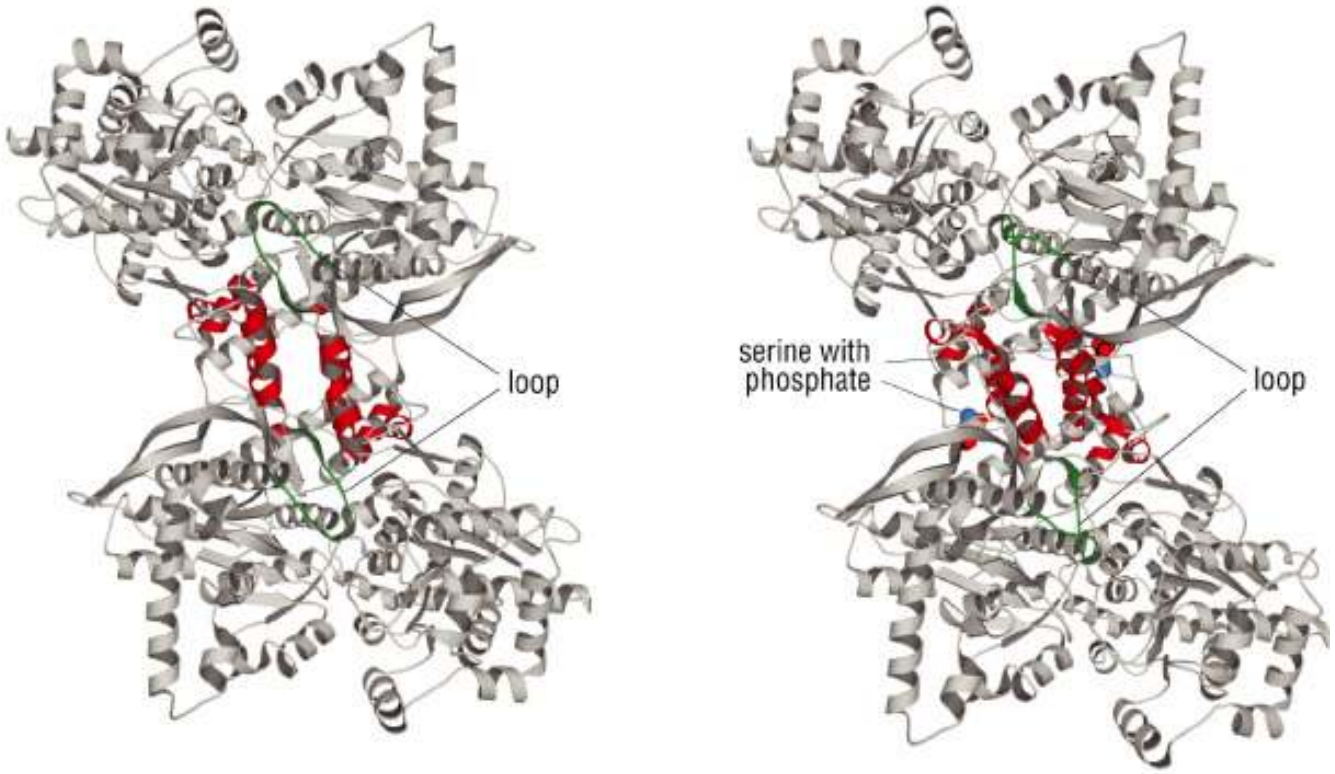


Examples of mammalian enzymes whose catalytic activity is altered by covalent phosphorylation-dephosphorylation

	Activity State	
Enzyme	**Low**	**High**
Acetyl-CoA carboxylase	EP	E
Glycogen synthase	EP	E
Pyruvate dehydrogenase	EP	E
HMG-CoA reductase	EP	E
Glycogen phosphorylase	E	EP
Citrate lyase	E	EP
Phosphorylase b kinase	E	EP
HMG-CoA reductase kinase	E	EP

Abbreviations: E, dephosphoenzyme; EP, phosphoenzyme.

Glycogen phosphorylase is modified by phosphorylation of Ser14

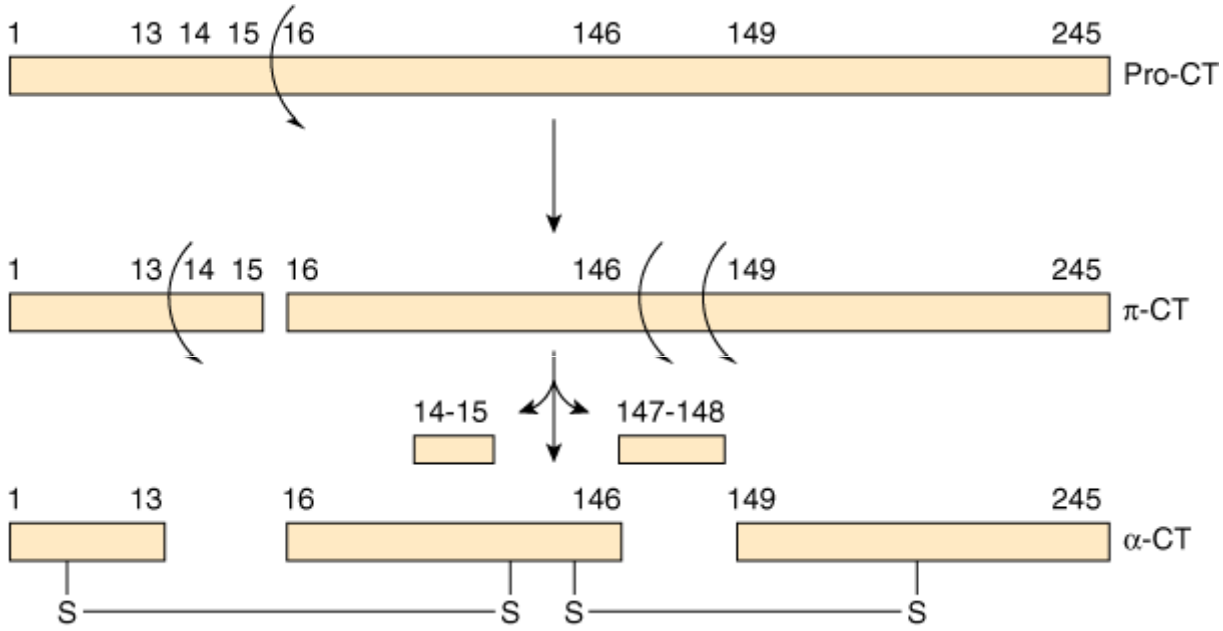


Phosphorylation changes the enzyme conformation and thus the enzyme activity

Zymogens (proenzymes) are inactive precursors of enzymes

Synthesis site	Zymogen	Active enzyme
Stomach	Pepsinogen	Pepsin
Pancreas	Chymotrypsinogen	Chymotrypsin
Pancreas	Trypsinogen	Trypsin
Pancreas	Procarboxypeptidase	Carboxypeptidase
Pancreas	Proelastase	Elastase

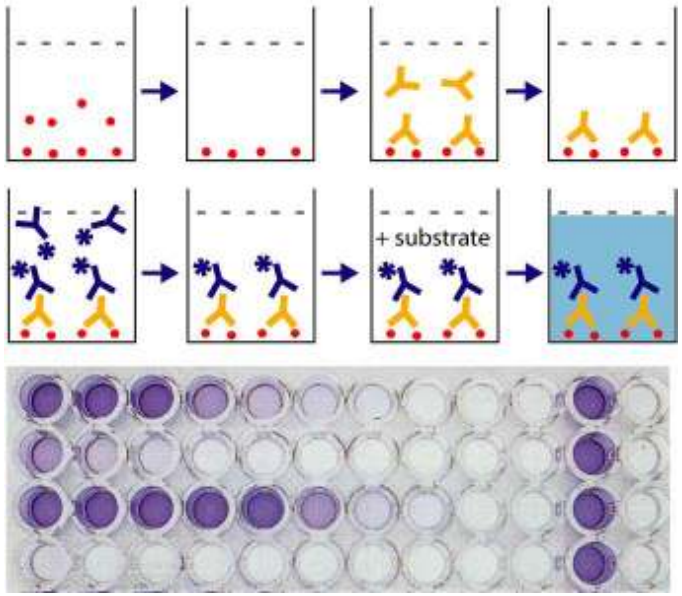
Activation of enzyme requires selective proteolysis



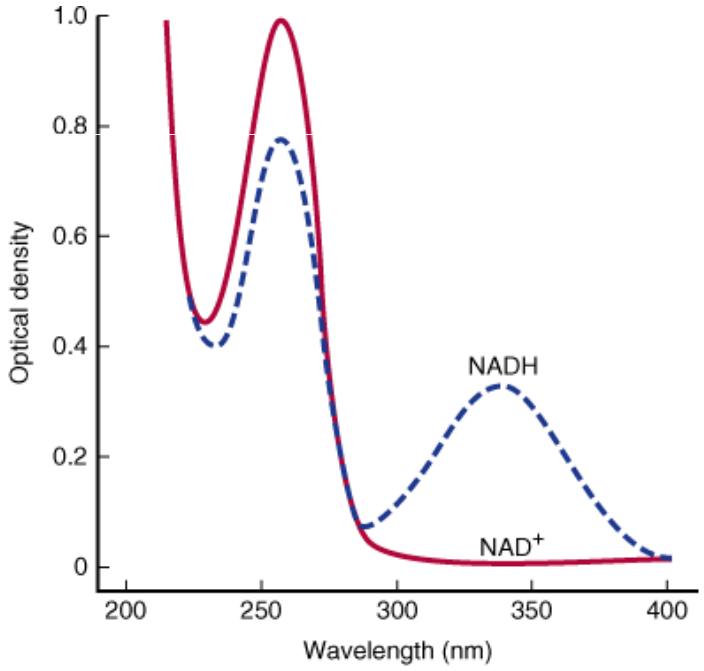
Successive proteolysis forms prochymotrypsin (pro-CT), π-chymotrypsin (π-Ct), and ultimately α-chymotrypsin (α-CT), an active protease whose three peptides (A, B, C) remain associated by covalent inter-chain disulfide bonds.

The catalytic activity of enzymes

- The catalytic activity of enzymes reveals their presence, facilitates their detection, and provides the basis for enzyme-linked immunoassays (ELISA)



- Many enzymes can be assayed spectrophotometrically by coupling them to an NAD(P)+-dependent dehydrogenase.



High-throughput screening

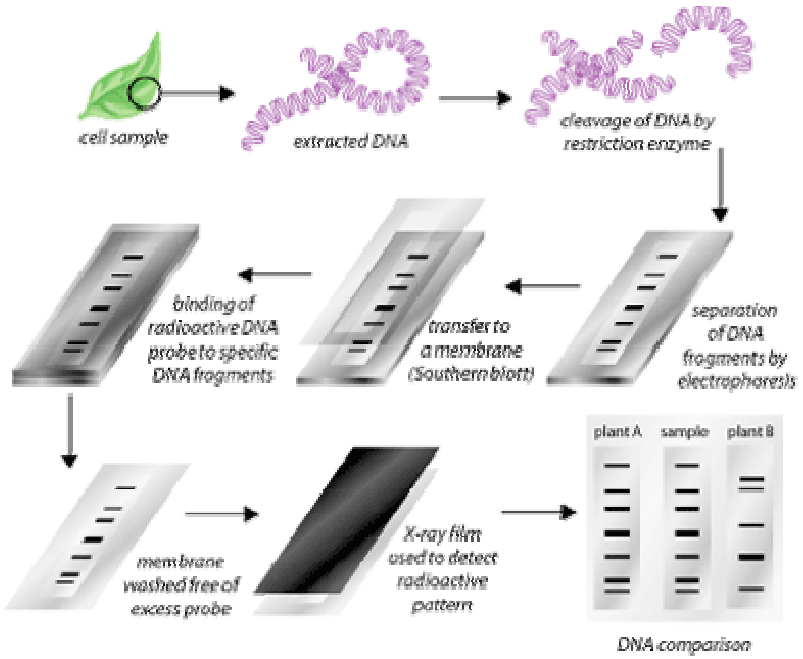
- Combinatorial chemistry generates extensive libraries of potential enzyme activators and inhibitors that can be tested

Principal serum enzymes used in clinical diagnosis

Serum Enzyme	Major Diagnostic Use
Aminotransferases	
Aspartate aminotransferase (AST, or SGOT)	Myocardial infarction
Alanine aminotransferase (ALT, or SGPT)	Viral hepatitis
Amylase	Acute pancreatitis
Ceruloplasmin	Hepatolenticular degeneration (Wilson's disease)
Creatine kinase	Muscle disorders and myocardial infarction
-Glutamyl transferase	Various liver diseases
Lactate dehydrogenase isozyme 5	Liver diseases
Lipase	Acute pancreatitis
Phosphatase, acid	Metastatic carcinoma of the prostate
Phosphatase, alkaline (isozymes)	Various bone disorders, obstructive liver diseases

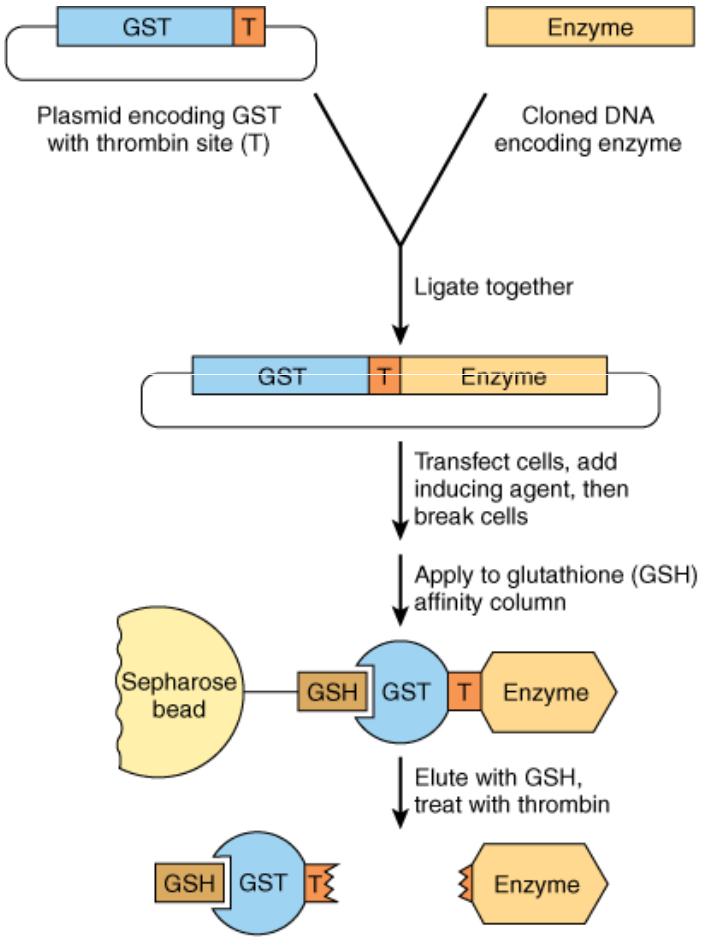
Enzymes facilitate diagnosis of genetic and infectious diseases

RFLP



PCR

Recombinant DNA provides an important tool for studying enzymes



Ribozymes

- Several ribozymes can cut and re-splice the phosphodiester bonds of RNA.
- In the ribosome rRNA is primarily responsible for catalysis