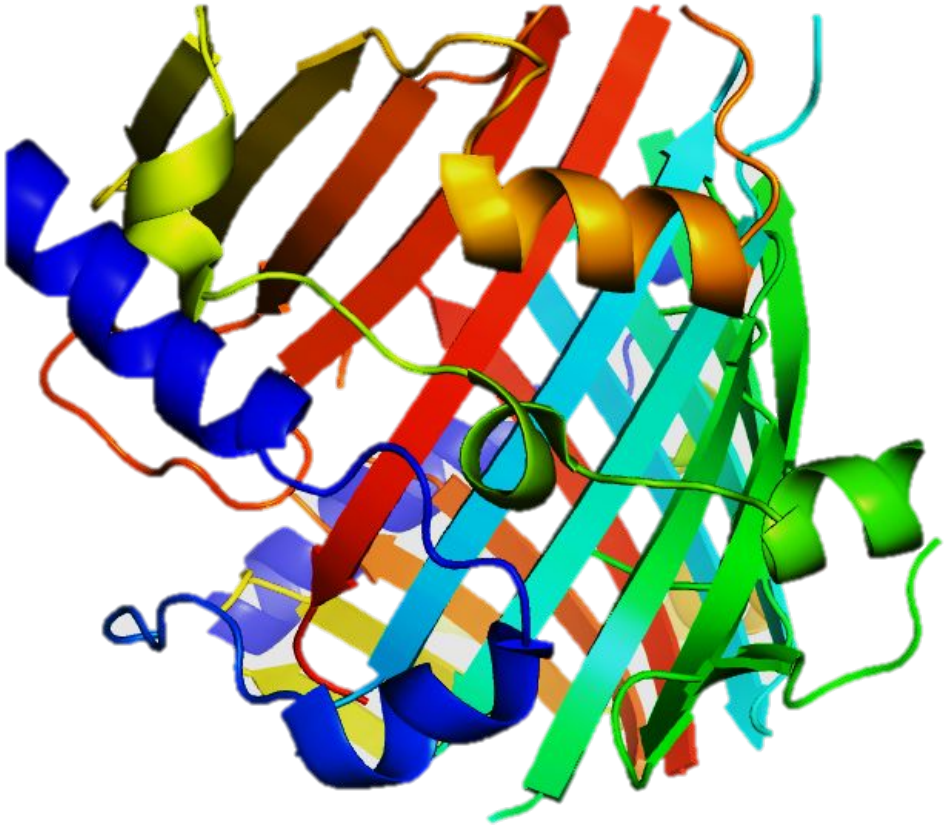
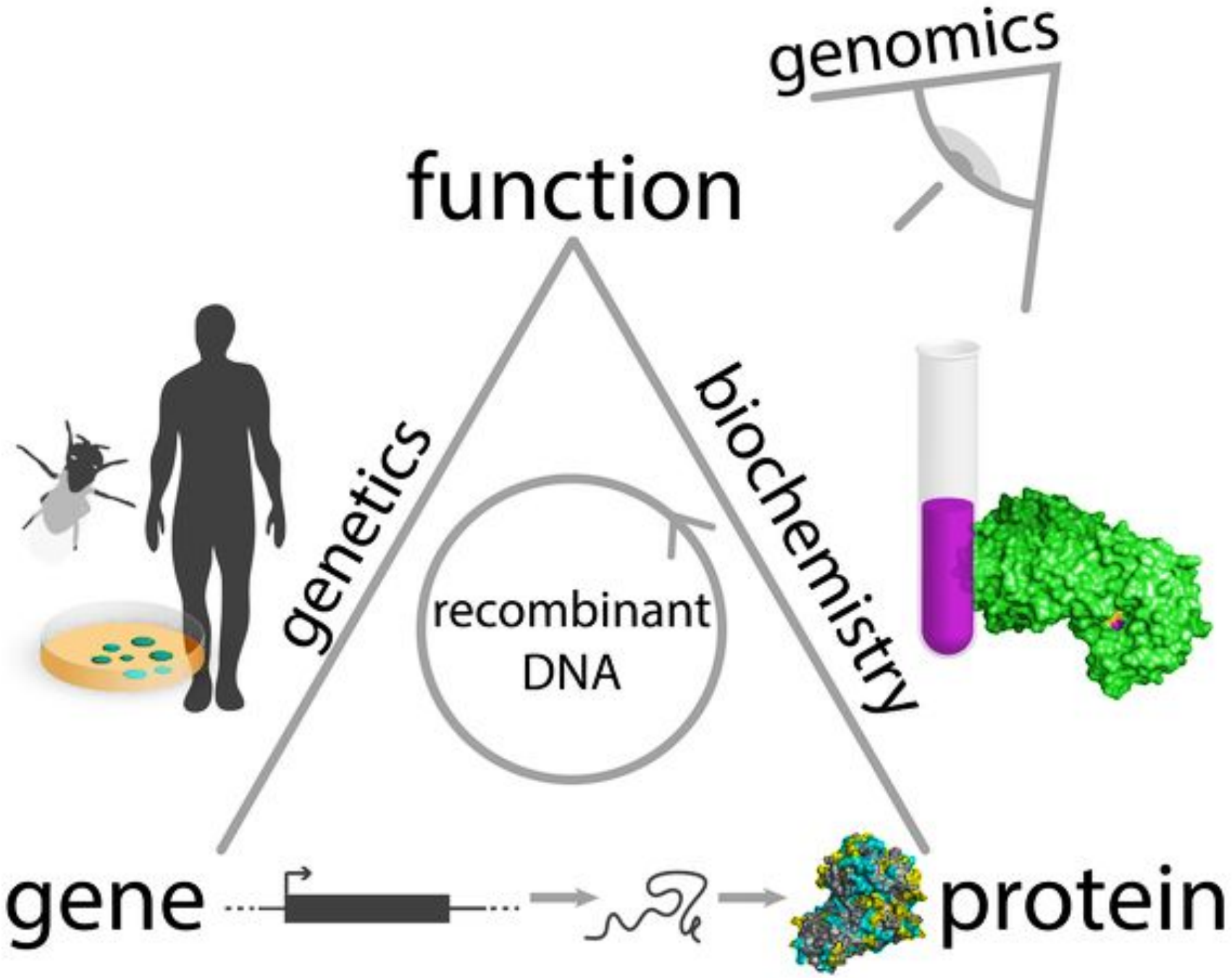
Protein Chemistry




genomics
function
genetics
biochemistry
recombinant
DNA
gene
protein

Essential amino acids

- valine, leucine, isoleucine, lysine, methionine, threonine, tryptophan, phenylalanine

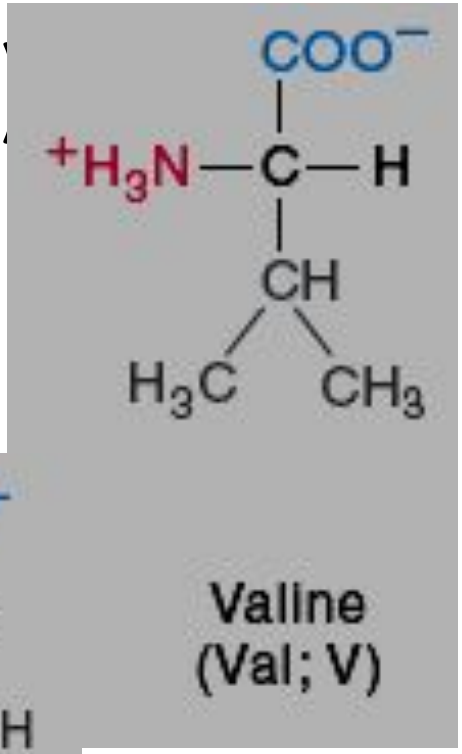
Semi-essential amino acids

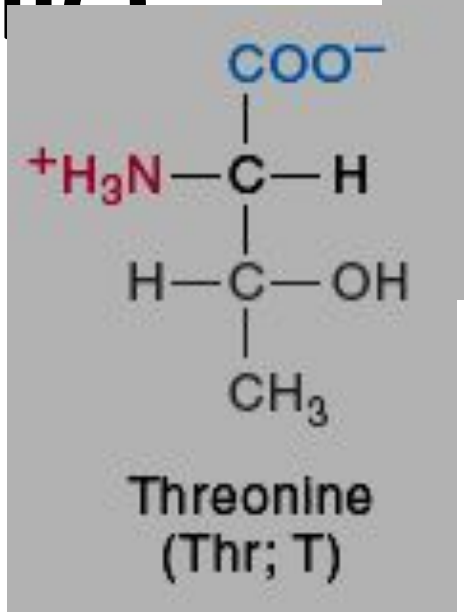
- arginine and histidine

Amino acids classification

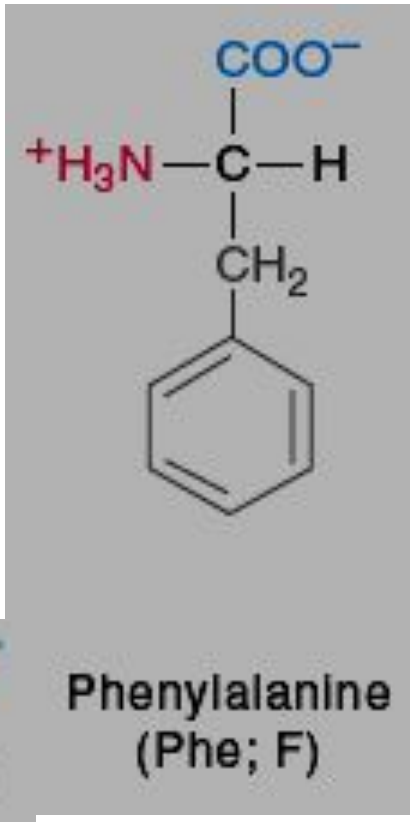
1. Non-polar (hydrophobic)

2. Polar (hydrophilic)



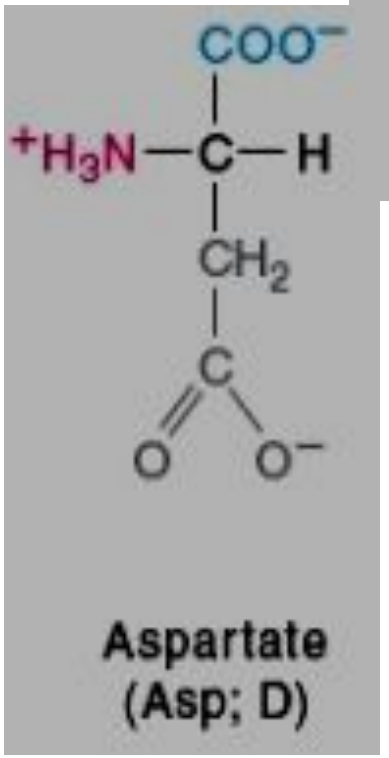


3. Aromatic (mainly non-polar)



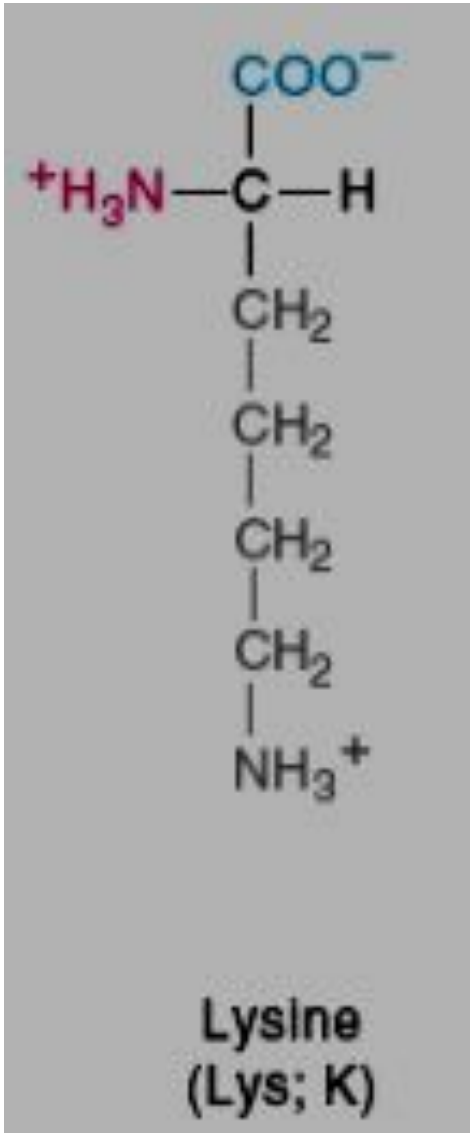
Phenylalanine
(Phe; F)

4. Negatively charged



Aspartate
(Asp; D)

5. Positively charged

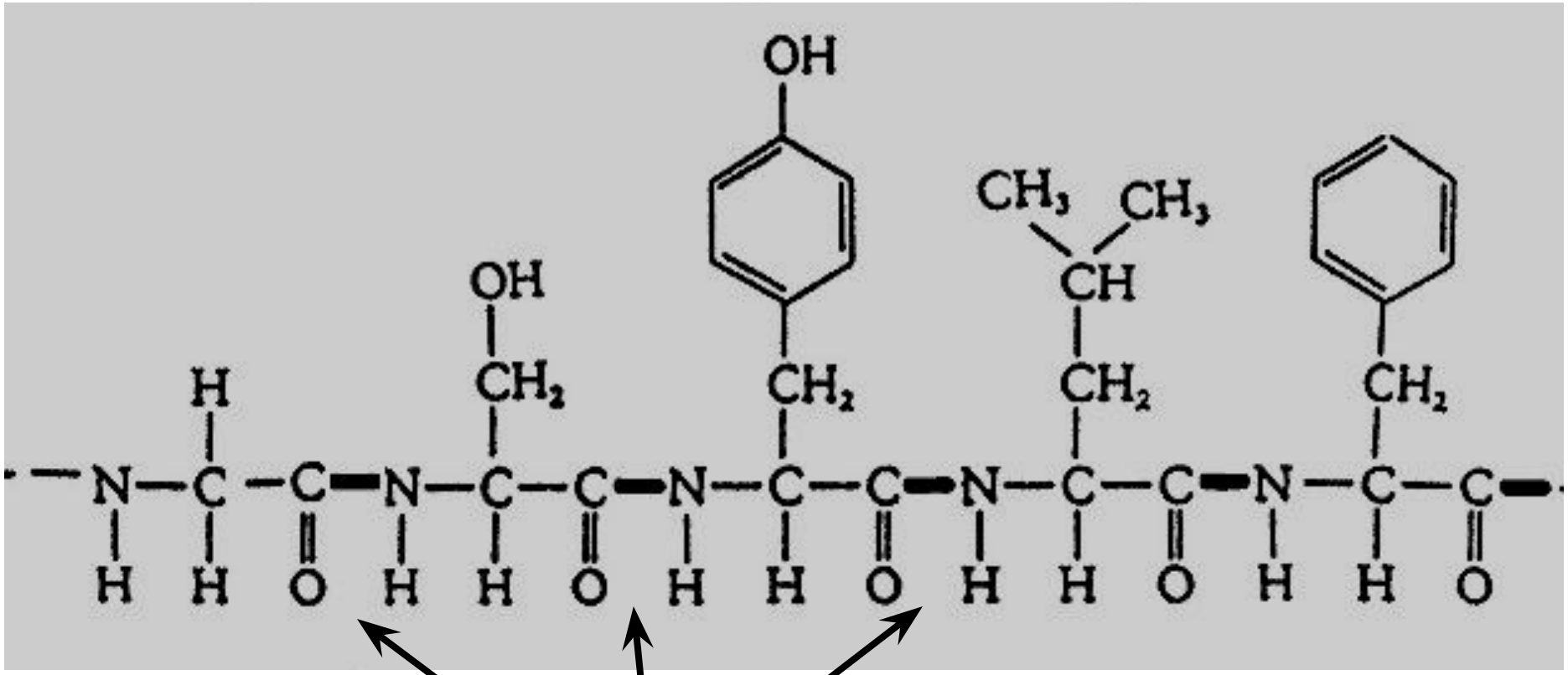


Acid-base properties of amino acids

Amino acid	pK_A (a-COOH): $COOH \rightleftharpoons COO^- + H^+$	pK_A (a-NH_3^+): $NH_3^+ \rightleftharpoons NH_2 + H^+$	p*I*
Gly	2,34	9,60	6,20
Ala	2,34	9,60	6,11
Val	2,29	9,72	6,00
Leu	2,36	9,60	6,04
Ser	2,21	9,15	5,68

$$pI = \frac{pK_1 + pK_2}{2}$$

The primary structure of protein



The determination of the primary structure

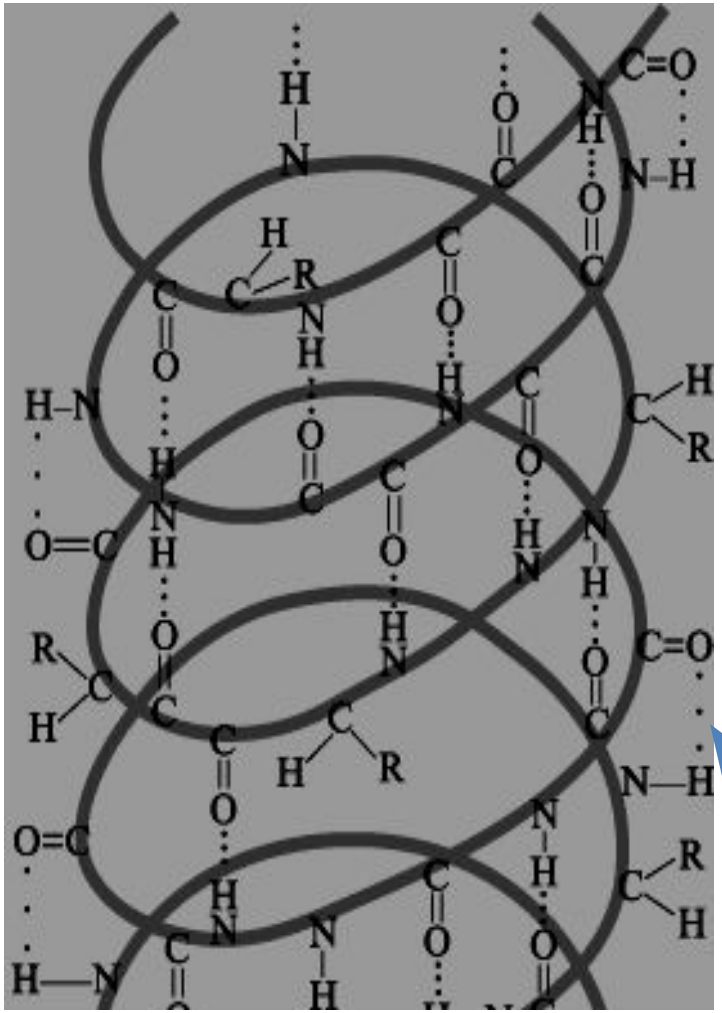
	Method	Reagent
N-terminal	Sanger' s method	2,4-Dinitrofluorobenzene
	Edman's metod	Phenylisithiocianate
C-terminal	Acabory's method	Hydrasin
	Enzymatic	Carboxypeptidase

The determination of the primary structure

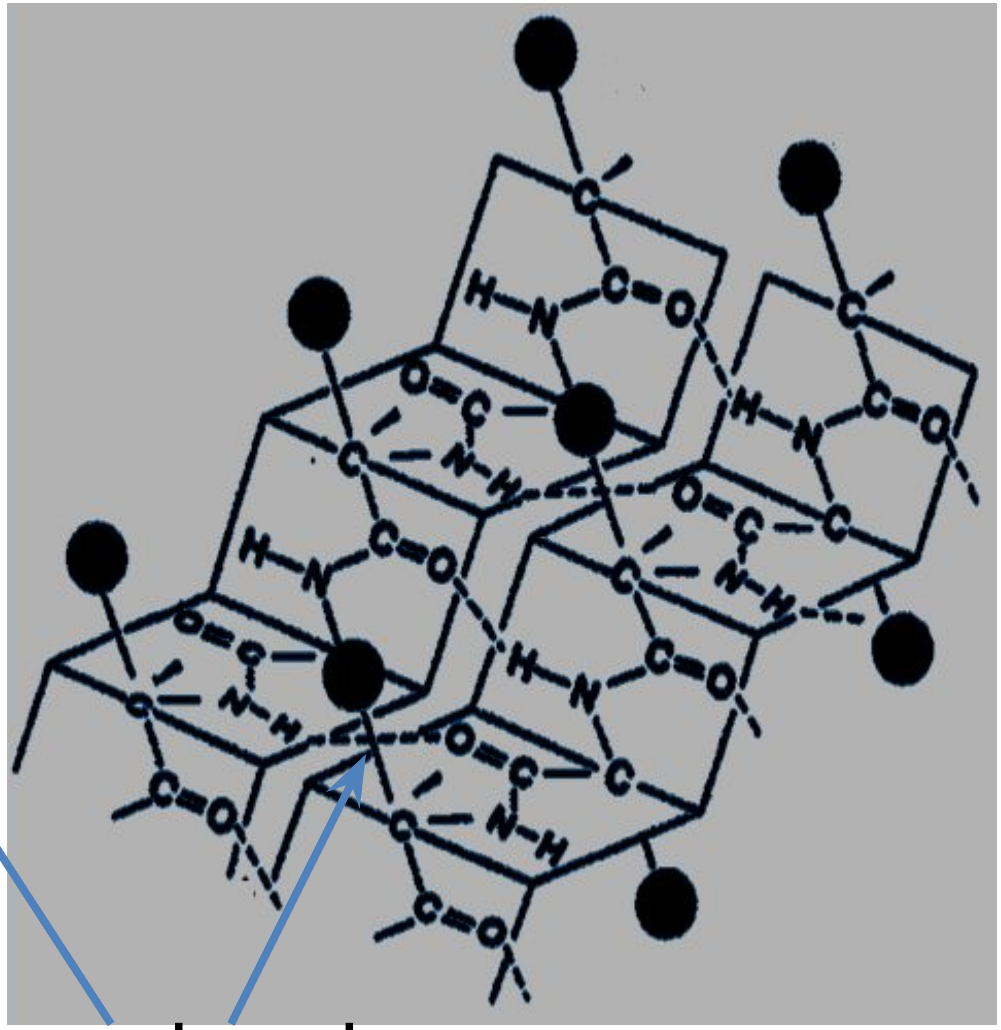
Reagent	Amino acid residues
Cyanogen bromide (CNBr)	Met
Hydroxylamine	Asp – Gly
N-bromosuccinimide	Trp
Pepsin	Phe, Tyr, Glu
Trypsin	Arg, Lys
Chymotrypsin	Trp, Tyr, Phe

The secondary structure of protein

α-helix



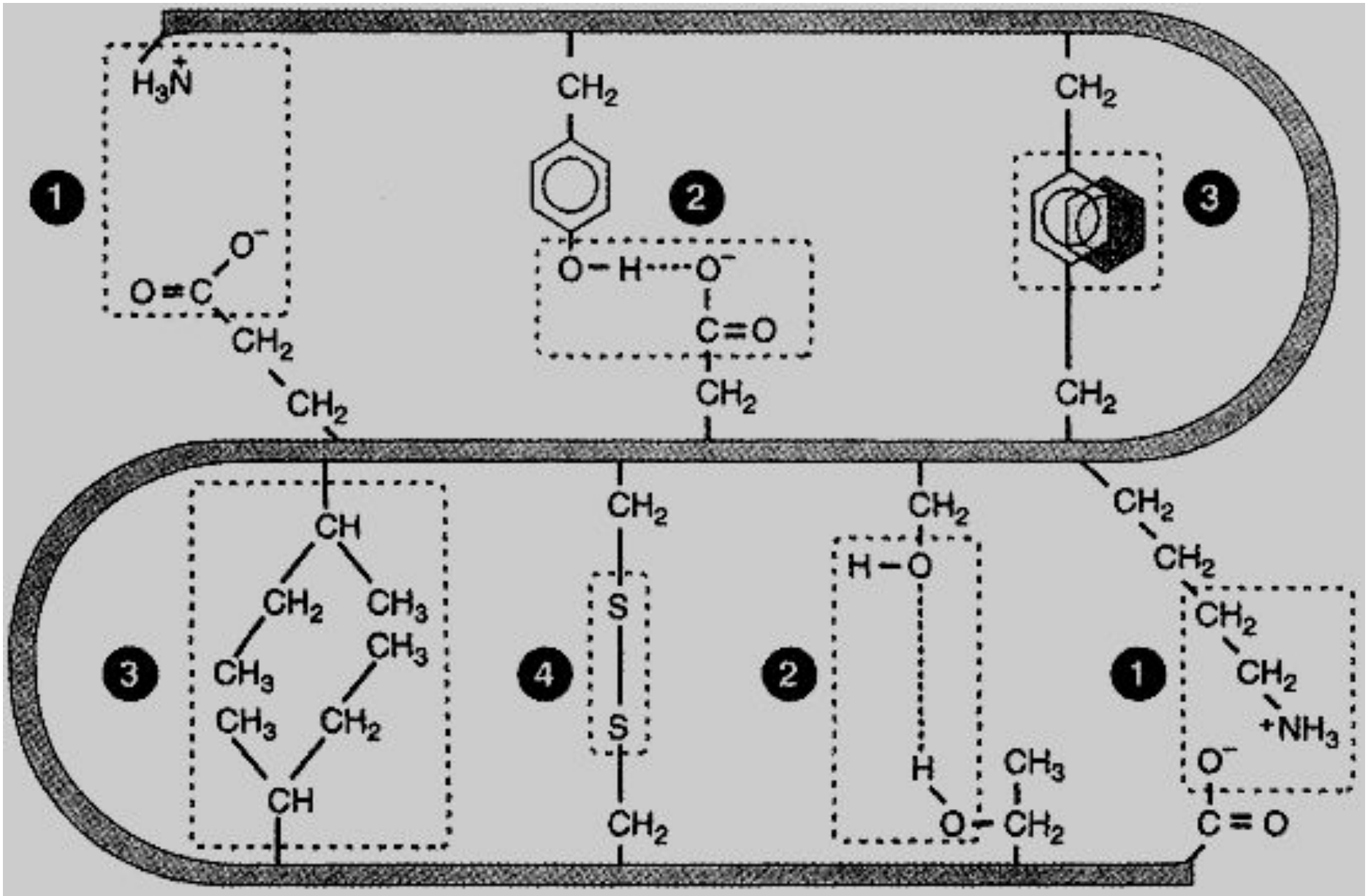
β-pleated sheet



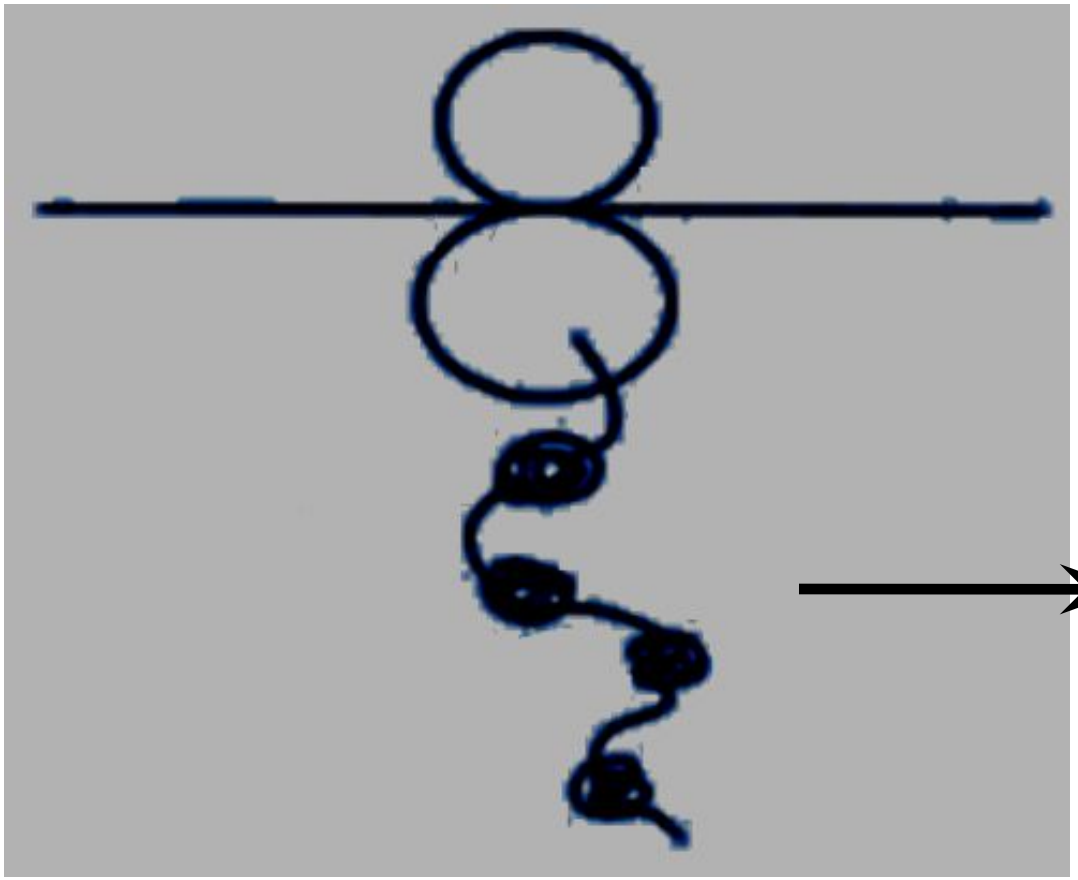
hydrogen bonds

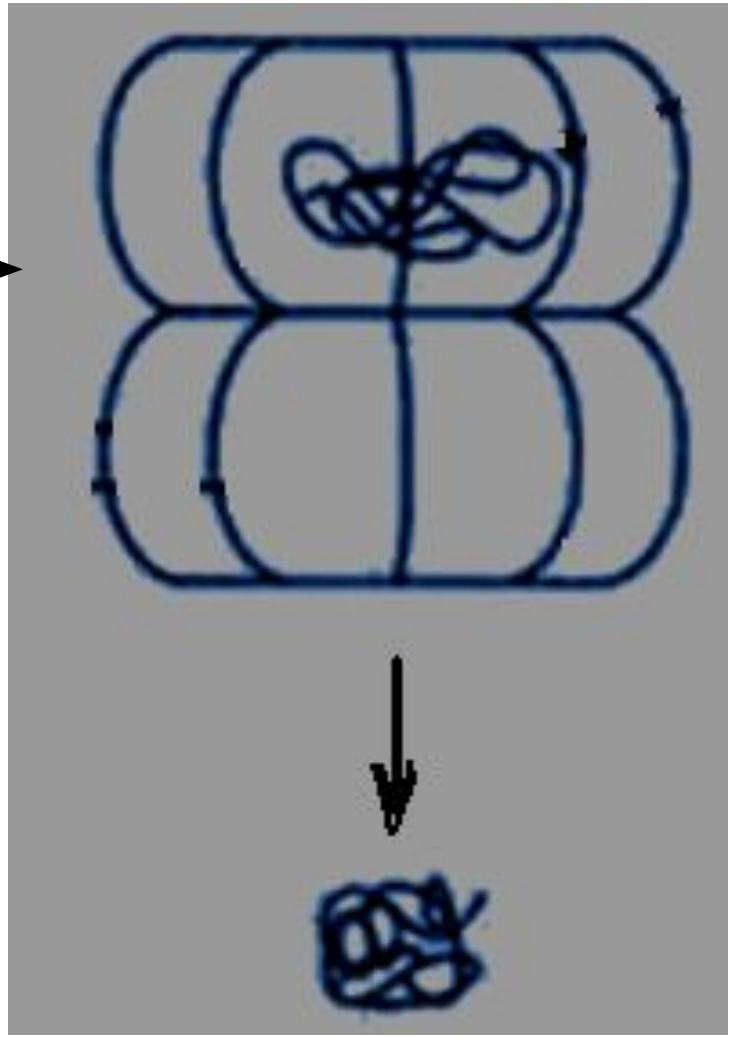
The tertiary structure of myoglobin

Types of bonds between amino acid radicals



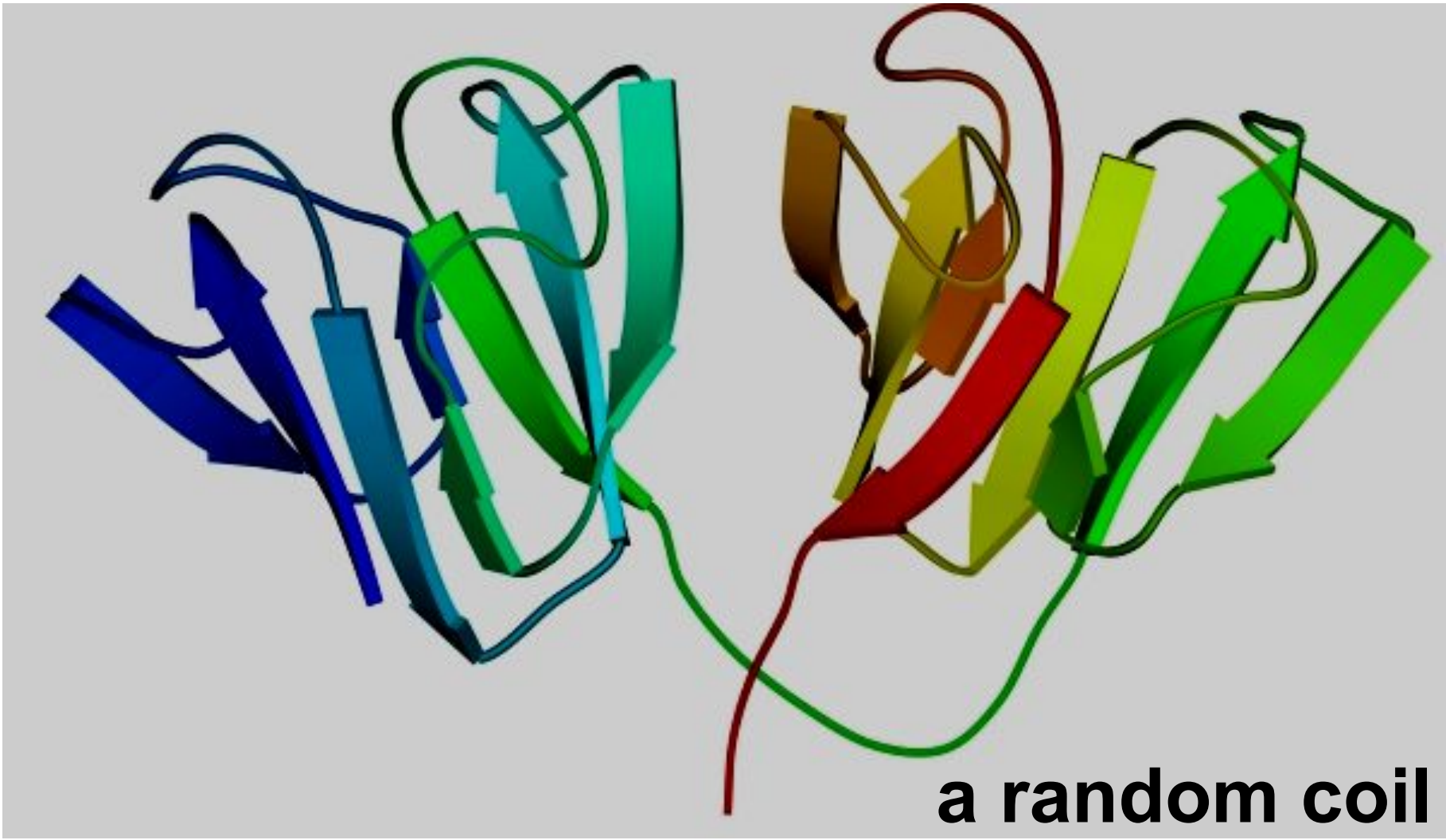
Chaperone



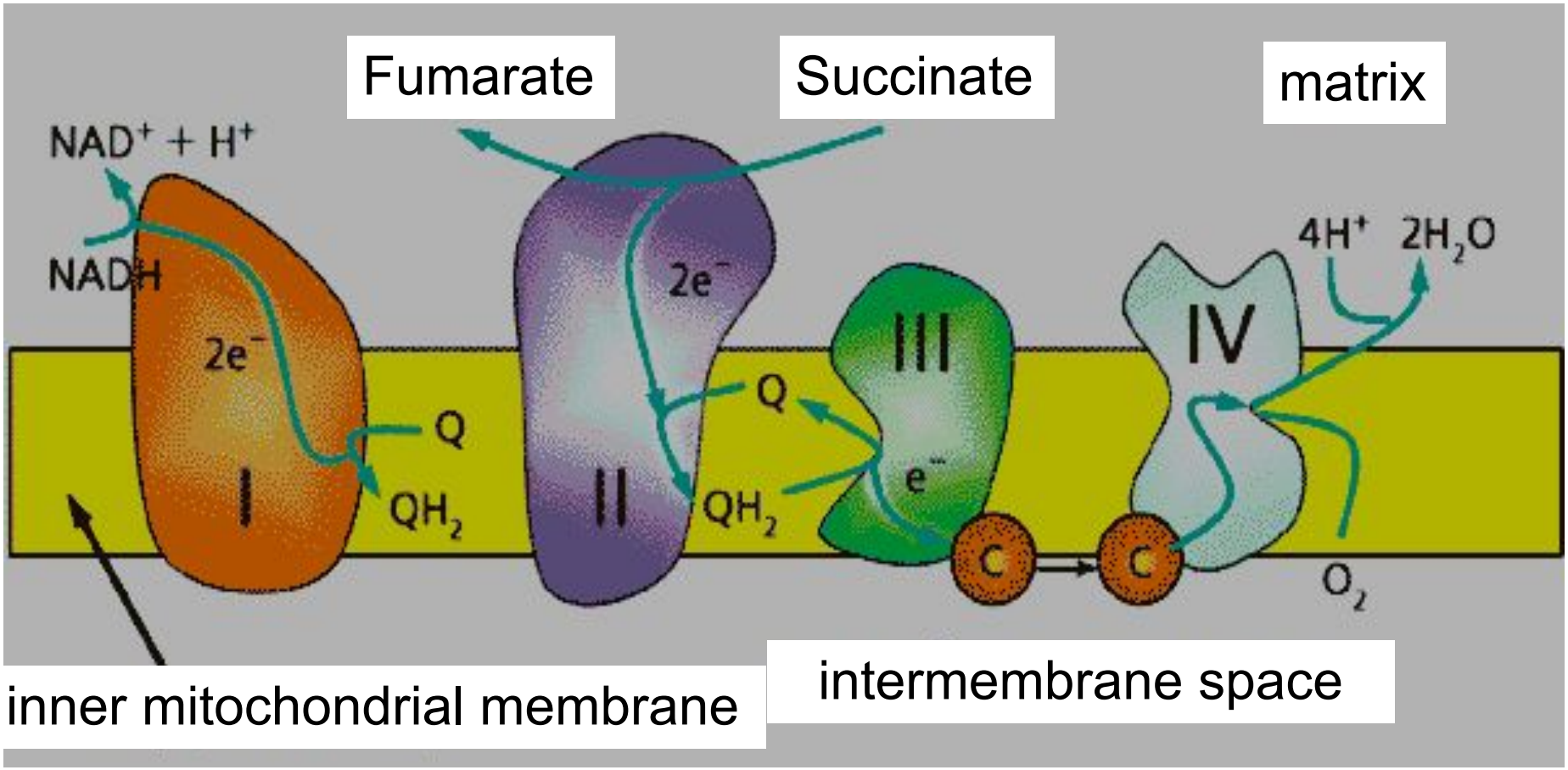


Participation
of chaperones
in protein folding

The globular domains in the g-crystallin
(protein of human's eye lens)



The quaternary structure of hemoglobin

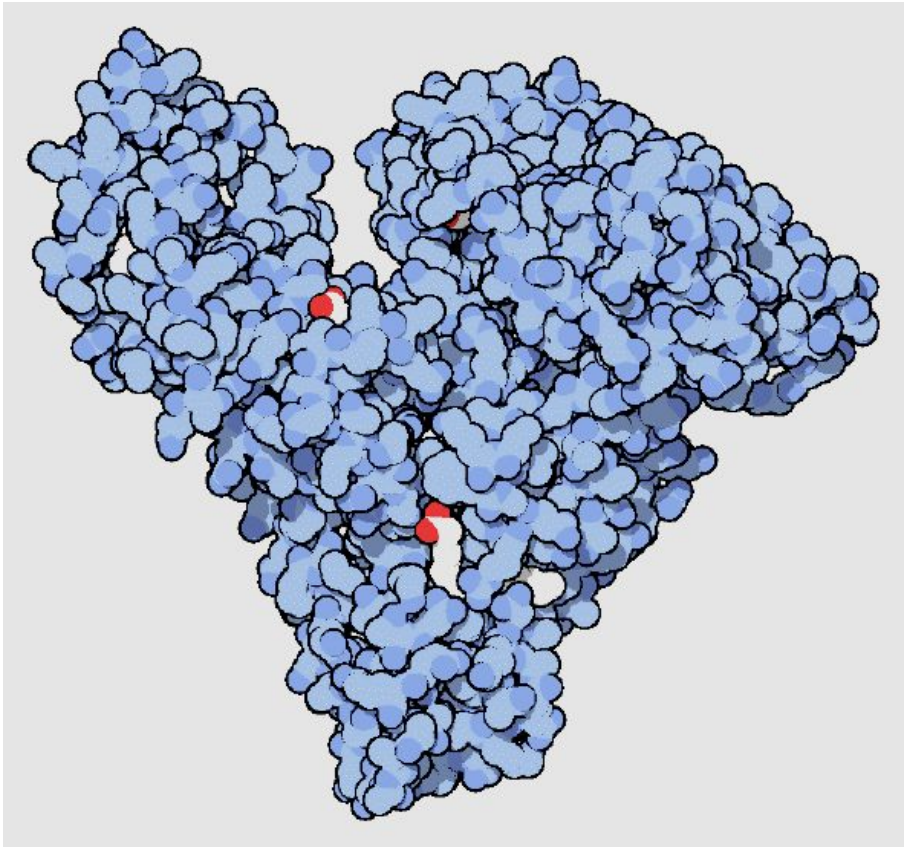


I, II, III and IV – mitochondrial respiratory chain complexes (the electron transport chain)

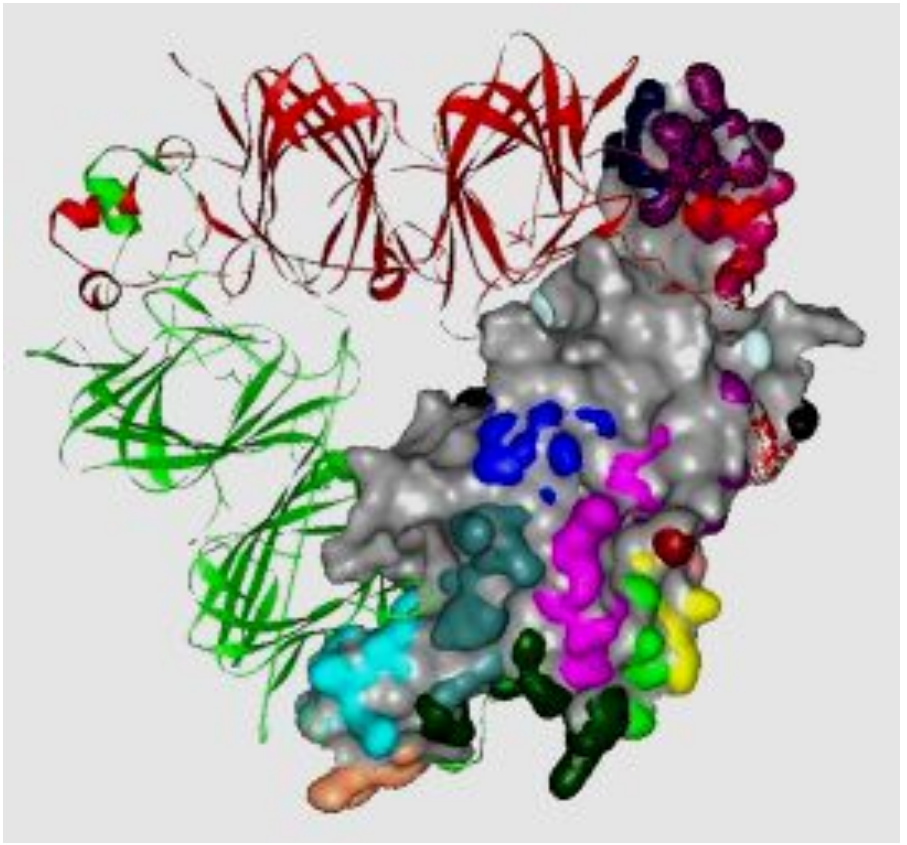
Classification of proteins

Simple proteins

Albumins and globulins

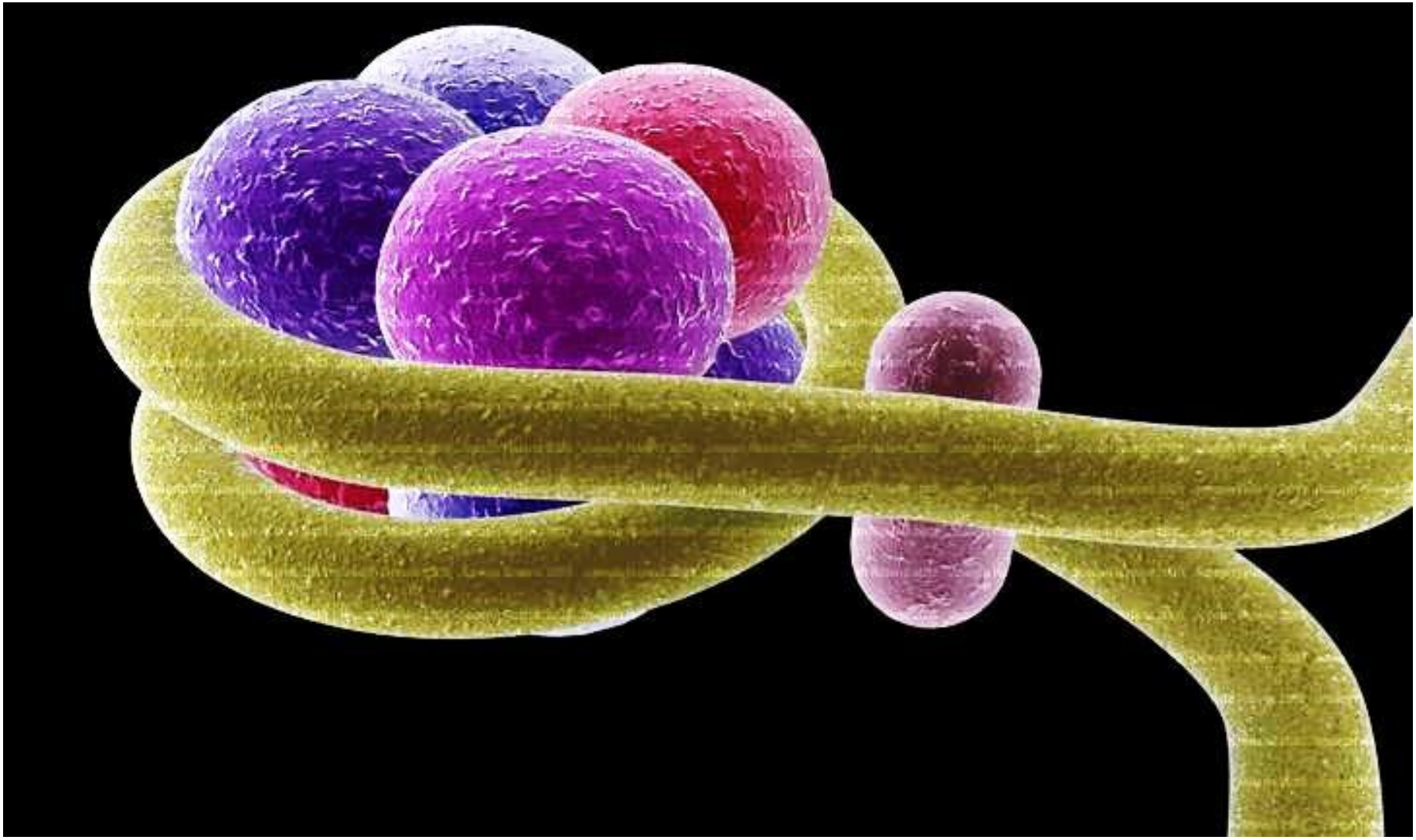


Serum albumin

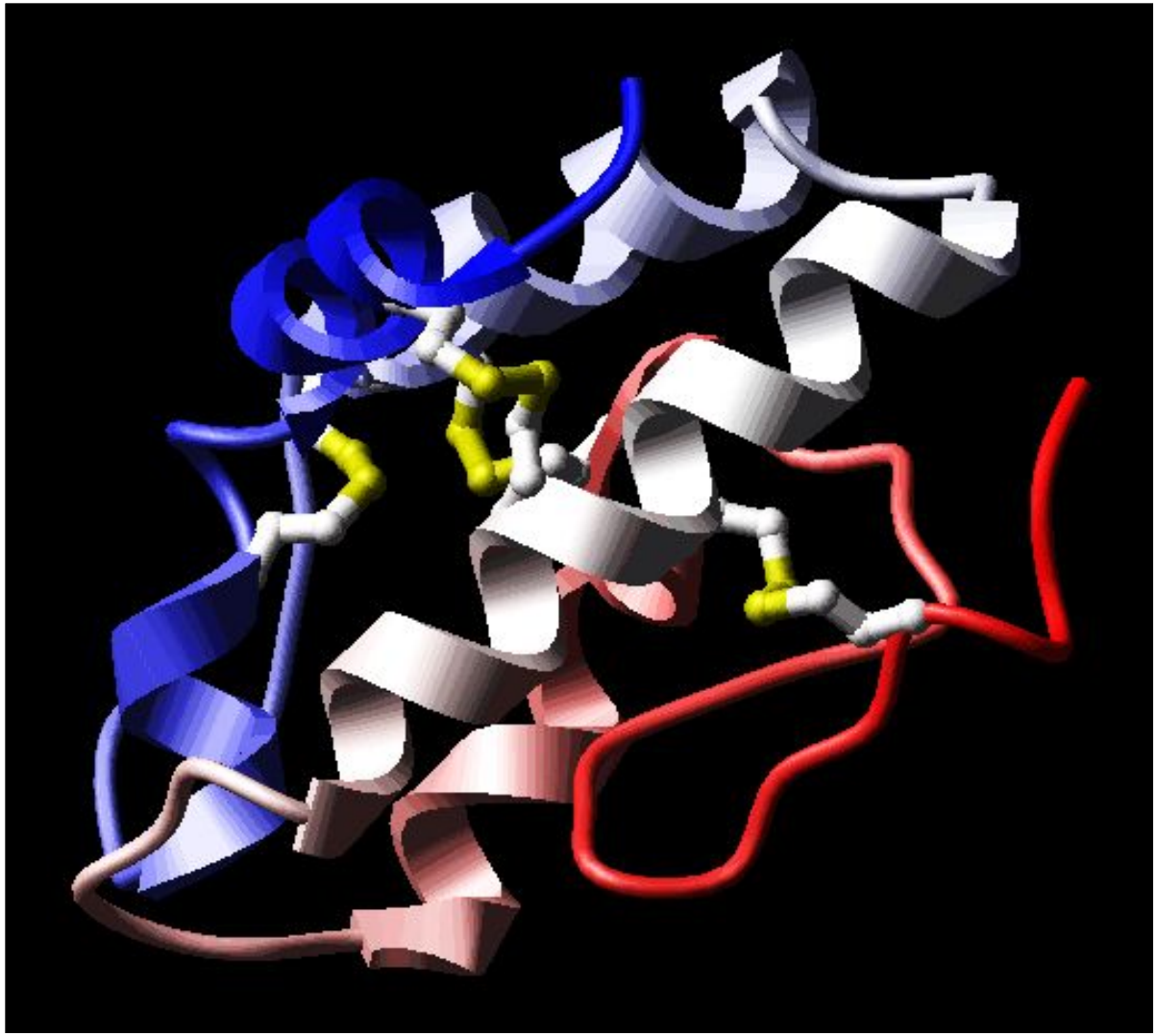


Cashew globulin - a powerful allergen

Hystones and DNA



Prolamin

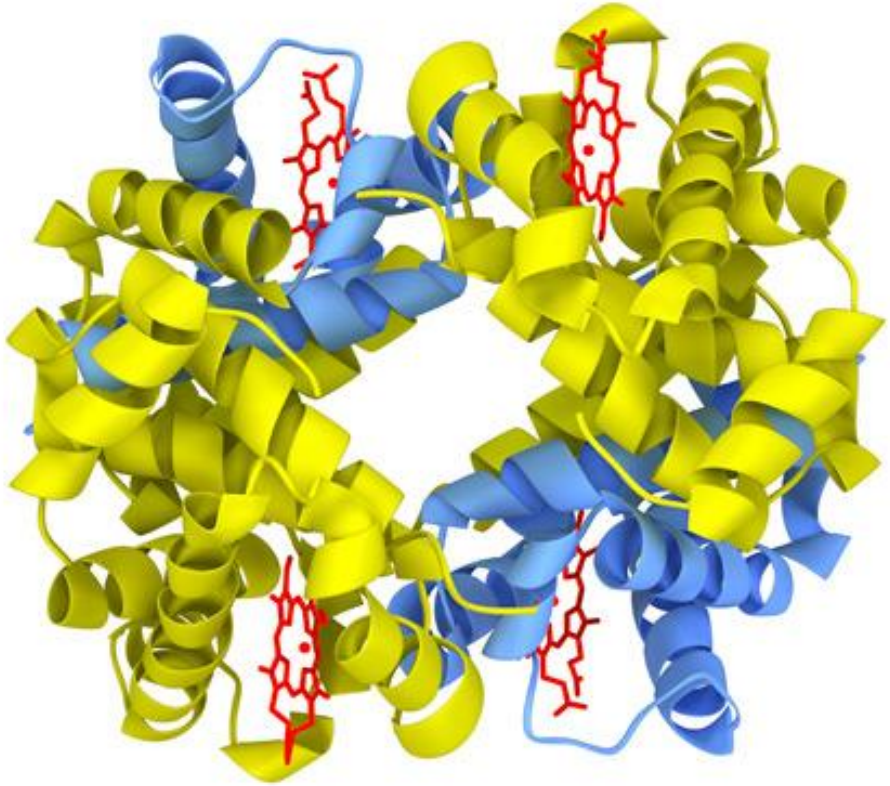


Conjugative proteins

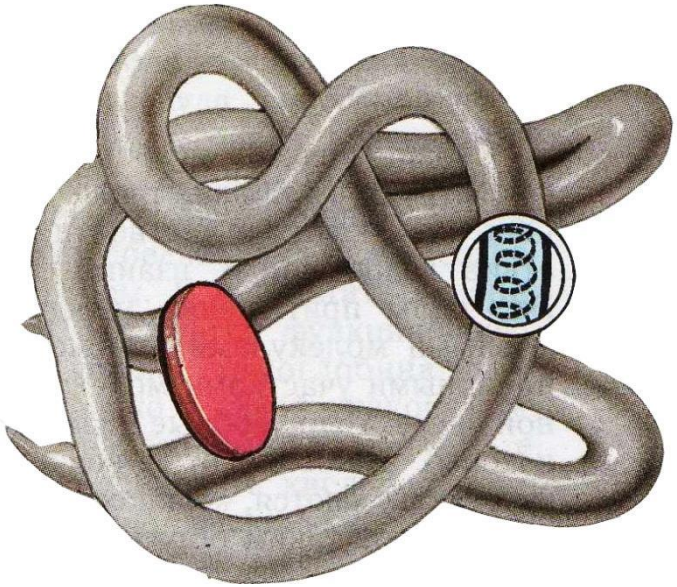
Chromoproteins

Hemoproteins

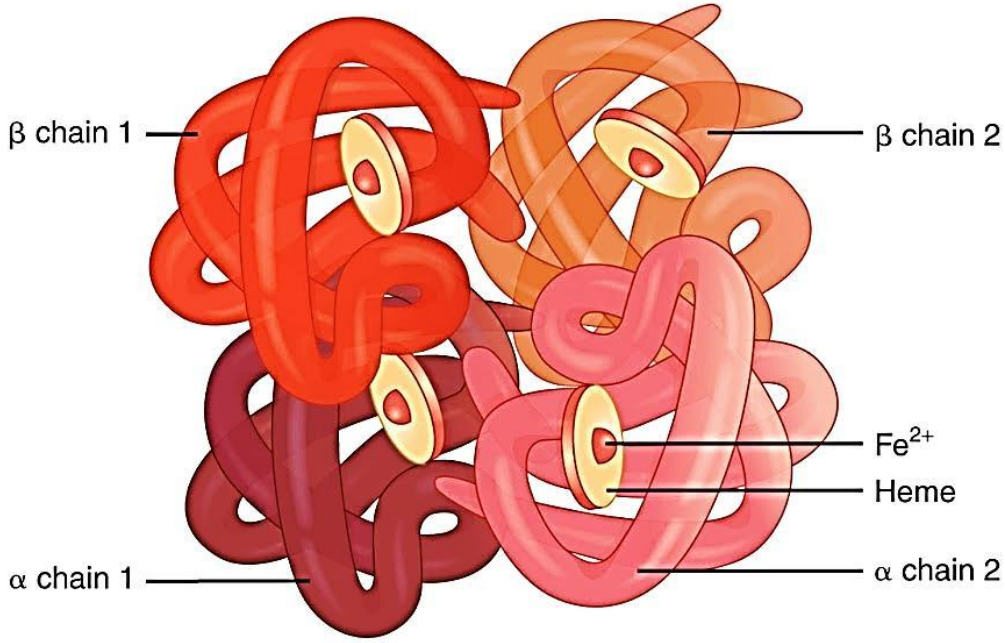
Hemoglobin



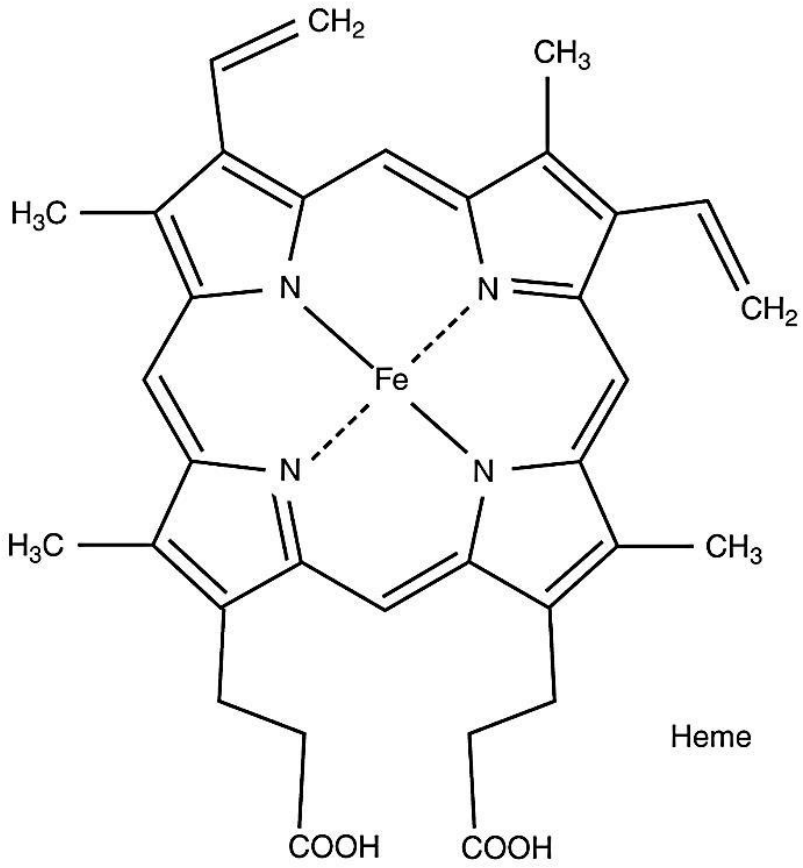
Myoglobin



Hemoglobine structure

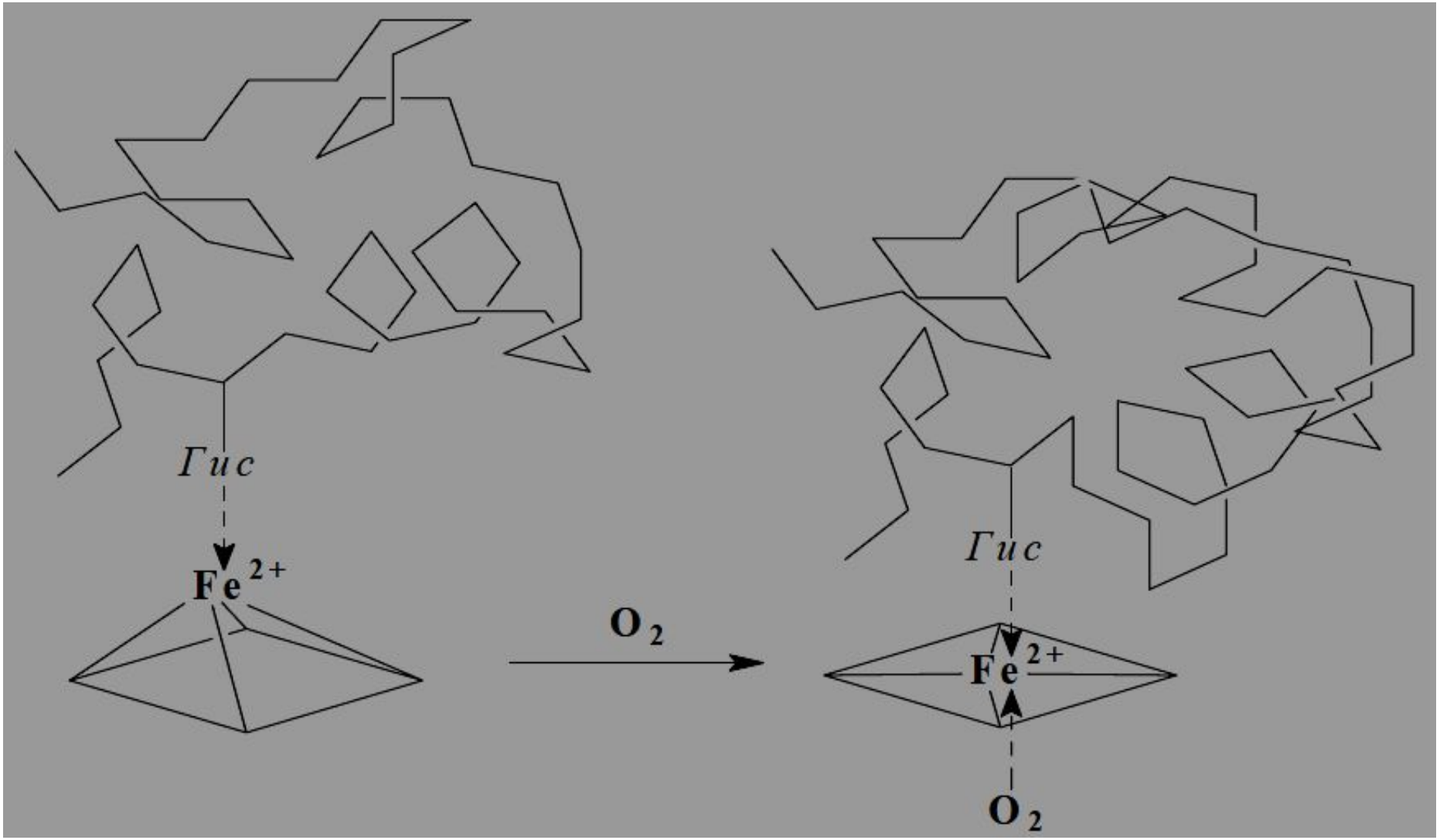


(a)



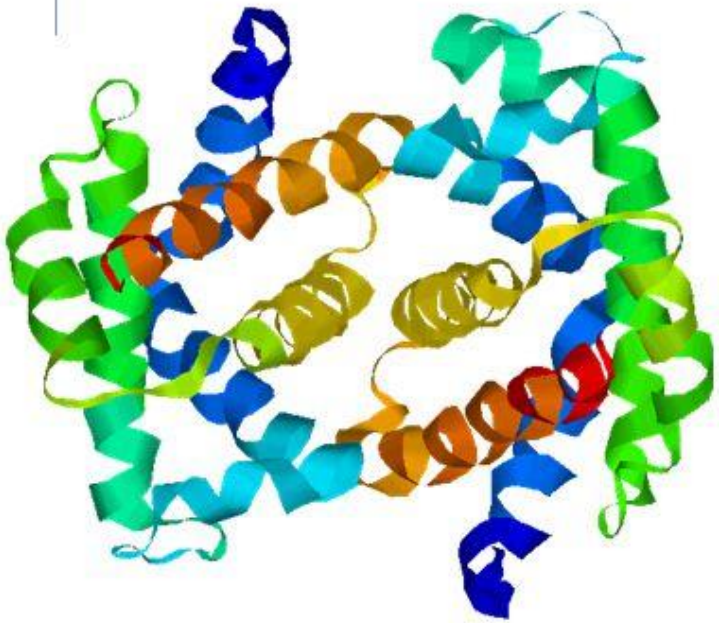
(b)

Bindig of oxygen by hemoglobin

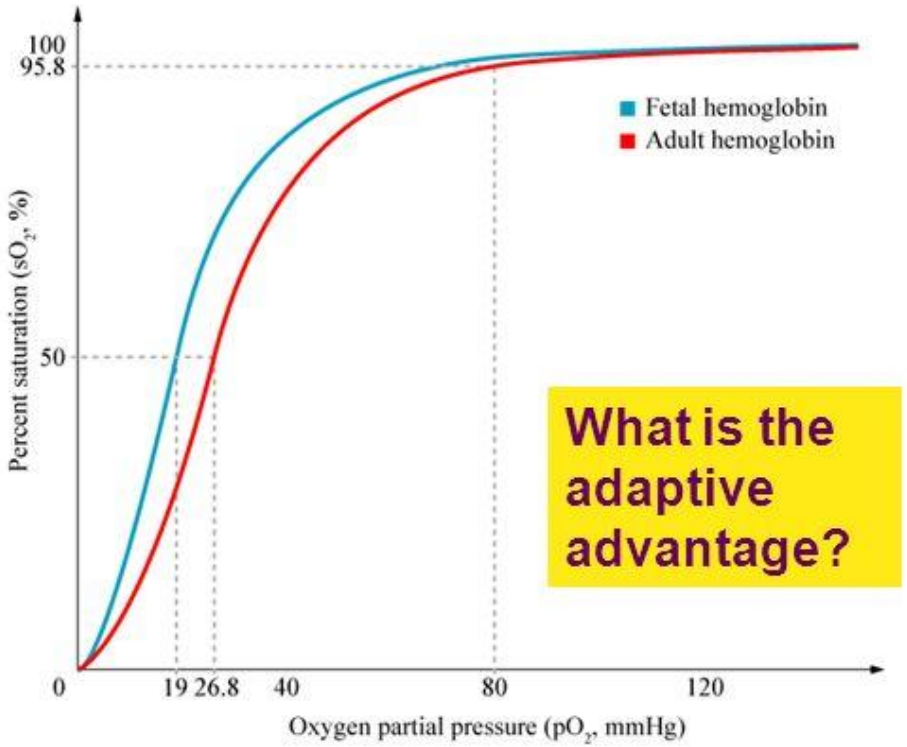


Fetal hemoglobin

- HbF has greater affinity to O_2 than Hb
  - low O_2% by time blood reaches placenta
  - fetal Hb must be able to bind O_2 with greater attraction than maternal Hb

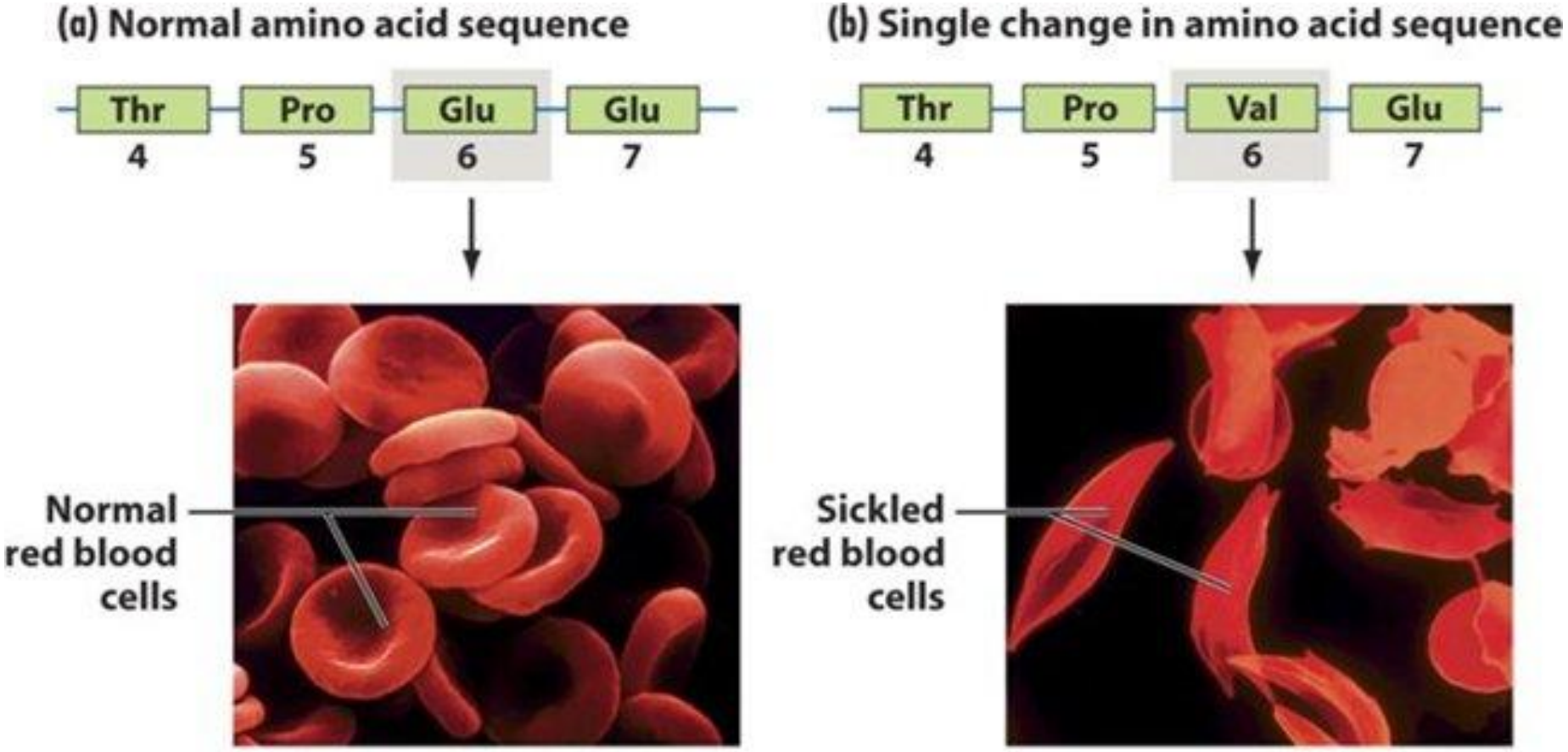


2 alpha & 2 gamma units

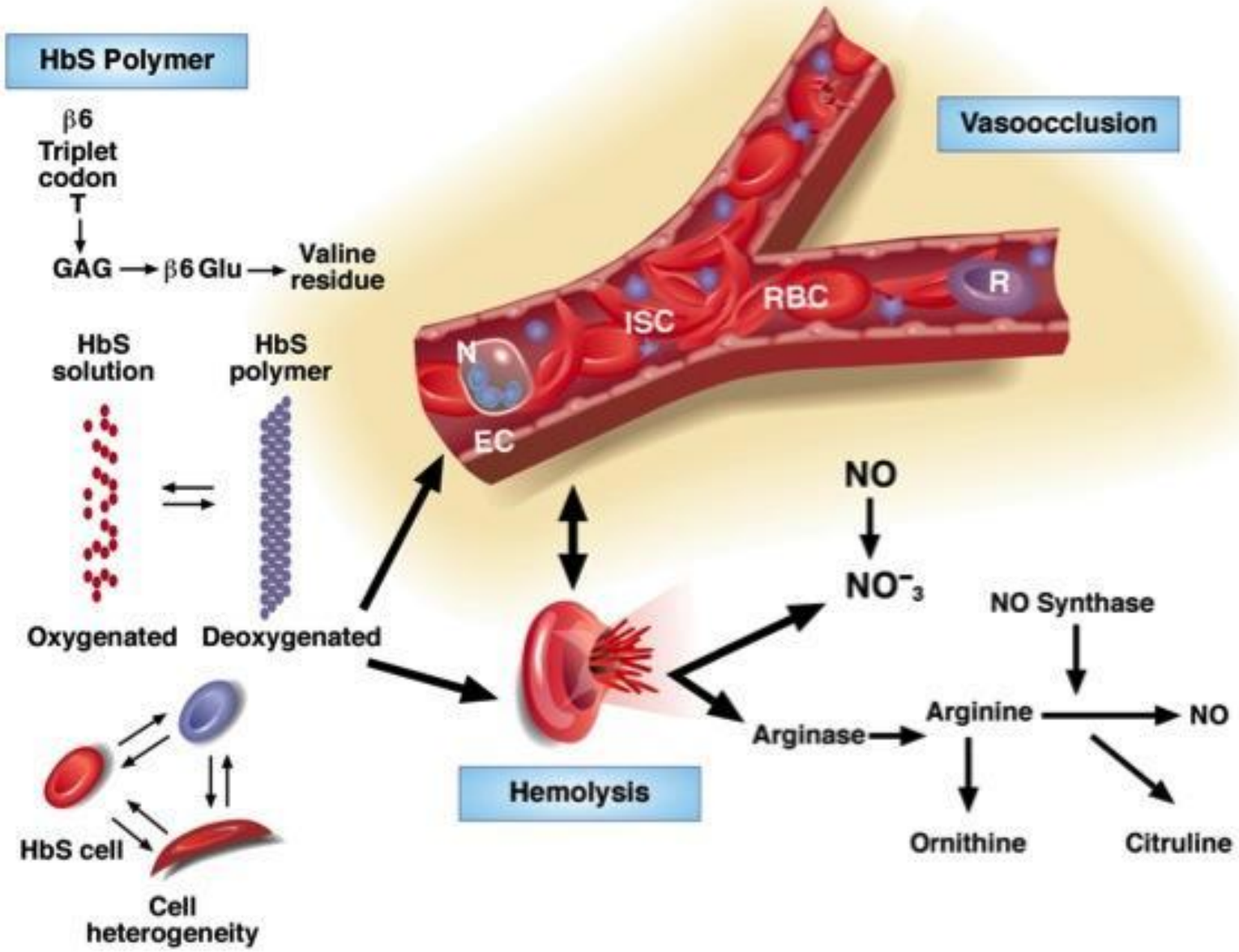


What is the adaptive advantage?

Hemoglobinopathies
Sickle cell anemia



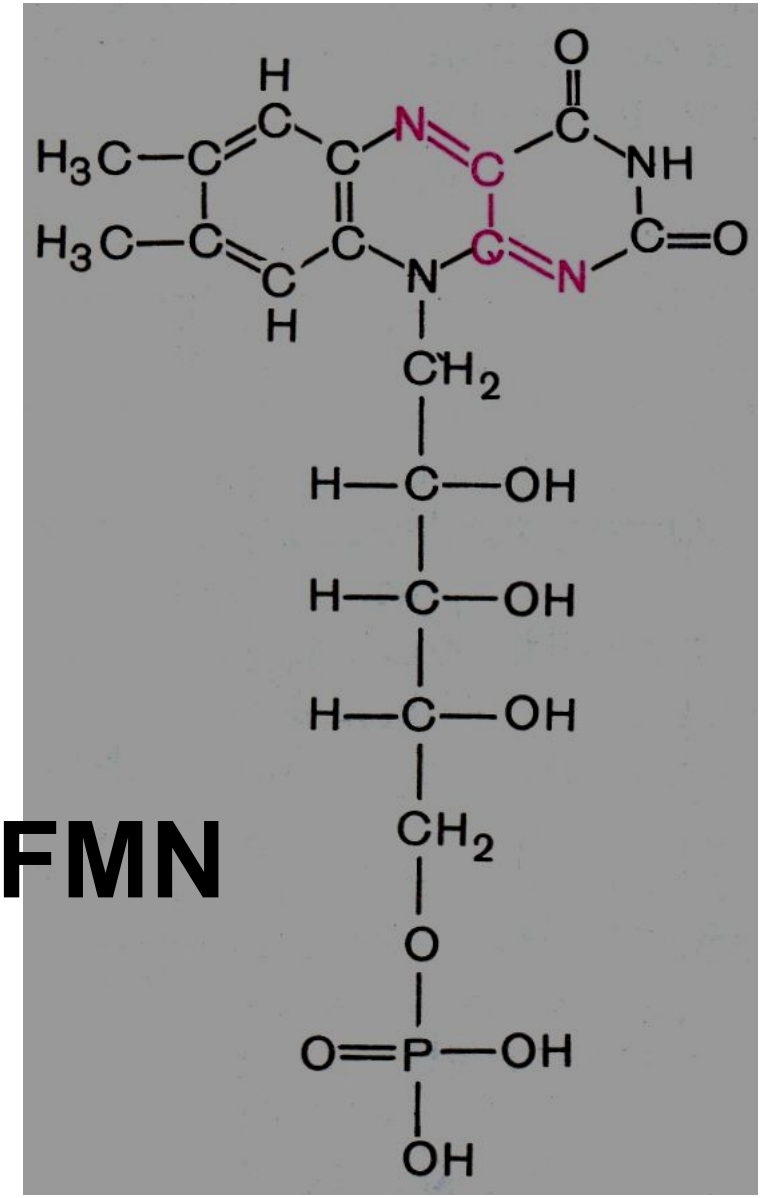
Pathophysiology of Sickle Cell Disease



Abnormal hemoglobins

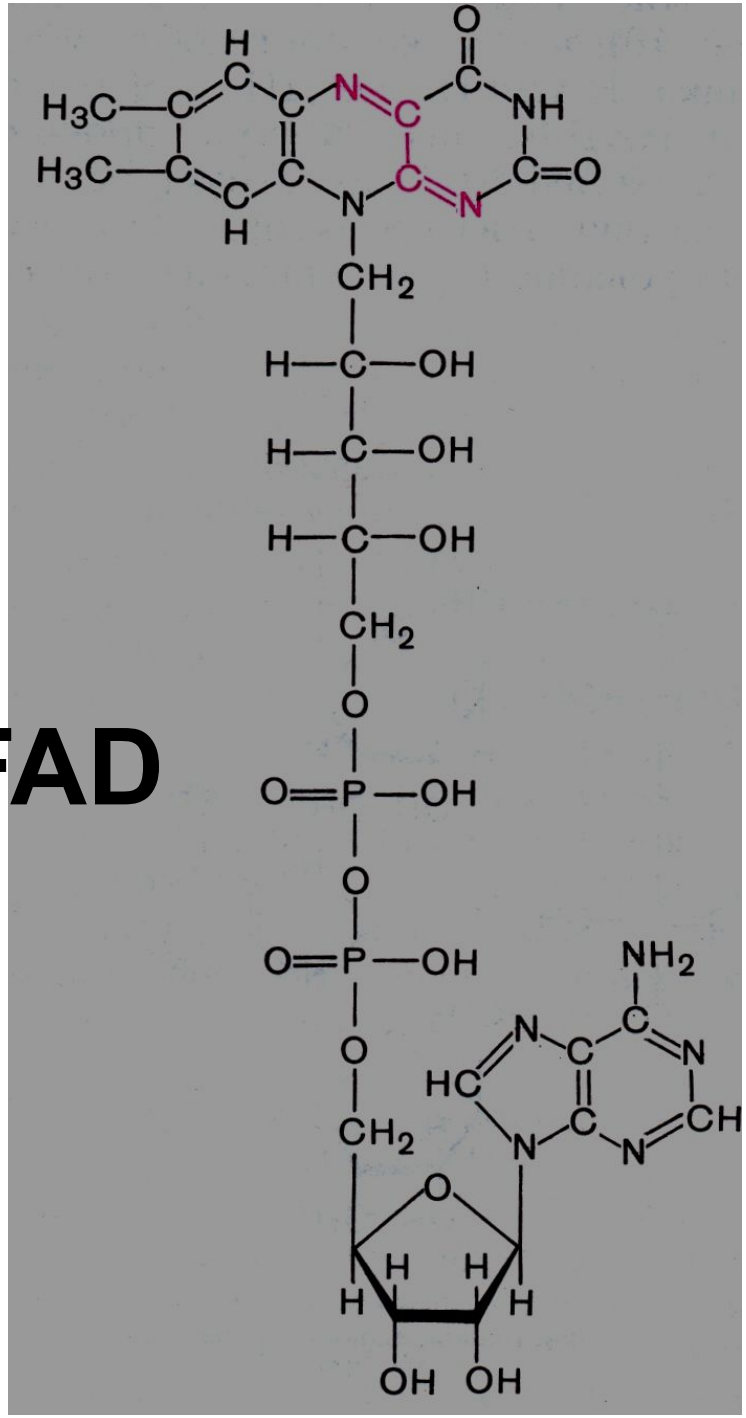
Type	Compo-si tion	Norm	Replace-ment
HbC	$\alpha_2\beta_2$	Gly 6 in β	Lis
HbD	$\alpha_2\beta_2$	Ley 28 in β	Gly
HbH	β_4		

Flavoproteins

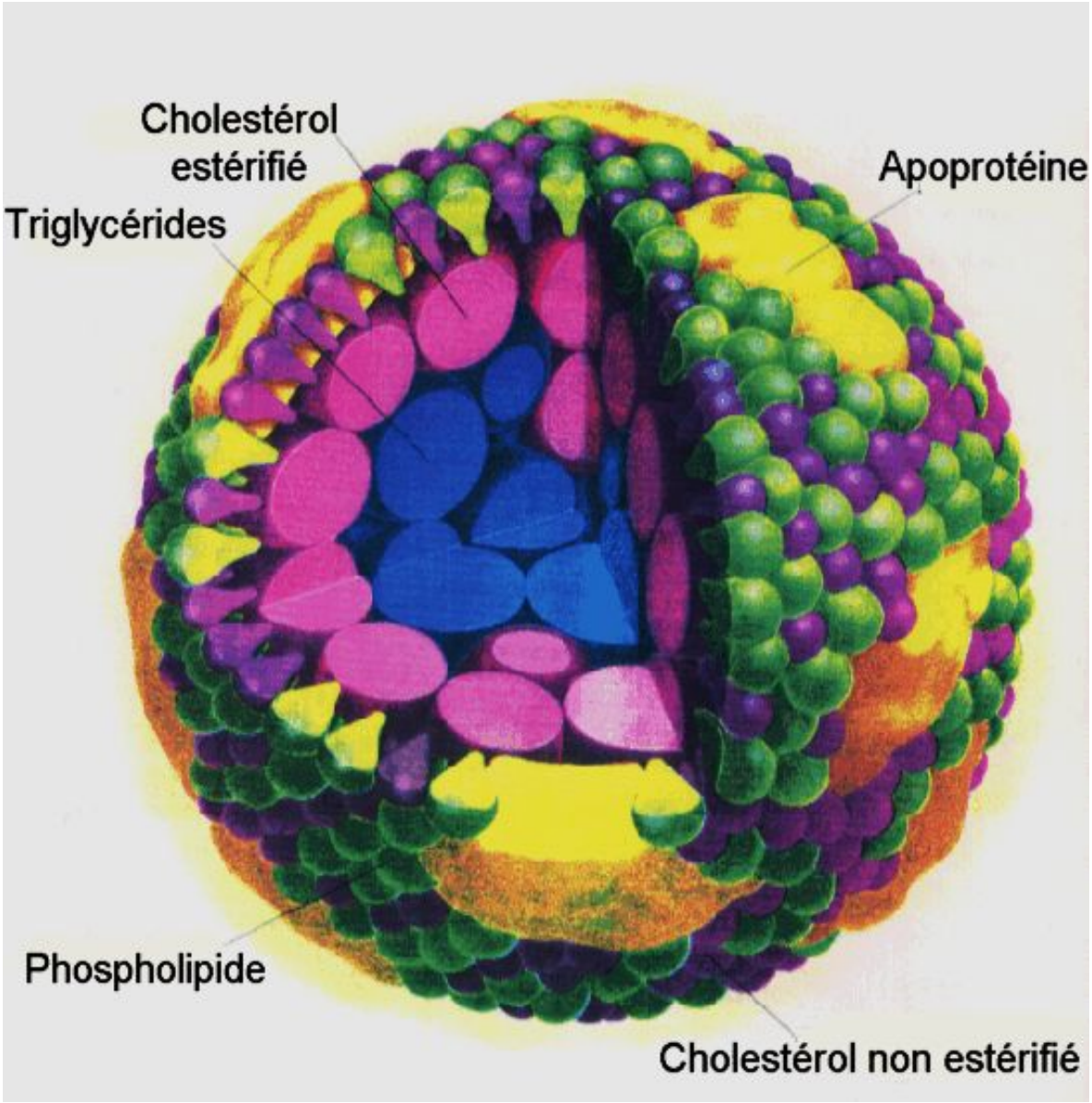


FMN

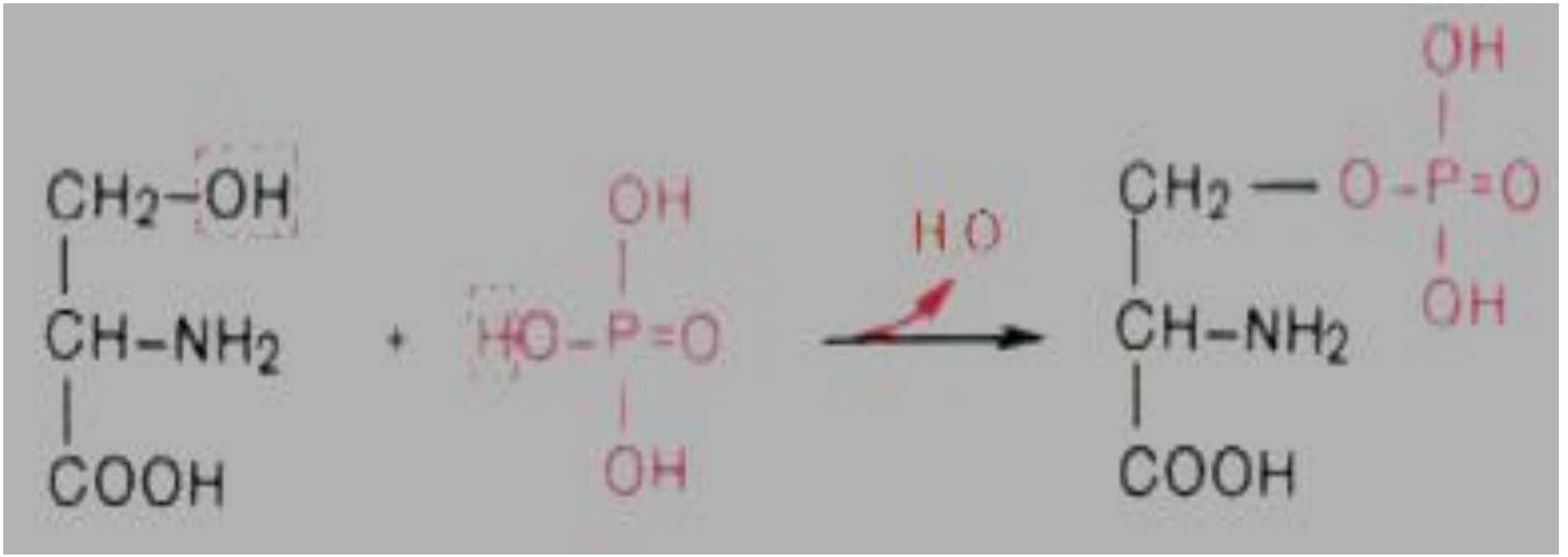
FAD



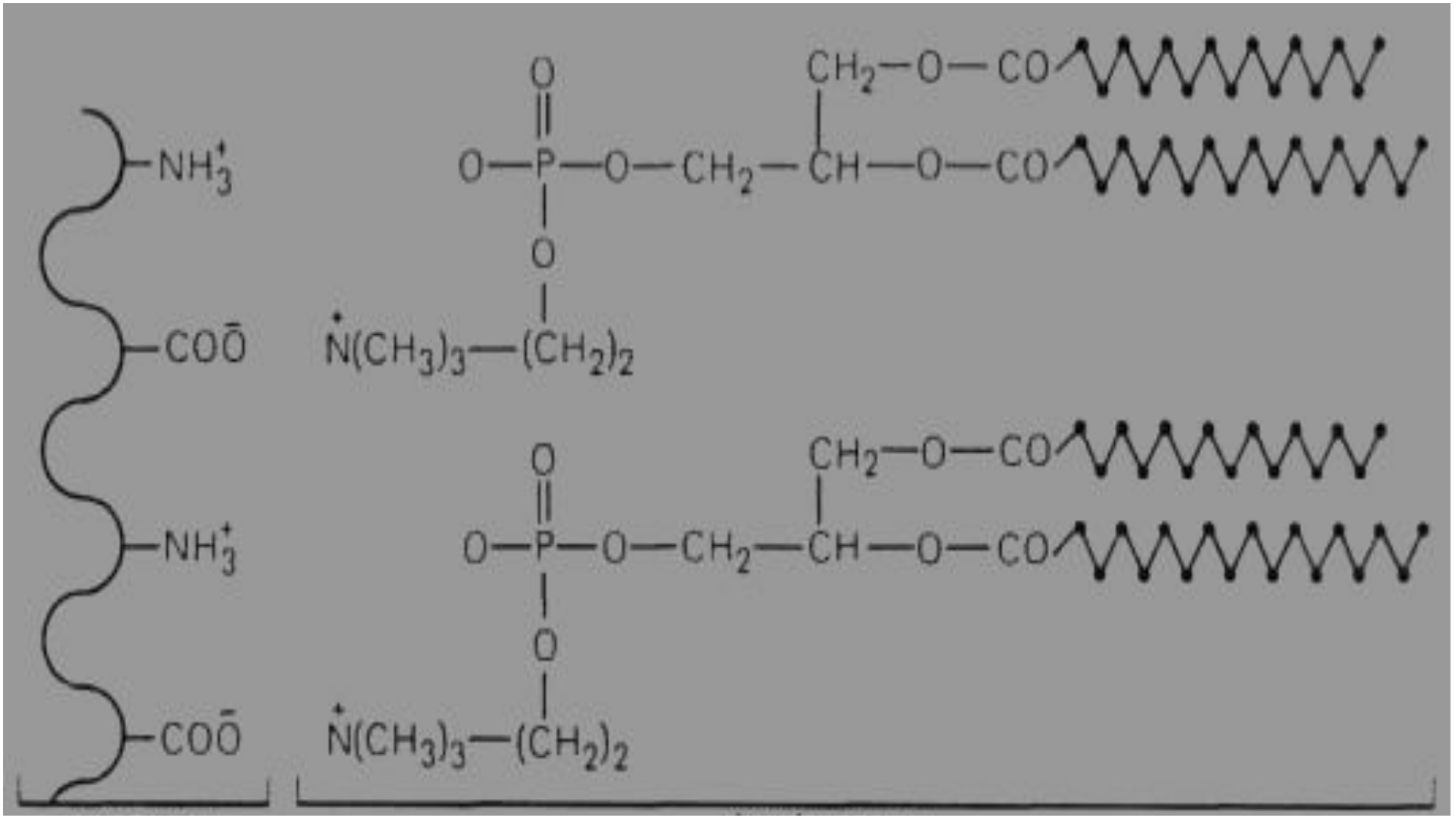
Lipoprotein structure



Covalent bond formation in phosphoprotein

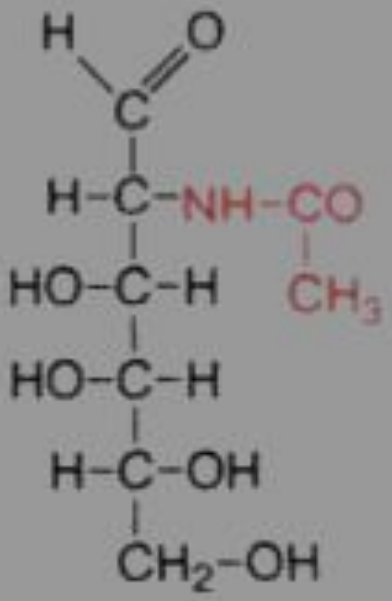


Ionic bond formation in phosphoprotein

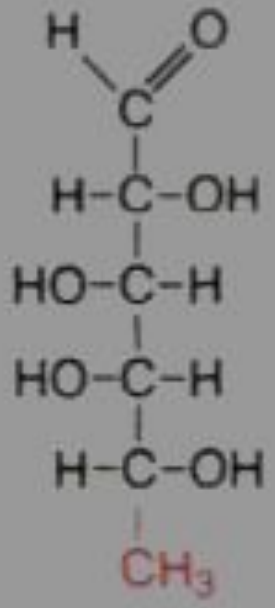


Glycoproteins

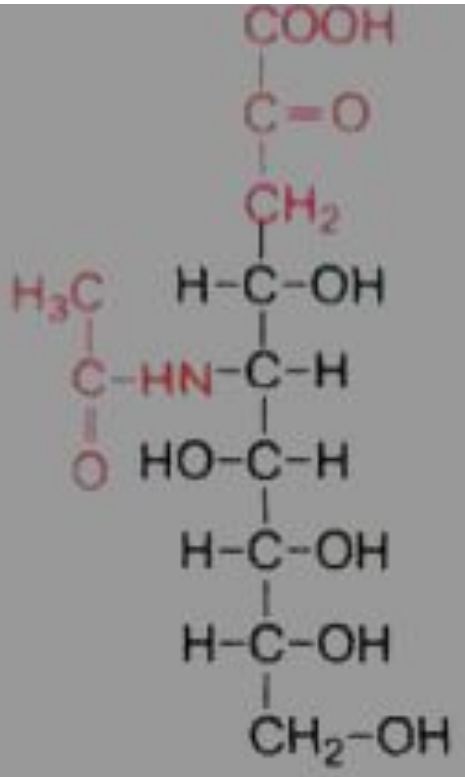
Terminal carbohydrate



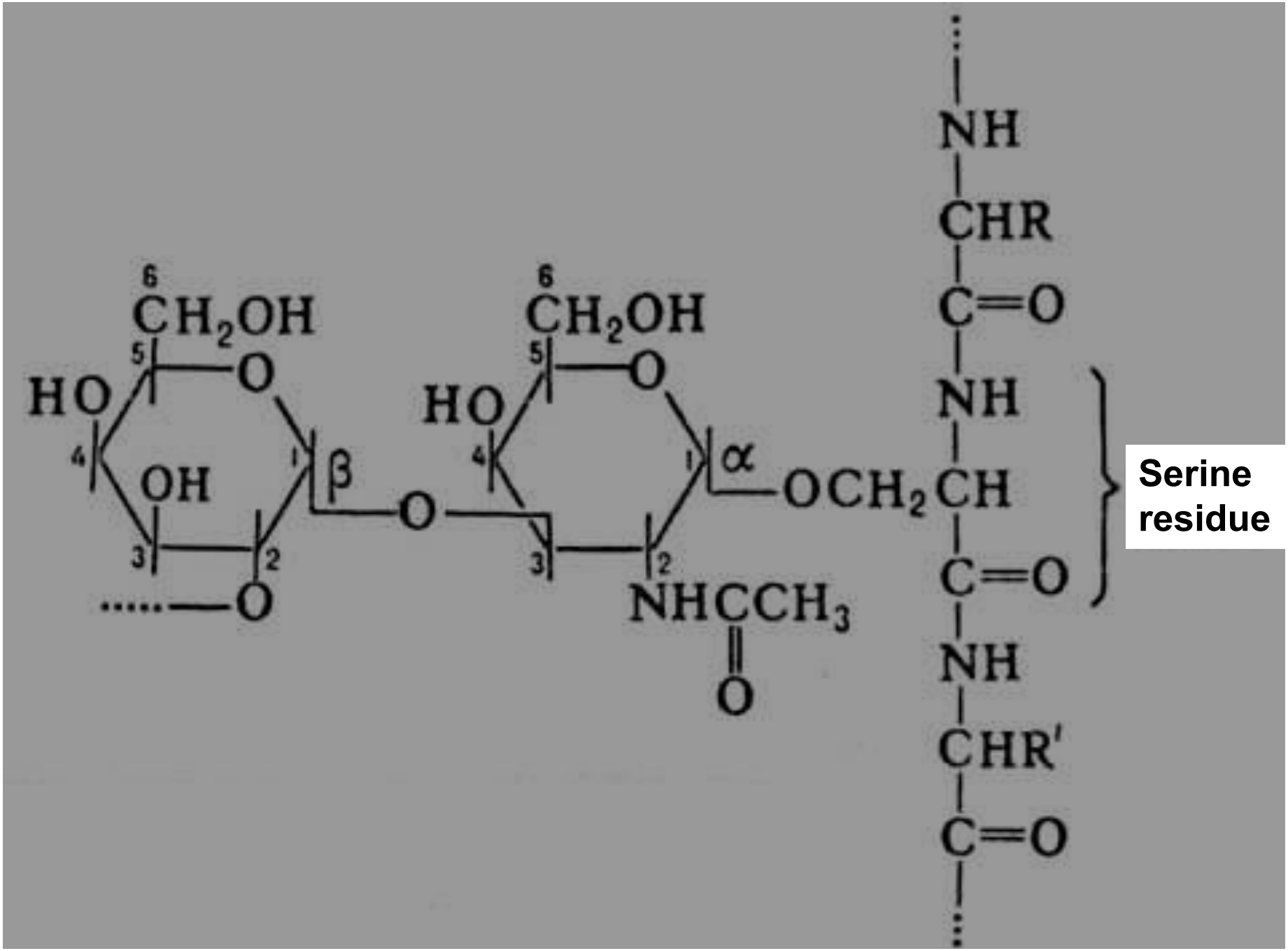
N-acetylgalactosamine

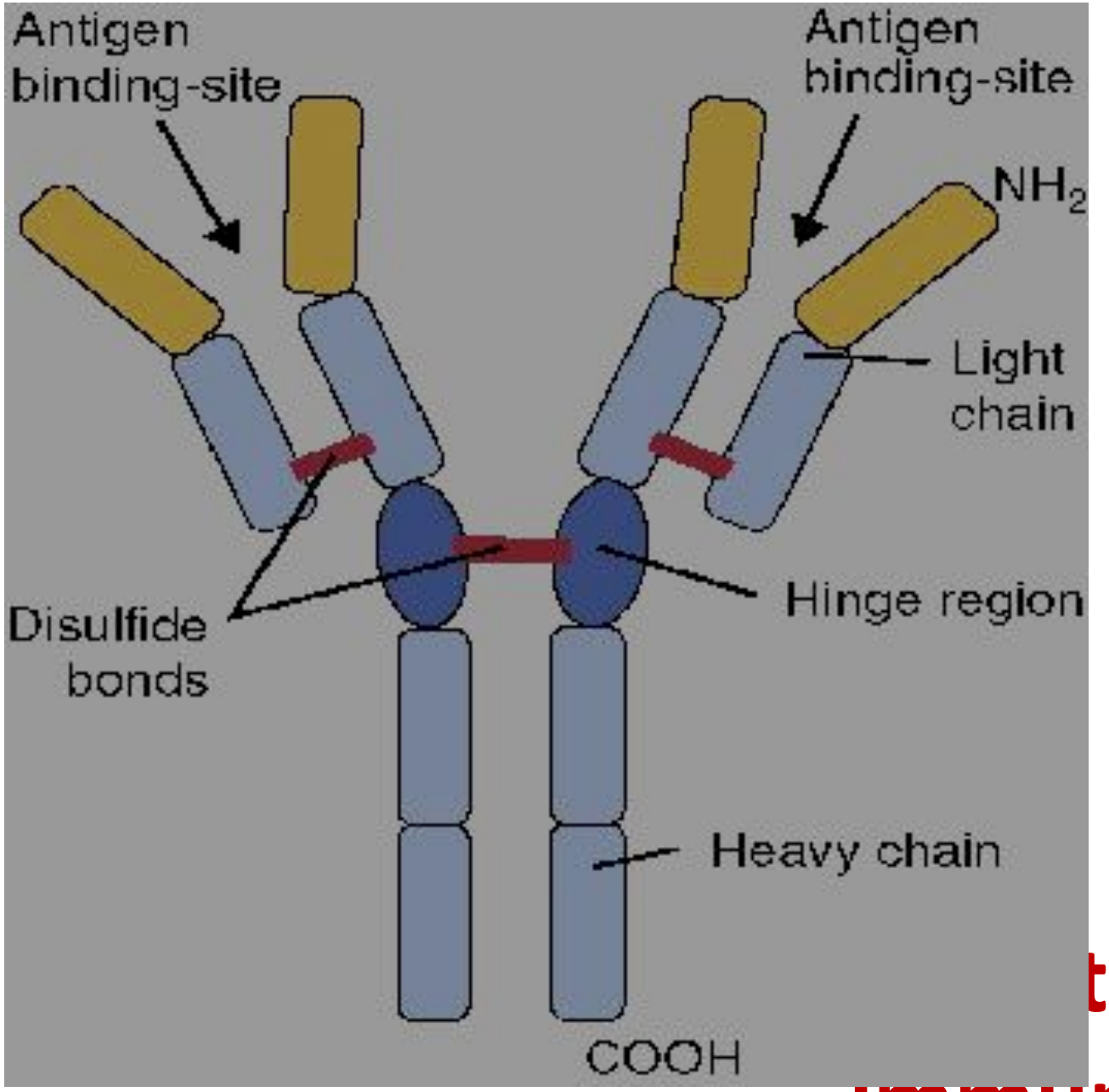


fucose



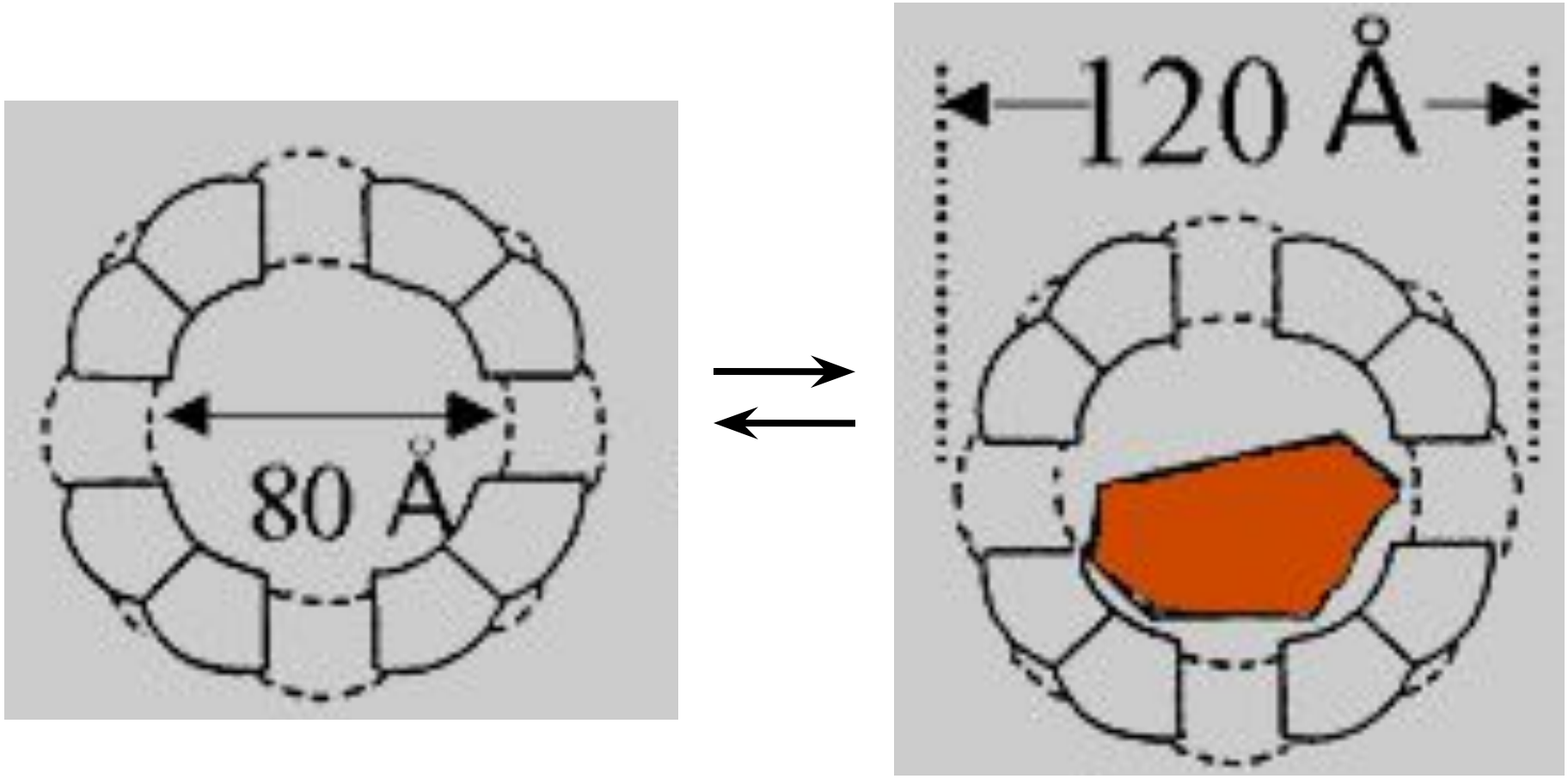
sialic acid


Serine residue



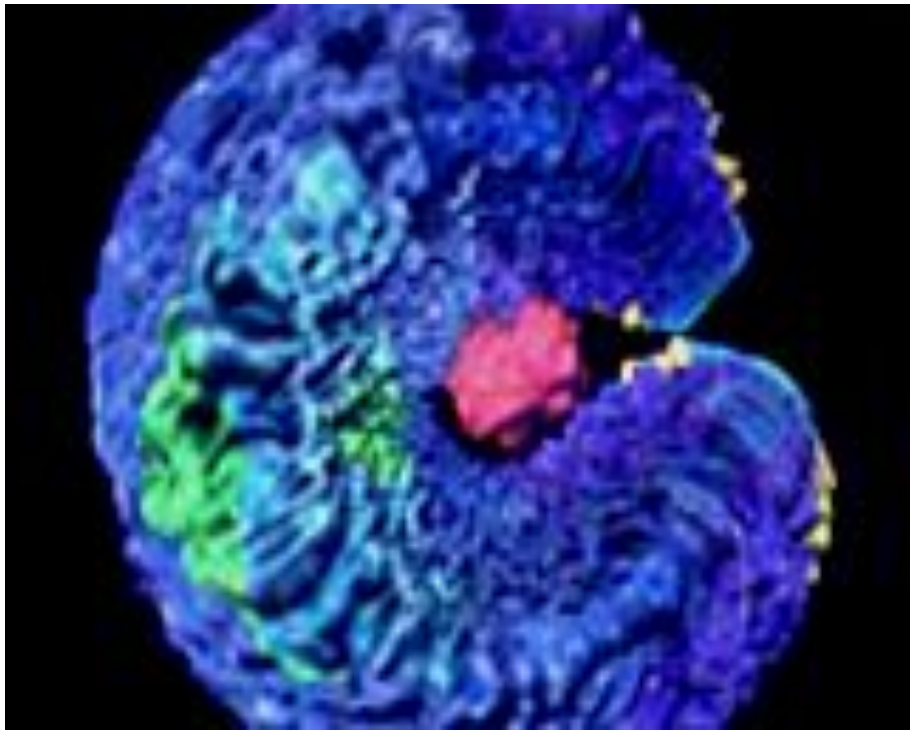
tructure of immunoglobulin

Metalloproteins

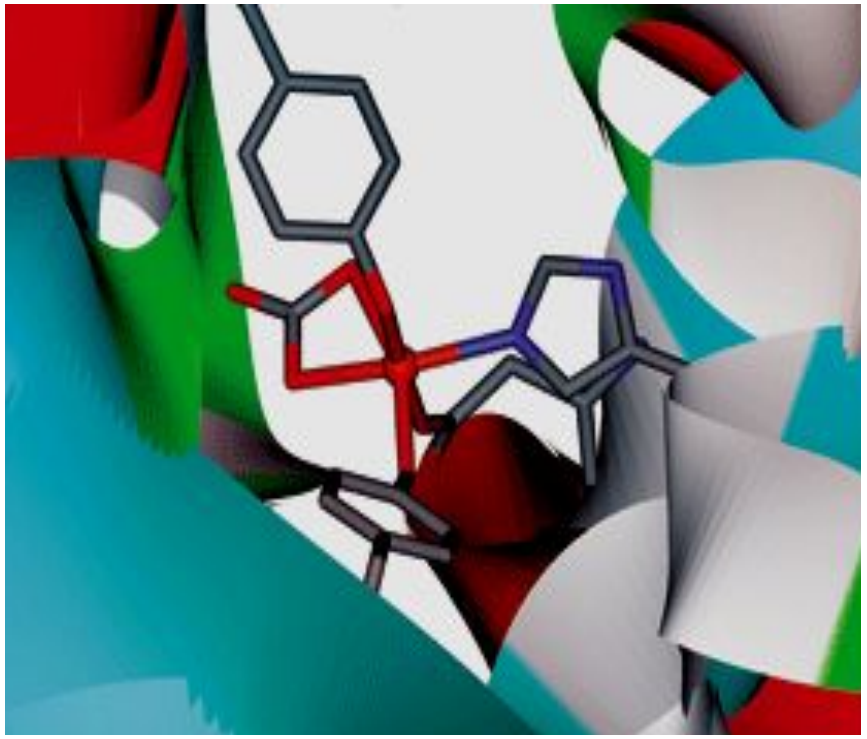


Apoferritin **Ferritin**

Metalloproteins



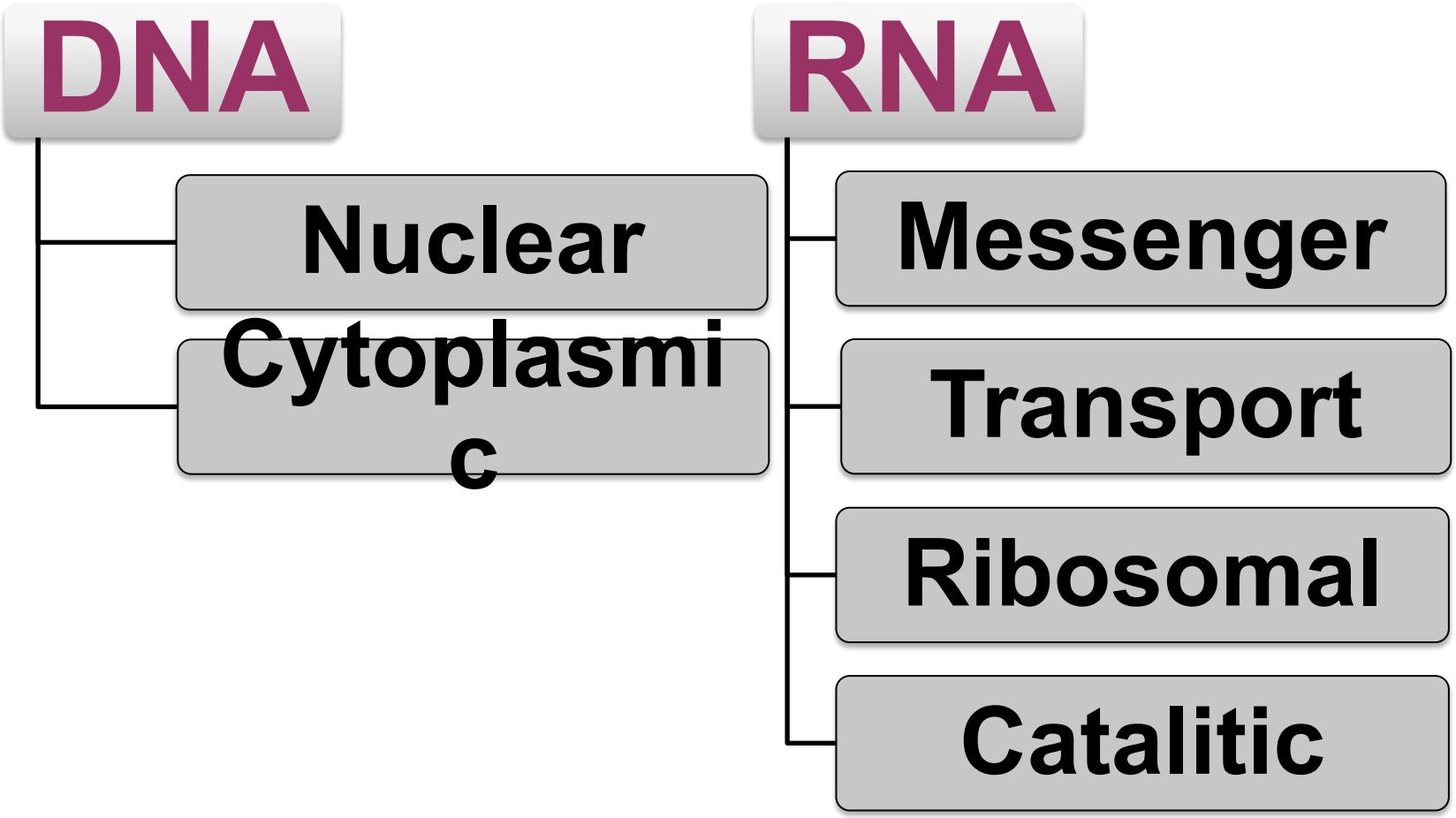
Transferrin



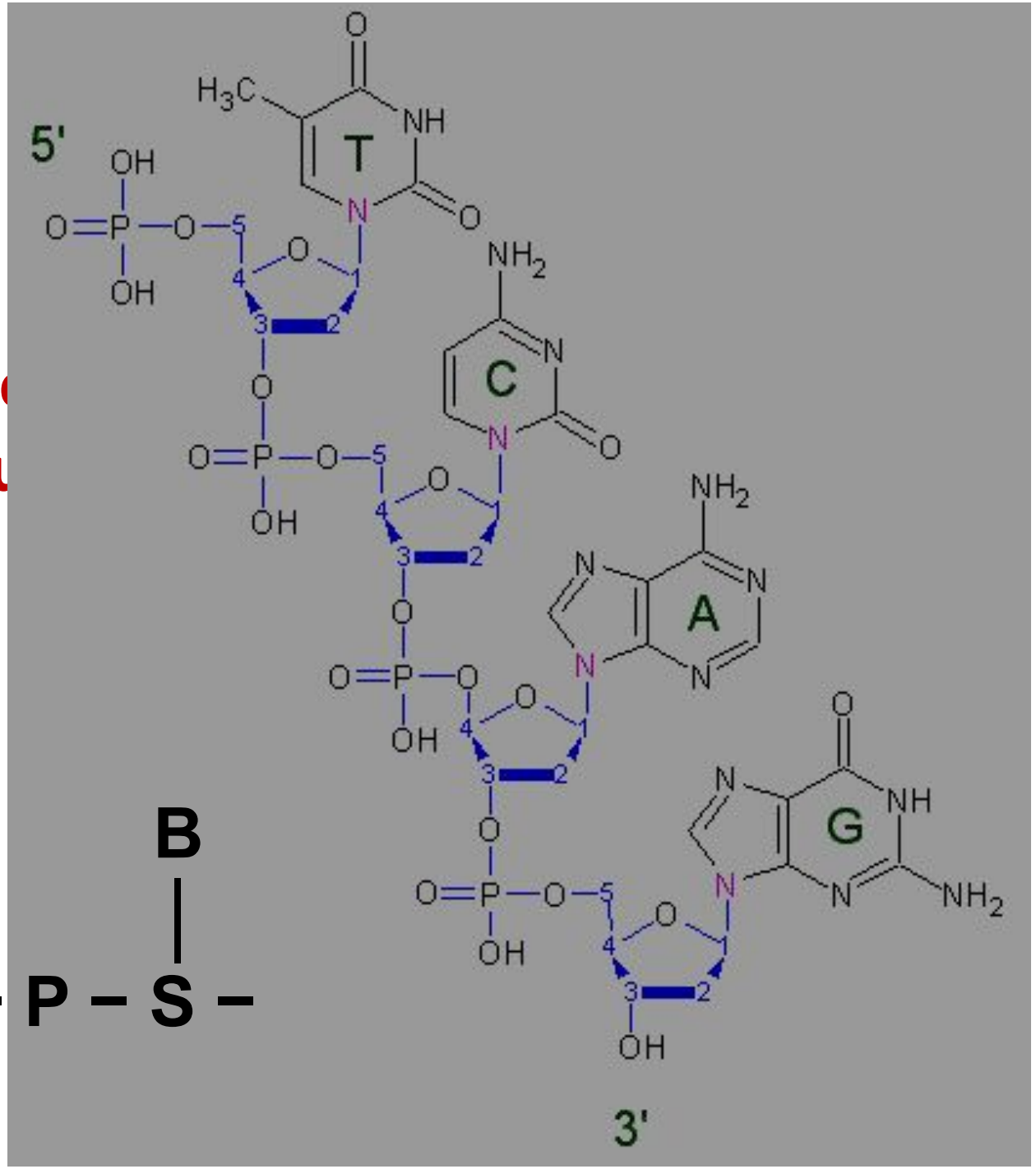
Linking center in transferrin

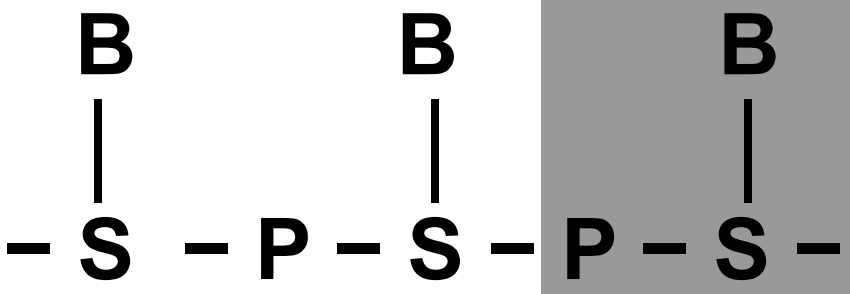
Nucleoproteins

Nucleic Acids



DNA polynucle
chain structu





Chargaff's rules

A = T, G = C

A + G = C + T

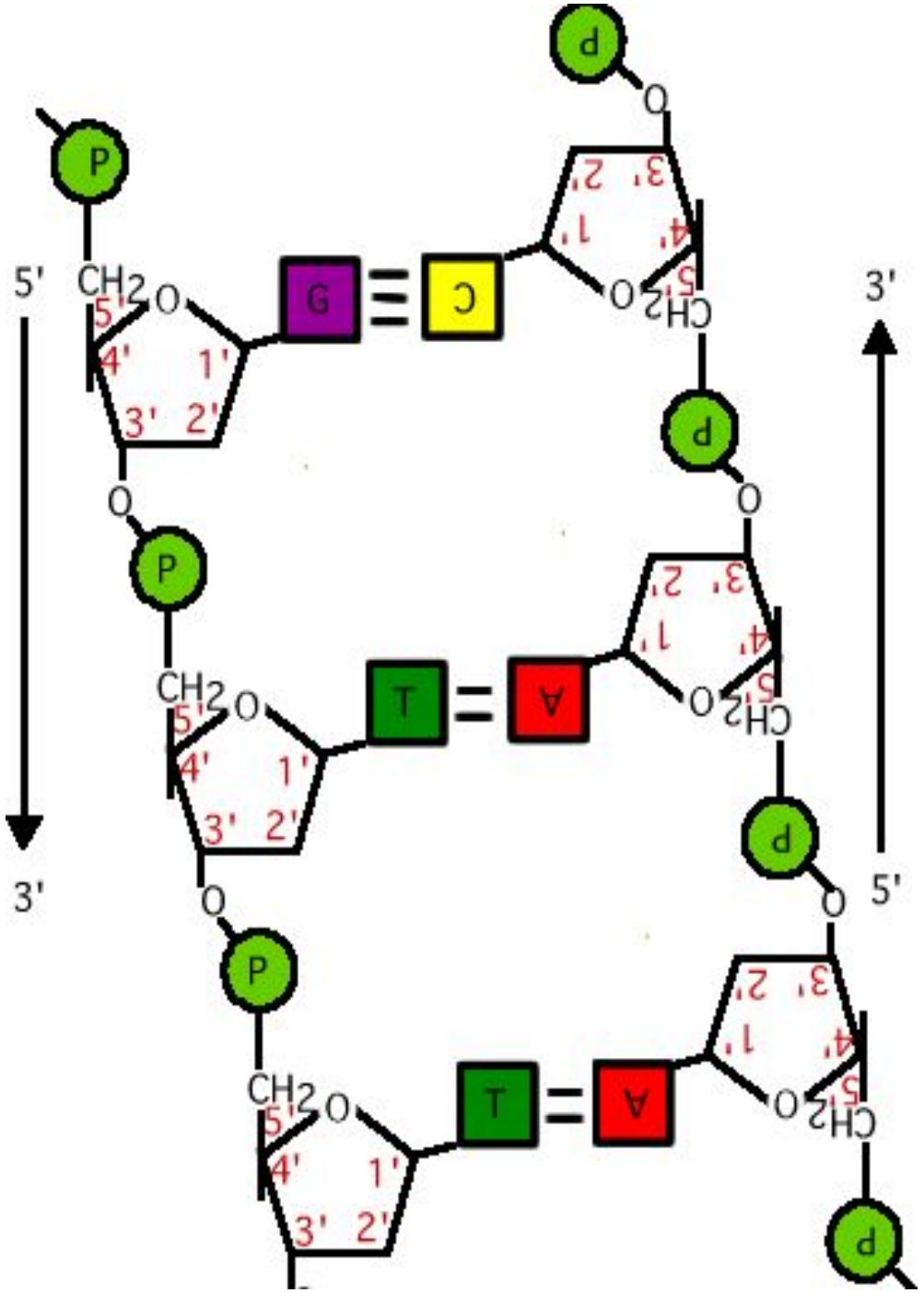
A + C = G + T

(G + C) / (A + T) = 0,54 - 0,94

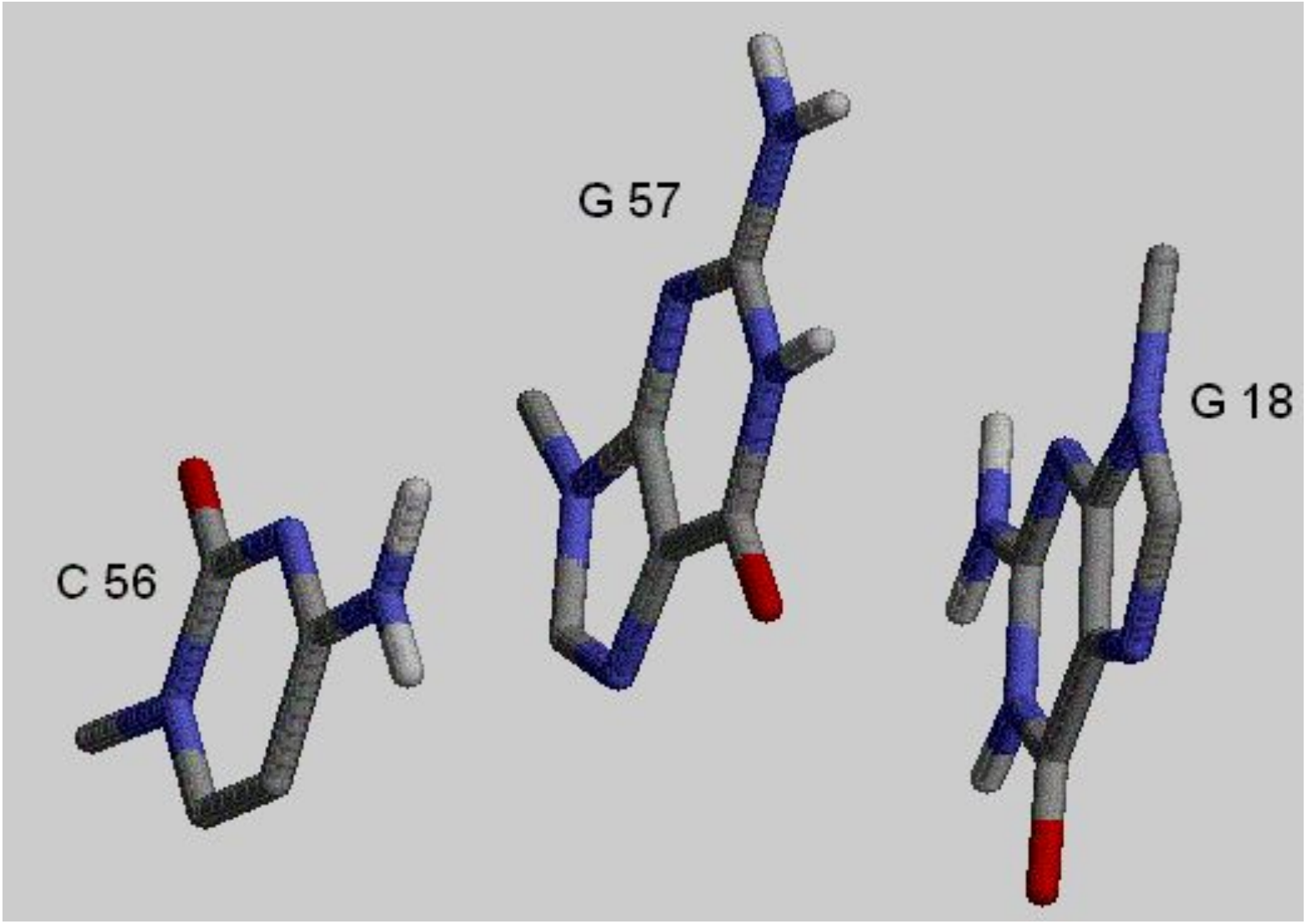
(in animals)

= 0,45-2,57 (in microorganisms)

Complementary chains of DNA



Stacking interaction



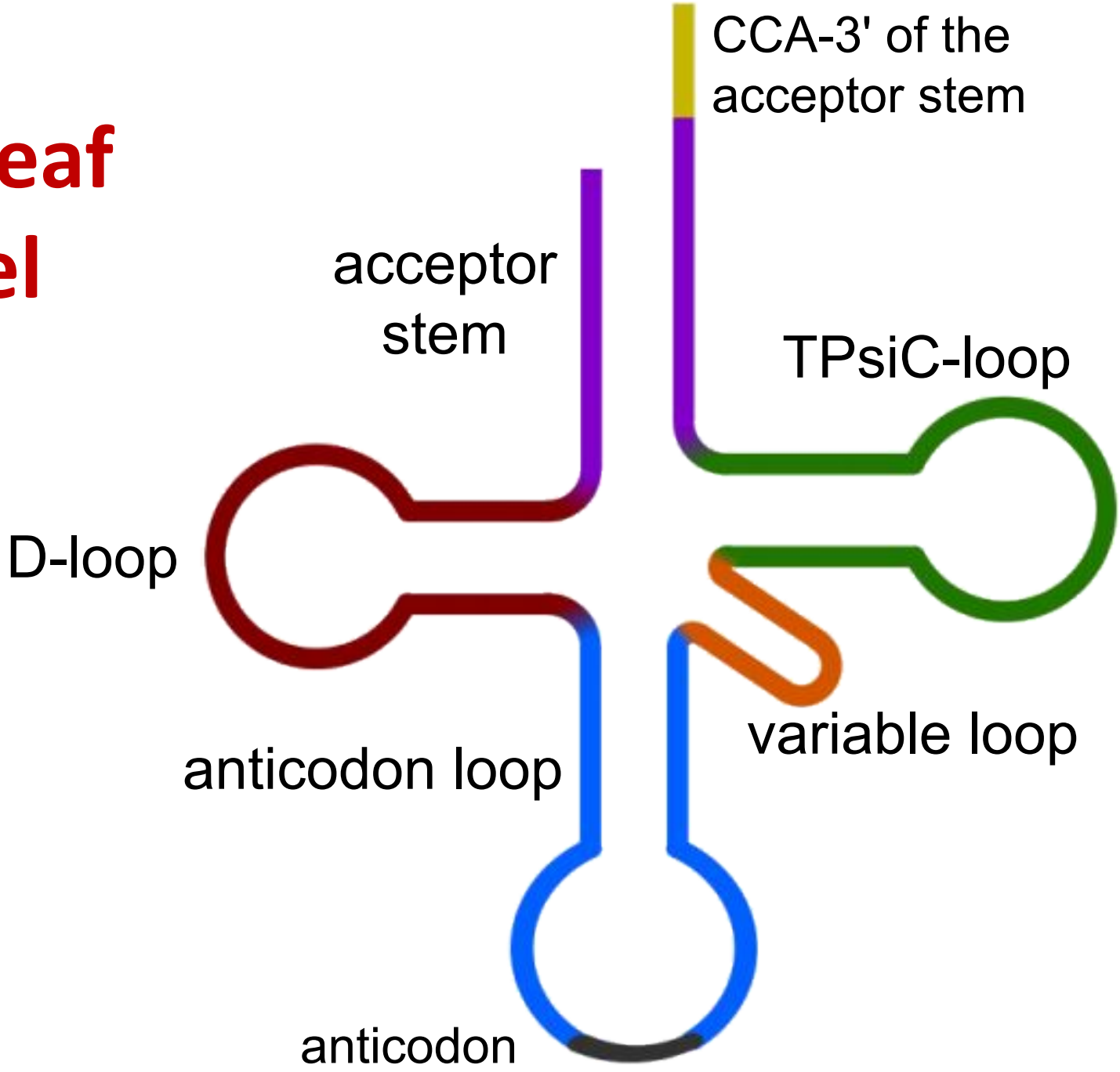
The intensity of stacking

Purine – Purine >

> Pyrimidine – Purine >

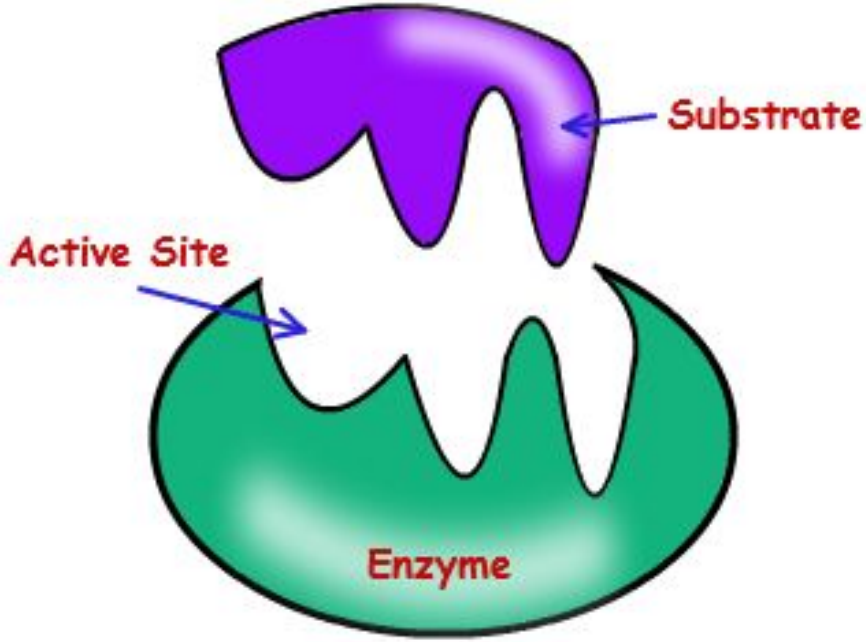
> Pyrimidine – Pyrimidine

Cloverleaf model

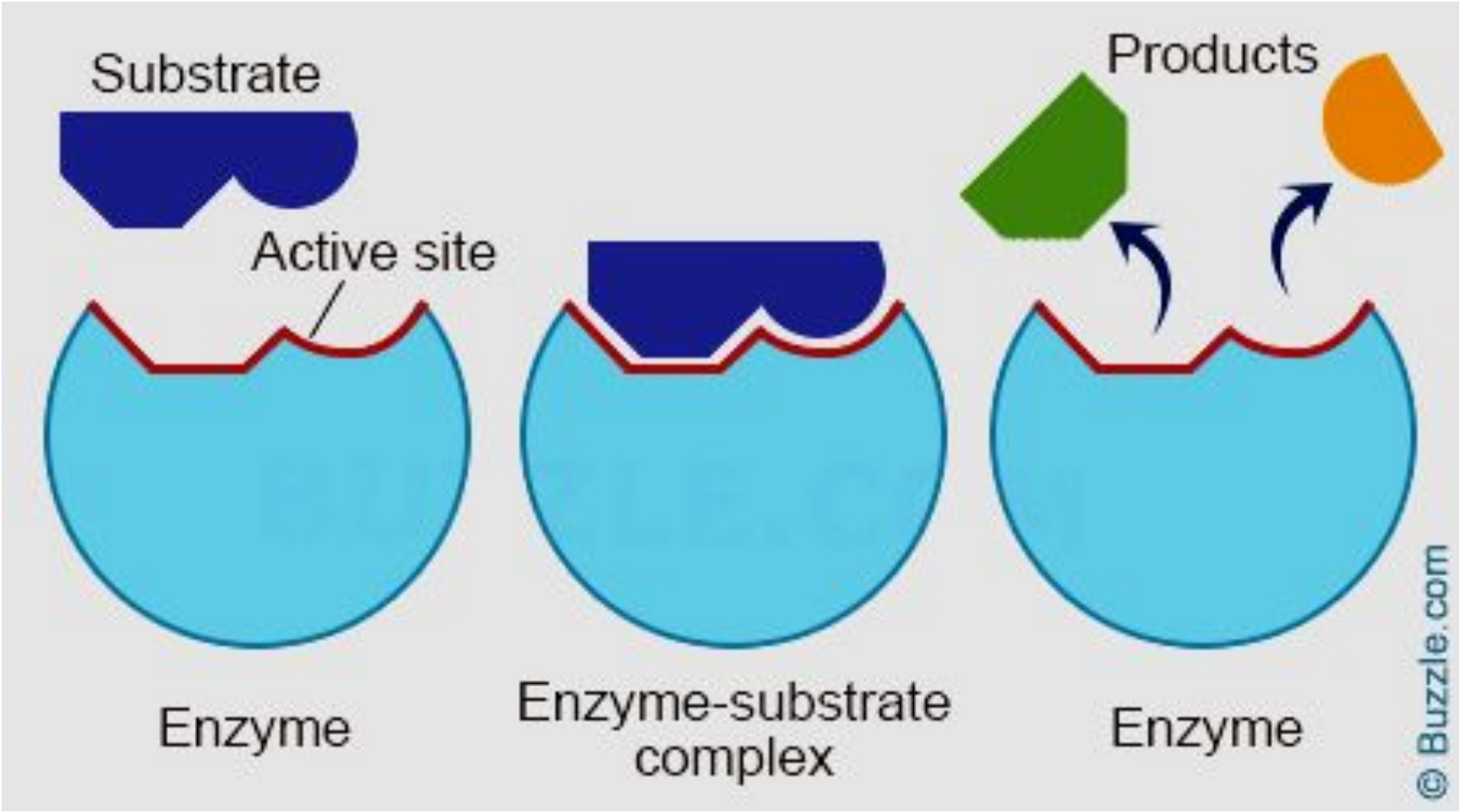


t-RNA: L-shaped

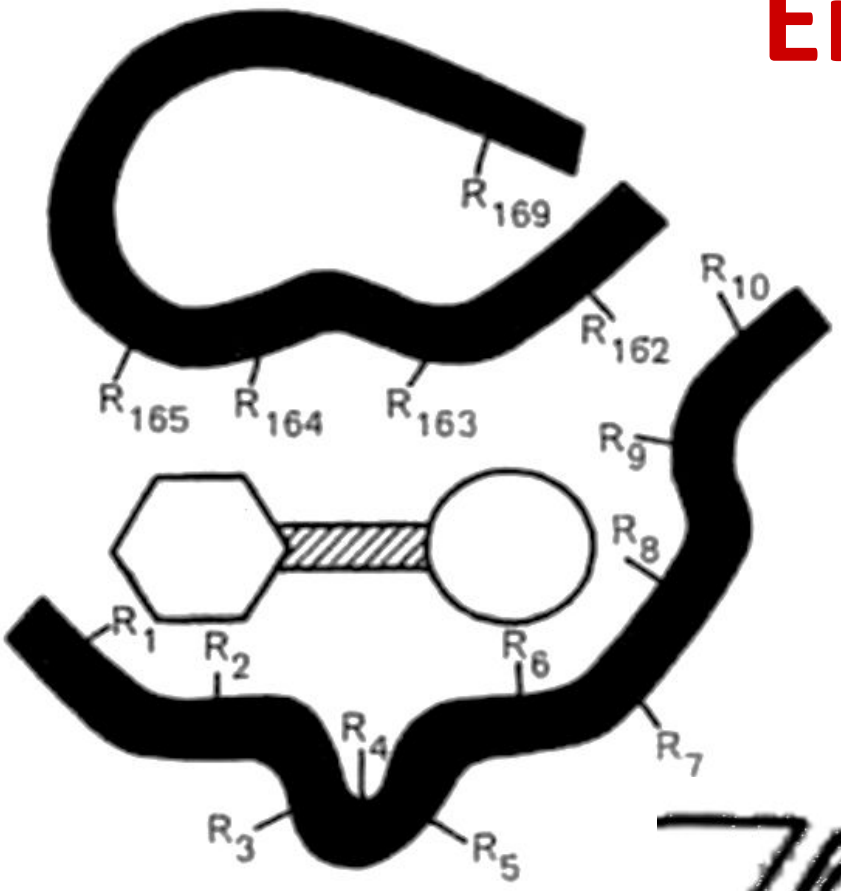
Biochemistry of enzymes

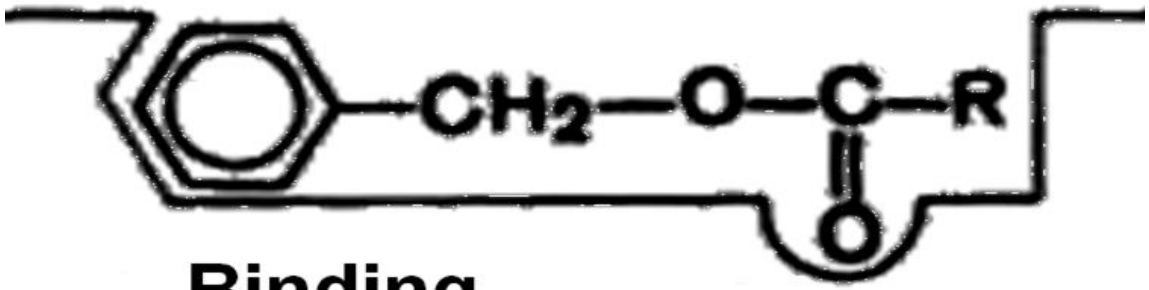


Enzymes are biological catalysts



Enzyme active site

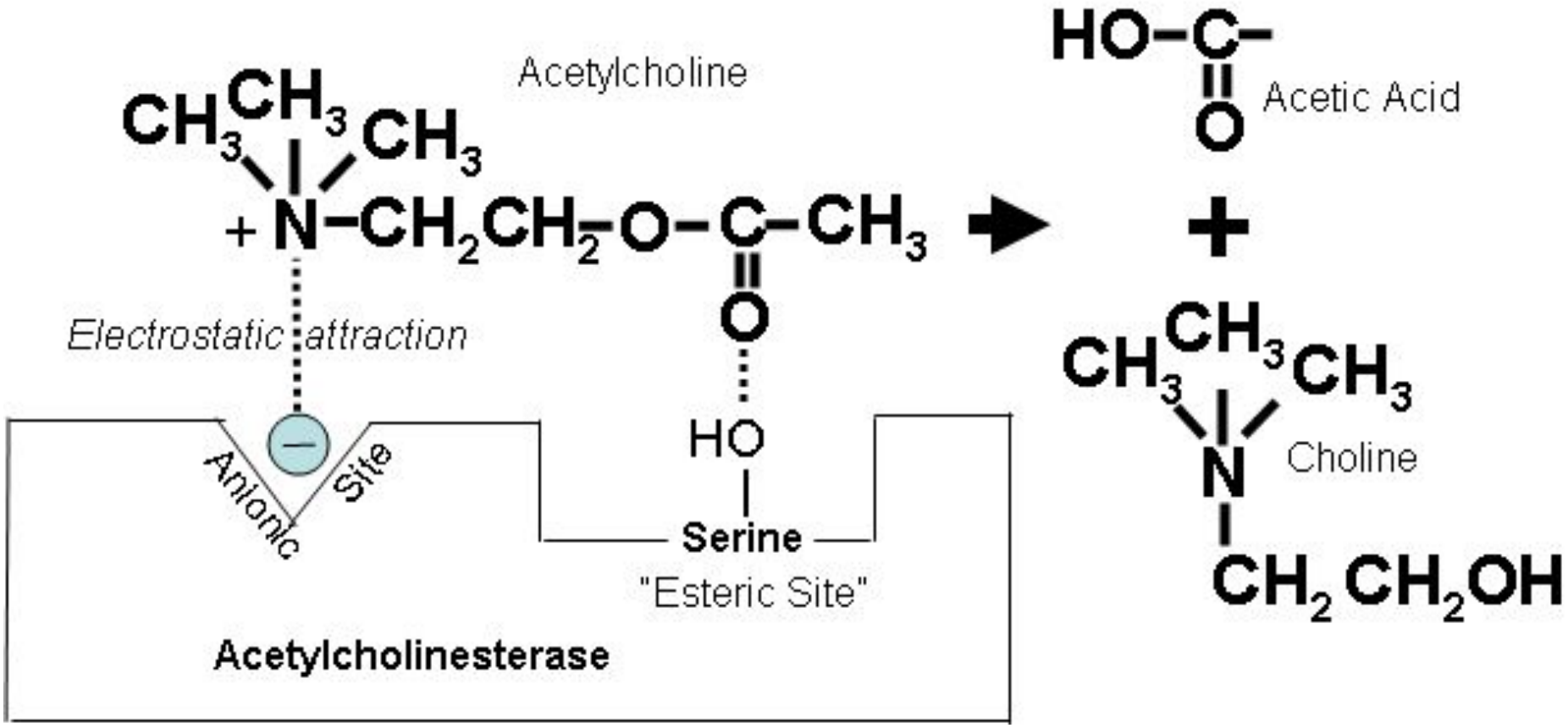


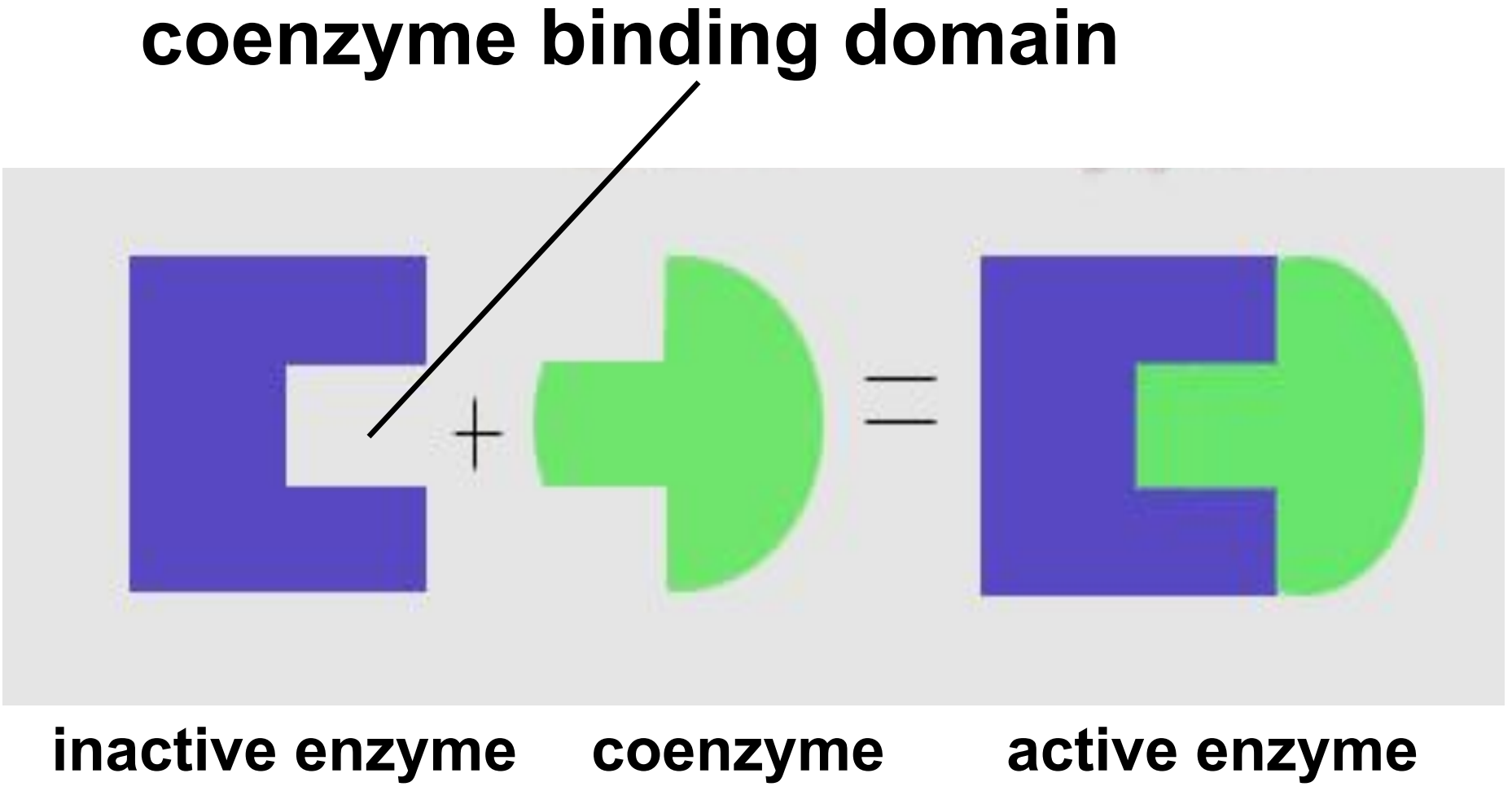


Binding site

Catalytic site

Enzyme active site



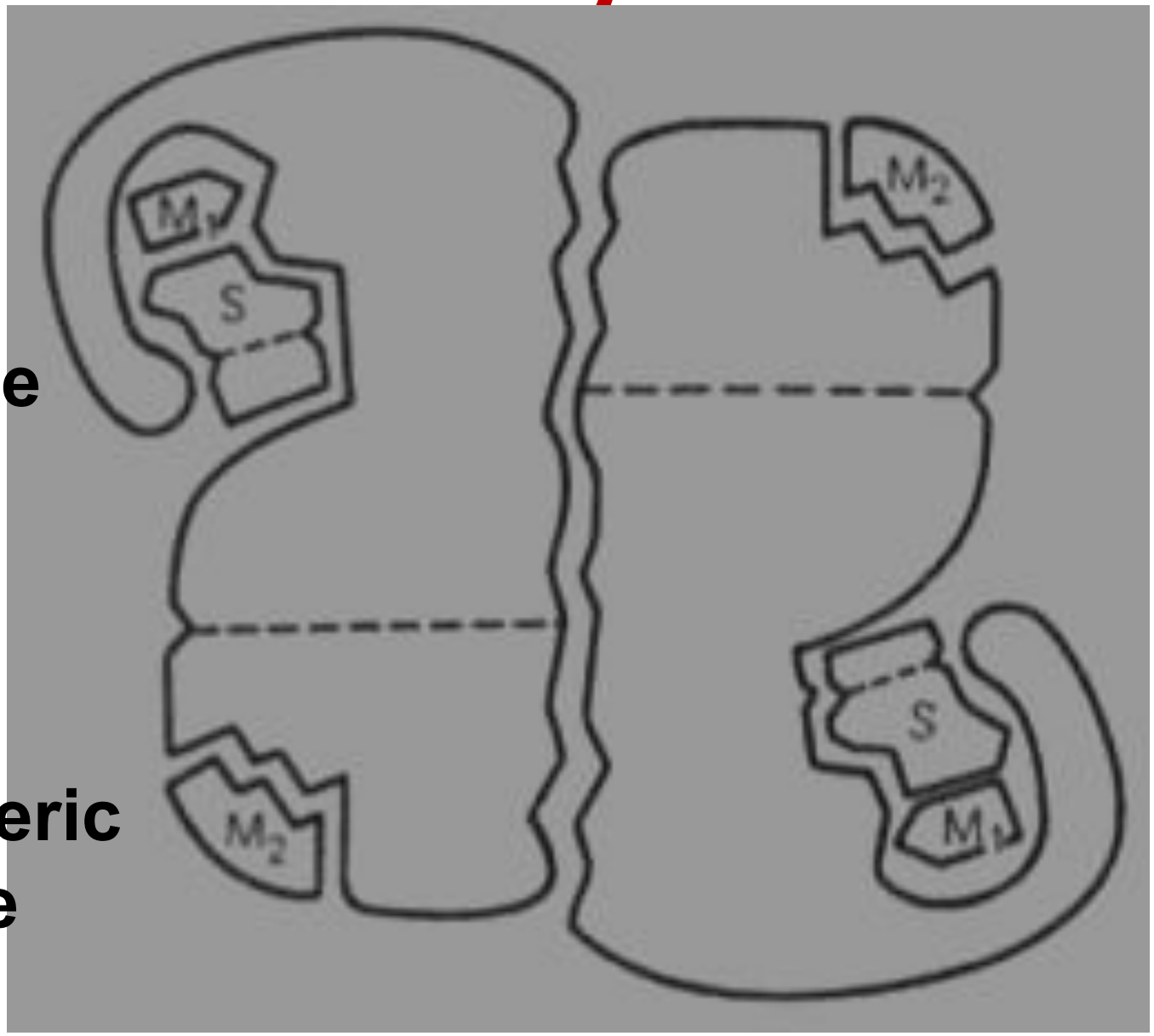

coenzyme binding domain
inactive enzyme
coenzyme
active enzyme

Coenzyme	The overall role	Vitamin precursor
Coenzyme A	Activation and transfer of acyl groups	Pantothenic acid
Pyridoxal phosphate	transfer of amino groups	Pyridoxine - Vitamin B_6
FAD	Transfer of hydrogen (electrons)	Riboflavin - Vitamin B_2

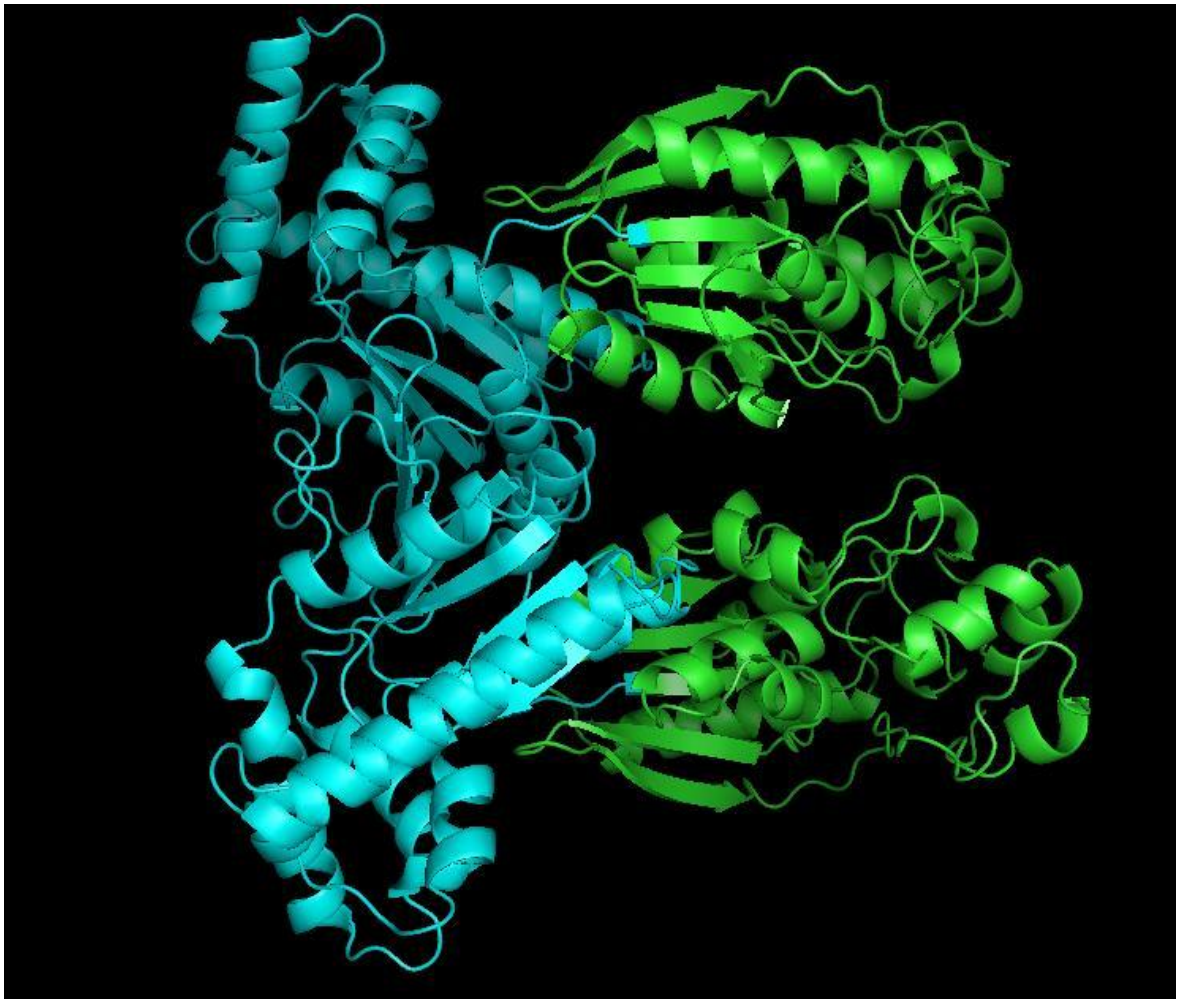
Allosteric enzyme

Active site

Allosteric site

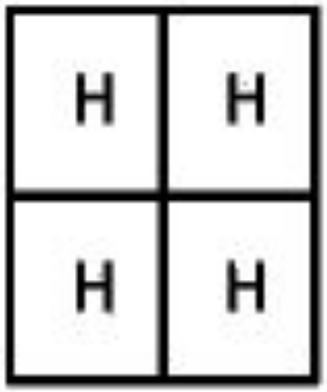


Bifunctional enzyme



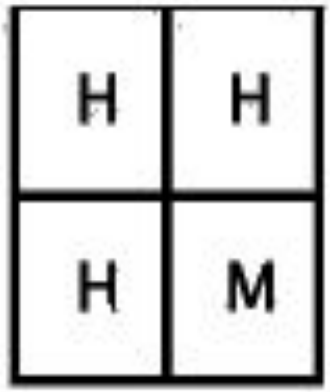
Kinase domain **Phosphatase domain**

Isozymes of lactate dehydrogenase



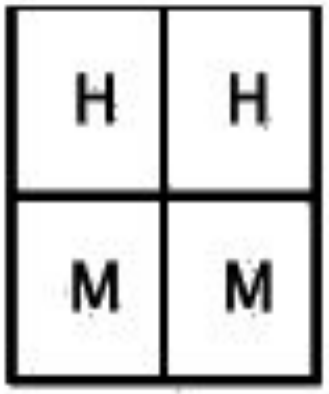
LDH_1

(H_4)



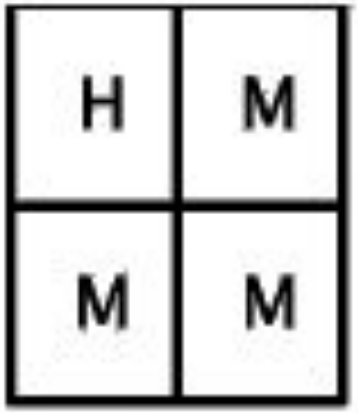
LDH_2

(H_3M)



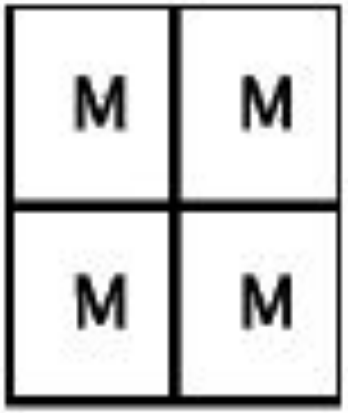
LDH_3

(H_2M_2)



LDH_4

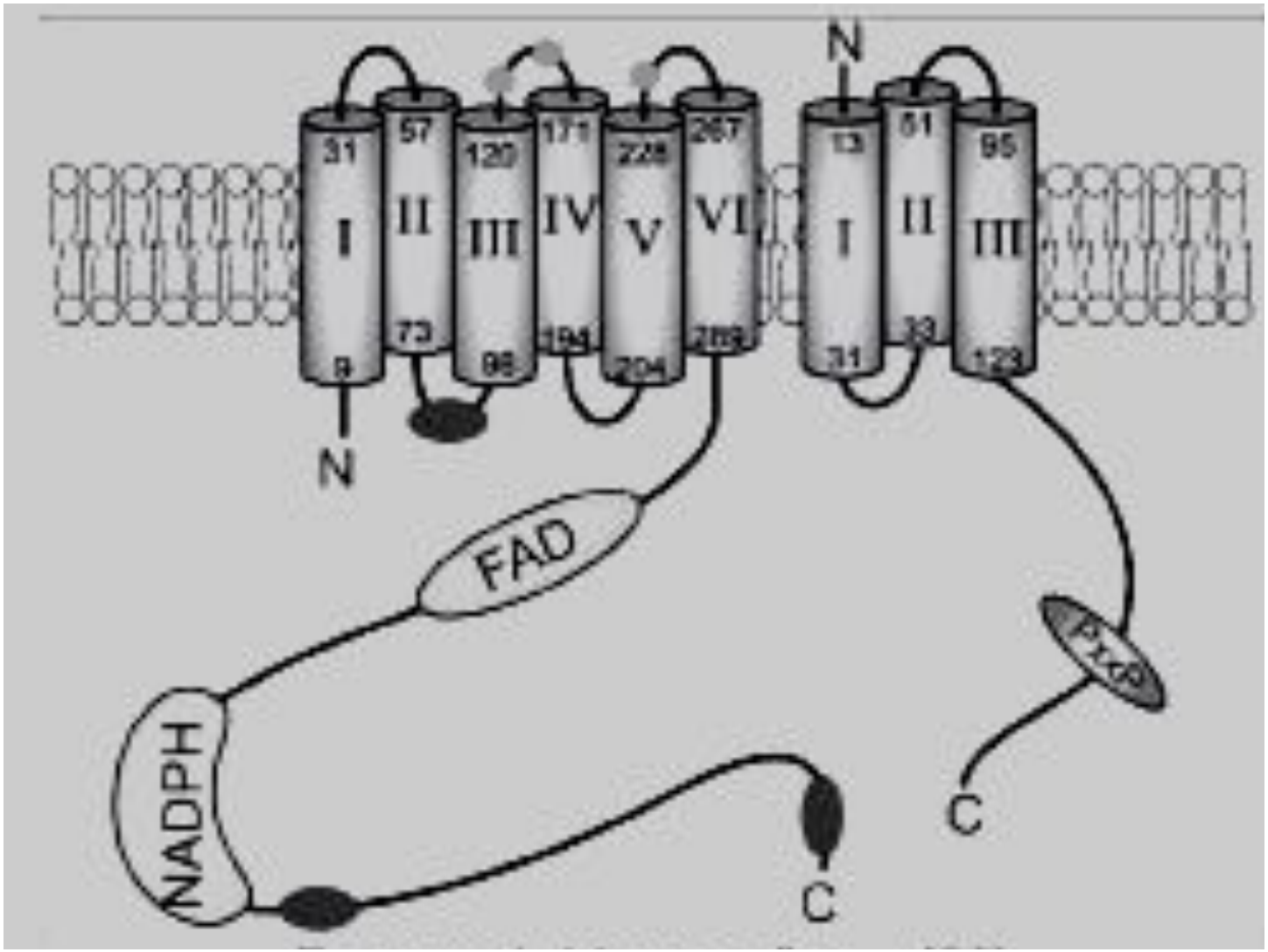
(H_1M_3)



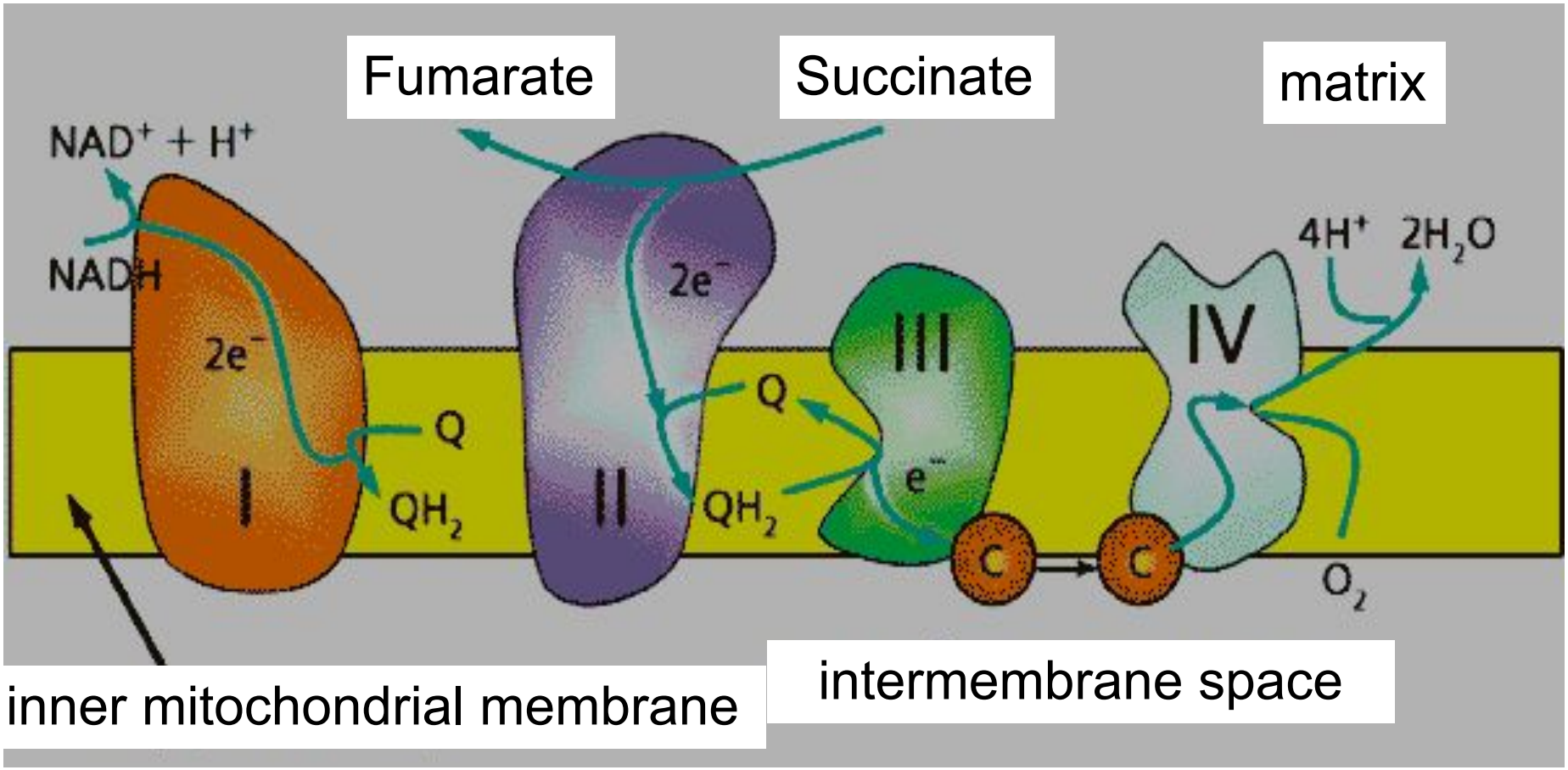
LDH_5

(M_4)

Multimolecular enzyme systems



NADPH oxidase

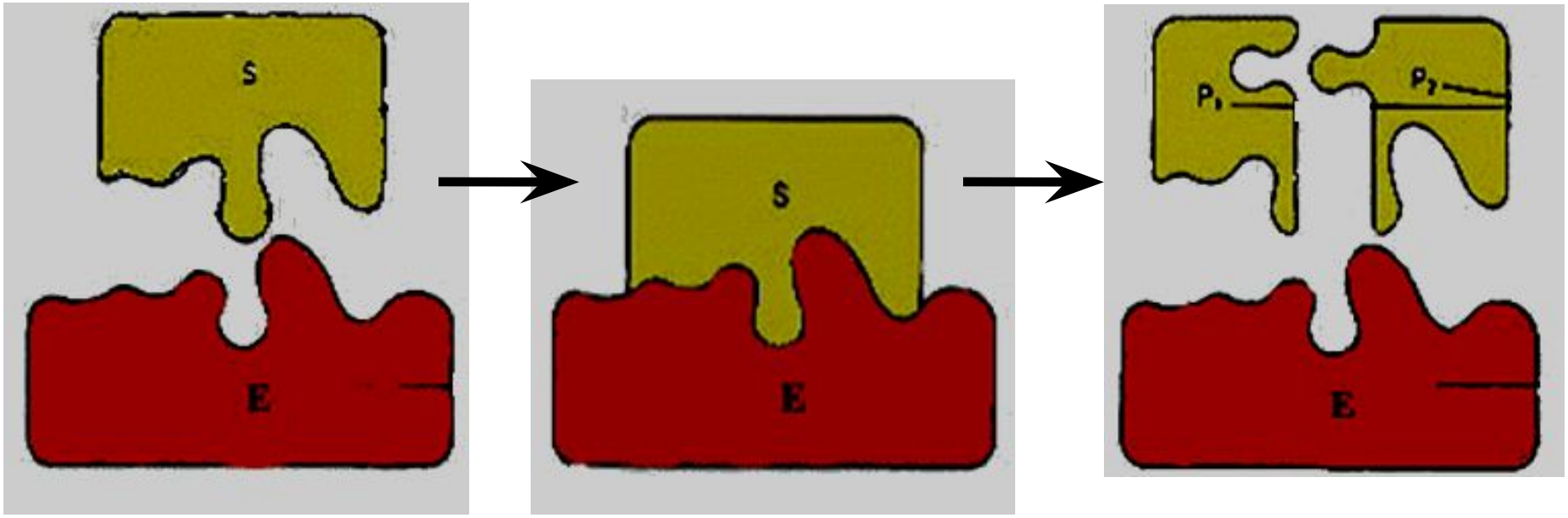


I, II, III and IV – mitochondrial respiratory chain complexes (the electron transport chain)

Hermann Emil Fischer (1852 - 1919)

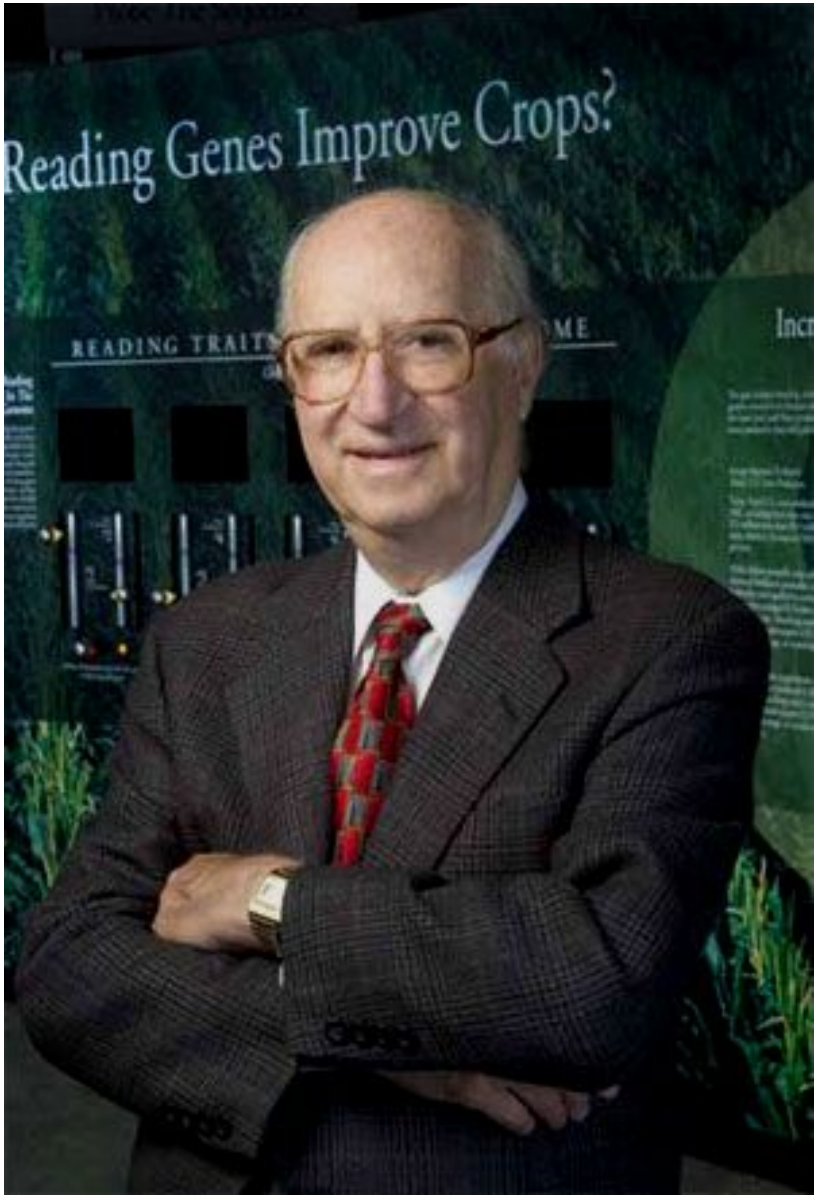
Lock-and-key model

by Fisher



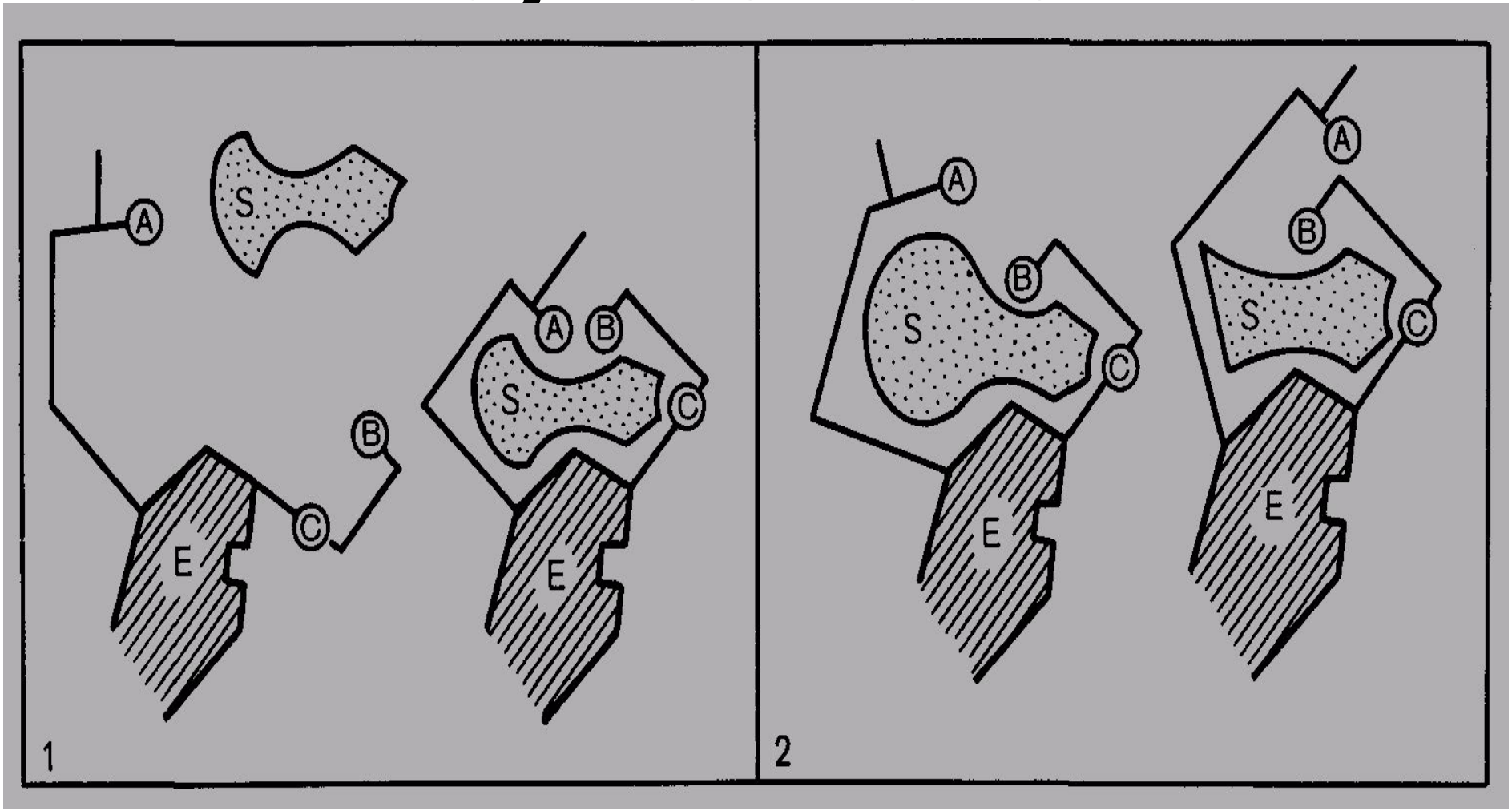
E + S → E-S → E + P

Daniel Koshland (1920 - 2007)

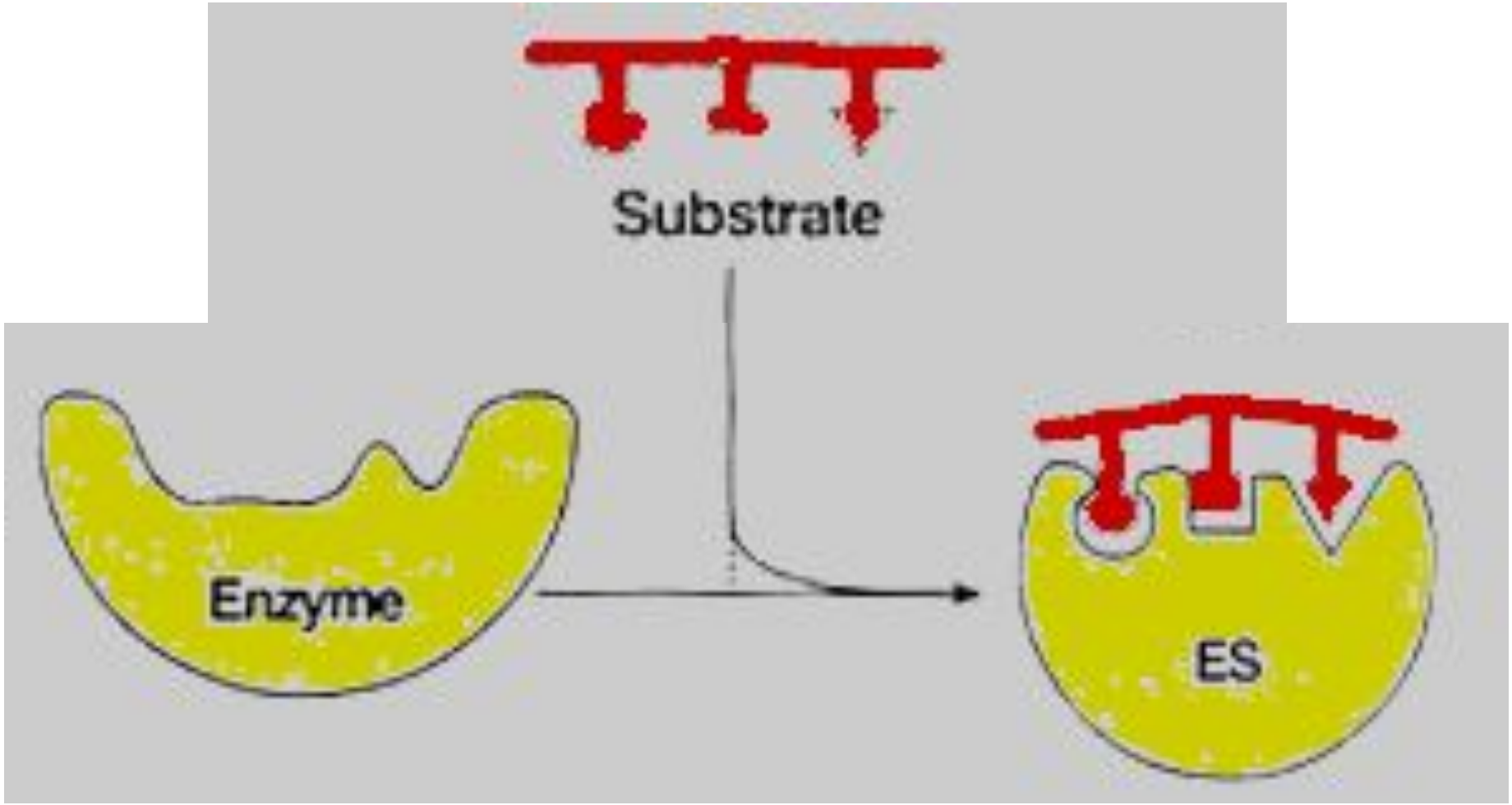


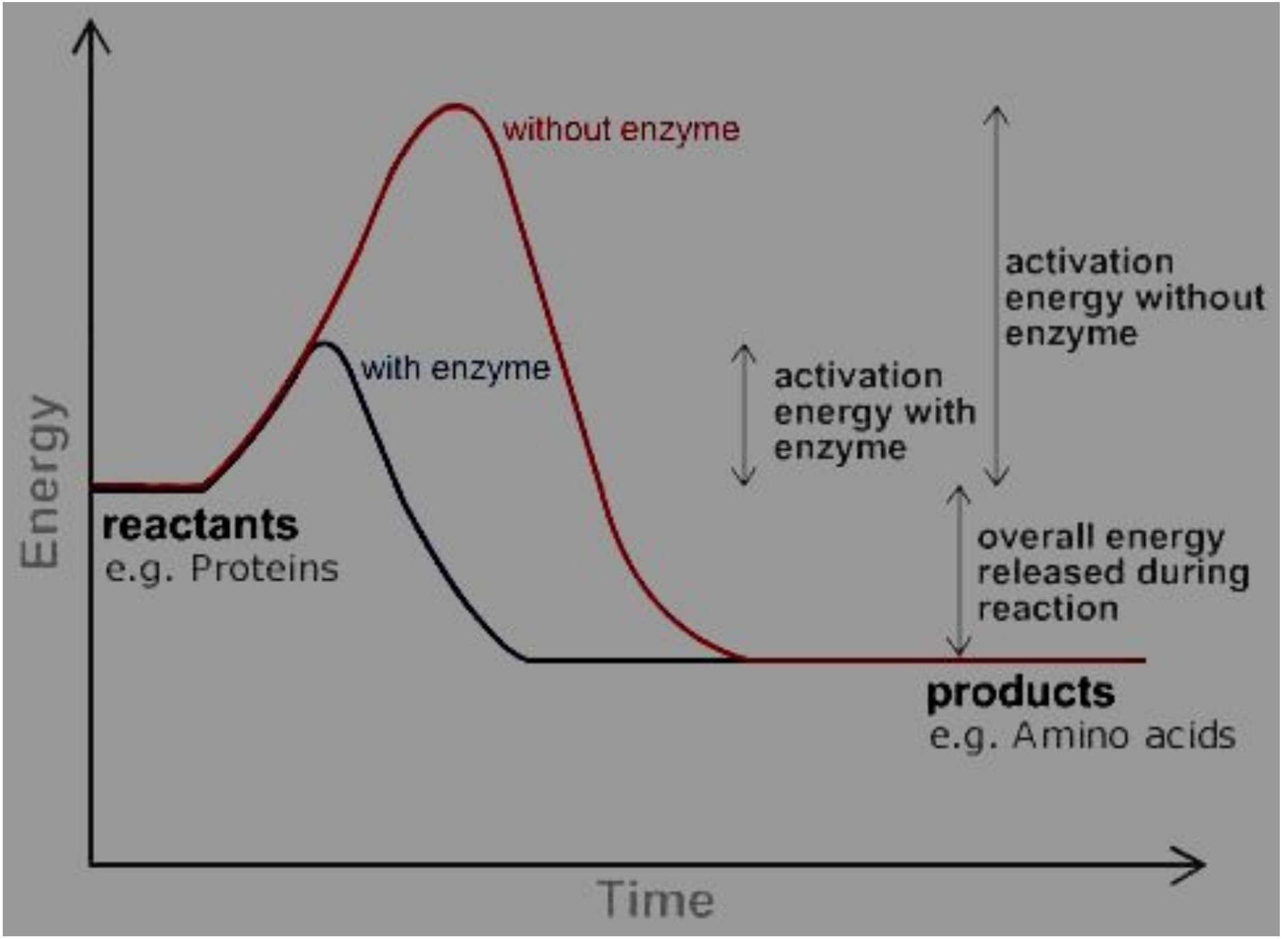
Induced-fit theory

by Koshlend



Substrate strain theory




without enzyme
with enzyme
activation energy without enzyme
activation energy with enzyme
overall energy released during reaction
reactants
e.g. Proteins
products
e.g. Amino acids
Energy
Time

Enzyme kinetics

Leonor Michaelis

Maud Leonora Menten

$$E + S \underset{k_{-1}}{\overset{k_{+1}}{\rightleftarrows}} ES \xrightarrow{k_{+2}} E + P,$$

$$K_S = \frac{[E][S]}{[ES]} = \frac{k_{-1}}{k_{+1}}$$

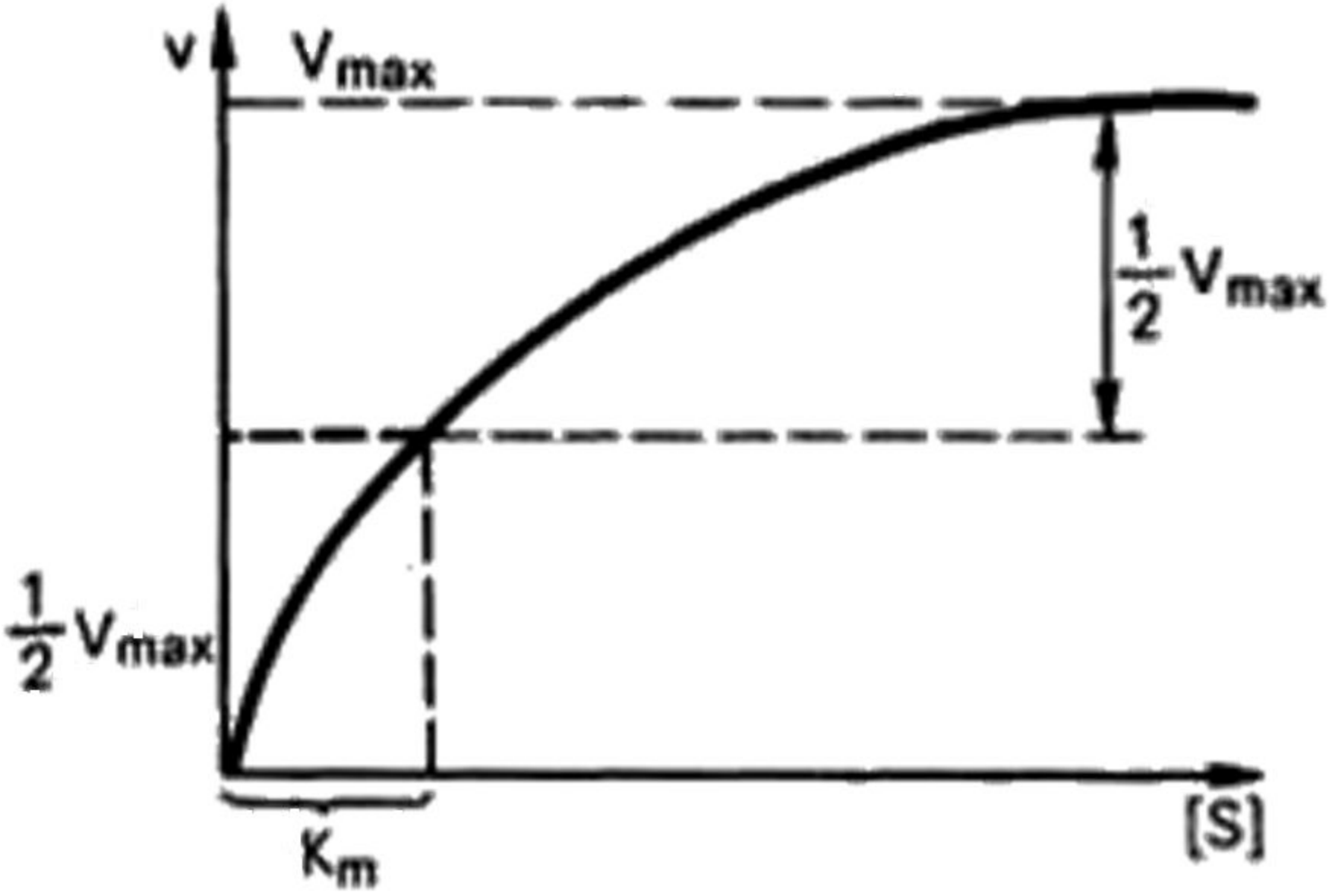
Michaelis – Menten equation

$$\vartheta = \frac{V_{max}[S]}{K_S + [S]}$$

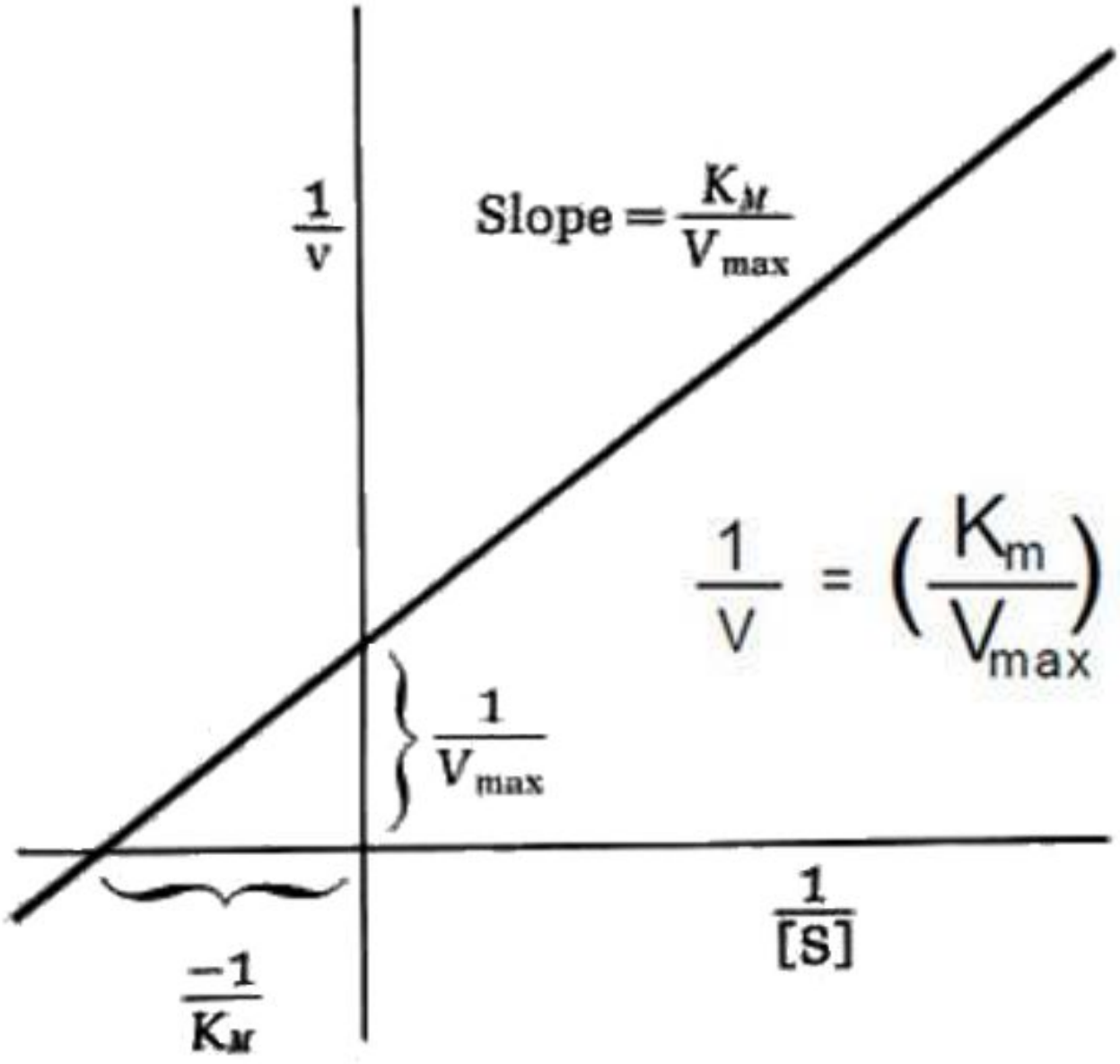
Briggs – Haldane equation:

$$\vartheta = \frac{\vartheta_{\max}[S]}{K_m + [S]}$$

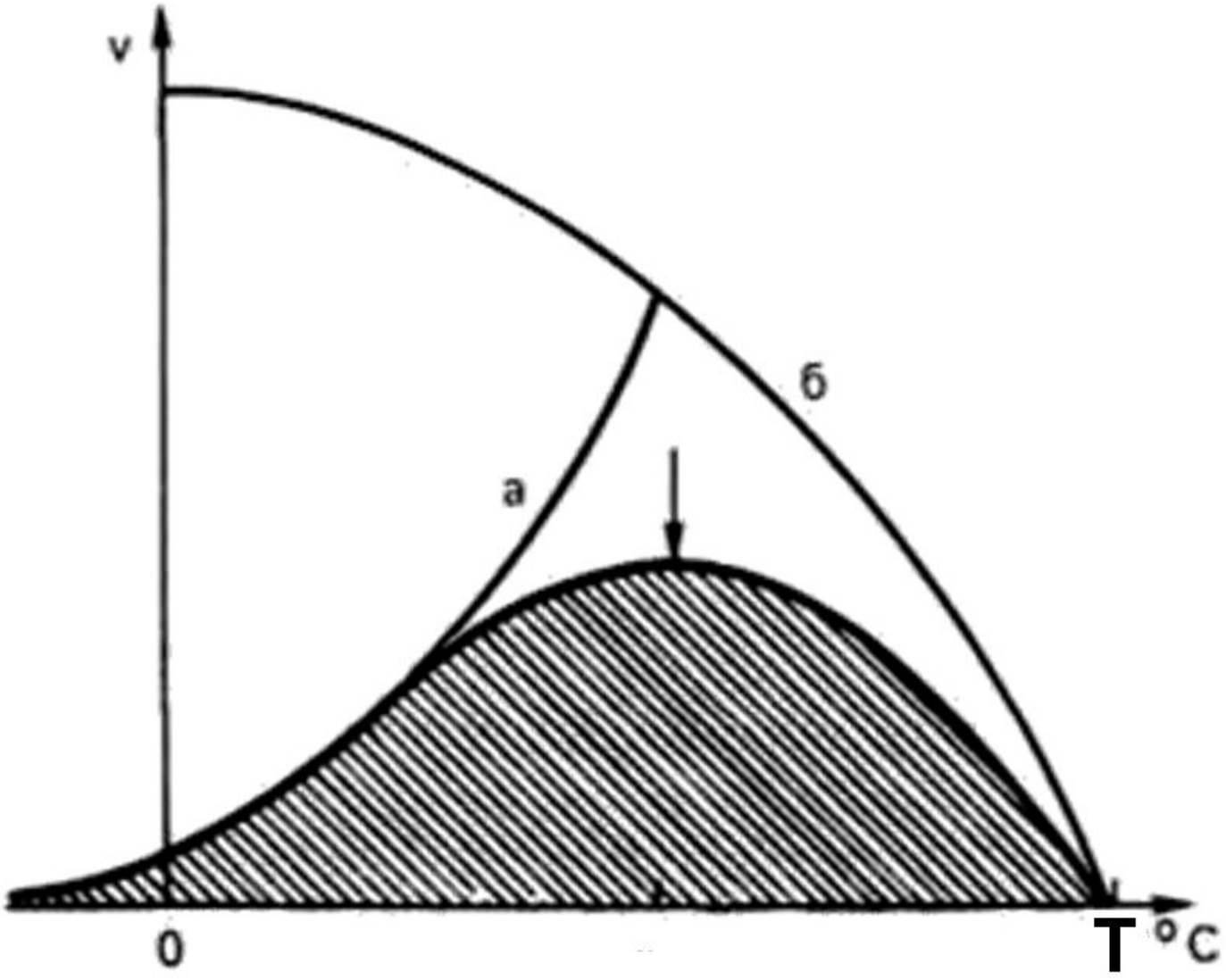
$$K_m = K_s + \frac{k_{+2}}{k_{+1}}$$


v
Vmax
1/2 Vmax
1/2 Vmax
[S]
Km

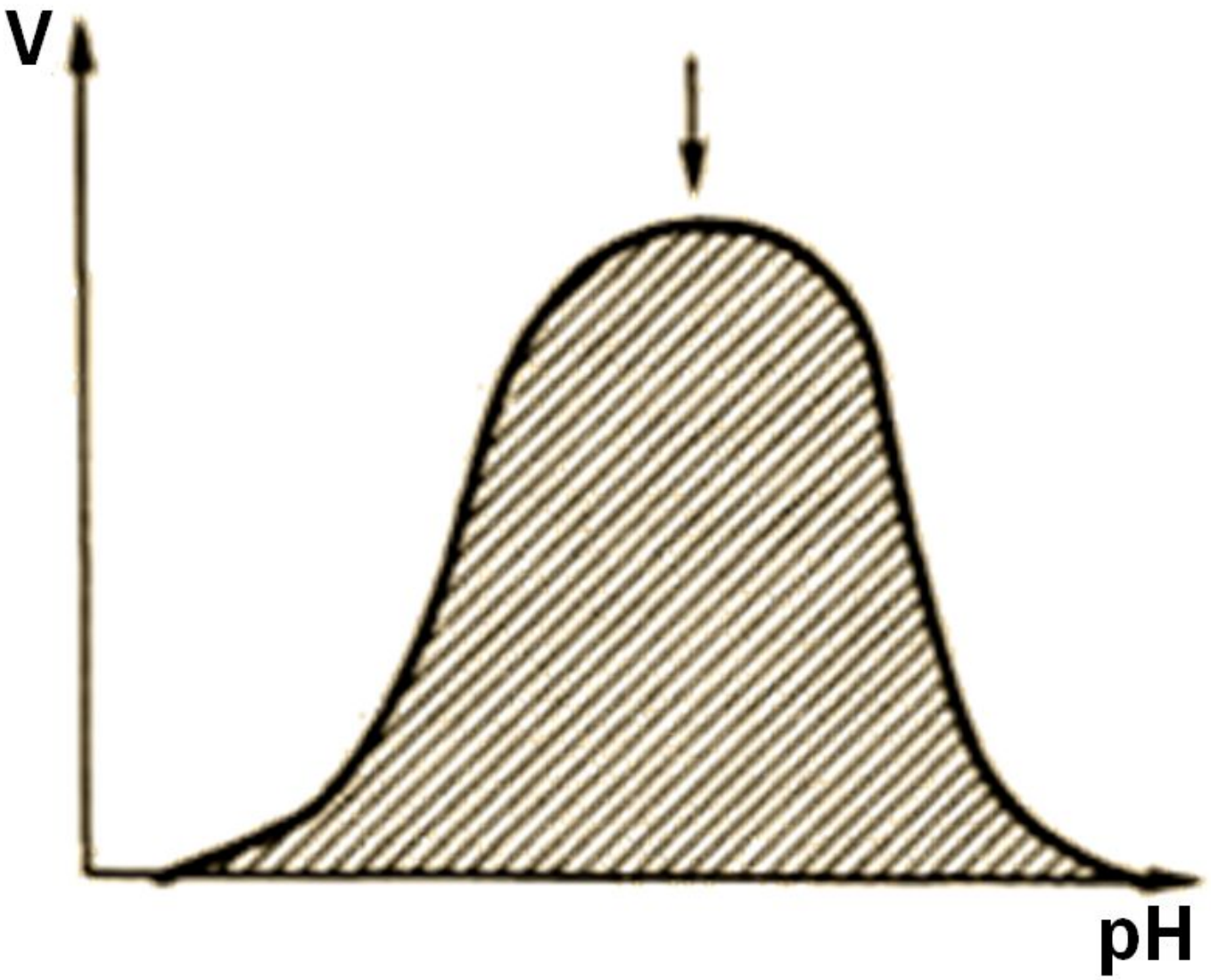
Lineweaver – Burk plot

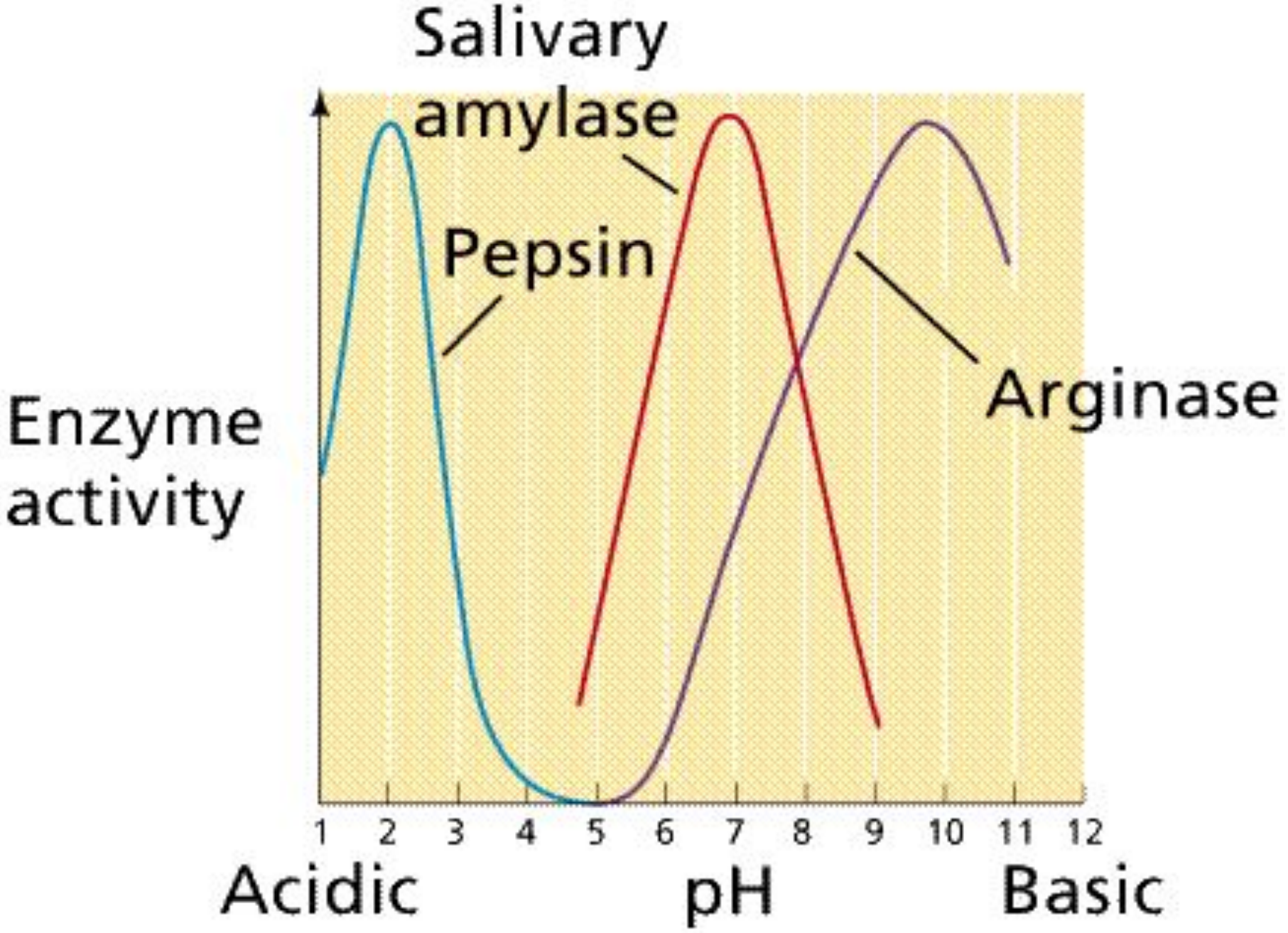


Enzymes are sensitive to temperature

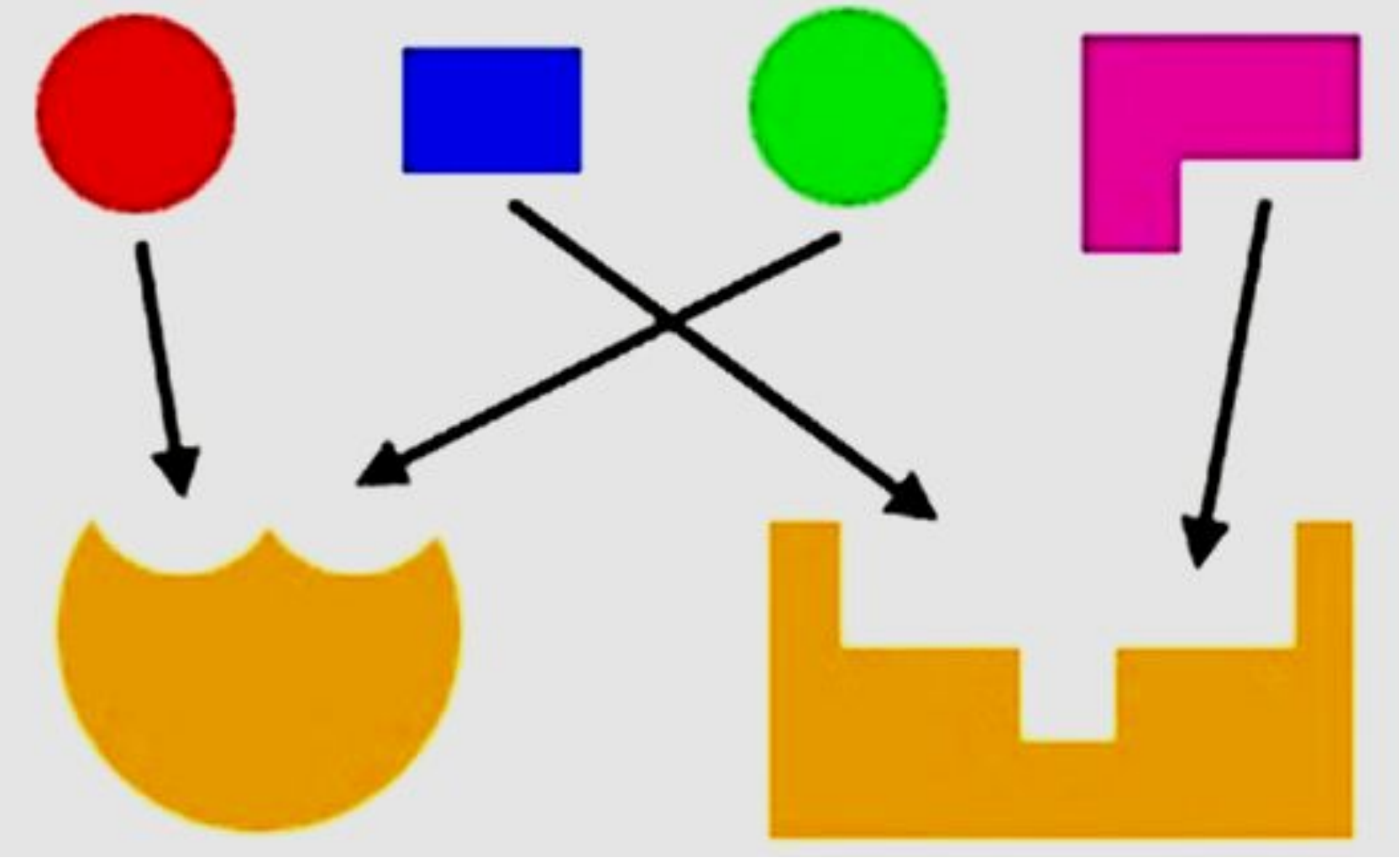


Enzymes are sensitive to pH

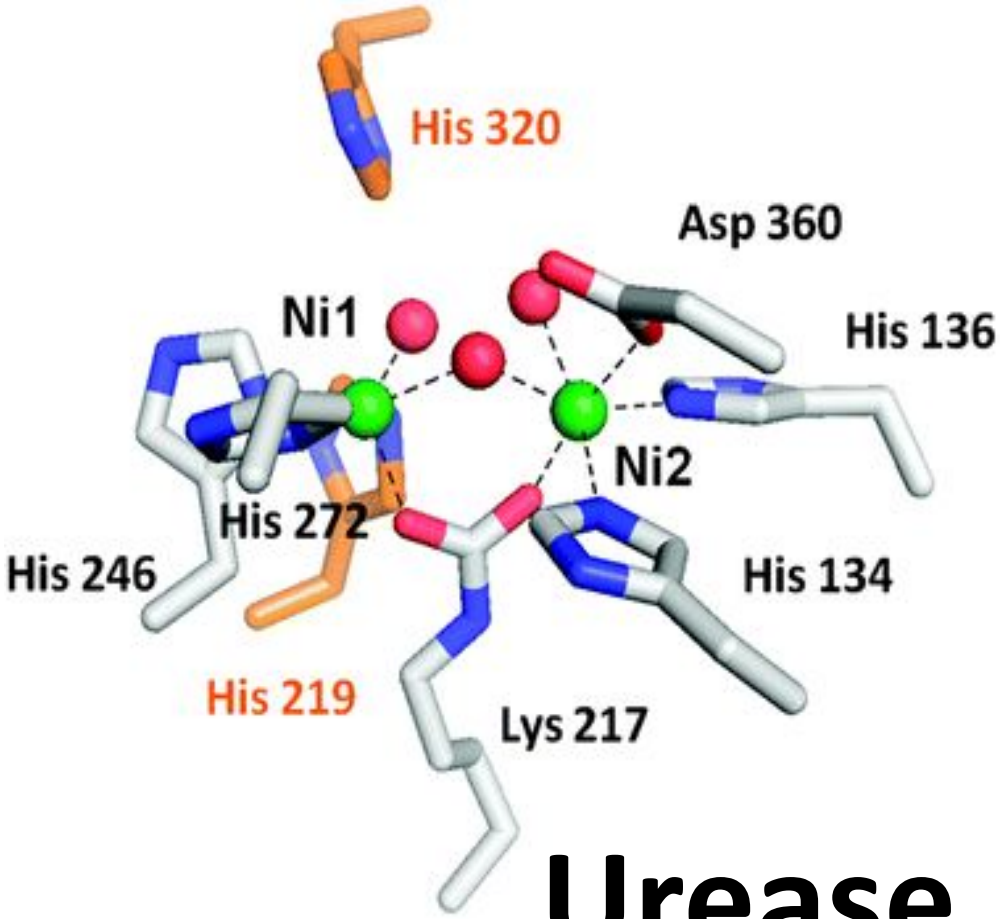


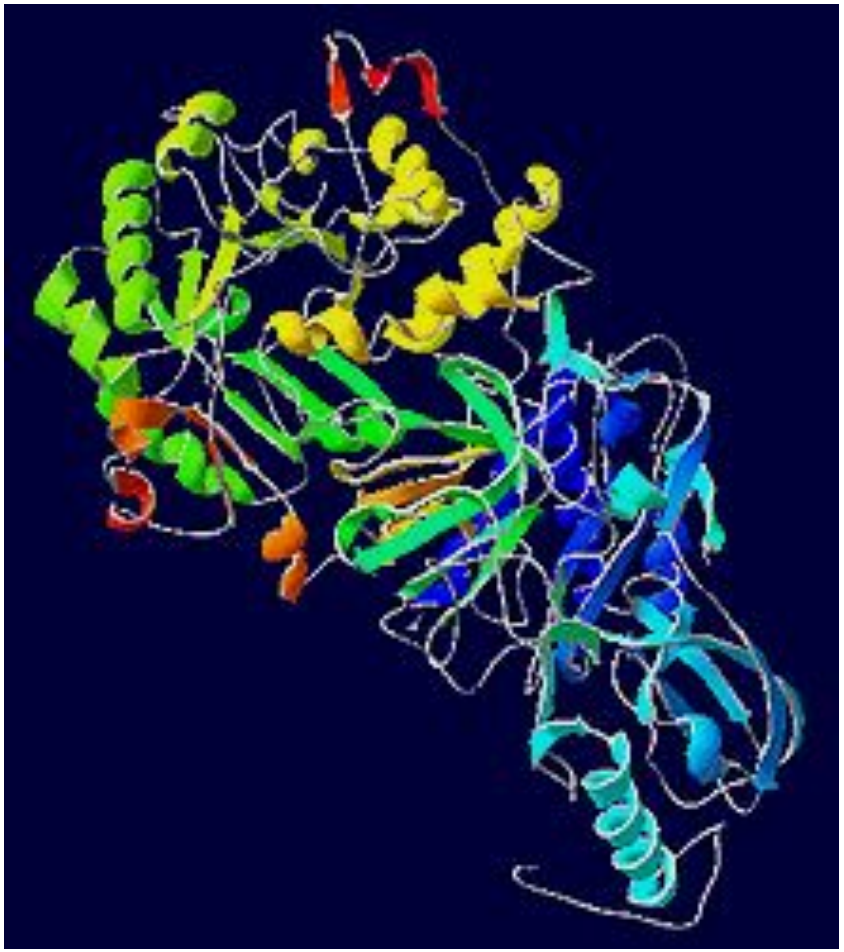

Salivary amylase
Pepsin
Arginase
Enzyme activity
1 2 3 4 5 6 7 8 9 10 11 12
Acidic
pH
Basic

Enzymes are very specific and only work with certain substrates



Enzymes with absolute specificity

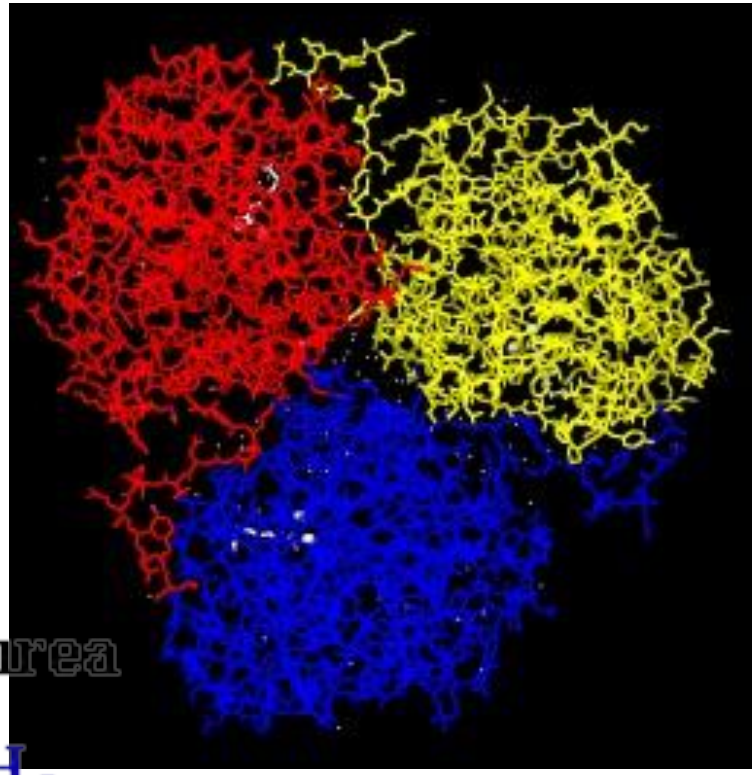


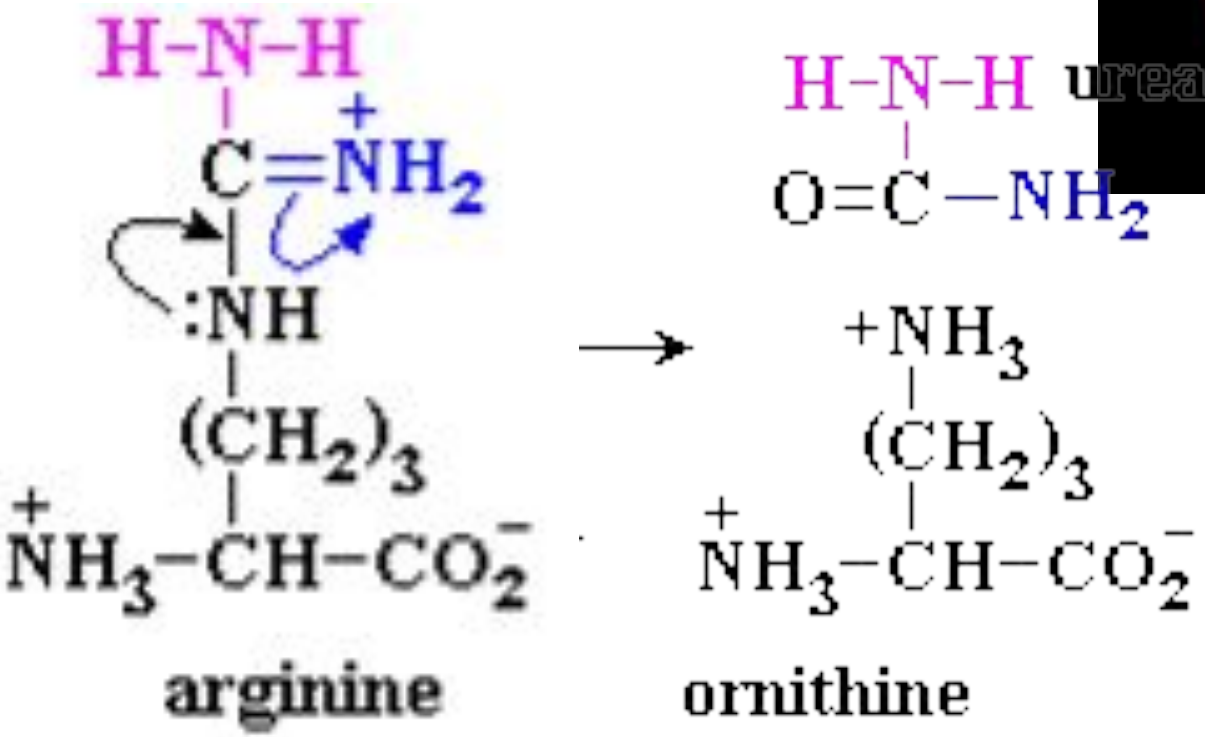


Urease

$$CO(NH_2)_2 + 2H_2O = H_2O + CO_2 +$$

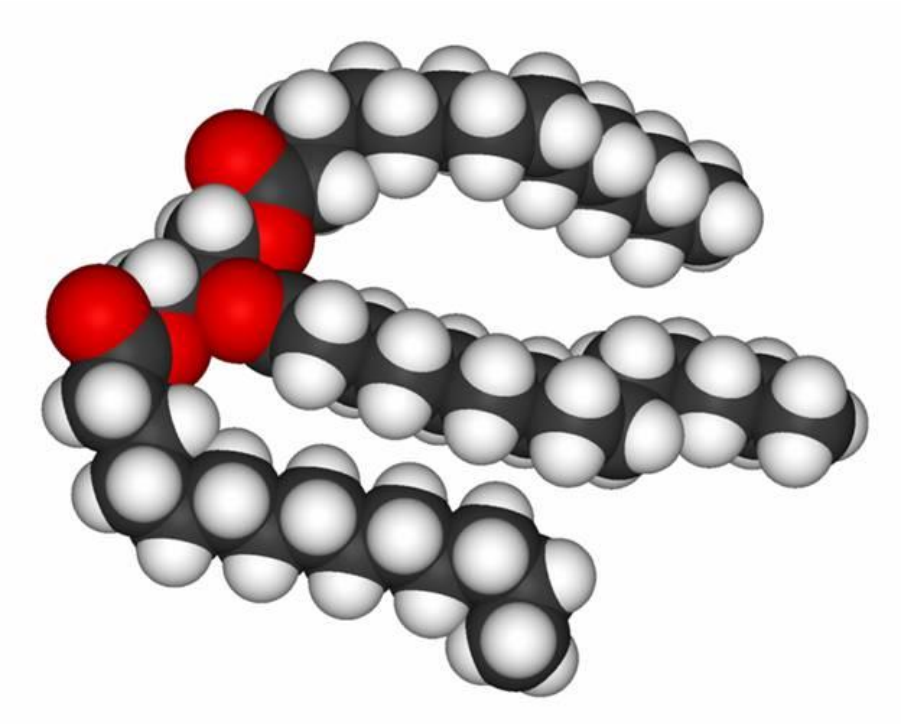
Arginase



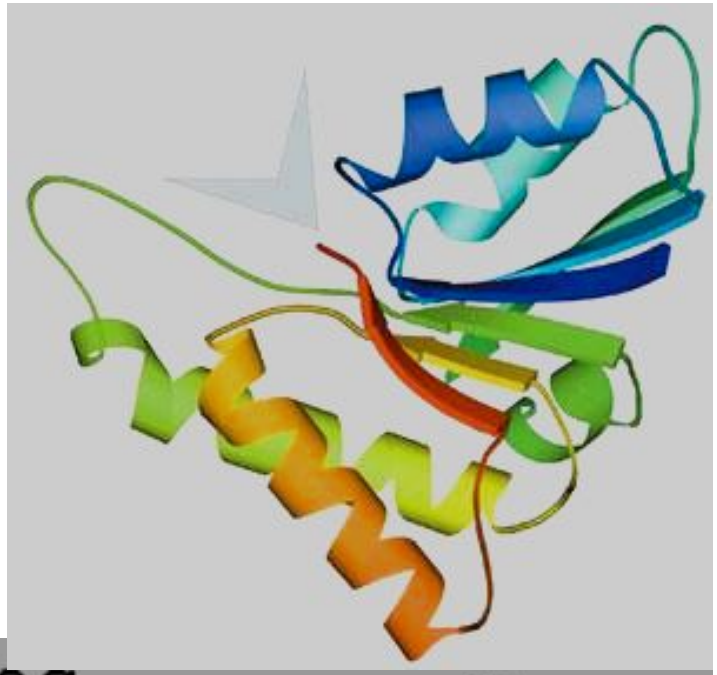

H-N-H
C=NH2
:NH
(CH2)3
NH3-CH-CO2
H-N-H
O=C-NH2
urea
+NH3
(CH2)3
NH3-CH-CO2
arginine
ornithine

Enzymes with relative (group) specificity

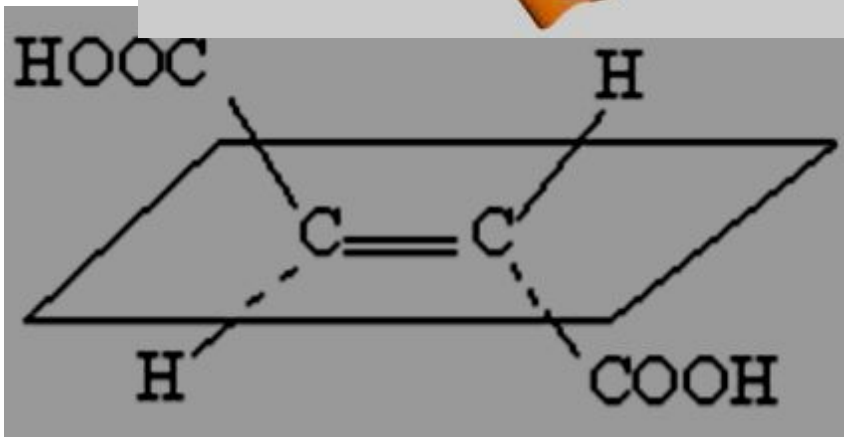
Pancreatic lipase



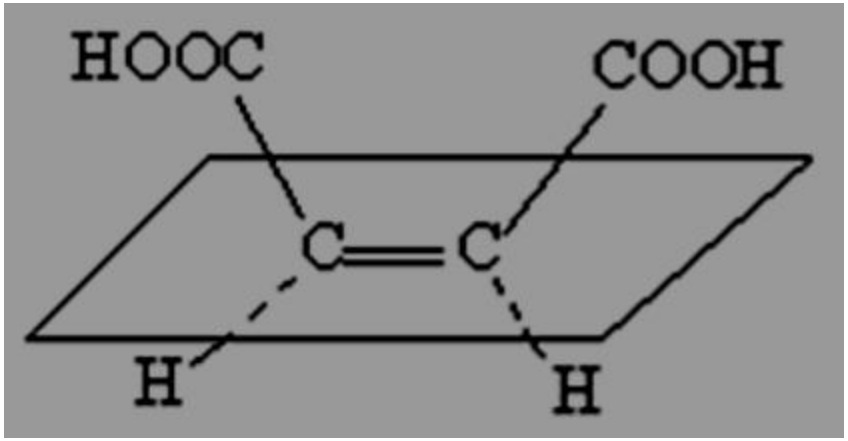
Stereo specificity



Fumarase



fumaric acid



maleic acid

Enzyme Classifcation

Group	Reaction catalyzed	Example(s)
EC 1 Oxido-reduc tases	Oxidation/reduction reactions; transfer of H and O atoms or electrons from one substance to another	Dehydro-gena se, oxidase
EC 2 Transferases	Transfer of a functional group from one substance to another.	Transaminase , kinase
EC 3 Hydrolases	Formation of two products from a substrate by hydrolysis	Lipase, peptidase

Enzyme Classifcation

Group	Reaction catalyzed	Example(s)
EC 4 Lyases	Non-hydrolytic addition or removal of groups from substrates. C-C, C-N, C-O or C-S bonds may be cleaved	Decarboxylase
EC 5 Isomerases	Intramolecule rearrangement, i.e. isomerization within a single molecule	Glucose-6-phosphate isomerase
EC 6 Ligases	Join together two molecules by synthesis of new C-O, C-S, C-N or C-C bonds with breakdown of ATP	Carboxylase

Enzyme Classifcation

The **Enzyme Commission number (EC number)** is a numerical classification scheme for enzymes, based on the chemical reactions they catalyze.

Tripeptide aminopeptidase

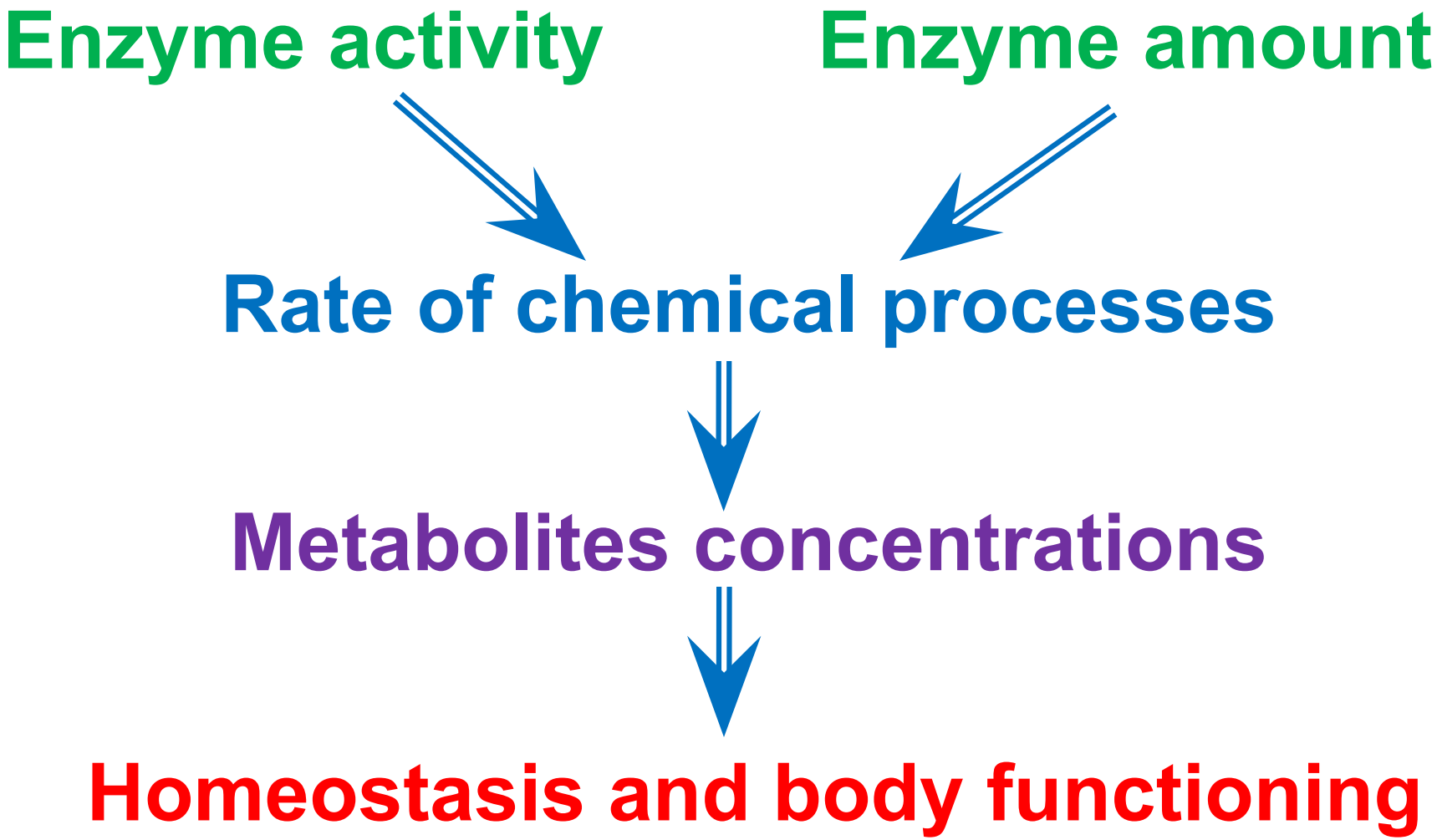
EC 3.4.11.4 ← cleave off the N-terminal end from a tripeptide

Hydrolase

act on peptide bonds

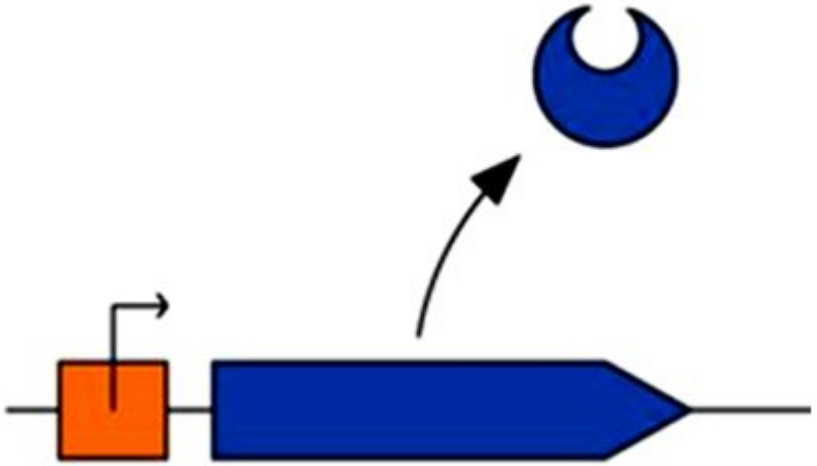
cleave off the N-terminal amino acid from a polypeptide

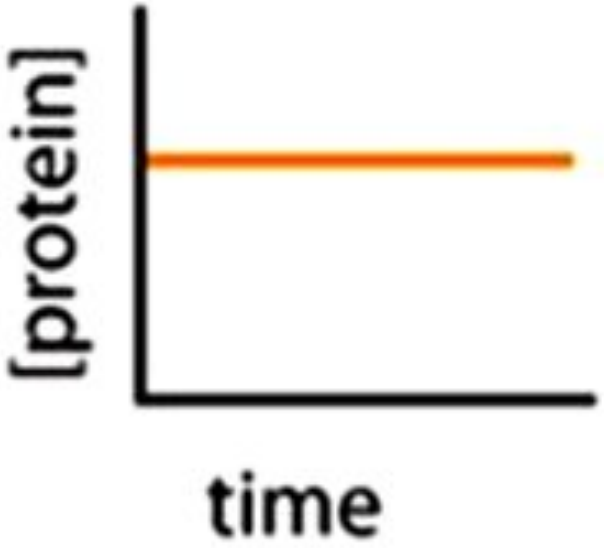
Metabolism regulation



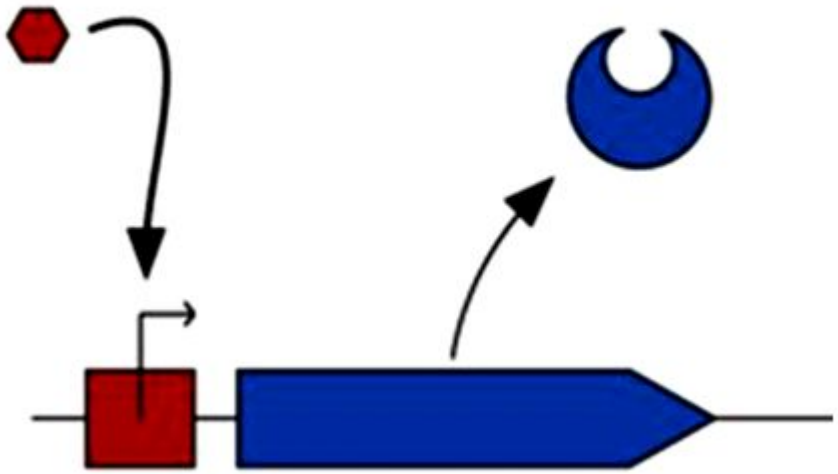
Regulation of enzyme synthesis

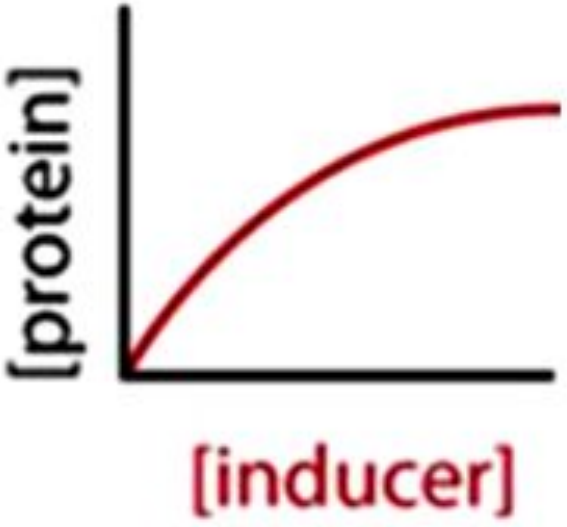
Constitutive





Inducible





Regulation of enzyme activity

Enzymatic activity

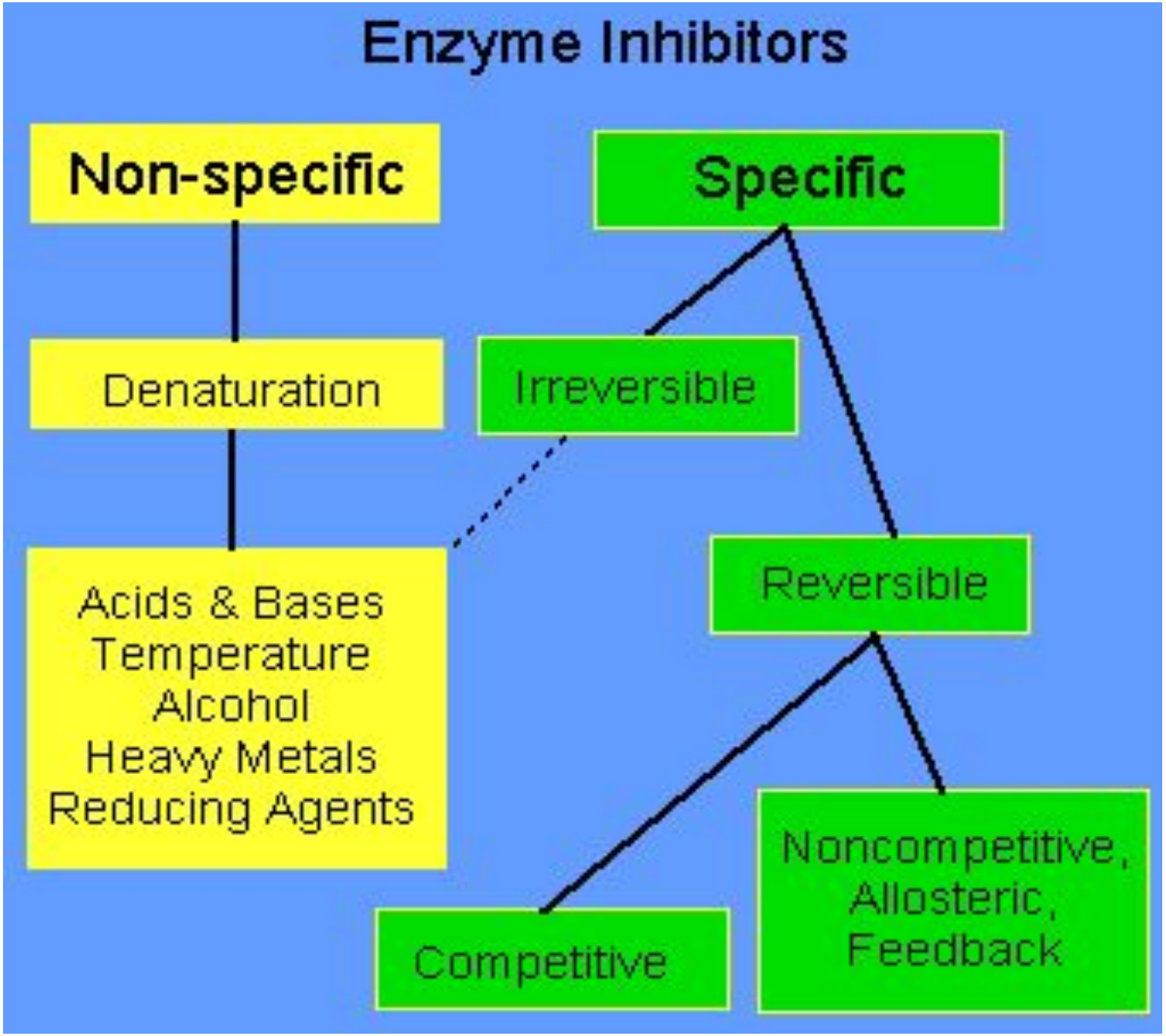
IU = 1mcmole/min

1 kat = 1 mole/sec

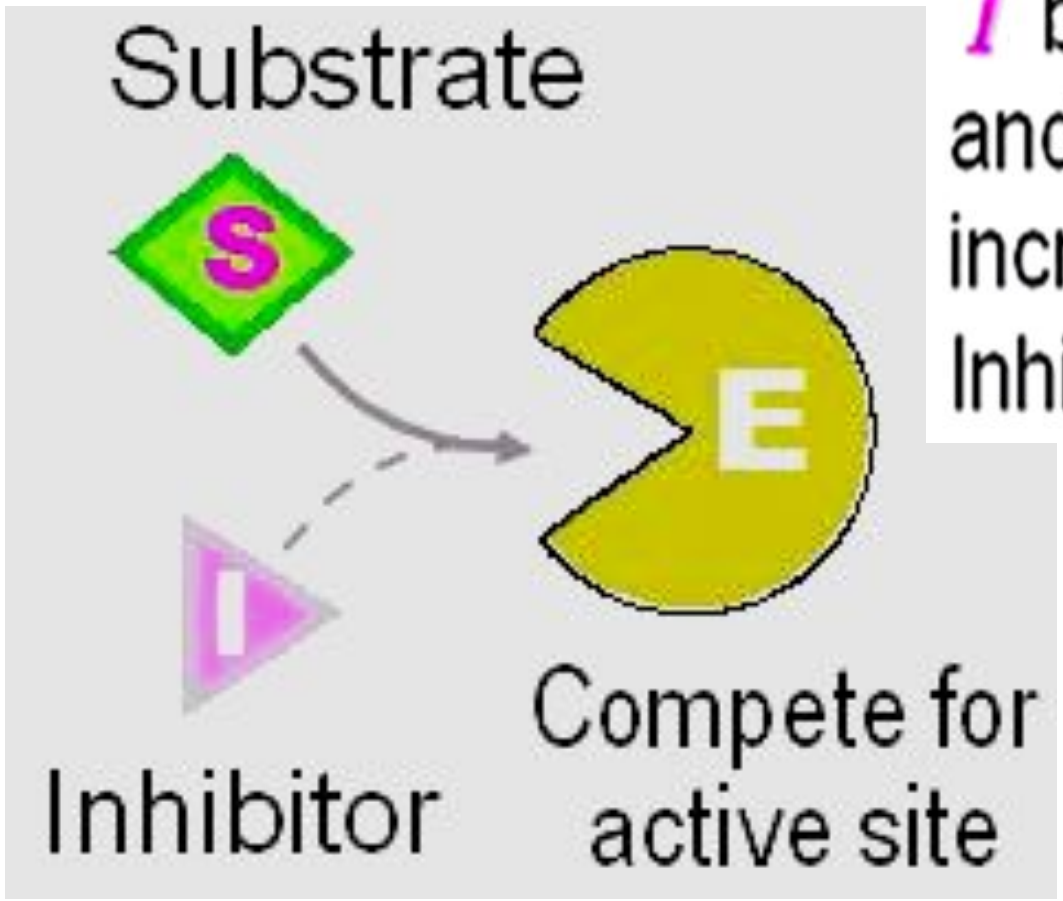
1 IU = 16.67 nkat

Enzymes activators

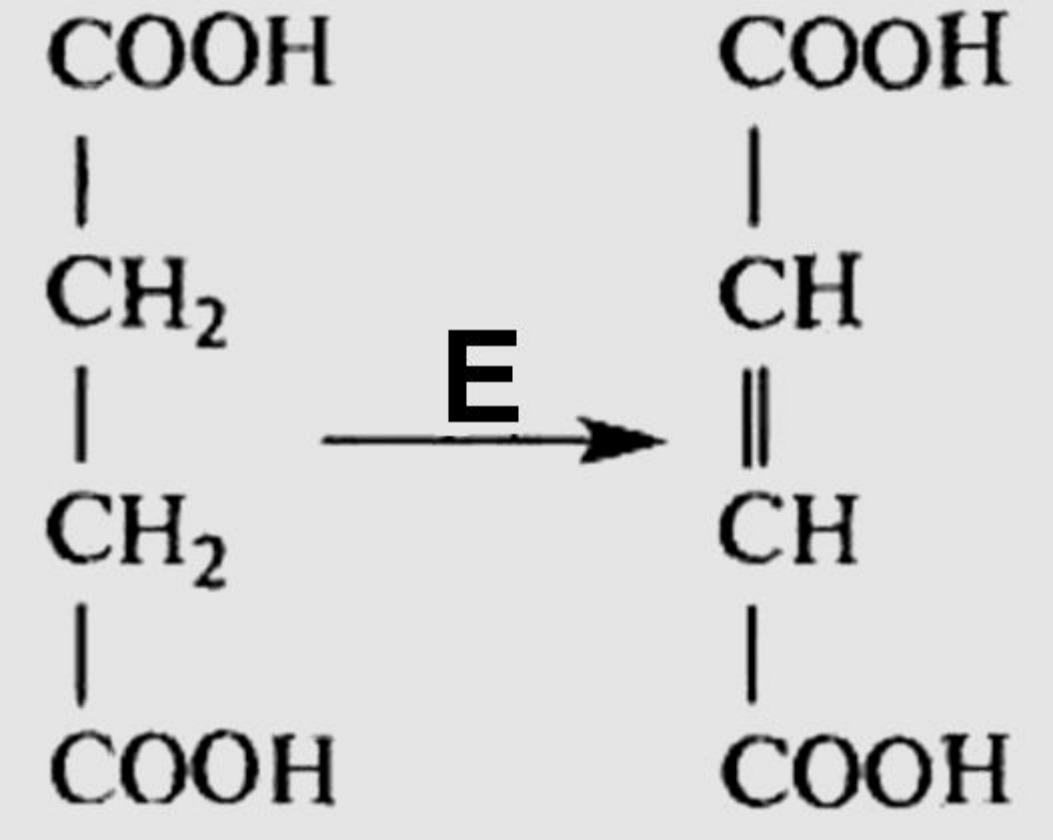
Enzyme	Activator
Cytochromes	Fe^{2+}
Amylase	Ca^{2+}, Cl^{-}
Cholinesterase	Mn^{2+}
Pancreatic lipase	Bile salts


Enzyme Inhibitors
Non-specific
Specific
Denaturation
Irreversible
Acids & Bases
Temperature
Alcohol
Heavy Metals
Reducing Agents
Reversible
Competitive
Noncompetitive,
Allosteric,
Feedback

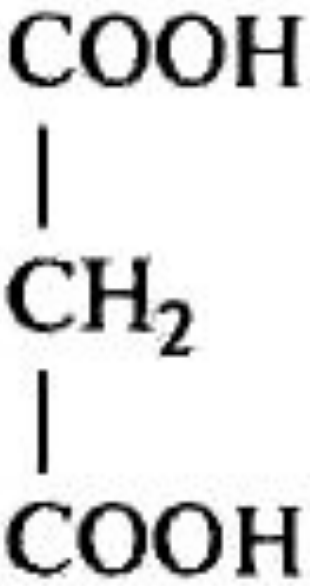
Competitive inhibition



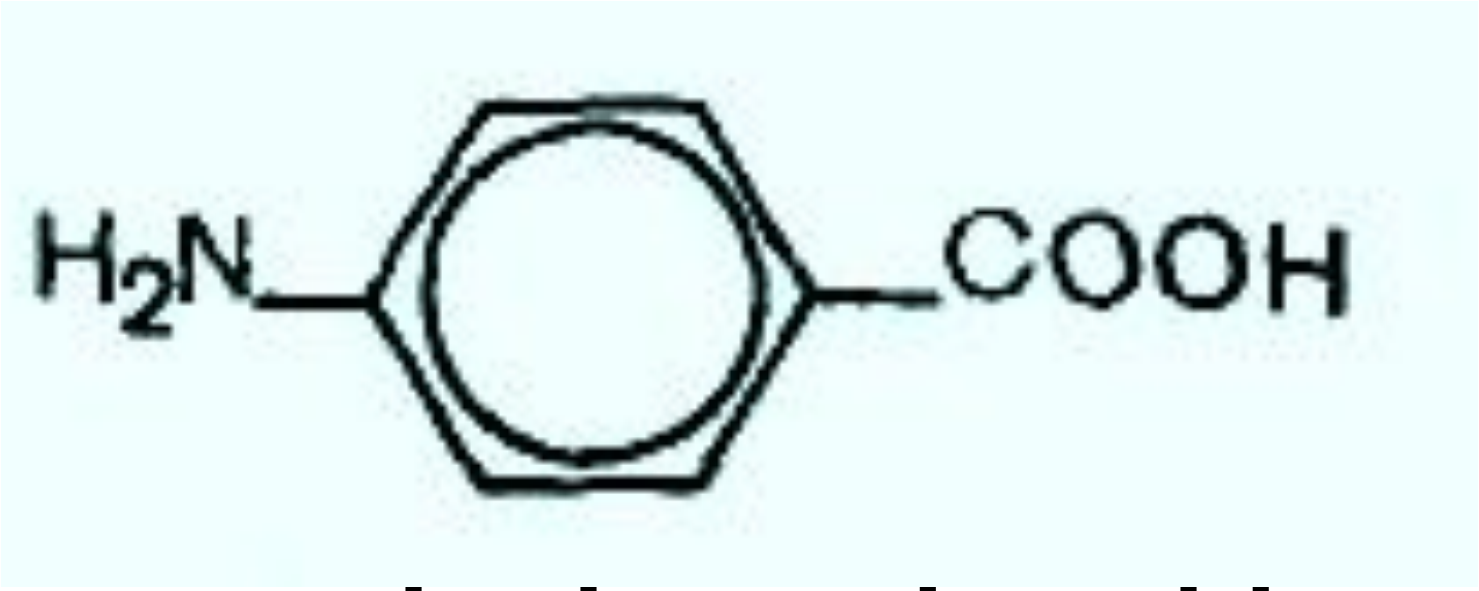
I binds to free E only, and competes with S; increasing [S] overcomes Inhibition by *I*.



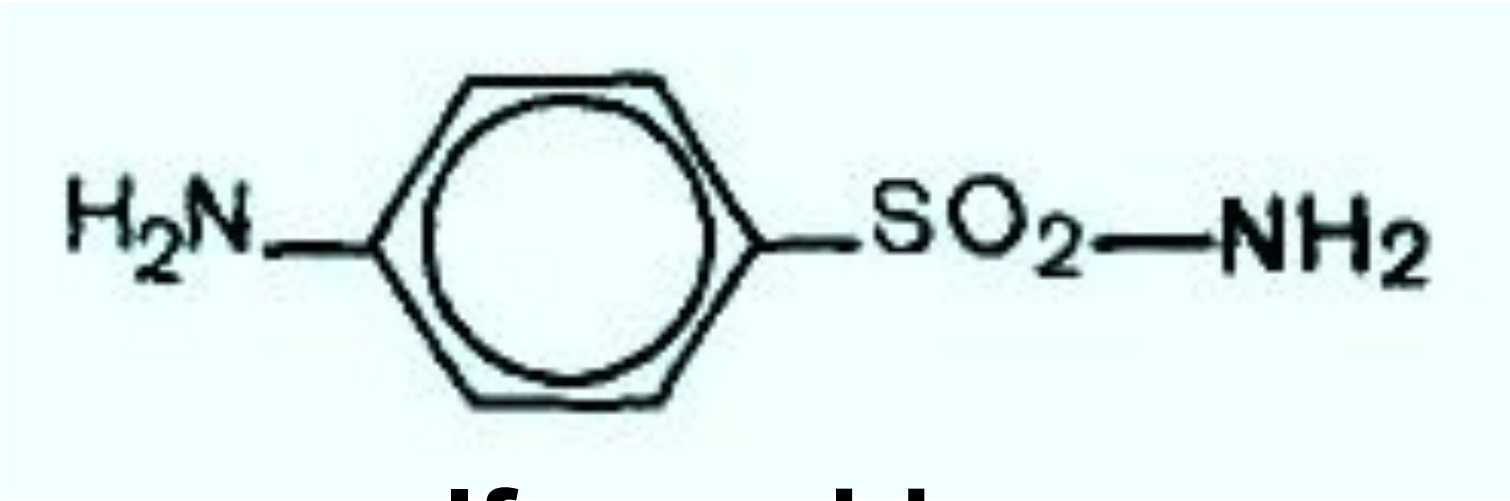
succinate fumarate



malonate



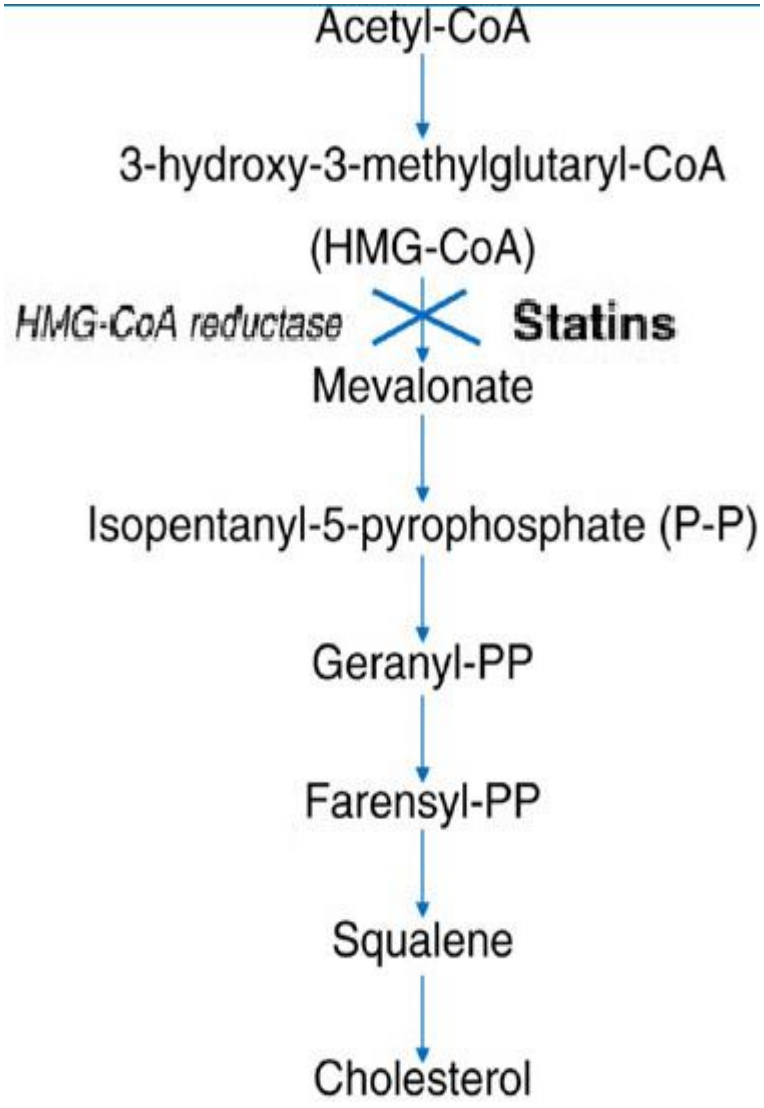
p-aminobenzoic acid

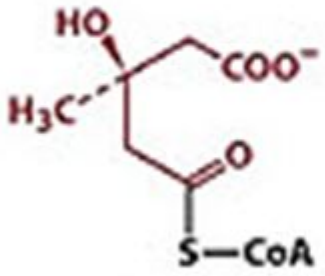


sulfonamide

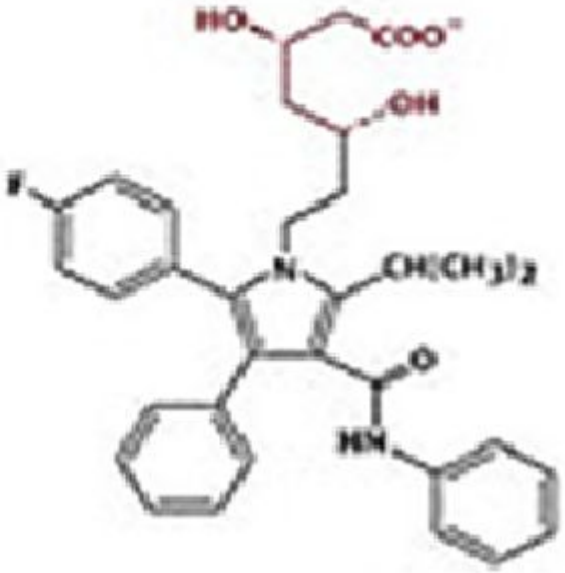
HMG-CoA REDUCTASE INHIBITORS (Statins)

- Statins are analogs of 3-OH-3-methylglutarate (HMG).



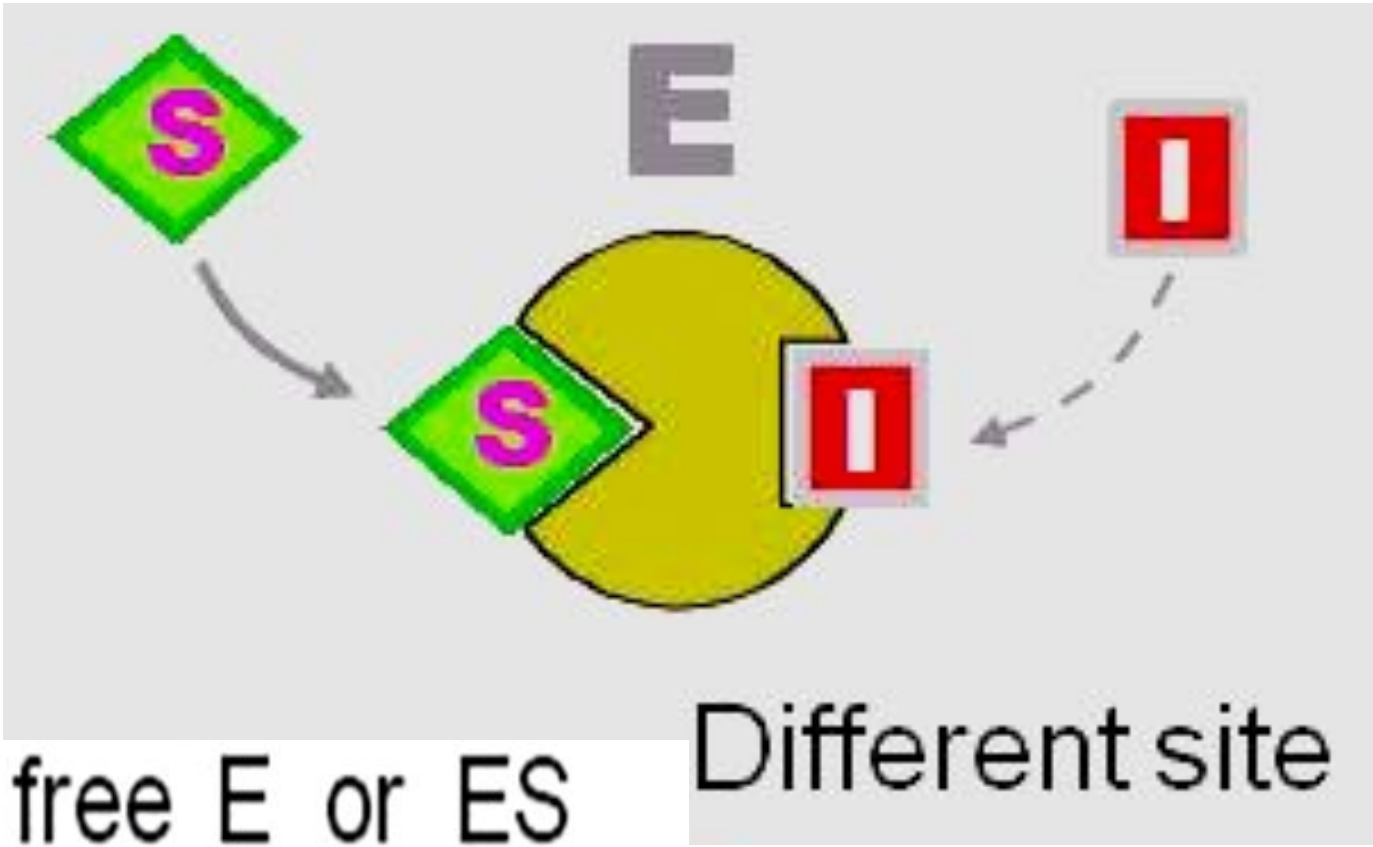


HMG-CoA

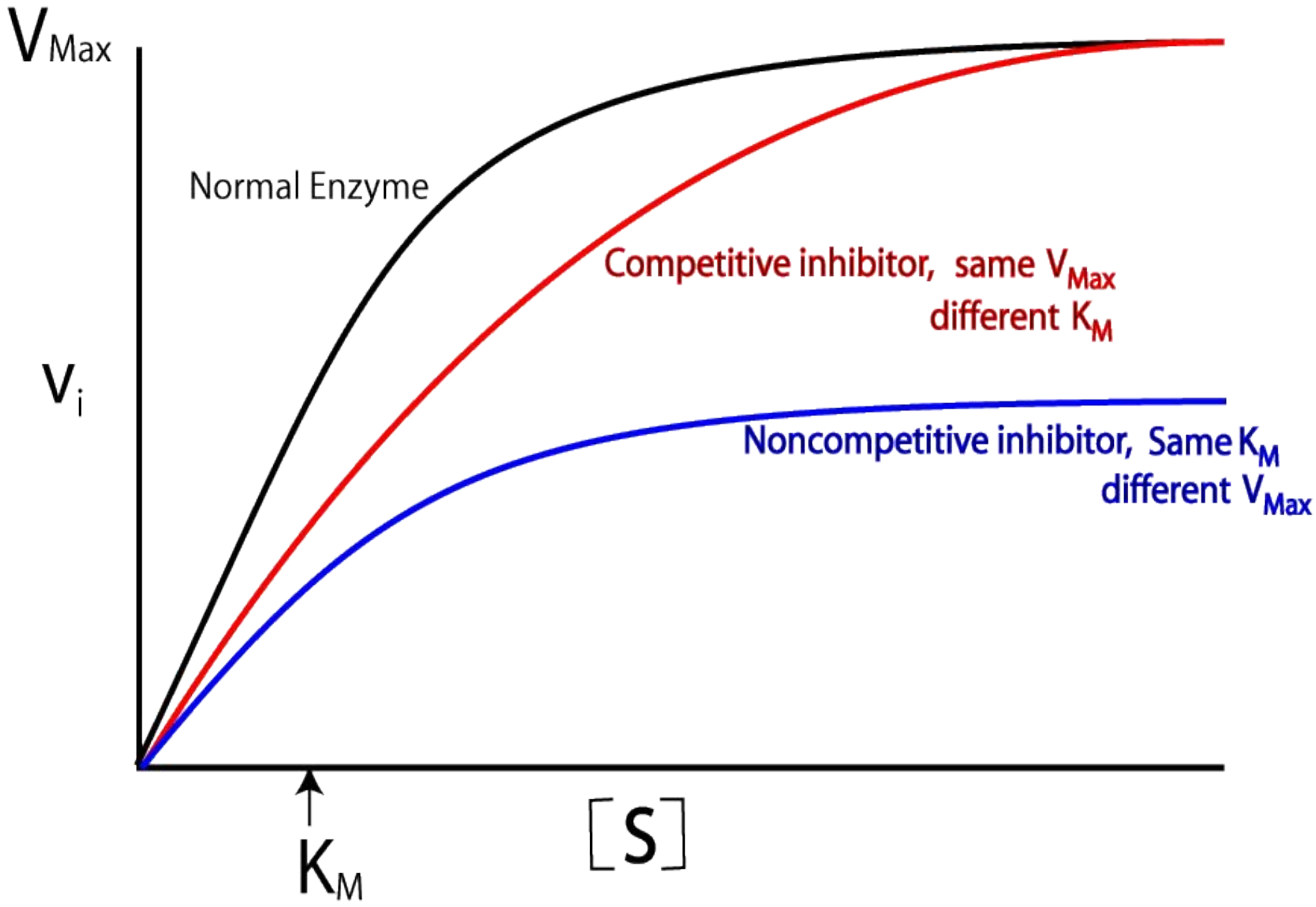


Atorvastatin

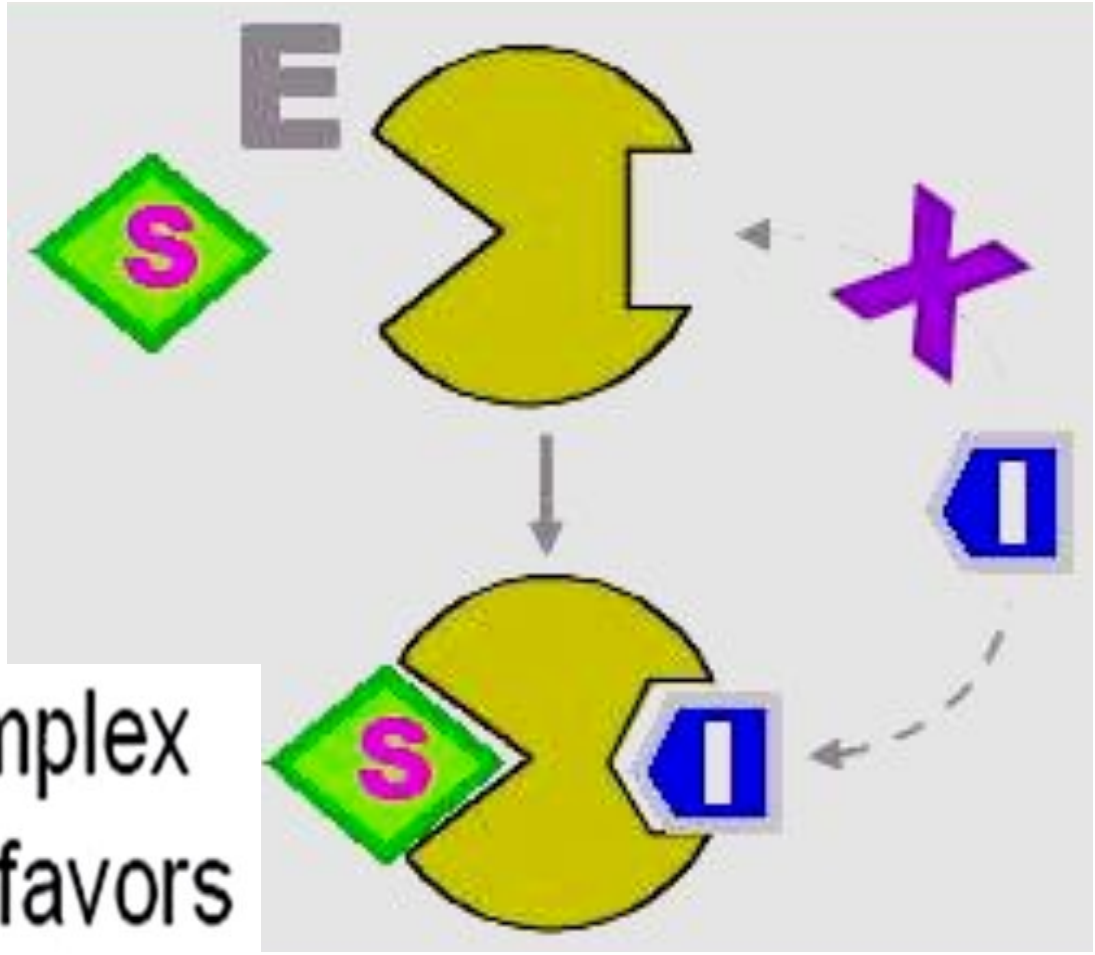
Non-competitive inhibition



I binds to free E or ES complex; Increasing [S] can not overcome *I* inhibition.

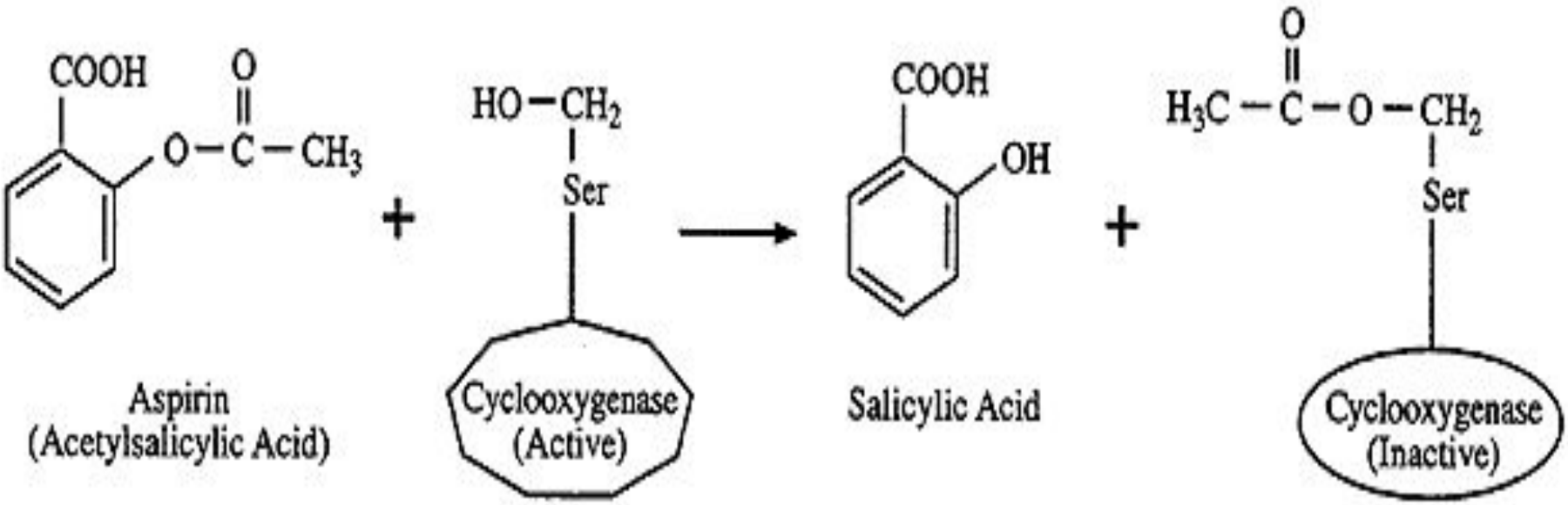

V_{Max}
Normal Enzyme
Competitive inhibitor, same V_{Max}
different K_M
V_i
Noncompetitive inhibitor, Same K_M
different V_{Max}
K_M
[S]

Un-competitive inhibition

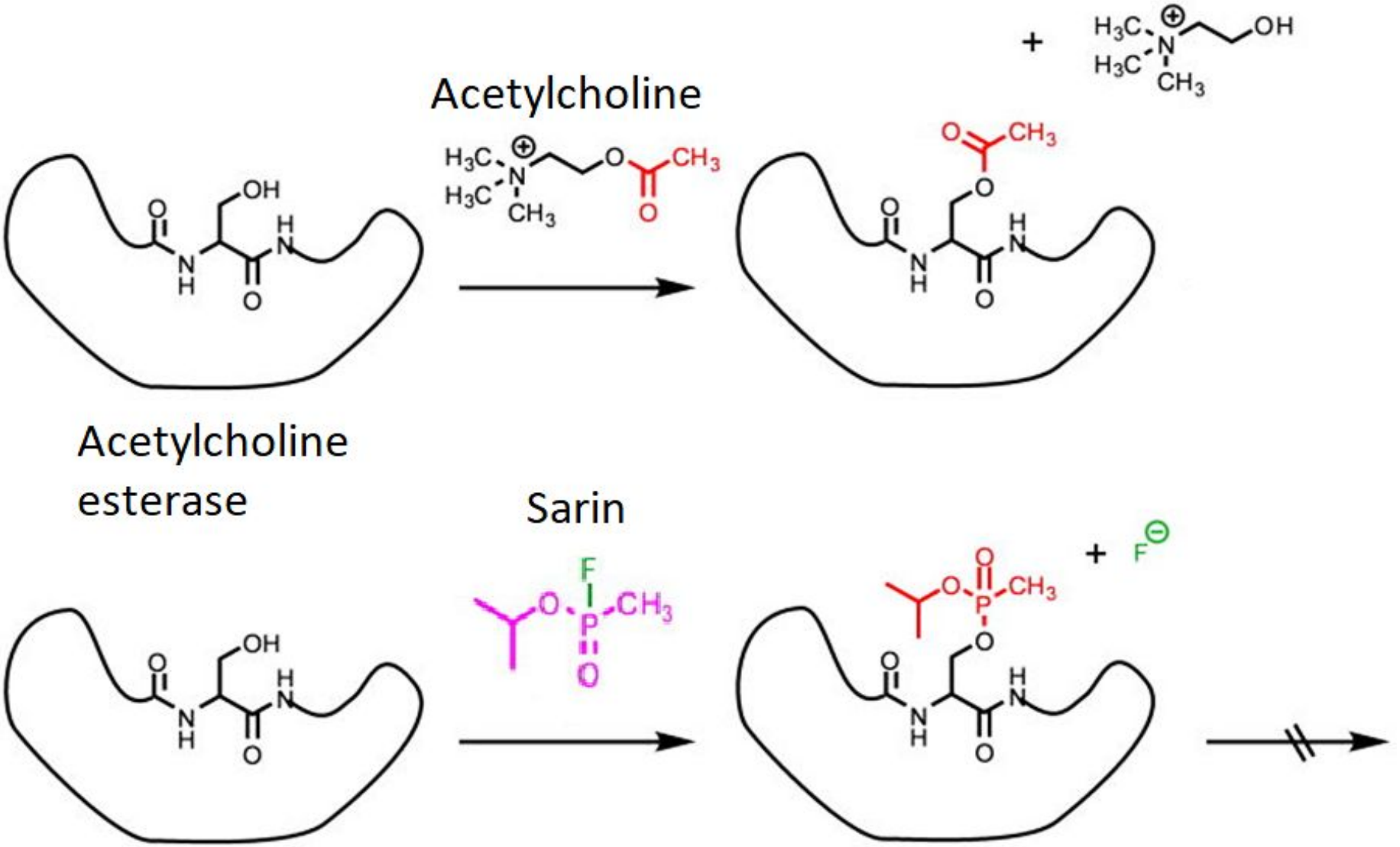


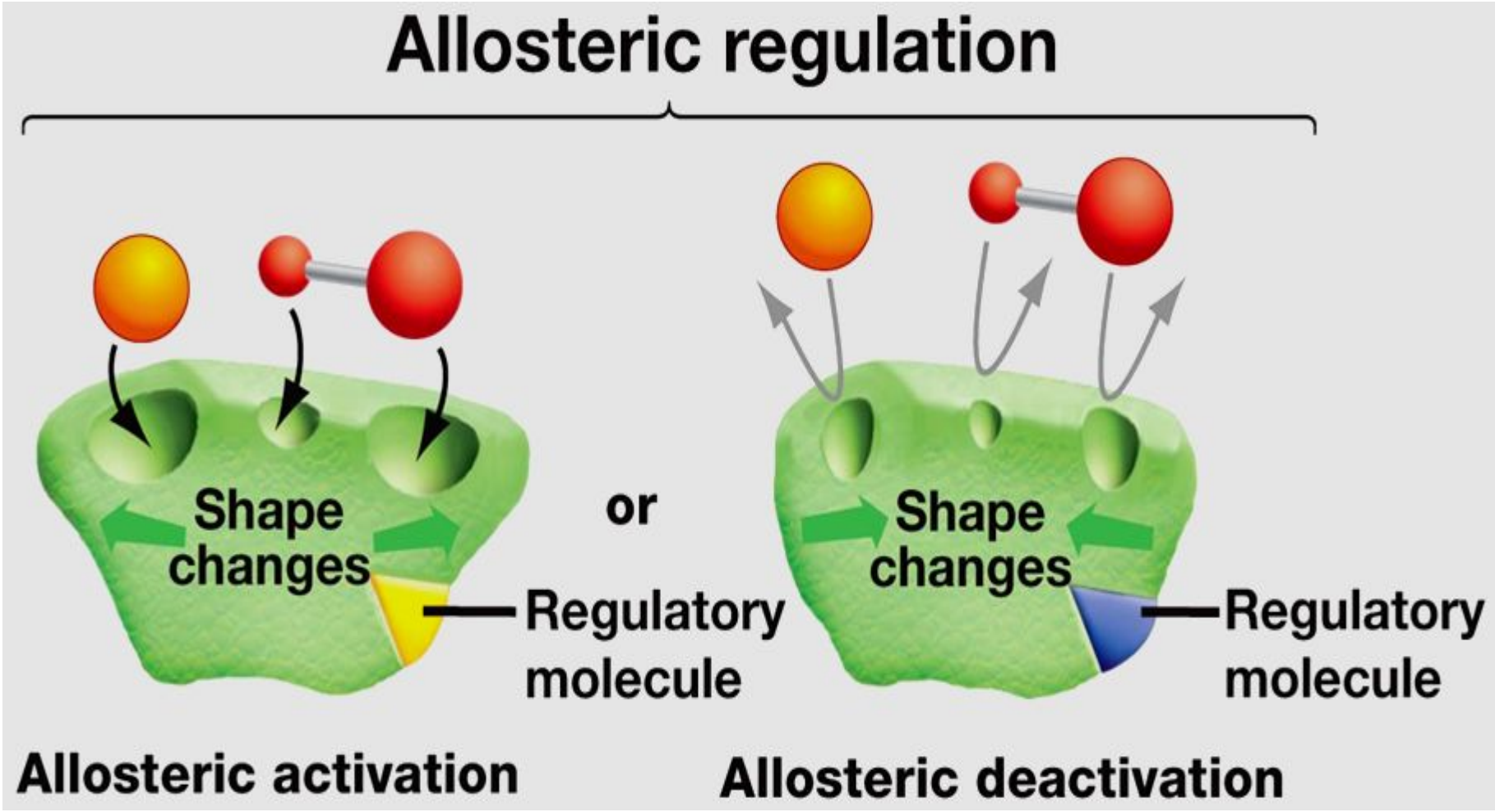
I binds to ES complex only, increasing [S] favors the inhibition by *I*.

Irreversible competitive inhibition



Irreversible competitive inhibition




Allosteric regulation
Shape changes
Regulatory molecule
or
Shape changes
Regulatory molecule
Allosteric activation
Allosteric deactivation

Feedback inhibition