

Introduction to Molecular and Cellular Biology

LECTURES 5-6:

Introduction to cell chemistry and biosynthesis III



LECTURES 5-6: INTRODUCTION TO CELL CHEMISTRY AND BIOSYNTHESIS III

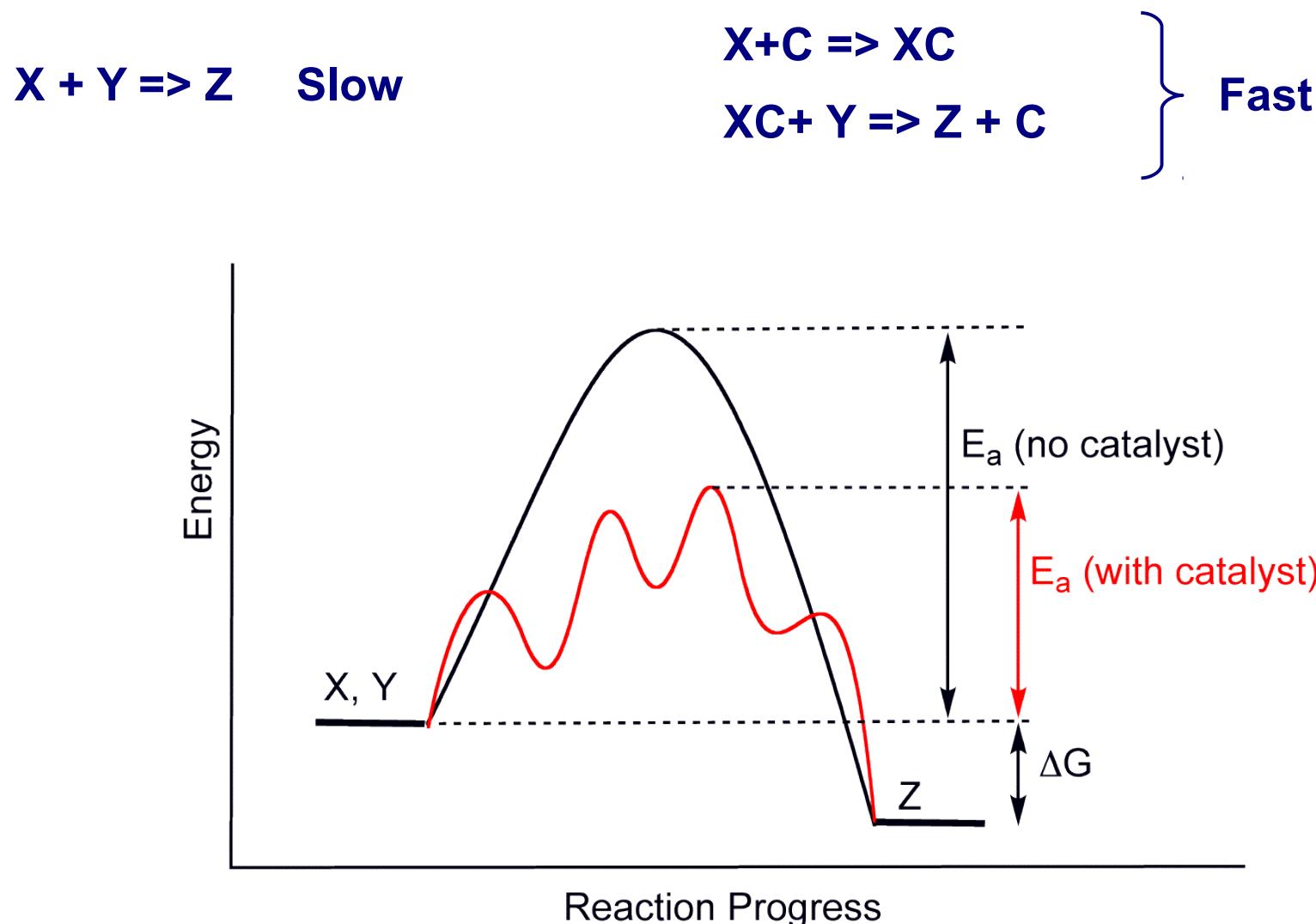
ENZYMES

- Basic concepts:
 - enzymatic catalysis
 - substrate, product, intermediate
 - reaction conditions
 - classification of enzymes
- Enzyme kinetics
- Examples of enzyme mechanisms (chymotrypsin, hexokinase, enolase, lysozyme)
- Regulation of enzymes
- Localization of enzymes and their function:
 - Phosphatase
 - Acyltransferase



CATALYSIS

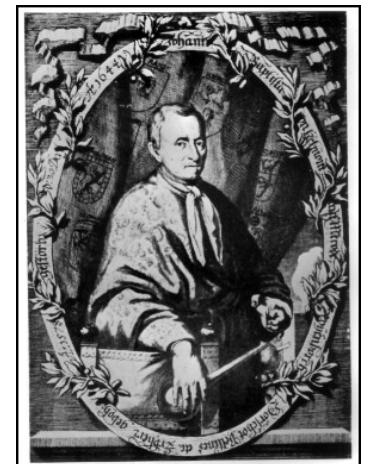
Catalysis: increase of the rate of chemical reaction due to participation of an additional substance (which is neither substrate nor product).



HISTORY

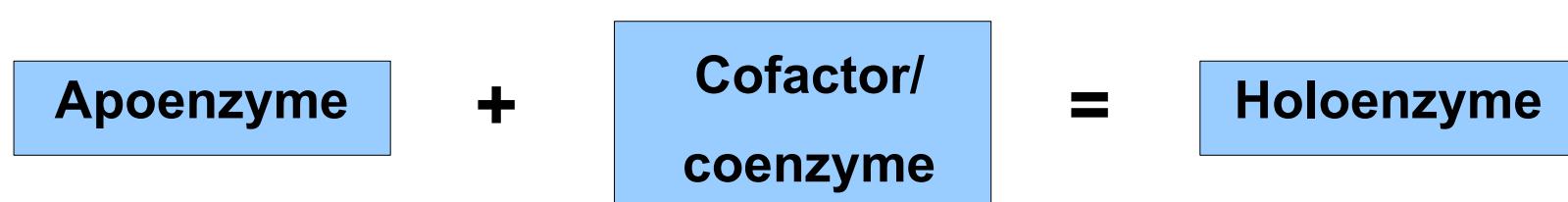
Enzyme is a macromolecular biological catalyst.

- “Ferment”, suggested by van Helmont (1580-1644), related to digestion
- XVIII-beginning XIX century:
 - starch+saliva=>sugar
 - gastric acid digests meat
- Louis Pasteur: fermentations is catalyzed by “ferments”
- Kühne: “enzyme”
- 1897: Buchner “Alcohol fermentation without yeast” => NP
- 1926: crystallization of the urease by Sumner
- 1930s: crystallization of pepsin and trypsin



INTRODUCTION

- Enzymatic catalysis is indispensable for life functions.
- Most of enzymes are proteins (around enzymatic 5000 reactions are known).
- Enzyme is natively folded if it is able to carry out its function and *vice versa*.
- All structural levels are important for enzyme's functional integrity.
- Cofactor: an additional inorganic component of the enzyme (Fe^{2+} , Mg^{2+} etc.)
- Coenzyme: an additional organic/metalloorganic component of the enzyme.
- Prosthetic group: a coenzyme or metal ion bound (covalently/non-covalently) to the enzyme.
- Holoenzyme: complex enzyme.
- Apoenzyme/apoprotein: protein part of holoenzyme.



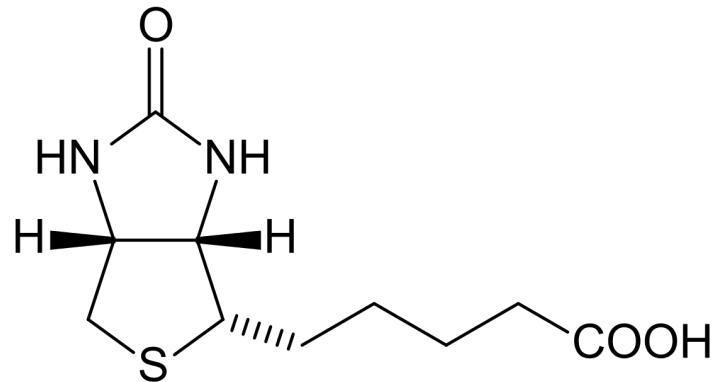
COFACTORS: EXAMPLES

Cofactors Enzymes

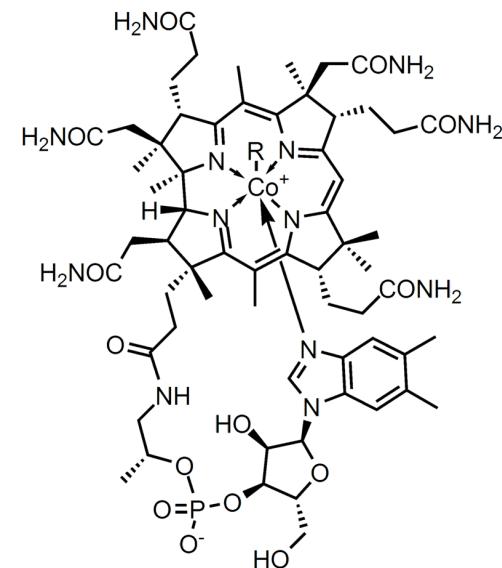
Cu^{2+}	Cytochrome oxidase
Fe^{2+} or Fe^{3+}	Cytochrome oxidase, catalase, peroxidase
K^+	Pyruvate kinase
Mg^{2+}	Hexokinase, glucose 6-phosphatase, pyruvate kinase
Mn^{2+}	Arginase, ribonucleotide reductase
Mo	Dinitrogenase
Ni^{2+}	Urease
Se	Glutathione peroxidase
Zn^{2+}	Carbonic anhydrase, alcohol dehydrogenase, carboxypeptidases A and B

COENZYMES: EXAMPLES

Coenzyme	Examples of chemical groups transferred	Dietary precursor in mammals
Biocytin	CO ₂	Biotin
Coenzyme A	Acyl groups	Pantothenic acid and other compounds
5'-Deoxyadenosylcobalamin (coenzyme B ₁₂)	H atoms and alkyl groups	Vitamin B ₁₂
Flavin adenine dinucleotide	Electrons	Riboflavin (vitamin B ₂)
Lipoate	Electrons and acyl groups	Not required in diet
Nicotinamide adenine dinucleotide	Hydride ion (:H ⁻)	Nicotinic acid (niacin)
Pyridoxal phosphate	Amino groups	Pyridoxine (vitamin B ₆)
Tetrahydrofolate	One-carbon groups	Folate
Thiamine pyrophosphate	Aldehydes	Thiamine (vitamin B ₁)



Biotin



R = 5'-deoxyadenosyl, Me, OH, CN

Vitamin B₁₂

CLASSIFICATION OF ENZYMES

Nomenclature: substrate-related + “ase” (RNase) or trivial (pepsin)

E.C. number: Enzyme Commission number X.Y.W.Z.

Glucose phosphotransferase:



E.C. 2.7.1.1.:

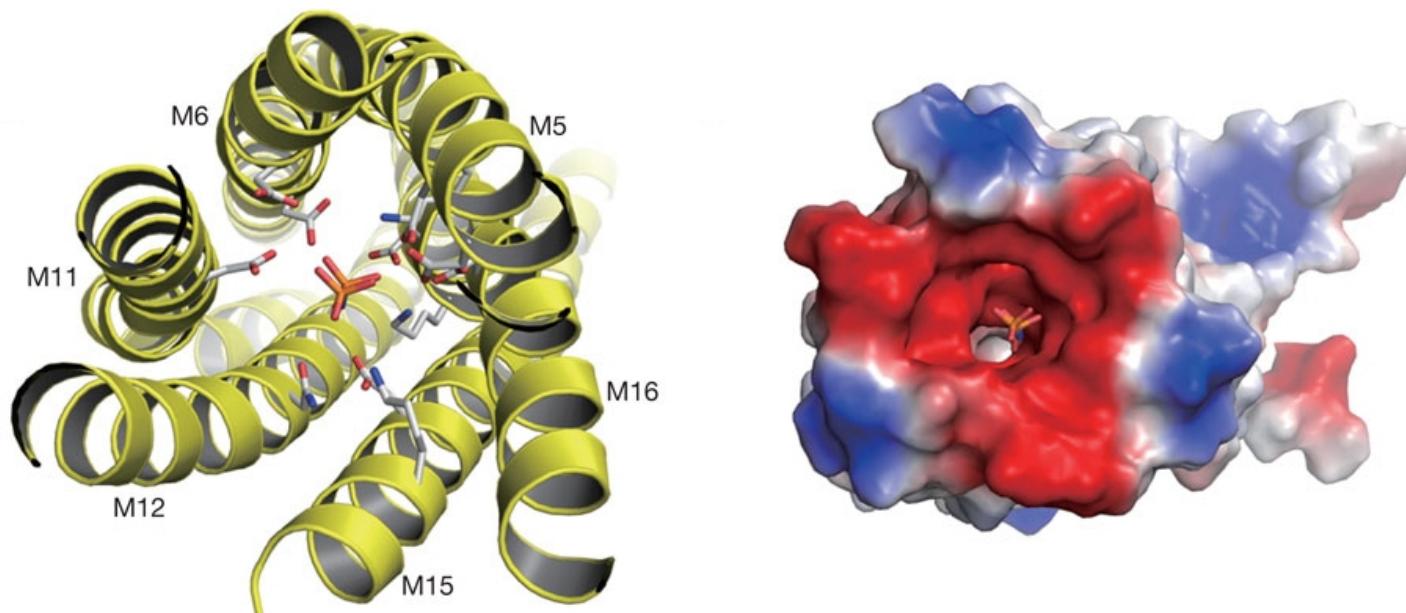
2. transferase; 7. phosphotransferase;

1. hydroxyl group is an acceptor ; 1. D-glucose is the P group acceptor.

No.	Class	Type of reaction catalyzed
1	Oxidoreductases	Transfer of electrons (hydride ions or H atoms)
2	Transferases	Group transfer reactions
3	Hydrolases	Hydrolysis reactions (transfer of functional groups to water)
4	Lyases	Addition of groups to double bonds, or formation of double bonds by removal of groups
5	Isomerases	Transfer of groups within molecules to yield isomeric forms
6	Ligases	Formation of C—C, C—S, C—O, and C—N bonds by condensation reactions coupled to ATP cleavage

BASIC CONCEPTS OF ENZYME CATALYSIS

- Substrate(s): the substance(s) before the reaction starts.
- Products(s): the substance(s) after the reaction starts.
- Enzyme active site: the enzyme residues localized in the place where the reaction occurs.
- Substrate is bound at the active site of the enzyme.



Pyrophosphatase with the substrate pyrophosphate (PDB ID: 2AUU)

ENZYME CATALYSIS: ENERGETICS



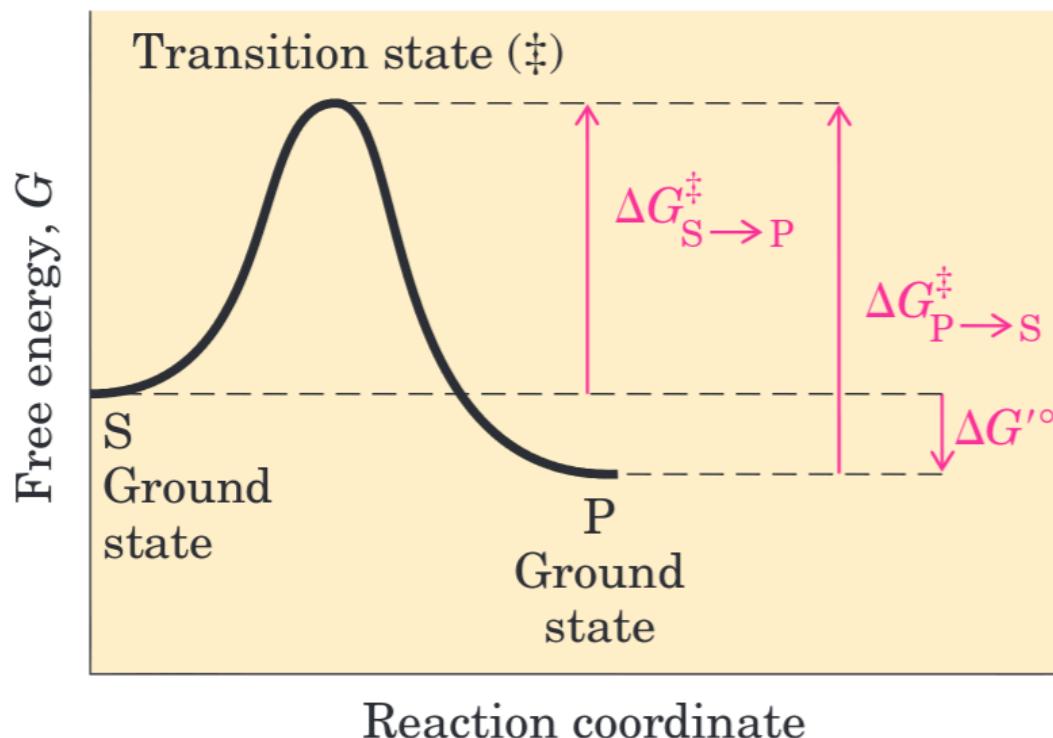
E: enzyme

S: substrate

P: product

ES, EP: transient complexes

The rate is increased,
but not the equilibrium!

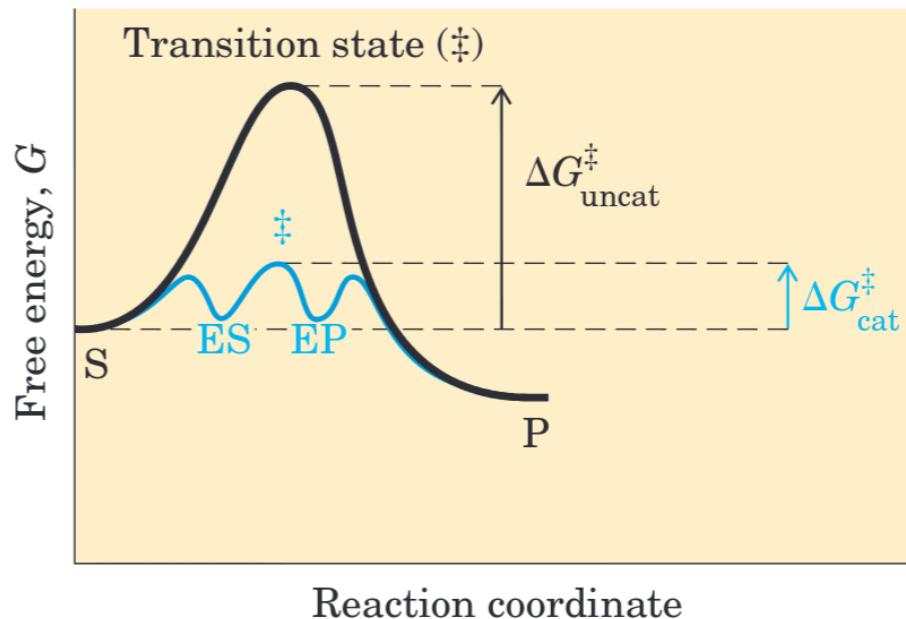


Ground state: starting point in the free energy diagram

Standard conditions: 298K, 1atm, 1M, pH7

ENZYME CATALYSIS: ENERGETICS

- Barrier height $\Delta G^{\ddagger}_{S \rightarrow P}$ defines the rate of the reaction from S to P.
- Transition state: the point where decay to S and P is equally probable.
- Transition state: local maximum in the curve from S to P.
- Transition state \neq ES or EP.
- $\Delta G^{\ddagger}_{S \rightarrow P}$ – activation energy.
- T increase $\Rightarrow \Delta G^{\ddagger}_{S \rightarrow P}$ decrease.
- Catalysis lowers $\Delta G^{\ddagger}_{S \rightarrow P}$.
- The same enzyme catalyzes $S \rightleftharpoons P$.



- Multiple step reaction involves reaction intermediates (ES, EP).
- The rate is dependent on maximum of intermediate energy (rate-limiting step).
- Different rates at different steps \Rightarrow sophisticated regulation.

REACTION RATE AND EQUILIBRIA

➤ Equilibria: ΔG°

➤ Rate: ΔG^\ddagger

➤ $K'_{\text{eq}} = [\text{P}]/[\text{S}]$

➤ $\Delta G^{\circ} = -RT \ln K'_{\text{eq}}$

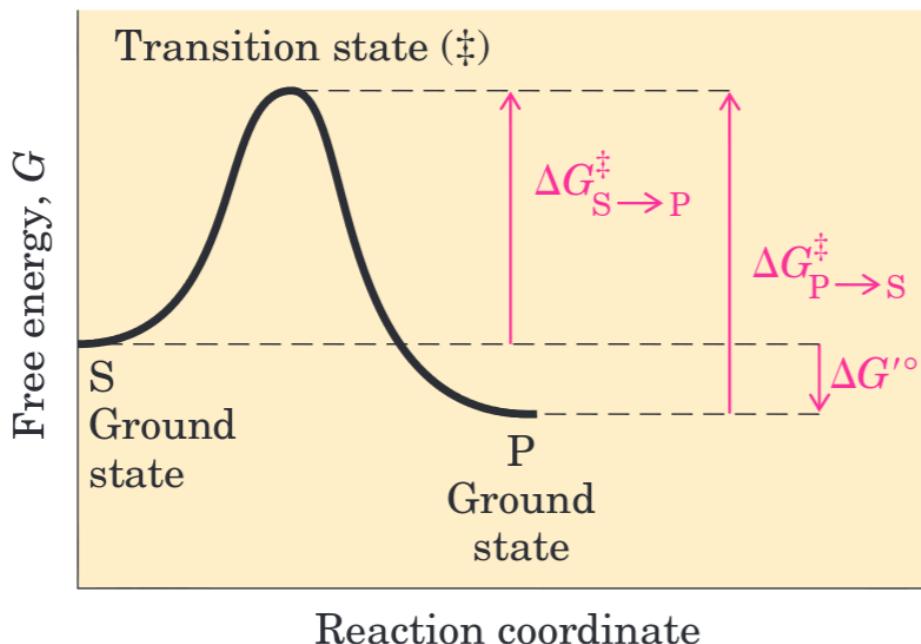
➤ First order reaction S=>P:

rate $V=k[\text{S}]$, $k(\text{s}^{-1})$

➤ $\text{S}_1 + \text{S}_2 \Rightarrow \text{P}$

rate $V=k[\text{S}_1][\text{S}_2]$, $k(\text{M}^{-1}\text{s}^{-1})$

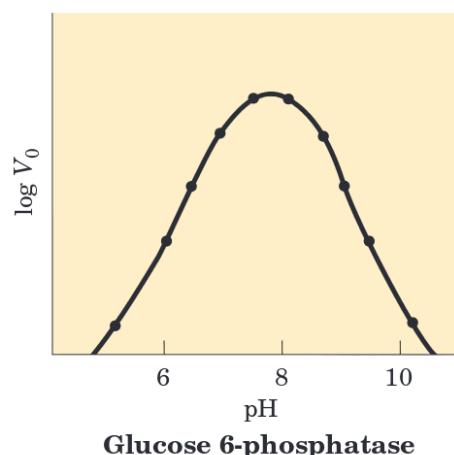
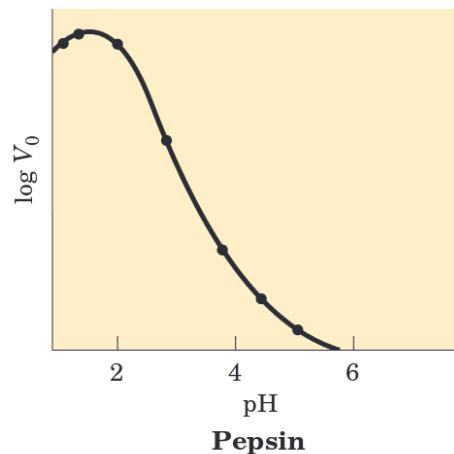
➤ $k = k_B T/h \exp(-\Delta G^\ddagger/RT)$



K'_{eq}	$\Delta G'^{\circ} (\text{kJ/mol})$
10^{-6}	34.2
10^{-5}	28.5
10^{-4}	22.8
10^{-3}	17.1
10^{-2}	11.4
10^{-1}	5.7
1	0.0
10^1	-5.7
10^2	-11.4
10^3	-17.1

REACTION CONDITIONS

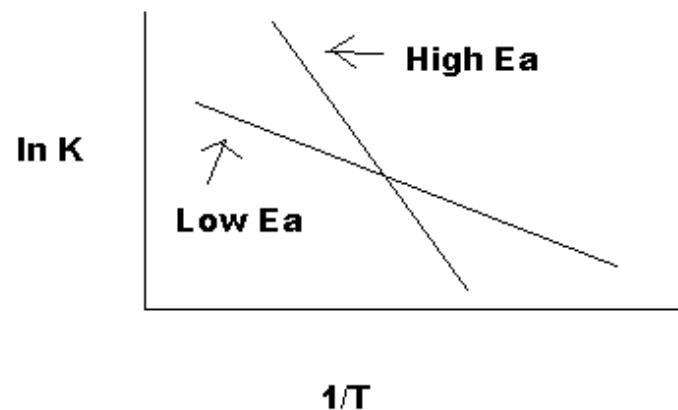
- Substrate concentration
- Enzyme concentration
- pH: protonation state, H⁺ concentration, chemical potential
- Temperature: Arrenius equation
- Co-factors
- Activators
- Inhibitors



$$k = A e^{-\frac{E_a}{RT}} \quad \text{or} \quad \ln k = -\frac{E_a}{RT} + \ln A$$

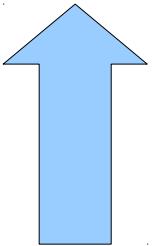
Where:

- k = Chemical Reaction Rate
 A = Pre-exponential Factor
 E_a = Activation Energy
 R = Gas Constant
 T = Temperature in Kelvin



CATALYTIC POWER OF ENZYMES

- Enormous increase of the rate
- High specificity

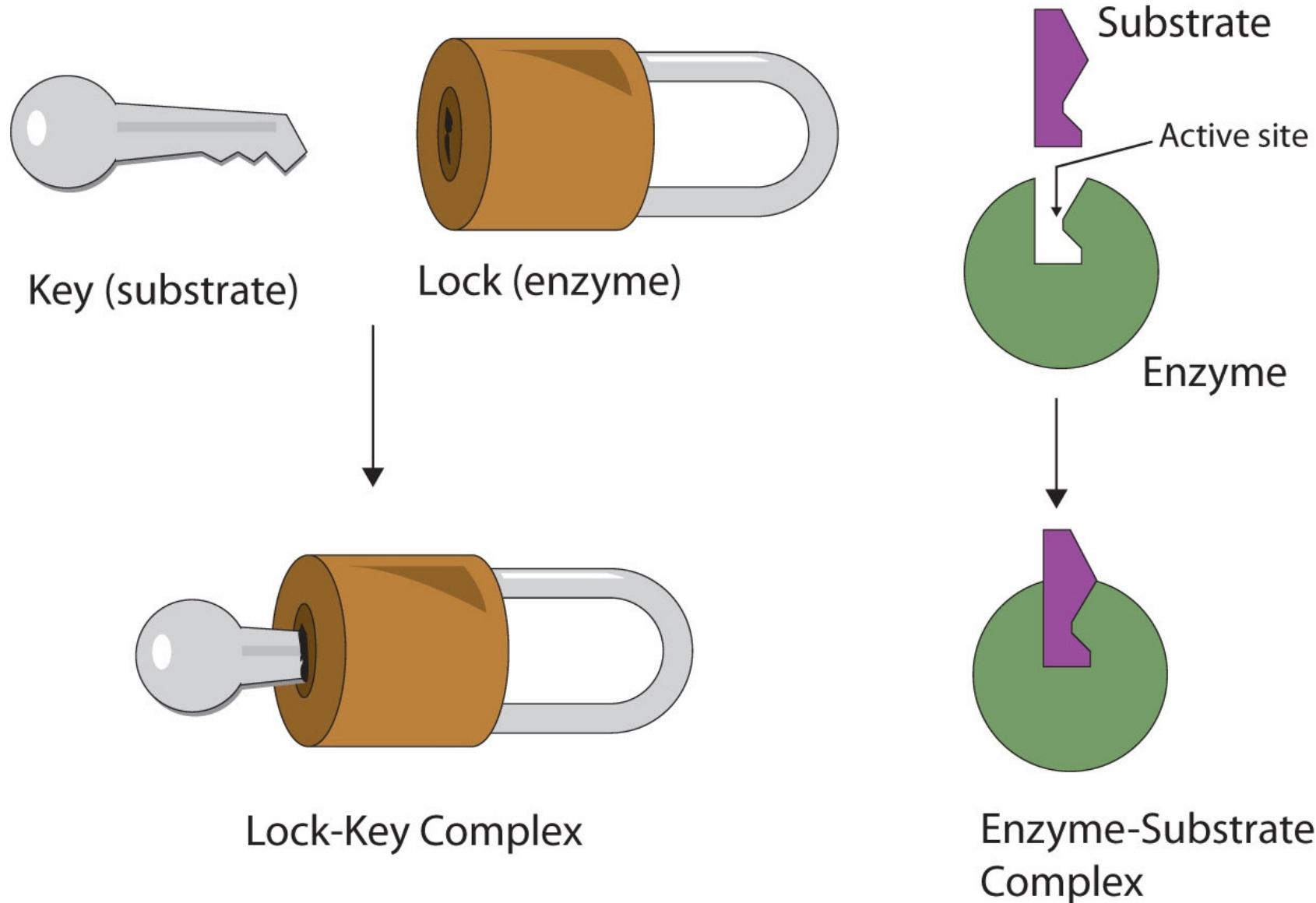


Rates increase by enzymes

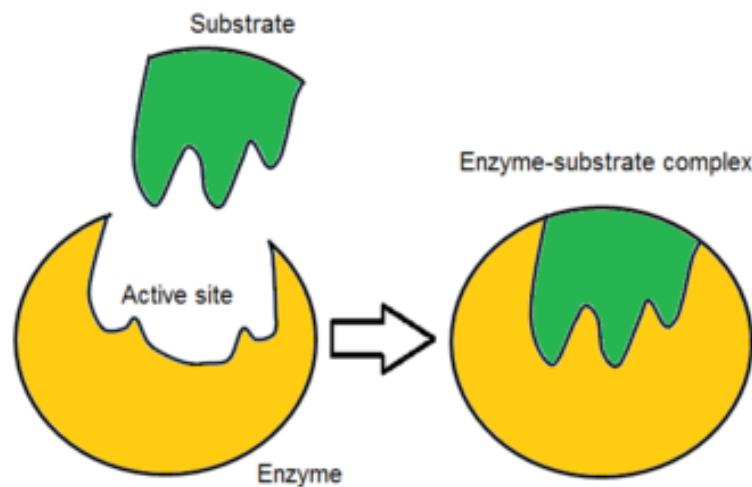
Cyclophilin	10^5
Carbonic anhydrase	10^7
Triose phosphate isomerase	10^9
Carboxypeptidase A	10^{11}
Phosphoglucomutase	10^{12}
Succinyl-CoA transferase	10^{13}
Urease	10^{14}
Orotidine monophosphate decarboxylase	10^{17}

- Formation of transient covalent bonds
- Non-covalent interactions ($\Delta G_{\text{binding}}$):
 - major source of lowering ΔG^\ddagger
 - structural complementation
- In ES complex the structure is optimized

LOCK AND KEY CONCEPT

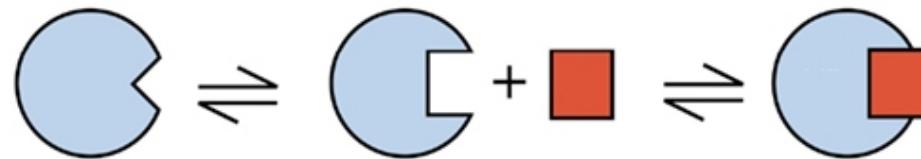


HAND AND GLOVE CONCEPT

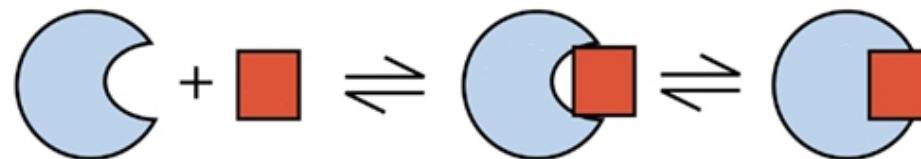


CONFORMATIONAL SELECTION VS. INDUCED FIT

Conformational selection

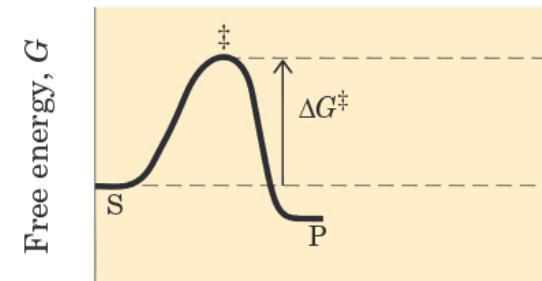
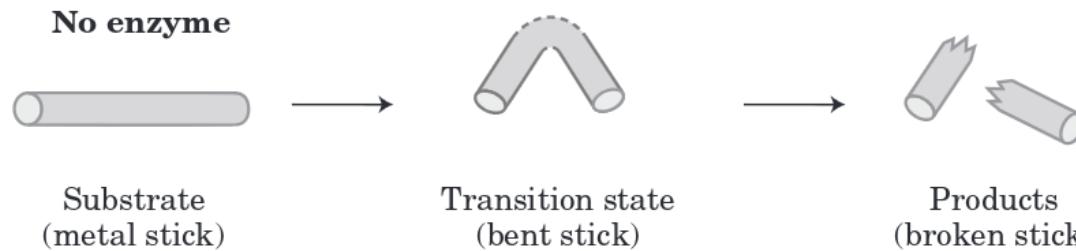


Induced fit

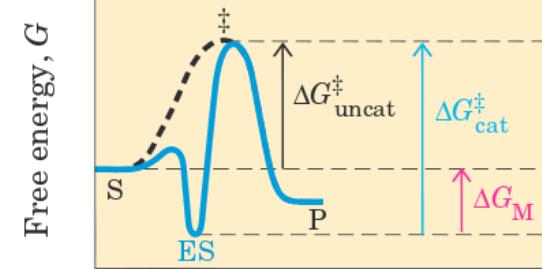
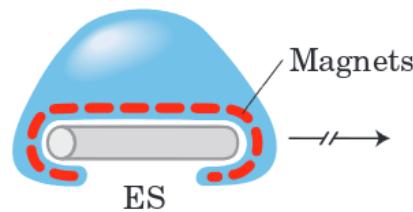


- **Conformational selection:** enzyme adapts the proper conformation prior to binding.
- **Induced fit:** binding induced the change of the conformation in the enzyme.
- **Reality:** mixed.

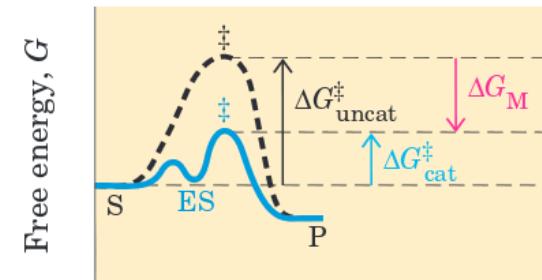
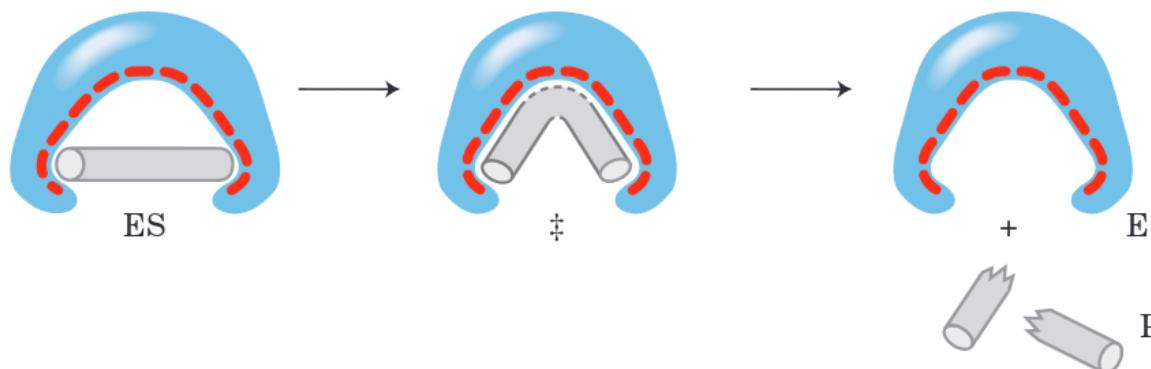
ENZYME COMPLEMENTARITY: SUBSTRATE VS. TRANSITION STATE



Enzyme complementary to substrate



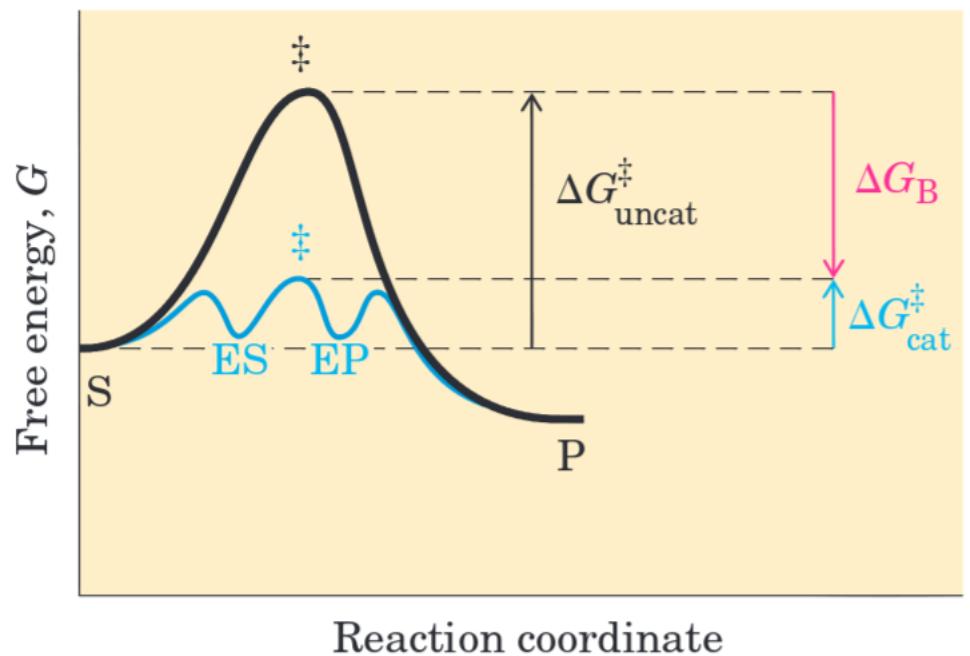
Enzyme complementary to transition state



Reaction coordinate

BINDING ENERGY IN THE CATALYSIS

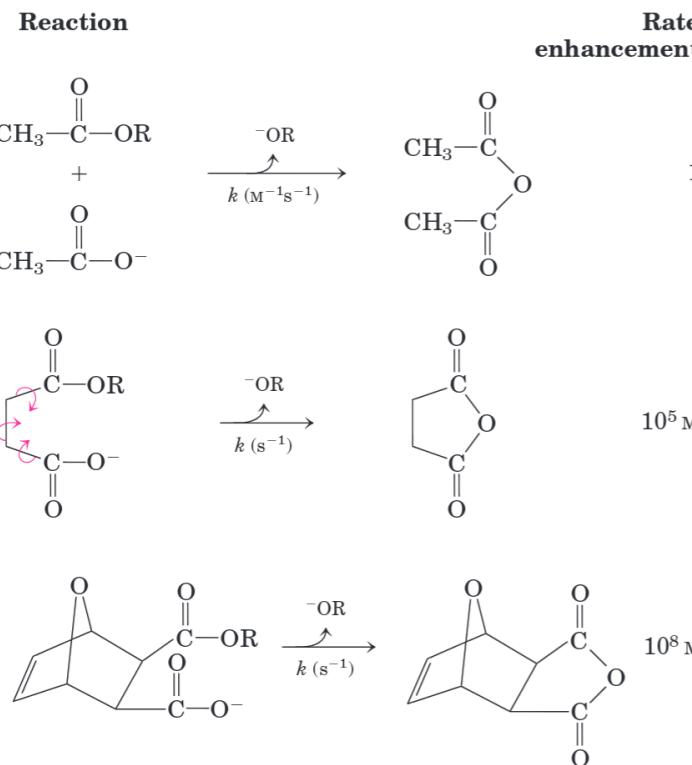
- Net activation energy is decisive for the rate: $\Delta G_B + \Delta G^\ddagger$.
- Non-covalent interactions between S and E should provide a substantial driving force for enzymatic catalysis.
- Cavity binding favours abundance/specificity of non-covalent interactions.
- Specificity: ability to discriminate the real substrate and others:
 - H-bonds
 - Salt bridges
 - van der Waals contacts



THERMODYNAMICS OF LOWERING A FREE ENERGY BARRIER

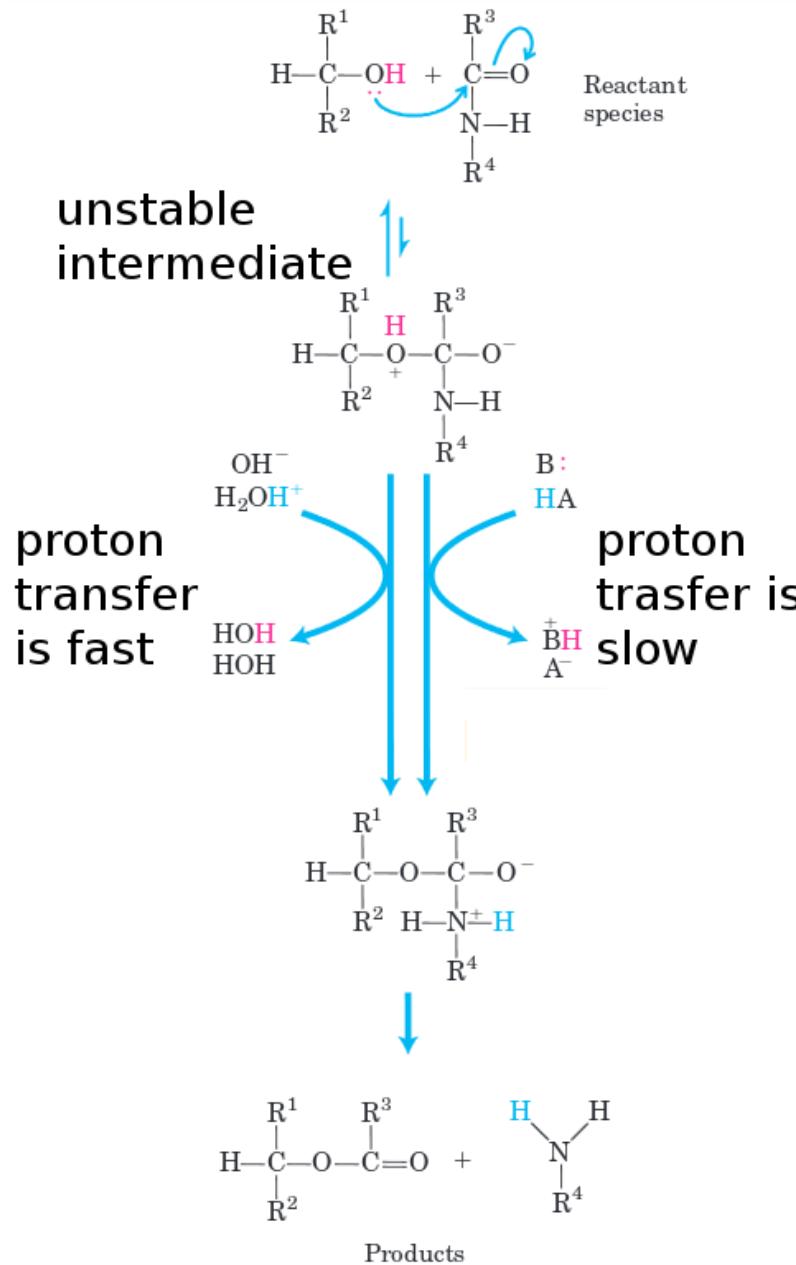
$$\Delta G = \Delta H - T\Delta S$$

- Entropy reduction
- Solvation/desolvation (solvent-solute, solvent-solvent interactions)
- Conformational changes/distortions of the substrate
- Proper orientation of catalytic functional groups



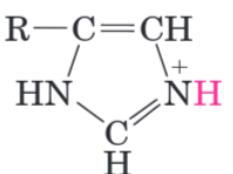
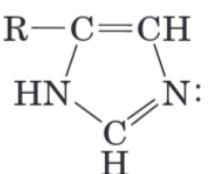
ACID-BASE CATALYSIS

➤ Acid/base catalysis: H_3O^+ , OH^- are involved in non-enzymatic case.

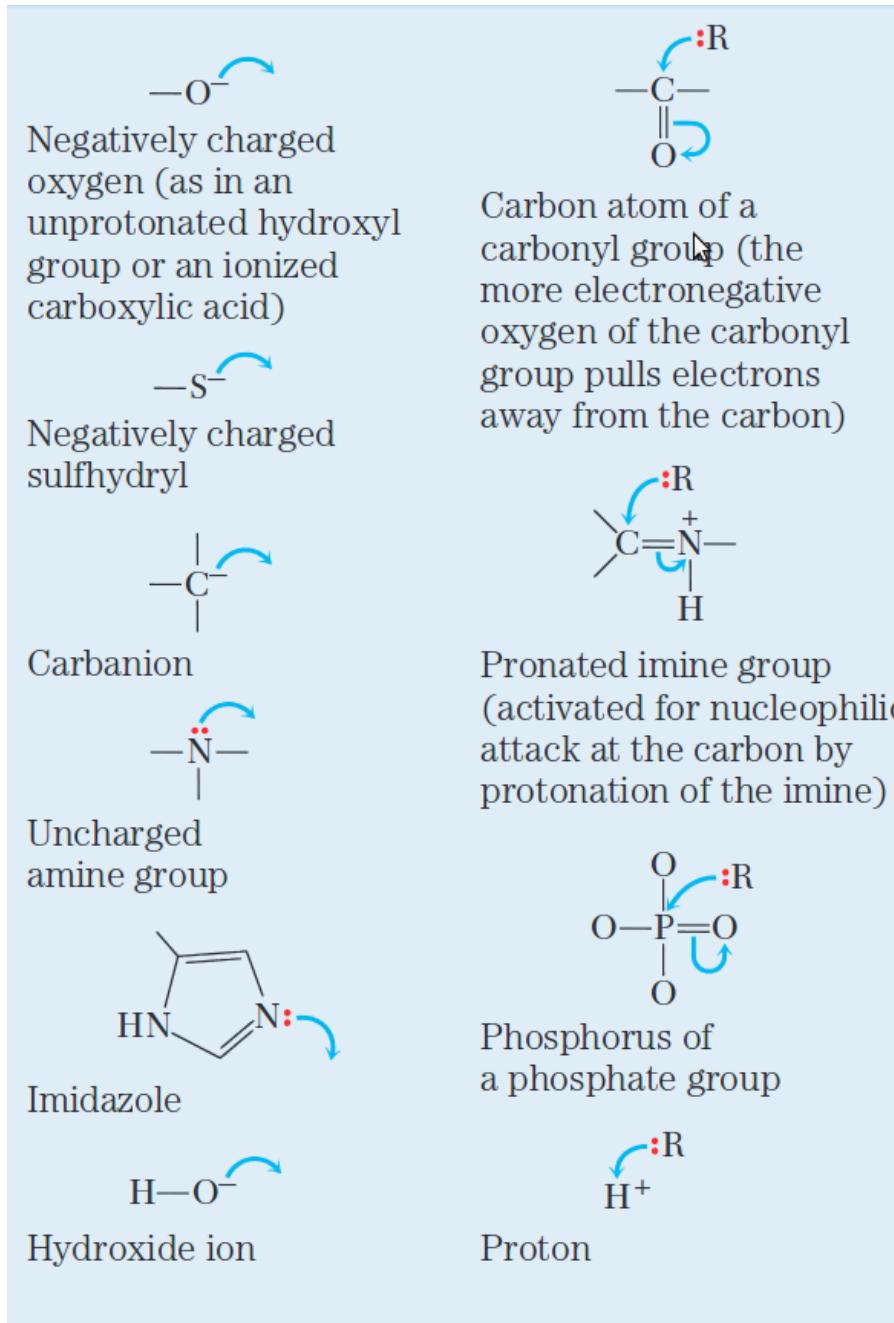


GENERAL ACID-BASE CATALYSIS

➤ General acid/base catalysis: proton transfer mediated by other molecules than water.

Amino acid residues	General acid form (proton donor)	General base form (proton acceptor)
Glu, Asp	R—COOH	R—COO [−]
Lys, Arg	R— ⁺ NH ₂	R—NH ₂
Cys	R—SH	R—S [−]
His		
Ser	R—OH	R—O [−]
Tyr		

NUCLEOPHILES AND ELECTROPHILES

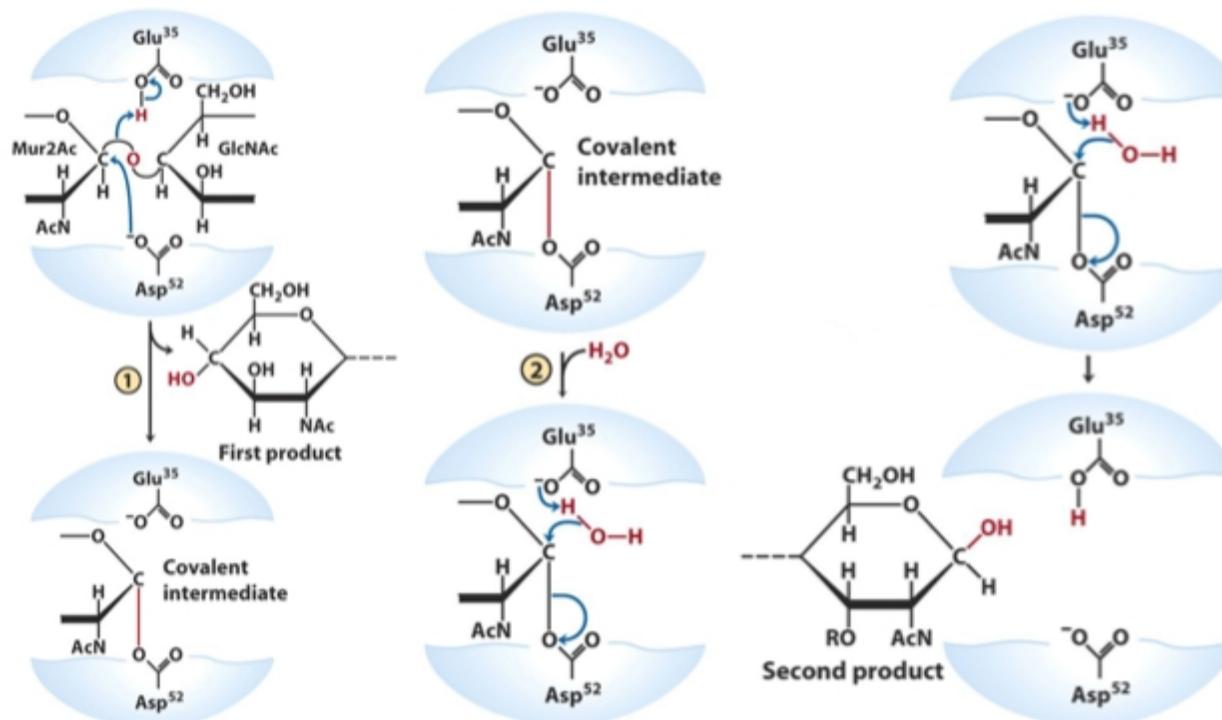


COVALENT CATALYSIS

Covalent bond is formed between S and E.

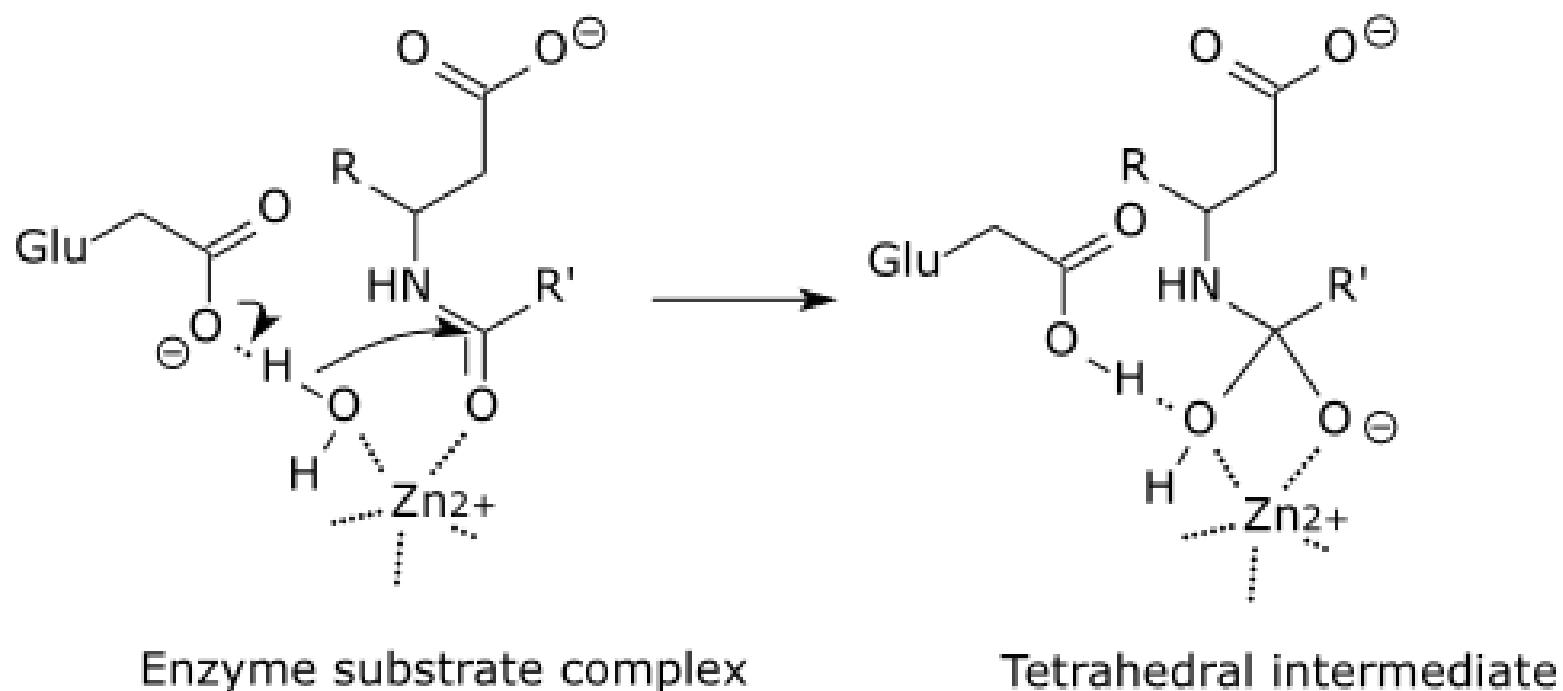


Together should be faster.



METAL ION CATALYSIS

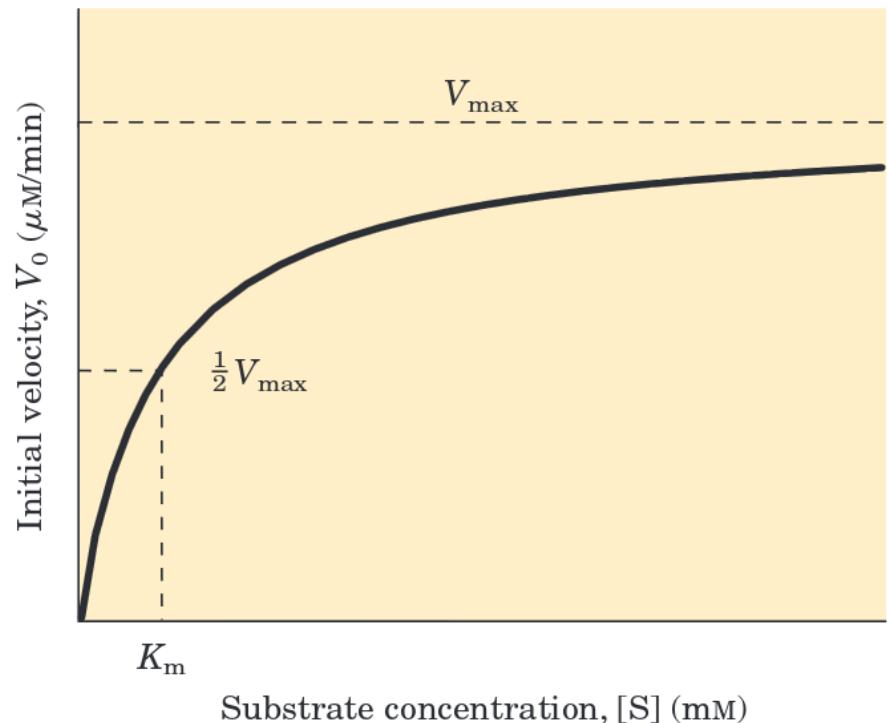
- Strong contribution to binding.
- Stabilization of the transient state.
- Oxidation-reduction reactions by changing its oxidation state:
 $\text{Cu}^+ \Rightarrow \text{Cu}^{2+}$, $\text{Fe}^{2+} \Rightarrow \text{Fe}^{3+}$ etc.



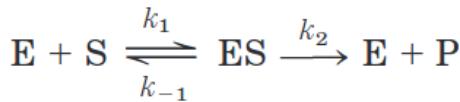
ENZYME KINETICS

Enzyme kinetics studies the rate of the reaction and its change in response to experimental parameters.

- $[S] \neq \text{const}$ during the reaction
- $S+E \Rightarrow ES$
- V_o is the initial rate of the reaction, where $[S] \gg [E]$ (usually $\sim 5\text{-}6$ orders)
- V_{\max} : all E is saturated
- Pre-steady state/steady state
- Steady state kinetics



MICHAELIS-MENTEN EQUATION



$$V_0 = k_2[ES]$$

Rate of ES formation = $k_1([E_t] - [ES])[S]$

Rate of ES breakdown = $k_{-1}[ES] + k_2[ES]$

$$k_1([E_t] - [ES])[S] = k_{-1}[ES] + k_2[ES]$$

$$k_1[E_t][S] - k_1[ES][S] = (k_{-1} + k_2)[ES]$$

$$k_1[E_t][S] = (k_1[S] + k_{-1} + k_2)[ES]$$

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

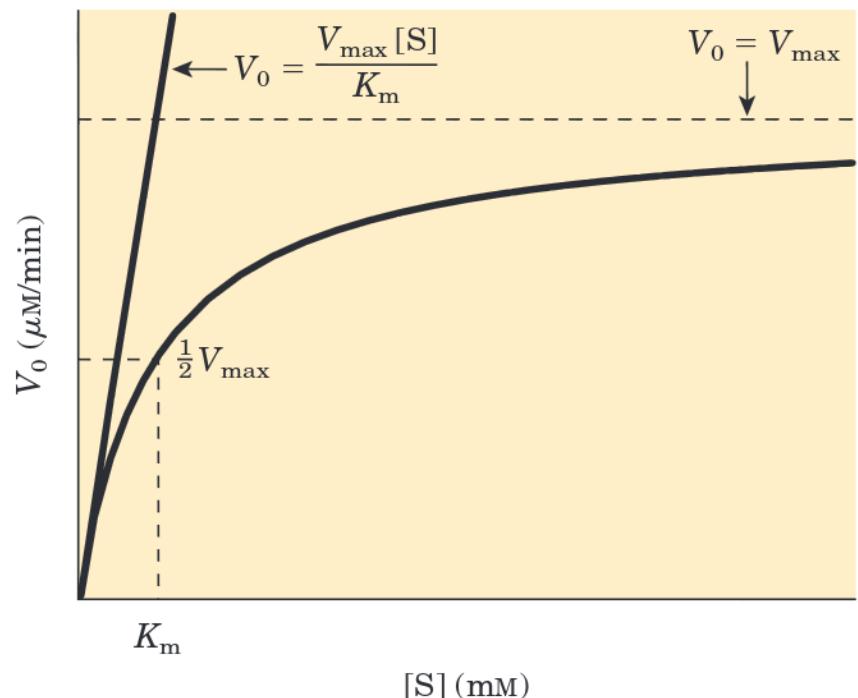
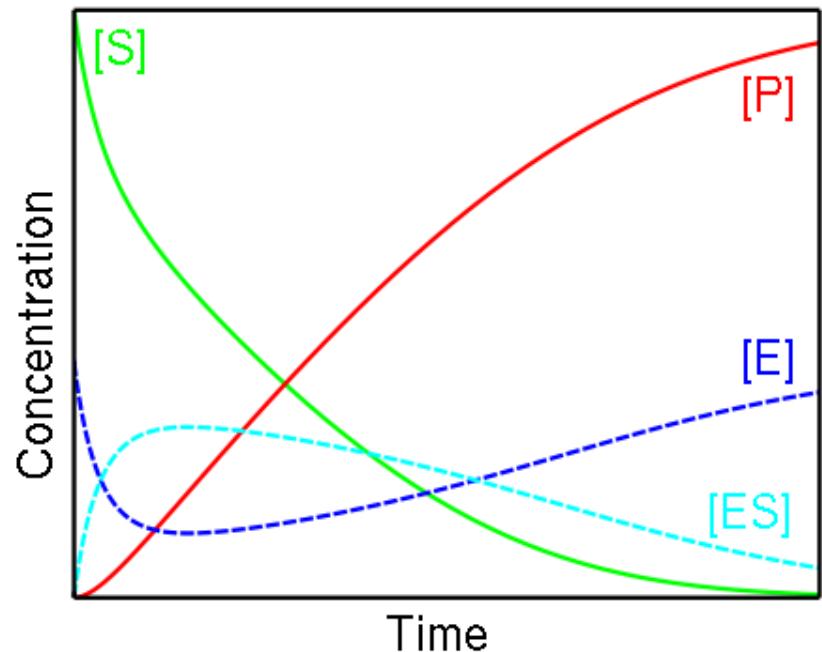
$$[ES] = \frac{[E_t][S]}{[S] + (k_2 + k_{-1})/k_1}$$

$$[ES] = \frac{[E_t][S]}{K_m + [S]} \quad K_m = \frac{k_2 + k_{-1}}{k_1}$$

$$V_0 = \frac{k_2[E_t][S]}{K_m + [S]}$$

$$V_0 = \frac{V_{\max} [S]}{K_m + [S]}$$

$$K_m = [S], \quad V_0 = \frac{1}{2}V_{\max}$$



MICHAELIS-MENTEN EQUATION

➤ Applicable to two-step simple reactions

➤ Steady-state kinetics

➤ Can be accessible from the experiment directly

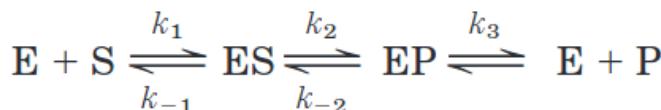
➤ Lineweaver-Burk equation

➤ If $k_2 \ll k_{-1}$, $K_m = K_d$

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

➤ If $k_2 \gg k_{-1}$, $K_m = k_2/k_1$

➤ EP \Rightarrow E + P is rate limiting



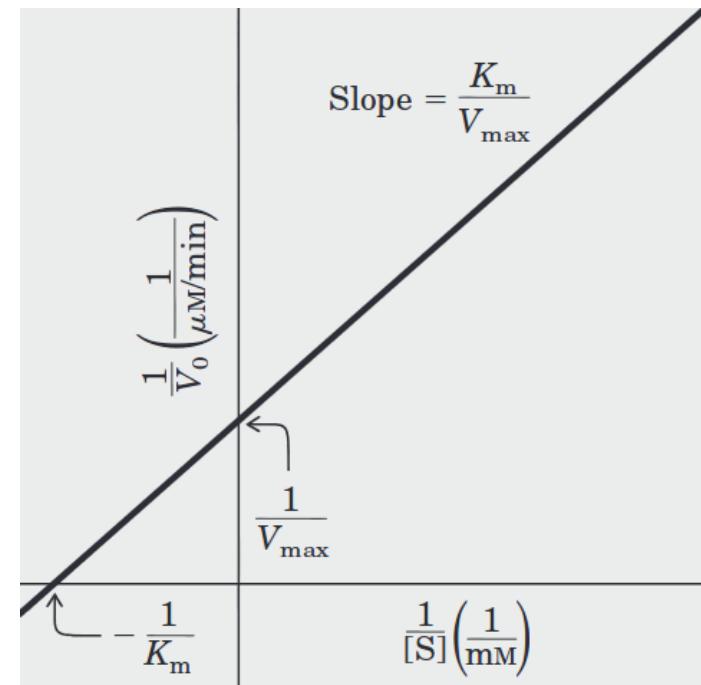
$$V_{\max} = k_3 [E_t]$$

$$k_{\text{cat}} = k_3$$

$$k_{\text{cat}} = V_{\max}/[E_t] \quad \text{Turnover number}$$

$$V_0 = \frac{k_{\text{cat}} [E_t][S]}{K_m + [S]}$$

$$\frac{1}{V_0} = \frac{K_m}{V_{\max}[S]} + \frac{1}{V_{\max}}$$



Enzyme	Substrate	$k_{\text{cat}} (\text{s}^{-1})$
Catalase	H_2O_2	40,000,000
Carbonic anhydrase	HCO_3^-	400,000
Acetylcholinesterase	Acetylcholine	14,000
β -Lactamase	Benzylpenicillin	2,000
Fumarase	Fumarate	800
RecA protein (an ATPase)	ATP	0.4

K_M AND k_{cat} FULLY DESCRIBE THE CATALYSIS

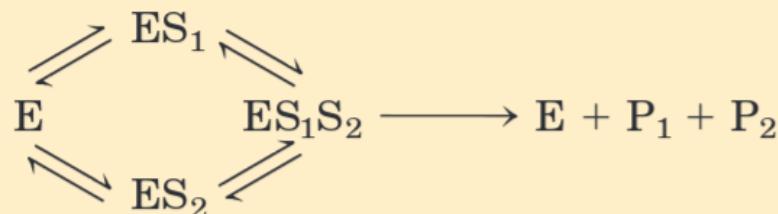
Specificity constant: k_{cat}/K_M ; If $[S] \ll K_M$ then: $V_0 = k_{cat}/K_M [E_t][S]$

BINDING OF SEVERAL SUBSTRATES

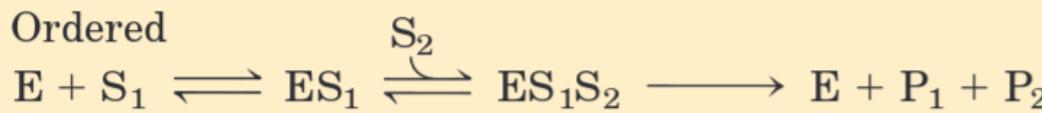
Hexokinase: ATP + Glucose \Rightarrow ADP + Glucose-6P

Enzyme reaction involving a ternary complex

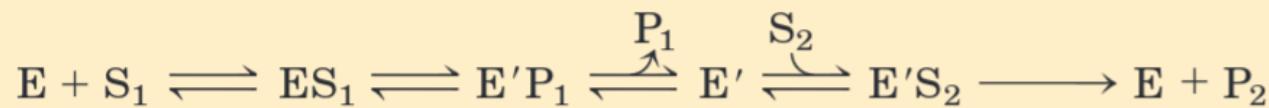
Random order



Ordered

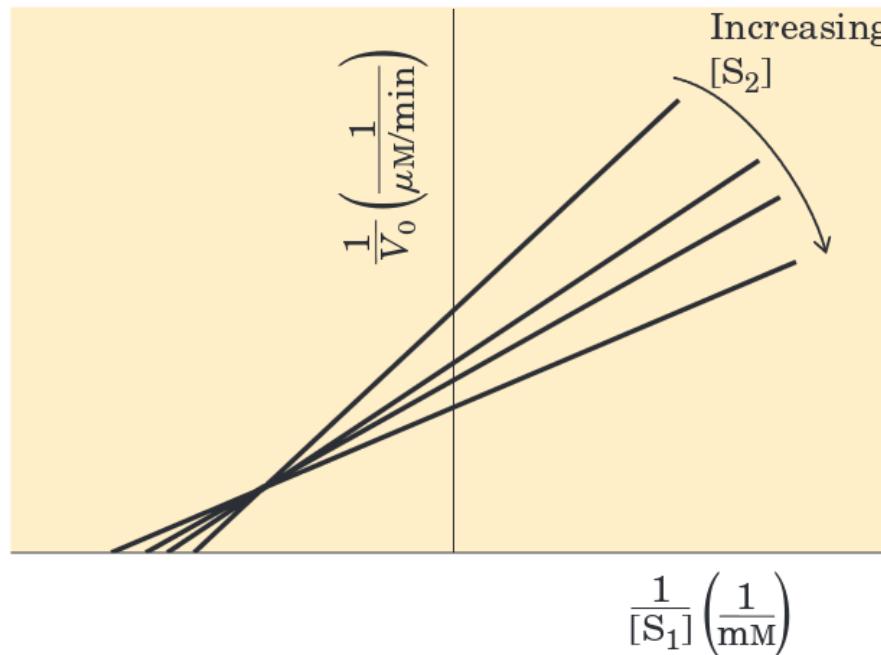


Enzyme reaction in which no ternary complex is formed

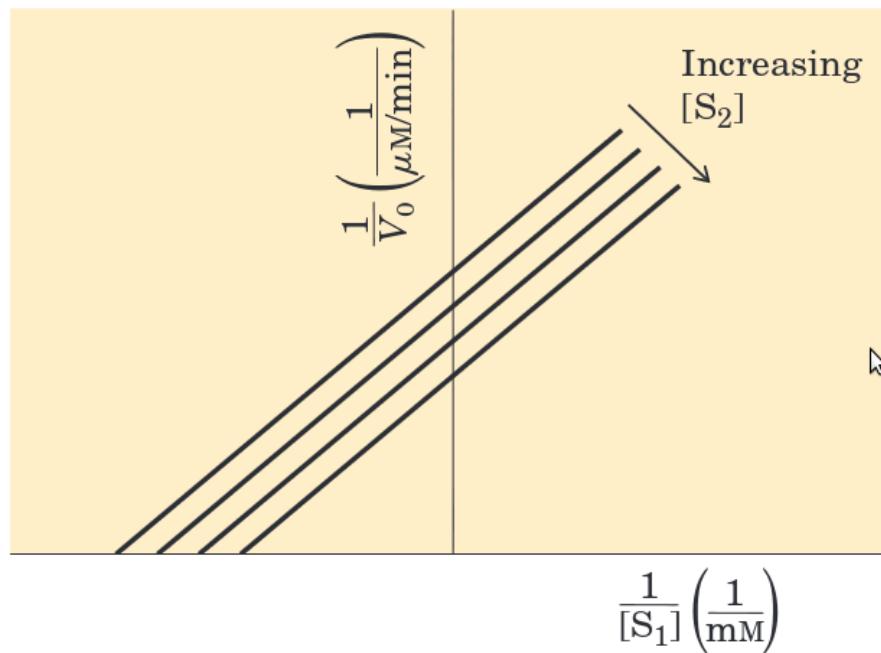


BINDING OF SEVERAL SUBSTRATES

Ternary complex



Ping-pong mechanism

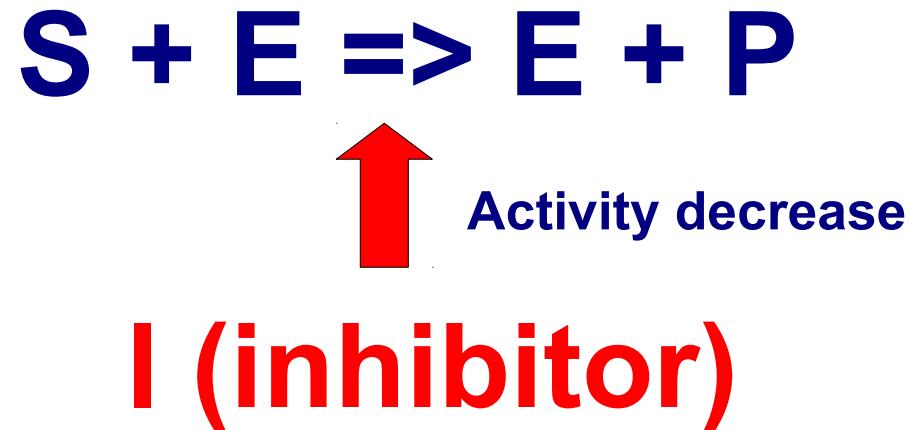


INHIBITION

Inhibition: binding a substance (inhibitor) decreases enzyme's activity

Reversible: when inhibitor is removed the enzyme restores its activity

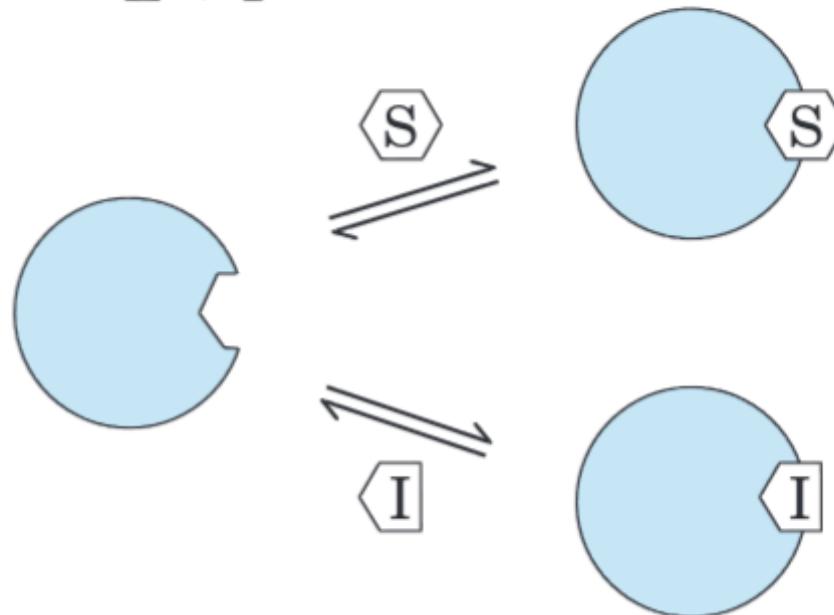
Irreversible: when inhibitor is removed the enzyme does not restore its activity (damage to the enzyme, covalent bonds etc.)



REVERSIBLE INHIBITION: COMPETITIVE



+
I
 $\downarrow K_I$
EI

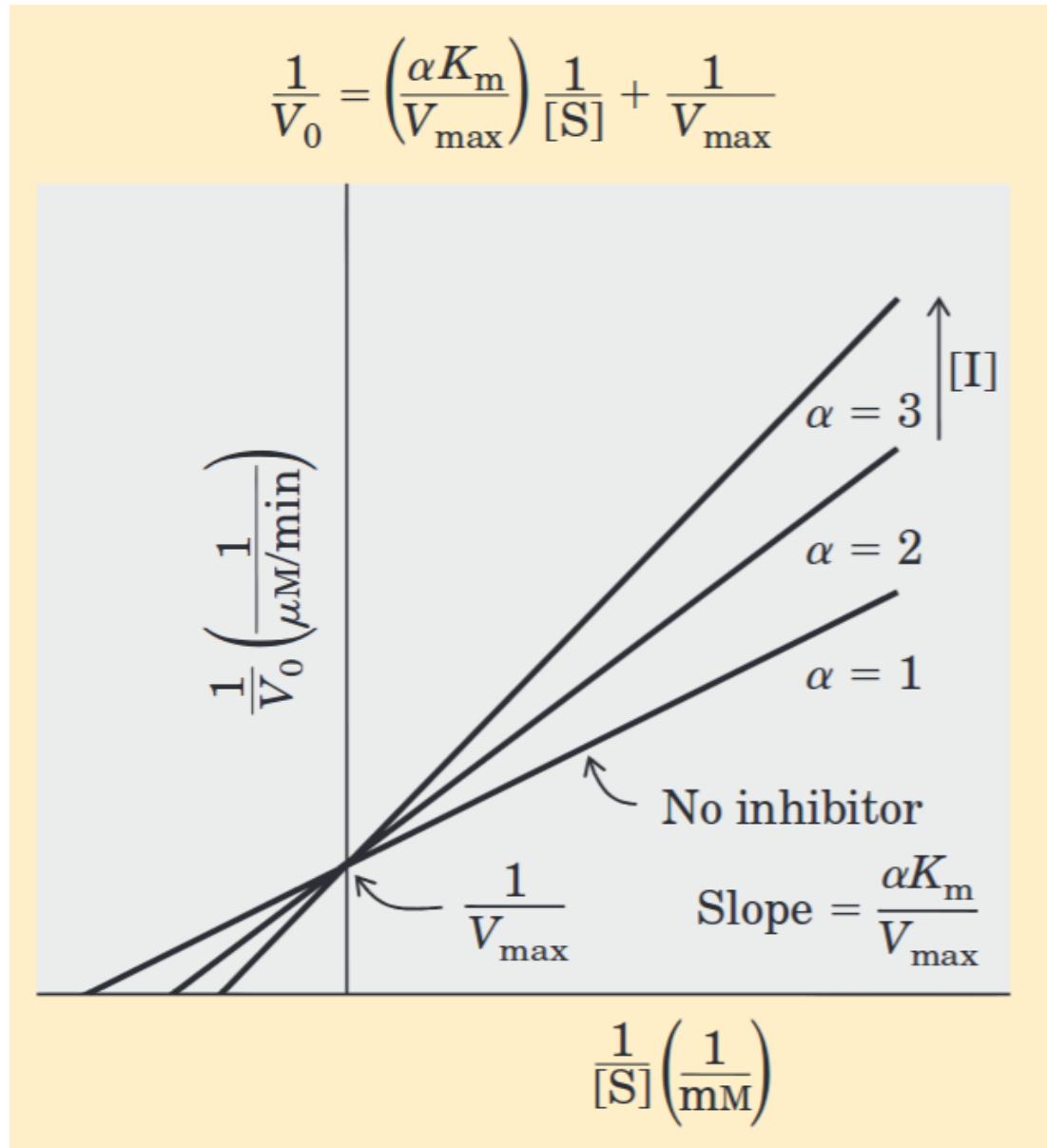


$$V_0 = \frac{V_{\max} [S]}{\alpha K_m + [S]}$$

$$\alpha = 1 + \frac{[I]}{K_I} \quad \text{and} \quad K_I = \frac{[E][I]}{[EI]}$$

- Inhibition can be compensated by addition of S.
- S and I have similar binding properties.

REVERSIBLE INHIBITION: COMPETITIVE



Example: methanol-ethanol relationship

REVERSIBLE INHIBITION: NONCOMPETITIVE

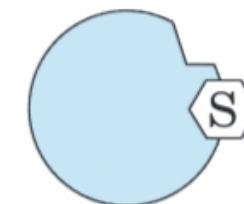
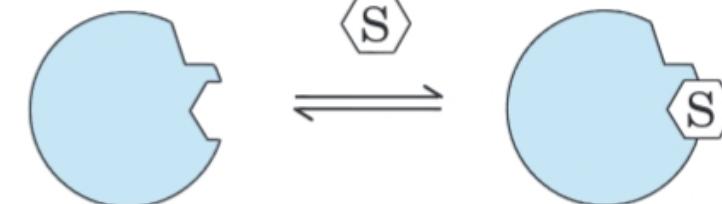


+

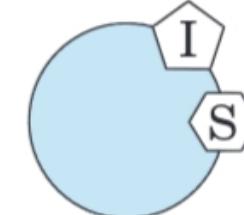
I

$$\begin{array}{c} 1 \\ \parallel \\ K_I' \\ \downarrow \end{array}$$

ESI



$$\begin{array}{c} 1 \\ \parallel \\ \downarrow \\ I \end{array}$$



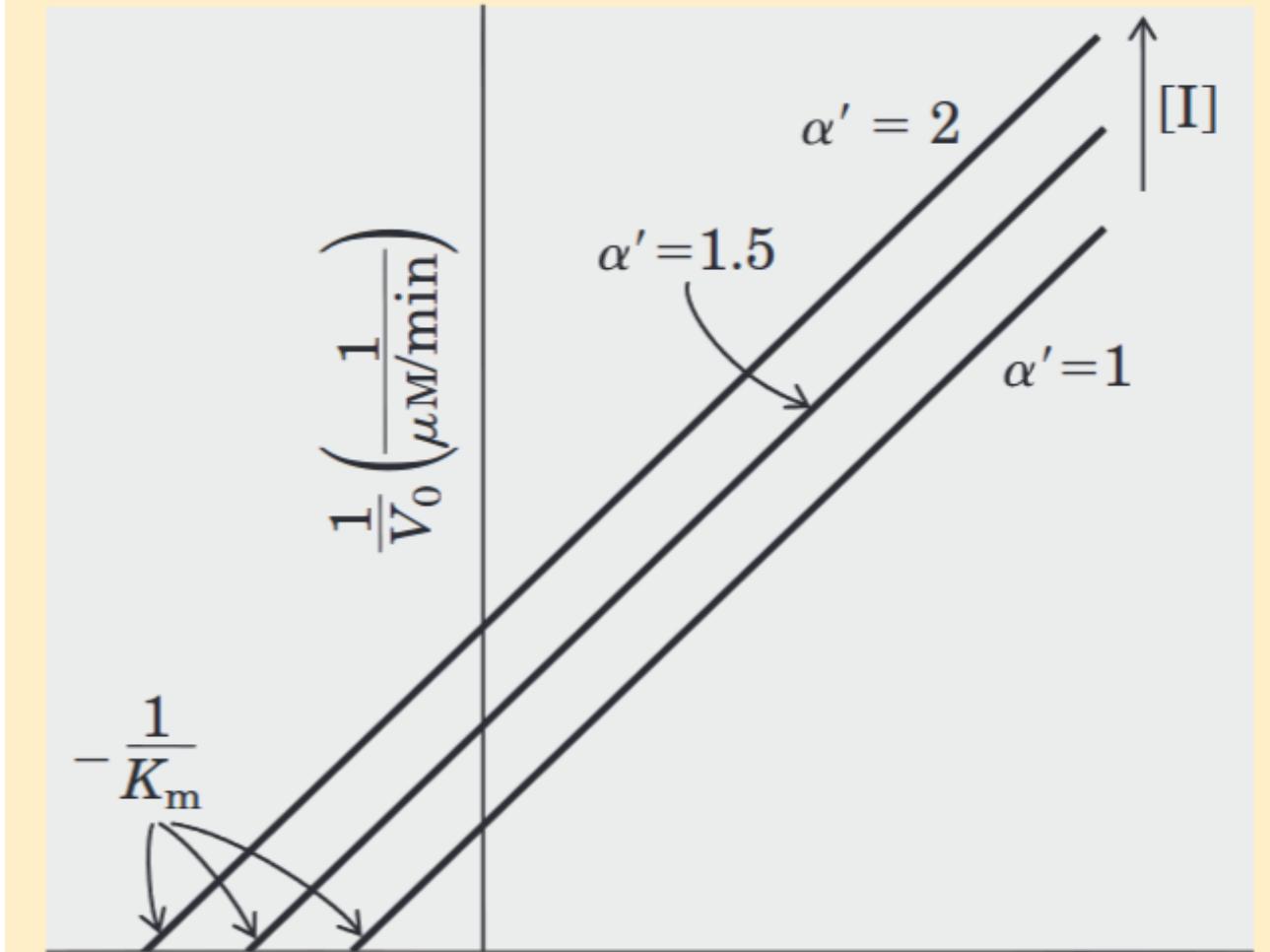
$$V_0 = \frac{V_{\max} [S]}{K_m + \alpha'[S]}$$

$$\alpha' = 1 + \frac{[I]}{K_I'} \quad \text{and} \quad K_I' = \frac{[ES][I]}{[ESI]}$$

- Inhibition cannot be compensated by addition of S.
- S and I have different binding properties.
- Allosteric effects can be important.

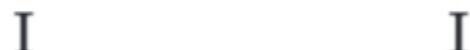
REVERSIBLE INHIBITION: NONCOMPETITIVE

$$\frac{1}{V_0} = \left(\frac{K_m}{V_{max}} \right) \frac{1}{[S]} + \frac{\alpha'}{V_{max}}$$



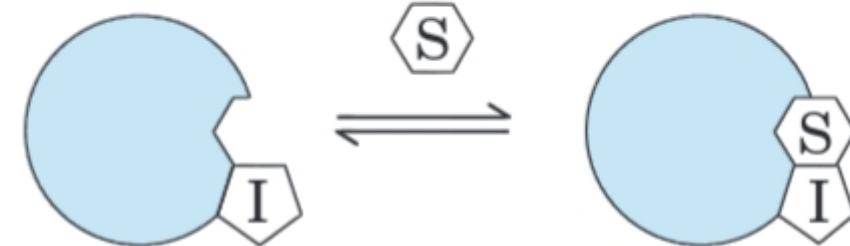
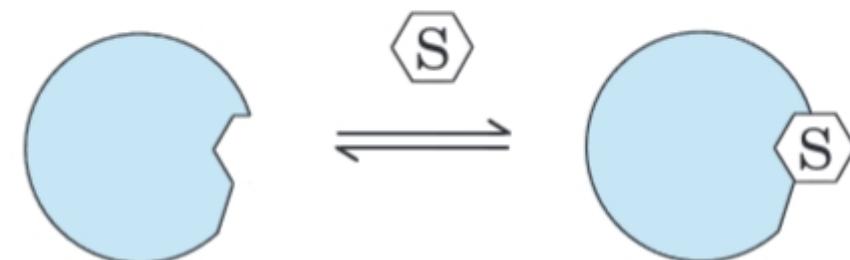
$$\frac{1}{[S]} \left(\frac{1}{\text{mM}} \right)$$

REVERSIBLE INHIBITION: MIXED



$$\Downarrow K_I$$

$$\Downarrow K'_I$$

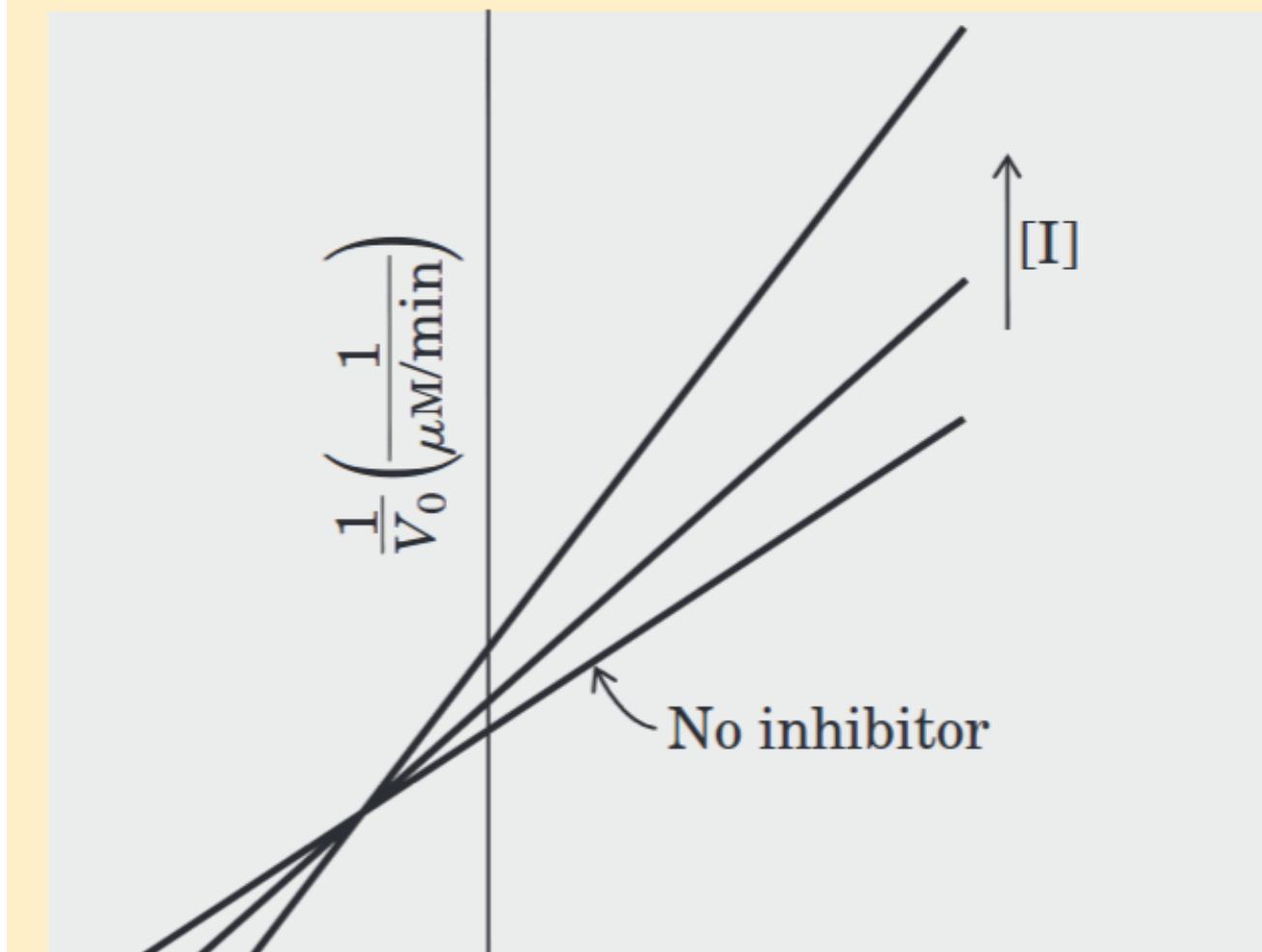


$$V_0 = \frac{V_{\max} [S]}{\alpha K_m + \alpha' [S]}$$

➤ If $\alpha=\alpha'$ it is noncompetitive inhibition.

REVERSIBLE INHIBITION: MIXED

$$\frac{1}{V_0} = \left(\frac{\alpha K_m}{V_{max}} \right) \frac{1}{[S]} + \frac{\alpha'}{V_{max}}$$



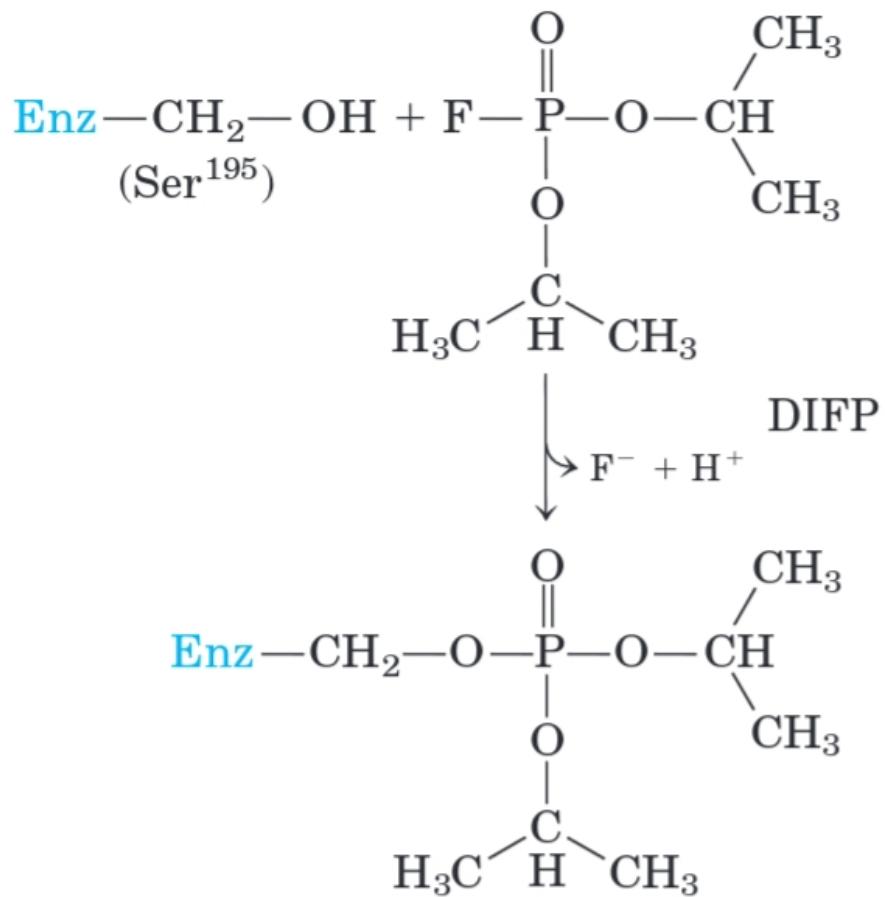
$$\frac{1}{[S]} \left(\frac{1}{\text{mM}} \right)$$

REVERSIBLE INHIBITION: SUMMARY

Inhibitor type	Apparent V_{max}	Apparent K_m
None	V_{max}	K_m
Competitive	V_{max}	αK_m
Uncompetitive	V_{max}/α'	K_m/α'
Mixed	V_{max}/α'	$\alpha K_m/\alpha'$

- Uncompetitive and mixed types of inhibition are for enzymes with two or more substrates.
- Product can be an inhibitor: feedback.

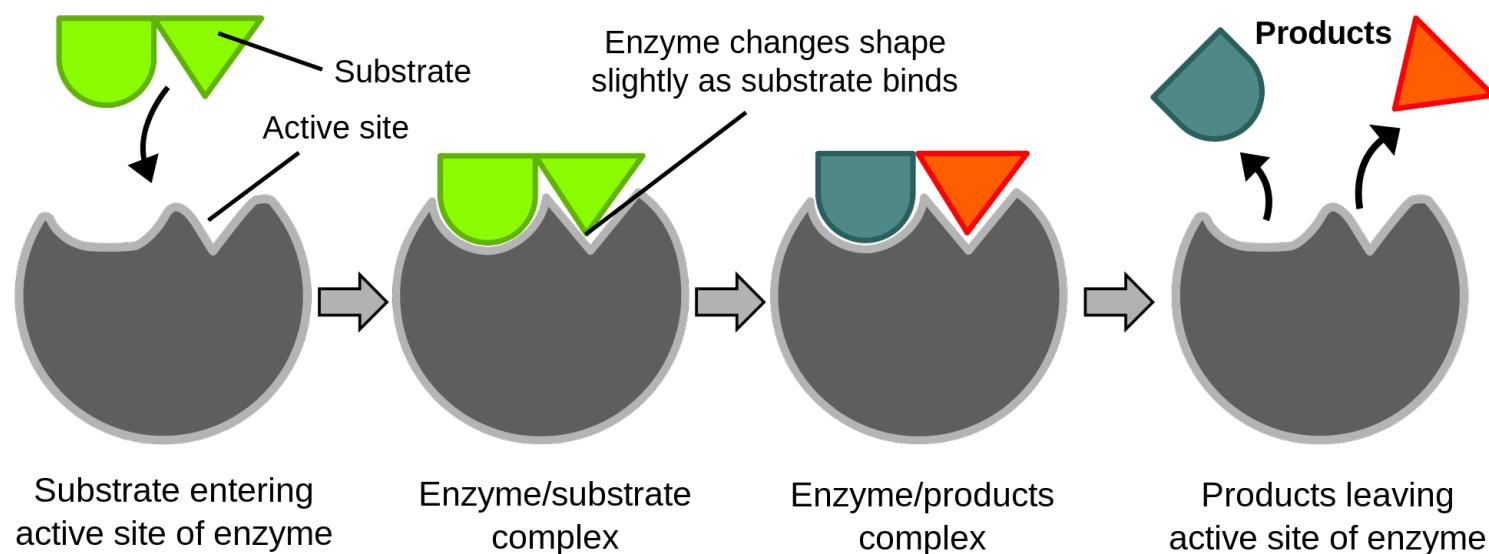
IRREVERSIBLE INHIBITION



Suicide inactivators: get covalently bound to the active site and do not unbind.

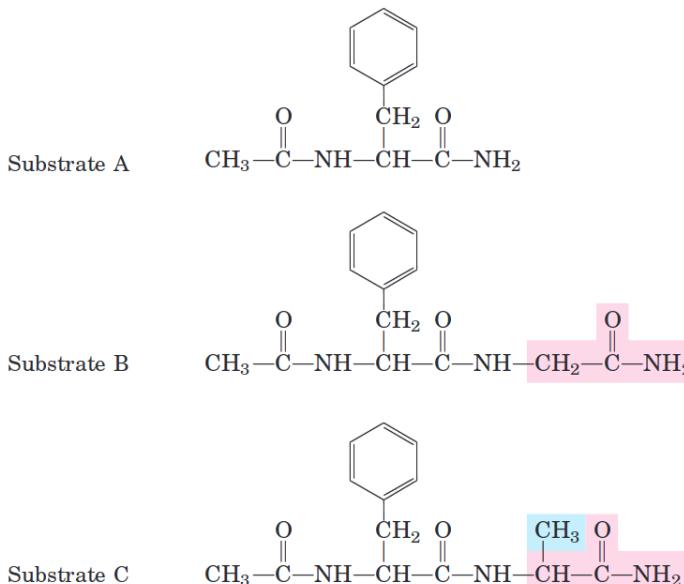
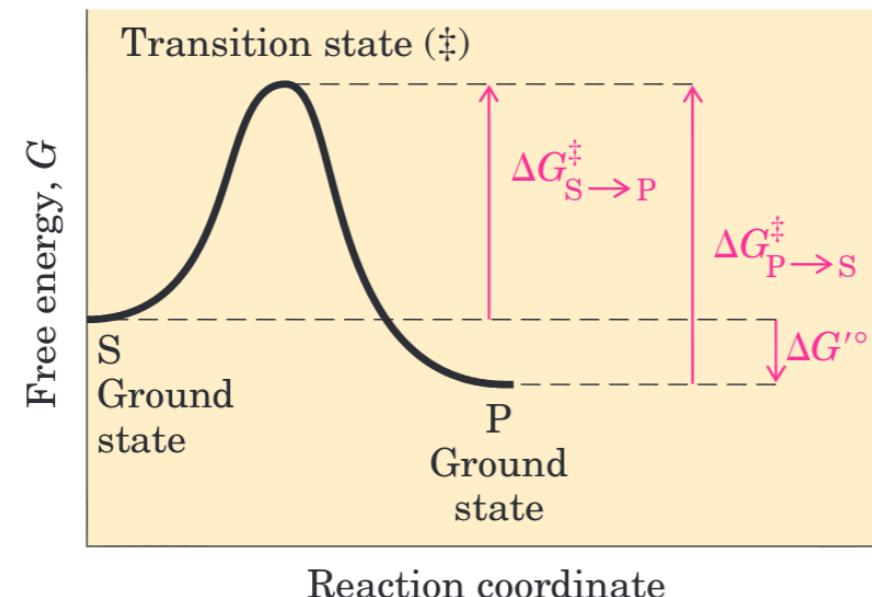
MECHANISM OF ENZYMATIC REACTIONS

- Sequential order of the events in the active site
- Structure of intermediate and all transition states
- The rates of interconversion between intermediates
- The structural relationship between the enzyme and each intermediate
- All energetic differences in the system



ENZYME-TRANSITION STATE COMPLEMENTARITY: STRUCTURE-ACTIVITY CORRELATION

- Transition state is very unstable.
- Binding of S-E_{TS} is weaker than in ES.
- Modification of S groups interacting with E in TS should change k_{cat} but not K_m.
- Enzyme engineering strategy.

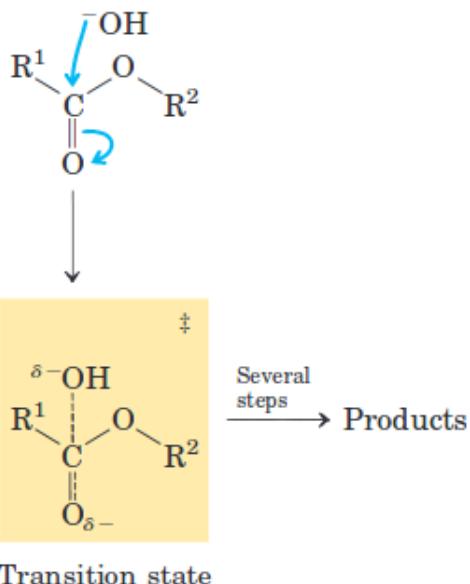


k_{cat} (s ⁻¹)	K_m (mM)	k_{cat}/K_m (M ⁻¹ s ⁻¹)
0.06	31	2
0.14	15	10
2.8	25	114

ENZYME-TRANSITION STATE COMPLEMENTARITY: TRANSITION STATE ANALOGS

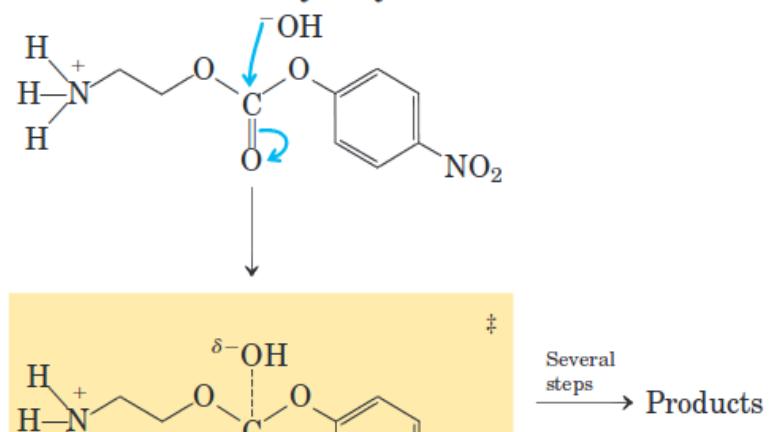
- TS-analogs bind strongly in TS-state.
- TS-analogs inhibit the reaction.
- Complexes with TS analogs can be crystallized.
- Catalytic antibody principle.

Ester hydrolysis

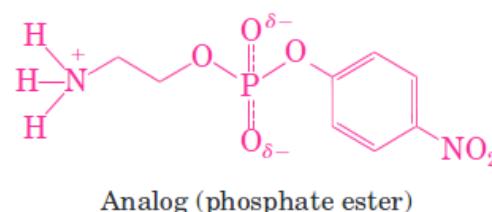


Analog (phosphonate ester)

Carbonate hydrolysis



Transition state



Analog (phosphate ester)

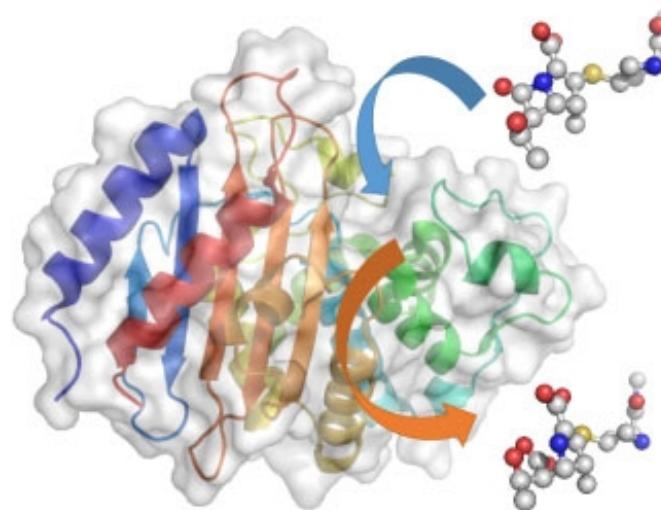
EXAMPLES OF ENZYME MECHANISMS

➤ Chymotrypsin

➤ Hexokinase

➤ Enolase

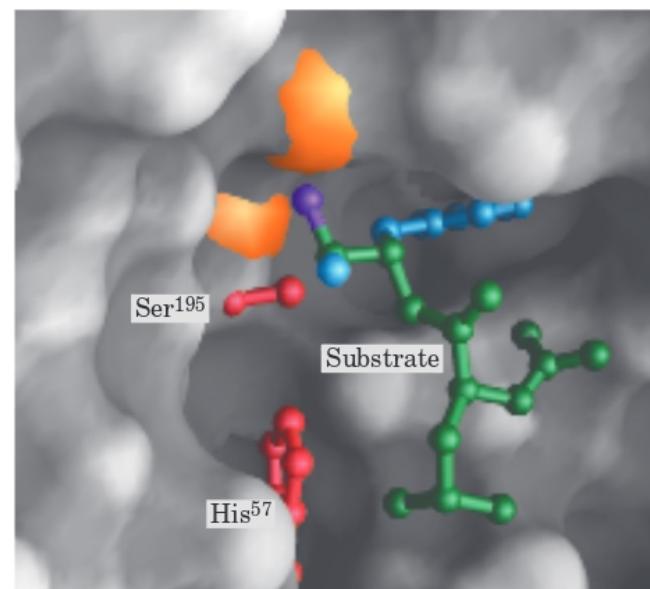
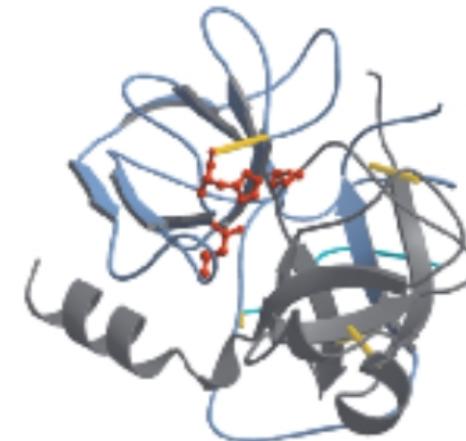
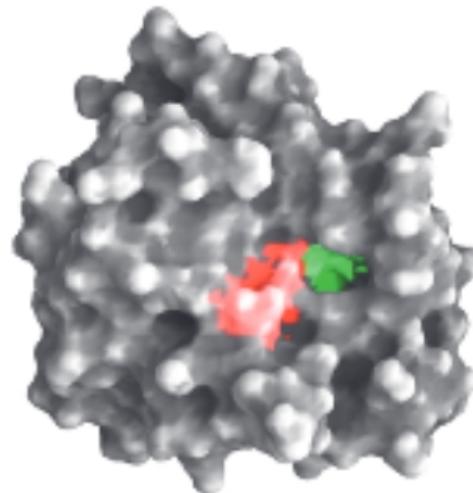
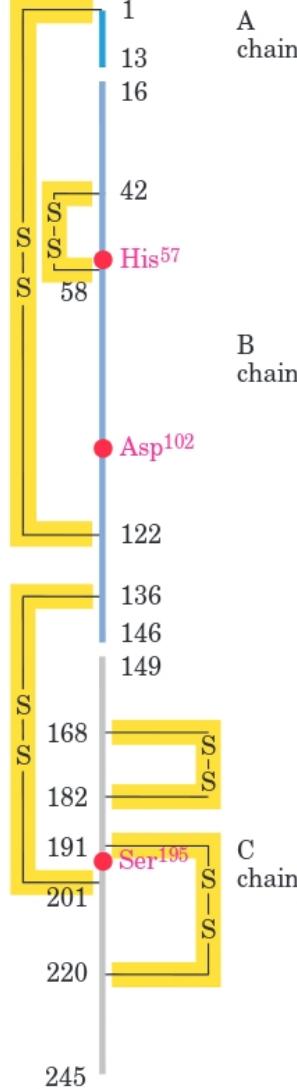
➤ Lysozyme



CHYMOTRYPSIN

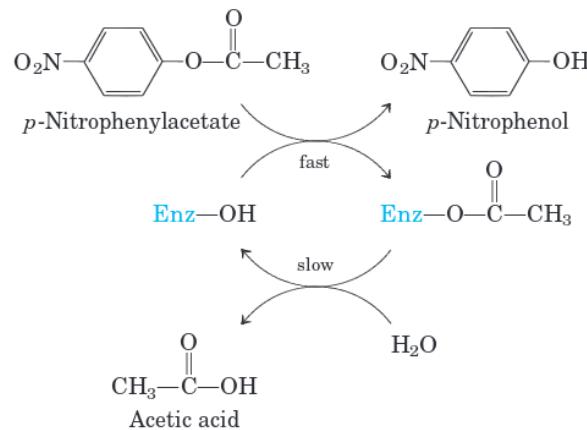
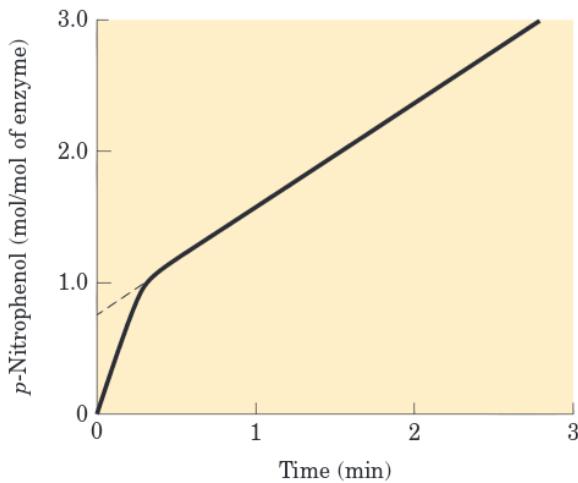
EC 3.4.21.1. Protease responsible for the cleavage of peptide bonds

Trp/Tyr/Phe-X. 10^9 of rate increase.

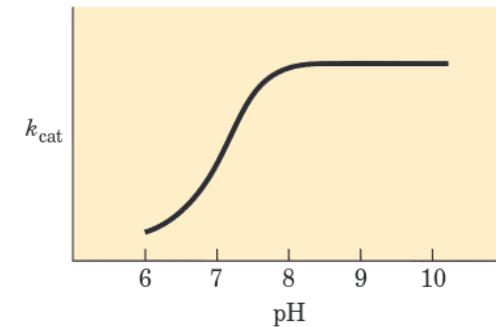
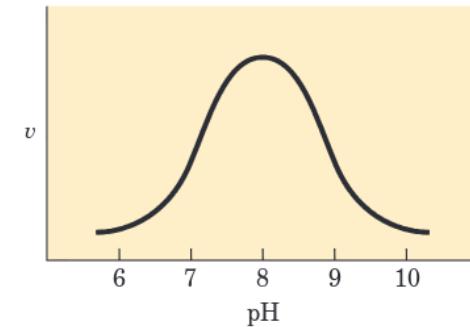


CHYMOTRYPSIN MECHANISM ELUCIDATION

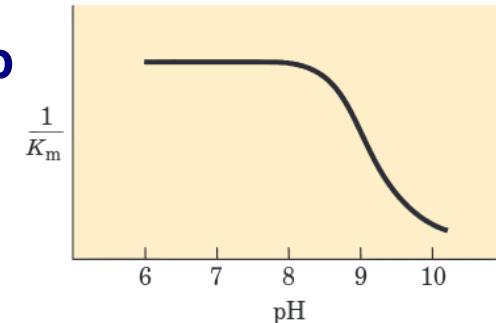
- Acylation phase: peptide bond is broken and ester linkage is formed.
- Deacylation phase: ester linkage is hydrolyzed.
- Kinetics propose the mechanism.



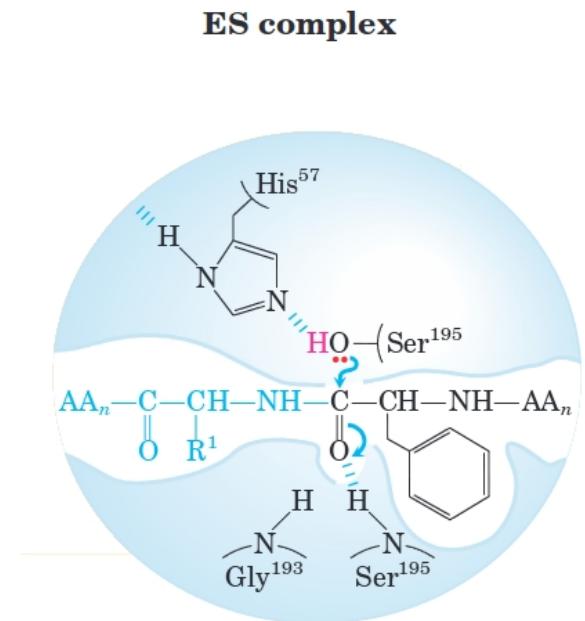
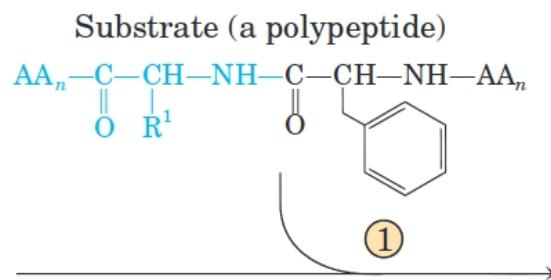
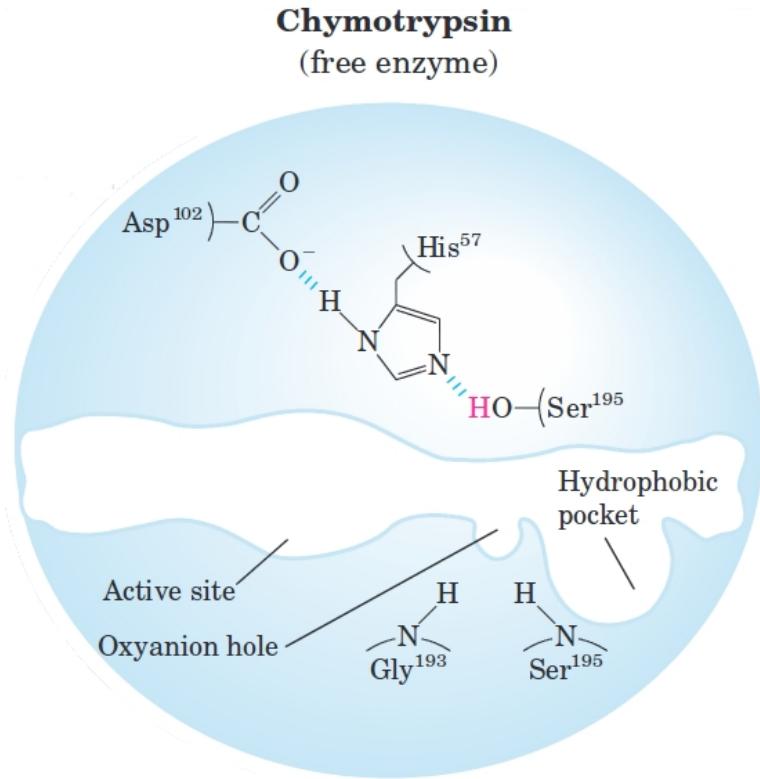
**His⁵⁷
protonation**



**Amino group
of Ile¹⁶**

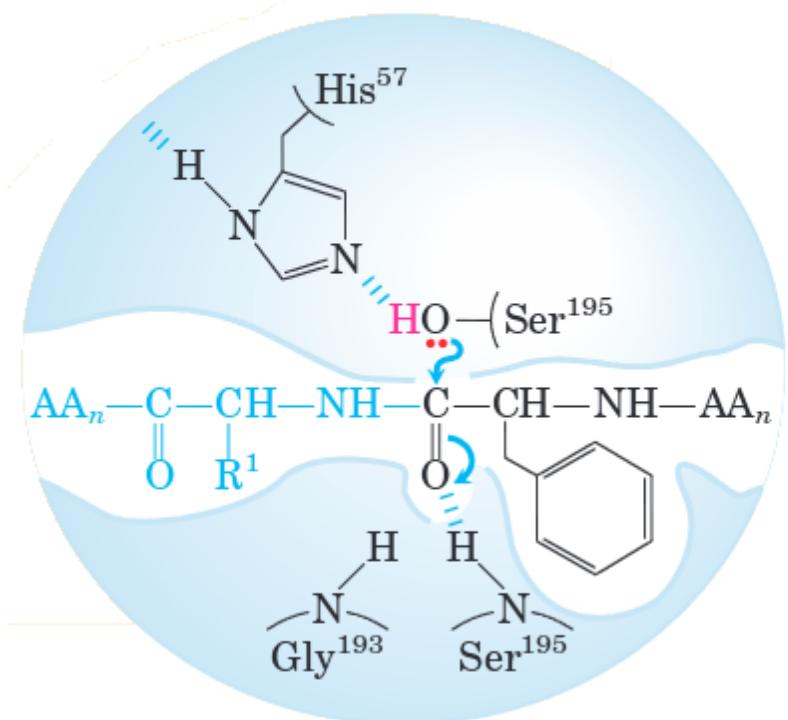


CHYMOTRYPSIN MECHANISM. STEP 1.



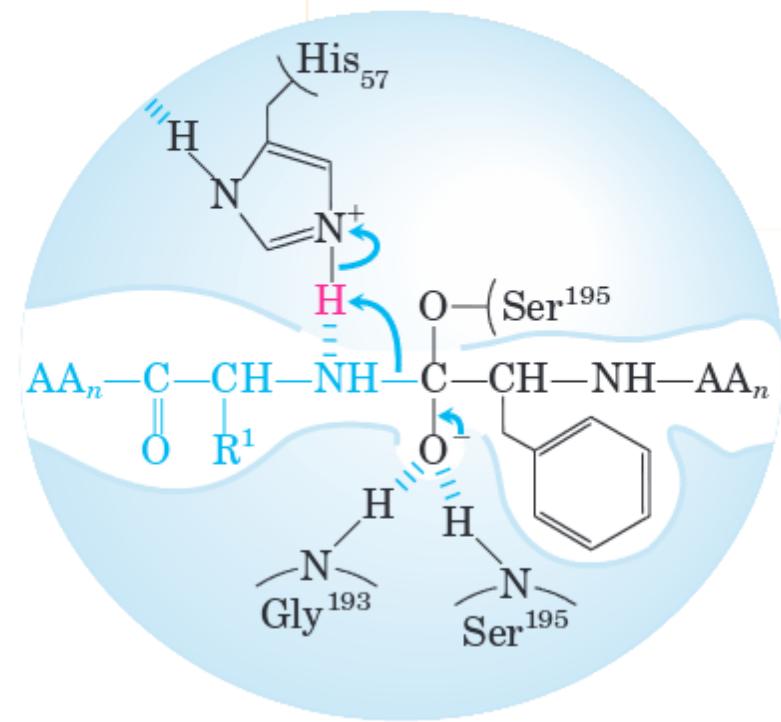
CHYMOTRYPSIN MECHANISM. STEP 2.

ES complex

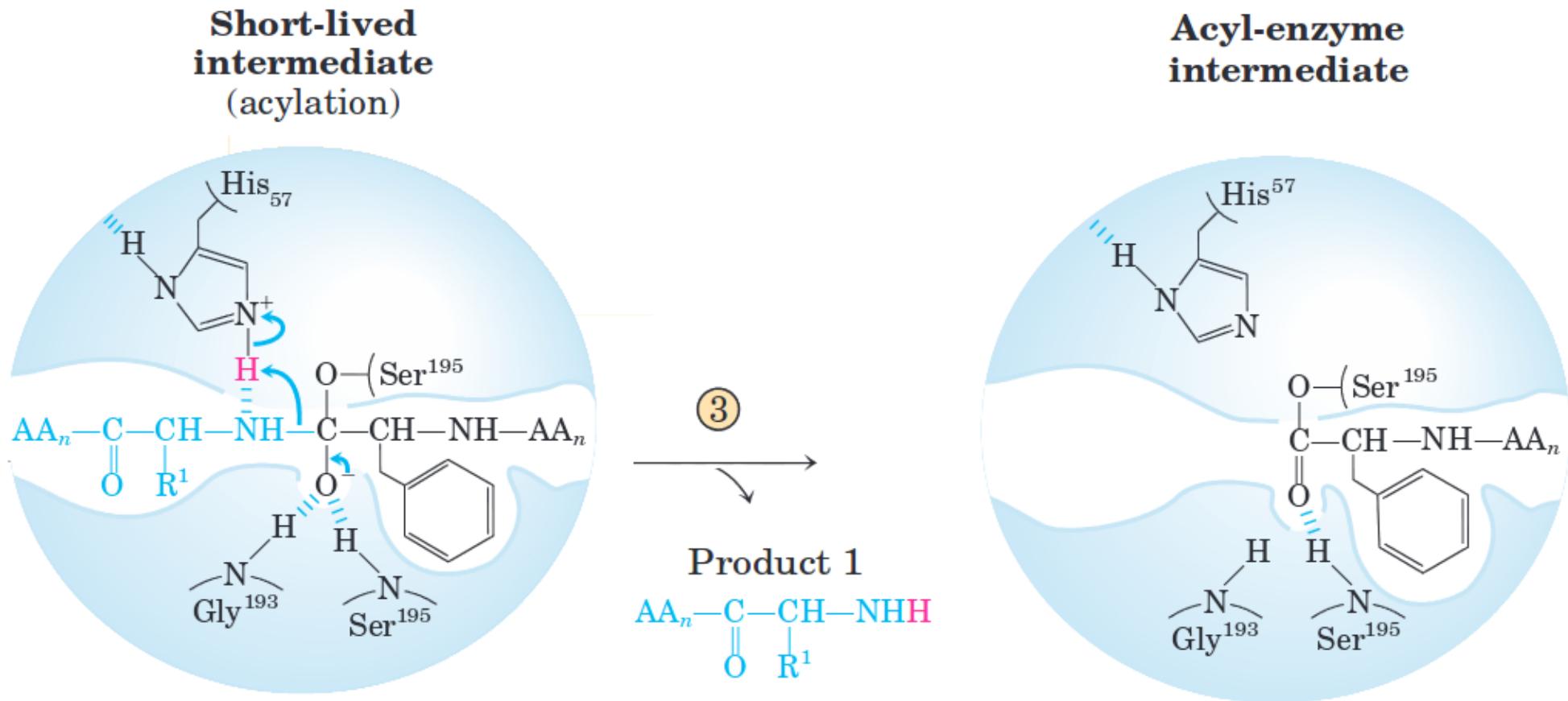


② →

**Short-lived intermediate
(acylation)**

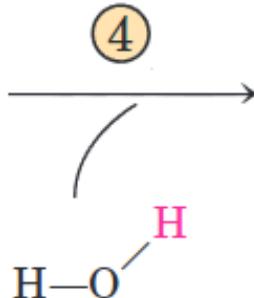
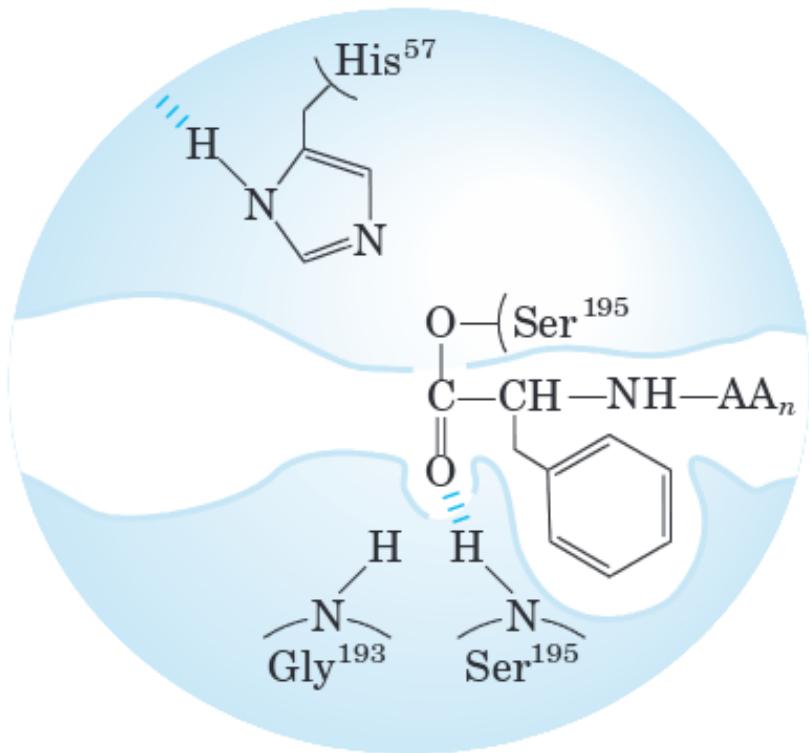


CHYMOTRYPSIN MECHANISM. STEP 3.

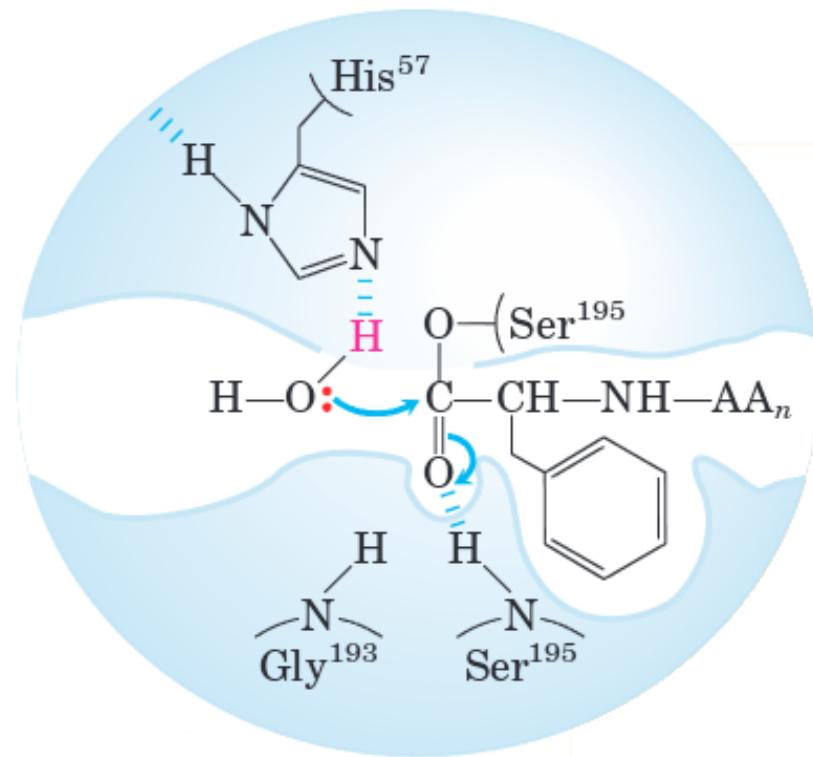


CHYMOTRYPSIN MECHANISM. STEP 4.

Acyl-enzyme
intermediate

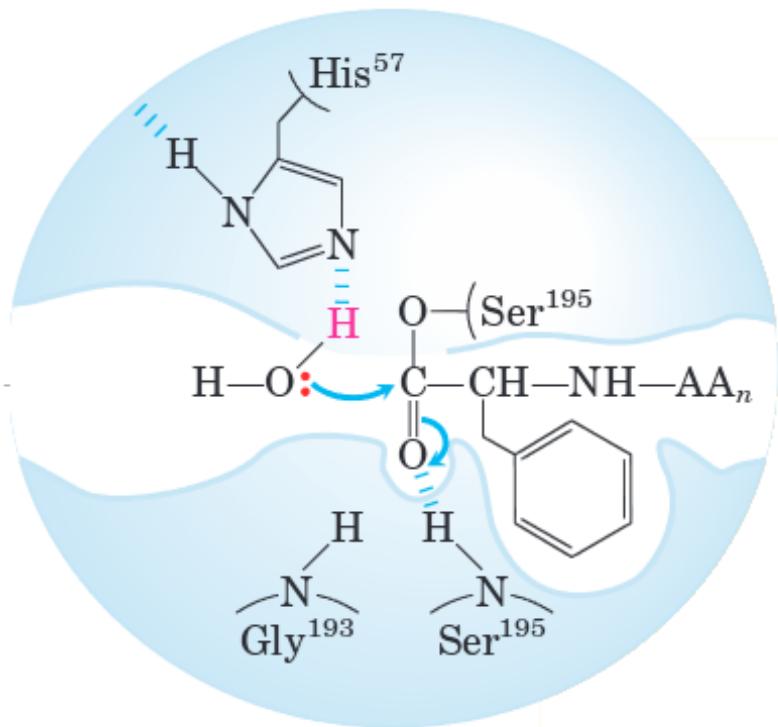


Acyl-enzyme
intermediate



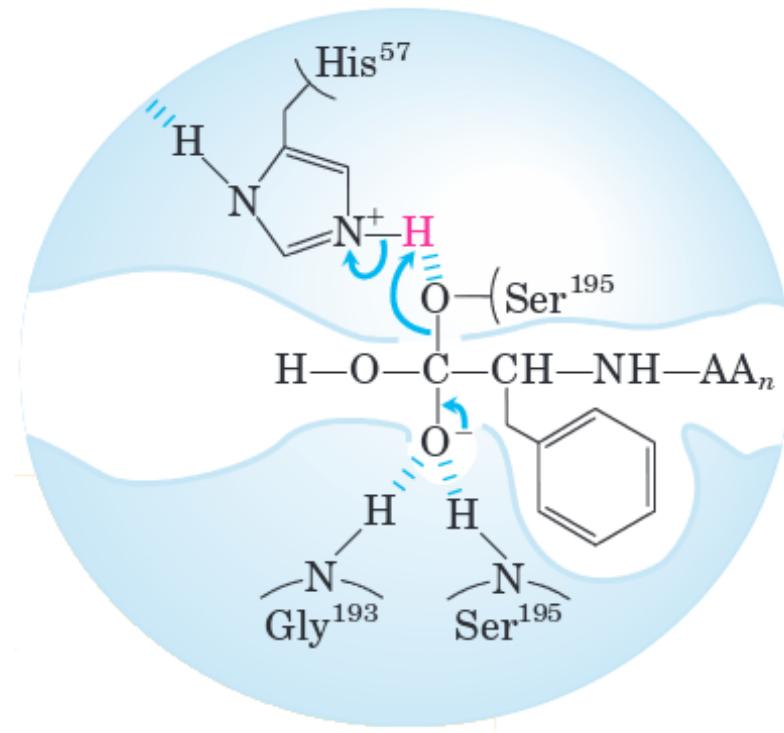
CHYMOTRYPSIN MECHANISM. STEP 5.

Acyl-enzyme
intermediate



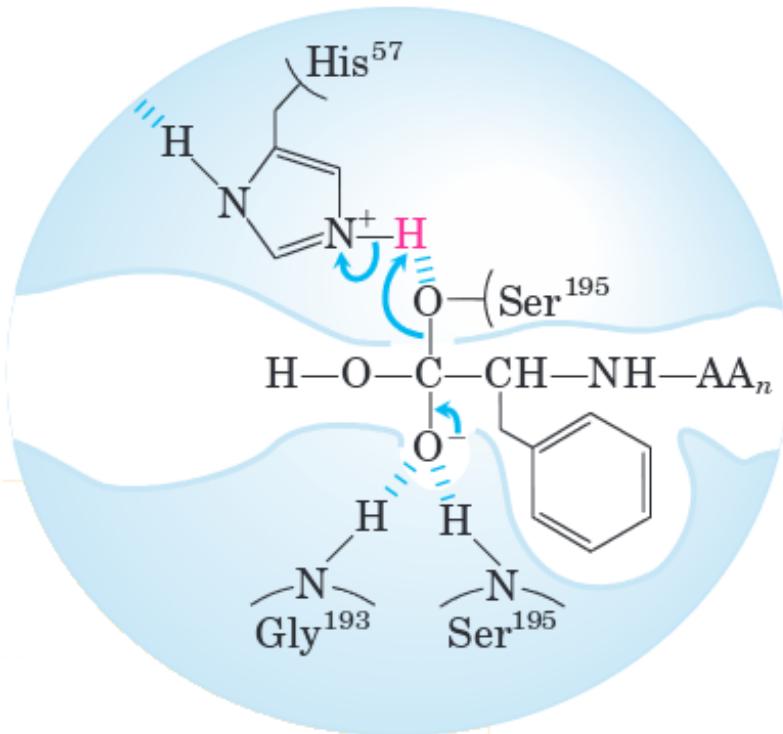
5 →

Short-lived
intermediate
(deacylation)



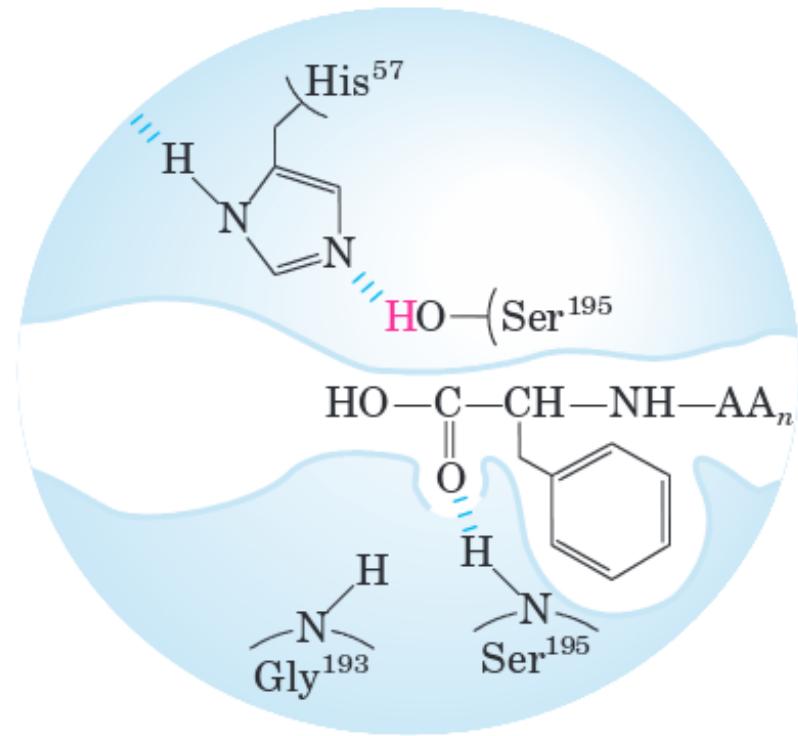
CHYMOTRYPSIN MECHANISM. STEP 6.

Short-lived intermediate
(deacylation)

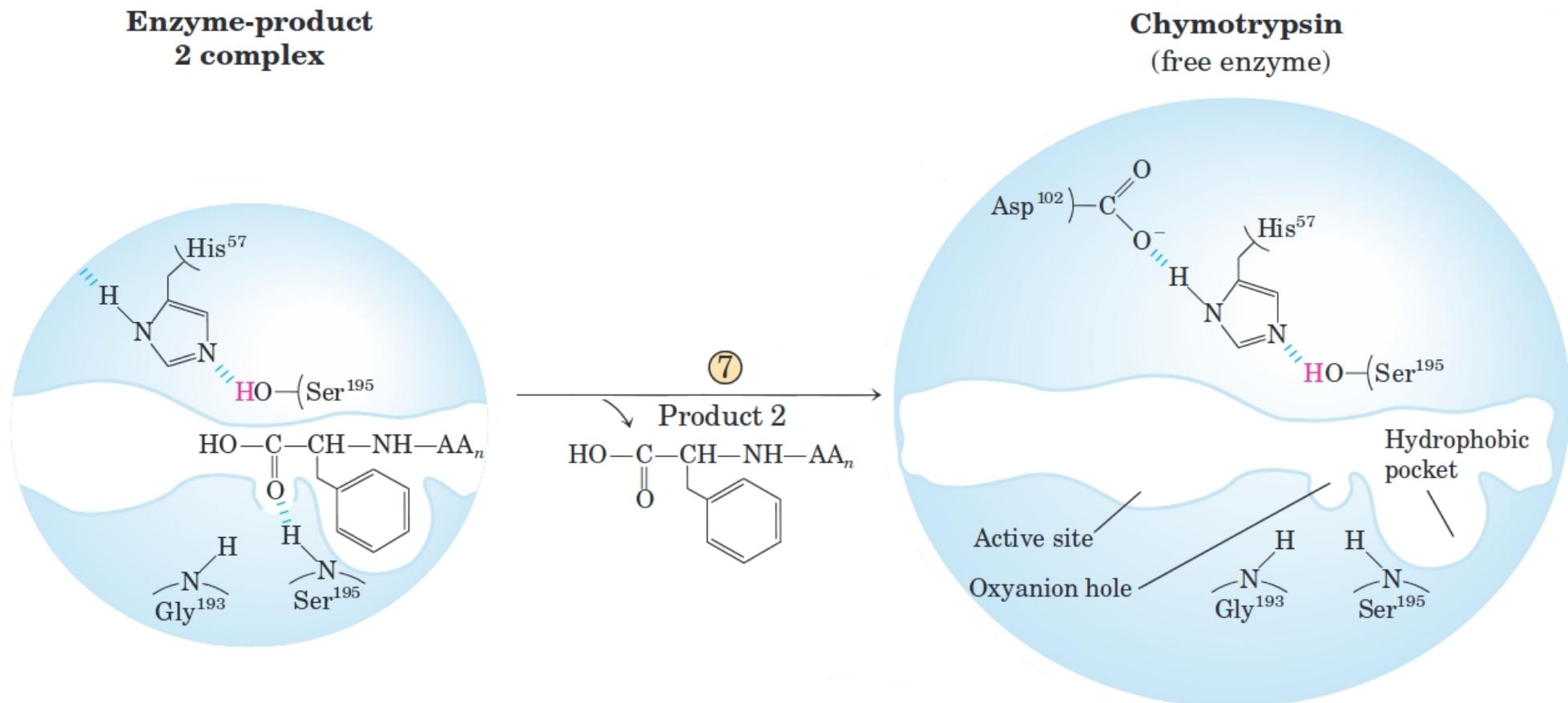


⑥

Enzyme-product 2 complex



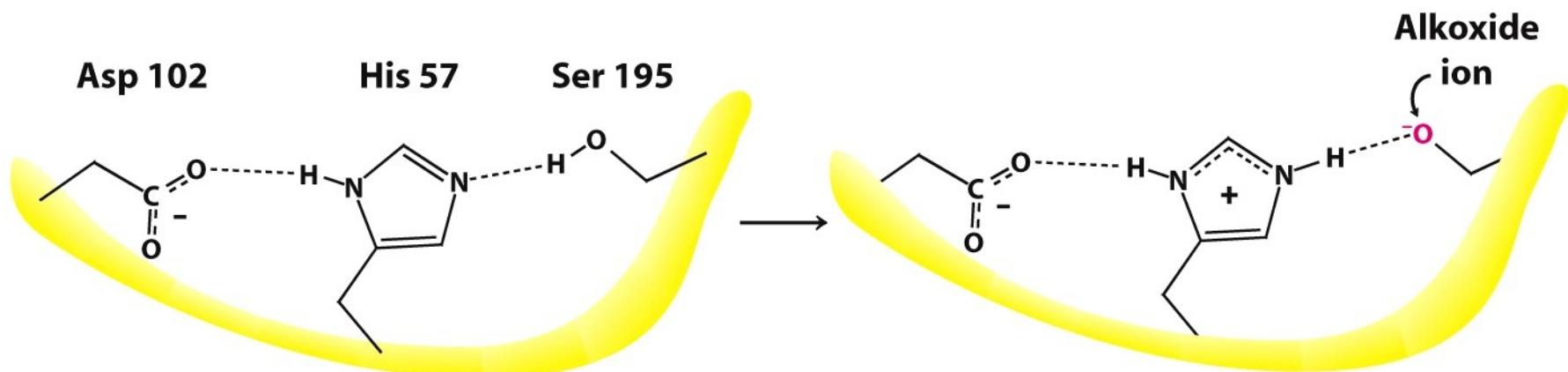
CHYMOTRYPSIN MECHANISM. STEP 7.



CATALYTICAL TRIAD

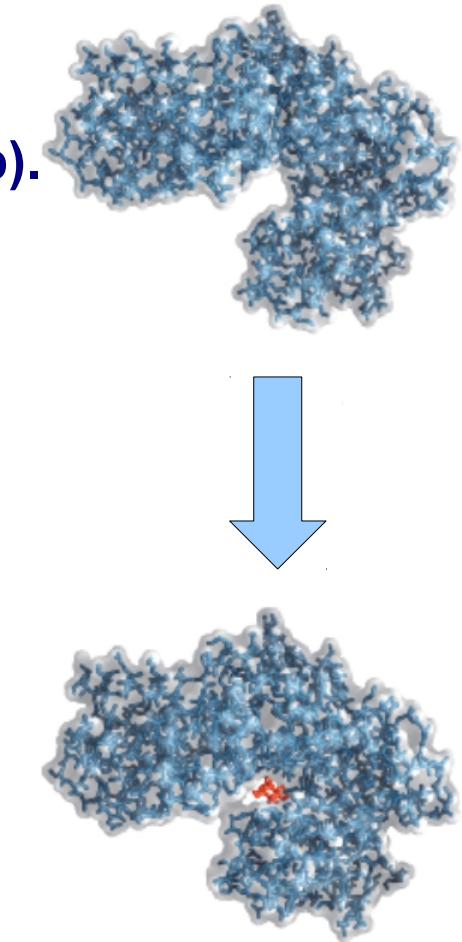
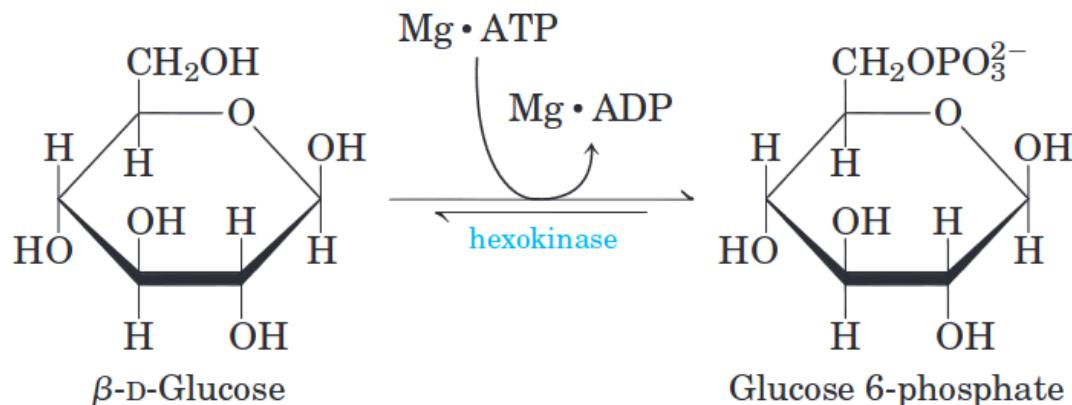
Asp¹⁰² is needed for adjusting pK_a of His⁵⁷

Asp¹⁰², His⁵⁷ and Ser¹⁹⁵: catalytical triad

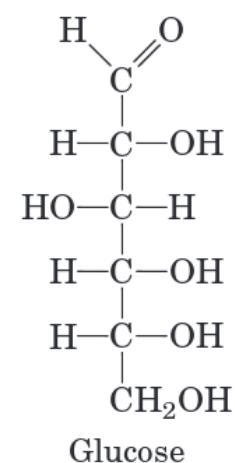
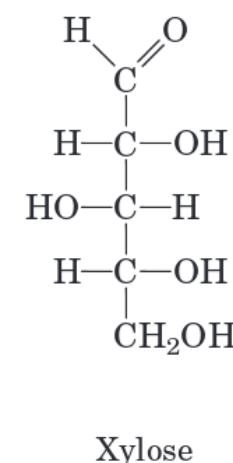


HEXOKINASE

E.C. 2.7.1.1. Phosphorylation of glucose (1st glycolysis step).

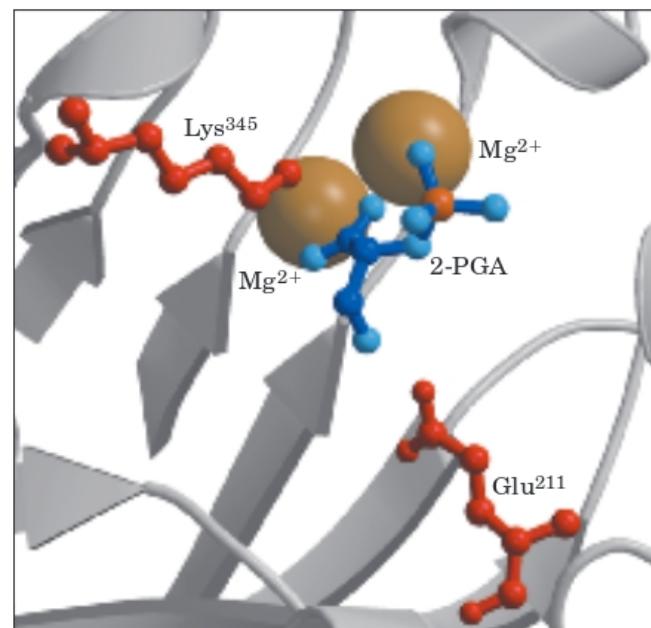
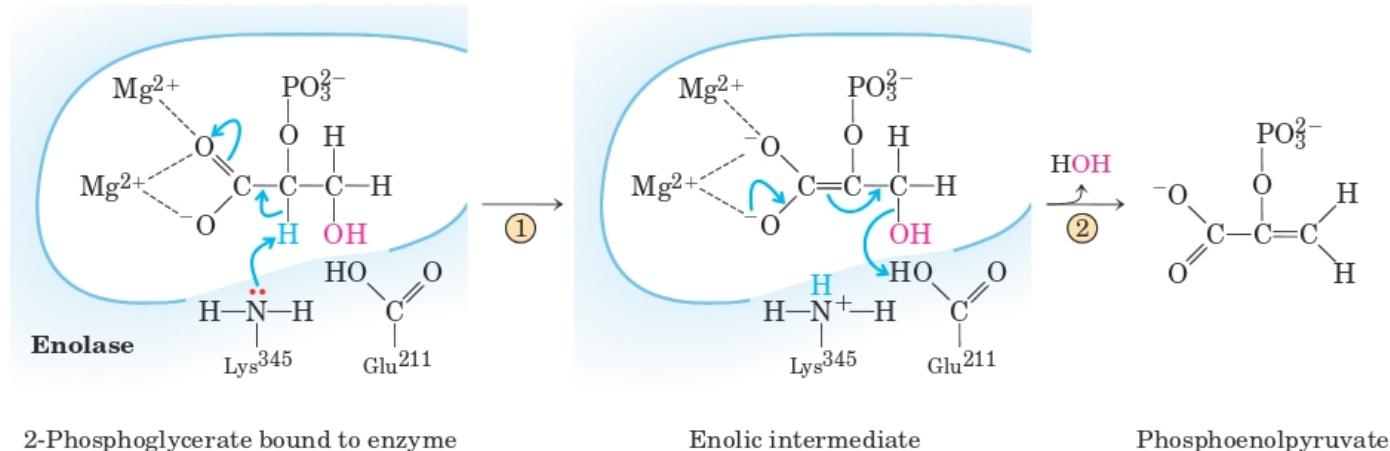


- Water can be a substrate.
- Allosteric regulation by glucose binding.
- Addition of xylose =>
conformational change, water phosphorylation



ENOLASE

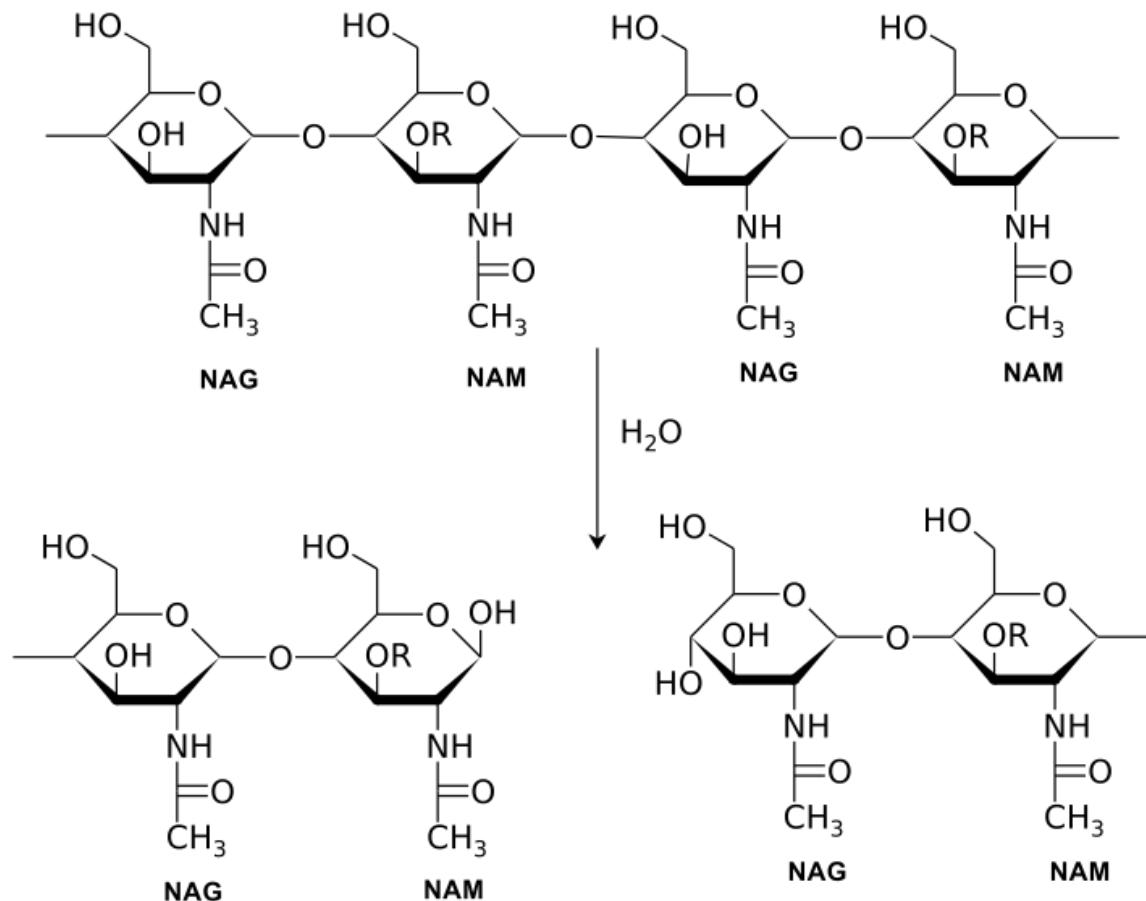
E.C. 4.2.1.11. Dehydration of 2-phosphoglycerate to phosphoenolpyruvate
(glycolysis 9th step). Mg²⁺ play a crucial role.



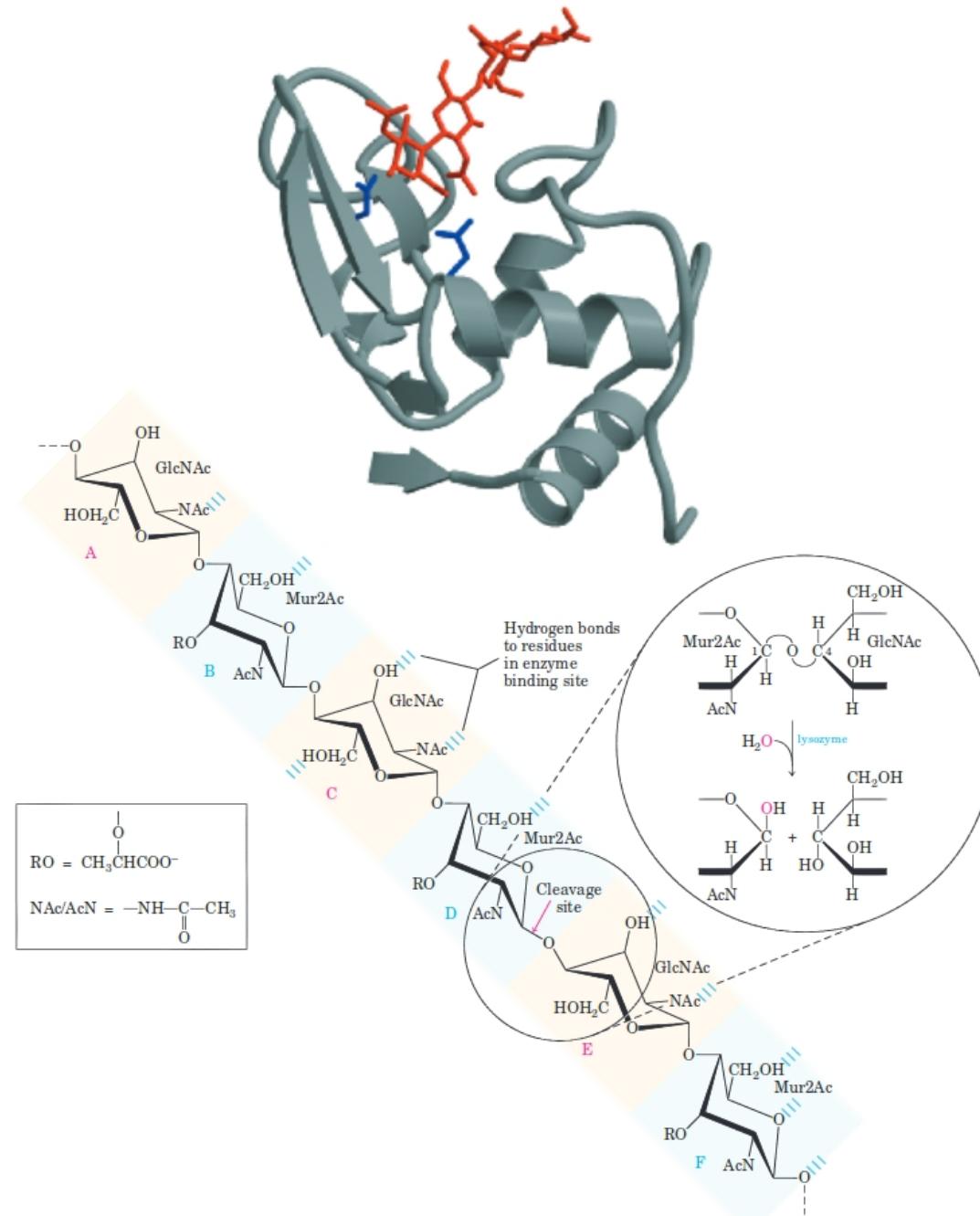
LYSOZYME

E.C. 3.2.1.17. N-acetylmuramide glycanhydrolase.

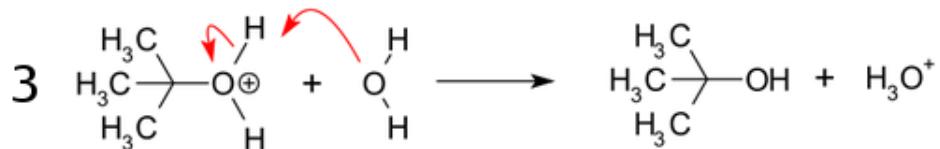
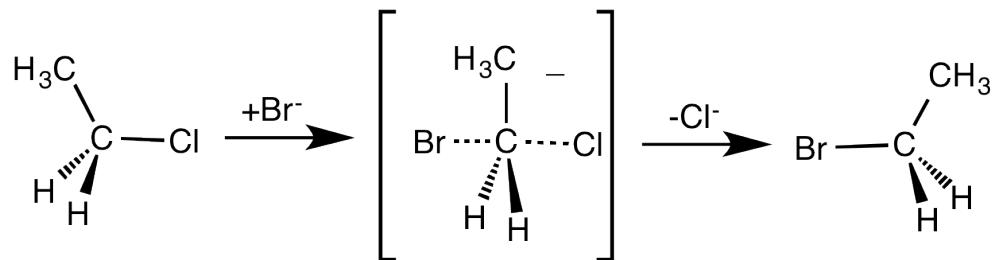
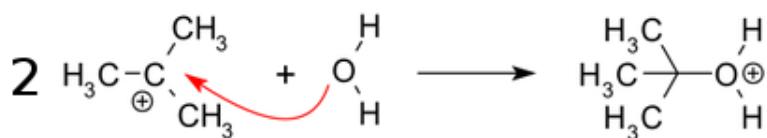
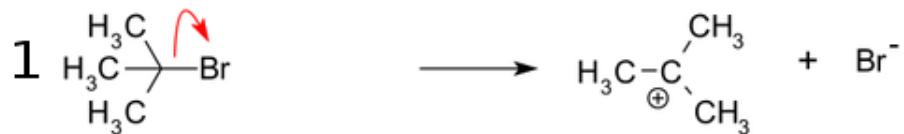
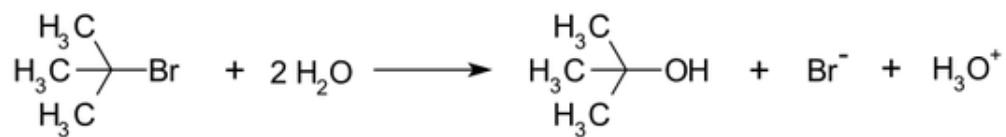
- Found in tears and egg whites.
- Destroys bacterial walls (cuts peptidoglycan).
- First crystallized ever enzyme (1965).



LYSOZYME

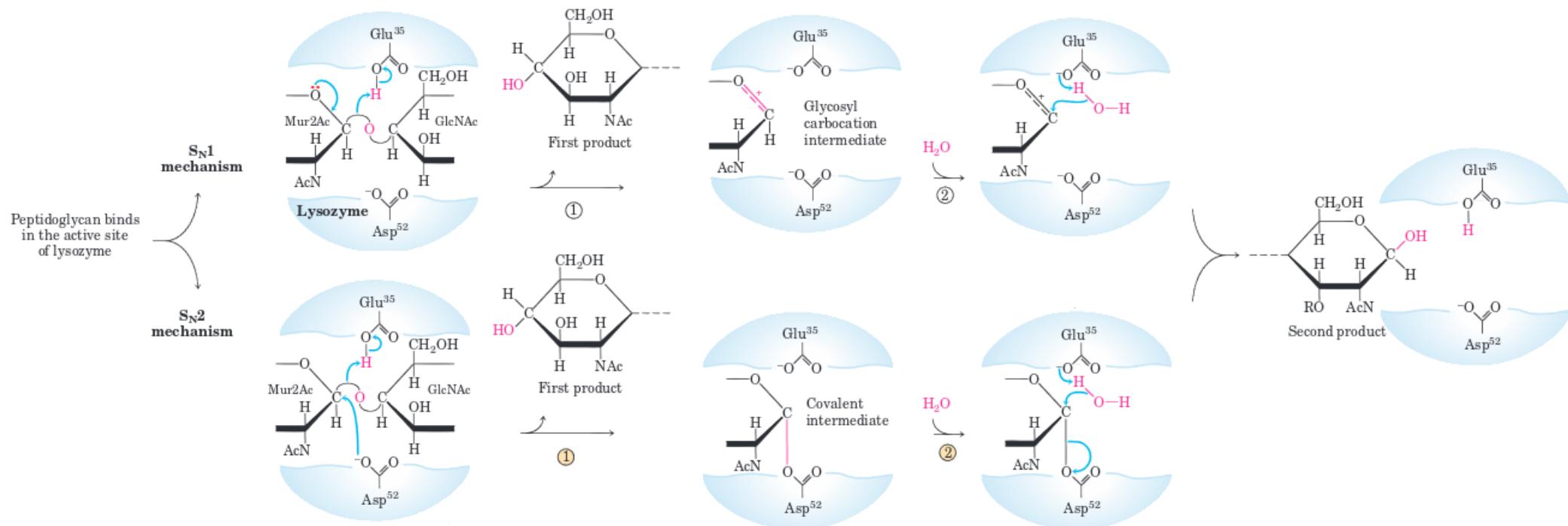


S_N1 VS S_N2



- S_N1 : nucleophilic substitution 1 (unimolar rate-determining step).
- S_N2 : nucleophilic substitution 2 (dimolar rate-determining step)

LYSOZYME: S_N1 VS S_N2



Mutations and anomeric-sensitive substitutions were used to study the mechanism.

REGULATORY ENZYMES

Regulatory enzymes exhibit increased/decreased activity in the metabolic pathways depending on the certain signals.

- Allostery: modulators and effectors
- Covalent modification
- Binding to regulatory proteins (reversible)
- Proteolytic cleavage (irreversible)
- Combination of several mechanisms

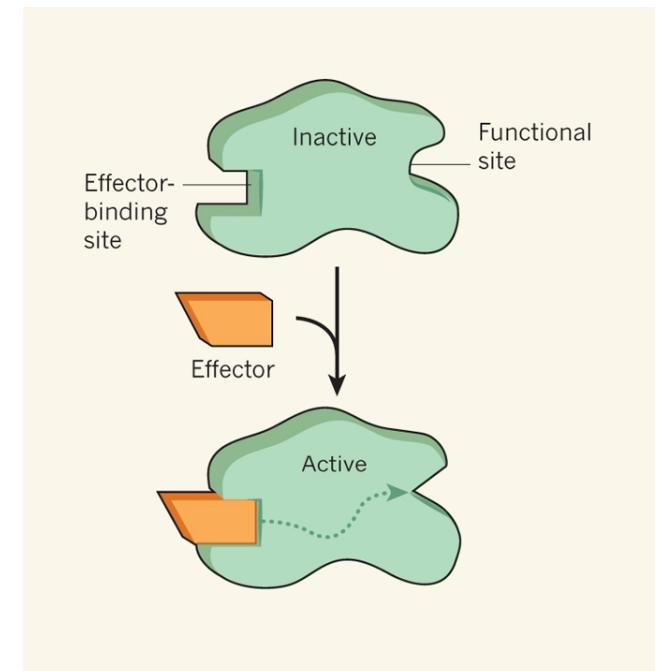
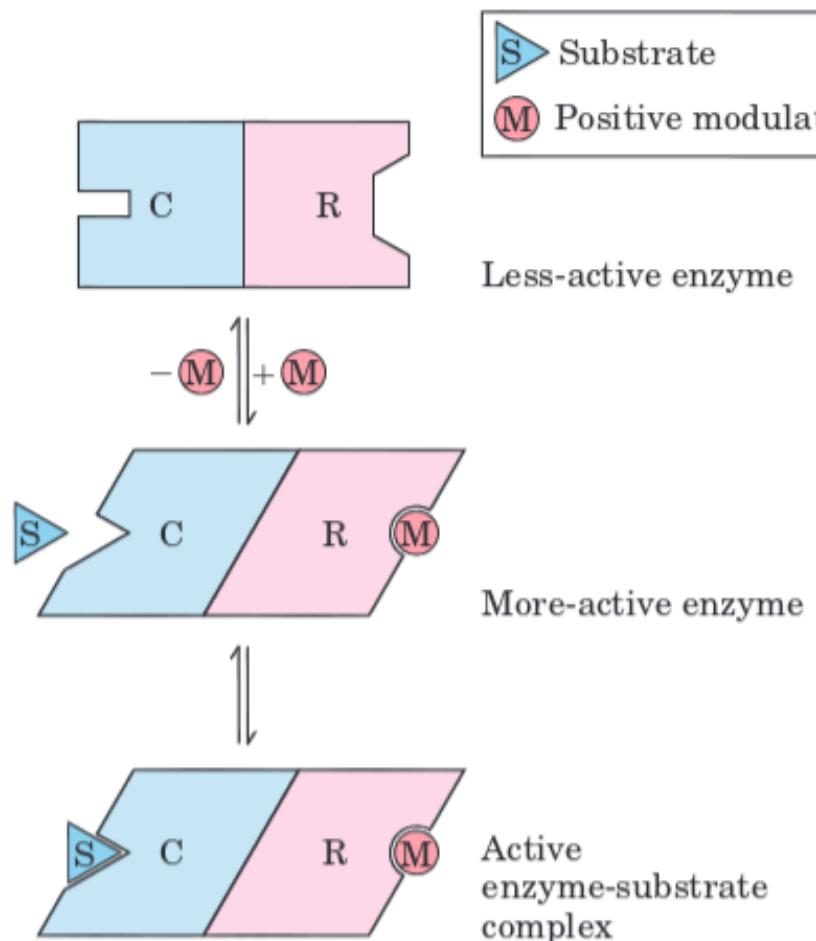


ALLOSTERIC REGULATION

Allosteric regulation: regulation by binding effector molecule at the site different from the active site (unless homotropic).

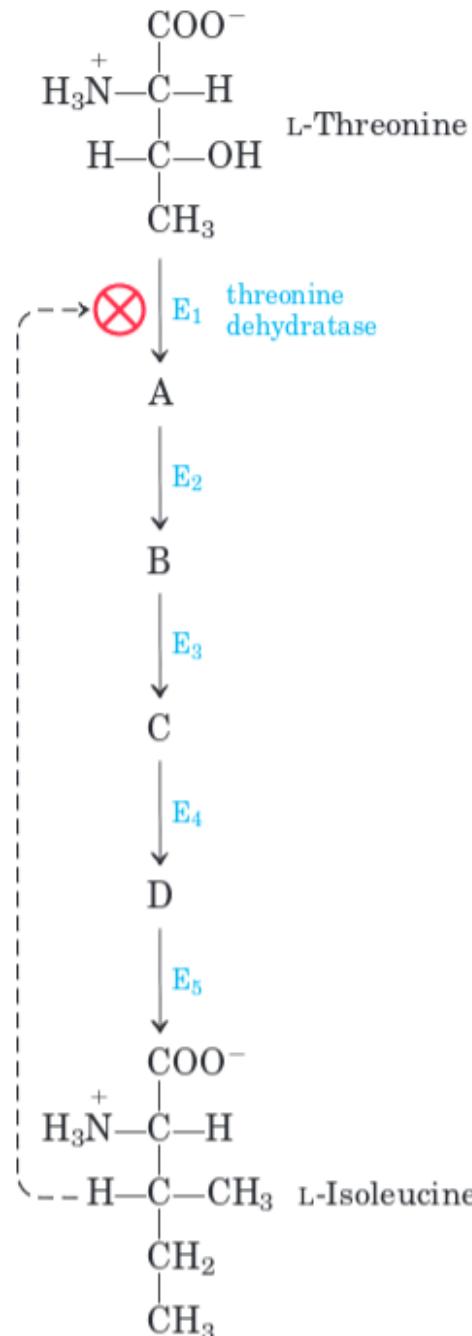
➤ Inhibitory

➤ Stimulatory



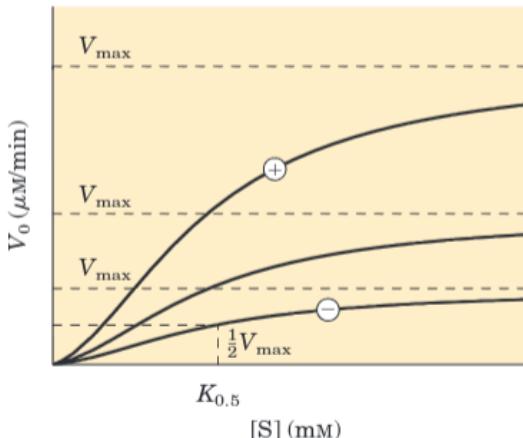
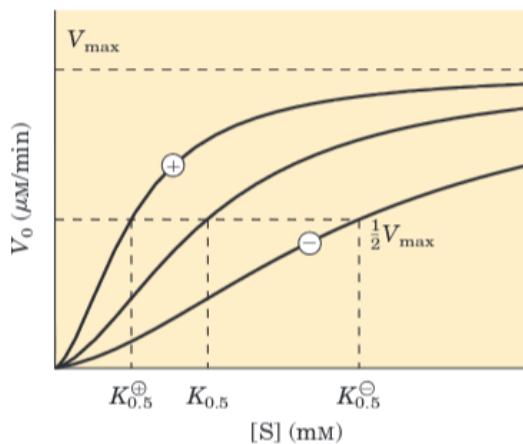
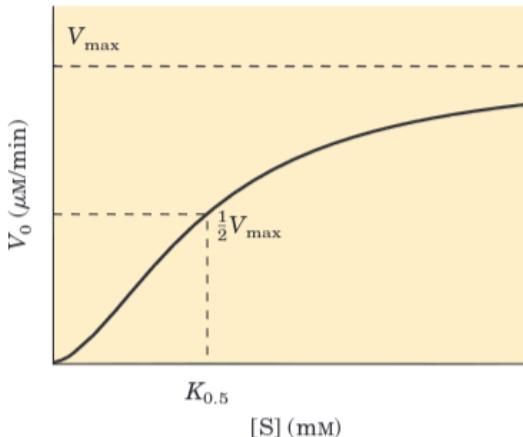
FEEDBACK REGULATION

- Regulation by the product
- Non-covalent
- Example: L-Thr=>L-Ile



KINETICS IN ALLOSTERY

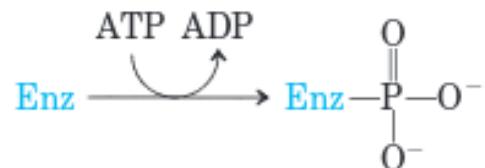
- Michaelis-Menton kinetics is affected.
- Homotropic enzyme: S is the activator.
- Homotropic enzyme: several subunits.



REVERSIBLE COVALENT MODIFICATIONS

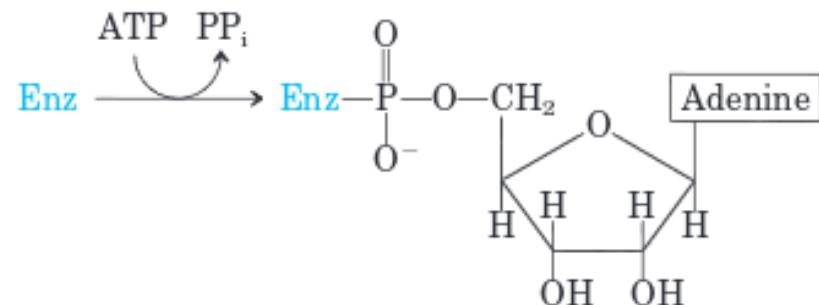
Phosphorylation

(Tyr, Ser, Thr, His)



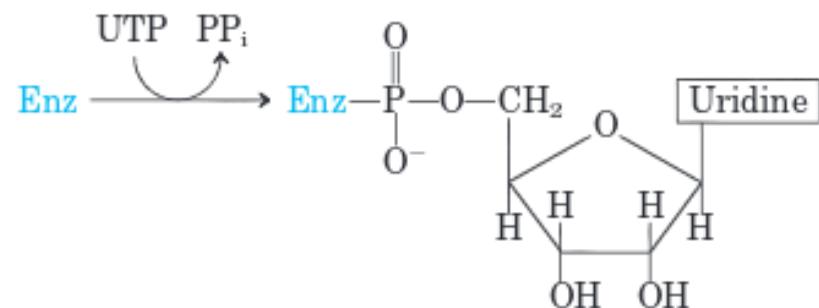
Adenylylation

(Tyr)



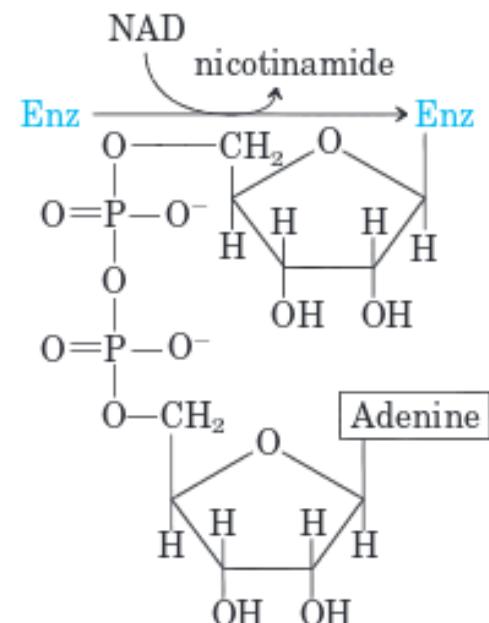
Uridylylation

(Tyr)



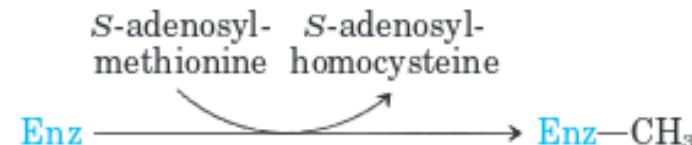
ADP-ribosylation

(Arg, Gln, Cys, diphthamide—a modified His)



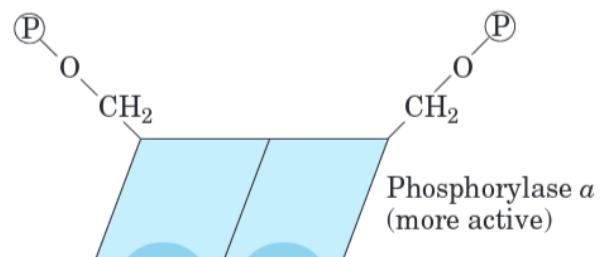
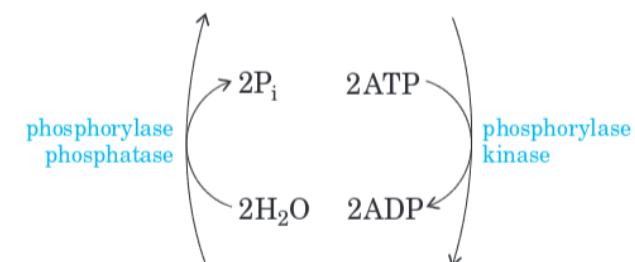
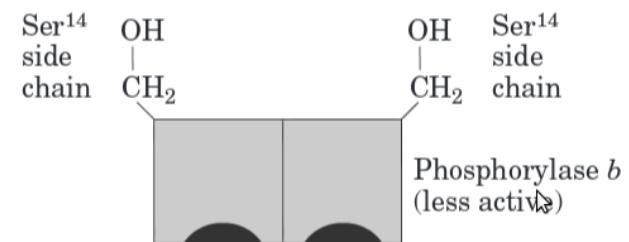
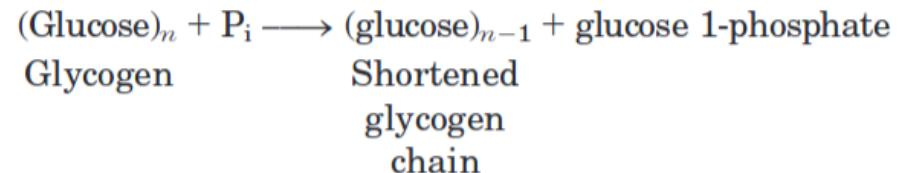
Methylation

(Glu)



PHOSPHORYLATION

- Ser-OH, Thr-OH, Tyr-OH
- Bulky charged group, H-bond acceptor
- Kinase/phosphatase
- Example: glycogen phosphorylase:
 - form a (more active)
 - form b (less active)
 - dramatic structural changes
 - electrostatics-driven



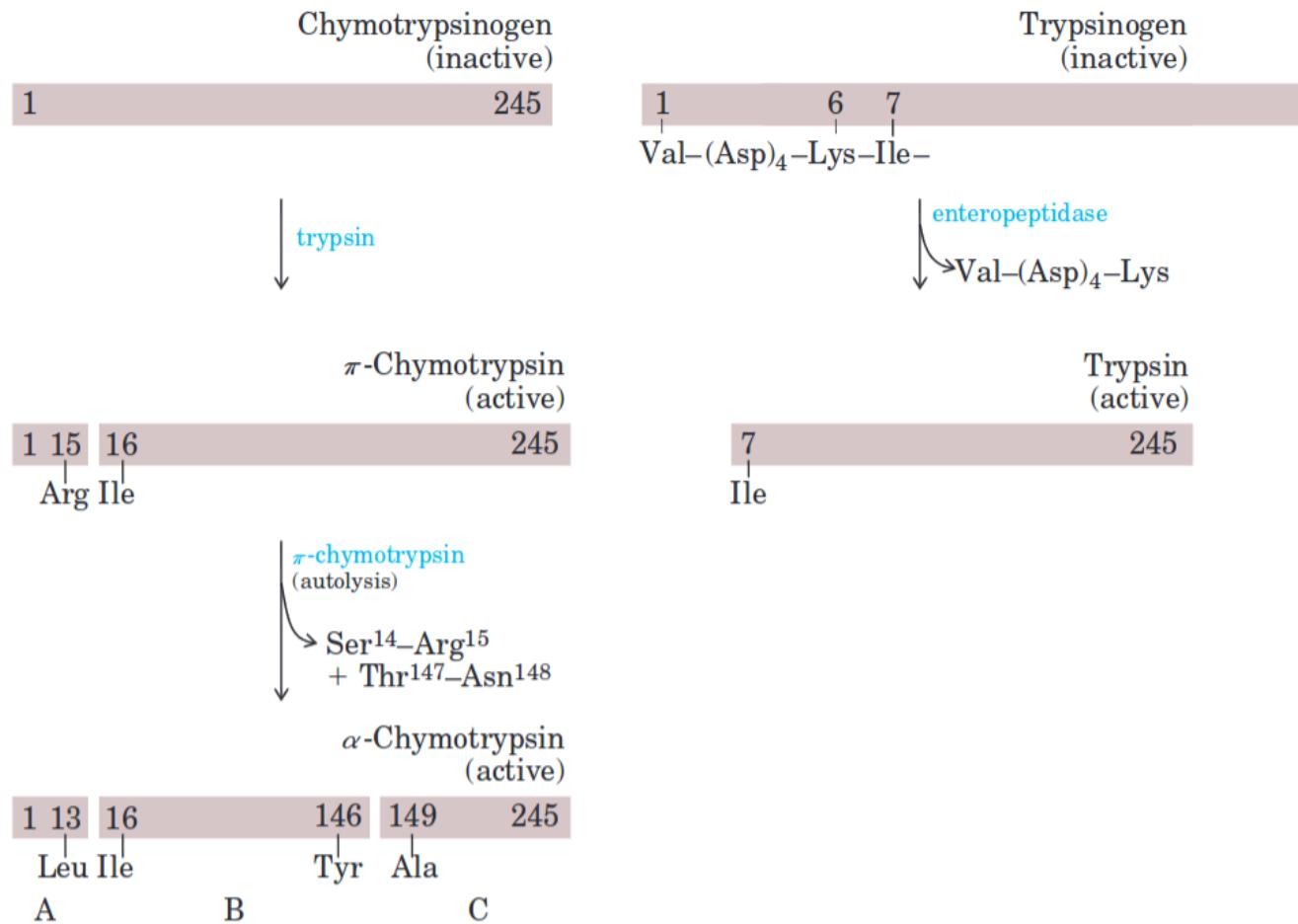
REGULATION OF PHOSPHORYLATION

- Sequence motifs
- Structural accessibility
- Hierarchical
- Cumulative

Protein kinase	Consensus sequence and phosphorylated residue
Protein kinase A	-X-R-(R/K)-X-(S/T)-B-
Protein kinase G	-X-R-(R/K)-X-(S/T)-X-
Protein kinase C	-(R/K)-(R/K)-X-(S/T)-B-(R/K)-(R/K)-
Protein kinase B	-X-R-X-(S/T)-X-K-
Ca ²⁺ /calmodulin kinase I	-B-X-R-X-X-(S/T)-X-X-X-B-
Ca ²⁺ /calmodulin kinase II	-B-X-(R/K)-X-X-(S/T)-X-X-
Myosin light chain kinase (smooth muscle)	-K-K-R-X-X-S-X-B-B-
Phosphorylase b kinase	-K-R-K-Q-I-S-V-R-
Extracellular signal-regulated kinase (ERK)	-P-X-(S/T)-P-P-
Cyclin-dependent protein kinase (cdc2)	-X-(S/T)-P-X-(K/R)-
Casein kinase I	-(Sp/Tp)-X-X-(X)-(S/T)-B
Casein kinase II	-X-(S/T)-X-X-(E/D/Sp/Yp)-X-
β-Adrenergic receptor kinase	-(D/E) _n -(S/T)-X-X-X-
Rhodopsin kinase	-X-X-(S/T)-(E) _n -
Insulin receptor kinase	-X-E-E-E-Y-M-M-M-M-K-K-S-R-G-D-Y-M-T-M-Q-I-G-K-K-K-
	<i>L-P-A-T-G-D-Y-M-N-M-S-P-V-G-D-</i>
Epidermal growth factor (EGF) receptor kinase	<i>-E-E-E-E-Y-F-E-L-V-</i>

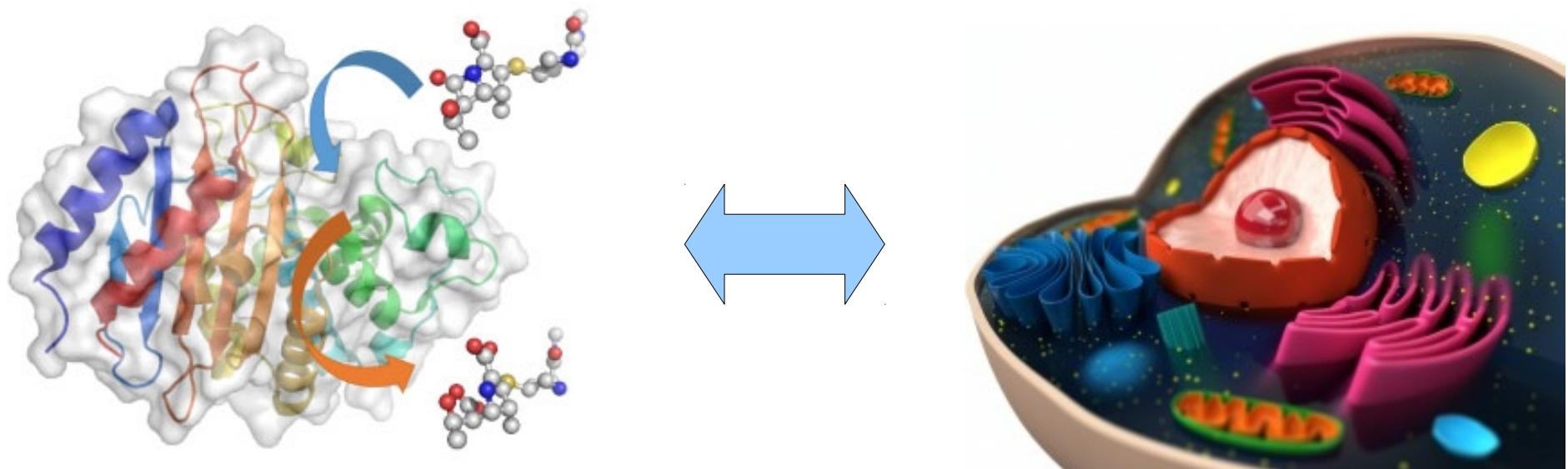
PROTEOLYTIC CLEAVAGE

- Zymogen: non-active longer precursor.
 - trypsin/trypsinogen, chymotrypsin/chymotrypsinogen
- Proenzymes: N-terminal
 - trombin/protrombin



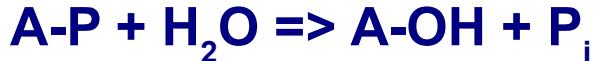
CYTOCHEMICAL LOCALIZATION OF ENZYMES AND THEIR FUNCTION

- Phosphatase
- Acyltransferase

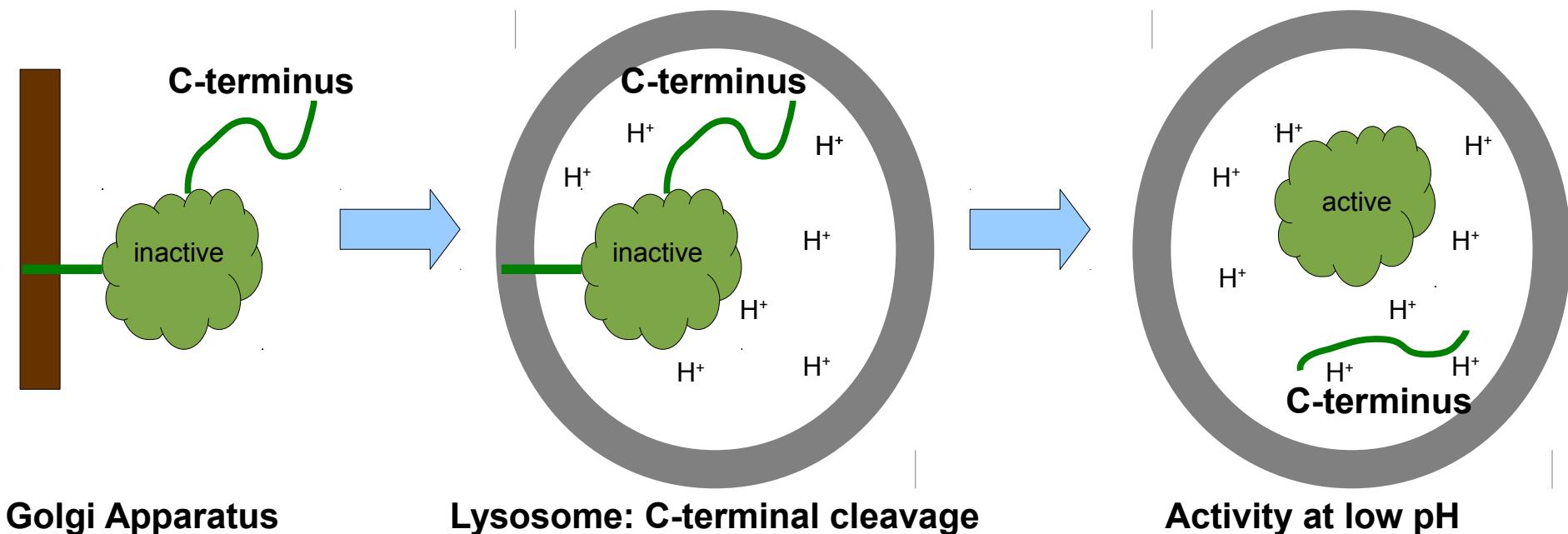


PHOSPHATASE

E.C. 3.1.3.2. Lysosomal acid phosphatase

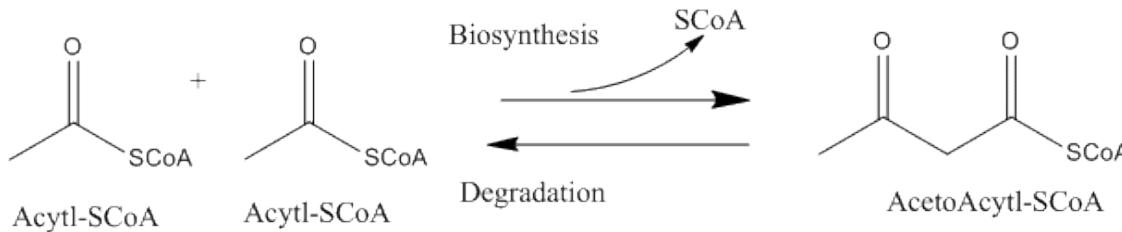


- Pathway ER => Golgi => lysosomes
- Synthesized as membrane-anchored protein
- Processed within the lysosomes
- Active at acidic pH in the lysosomes but not in Golgi Appratus

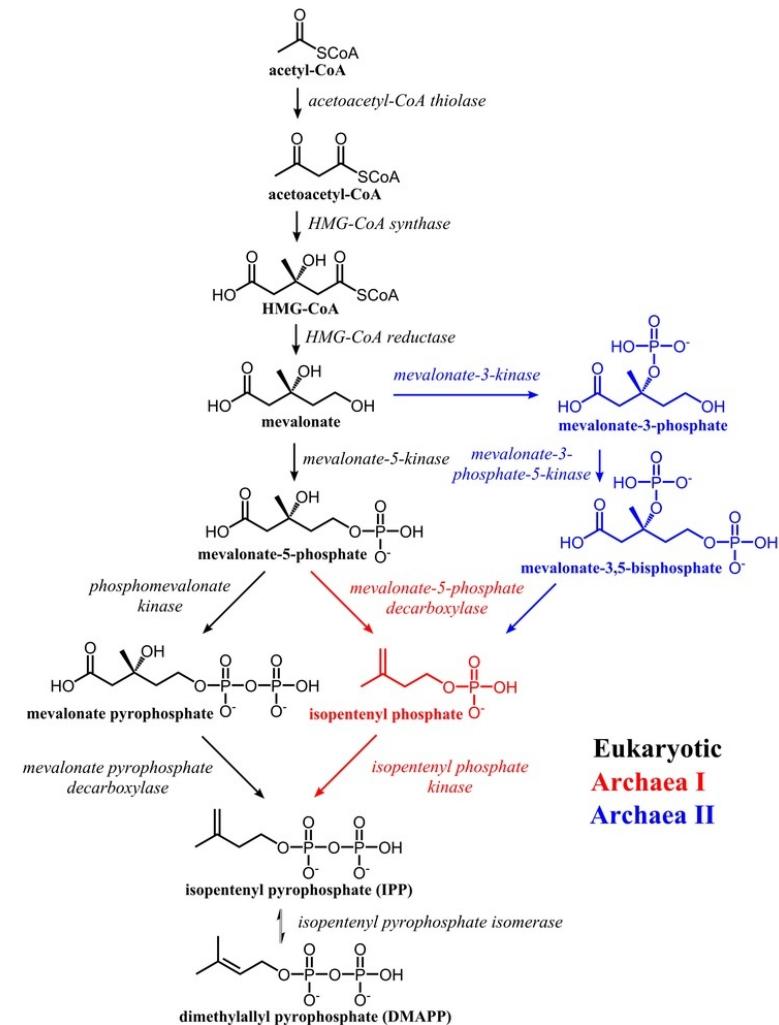


ACYLTRANSFERASE

E.C> 2.3.1.9. Acetyl-coenzyme A acetyltransferases (ACAT, thiolase)



Mevalonate Pathway



➤ Fatty-acid oxidation in peroxisomes and mitochondria

➤ Mevalonate pathway in peroxisomes and cytoplasm

Eukaryotic
Archaea I
Archaea II

LECTURES 5-6: INTRODUCTION TO CELL CHEMISTRY AND BIOSYNTHESIS III

ENZYMES

- Basic concepts:
 - enzymatic catalysis
 - substrate, product, intermediate
 - reaction conditions
 - classification of enzymes
- Enzyme kinetics
- Examples of enzyme mechanisms (chymotrypsin, hexokinase, enolase, lysozyme)
- Regulation of enzymes
- Localization of enzymes and their function:
 - Phosphatase
 - Acyltransferase

