Biochemistry of Enzyme Action

1

The structure and properties

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Content

Intoduction

- Life depends on the existence of powerful and specific catalysts: the enzymes. Almost every biochemical reaction is catalyzed by an enzyme.
- With the exception of a few catalytic RNAs, all known enzymes are proteins. Many require nonprotein coenzymes or cofactors for their catalytic function.
- Enzymes are classified according to the type of reaction they catalyze. All enzymes have formal E.C. numbers and names, and most have trivial names.

General Characteristics of Enzymes

Enzymes are biochemical catalysts.

Enzyme is a protein molecule that speeds up chemical reactions in all living things.

- Without enzymes, these reactions would occur too slowly or not at all, and no life would be possible.

- The word *enzyme* comes from two Greek words meaning *in yeast*.
  - Many scientists who studied enzymes in the 1800's studied reactions caused by yeast enzymes
- Historical background
- Properties of enzymes
- Structure of enzymes

Historical Background of Enzymology (1)

- **In ancient times**, using enzymatic technology (bakery, wine-making, processing of hides and others.)
- **XVIII century** - R. Reaumur, L. Spallanzani: described digestion in birds.
- **1814** - K. Kirchhoff showed the characteristic of catalytic hydrolysis of starch during grain germination.
- 1835 **Jon Berzelius:** coined the term "*catalysis*";
- 1850 – 1860 **Louis Pasteur:** no fermentation in heated flask;
  - **The middle of the XIX century** the dispute Yu Liebig and Louis Pasteur: *"organized"* and *"unorganized"* ferments.
- **1878** - F. Kuehne coined the term **"enzyme".**

Historical Background of Enzymology (2)

- **1871** – M.M. Manasseina, and then 21 years later E. Buchner showed that cell extract capable of catalyzing.
  - They grinded yeast with sand, filtered, and then observed fermentation.
- **1894** – E. Fischer has created "lock and key" model.
- **1913** – L. Michaelis and M. Menten created the theory of enzymatic catalysis.
- **1929 -** J. Sumner demonstrated the protein nature of enzymes (urease, and others).
  - He used the enzyme extract from beans.
- **1963 -** the primary structure of RNA-ase was studied.
- **1968 -** M. Merrifield: artificial synthesis of RNA-ase.
- **This time -** the work on the synthesis of artificial enzymes.

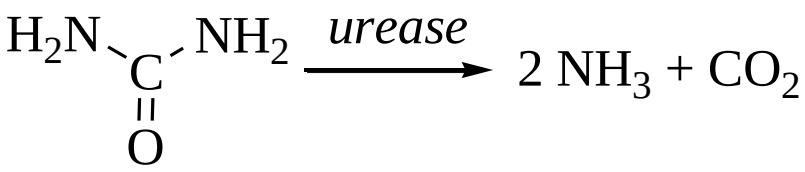
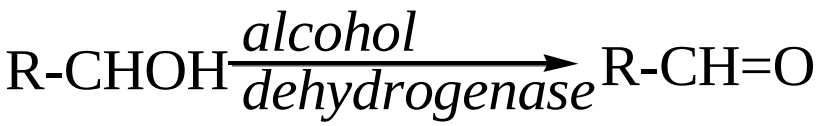
Properties of Enzymes

- **Like any catalyst** enzymes:
  - Neither consumed nor produced during the course of a reaction.
  - Do not cause reactions to take place.
  - Do not alter the free energies of the substrates or the products.
  - Alter the rate but not the equilibrium constant of reactions.
- **Differences**. Enzymes:
  - Are proteins.
  - More effective
  - Highly specific
  - Function within a moderate pH and temperature
  - Are capable of being regulated.

Enzyme Specificity

- **Stereospecificity** (optical specificity)
  - L-amino acid oxidase and D-amino acid oxidase.
- **Reaction specificity**
  - Amino acids: transamination, oxidative deamination, decarboxylation etc.
- **Substrate specificity**
  - **Absolute**: urease cleaves urea, glucokinase acts on glucose
  - **Relative**: trypsin hydrolyses peptide bonds at Arg, Lys. Glycosidases, lipases.
  - **Broad specific**: hexokinase acts on glucose, mannose, and glucosamine and not on galactose.

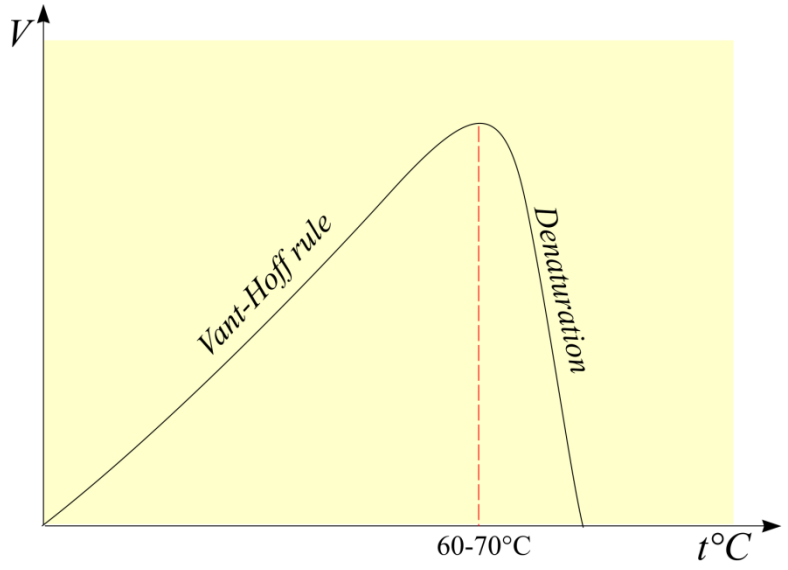
Range of enzyme specificity

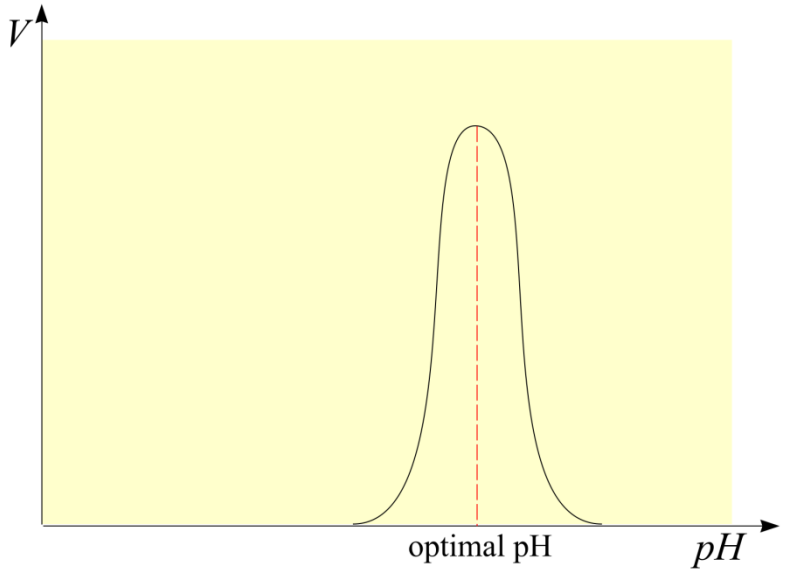
Decreasing specificity	Examples
Absolute, one unique substrate (urease)	
Stereochemical, one enantiomer as substrate	
Group or unction specificity	
Low	

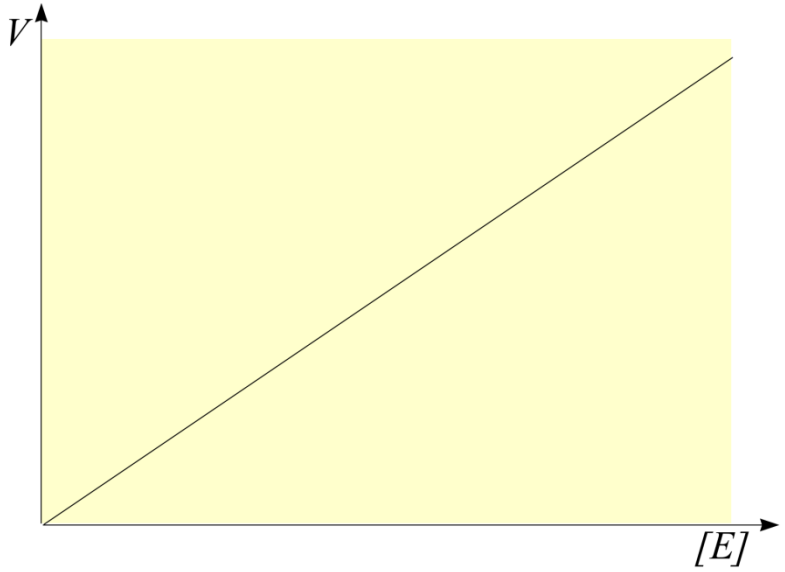
Measures of Enzyme Activity

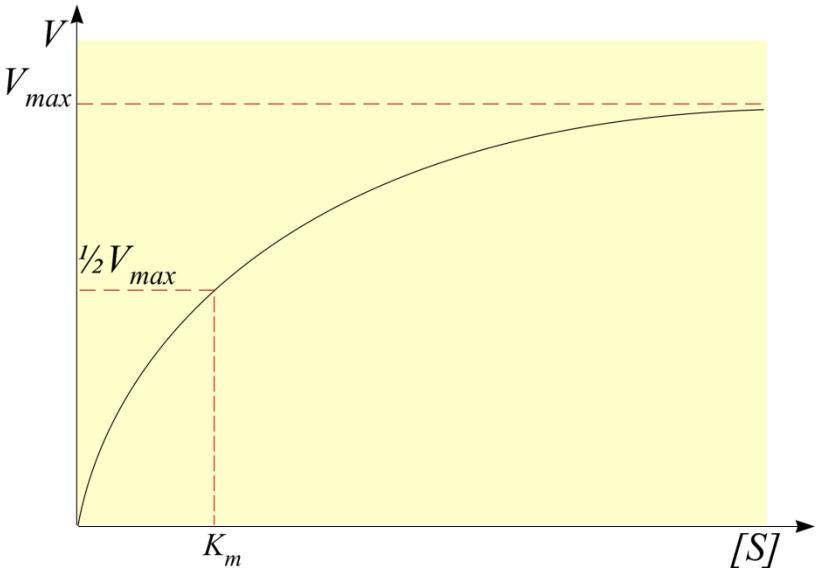
- The International Commission on Enzymes defines **One International Unit** of enzyme *as the amount that catalyzes the formation of one micromole of product in one minute*.
  - (Because enzymes are very sensitive to factors such as pH, temperature, and ionic strength, the conditions of assay must be specified.)
  - Another definition for units of enzyme activity is the **katal.** One katal is *that amount of enzyme catalyzing the conversion of one mole of substrate to product in one second*.
    - Thus, one katal equals 6×10^7 international units.
- **Specific activity**: μmol of substrate/(minute x mg of enzyme).
- **Turnover number:** (k_{cat}) – number of substrate molecules metabolized per enzyme molecule per unit time with units of min^{-1} or s^{-1}.

Enzyme Activity Influencing Factors









Values of k_{cat} (Turnover Number) for Some Enzymes

Enzyme	k_{cat} (sec^{-1})
Catalase	40,000,000
Carbonic anhydrase	1,000,000
Acetylcholinesterase	14,000
Penicillinase	2,000
Lactate dehydrogenase	1,000
Chymotrypsin	100
DNA polymerase I	15
Lysozyme	0.5

Enzyme Nomenclature

- Trivial names
  - Pepsin, trypsin.
- Rational names: the suffix-*ase* added to the name of the substrate upon which they acted
  - urease, amylase, glucose-6-phosphatase.
- Systematic naming of International Union of Biochemistry (IUB)
  - Six major classes: (1) oxidoreductases, (2) transferases, (3) hydrolases, (4) lyases, (5) isomerases, (6) ligases (synthetases).
  - Each enzyme has a code number (EC): 1 - class, 2 – subclass, 3 – subsubclass, 4 – specific enzyme.

Enzymes Are Named for the Types of Reactions They Catalyze

EC	Name of Class	Type of Reaction	Example
1	**Oxidoreductases**	Transfer of electrons, hydrogen and hydride ions	$A^{e-} + B \rightarrow A + B^{e-}$
2	**Transferases**	Transfer of a functional group (e.g., acyl group)	A—B + C →A + B—C
3	**Hydrolases**	Cleave C—O, N , or S bonds by adding H_2O.	A—B + H_20→ AH + BOH
4	**Lyases**	Add groups to or from double bonds	XA—BY → AB + X=Y
5	**Isomerases**	Transfer groups within the same molecule	XA—BY → YA—BX
6	**Ligases**	Join molecules (e.g., nucleotides) using ATP, etc	A + B → A—B

Structure of Enzymes

- **Simple** – consists of amino acids only.
- **Conjugated** – contain non-protein small organic or inorganic molecule known as a **cofactor**. Cofactors may be metal ions or organic molecules referred to as **coenzymes**.
  - Coenzymes – non-covalently attached to an enzyme.
    - Many coenzymes are vitamins or contain vitamins as part of their structure.
  - Prosthetic group - covalently attached to an enzyme.

Coenzymes

- Usually coenzymes are actively involved in the catalytic reaction of the enzyme, often serving as intermediate carriers of functional groups in the conversion of substrates to products.
- The catalytically active complex of protein and prosthetic group is called the **holoenzyme**.
- The protein without the prosthetic group is called the **apoenzyme**; it is catalytically inactive.

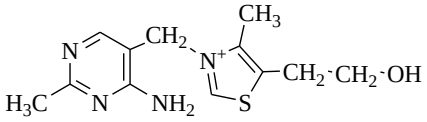
Metal Ions and Some Enzymes That Require Them

Metal Ion	Enzyme	Metal Ion	Enzyme
Fe^{2+} or Fe^{3+}	Cytochrome oxidase Catalase Peroxidase	**Mn^{2+}**	Arginase
Cu^{2+}	Cytochrome oxidase	**K^{+}**	Pyruvate kinase (also requires Mg^{2+})
Zn^{2+}	DNA polymerase Carbonic anhydrase	**Ni^{2+}**	Urease
	Alcohol dehydrogenase	**Mo**	Nitrate reductase
Mg^{2+}	Hexokinase Glucose-6-phosphatase	**Se**	Glutathione peroxidase

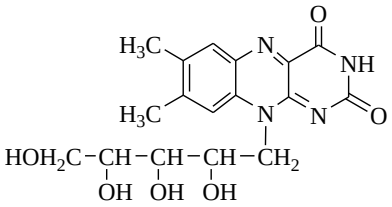
Coenzymes Serving as Transient Carriers of Specific Atoms or Functional Groups

Coenzyme	Entity Transferred	Representative Enzymes Using Coenzymes
Thiamine pyrophosphate (TPP) Flavin adenine dinucleotide (FAD) Nicotinamide adenine dinucleotide (NAD)	Aldehydes Hydrogen atoms Hydride ion (H^-)	Pyruvate dehydrogenase Succinate dehydrogenase Alcohol dehydrogenase
Coenzyme A (CoA) Pyridoxal phosphate (PLP)	Acyl groups Amino groups	Acetyl-CoA carboxylase Aspartate aminotransferase
5'-Deoxyadenosylcobalamin (vitamin B_{12})	H atoms and alkyl groups	Methylmalonyl-CoA mutase
Biotin (biocytin)	CO_2	Propionyl-CoA carboxylase
Tetrahydrofolate (THF)	Other one-carbon groups	Thymidylate synthase

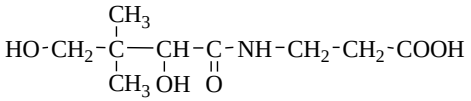
Some coenzymes



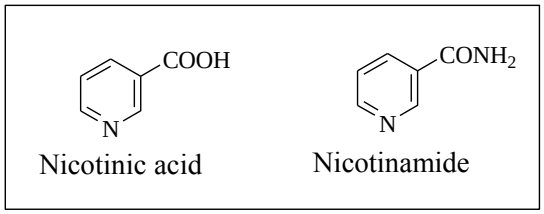
Thiamin (B_1)



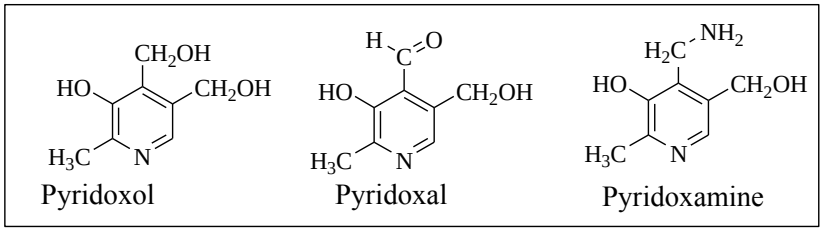
Riboflavin (B_2)



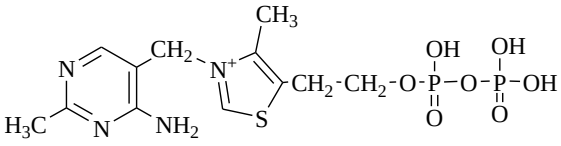
Pantothenic acid (B_3)



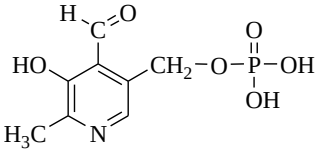
Vitamin PP (B_5)



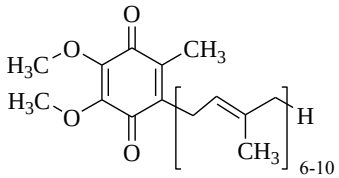
Pyridoxine, vitamin B_6



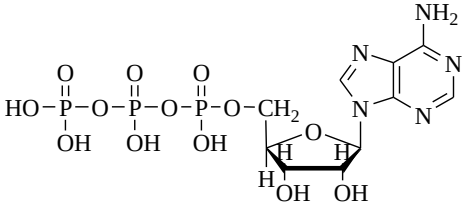
Thiamin pyrophosphate (TPP)
Thiamin diphosphate (TDP)



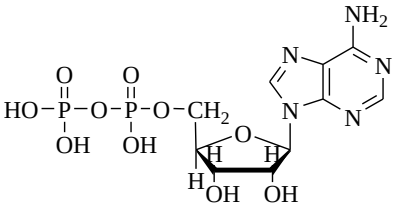
Pyridoxal phosphate (PLP)



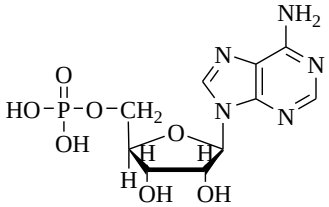
Coenzyme Q (CoQ, ubiquinone)



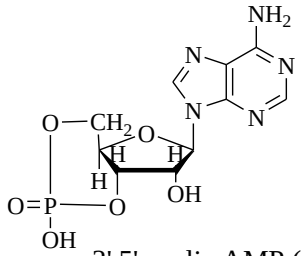
Adenosine tpiphosphate (ATP)



Adenosine diphosphate (ADP)

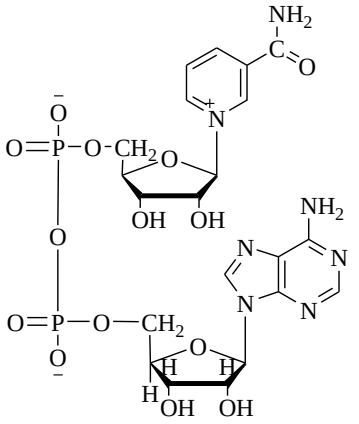


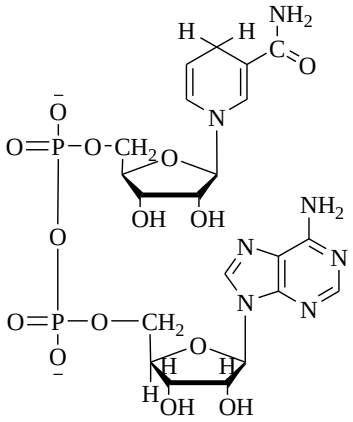
Adenosine monohosphate (AMP)

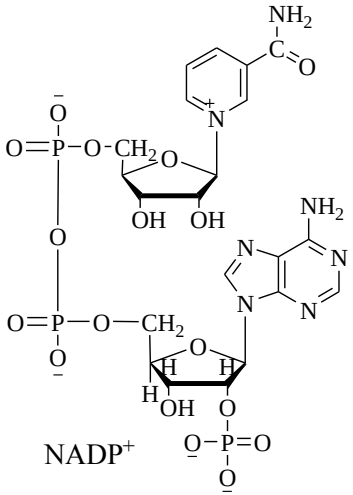


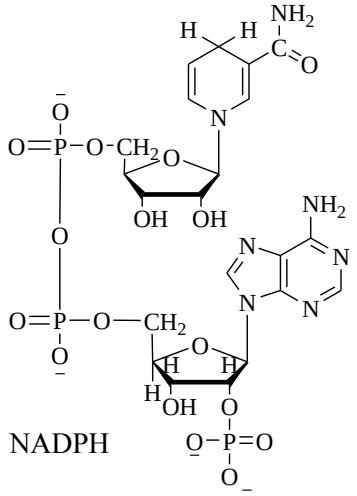
3',5' cyclic AMP (cAMP)

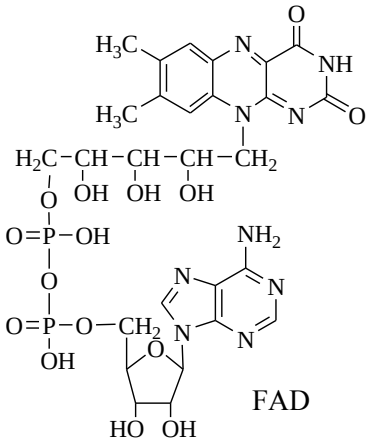
Coenzymes of dehydrogenases: pyridinic ($NAD(P)^+$), and flavic (FMN, FAD)

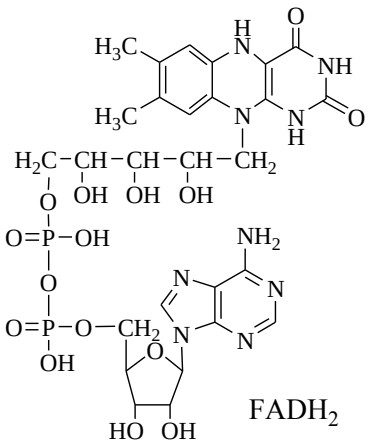


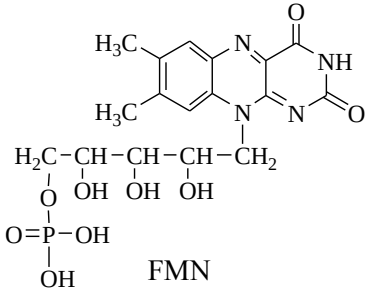


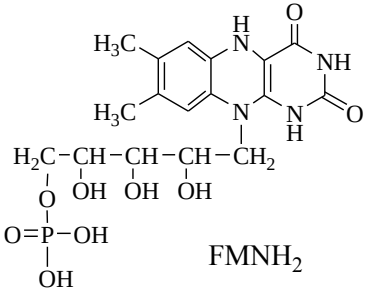








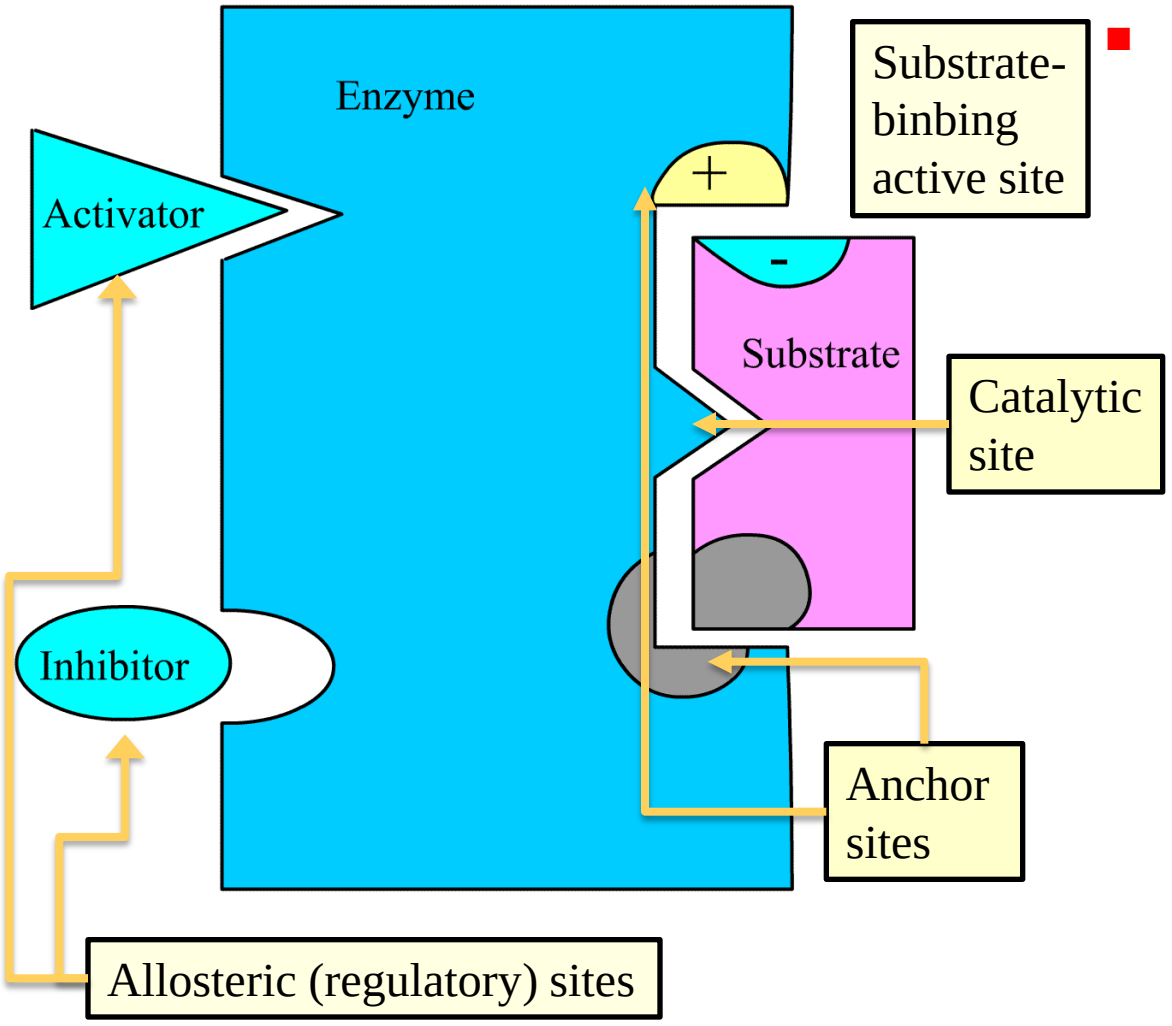




Monomeric and Oligomeric enzymes. Multienzyme Complexes

- **Monomeric** enzyme is made up of a single polypeptide.
- **Oligomeric** enzymes possess more than one polypeptide (subunit) chain.
- Certain **multienzyme complexes** possess specific sites to catalyze different reactions in a sequence.
  - The specificity of the enzyme is dependent on the apoenzyme.

Structure of enzyme: active site



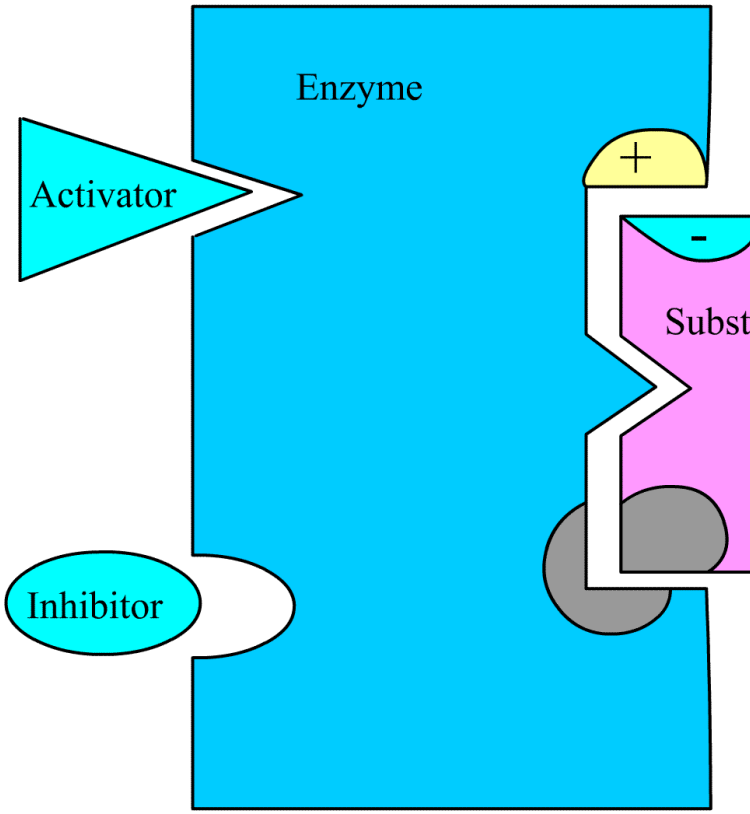
- Active site of enzyme is defined as the small region at which the ligand (substrate, or regulatory molecules) binds and participate in catalysis.
  - Substrate-binbing active site: binds substrate and convertes it to products, and releases them.
    - Catalytic site
    - Anchor sites.
  - Allosteric (regulatory) sites.

10 Salient Features of Active Site

1. Depends on native 3D-structure of the enzyme
2. Made up of amino acids (catalytic residues) which are far from each other
3. As cleft or crevices or pocked occupying a small region
4. Rather flexible
5. Possesses a substrate binding site and a catalytic site.
6. Cofactors are part of it
7. Substrates binds by weak noncovalent bonds
8. Determines enzyme specificity
9. Repeatedly found amino acids: **ser, asp, his, cys, lys, arg, glu, tyr**, etc.
10. Enzyme-substrate complex is formed; product is released

Allosteric Site

- Some enzymes has a regulatory site – allosteric (αλλοσ *gr*. – "another site")
- These enzymes are allosteric
- Activity of the enzymes depends on effector molecules – modulators or modifiers.
  - Positive or stimulatory effectors
  - Negative effectors
- Allosteric effectors catalyze key regulatory steps in biochemical pathways.



Mechanism of Enzyme Action

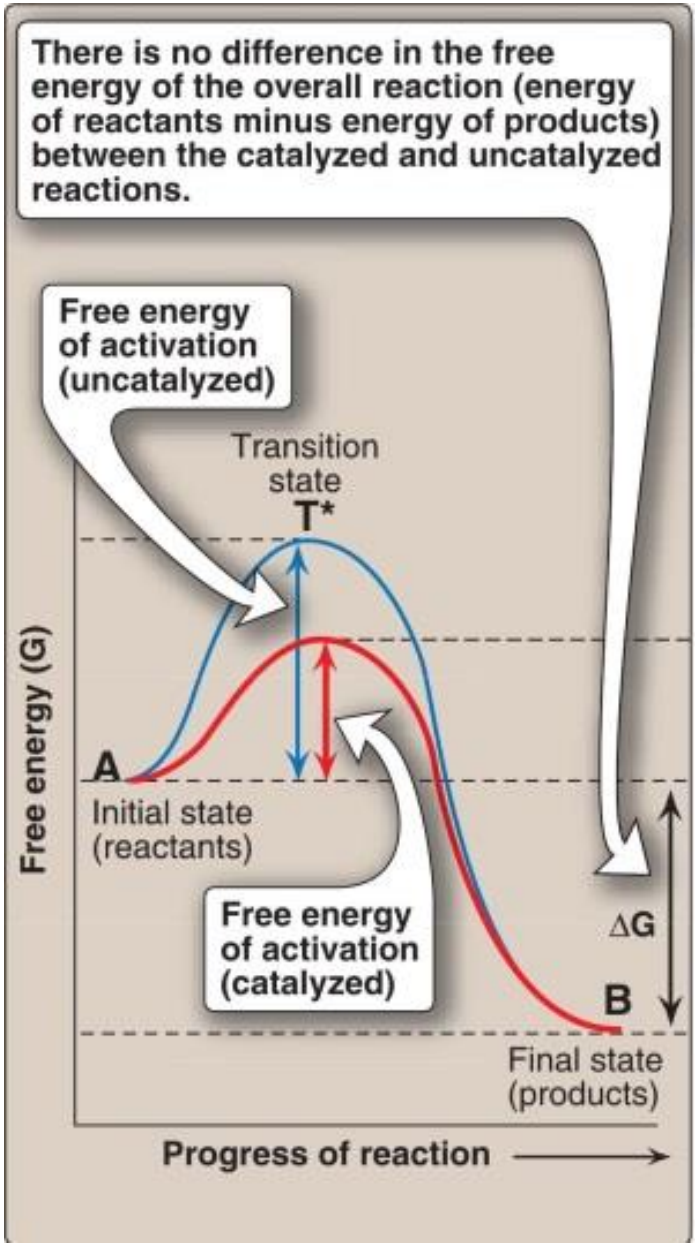
- Chemical reactions
- Enzyme-substrate complex formation
- Mechanism of enzyme catalysis
- Factors affecting enzyme activity
  - See flash
    - **Enzyme Specificity** "enzyme_binding[1].swf“
    - "catalysis_energy[1].swf“

Energy Changes Occurring During the Reaction

- Chemical reactions have an **energy barrier** separating the reactants and the products.
  - This barrier, called the **free energy of activation**, is the energy difference between that of the reactants and a high-energy intermediate that occurs during the formation of product.
- Changes in energy during the conversion of a molecule of reactant A to product B as it proceeds through the transition state (high-energy intermediate), T^*:

$$\mathbf{A \leftrightarrow T^* \leftrightarrow B}$$

- 1. Free energy of activation: The peak of energy is the difference in free energy between the reactant and T^*.



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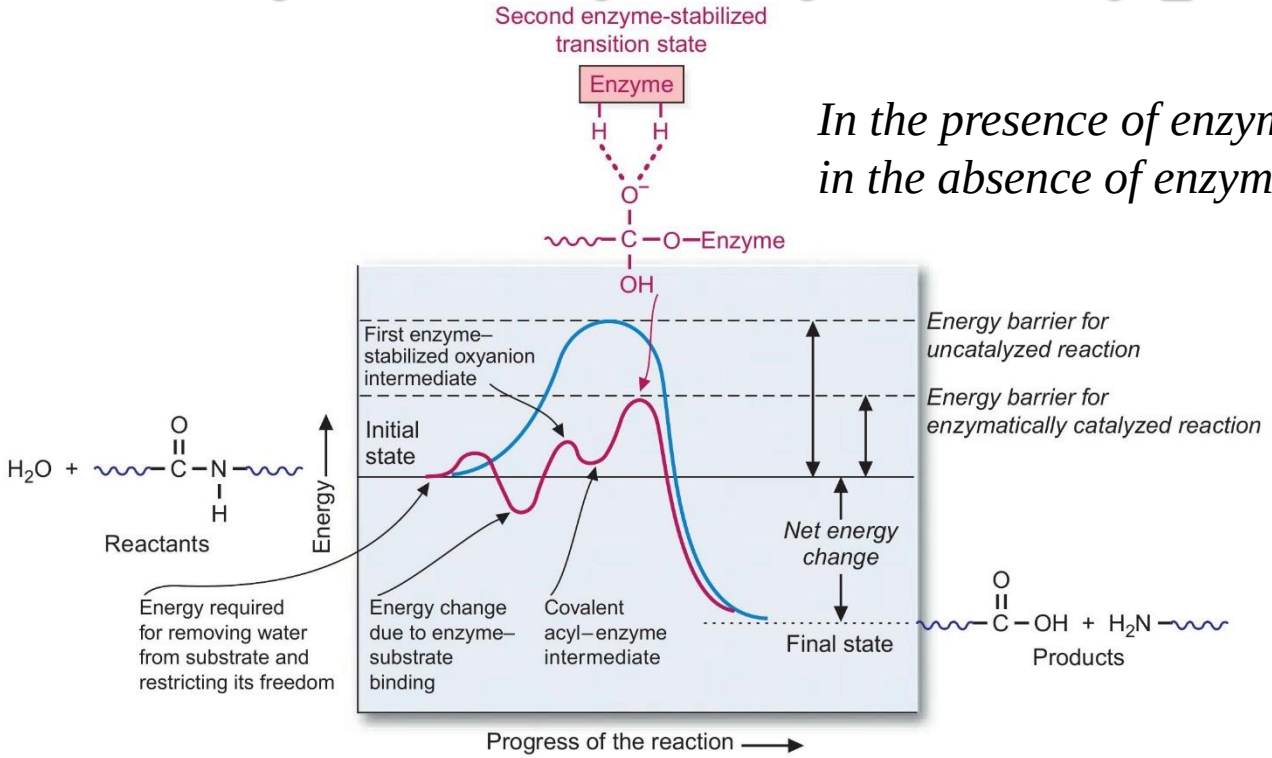
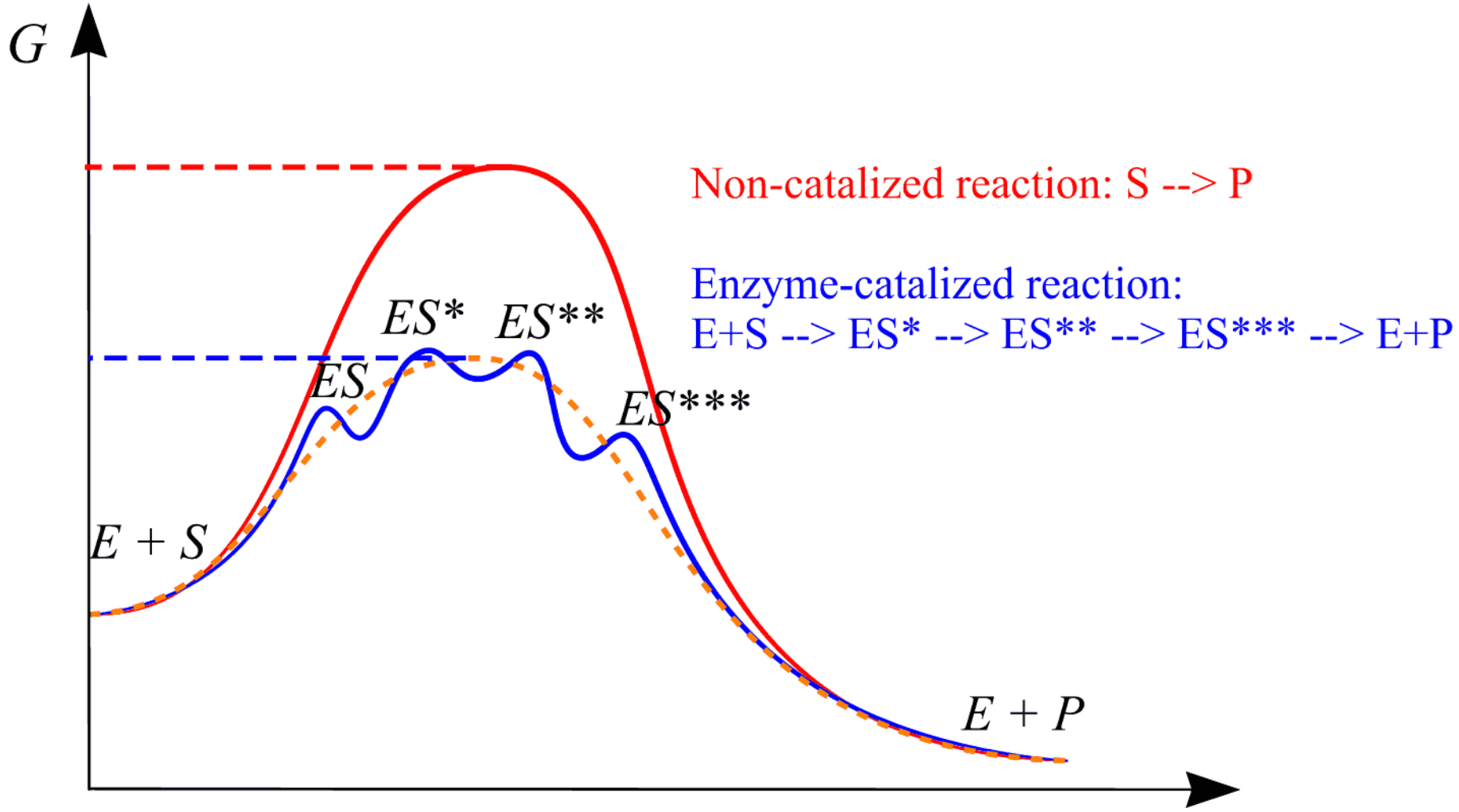
Energy diagram for the reaction catalyzed by chymotrypsin



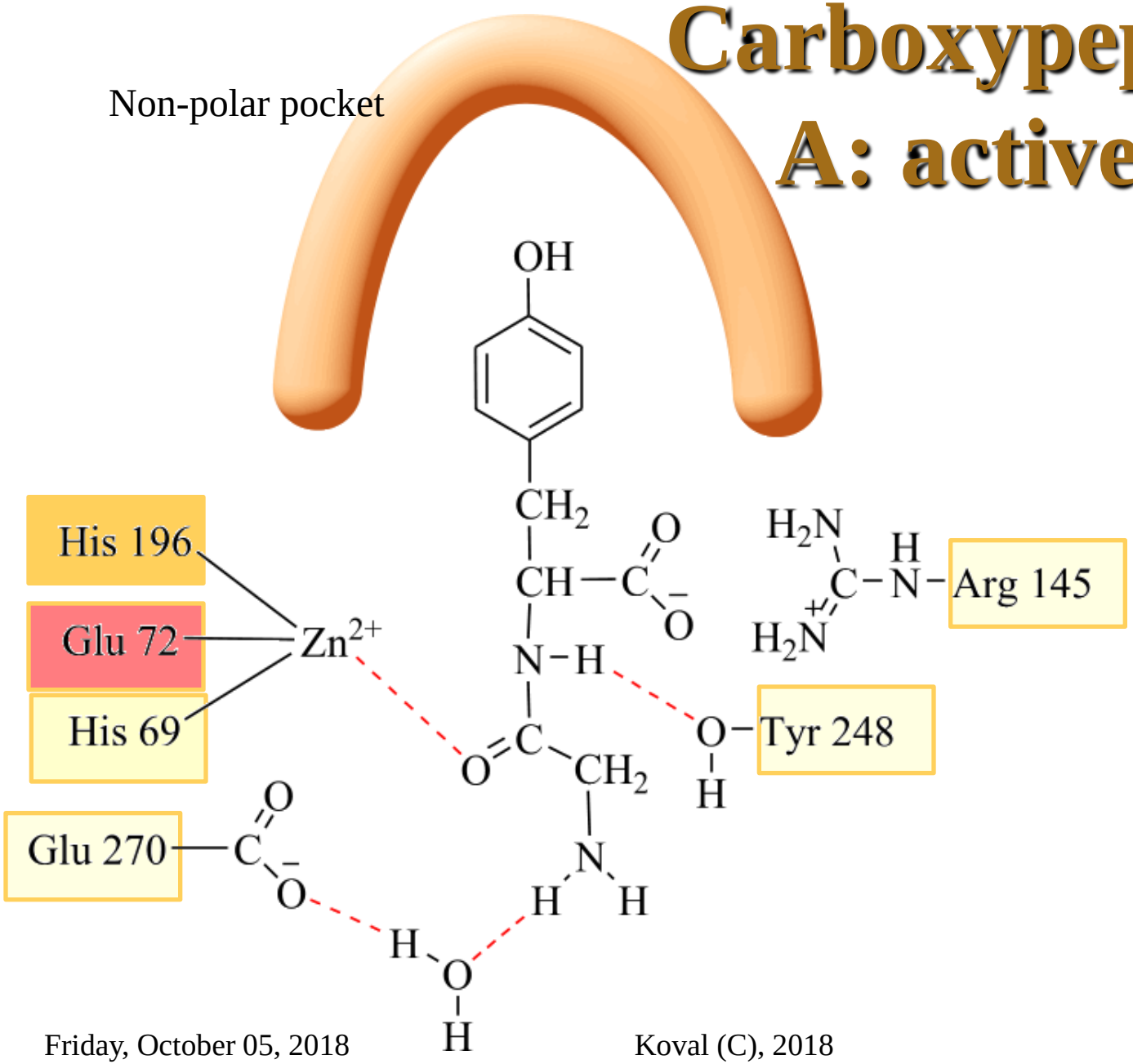
In the presence of enzyme (red); in the absence of enzyme (blue).

The energy barrier to the transition state is lowered in the enzyme-catalyzed reaction by the formation of additional bonds between the substrate and enzyme in the transition-state complex. The energy is provided by substrate binding to the enzyme. The enzyme does not, however, change the energy levels of the substrate or product.

Transition state intermediates

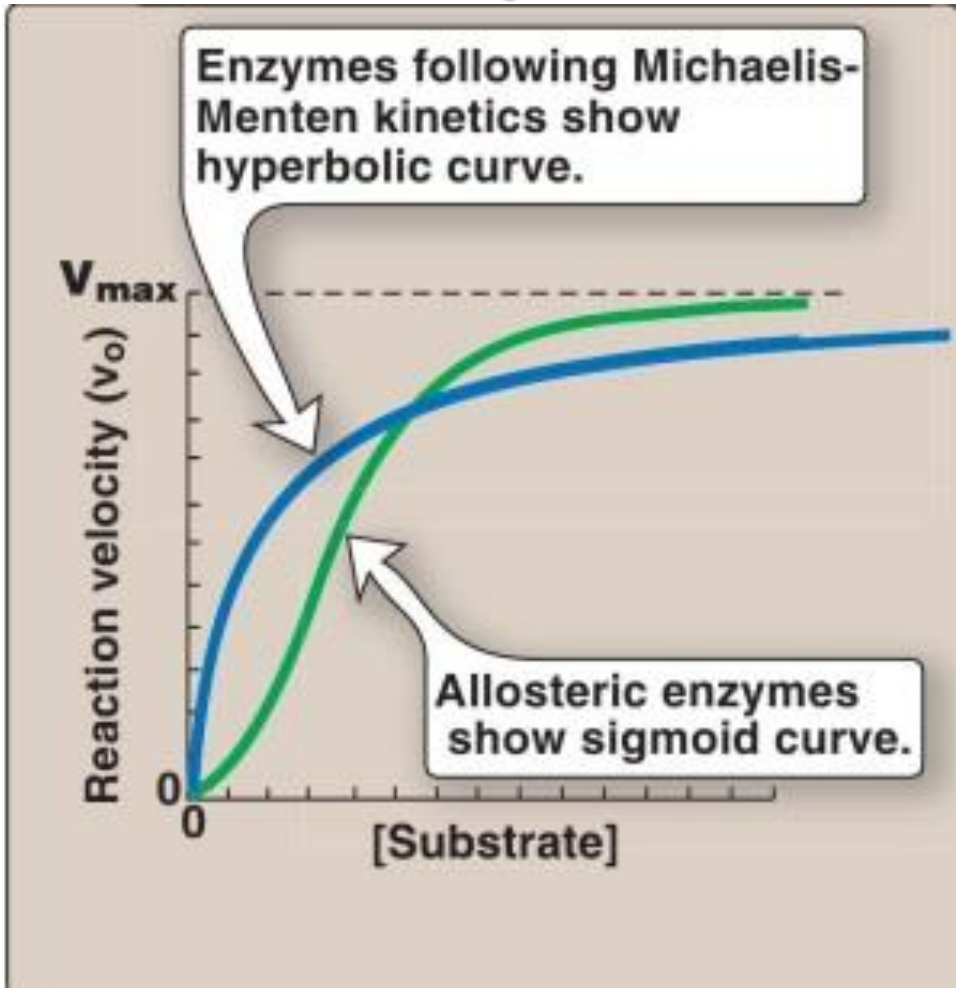


Carboxypeptidase A: active site



Effect of Substrate Concentration on Reaction Velocity

- **1. Maximal velocity:** The rate of an enzyme-catalyzed reaction increases with substrate concentration until a maximal velocity (V_{max}) is reached.
  - The leveling off of the reaction rate at high substrate concentrations reflects the saturation with substrate of all available binding sites on the enzyme molecules present.
- **2. Hyperbolic shape of the enzyme kinetics curve:**
- Most enzymes show **Michaelis-Menten kinetics**, in which the plot of initial reaction velocity (v_0) against substrate concentration ([S]), is **<u>hyperbolic</u>**.
  - In contrast, <u>allosteric enzymes</u> do not follow Michaelis-Menton kinetics and <u>show a sigmoidal curve</u>.



Effect of substrate concentration on reaction velocities

- Effect of substrate concentration on reaction velocities for two enzymes:
  - enzyme 1 with a small K_m, and
  - enzyme 2 with a large K_m.
- **Michaelis-Menten equation:**

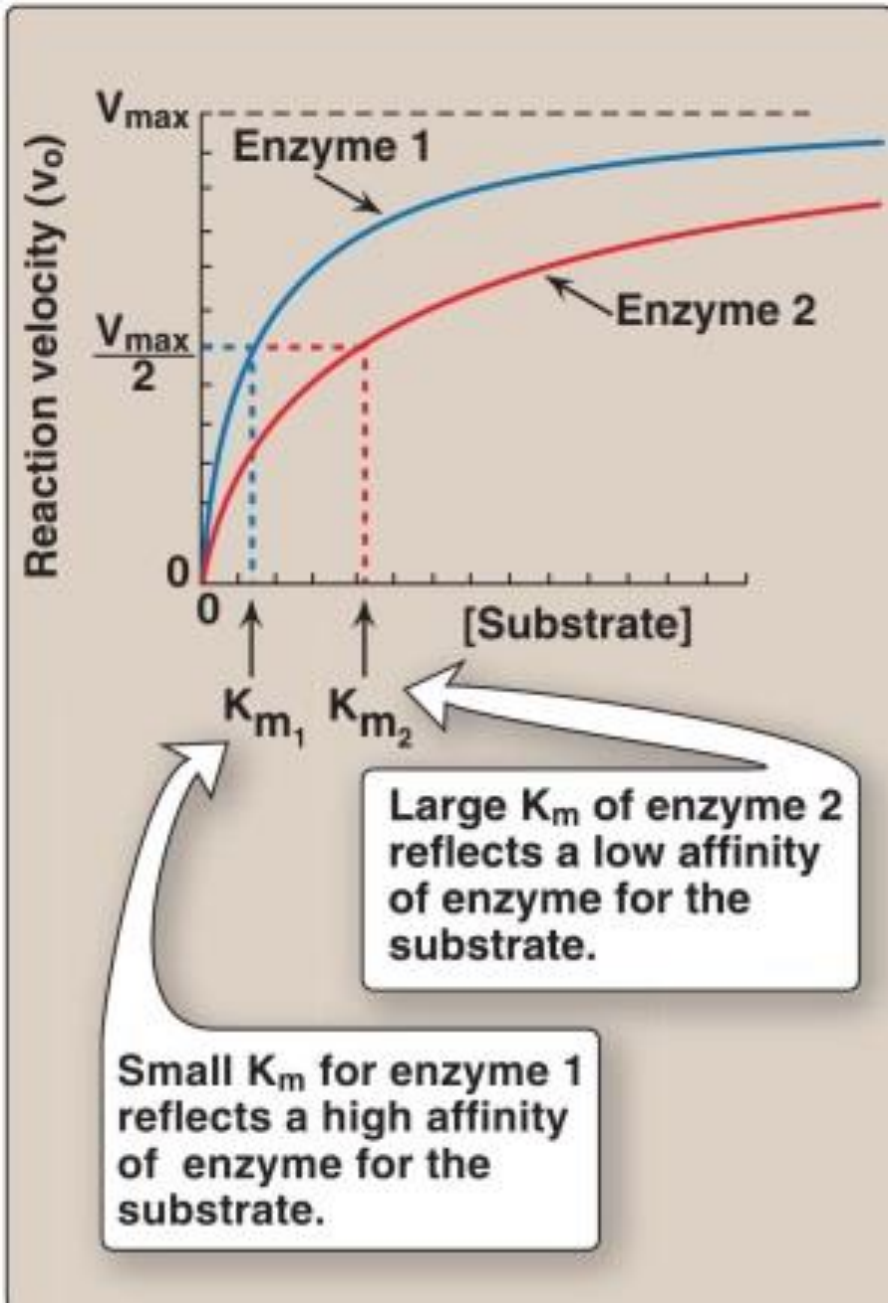
$$v_0 = \frac{V_{max} \times [S]}{K_m + [S]}$$

where v_0 = initial reaction velocity

V_{max} = maximal velocity

K_m = Michaelis constant

[S] = substrate concentration



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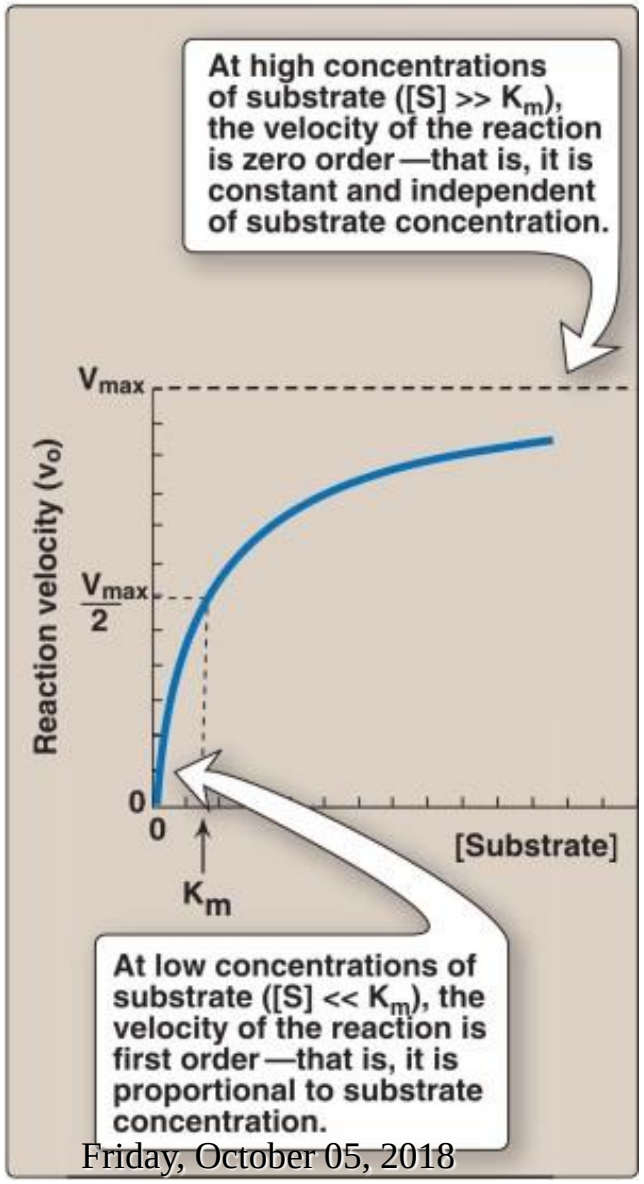
Order of Reaction

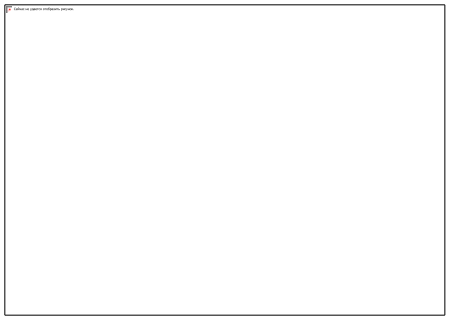


- When [S] is much less than K_m, the velocity of the reaction is approximately proportional to the substrate concentration.
  - The rate of reaction is then said to be first order with respect to substrate.
- When [S] is much greater than K_m, the velocity is constant and equal to V_{max}.
  - The rate of reaction is then independent of substrate concentration, and is said to be zero order with respect to substrate concentration.

Hyperbolas Versus Straight Lines

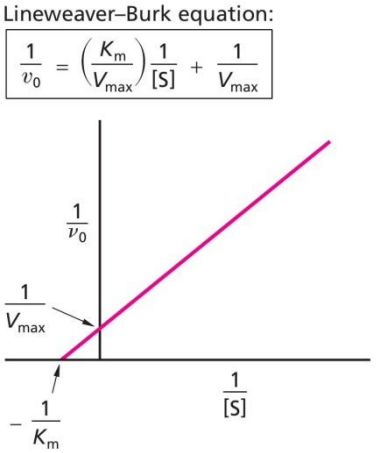
$$v_0 = \frac{V_{max} \times [S]}{K_m + [S]}$$

Michaelis-Menten equation (hyperbolic)
It is difficult to determine V_{max}.



Transformation of hyperbolic equation to linear one (like $y=mx+b$):

$$\frac{1}{v_0} = \frac{K_m + [S]}{V_{max}[S]} = \frac{K_m}{V_{max}[S]} + \frac{[S]}{V_{max}[S]}$$



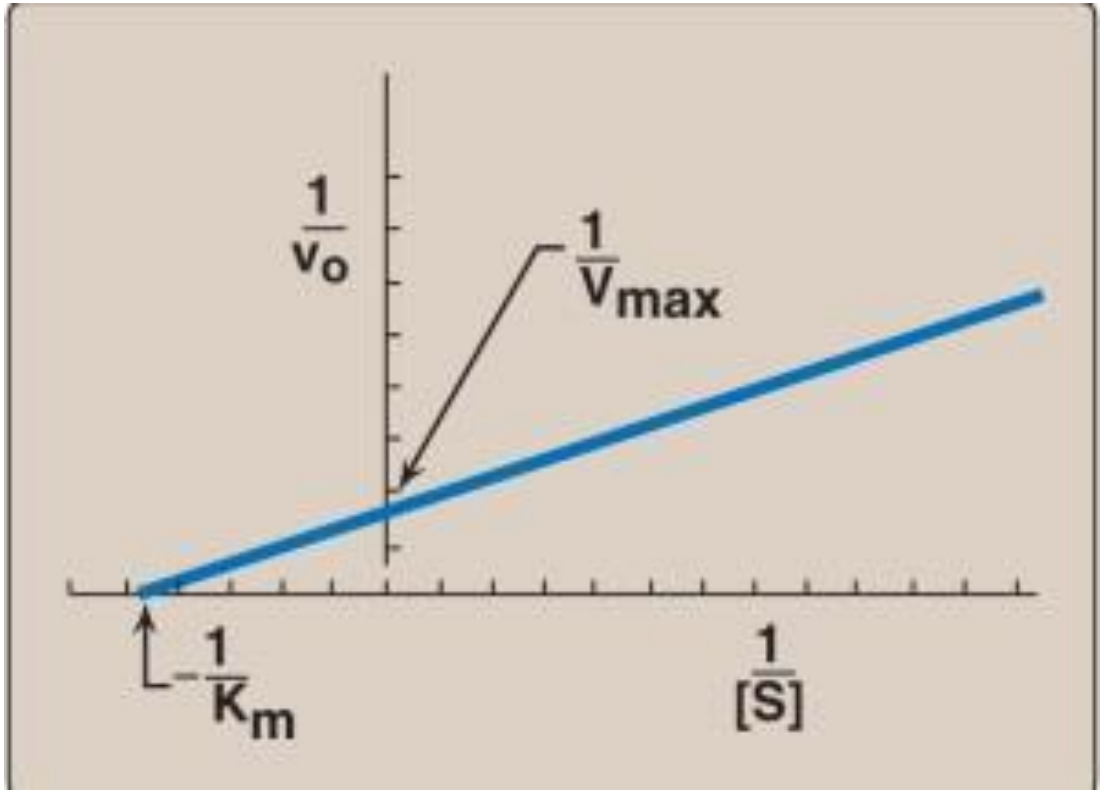
$$\frac{1}{v_0} = \frac{K_m}{V_{max}} \cdot \frac{1}{[S]} + \frac{1}{V_{max}}$$

$y = m x + b$

Double-reciprocal (**Lineweaver–Burk**) plot. This plot is derived from a linear transformation of the Michaelis-Menten equation.

Lineweaver–Burk Plot

- The original reason for this sort of transformation was to calculate K_m and V_{max} from experimental data. It was easier to plot the reciprocal values of v_0 and [S] and draw a straight line through the points in order to calculate the kinetic constants.
  - Nowadays, there are computer programs that can accurately fit the data to a hyperbolic curve and calculate the constants so the Lineweaver-Burk plot is no longer necessary for this type of analysis.
- Lineweaver-Burk plots to illustrate some general features of enzyme kinetics.



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Activators of Enzymes

- 2 categories of enzymes requiring metals
  - **Metal-activated enzymes**: the metal is not tightly held by the enzyme and can be exchanged easily with other ions
    - e.g. ATPase.
  - **Metalloenzymes**: these enzymes hold the metals rather tightly which are not readily exchanged,
    - e.g. alcohol dehydrogenase,
    - carbonic anhydrase,
    - alkaline phosphatase.

Enzyme Inhibition

- Inhibition can be:
  - **Irreversible**: inhibitors form covalent linkages with functional groups of the enzyme
    - Heavy metals - Hg, Pb; cyanide, β-mercuriobenzoate, diisopropylfluorphosphate, iodoacetate.
  - **Reversible**: inhibitors bond noncovalently, can dissociate.
    - Competitive
    - Uncompetitive
    - Noncompetitive
      - See flash "enzyme_inhibition[1].swf"

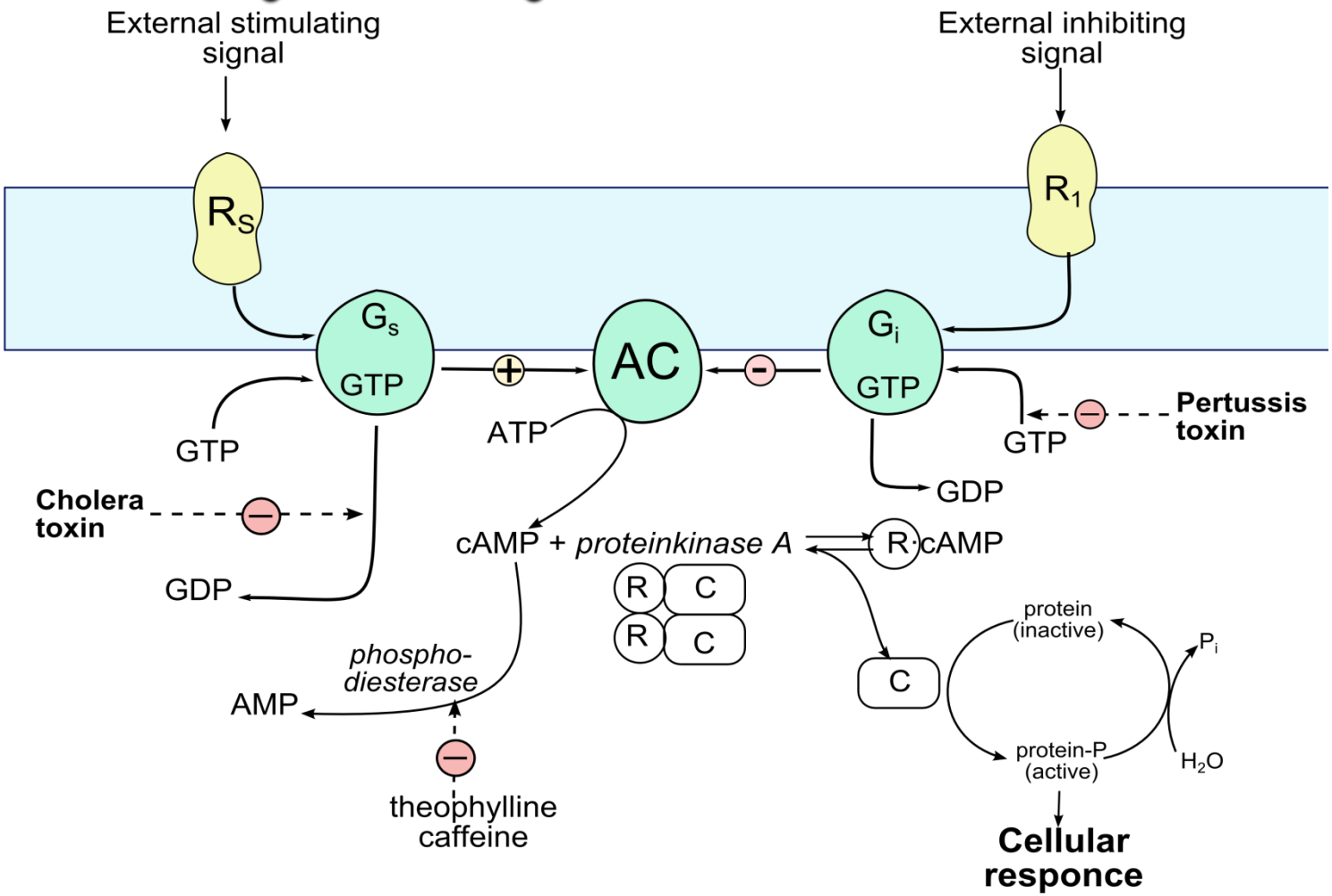
Medical Relevance of Enzyme Inhibitors

Inhibitor	Target Enzyme	Effect or Application
Allopurinol	Xanthine oxidase	Treatment of gout
Aspirin	Cyclooxygenase	Anti-inflammatory agent
5-Fluorouracil	Thymidylate synthetase	Antineoplastic agent
Lovastatin	HMG-CoA reductase	Cholesterol-lowering agent
Pargyline	Monoamine oxidase	Antihypertensive agent
Penicillin	transpeptidase	Antibacterial agent

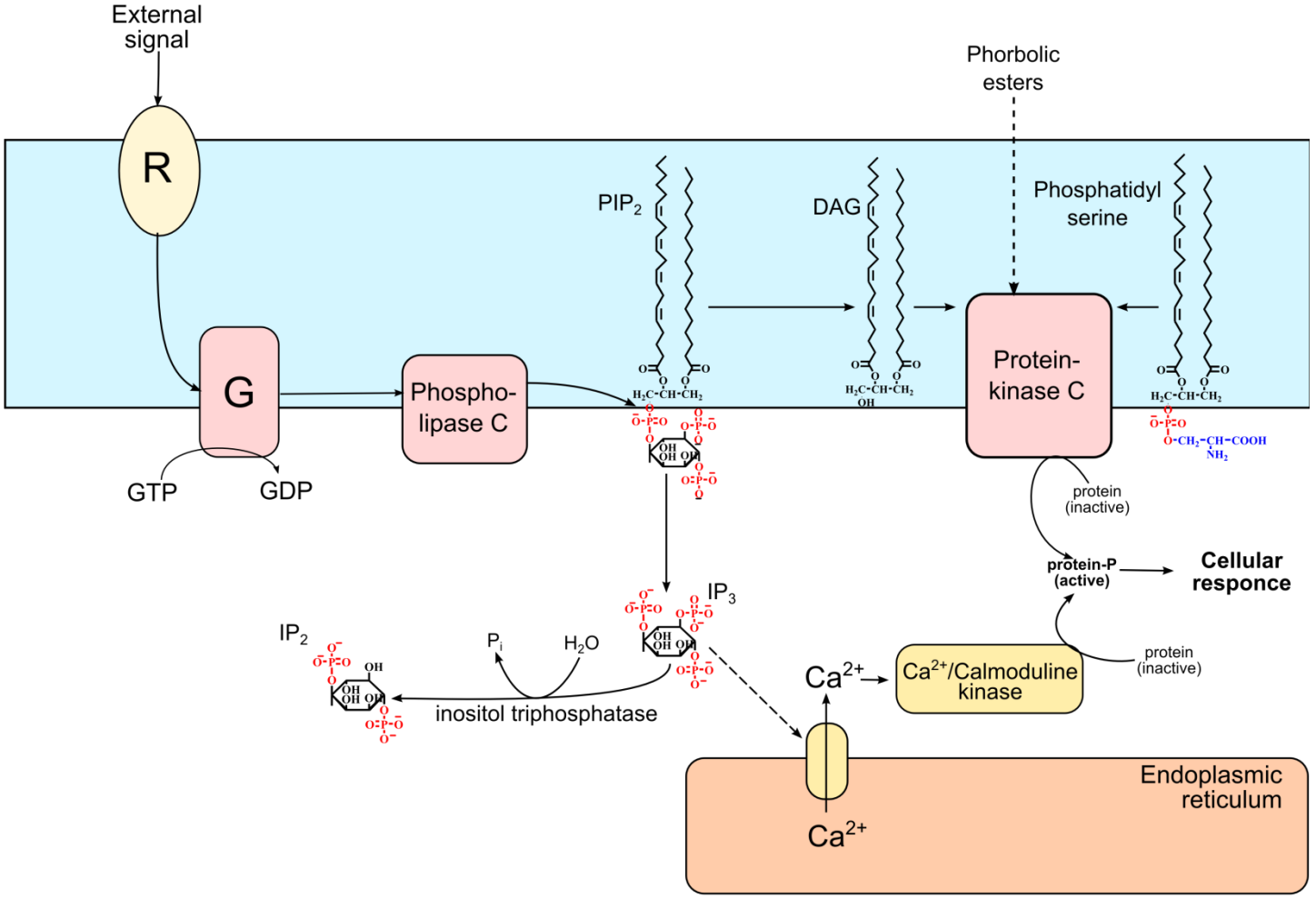
Enzyme Regulation

- **Covalent modification**
  - **Phosphorylation**: addition of phosphate group (at *Ser*, *Tyr*, *Thr*). Enhances or depresses activity.
    - Specific **kinases** phosphorylate.
    - Specific **phosphatases** dephosphorylate.
  - **Nucleotidylation**: addition of a nucleotide (adenosine)
  - **Methylation**
  - Acetylation
  - **Proteolytic cleavage**: proenzyme (zymogen) becomes active. Many proteases. Digestive enzymes; blood clotting.
- Allosteric regulation.
  - T and R conformation of enzymes.
  - Cooperativity:
    - Negative
    - Positive
- **Feedback** (by **end product**) **inhibition**

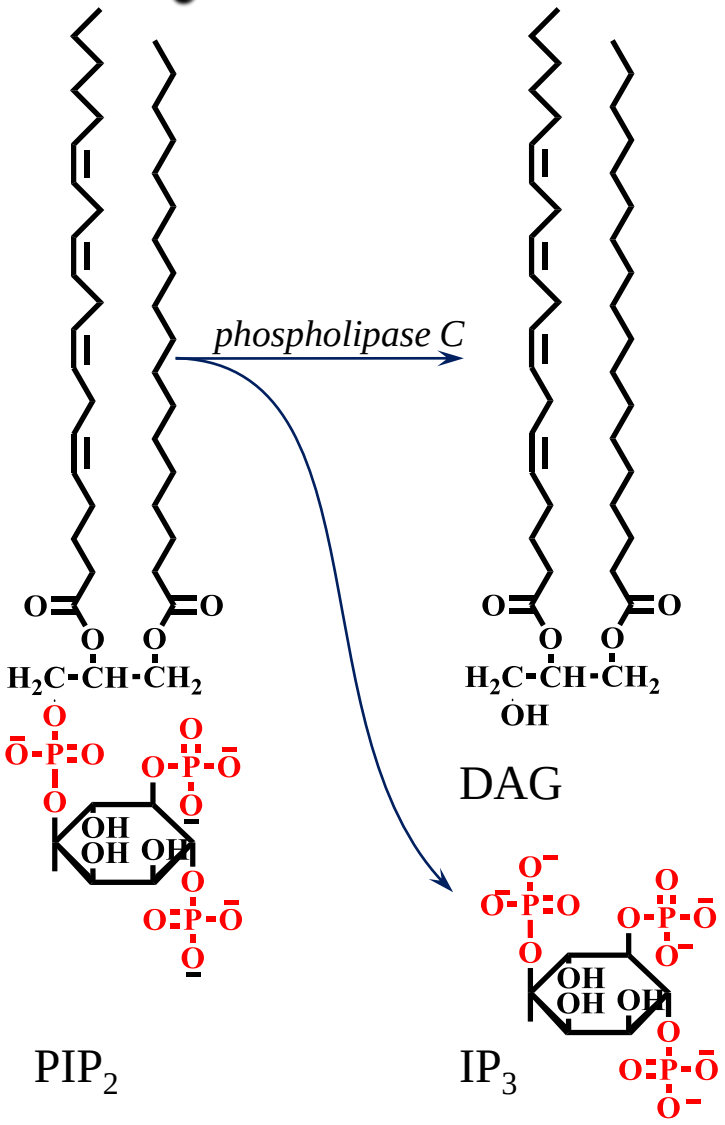
Hormonal regulation of enzymes
Adenylatecyclase mechanism



Hormonal regulation of enzymes: Inositol-3-phosphate mechanism



Key moment in Ca^{2+}-IP_3-mechanism



- PIP_2 – phosphatydyl inositol-2-phosphate,
- DAG – diacyl-glycerol,
- IP_3 - inositol-3-phosphate.
- Enzyme – phospholipase C (*aka phosphodiesterase*).

Ca^{2+}/Calmodulin kinase

Medical Enzymology

- Enzymopathology
- Enzymodiagnostics
- Enzymotherapy

Enzymopathology

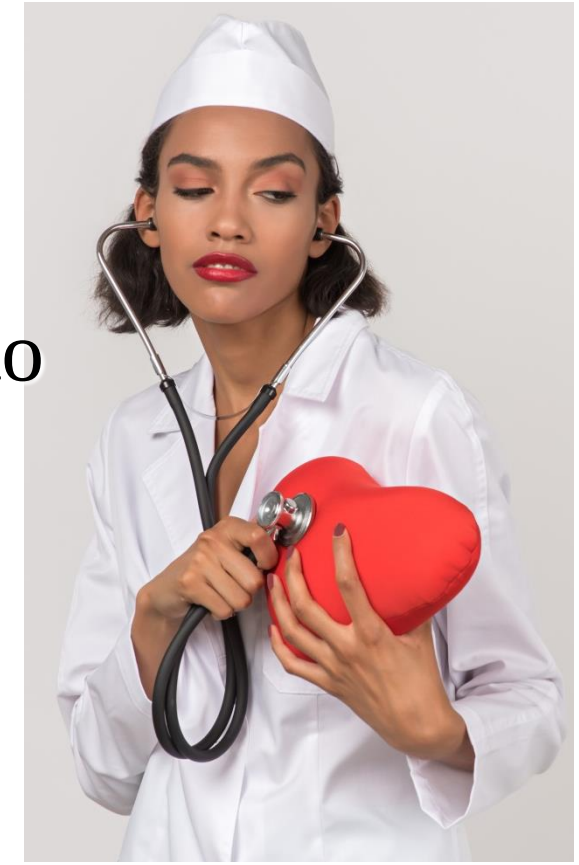
- ***Pokrovsky*** Enzymopathies classification:
  - **Primary**: inherited (e.g. PKU)
  - **Secondary**: non-inherited, acquired.
    - Alimentary: poor nutrition.
    - Toxic: poisoning with heavy metals (lead poisoning, mercury poisoning).

Enzymodiagnostics

- Enzymes in clinical diagnosis
  - Enzyme assays provide important information (presence and severity of a disease).
  - Enzyme assay allow to monitor a patient's response to therapy.
  - Genetic predispositions is possible to determine using enzyme assay.
- Several enzymes are used as reagent.

Plasma (Serum) Enzymes

- Elevation or depression of the levels of activity of specific enzymes may indicate either the presence of a disease or damage to a specific tissue.



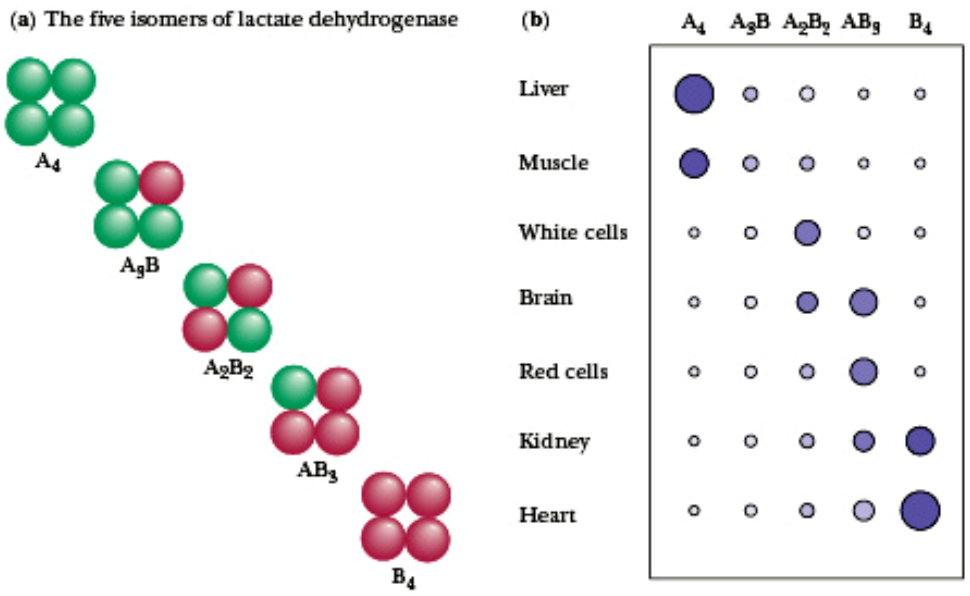
From photos.icons8.com

Some Enzymes Used for Clinical Diagnosis of Disease

Enzyme	Tissue source(s)	Diagnostic use
Aspartate aminotransferase (AST)	heart, skeletal muscle, liver, brain	myocardial infarction, liver disease
Alanine aminotransferase (ALT)	liver	liver disease, e.g. hepatitis (if ALT > AST)
Amylase	pancreas, salivary gland	acute pancreatitis, biliary obstruction
Creatine kinase (CK)	skeletal muscle, heart, brain	muscular dystrophy, myocardial infarction
Gamma glutamyl transferase (GGT)	liver	hepatitis, alcohol excess
Lactate dehydrogenase (LDH)	heart, liver, erythrocytes	myocardial infarction, lymphoma, hepatitis
Lipase	pancreas	acute pancreatitis, biliary obstruction
Alkaline phosphatase	bile ducts, osteoblasts	liver disease, bone tumors
Acid phosphatase	prostate	prostate cancer

Isozymes

- **Multiple forms of an enzymes, catalyzing the same reaction.**

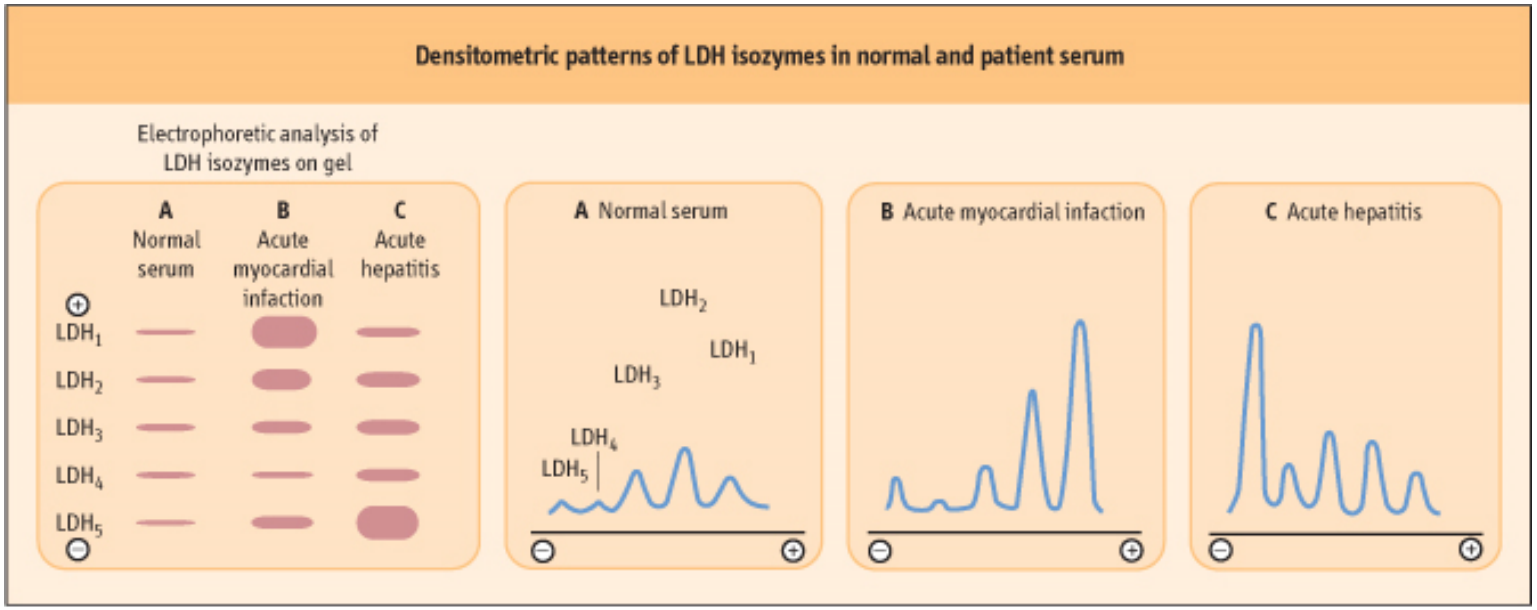


Lactate dehydrogenase:	
▪tetrameric, ▪2 subunints, ▪5 isoforms are possible:	LDH 1 (B4) LDH 2 (AB3) LDH 3 (A2B2) LDH 4 (A3B) LDH 5 (A4)

Subunit designation variants: A=M; B=H
(M – muscle, anaerobic izozyme
H – heart, aerobic izozyme)

There is also 3rd subunit is X – in testis.
Analogous to subunit A.

LDH Isozymes in Normal and Patient Serum



- **Densitometric patterns of the LDH isozymes in serum of patients diagnosed with myocardial infarction or acute hepatitis.**
  - Isozymes, differing slightly in charge, are separated by electrophoresis on cellulose acetate, visualized using a chromogenic substrate, and quantified by densitometry. Total serum LDH activity is also increased in these patients.
  - Since hemolysis releases LDH from red blood cells and affects diagnosis, blood samples should be treated with care.
  - The LDH measurements for the diagnosis of myocardial infarction have now been superseded by plasma troponin levels.

Example: Markers for Acute Myocardial Infarction

- Acute myocardial infarction (first 6 hr.):
  - Creatine kinase (CK-MB)
  - Cardiac troponin I (CTI) and cardiac troponin T (CTT).
    - *These are not true enzymes*
  - Brain Natriuretic Peptide (BNP).
    - *It is a reliable marker of ventricular function*
  - LDH and AST were previously used as markers of myocardial infarction.
    - *no more used in clinical practice.*

Vasudevan, 2011

Enzymodiagnostics: Enzymes as a reagents

Enzyme	Tested substance
Urease	Urea
Uricase	Uric acid
Glucose oxidase	Glucose
Peroxidase	Glucose, Cholesterol
Hexokinase	Glucose
Cholesterol oxidase	Cholesterol
Lipase	Triglycerides
Horse radish peroxidase	ELISA
Alkaline phosphatase	ELISA
Restriction endonuclease	Southern blot; RFLP
Reverse transcriptase	Polymerase chain reaction (RT=PCR)

Enzymotherapy

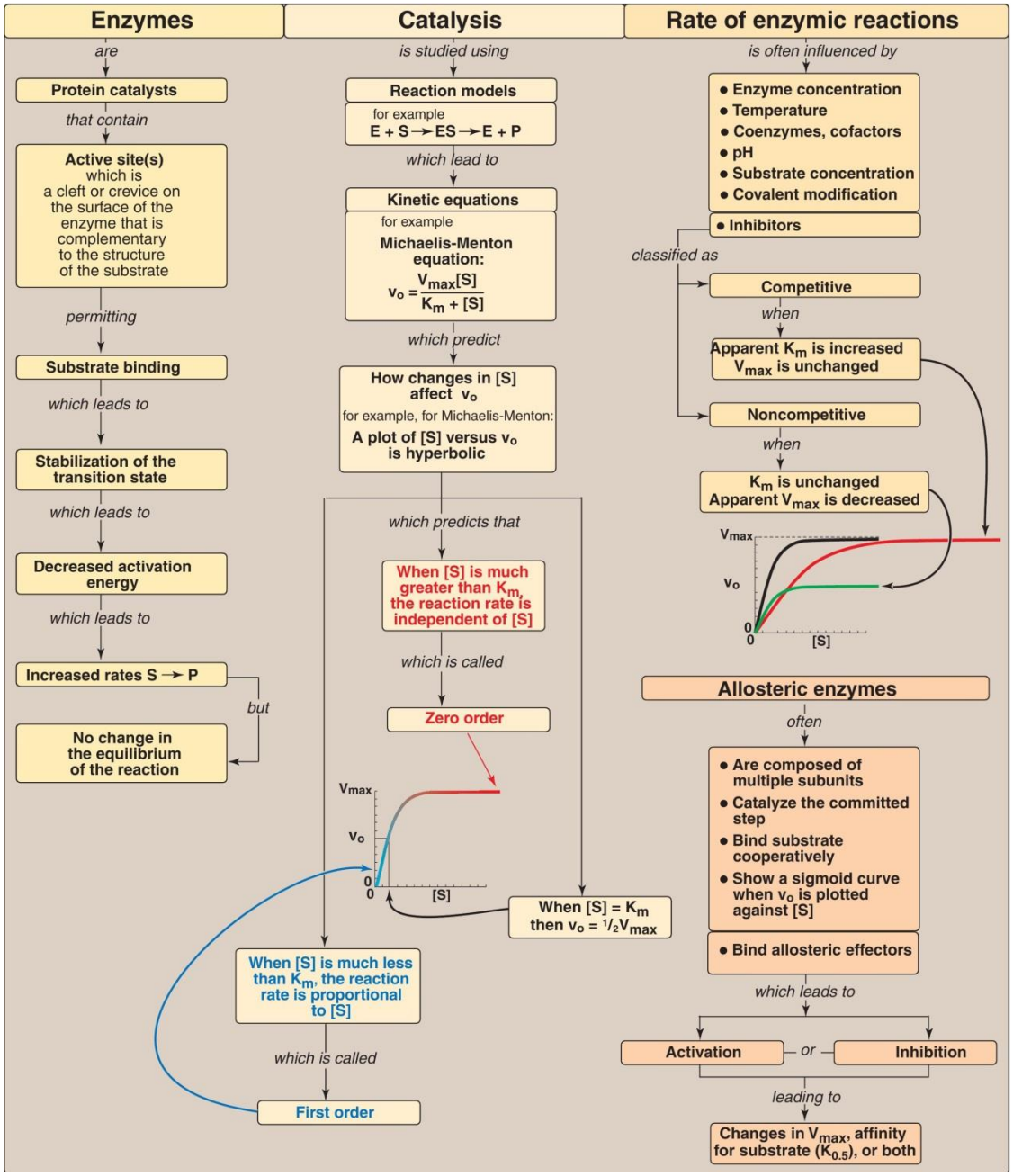
- **Festal, Pancreatin (trypsin + lipase)** – pancreatic insufficiency (orally);
- **Streptokinase, Urokinase** – in treatment of myocardial infarction (to lyse intravascular clot);
- **Streptodornase -** locally applied DNAse;
- **Asparaginase** – treatment of acute lymphoblastic leukemia;
- **Papain** – anti-inflammatory action;
- **α-1-antitrypsin** – AAT deficiency; emphysema.

Control of Enzyme Synthesis (1/2)

- Enzymes whose concentrations remain essentially constantover time are termed **constitutive enzymes.**
- By contrast, the concentrations of many other enzymes depend upon the presence of **inducers,** typically substrates or structurally related compounds, that initiate their synthesis.
  - *Escherichia coli* grown on glucose will, for example, only catabolize lactose after addition of a β-galactoside, an inducer that initiates synthesis of a *β-galactosidase* and a *galactoside permease*.
  - **Inducible enzymes** of humans include tryptophan pyrrolase, threonine dehydrase, tyrosine-α-ketoglutarate aminotransferase, enzymes of the urea cycle, HMG-CoA reductase, and cytochrome P450.

Control of Enzyme Synthesis (2/2)

- Conversely, an excess of a metabolite may curtail synthesis of its cognate enzyme via repression.
- Both induction and repression involve cis elements, specific DNA sequences located upstream of regulated genes, and trans-acting regulatory proteins.



Conclusion: Key Concept Map for the Enzymes

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Thank you
for your attention!