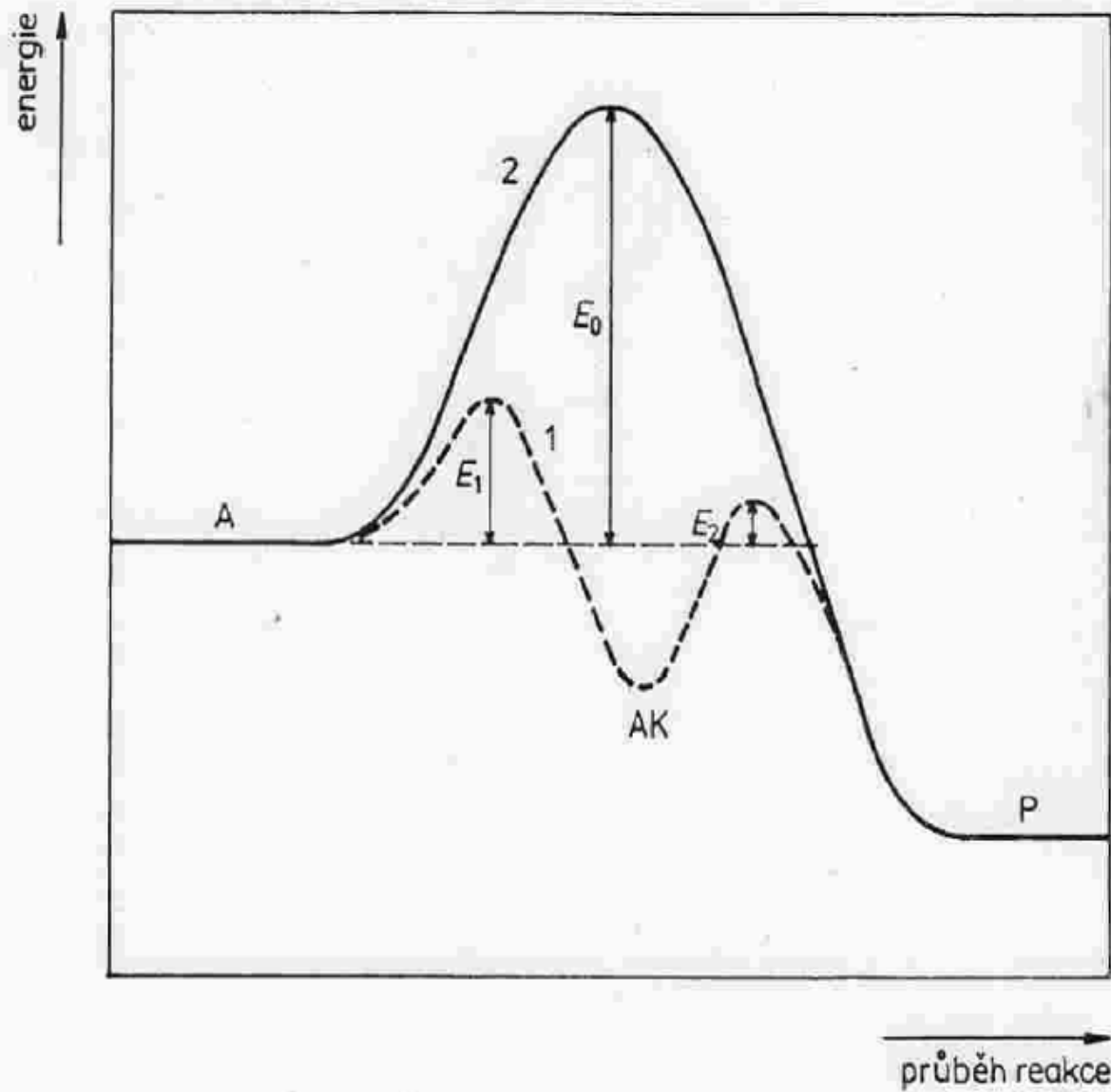
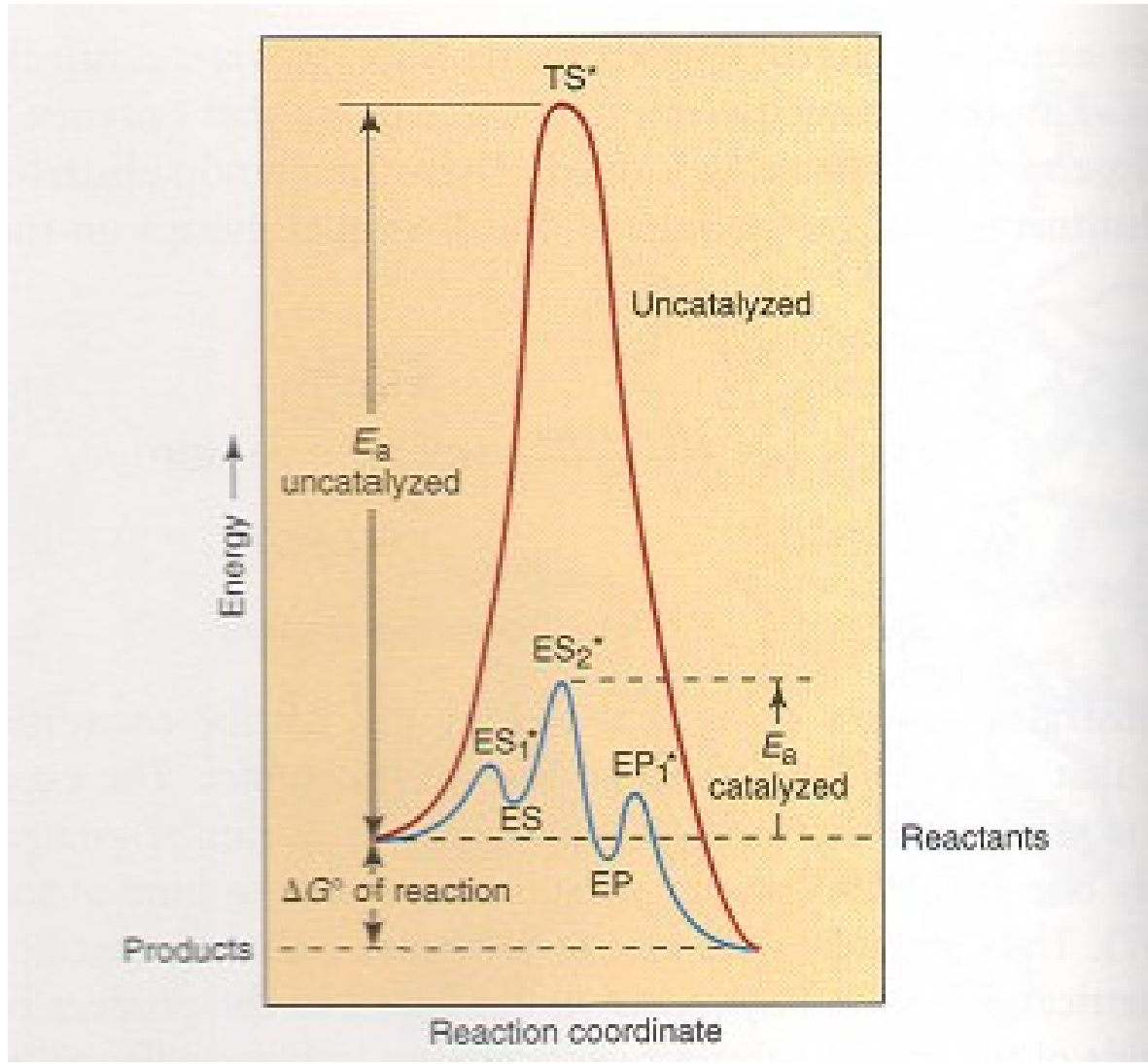


ENZYMOLOGIE

Katalýza - Berzelius 1838

- katalyzátor**
- **látky urychlující chemické reakce**
 - **nemění rovnováhu chemických reakcí**
 - **snížují aktivační energii**





Požadavky na biokatalyzátory :

A. Reakce musí probíhat cíleně.

B. Musí probíhat specificky

C. Jejich aktivita musí být přesně regulovaná

Biokatalyzátory

- Globulární bílkoviny – enzymy
- RNA - ribozymy

Historie poznání enzymů

- 1878 - KUHNEN - ENZYM - *En Zyme* - v kvasnicích
- 1860 - PASTEUR - *vis vitalis* - životní síla v kvasinkách
 - LIEBIG - *fermenty* - chemické látky
- 1897 - BUCHNER - extrakt kvasinek katalyzuje kvašení
- 1926 - SUMNER - bílkovinná povaha enzymů - ureasa

Enzymologie :

- studium struktury enzymů
- studium kinetiky enzymových reakcí
- studium reakčních mechanismů
- studium forem a lokalizace enzymů
- studium vztahu enzymů k patologii organismů
- praktické využití enzymů
- příprava a studium umělých enzymů

Názvosloví

1. triviální - *trypsin, pepsin, ptyalin*

2. název substrátu + asa - *lipasa, amylasa*

reakce + asa - *oxidasa, hydrolasa*

3. substrát + reakce - *alkoholdehydrogenasa*

substrát₁ + substrát₂ + reakce - *alkohol: NAD-oxidoreduktasa*

Enzymová nomenklatura

IUB 1961 - nejnovější 1984

1. OXIDOREDUKTASY - oxidačně redukční reakce
- *alkoholdehydrogenasa*

2. TRANSFERASY - přenos skupin
- *aspartátaminotransferasa*

3. HYDROLASA - hydrolytické štěpení (+ H₂O)
- *proteasy*

4. LYASY

- **nehydrolytické štěpení (bez H₂O)**
- *karbonátanhydrasa*

5. IZOMERASY

- **přesuny atomů a skupin**
- *glukosafosfátizomerasa*

6. LIGASY

- **vznik vazby za současného rozkladu ATP**
- *asparaginsynthetasa*

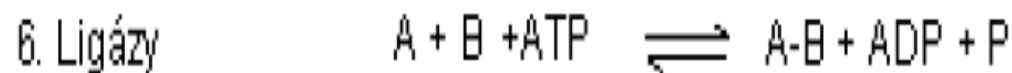
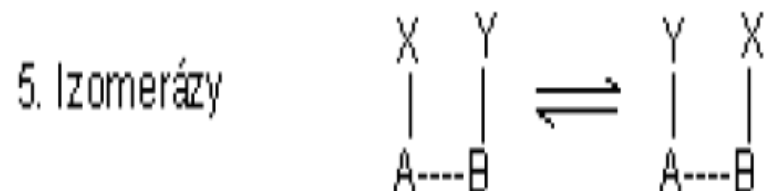
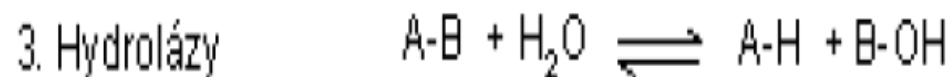
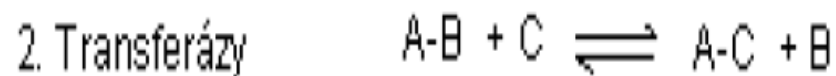
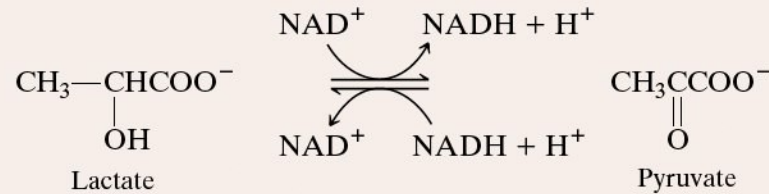


Table 5.2**An example of each class of enzyme**

1. Oxidoreductases



Common name: Lactate dehydrogenase

Official name: L-Lactate:NAD⁺ oxidoreductase

Official number: 1.1.2.3

2. Transferases

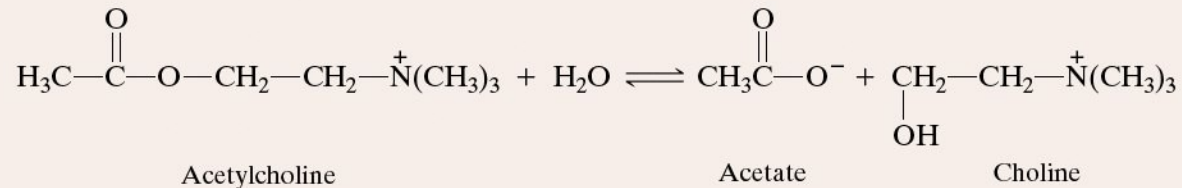
 $(dNMP)_n$ = DNA with n nucleotides $dNTP$ = deoxynucleoside triphosphate $(dNMP)_{n+1}$ = DNA with $n + 1$ nucleotides PP_i = Pyrophosphate

Common name: DNA polymerase

Official name: Deoxynucleoside triphosphate:DNA deoxynucleotidyltransferase (DNA-directed)

Official number: 2.7.7.7

3. Hydrolases



Common name: Acetylcholinesterase

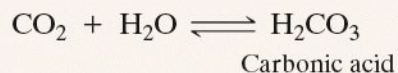
Official name: Acetylcholine acetylhydrolase

Official number: 3.1.1.7

Table 5.2

An example of each class of enzyme

4. Lyases

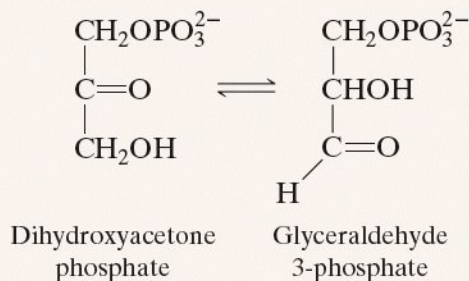


Common name: Carbonic anhydrase

Official name: Carbonate hydrolyase

Official number: 4.2.1.1

5. Isomerases

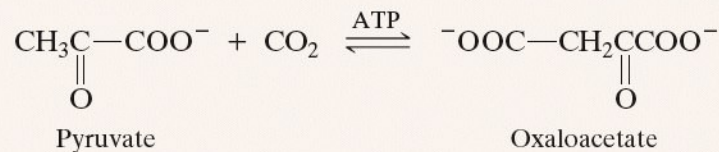


Common name: Triose phosphate isomerase

Official name: D-Glyceraldehyde-3-phosphate ketoisomerase

Official number: 5.3.1.1

6. Ligases



Common name: Pyruvate carboxylase

Official name: Pyruvate CO₂ ligase (ADP-forming)

Official number: 6.4.1.1

EC 1.1.1.27

1.

1.

1.

27

Enzyme Commission

Třída - *oxidoreduktasa*

Podtřída - *skupina CHOH*

Podpodtřída - *koenzym NAD*

Číslo enzymu

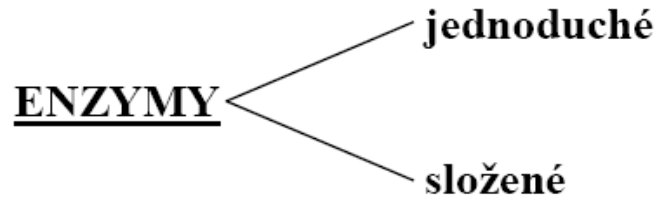
Vyjádřování aktivity enzymů :

- smluvené jednotky
- **IU - International Unit - mezinárodní jednotka (IUB 1961)**
- počet mikromolů přeměněného substrátu za minutu
- **kat - katal (IUB 1971)**
- počet molů přeměněného substrátu za sekundu

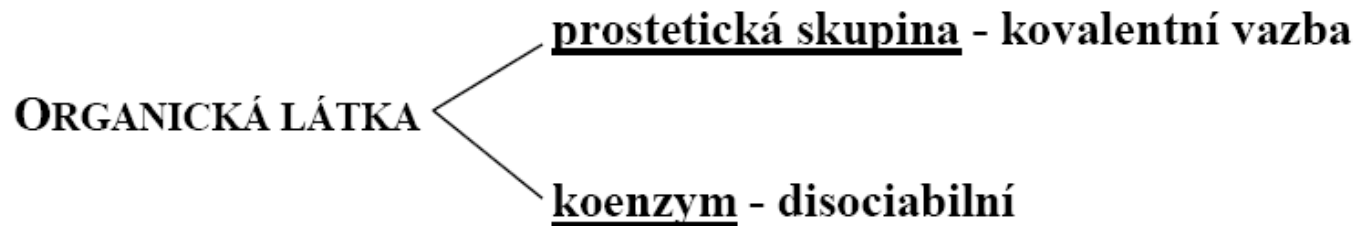
Specifická aktivita - aktivita vztažená na mg bílkoviny

Číslo přeměny - počet molů substrátu přeměněných molem enzymu za jednu sekundu

STRUKTURA ENZYMŮ



KOFAKTOR + APOENZYM → HOLOENZYM



Kofaktor - kovový ion nebo organická látka

METALOENZYMY

kovový ion	enzym
Zn^{2+}	alkoholdehydrogenasa alkalická fosfatasa karbonátanhydrasa
Mg^{2+}	fosfohydrolasy fosfotransferasy
Mn^{2+}	arginasa
$\text{Fe}^{2+}, \text{Fe}^{3+}$	cytochromy peroxidasa katalasa
$\text{Cu}^{2+}, \text{Cu}^{+}$	tyrosinasa diaminoxidasa

Table 6.2

Enzymes requiring metal ions as cofactors

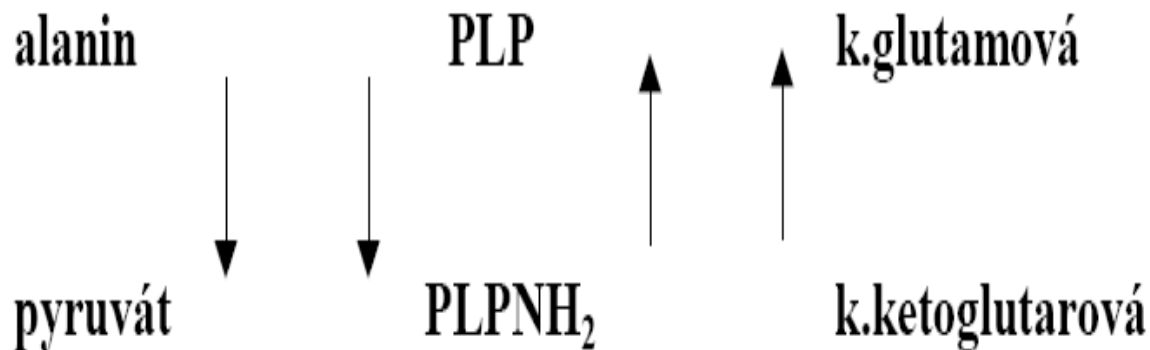
Enzyme	Metal Ion
Catalase, peroxidase, aconitase, and cytochrome oxidase	Fe ²⁺ and Fe ³⁺
Alcohol dehydrogenase, carboxypeptidase A, carboxypeptidase B, and DNA polymerase	Zn ²⁺
Cytochrome oxidase, lysyl oxidase, ascorbate oxidase, and superoxide dismutase	Cu ²⁺
Hexokinase and glucose-6-phosphatase	Mg ²⁺
Arginase	Mn ²⁺
Pyruvate kinase	K ⁺
Urease	Ni ²⁺
Nitrate reductase	Mo ⁴⁺ and Mo ⁶⁺
Carbonic anhydrase	Zn ²⁺ , Cd ²⁺

Table 6-2 Concepts in Biochemistry, 3/e

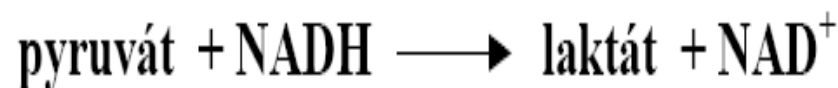
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Regenerace kofaktorů

1. *Prostetická skupina* se regeneruje na téže enzymové bílkovině :



2. *Koenzym* se odštěpí napojí se na jiný apoenzym a regeneruje se v jiné enzymové reakci :



KOFAKTORY A VITAMINY

VITAMIN - FUNK - “amin potřebný pro život”

Vitamin	Kofaktor	Funkce
<u>rozpustné ve vodě</u>		<u>přenos (reakce)</u>
thiamin - B₁	thiamindifosfát TPP	aldehydicke s.
riboflavin - B₂	FMN, FAD	H
k.nikotinová(nikotinamid)	NAD⁺, NADP	H
k.pantothenová	CoA	acylové s.
k.listová	k.listová	C₁ skupin
pyridoxin - B₆	pyridoxalfosfát	aminoskupiny
kobalamin - B₁₂	kobalamin	izomerace
k.askorbová - C	k.askorbová	hydroxylace
biotin - H	biotin	COOH
k. lipoová	k. lipoová	H
<u>rozpustné v tucích</u>		
karotenoidy - A		proces vidění
kalciferoly - D		metabolismus Ca
 tokoferoly - E		antioxidans
maftochinony - A		srážení krve

Table 6.1

Characteristics of vitamins and coenzymes

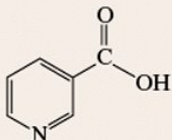
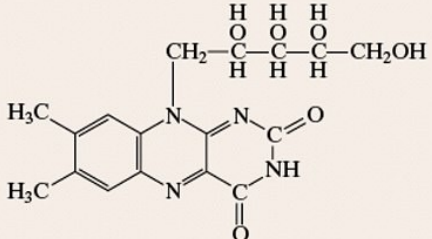
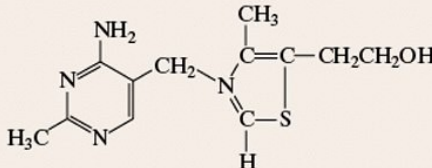
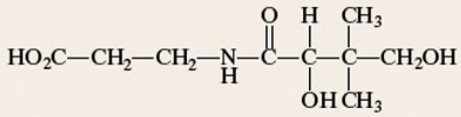
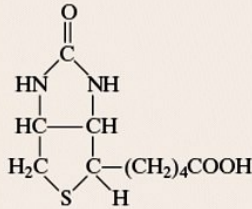
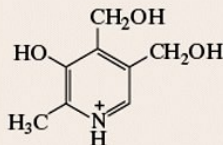
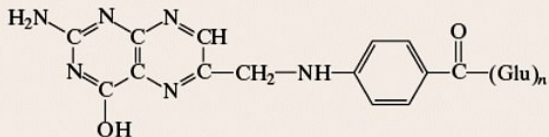
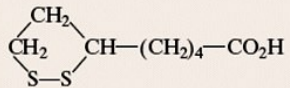
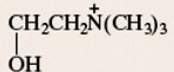
Name/Structure of Vitamin	Related Coenzyme	Reaction type (page numbers ^a)	Deficiency Disease
Water-Soluble Vitamins			
<p>Niacin</p> 	NAD ⁺ , NADP ⁺	Oxidation–reduction (pp. 485-494, 505-508, 515-524)	Pellagra
<p>Riboflavin (vitamin B₂)</p> 	FAD, FMN	Oxidation–reduction (pp. 485-494, 515-524)	Growth retardation
<p>Thiamine (vitamin B₁)</p> 	Thiamine pyrophosphate	Decarboxylation (pp. 461, 463, 487-494)	Beriberi
<p>Pantothenic acid (vitamin B₃)</p> 	Coenzyme A	Acyl group activation and transfer (pp. 440-441, 485-494, 563-571)	Dermatitis (chickens)

Table 6-1 part 1 Concepts in Biochemistry, 3/e
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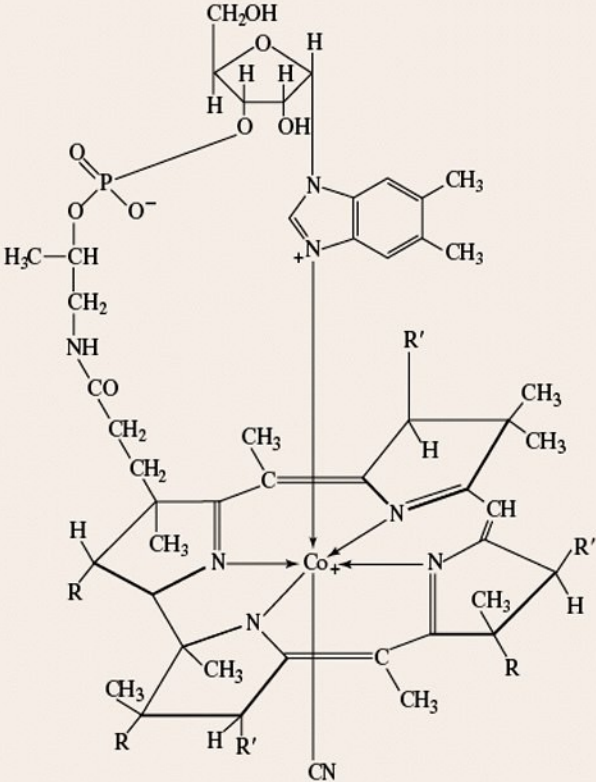
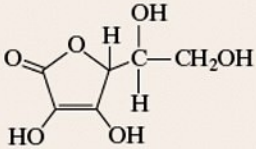
<p>Biotin</p> 	Biotinylated enzymes	CO ₂ activation and transfer (pp. 465-466)	Dermatitis (humans)
<p>Pyridoxine (vitamin B₆)</p> 	Pyridoxal phosphate	Amino group transfer (pp. 605-606)	Dermatitis (rats): neurological symptoms
<p>Folic acid</p> 	Tetrahydrofolate	Transfer of one carbon unit (pp. 600-601)	Anemias
<p>Lipoic acid (may not be a vitamin)</p> 	Attached to ε-NH ₂ group of Lys in protein	Acyl group activation and transfer (pp. 485-493)	Growth deficiencies
<p>Choline</p> 	?	(pp. 163, 171, 242-243)	Impaired brain development

(continued)

Table 6-1 part 2 Concepts in Biochemistry, 3/e
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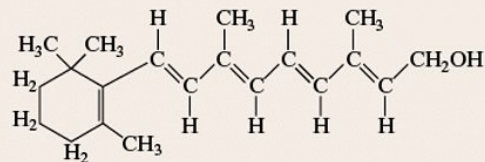
Table 6.1 (continued)

Characteristics of vitamins and coenzymes

Name/Structure of Vitamin	Related Coenzyme	Reaction type (page numbers ^d)	Deficiency Disease
<p>Water-Soluble Vitamins (continued)</p> <p>Cobalamin (vitamin B₁₂)</p> 	5'-Deoxyadenosylcobalamin	Methyl group transfer (pp. 570-571)	Pernicious anemia
<p>L-Absorbic acid (vitamin C)</p> 	L-Absorbic acid	Hydroxylation (pp. 105-107, 493)	Scurvy

Fat-Soluble Vitamins

trans-Retinol (vitamin A)

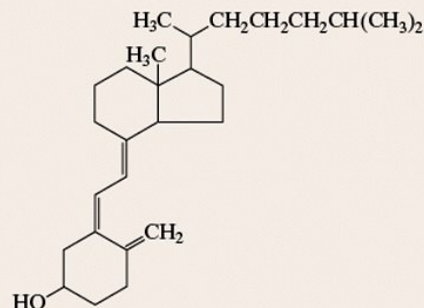


Associated with visual pigment

(pp. 123-125, 252)

Night blindness, other effects

Cholecalciferol (vitamin D₃)

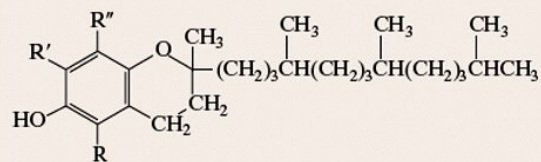


None

(pp. 252, 582-584)

Rickets

Tocopherol (vitamin E)



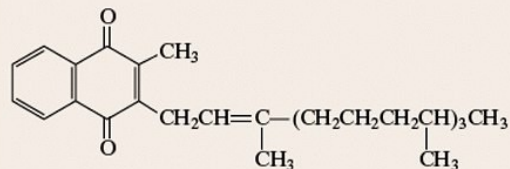
None

(p.252)

Reproductive and other problems in rats; uncertain in humans

(several variants, with R, R', R''=H or CH₃)

Phylloquinone (vitamin K₁)



None

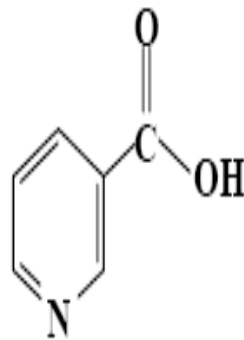
(pp. 252, 539-544)

Problems in blood clotting

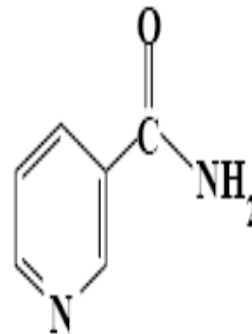
^a Page numbers listed here refer to page numbers in this book.

NIKOTINAMIDOVÉ KOENZYMY

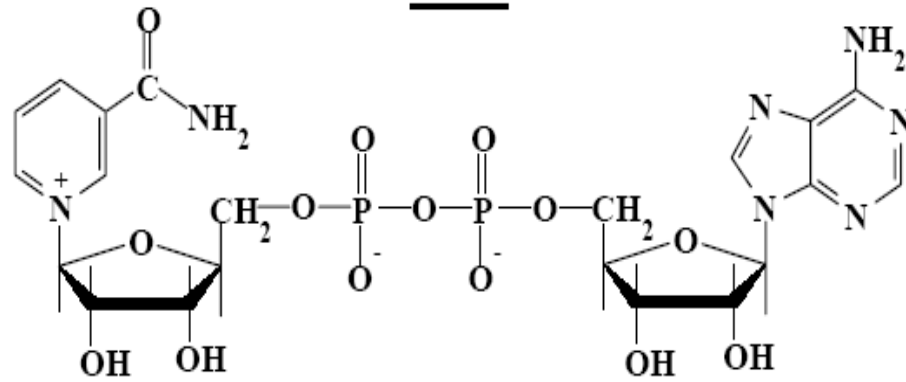
k. nikotinová



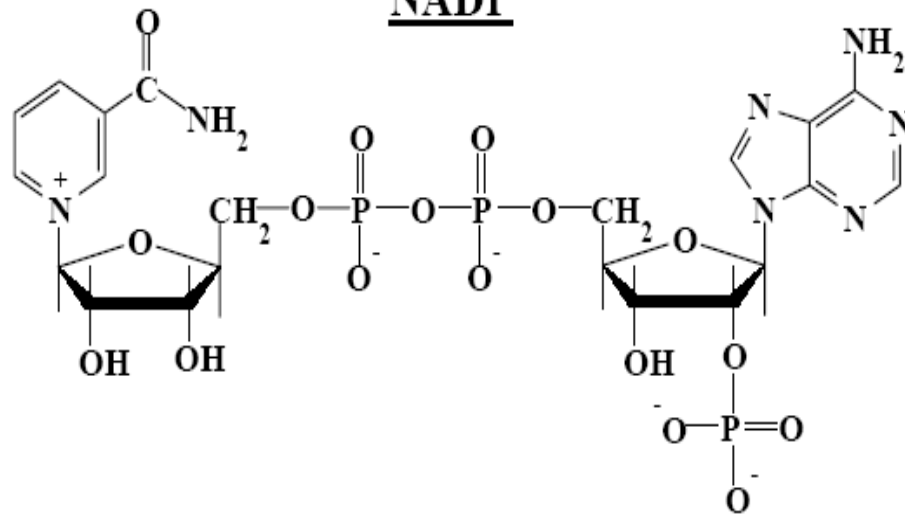
nikotinamid

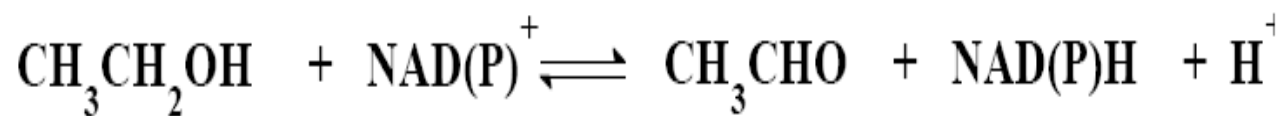
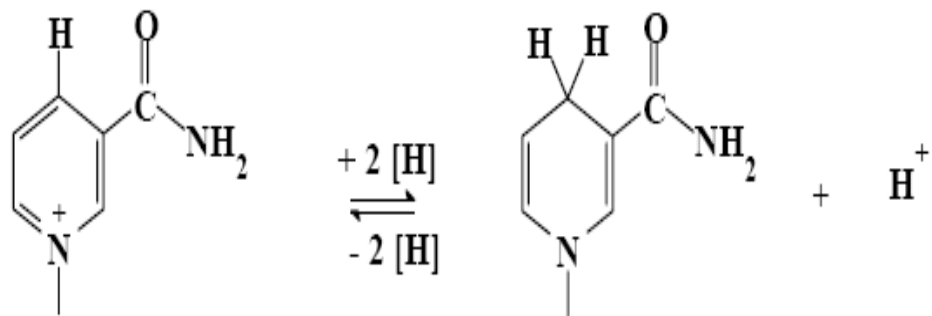


NAD⁺

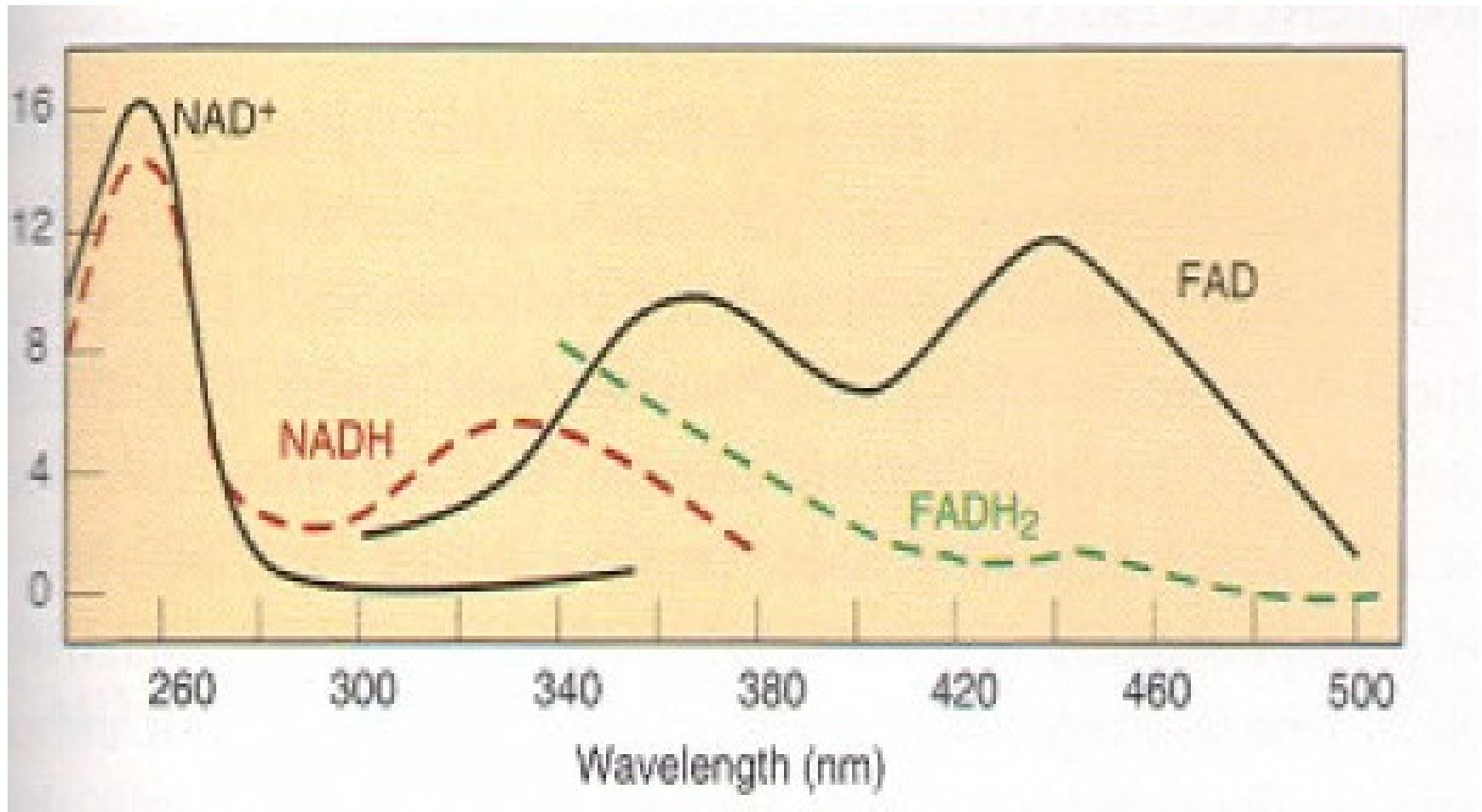


NADP⁺



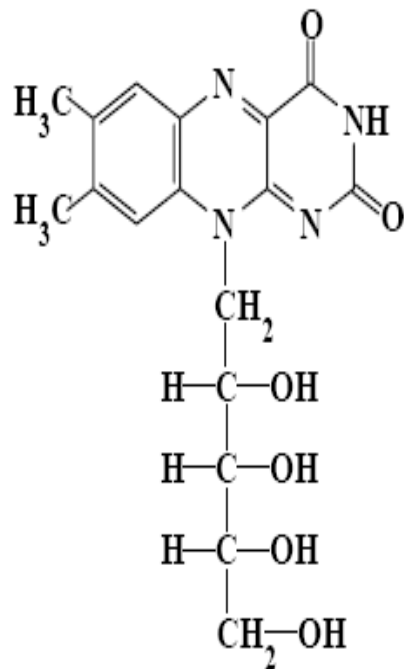


Warburgův optický test

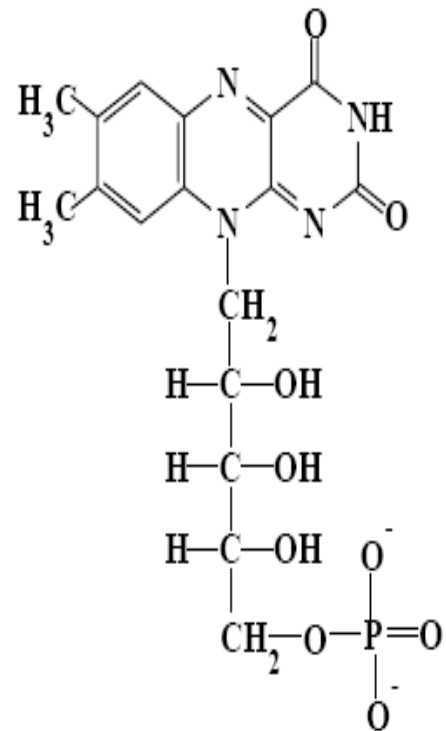


FLAVINOVÉ KOENZYMY

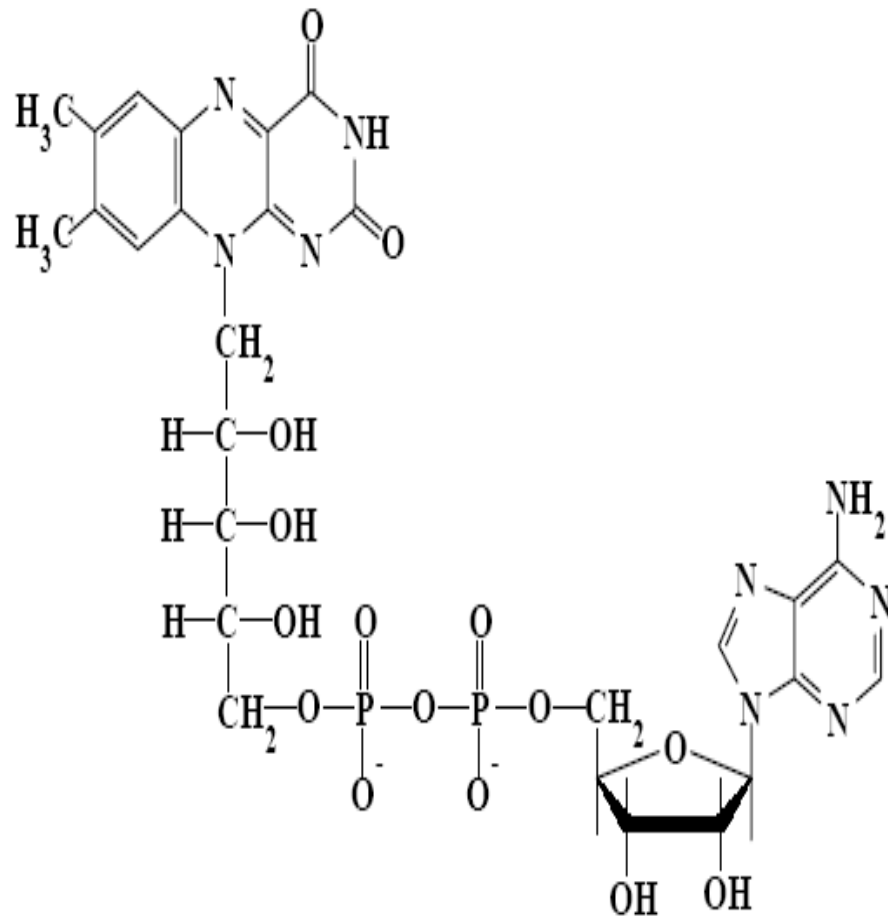
riboflavin

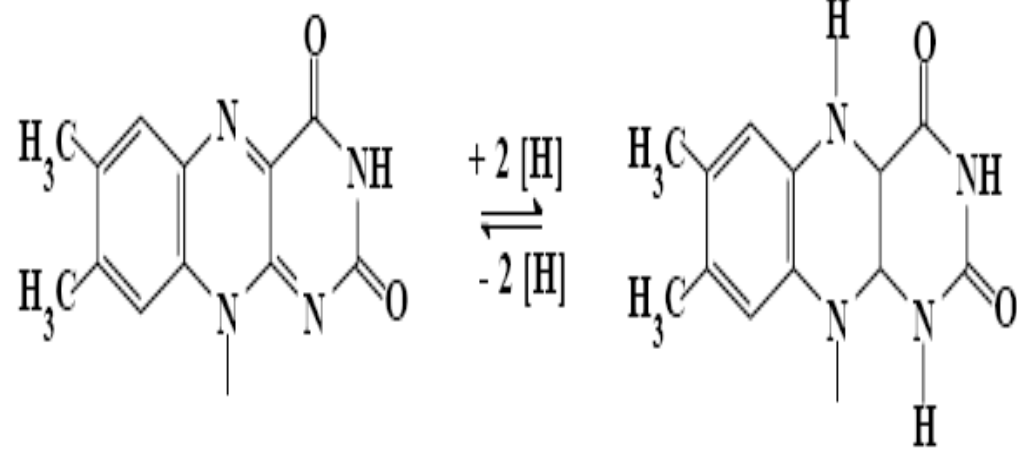


FMN

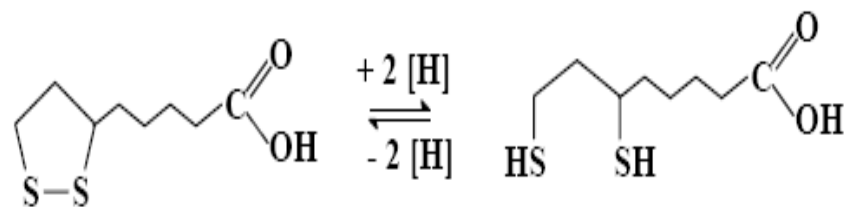


FAD

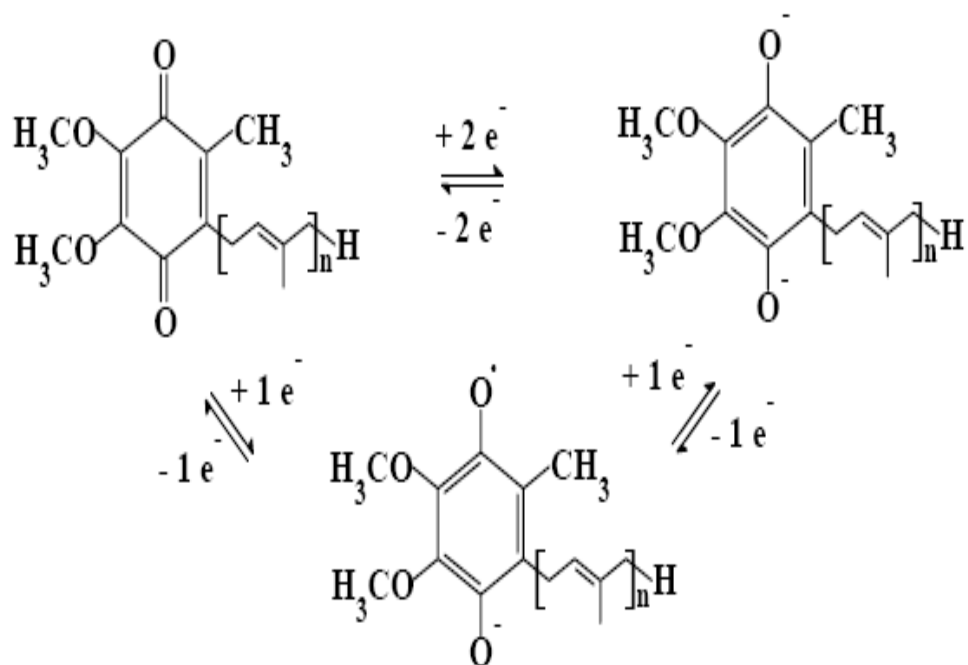




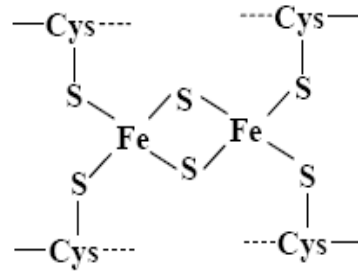
k.lipoová



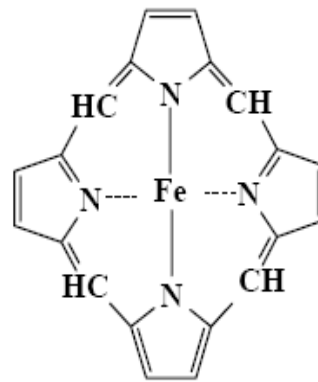
ubichinon



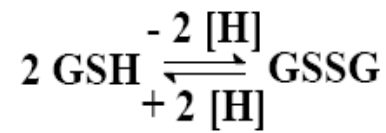
ferredoxin



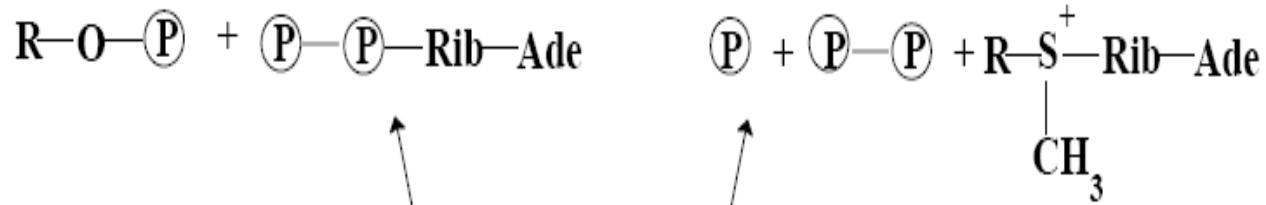
hem



glutathion

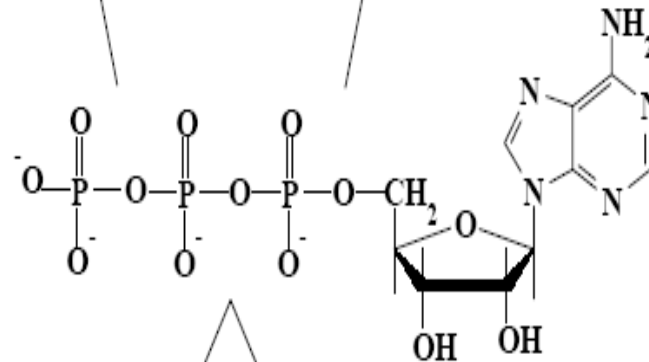


ATP



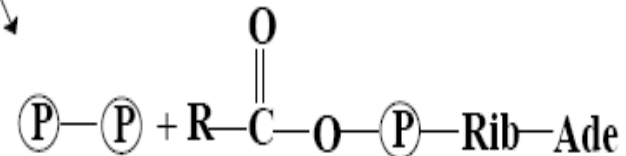
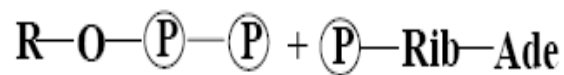
R-OH

R-S-CH₃

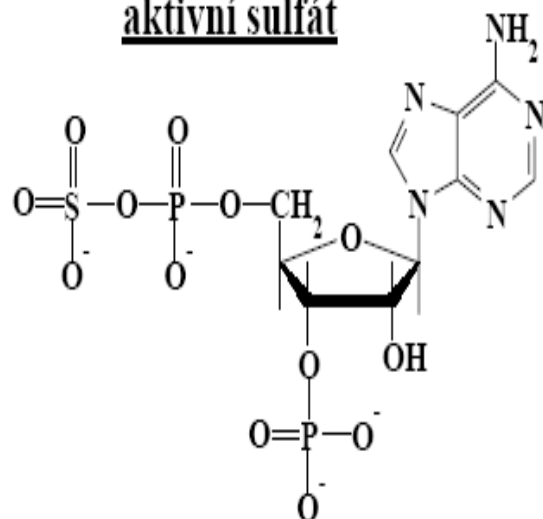


R-OH

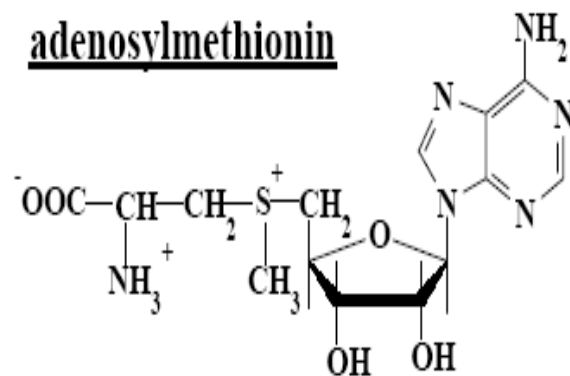
R-COOH



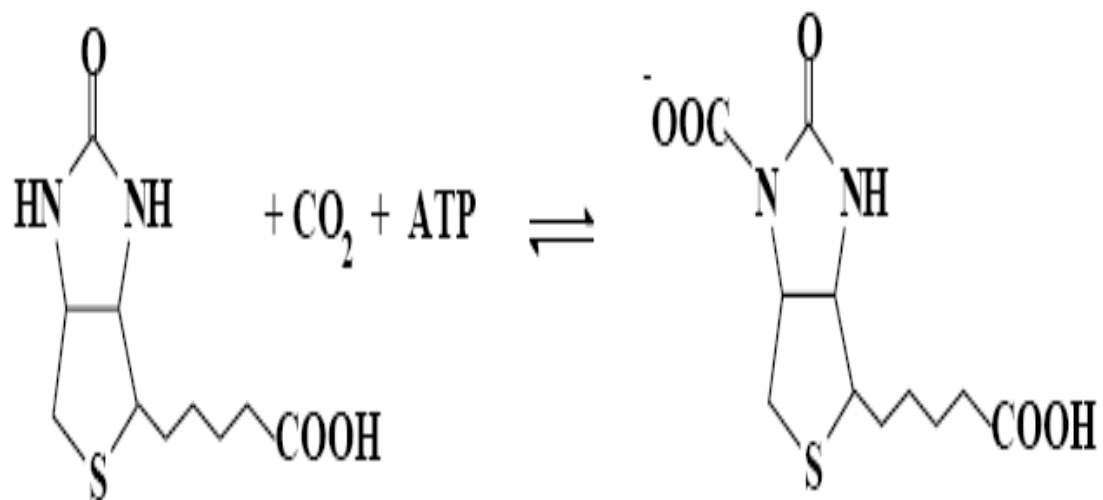
aktivní sulfát



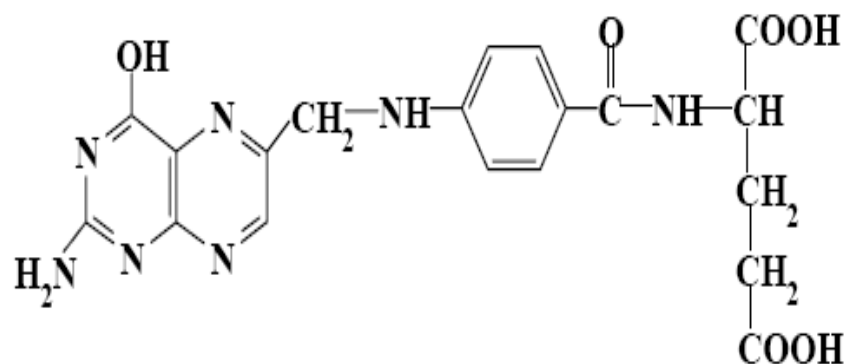
adenosylmethionin



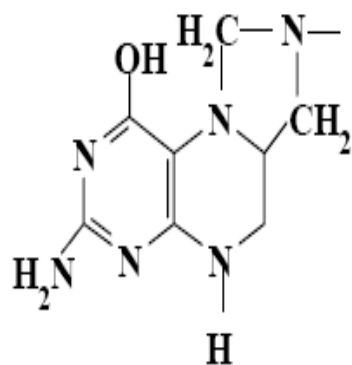
biotin



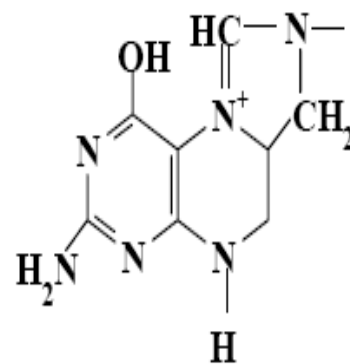
tetrahydrolistová k.



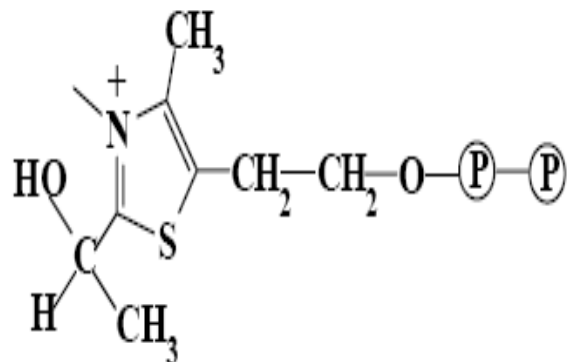
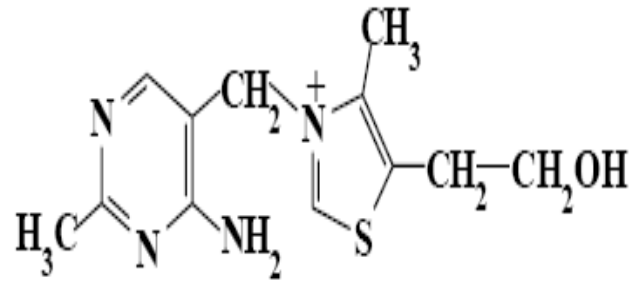
methylenetetrahydrolistová k.



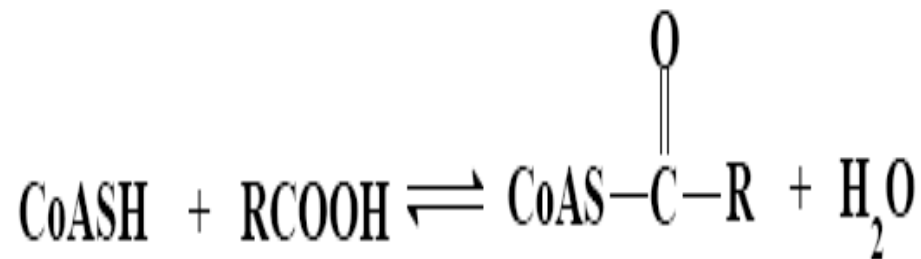
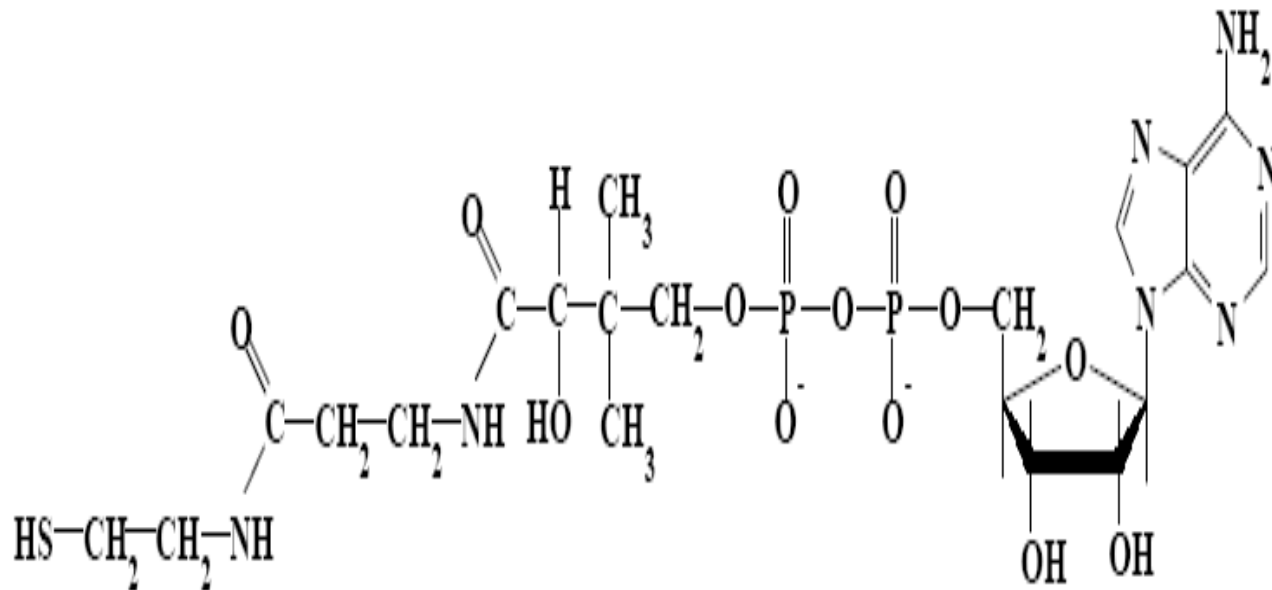
methenyltetrahydrolistová k.



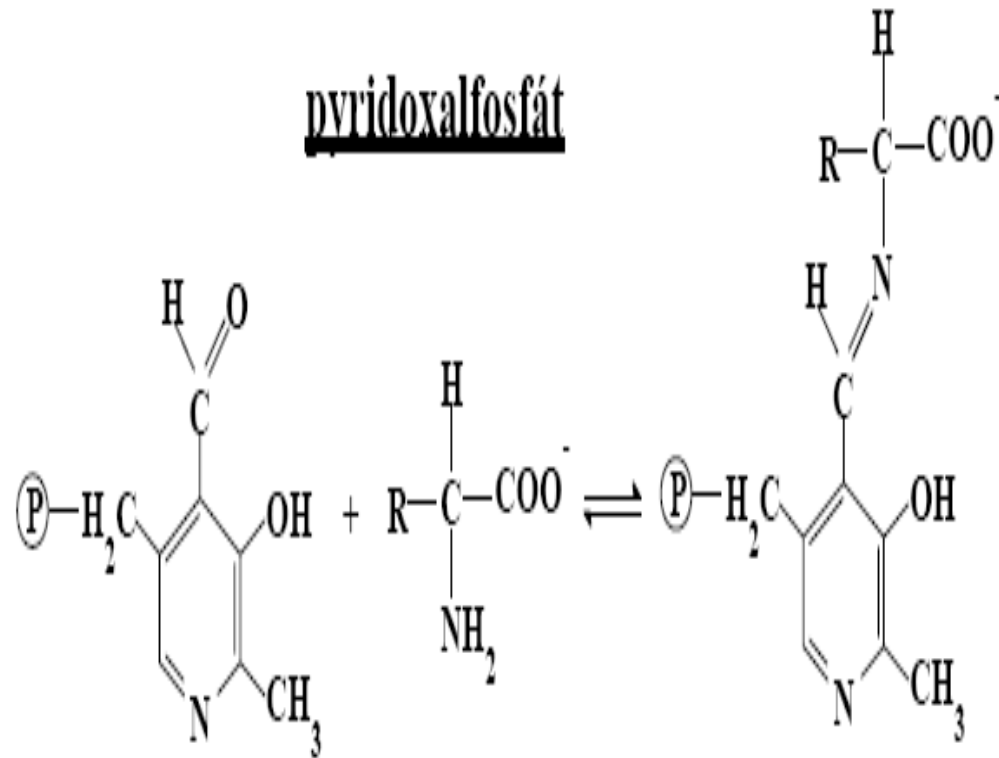
thiamin



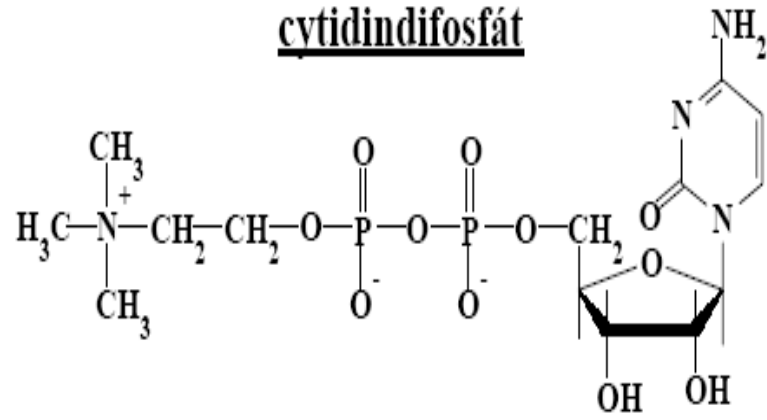
koenzym A - CoA - CoASH



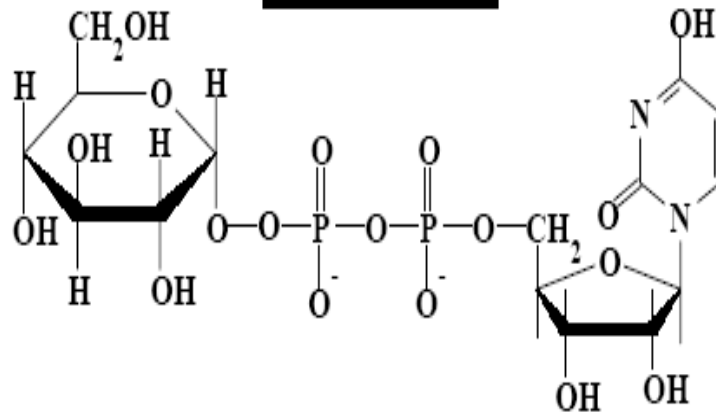
pyridoxalfosfát



cytidindifosfát



uridindifosfát



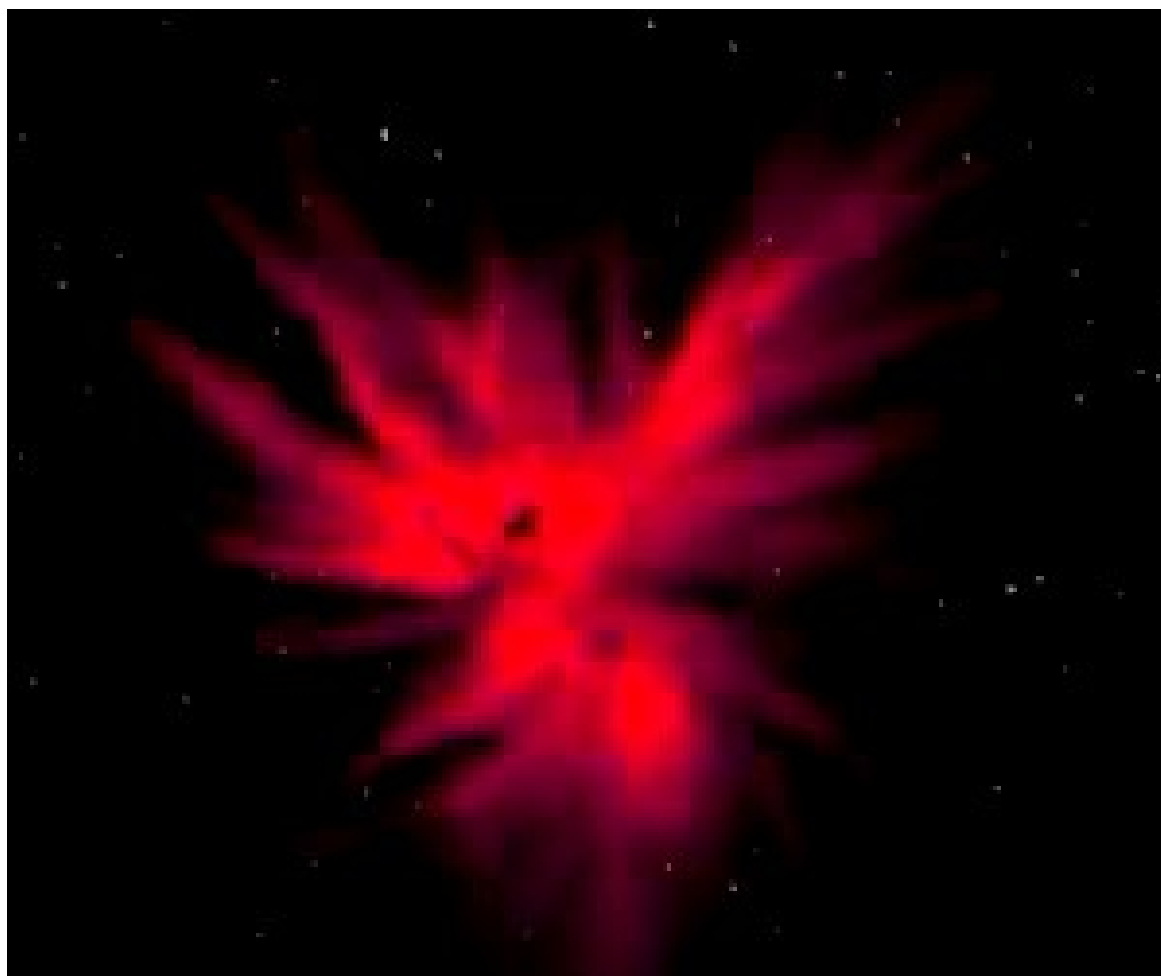
Lyasy a ligasy - bez kofaktoru nebo již popsáným kofaktorem TPP

Hydrolasy - bez kofaktoru

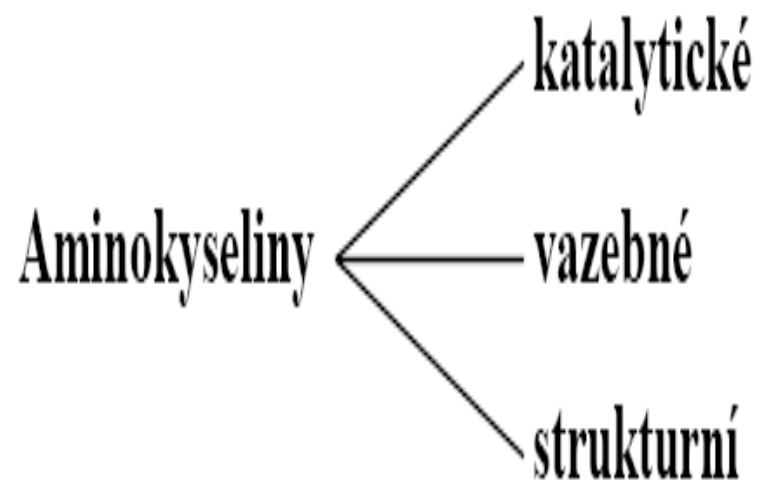
Izomerasy - většinou bez kofaktoru nebo kobalamin,

Enzymové bílkoviny

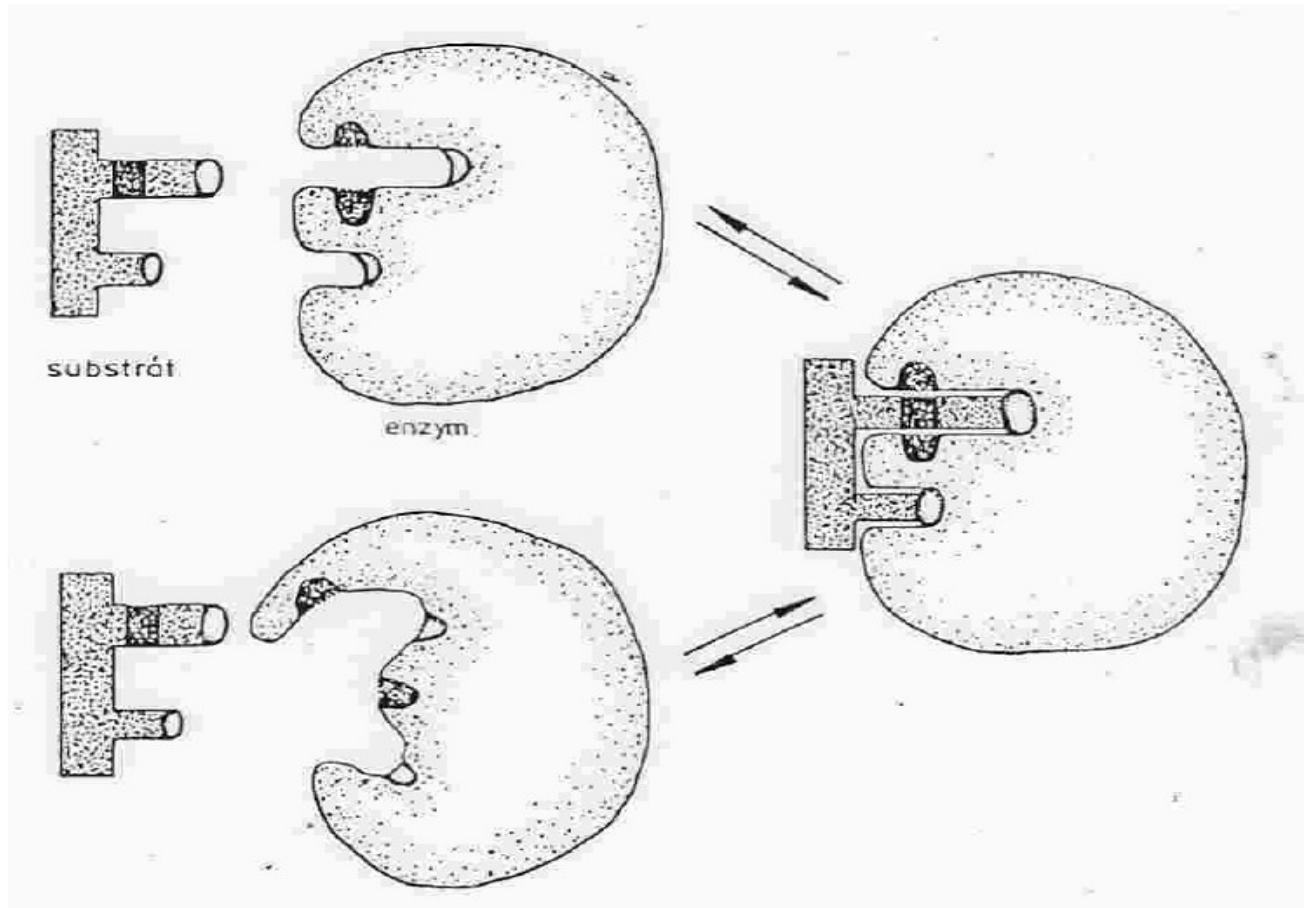
- monomerní
- oligomerní
- multienzymové komplexy



Aktivní místo enzymů

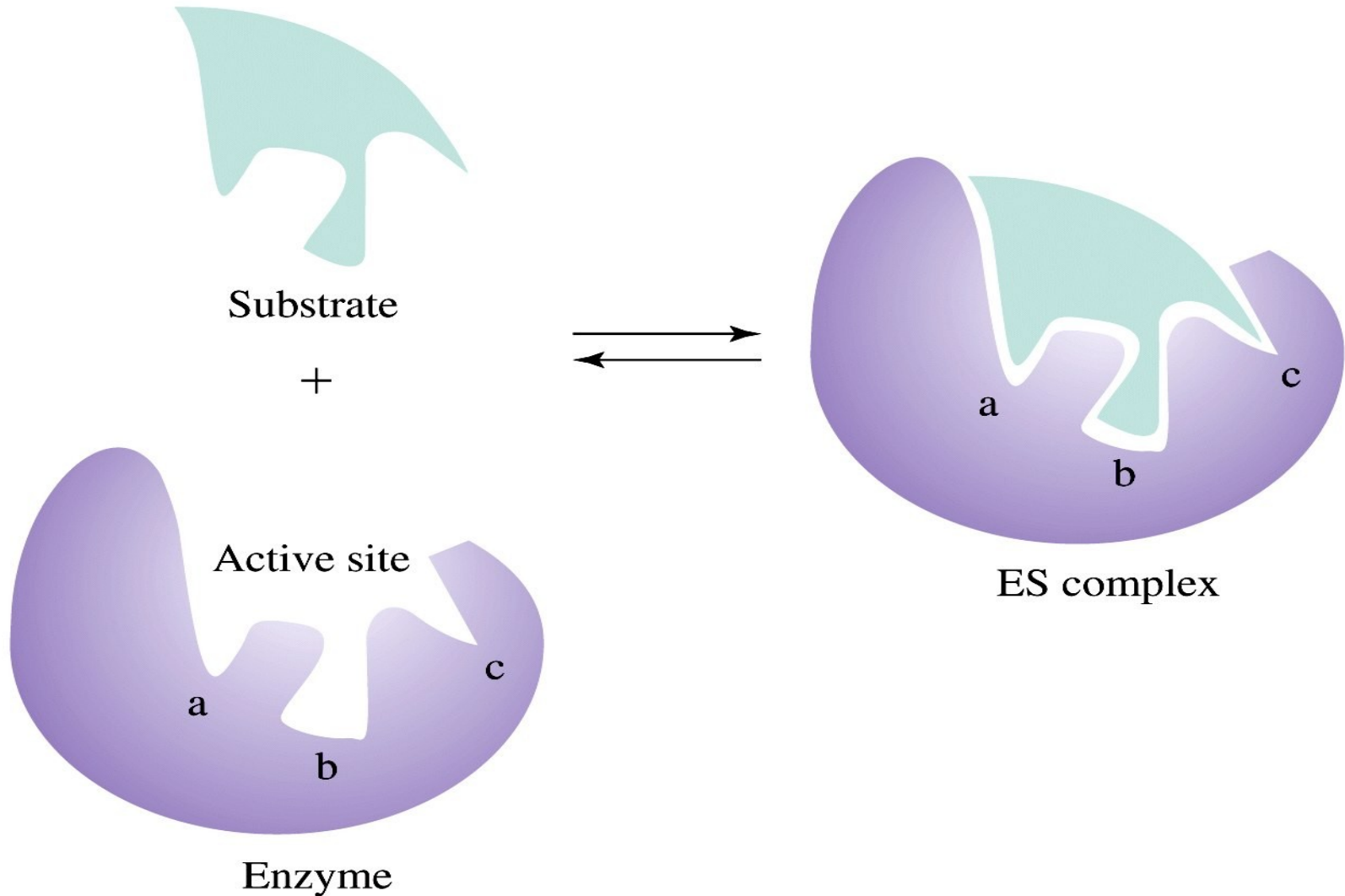


Fischer - 1894 - *teorie o zámku a klíči*

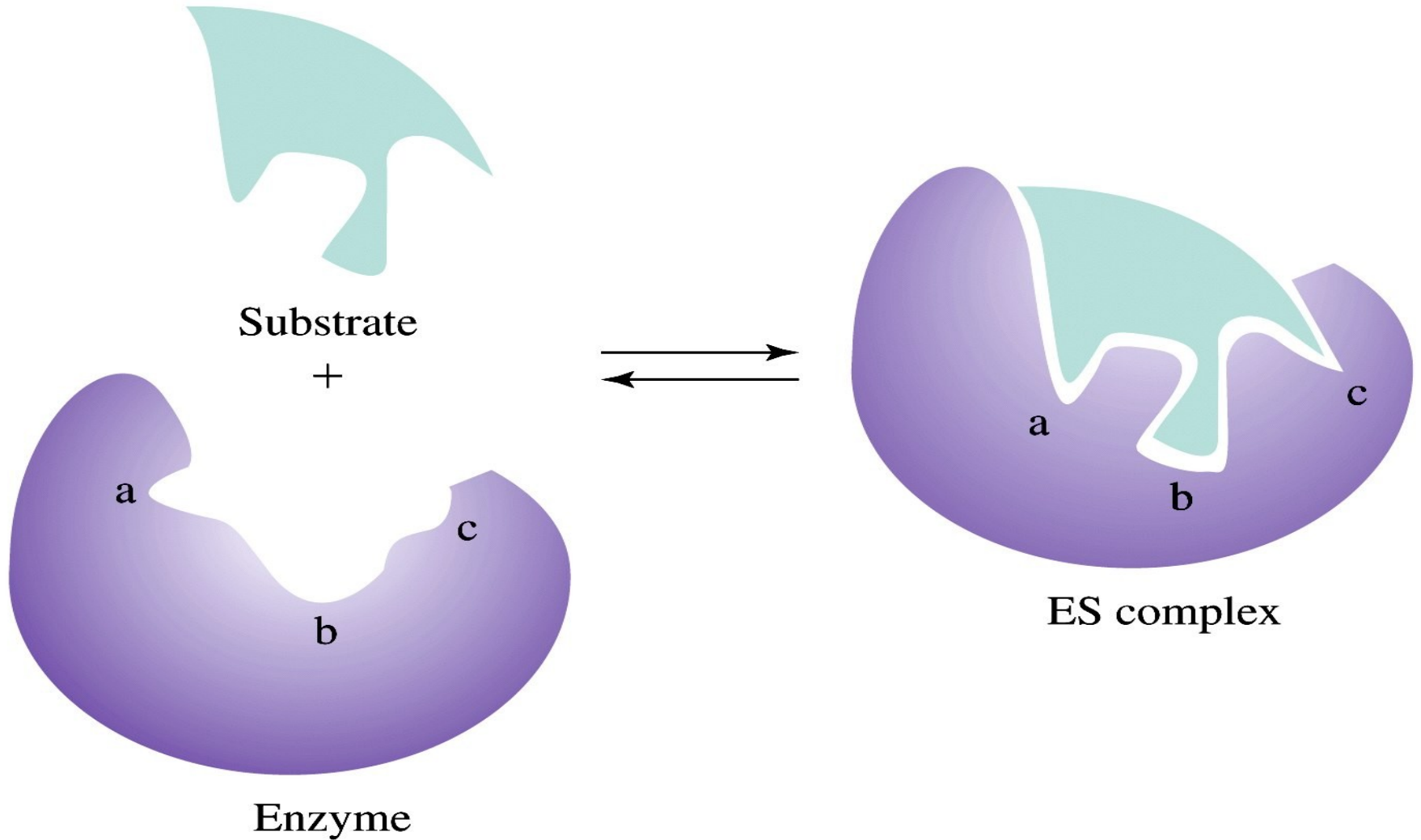


Koshland - 1959 - *teorie indukovaného přizpůsobení*

„Lock and key“ model



„Induced fit“ model



„Transition state“ model

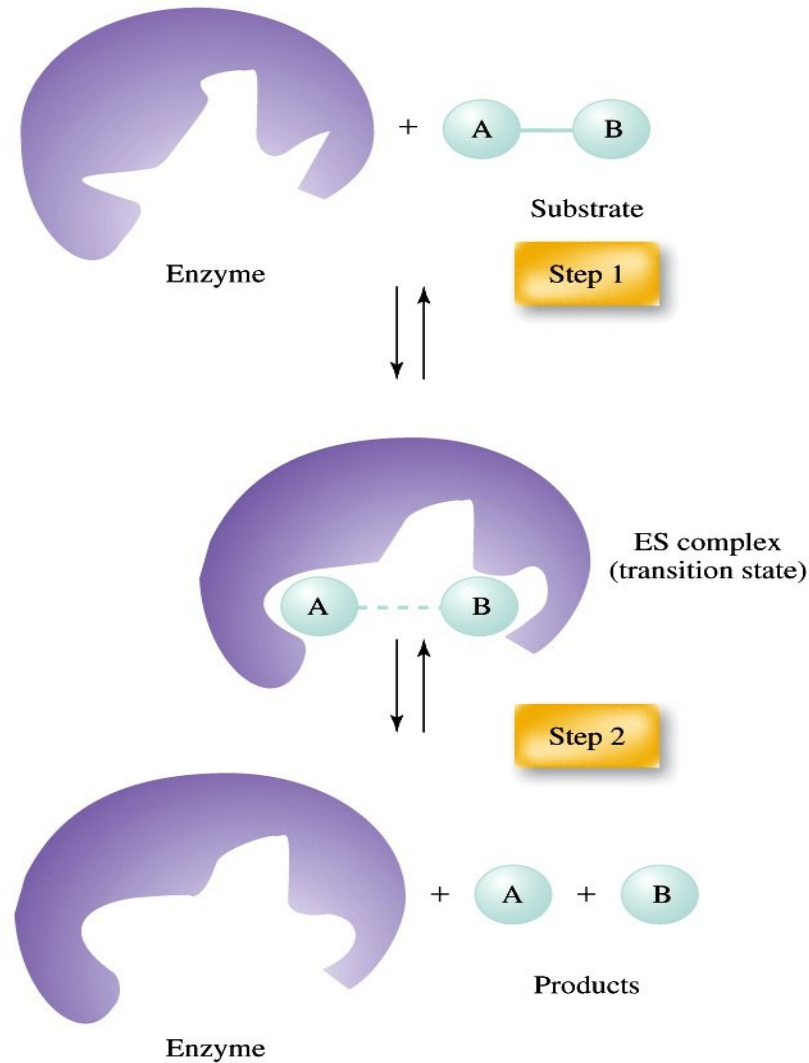


Figure 5-11 Concepts in Biochemistry, 3/e
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Aktivní místo

- Efekt přiblížení – překryv orbitalů
- Specifické mikroprostředí – pH, I, hydrofobita atd
- Dehydratace
- Koncentrační efekt - 10^5
- Vhodná orientace

Aktivační energie

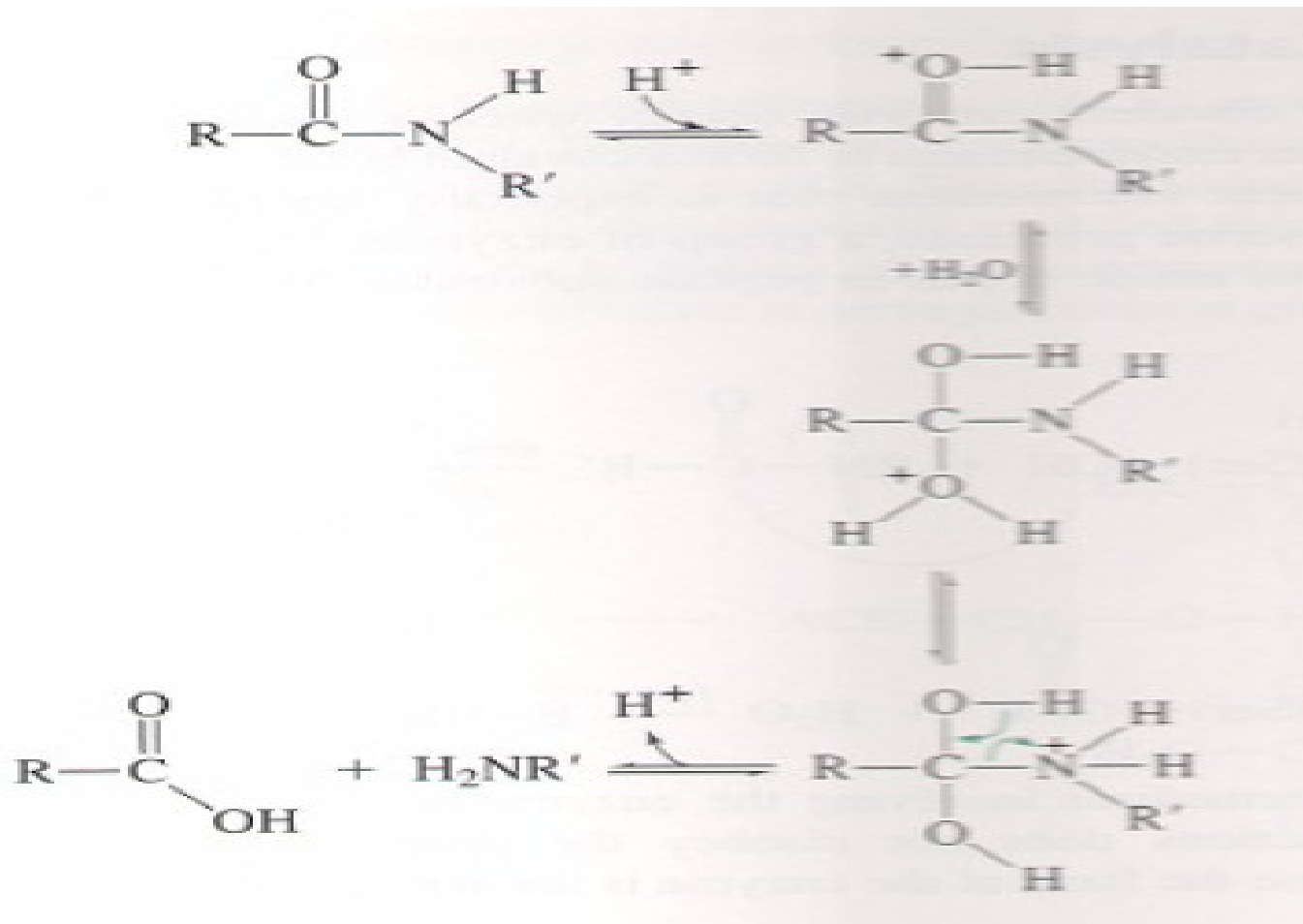


Uvolněna při vazbě substrátu na enzym

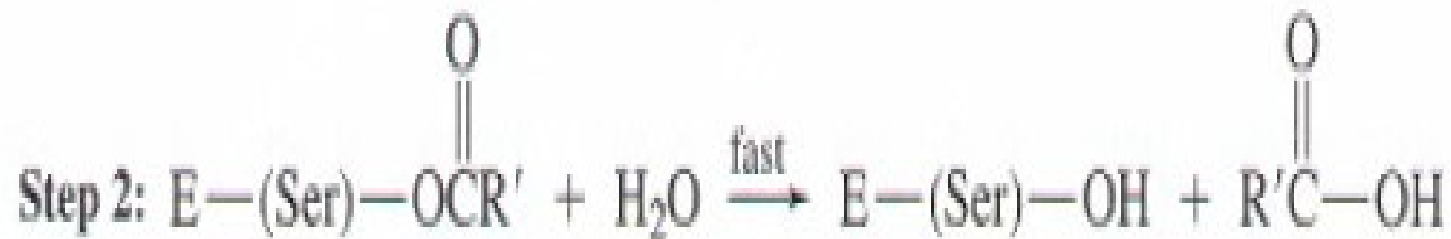
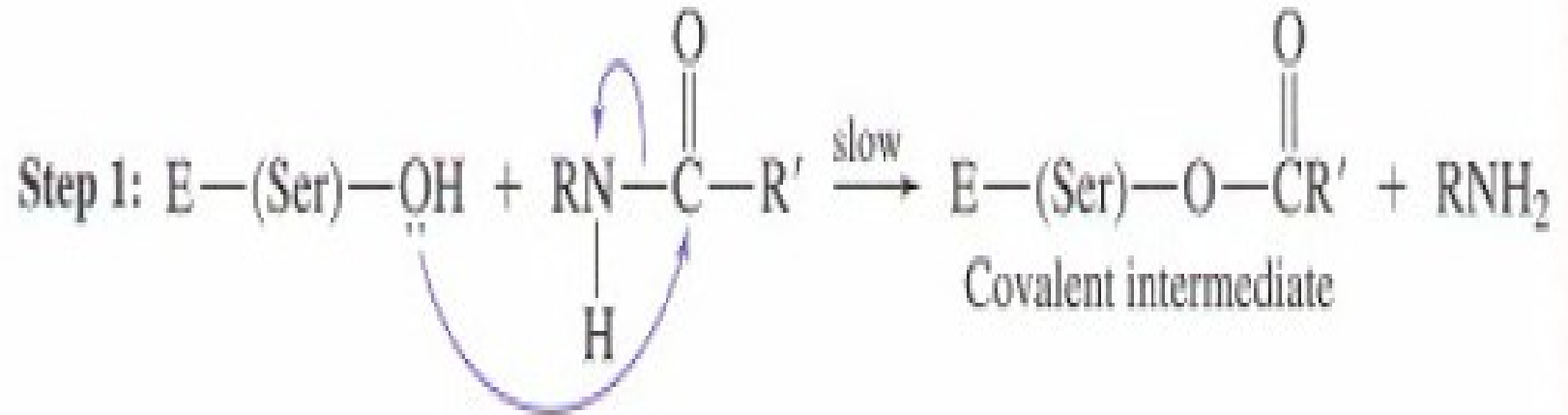
Mechanismus katalýzy

- Acidobazická
- Kovalentní
- Kovovými ionty
- Elektrostatická interakce
- Proximitní a orientační
- Přednostní vazbu přechodného komplexu

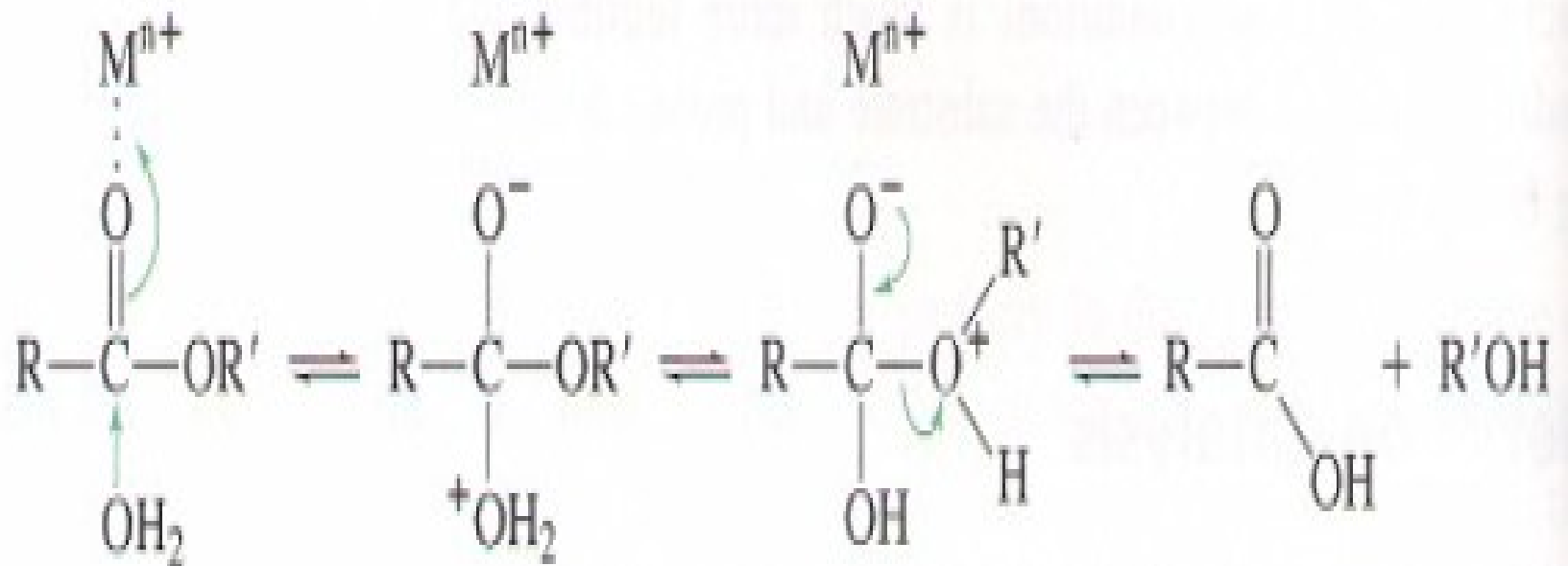
Acidobazická



Kovalentní

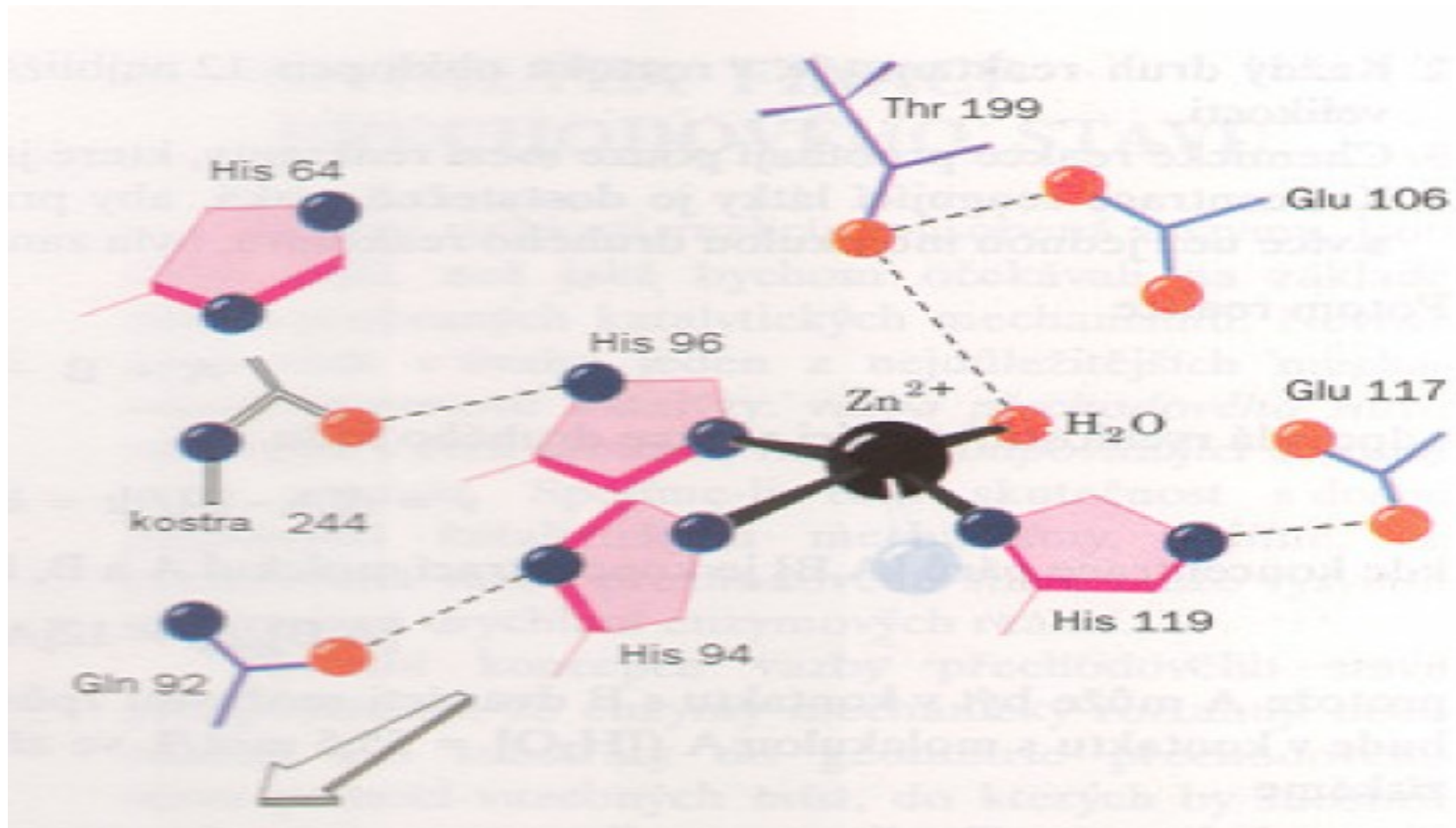


Kovovými ionty

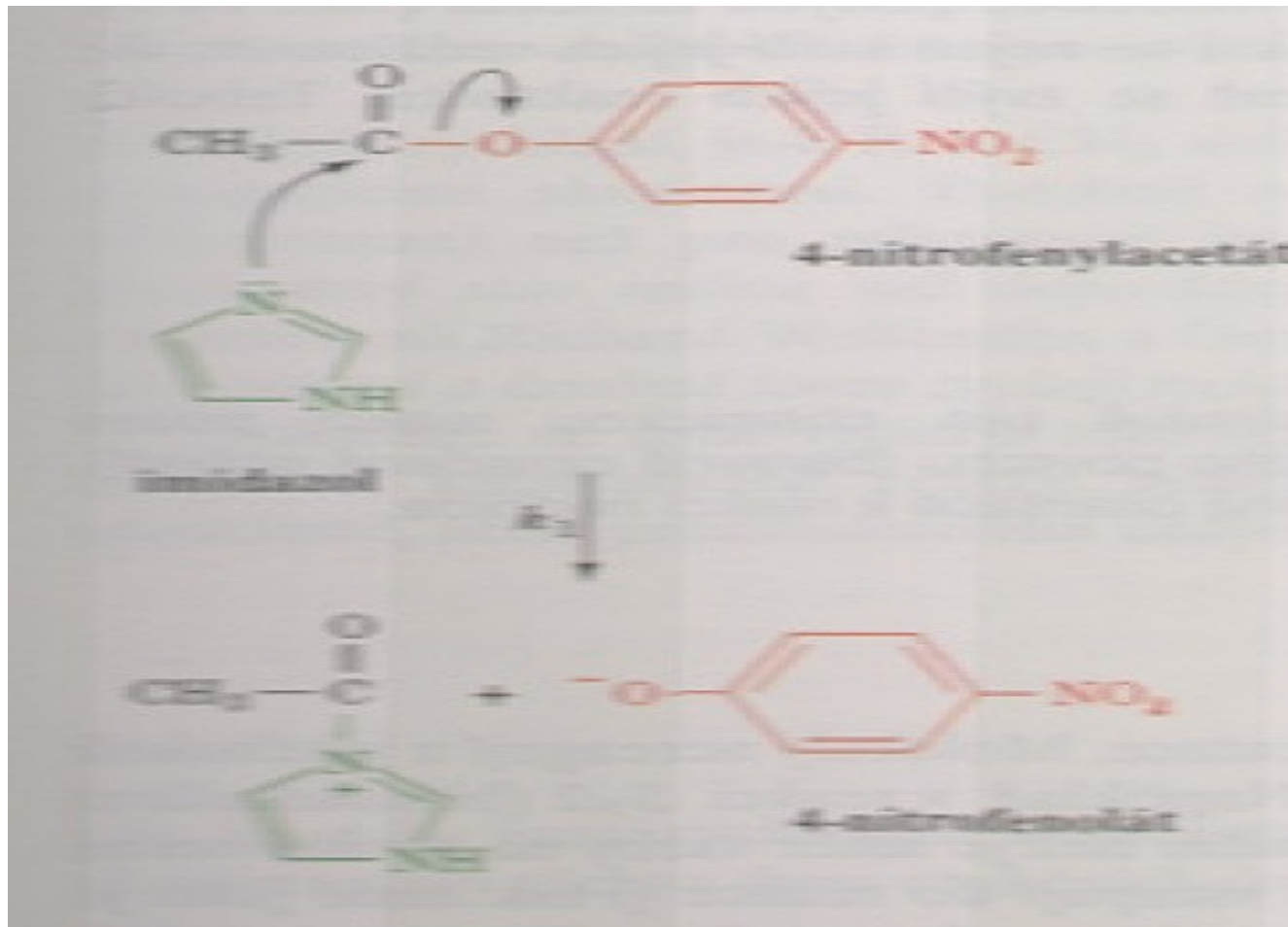


(b)

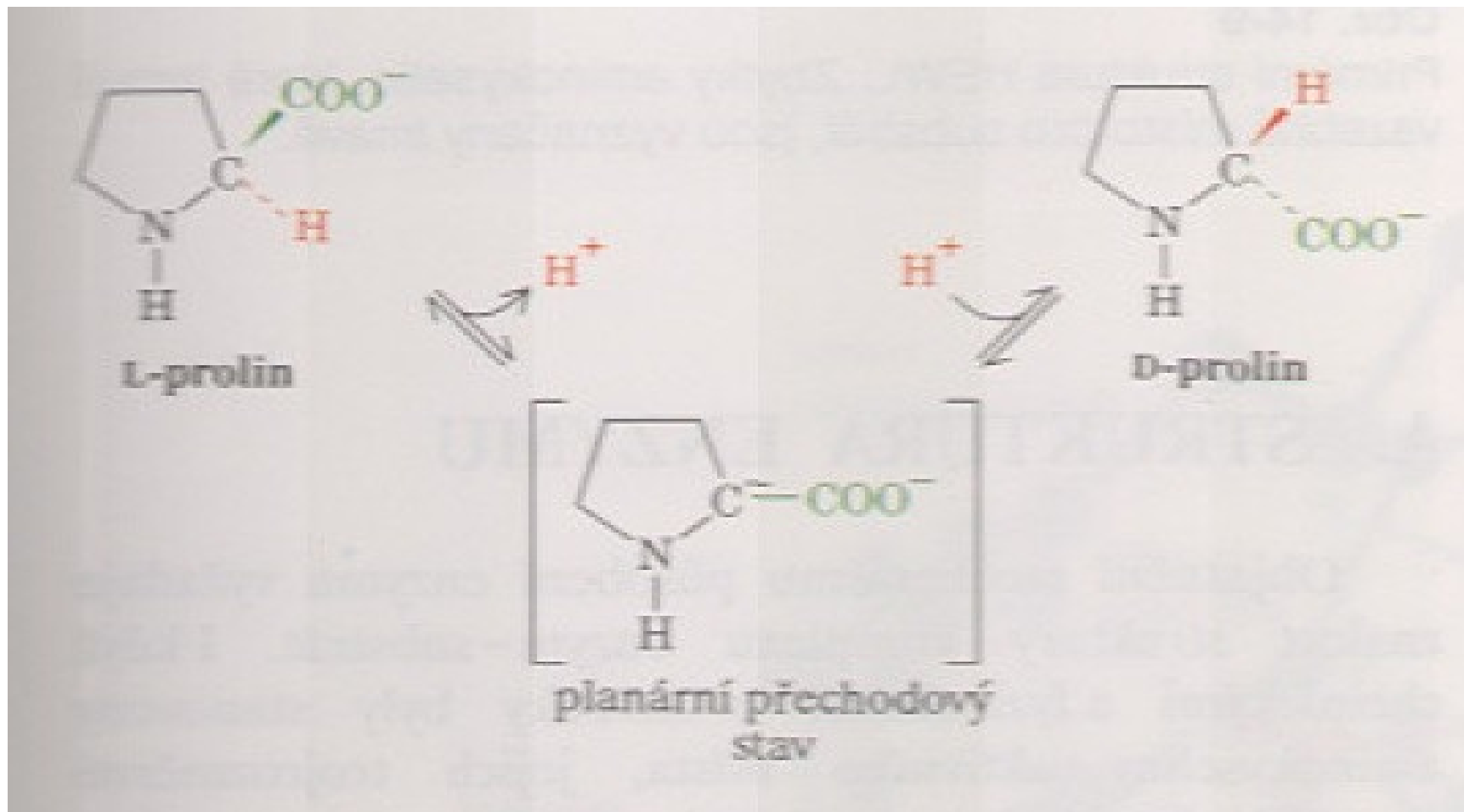
Elektrostatickou interakcí



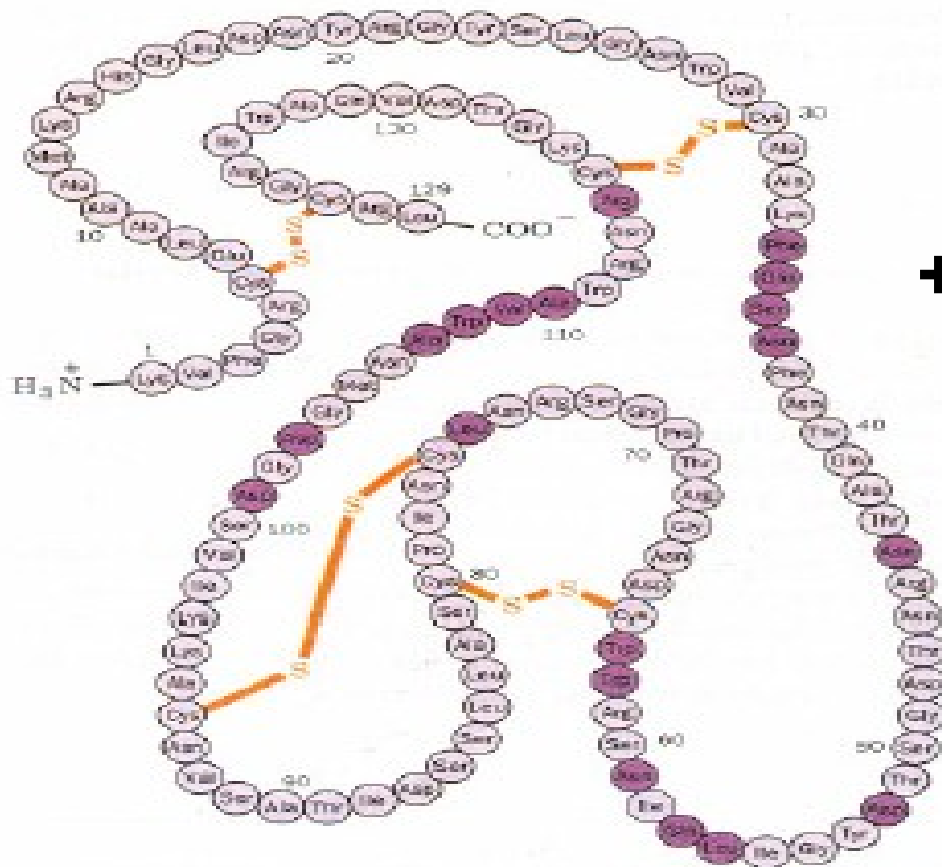
Proximitní a orientační



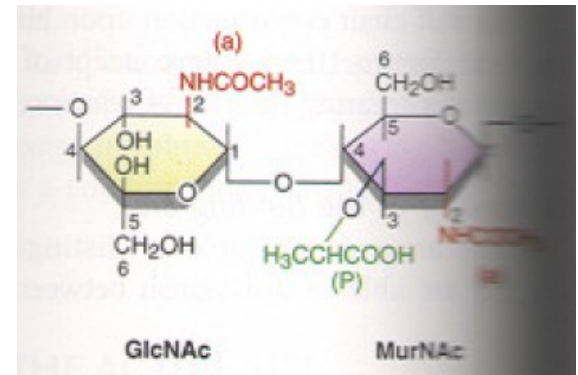
Přednostní vazbu přechodného komplexu



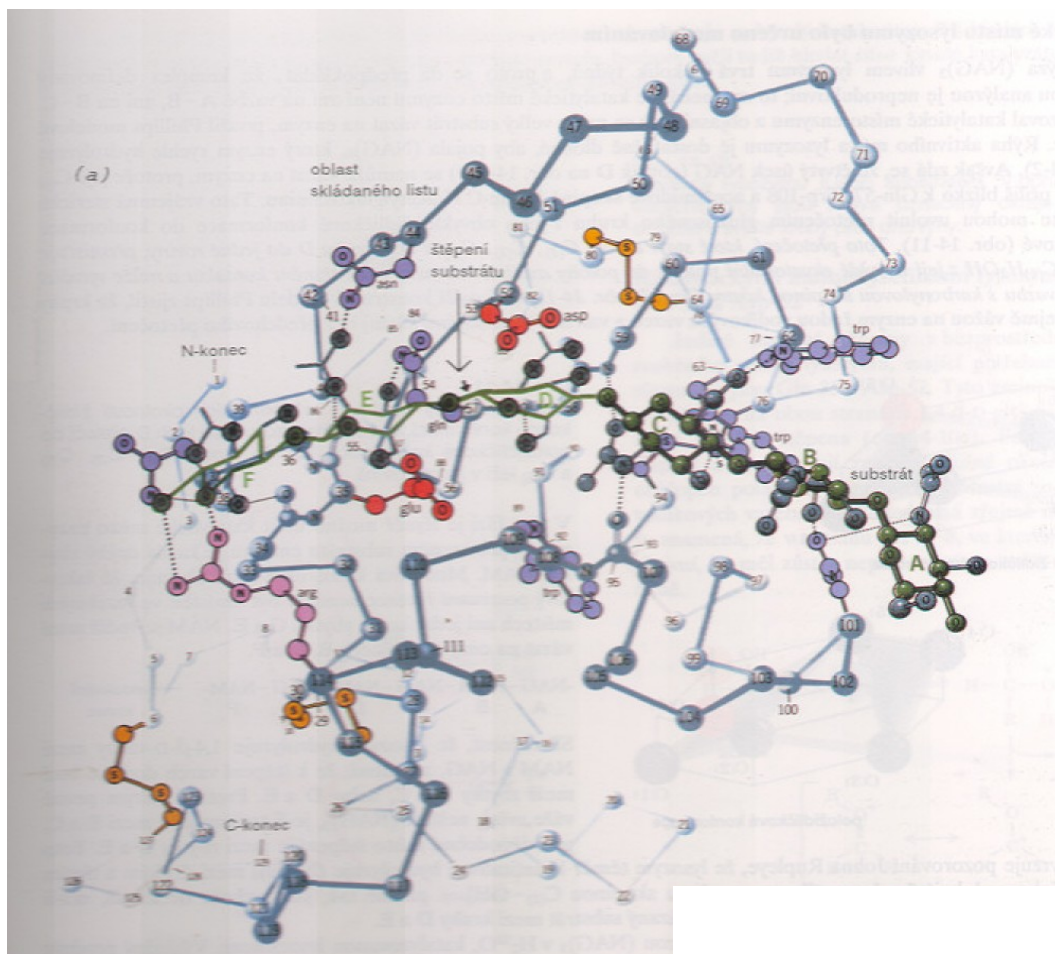
Lysozym



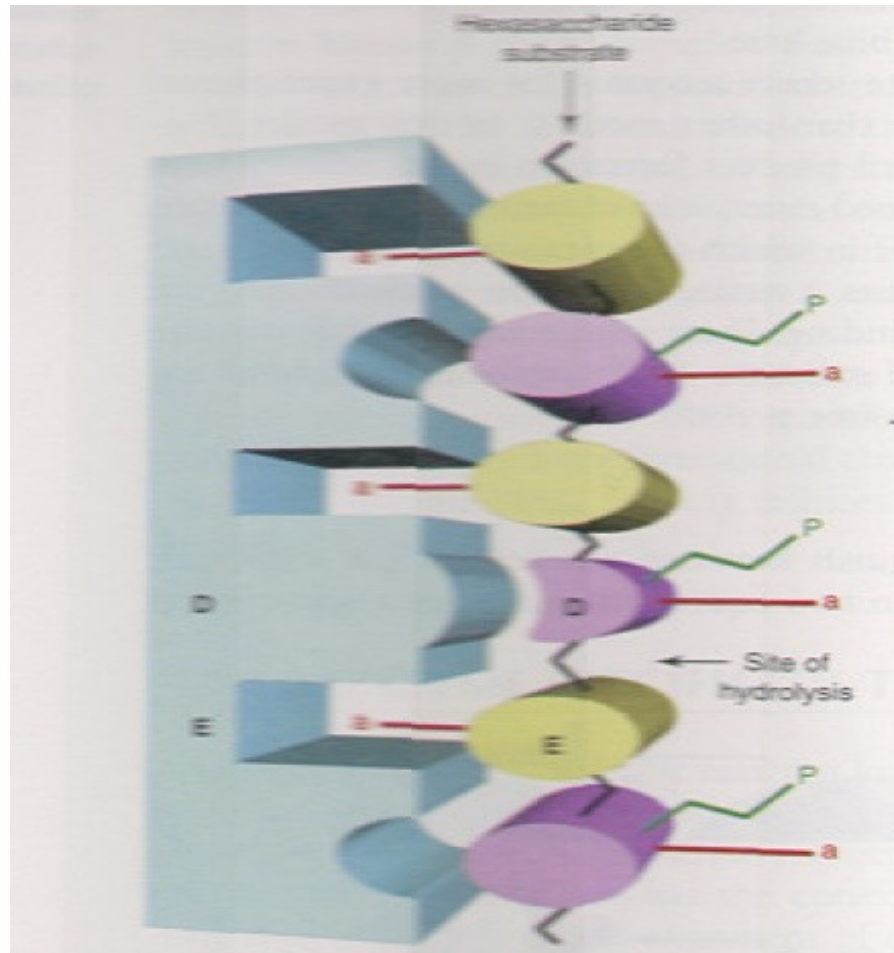
+



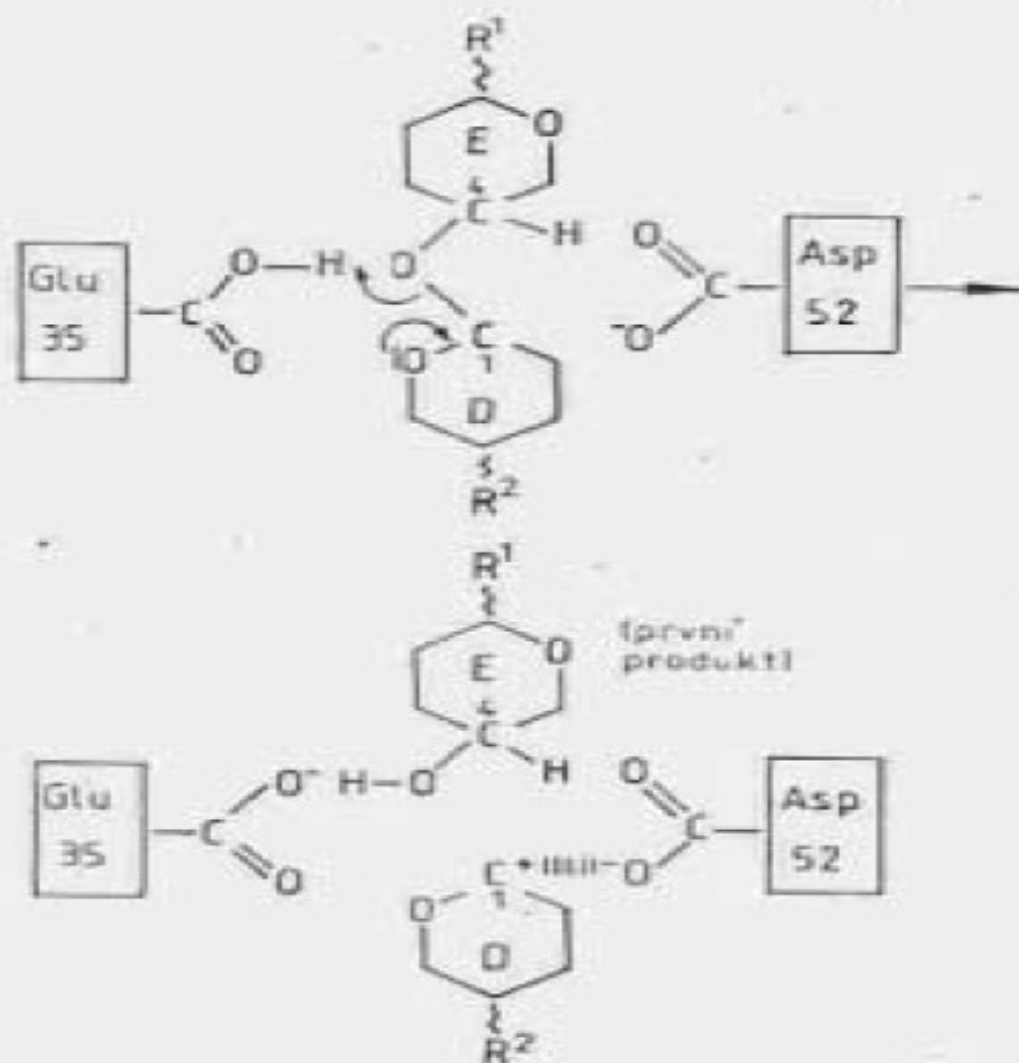
Lysozym

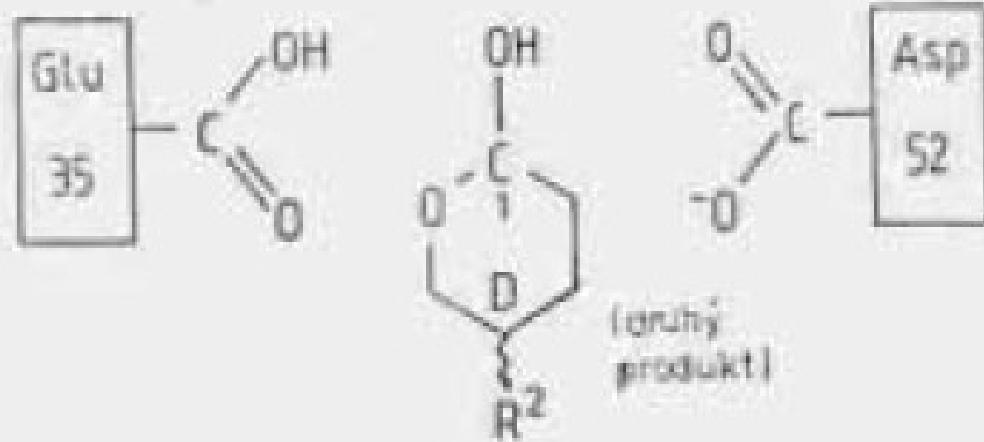
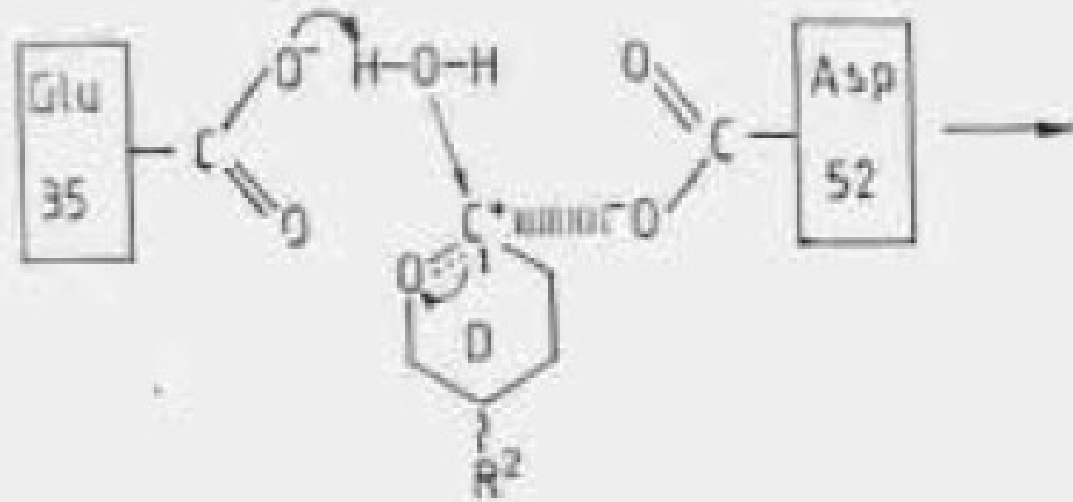


Lysozym

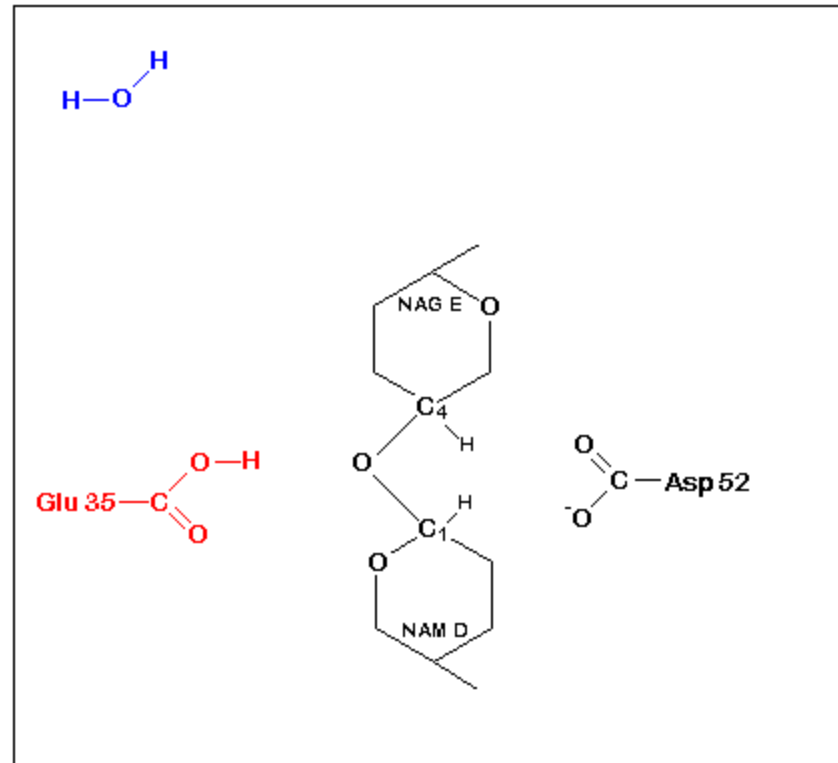


Mechanismus působení lysozymu





Lysozym



- Fosfotriosoisomerasa

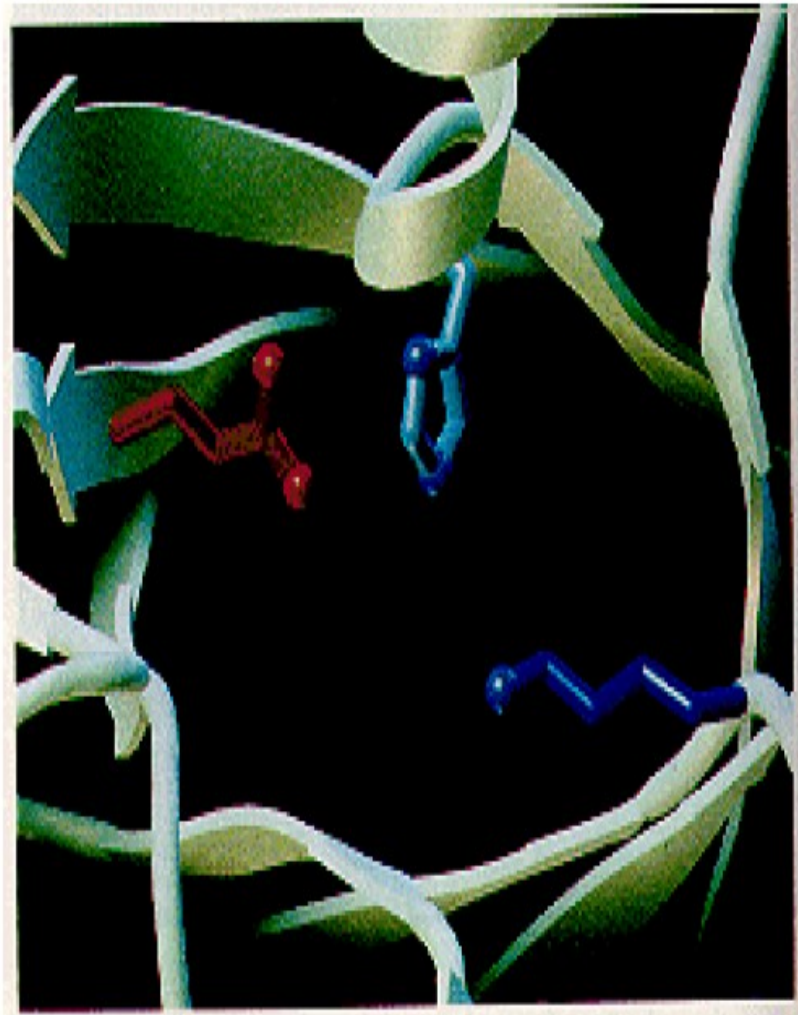


- Fosfotriosoisomerasa

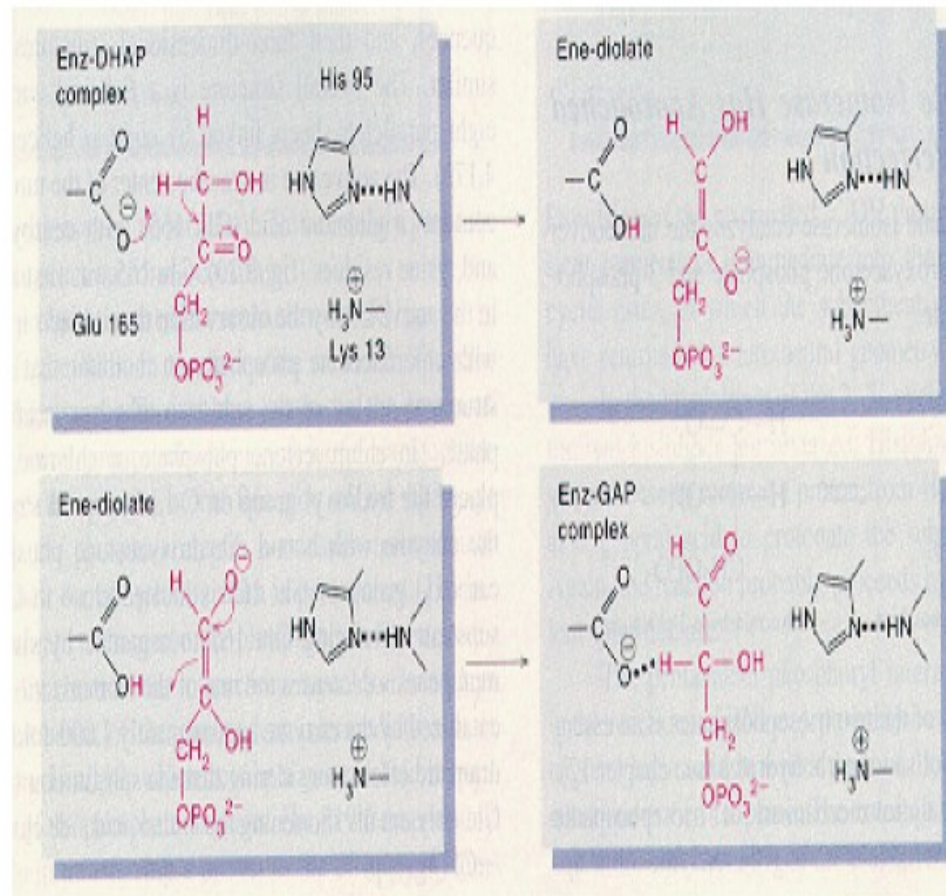
- Glu165

- His95

- Lys13



- Mechanismus
působení PTI



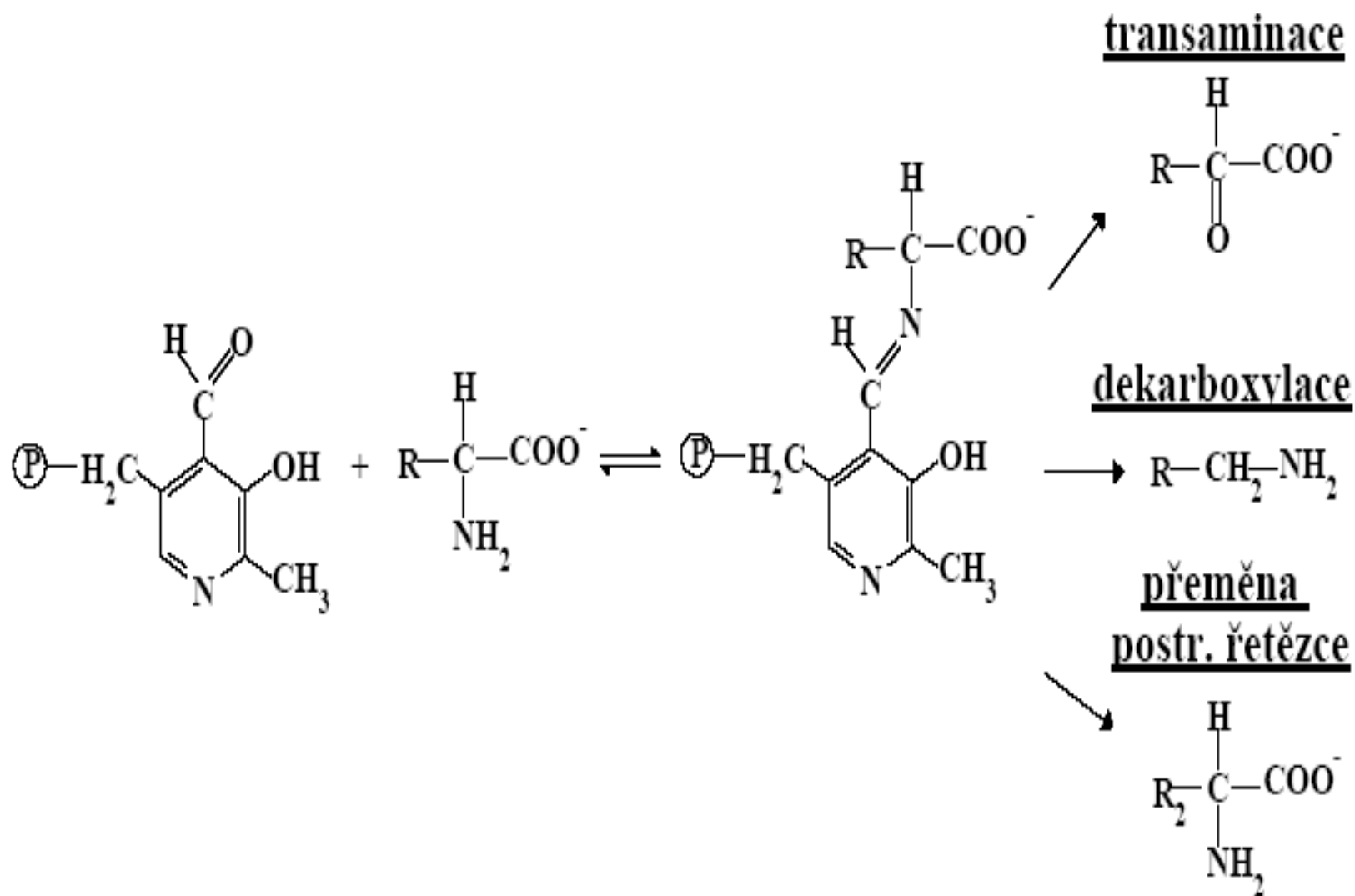
Specifita enzymové reakce

specifita reakční - účinku - jaká reakce proběhne

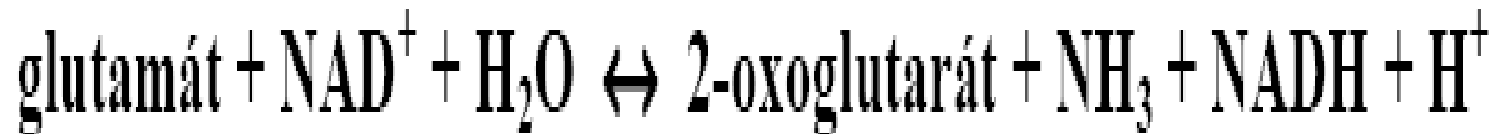
specifita substrátová - absolutní

- skupinová

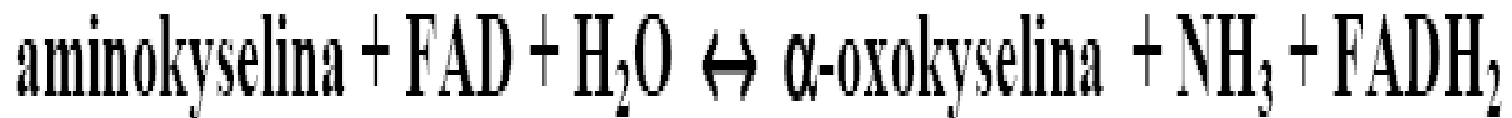
- stereospecifita

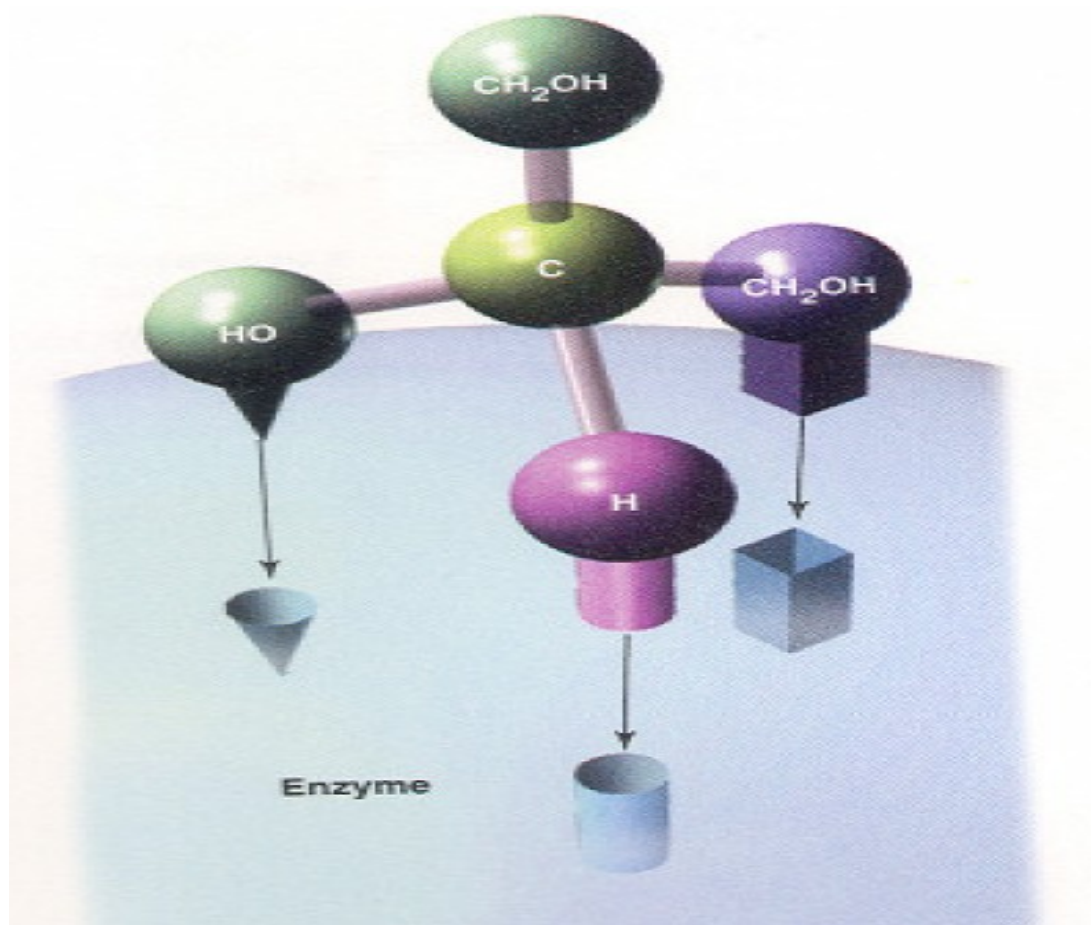


savci



vejcorodí





ENZYMOVÁ KINETIKA

Reakce s jedním substrátem

BROWN 1902

MICHAELIS MENTENOVÁ 1913

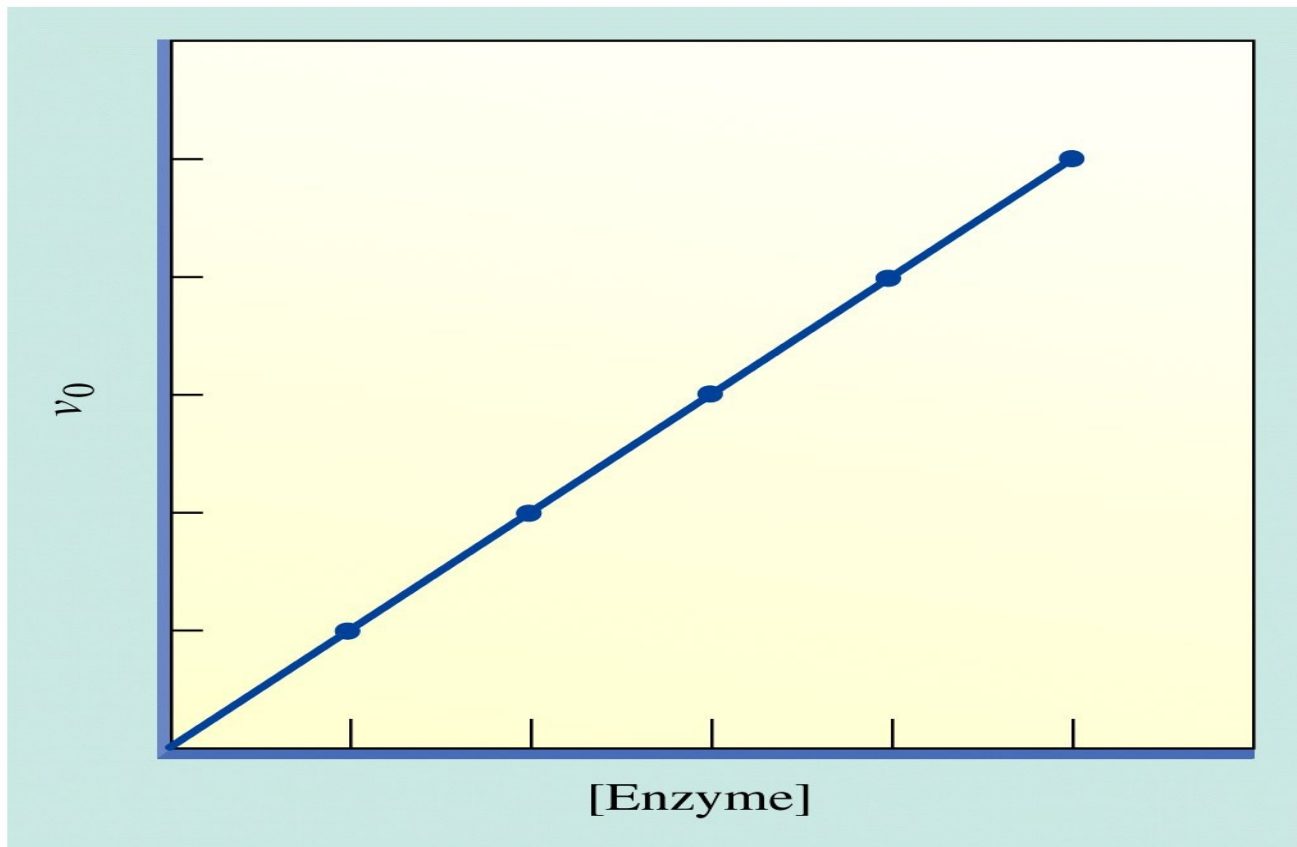
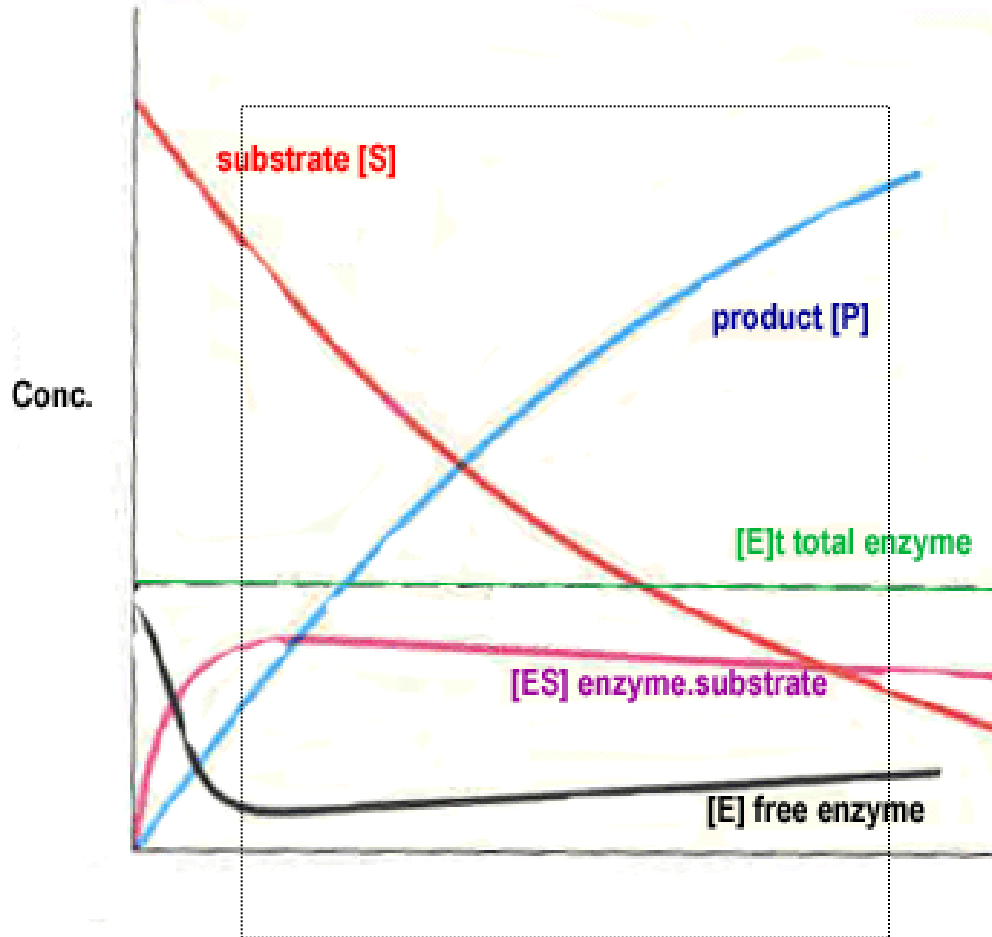
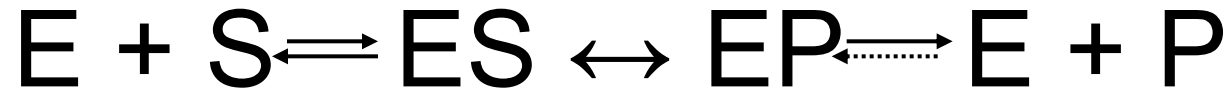


Figure 5-5 Concepts in Biochemistry, 3/e
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Ustálený stav



b) závislost počáteční rychlosti na koncentraci enzymu

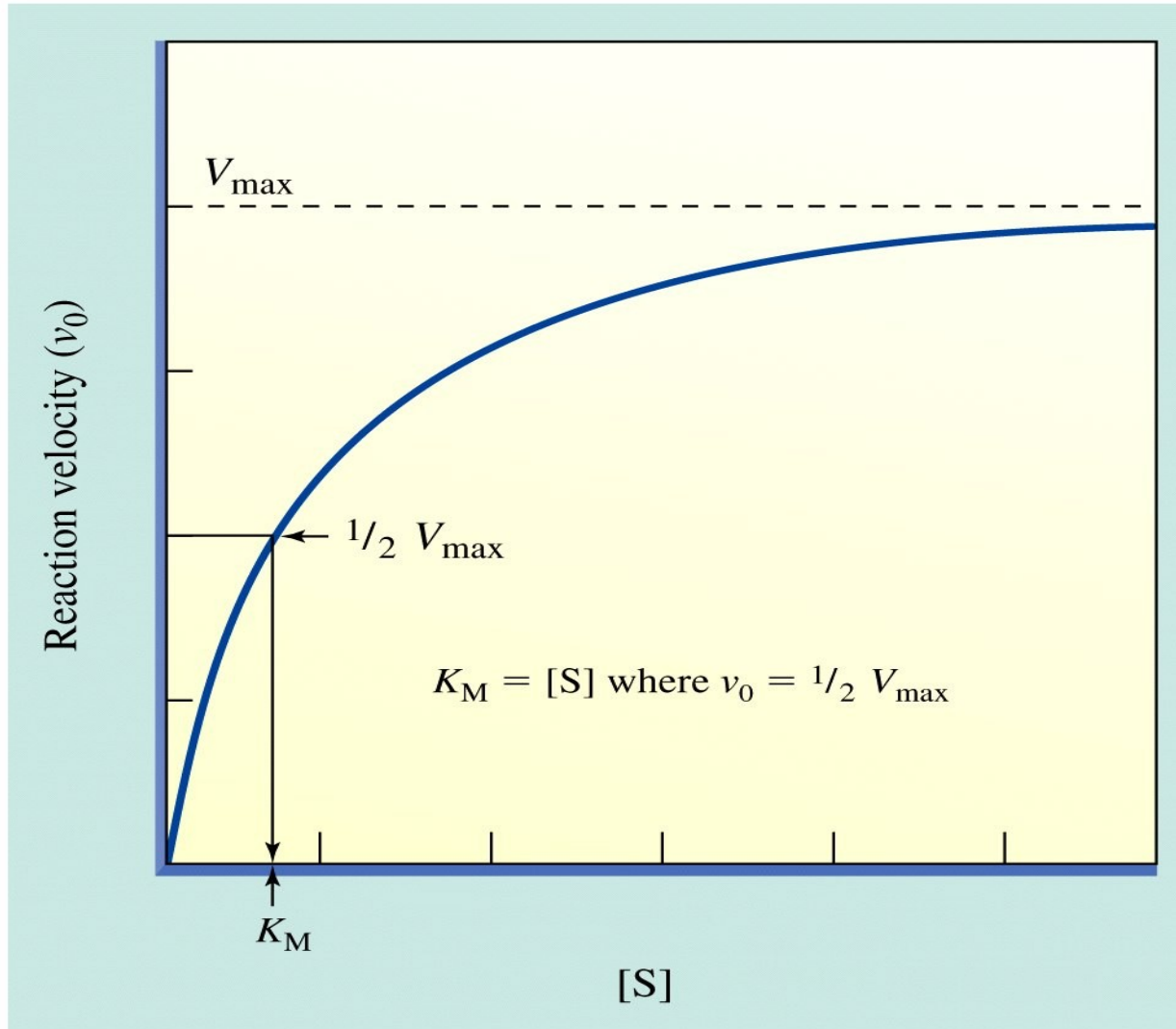


Figure 5-4 Concepts in Biochemistry, 3/e
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Rovnice Michaelis Mentenové

$$v = \frac{V \cdot [S]}{K_m + [S]}$$

v - počáteční reakční rychlost

V - maximální (limitní) reakční rychlost

K_m - Michaelisova konstanta

$$v = \frac{V \cdot [S]}{K_m + [S]}$$

$[S] \gg K_m$ $v = \frac{V \cdot [S]}{[S]} = V$

$[S] \ll K_m$ $v = \frac{V \cdot [S]}{K_m} = \text{konst.} \cdot [S]$

Stanovení K_m a V_{max}

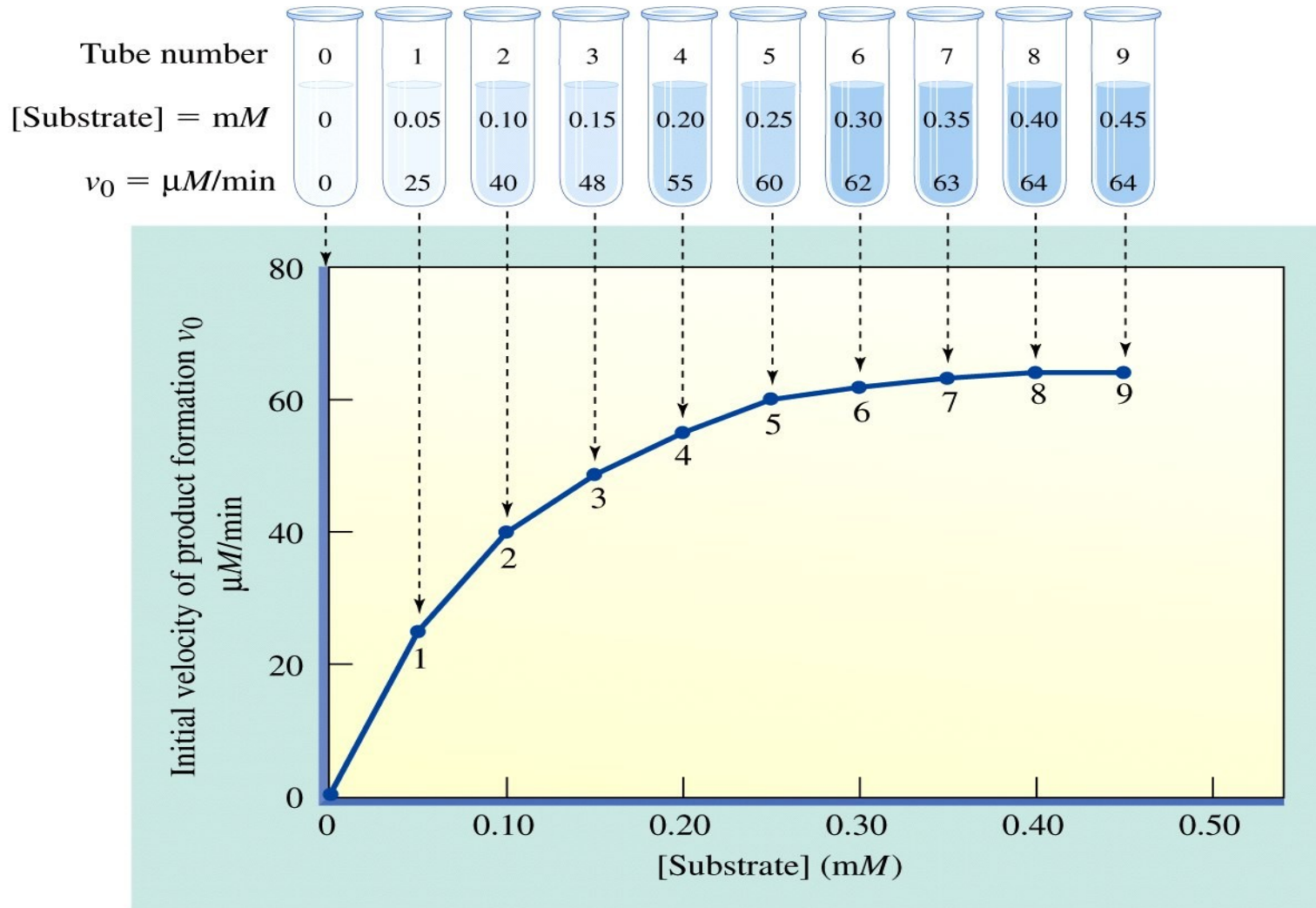


Figure 5-3 Concepts in Biochemistry, 3/e
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Stanovení K_M :

LINEWEAVER BURKE

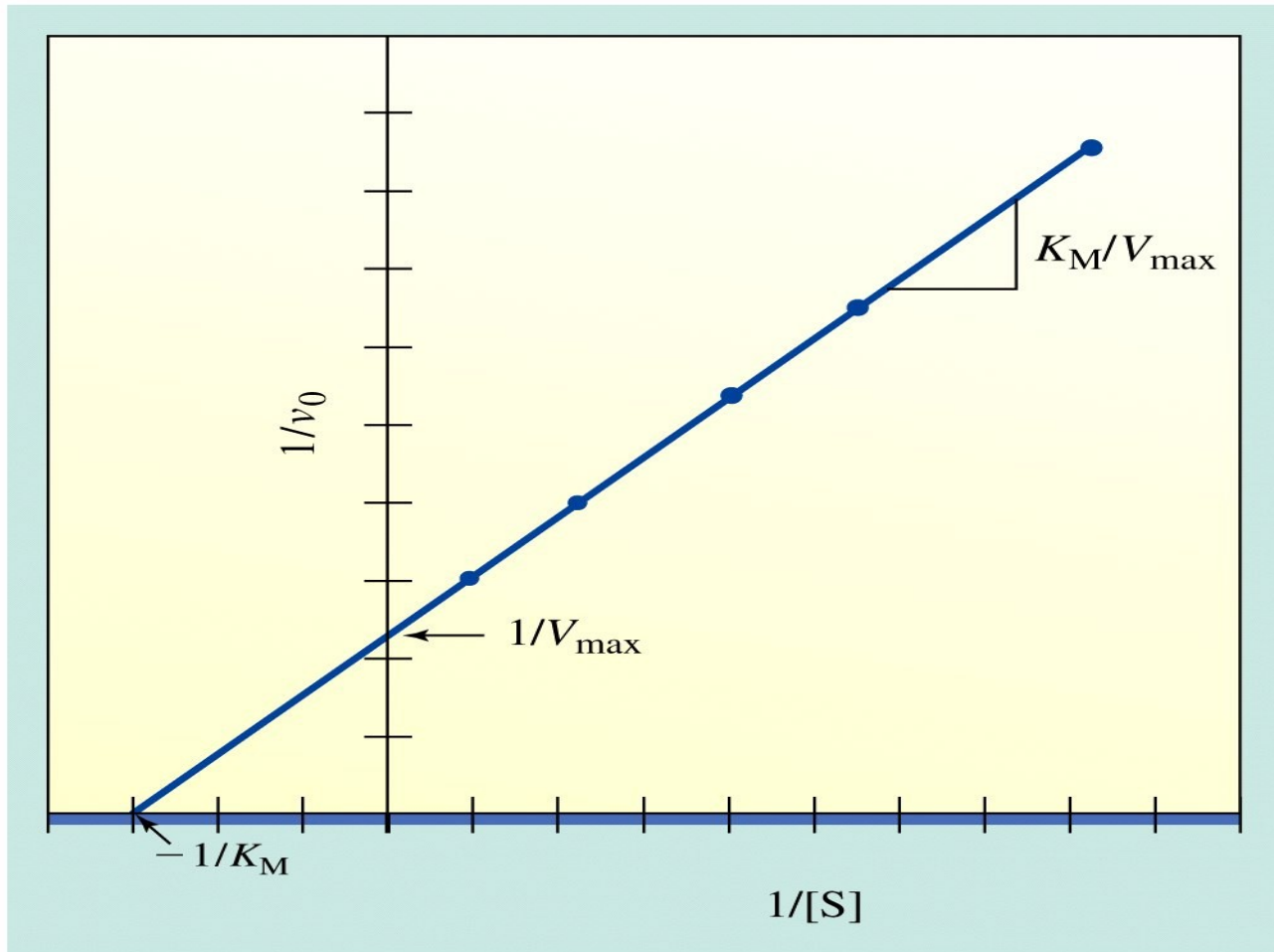


Table 5.3 **K_M values for some enzyme–substrate systems**

Enzyme	Substrate	K_M (mM)
Catalase	H ₂ O ₂	0.001
Hexokinase from brain	ATP	0.4
	D-Glucose	0.05
	D-Fructose	1.5
Carbonic anhydrase	HCO ₃ ⁻	9
Chymotrypsin	Glycyltyrosinylglycine	108
	<i>N</i> -Benzoyltyrosinamide	2.5
β-Galactosidase	Lactose	4.0
Penicillinase	Benzylpenicillin	0.050
Pyruvate carboxylase	ATP	0.060
	Pyruvate	0.40
	HCO ₃ ⁻	1.0
Ribulose-1,5-bisphosphate carboxylase (rubisco)	Ribulose-1,5-bisphosphate	0.028
	CO ₂	0.009
Ribulose-1,5-bisphosphate oxygenase (rubisco)	Ribulose-1,5-bisphosphate	0.028
	O ₂	0.535

Table 5.4**Turnover numbers, k_3 , for some enzymes**

Enzyme	Substrate	k_3 (sec ⁻¹)
Catalase	H ₂ O ₂	40,000,000
Carbonic anhydrase	HCO ₃ ⁻	400,000
Acetylcholinesterase	Acetylcholine	25,000
Penicillinase	Benzylpenicillin	2,000
Lactate dehydrogenase	Lactate	1,000
Chymotrypsin	Glycyltyrosinylglycine	100
DNA polymerase	DNA	15
Ribulose-1,5-bisphosphate carboxylase	Ribulose-1,5-bisphosphate + CO ₂	3.3
Ribulose-1,5-bisphosphate oxygenase	Ribulose-1,5-bisphosphate + O ₂	2.4

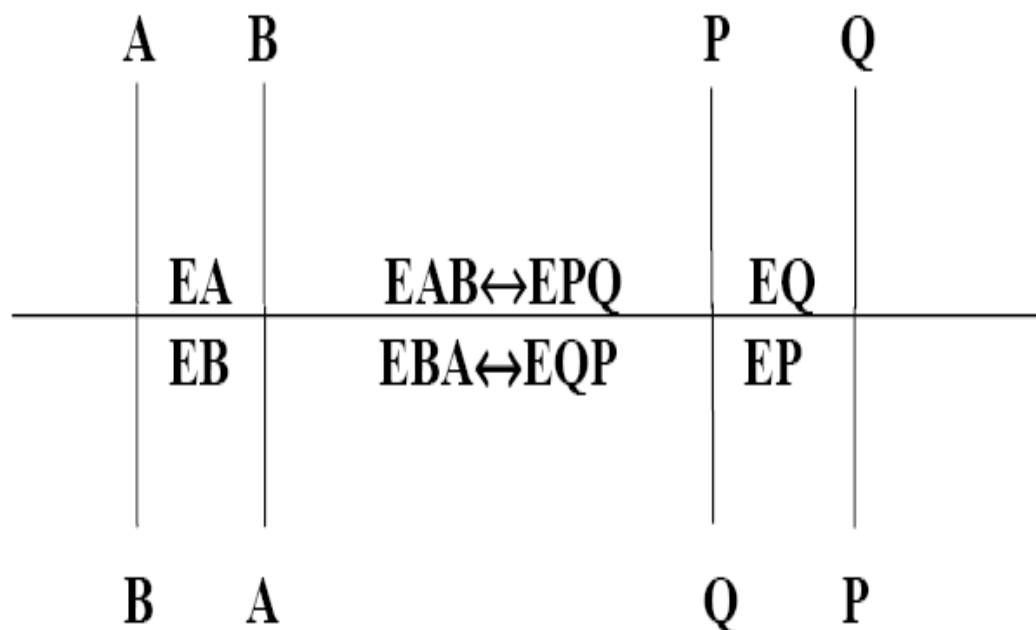
Table 5-4 Concepts in Biochemistry, 3/e
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Reakce se dvěma substráty

Mechanismy - CLELAND

Sekvenční :

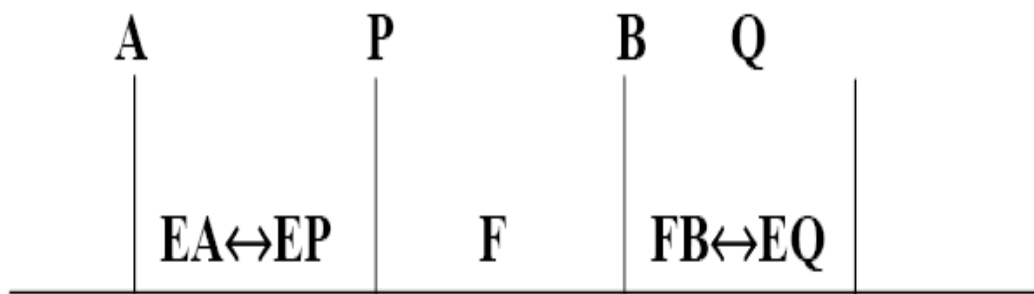
a) *náhodný*



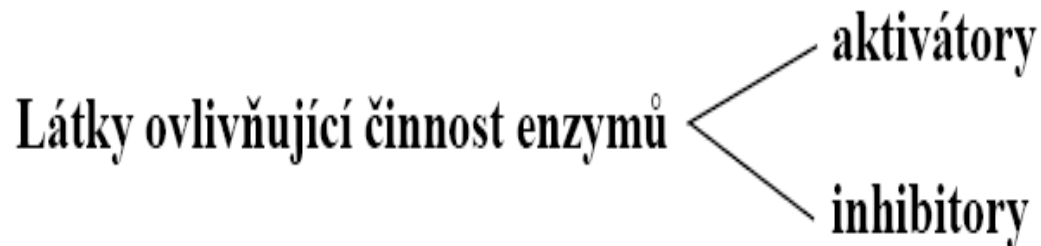
b) *uspořádaný*



Pingpongový



Látky ovlivňující činnost enzymů

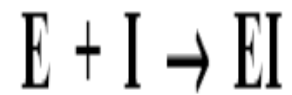


Aktivátory - zvyšují rychlost enzymové reakce

Inhibitory - snižují rychlost enzymové reakce

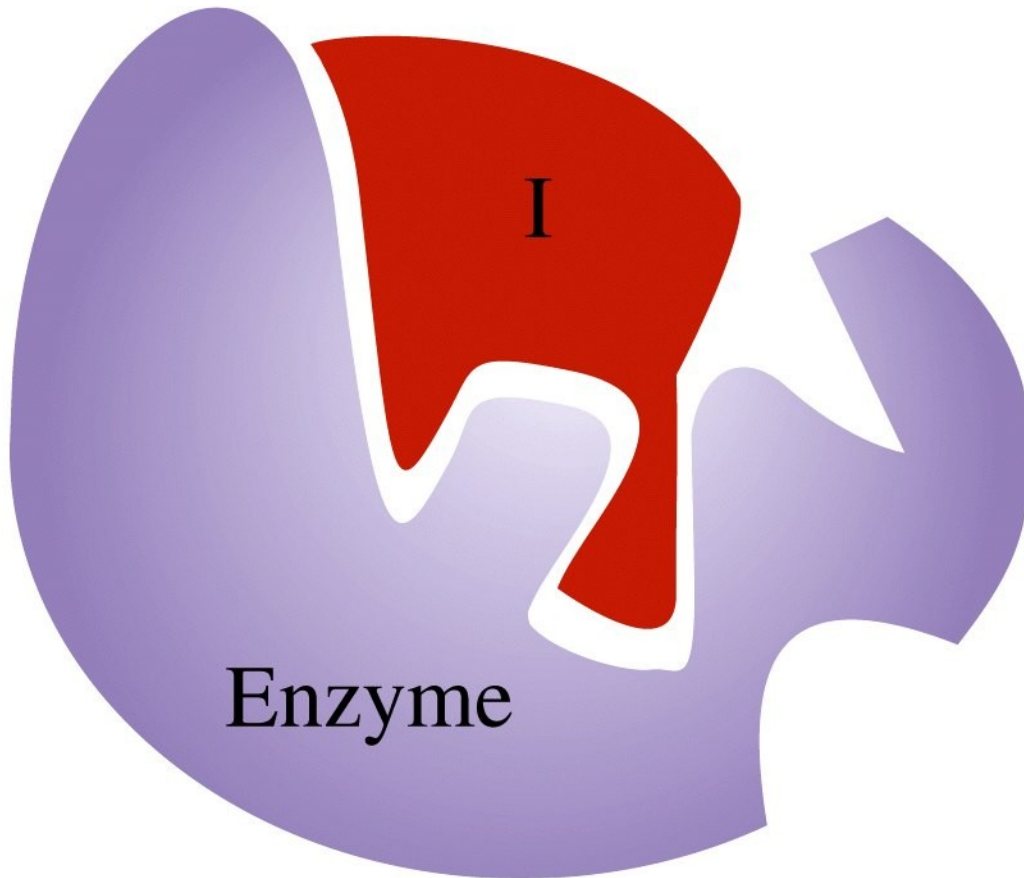
Inhibice

- Ireverzibilní inhibice



- Reverzibilní inhibice

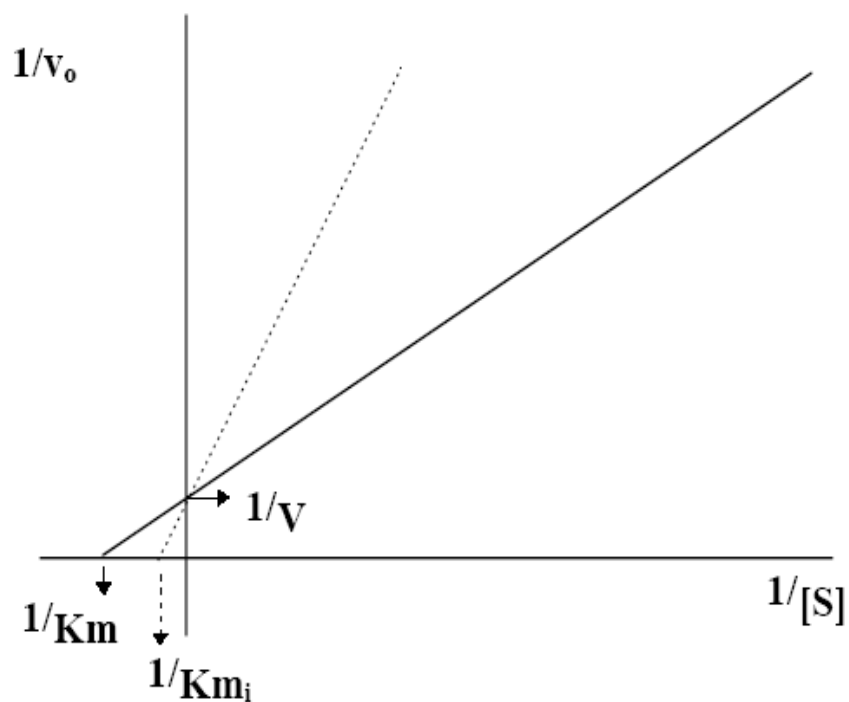
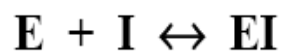
Kompetitivní inhibice



Competitive
(inhibitor binds
in active site)

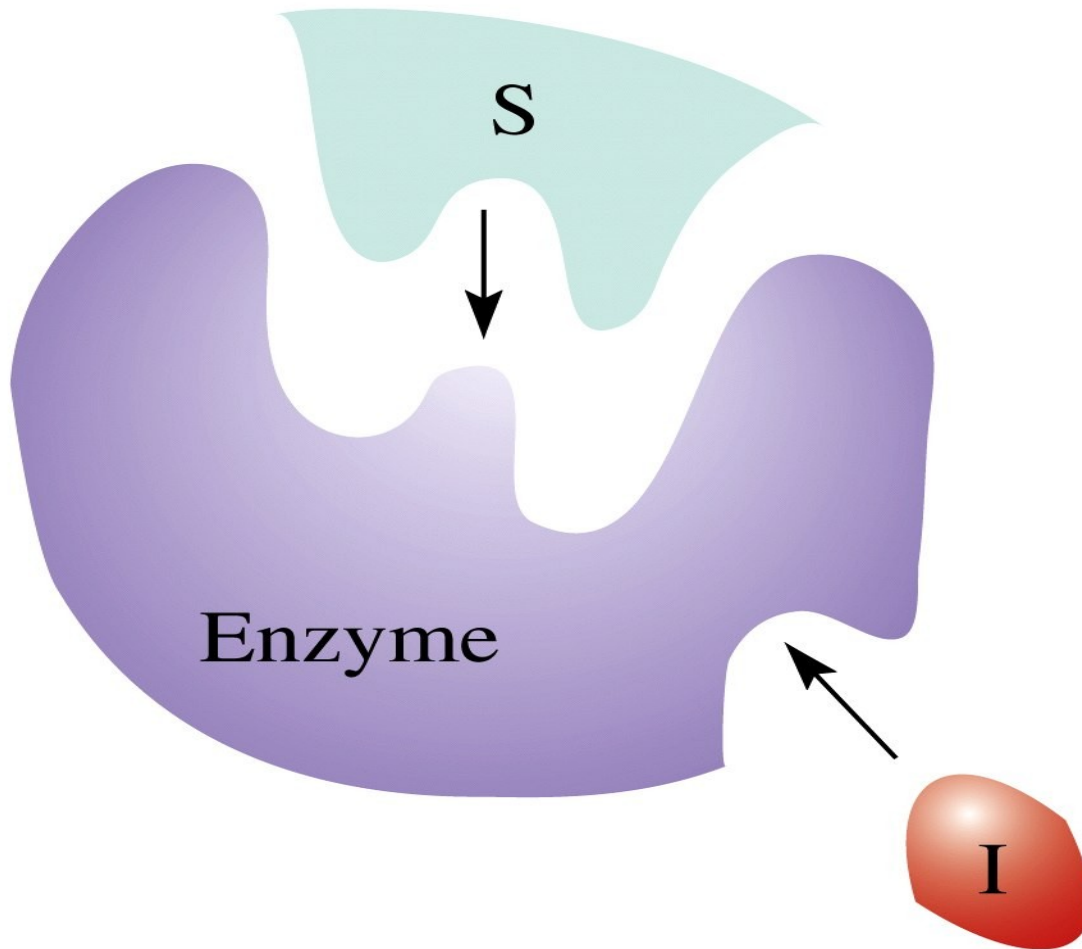
- Reverzibilní inhibice

Kompetitivní inhibice



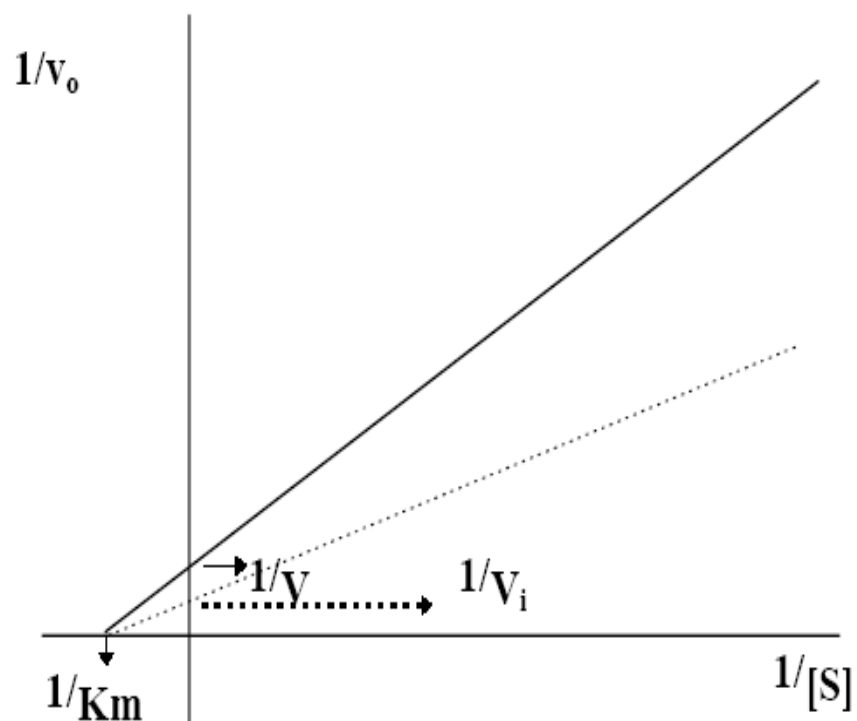
$$K_{m_i} > K_m \quad V_i = V$$

Nekompetitivní inhibice

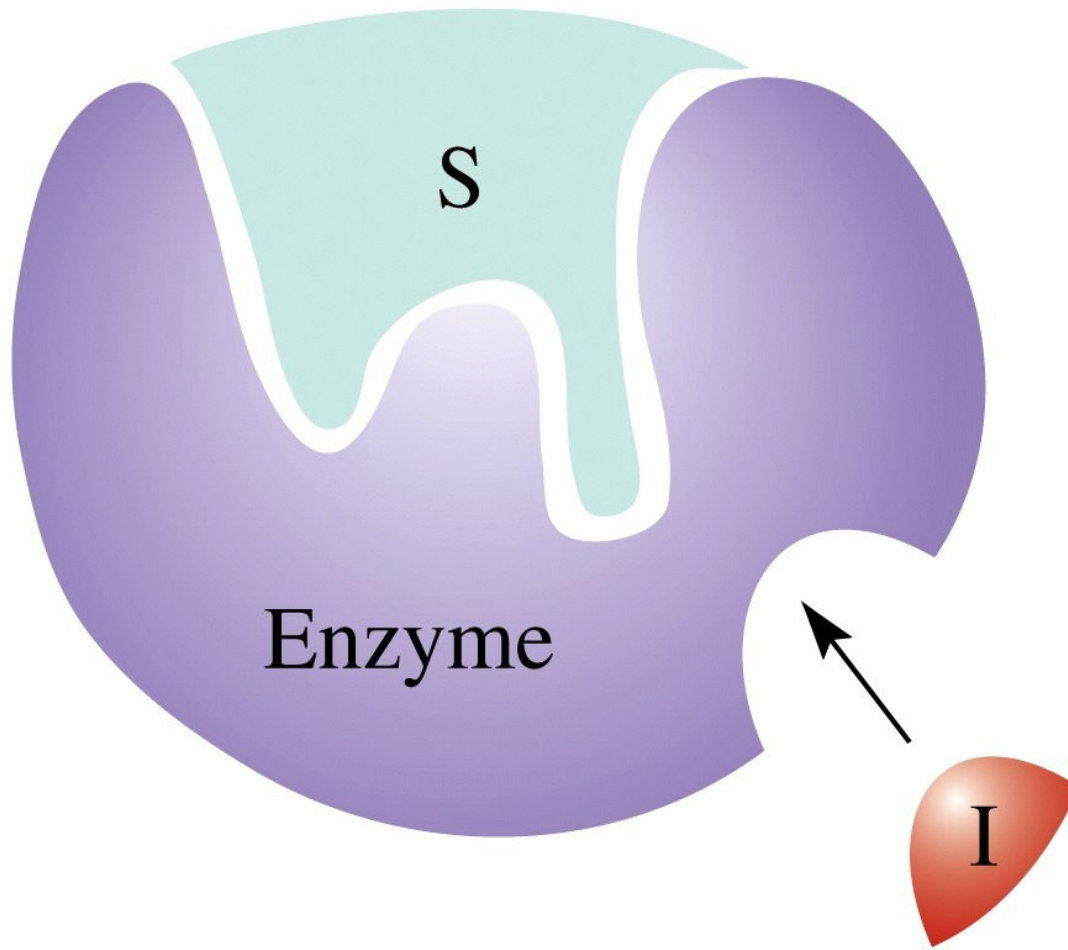


Noncompetitive
(inhibitor binds
at another site)

Nekompetitivní inhibice



$$K_{m_i} = K_m \quad V_i < V$$

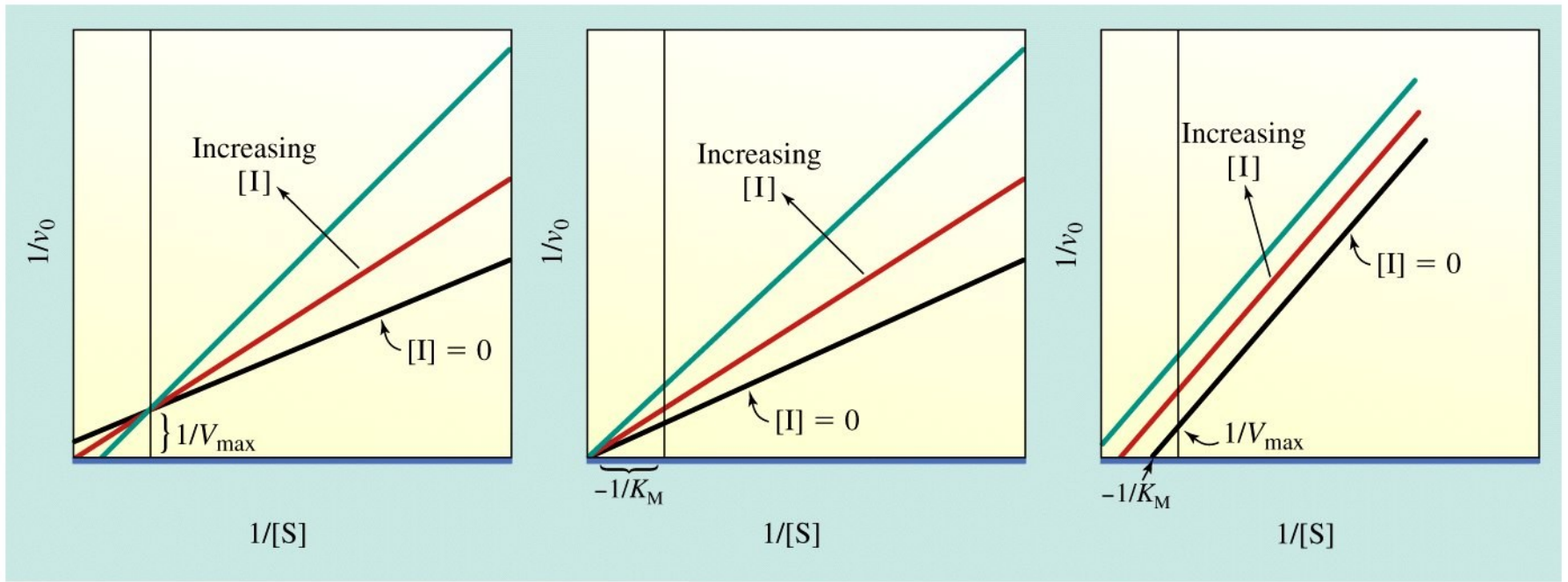


Uncompetitive
(inhibitor binds
after S binding)

Table 5.5**Kinetic characteristics of reversible inhibition**

Type of Inhibition	<i>Effect of Inhibition^a</i>		
	K_M	V_{\max}	K_M/V_{\max} (slope)
Competitive	Higher	Same	Increase
Uncompetitive	Lower	Lower	Same
Noncompetitive			
Pure	Same	Lower	Increase
Mixed	Higher	Lower	Increase

^a Compared to uninhibited reaction.



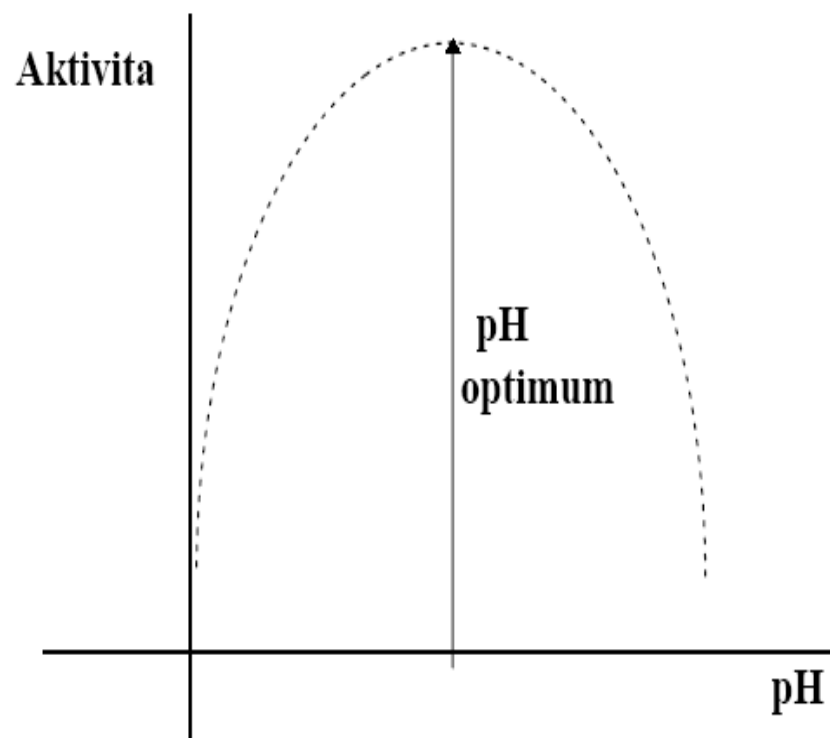
(a) Competitive inhibition

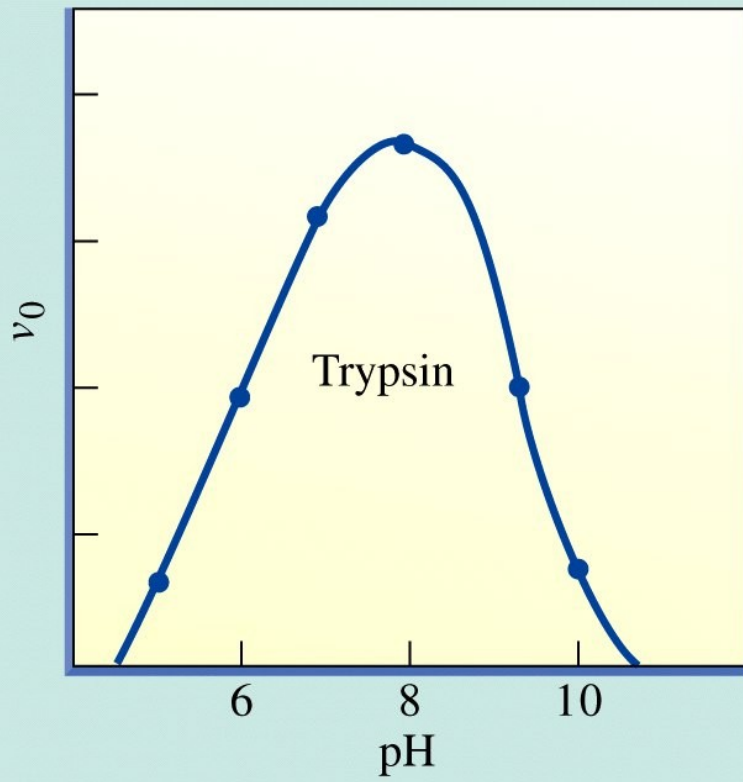
(b) Noncompetitive inhibition

(c) Uncompetitive inhibition

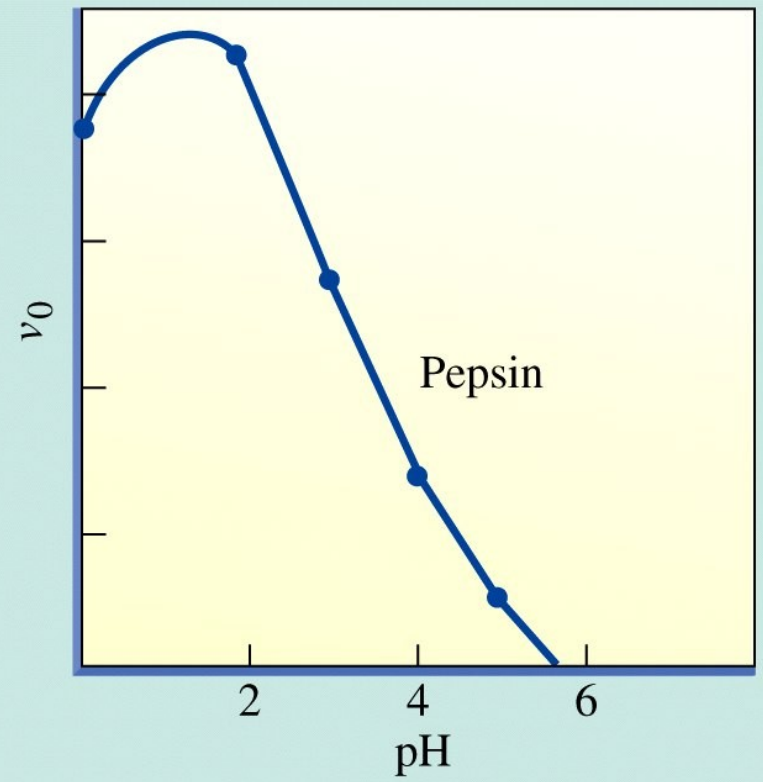
Fyzikálně chemické faktory
ovlivňující rychlost enzymové reakce

Vliv pH



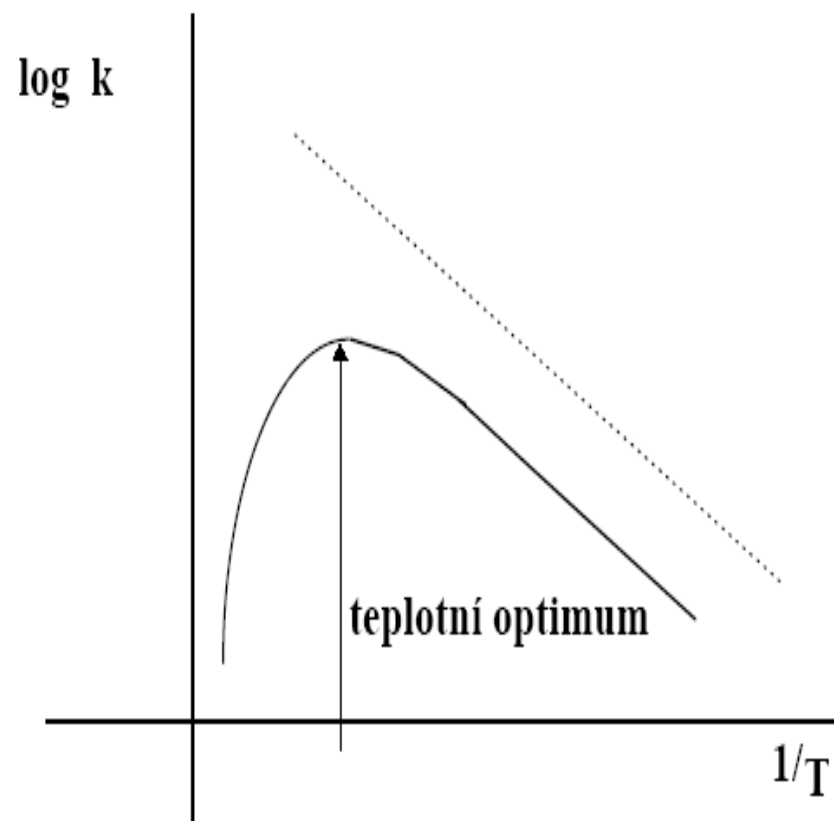


(a)



(b)

Vliv teploty



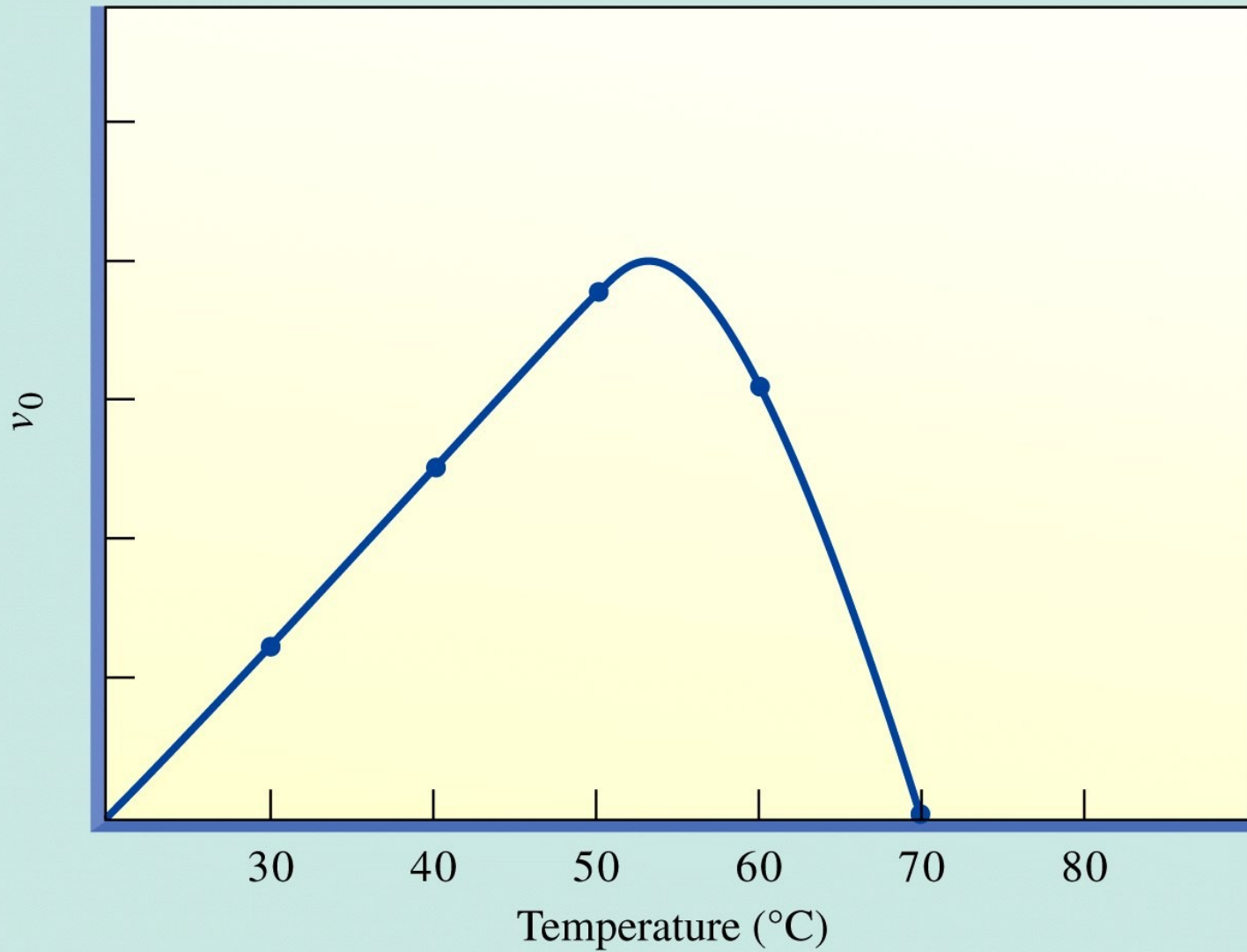
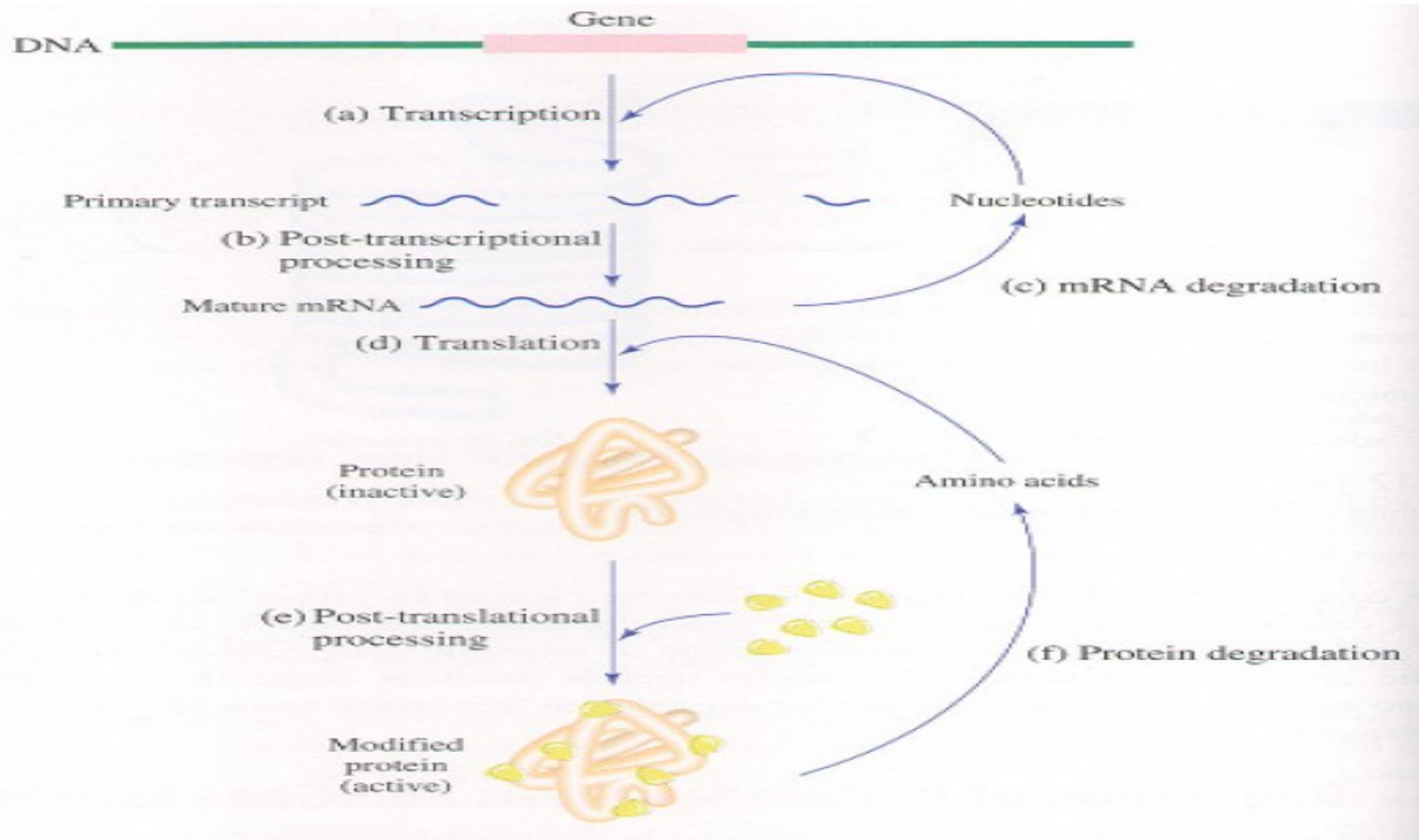


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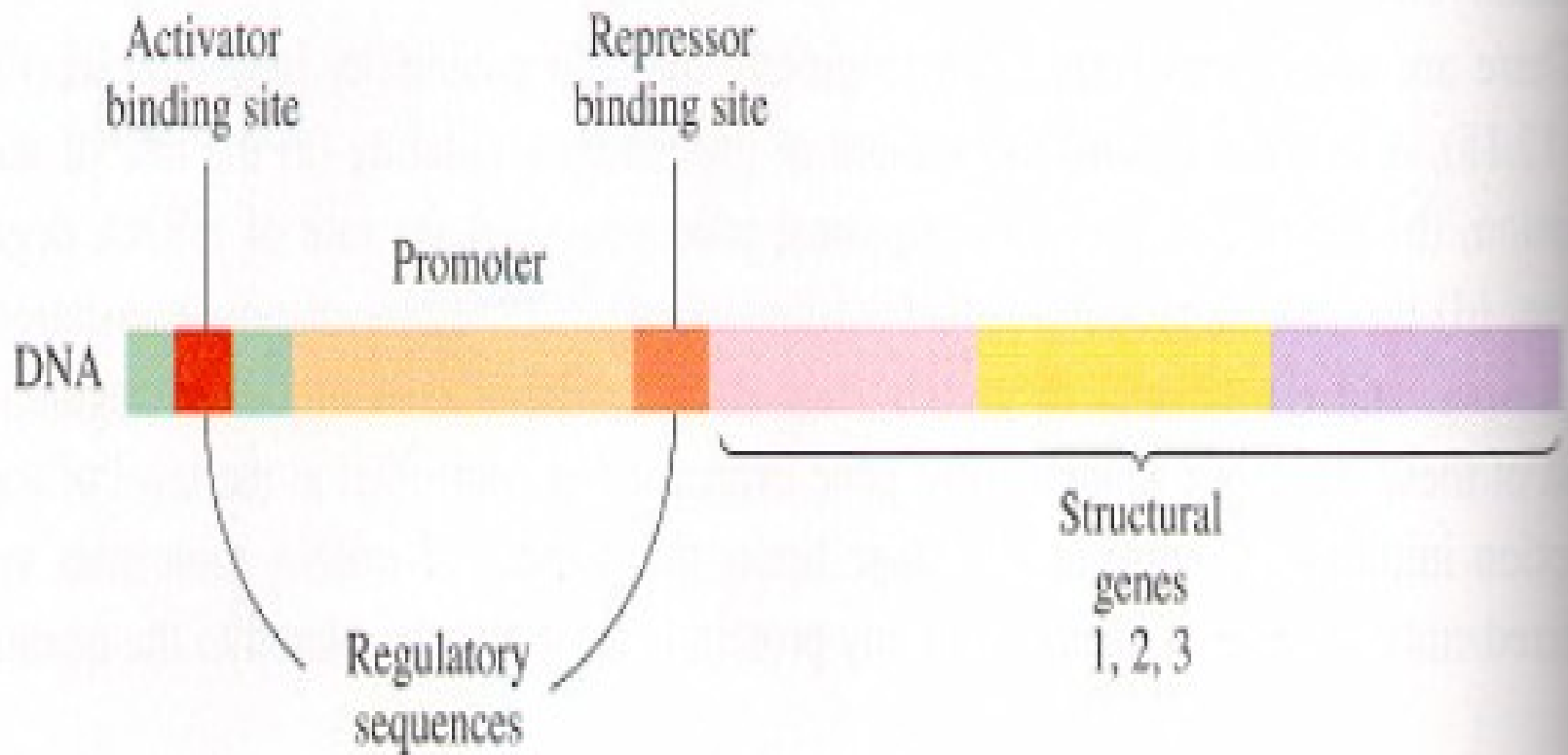
Regulace činnosti enzymu

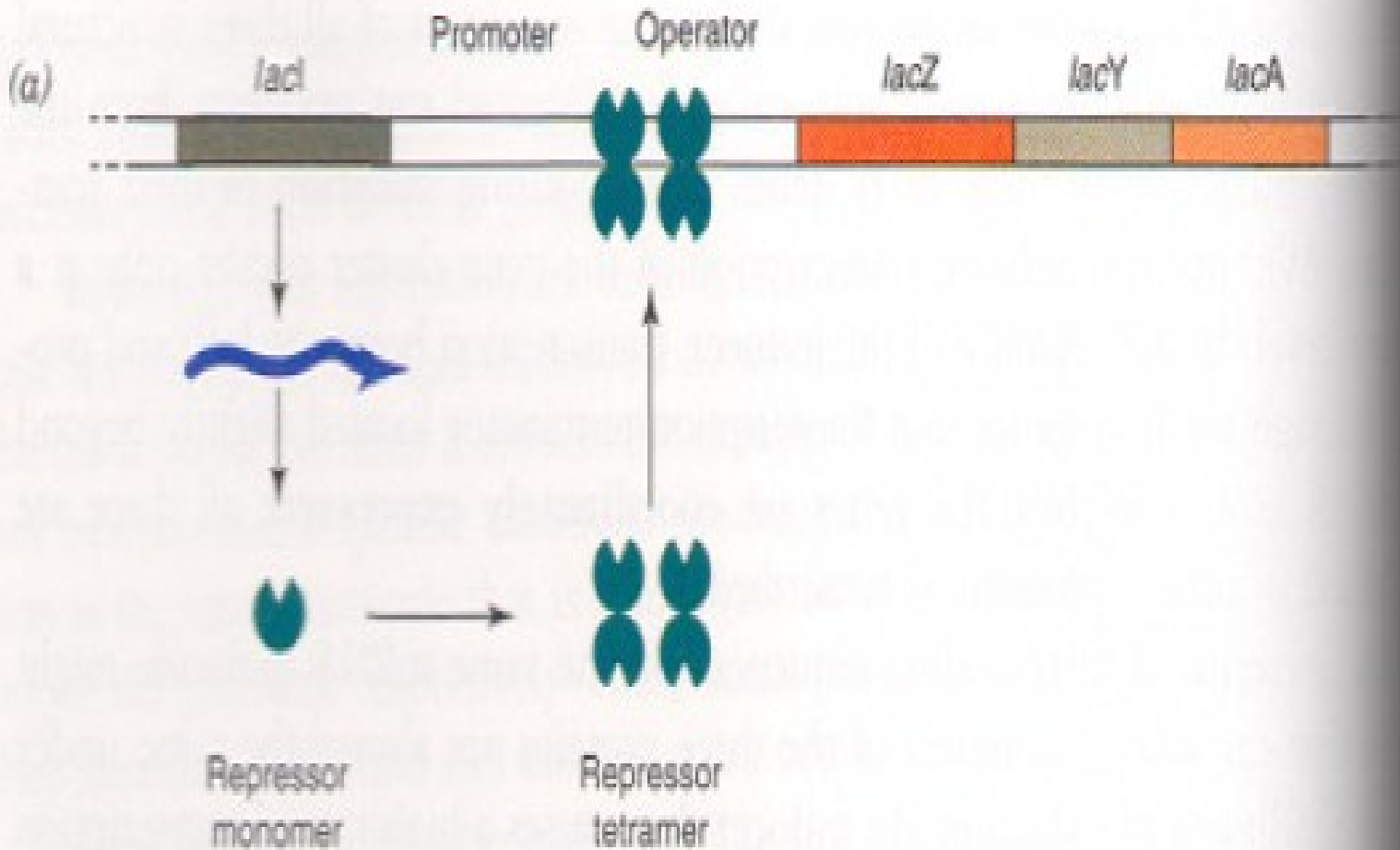
- Regulace koncentrace enzymu
- Allosterická regulace MONOD 1963
- Regulace zpětnou vazbou
- Regulace kovalentní modifikací
- Kompartmentace

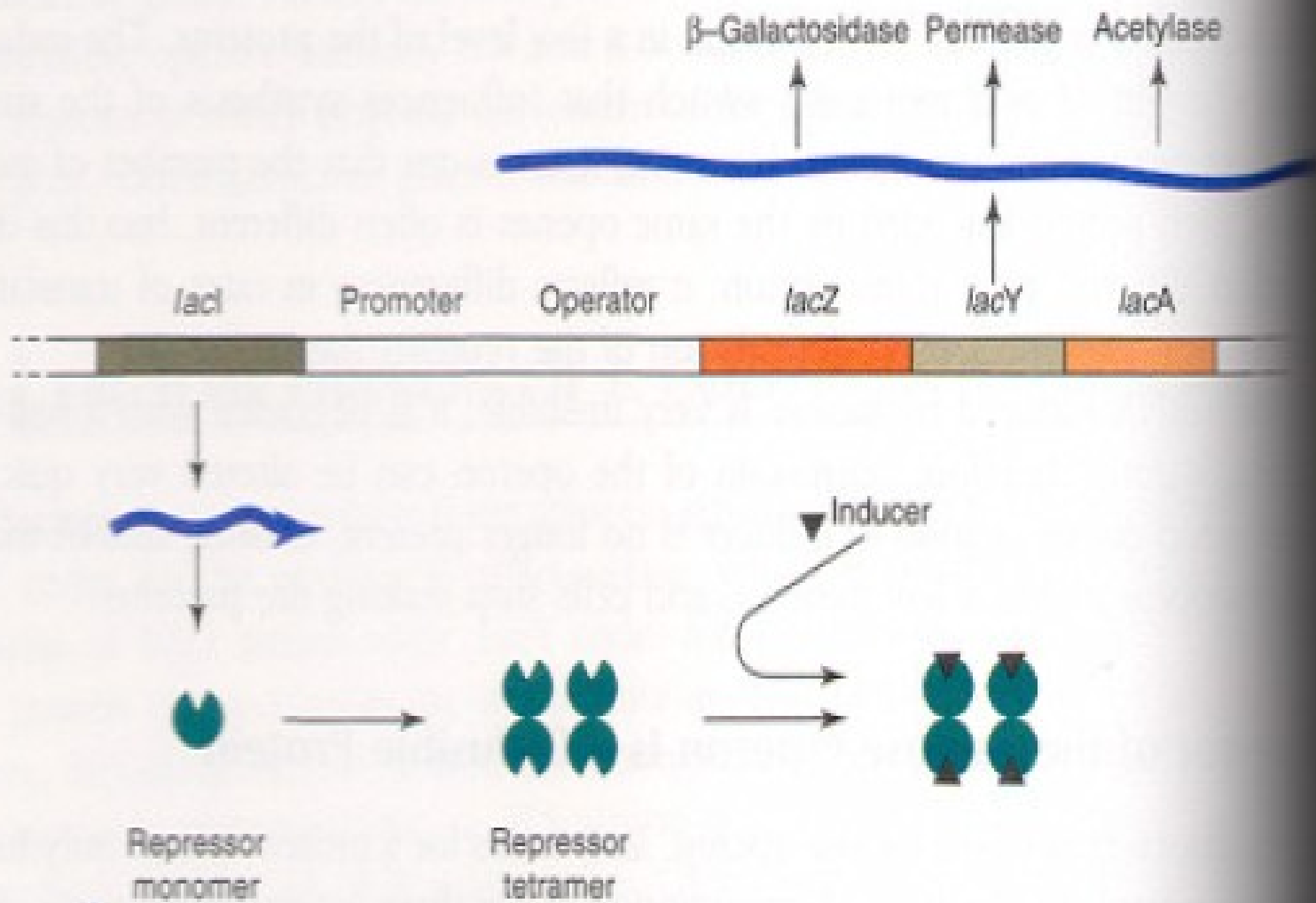
Regulace koncentrací enzymu



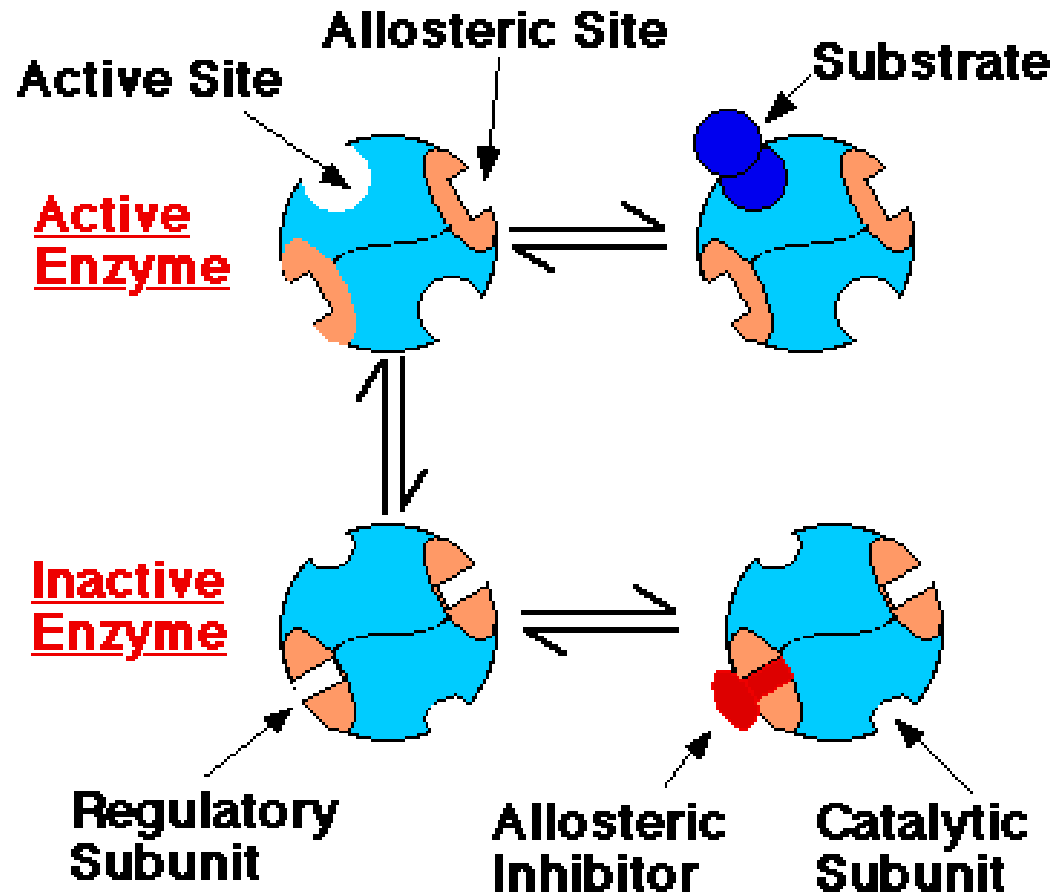
Operonový model



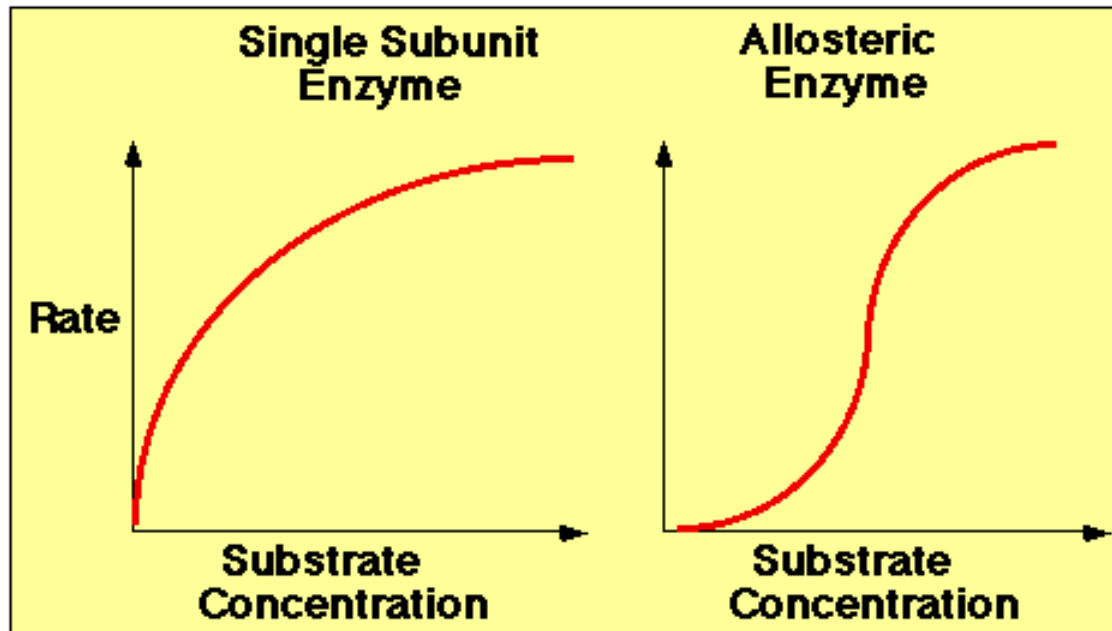




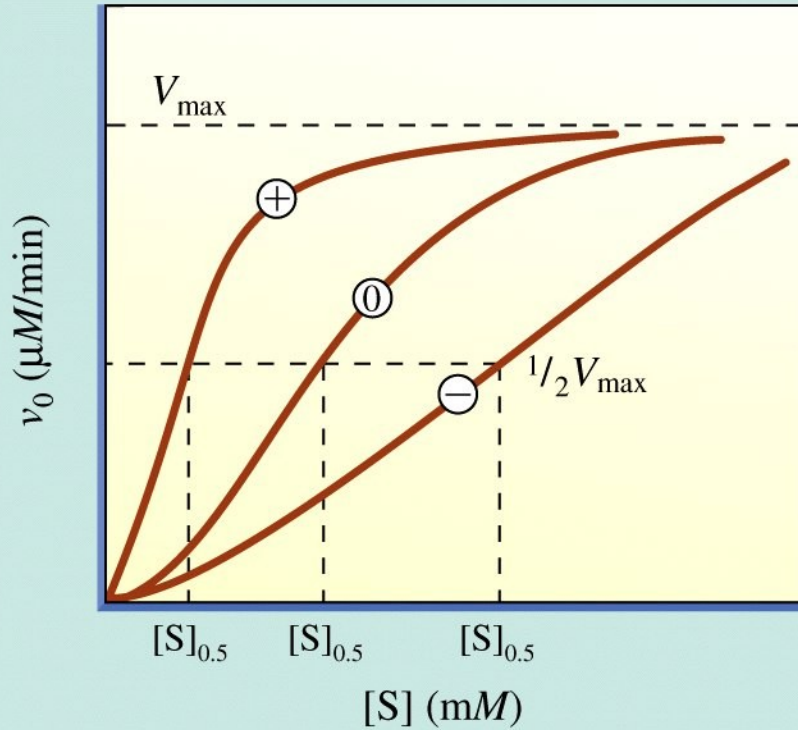
Allosterie



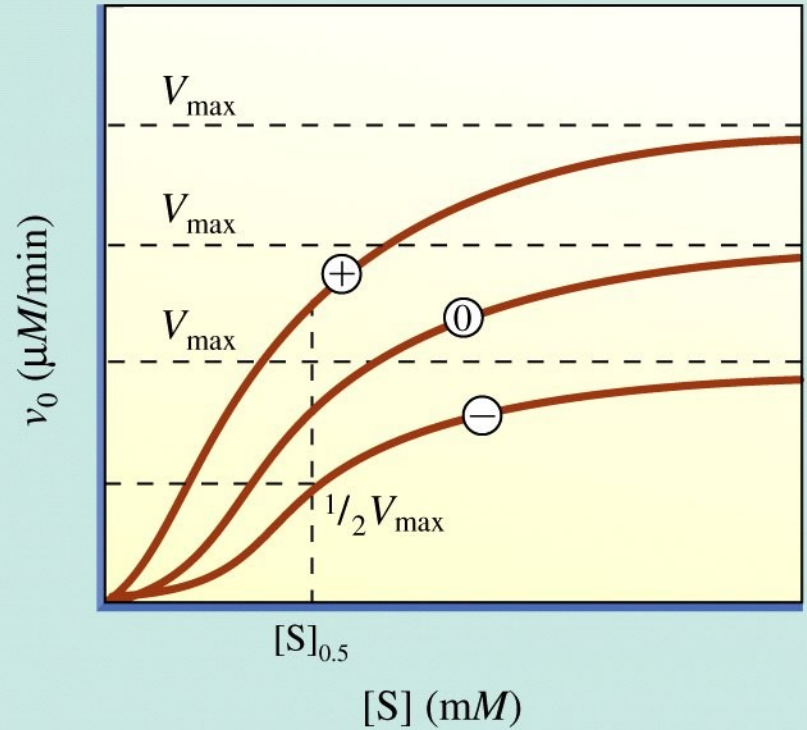
Allosterie



Allosterie

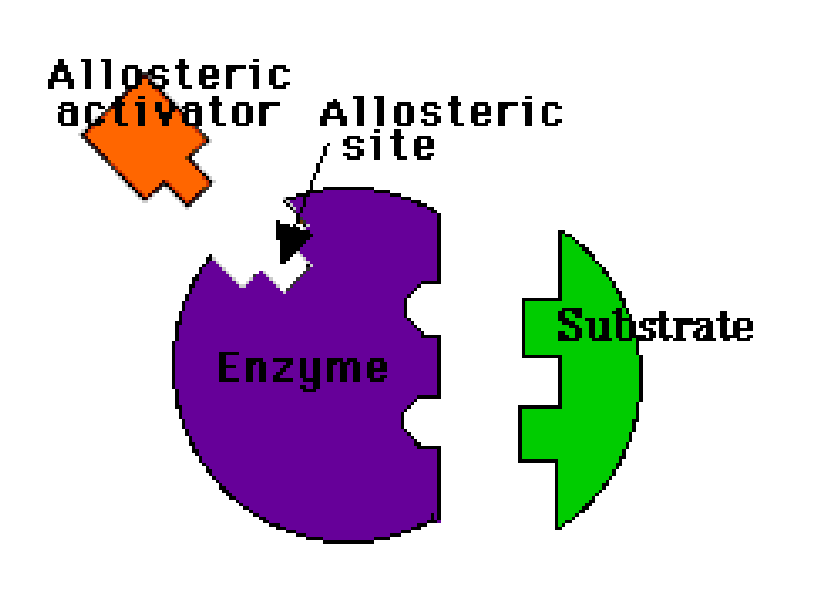


(a)

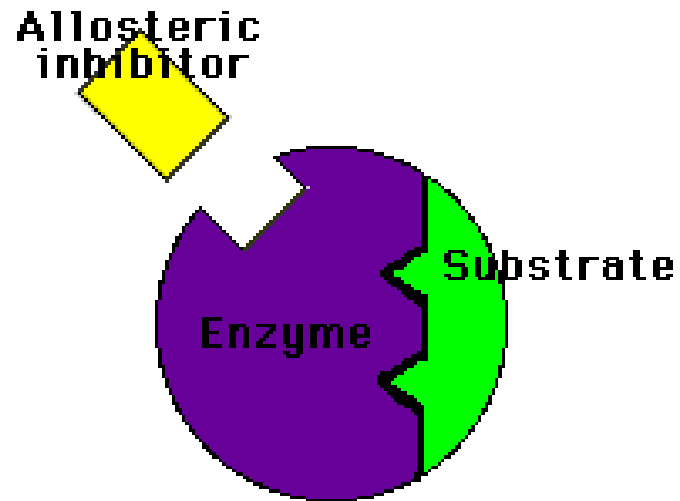


(b)

Allosterický aktivátor



Allosterický inhibitor



Symetrický model

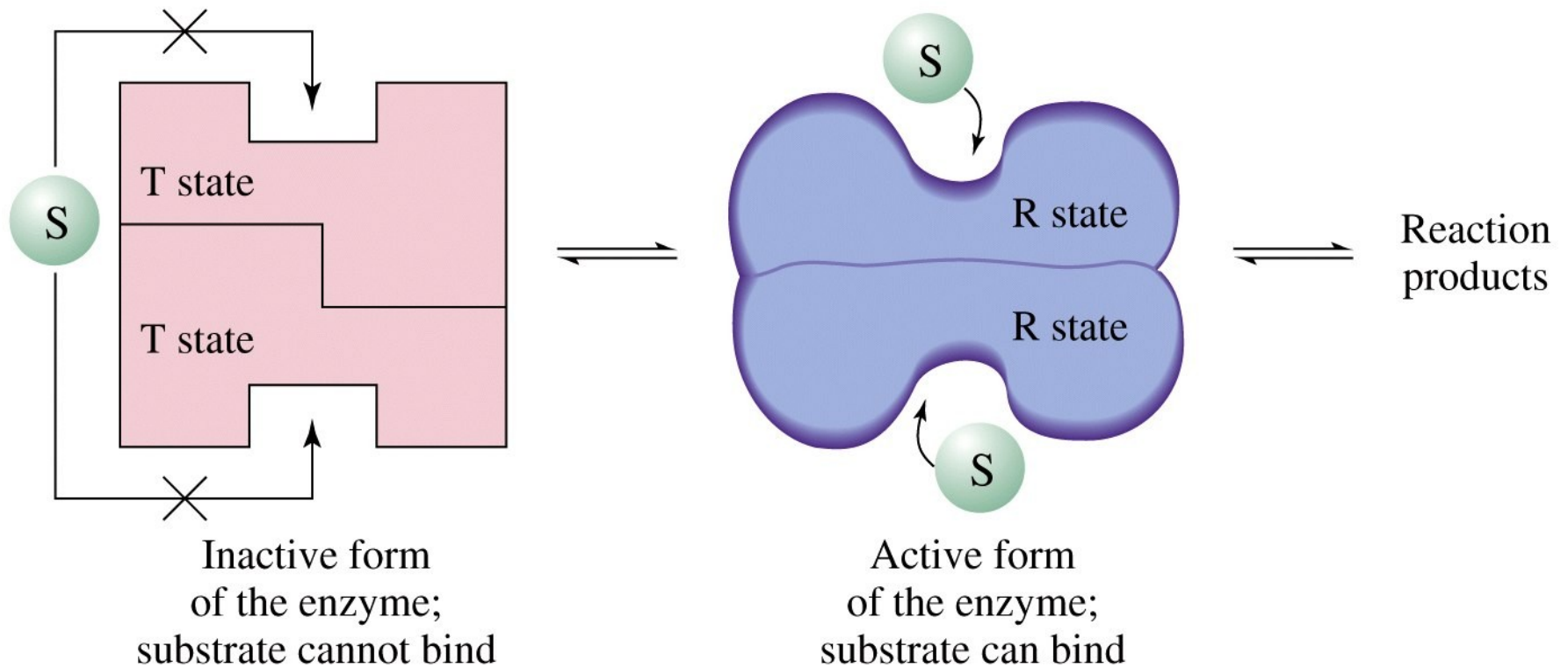


Figure 6-5 Concepts in Biochemistry, 3/e
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Sekvenční model

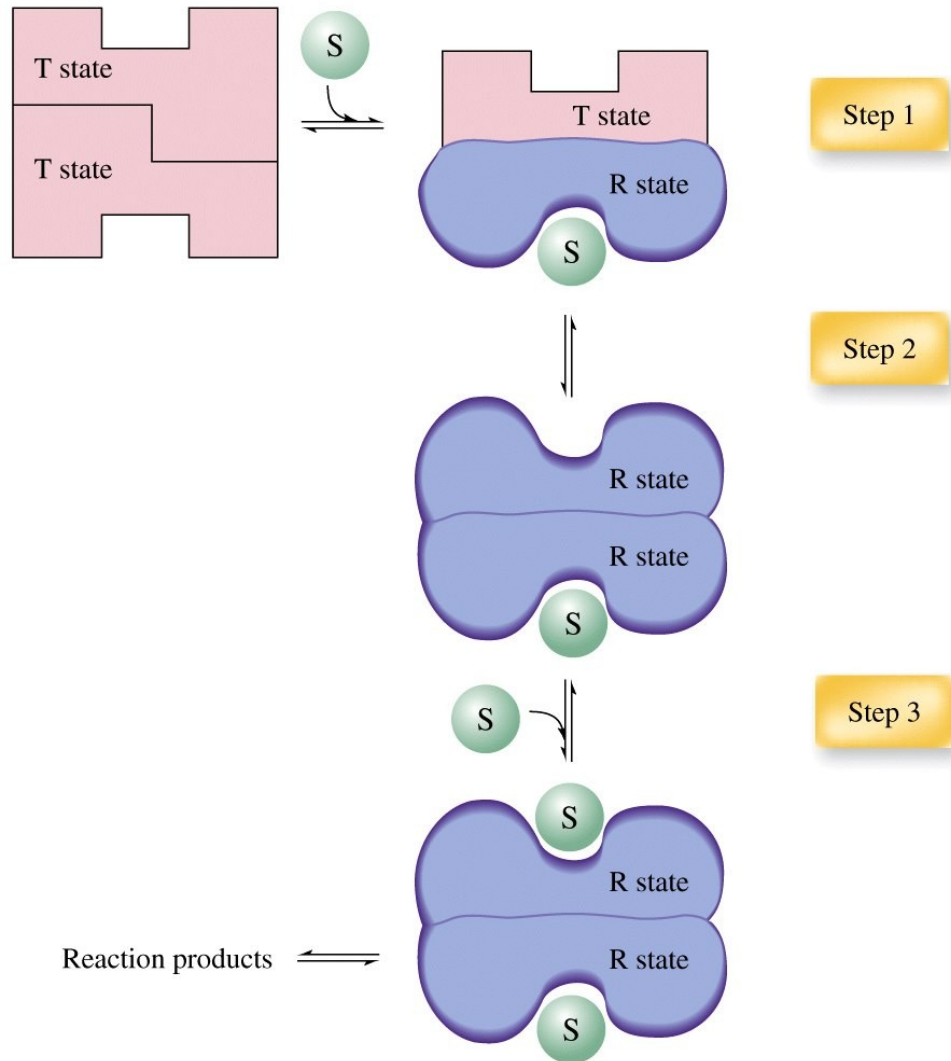
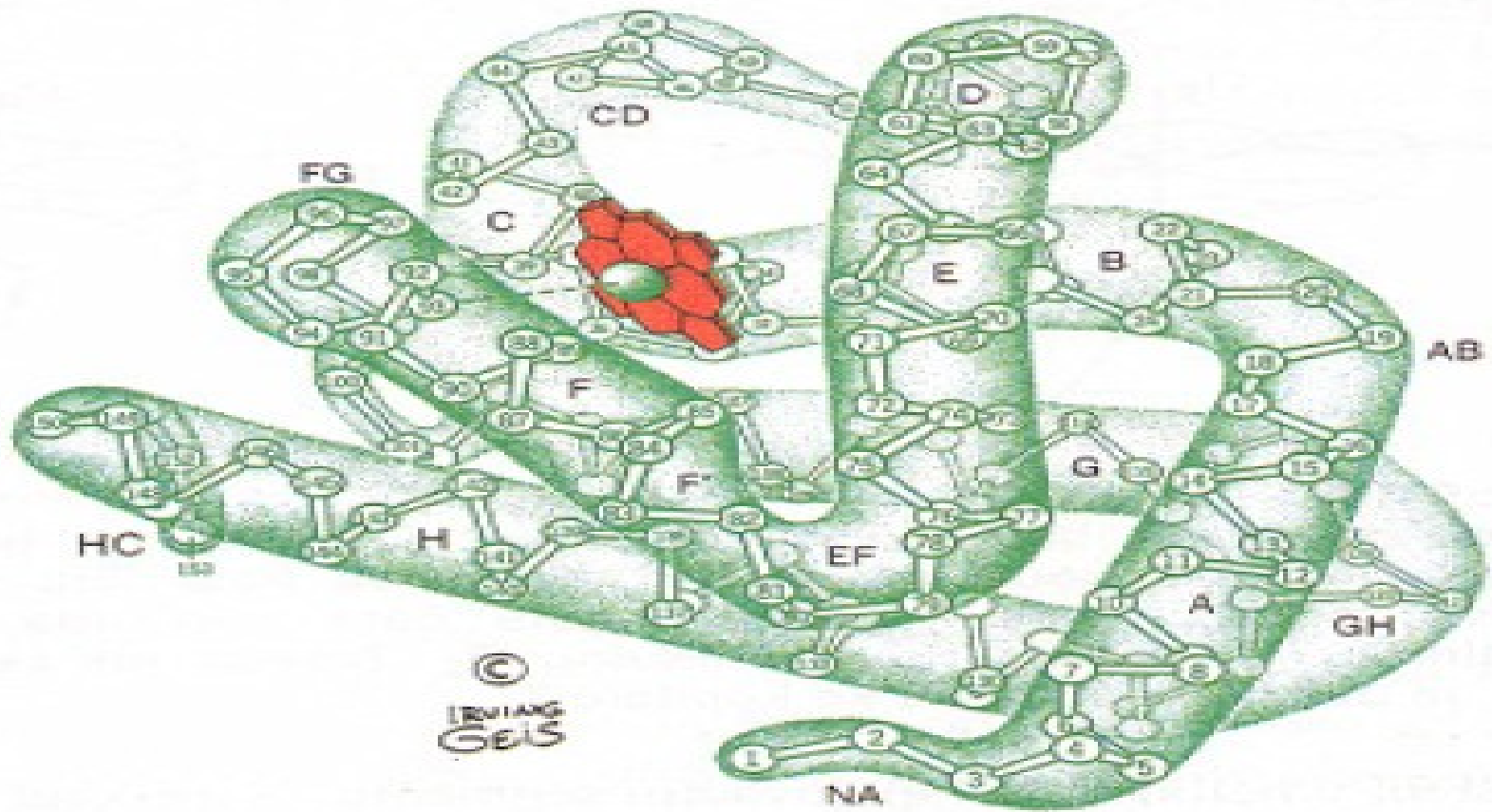
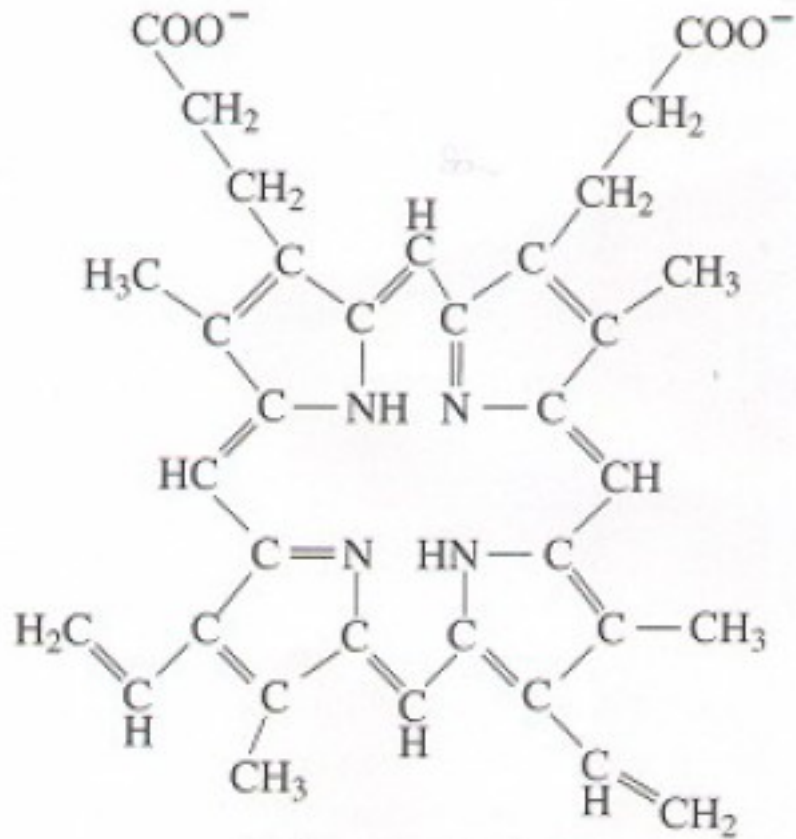


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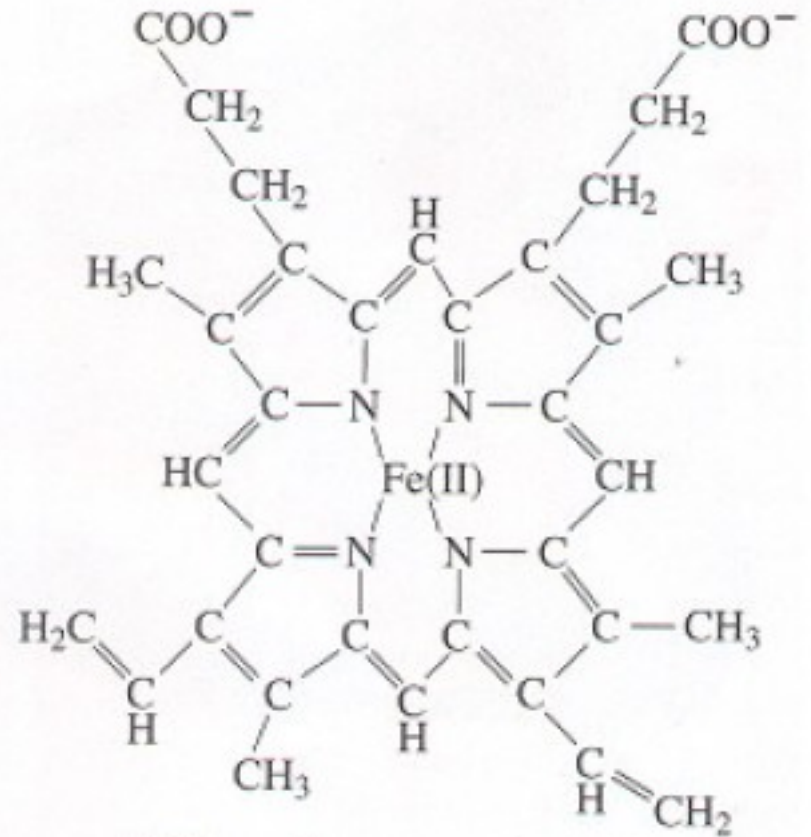
Myoglobin



Hem

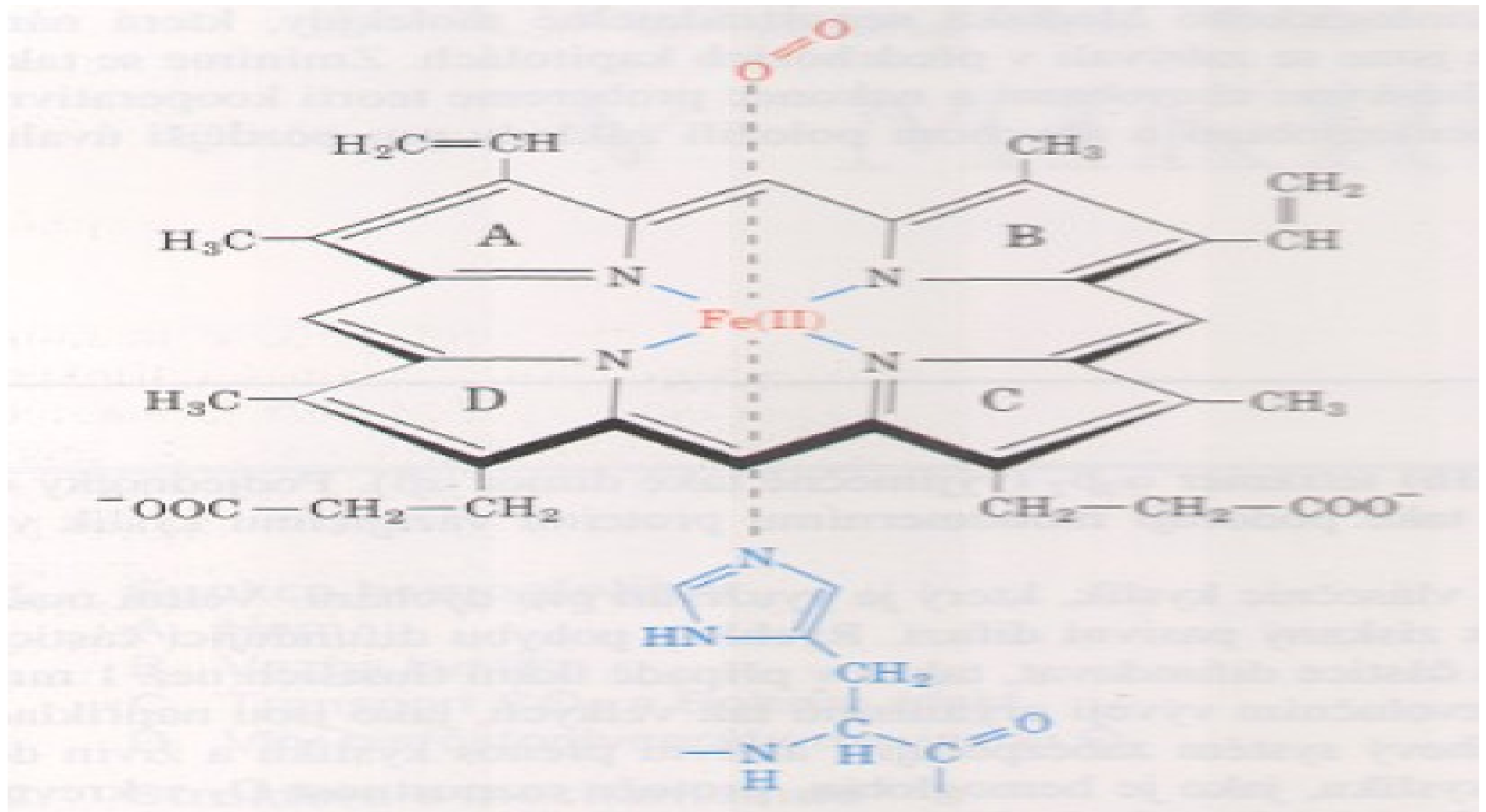


(a) Protoporphyrin IX

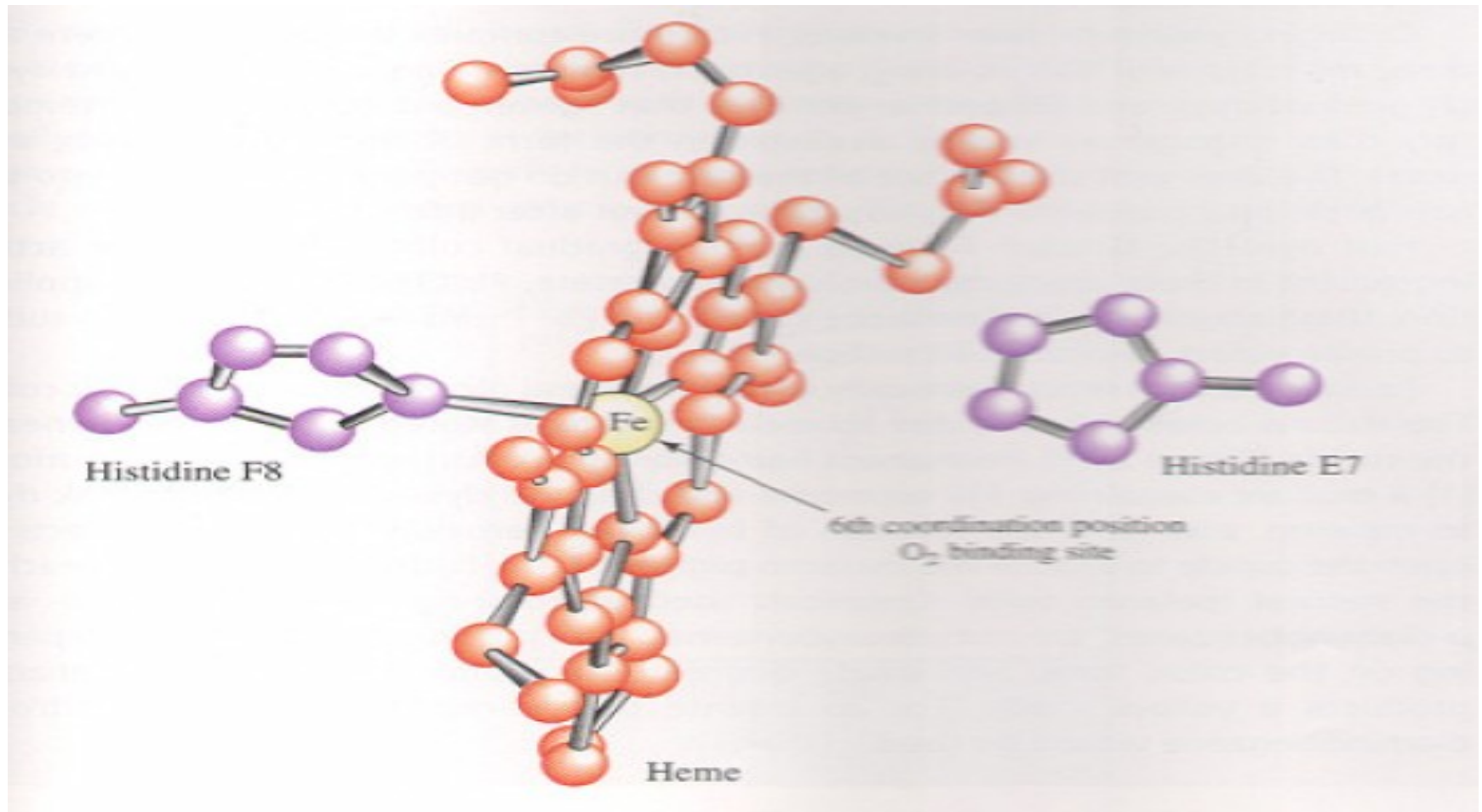


(b) Heme (Fe-protoporphyrin IX)

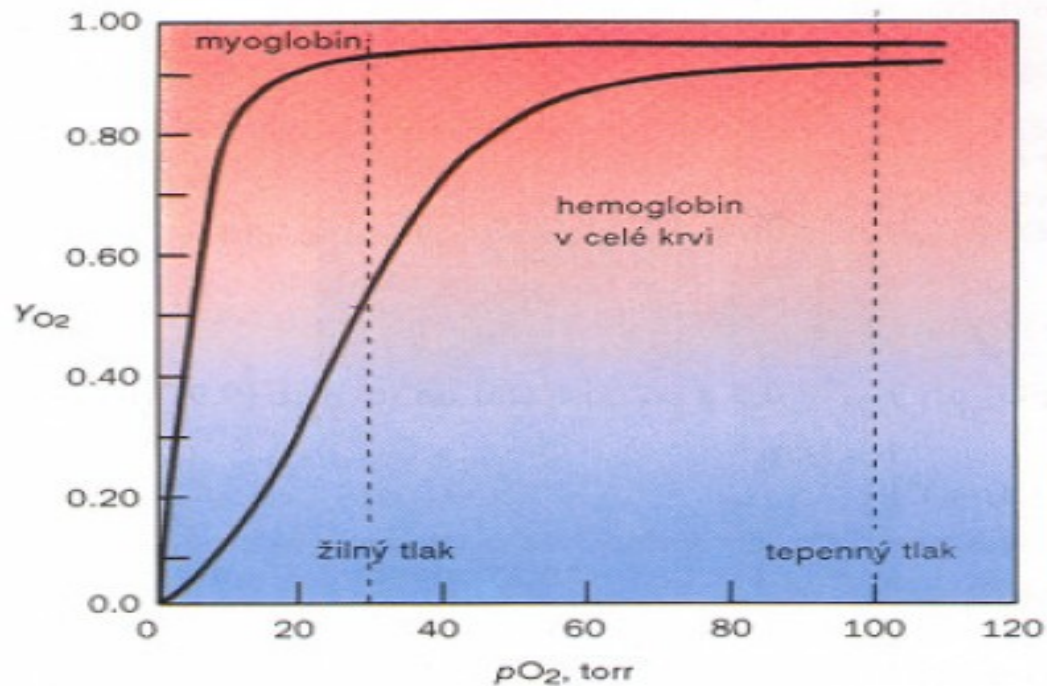
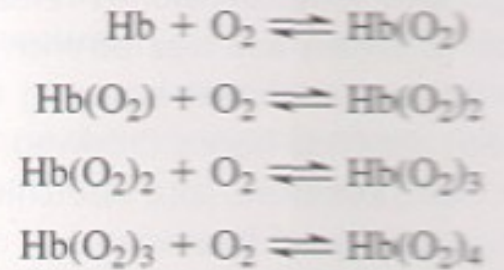
Vazba O₂ na Hb



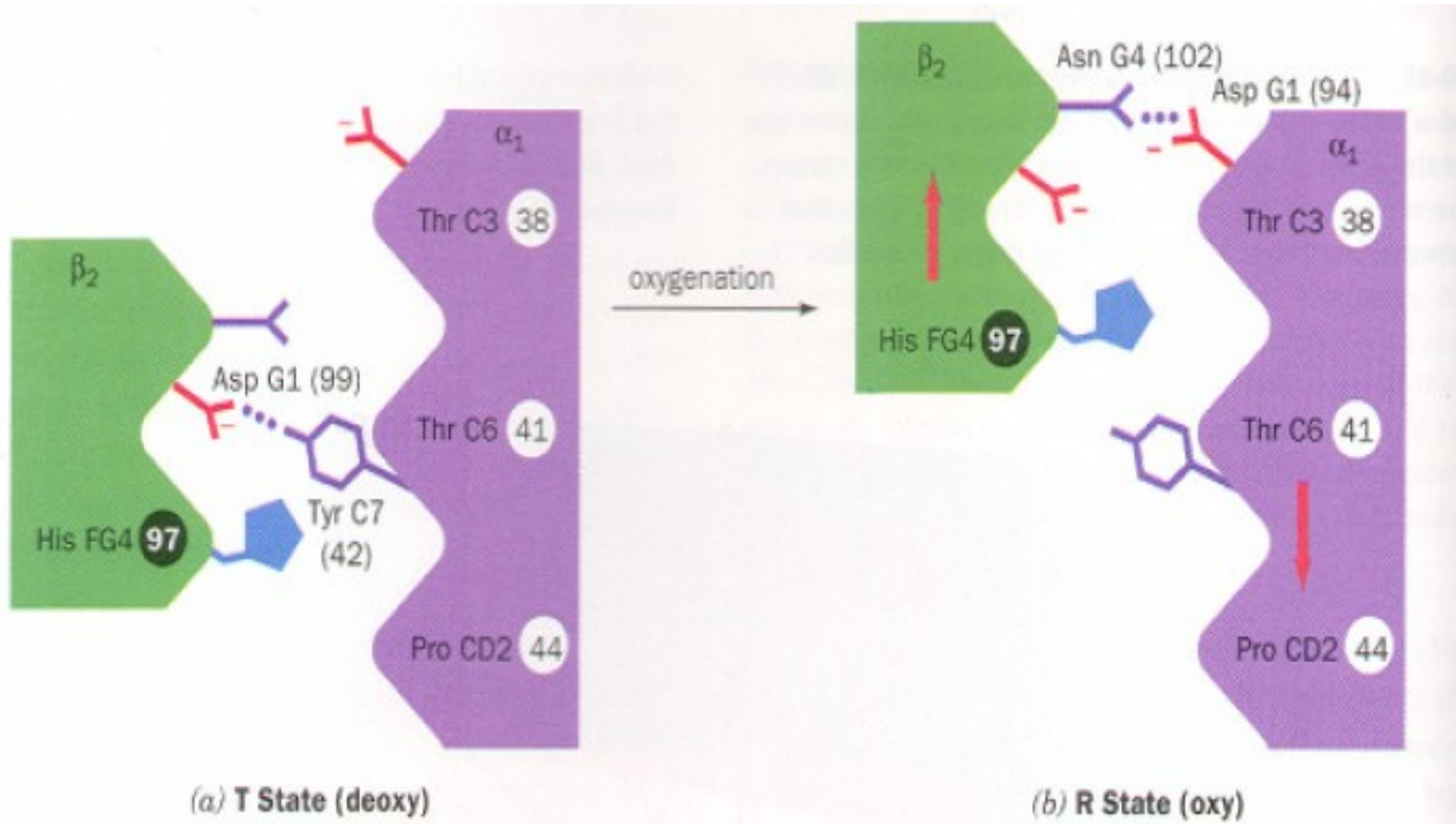
Vazba O₂ na Hb



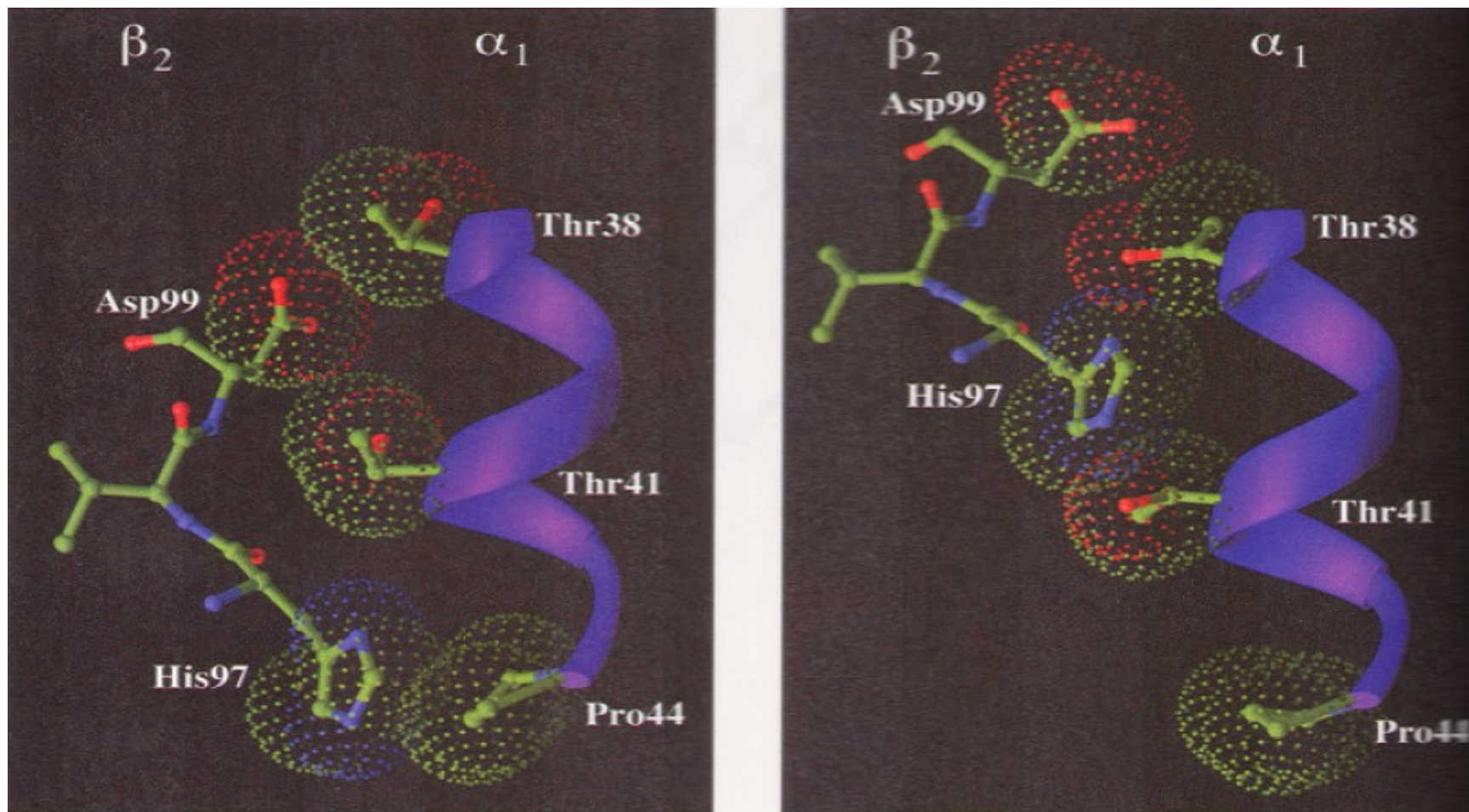
Hb versus Mb



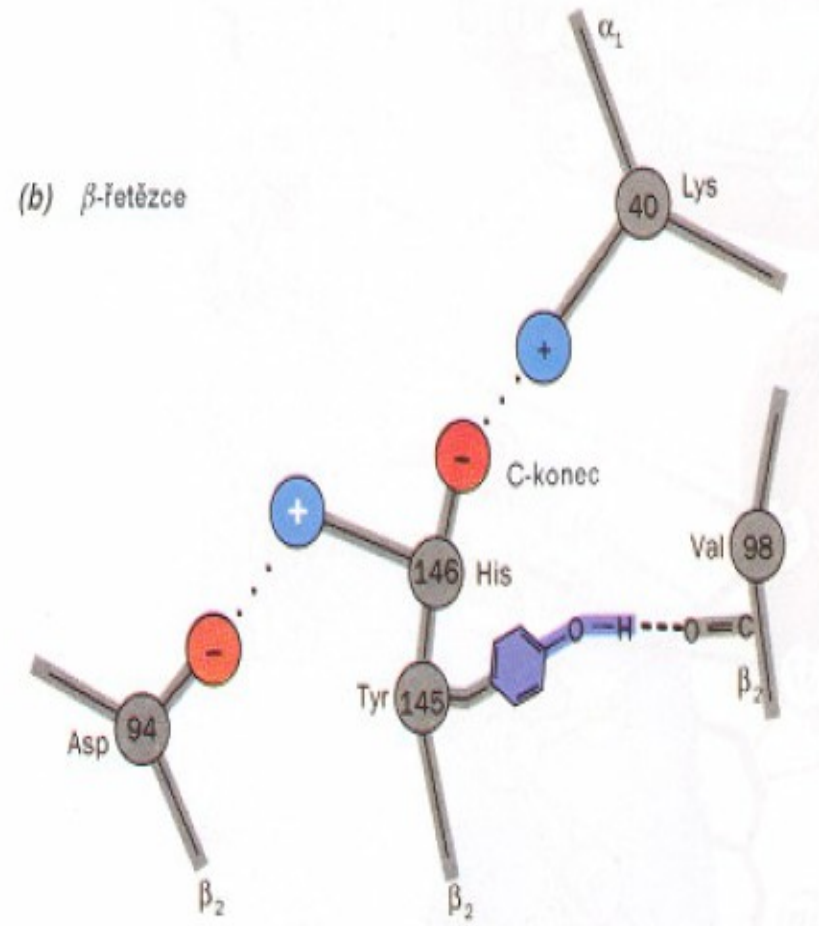
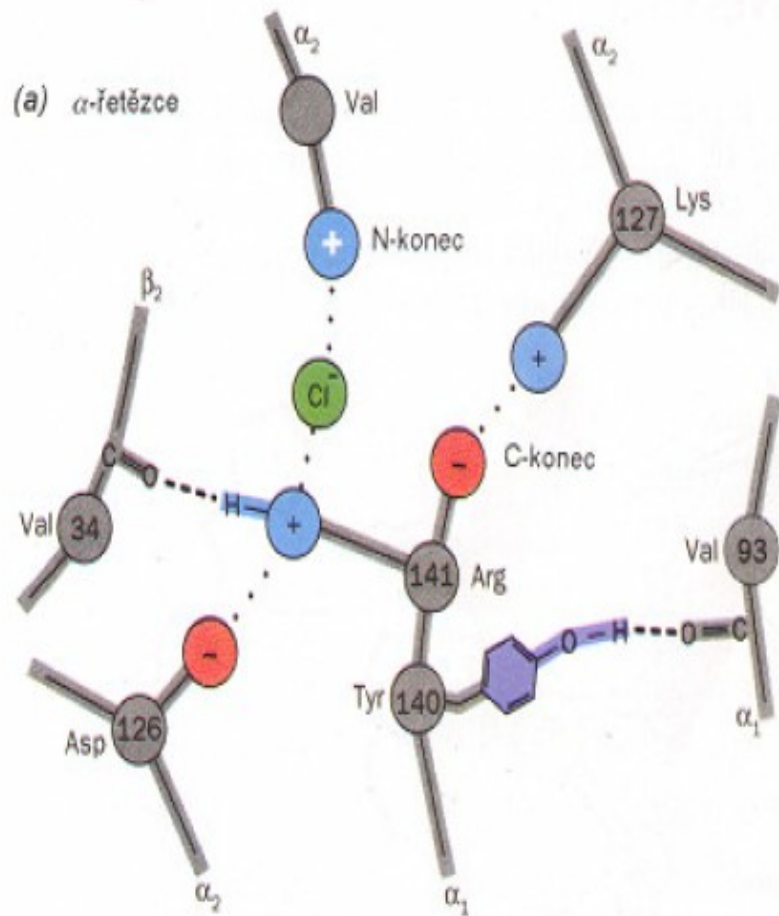
Solné můstky



Solné můstky



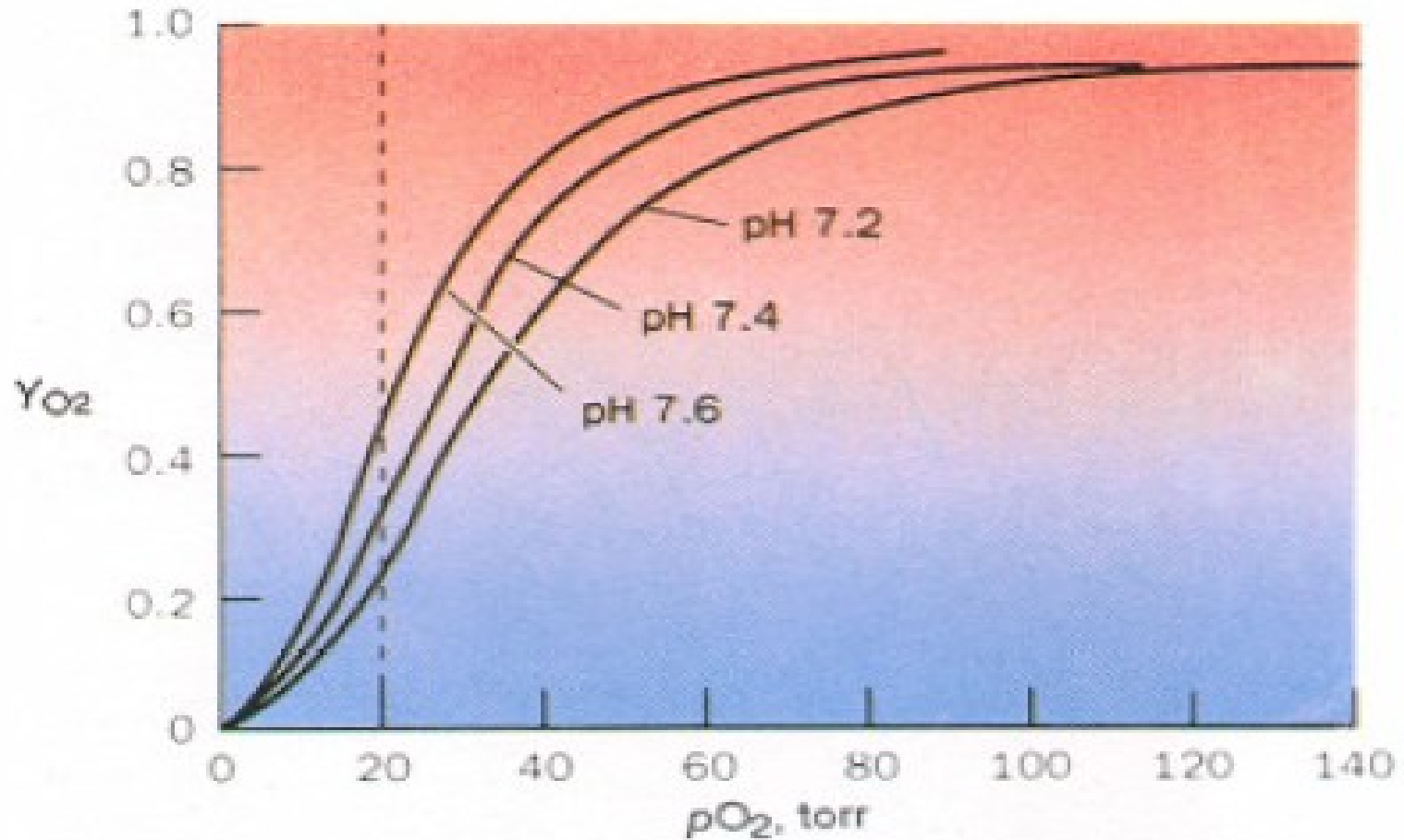
Solné můstky



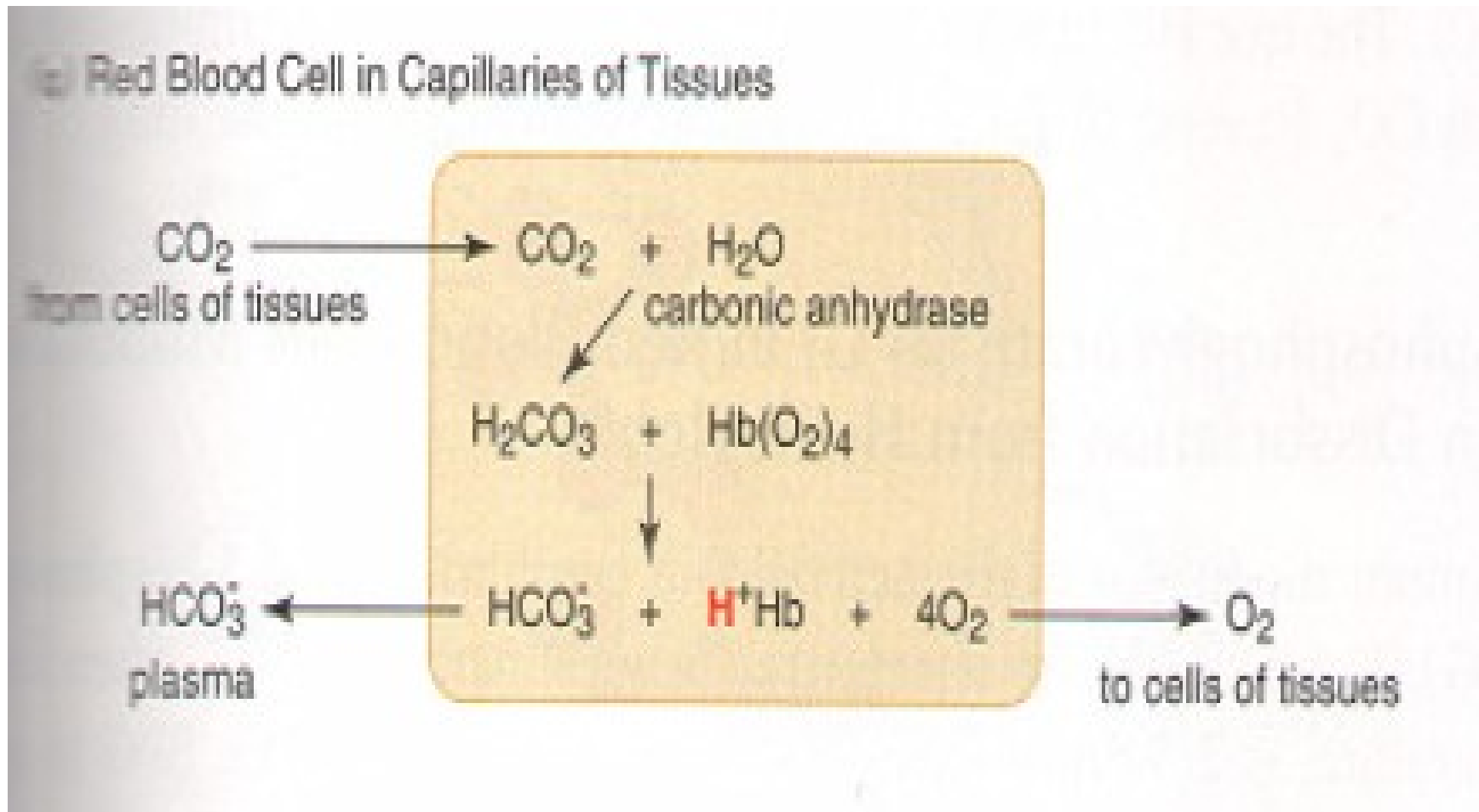
Bohrův efekt – vliv H^+ a CO_2



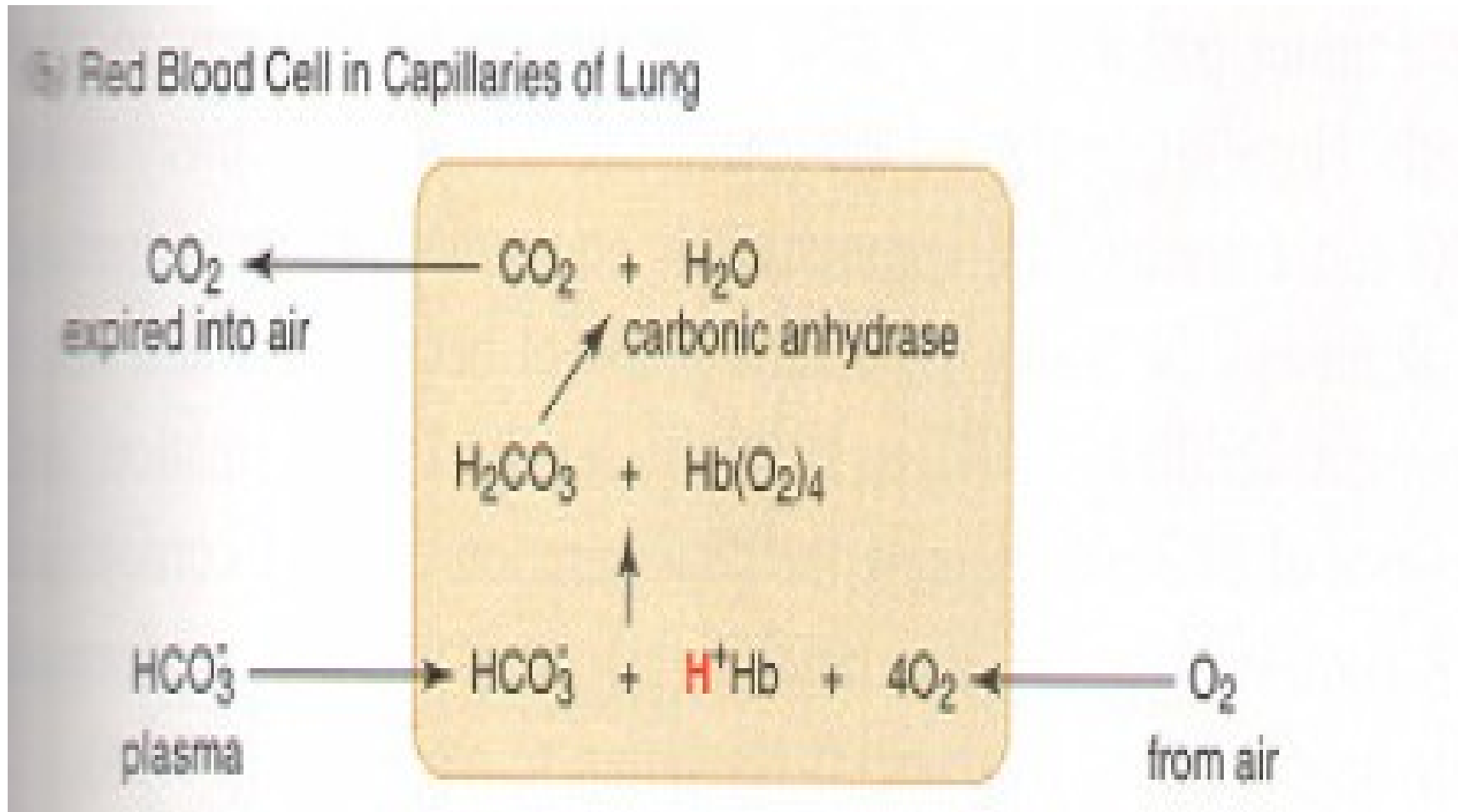
Bohrův efekt – vliv H^+ a CO_2



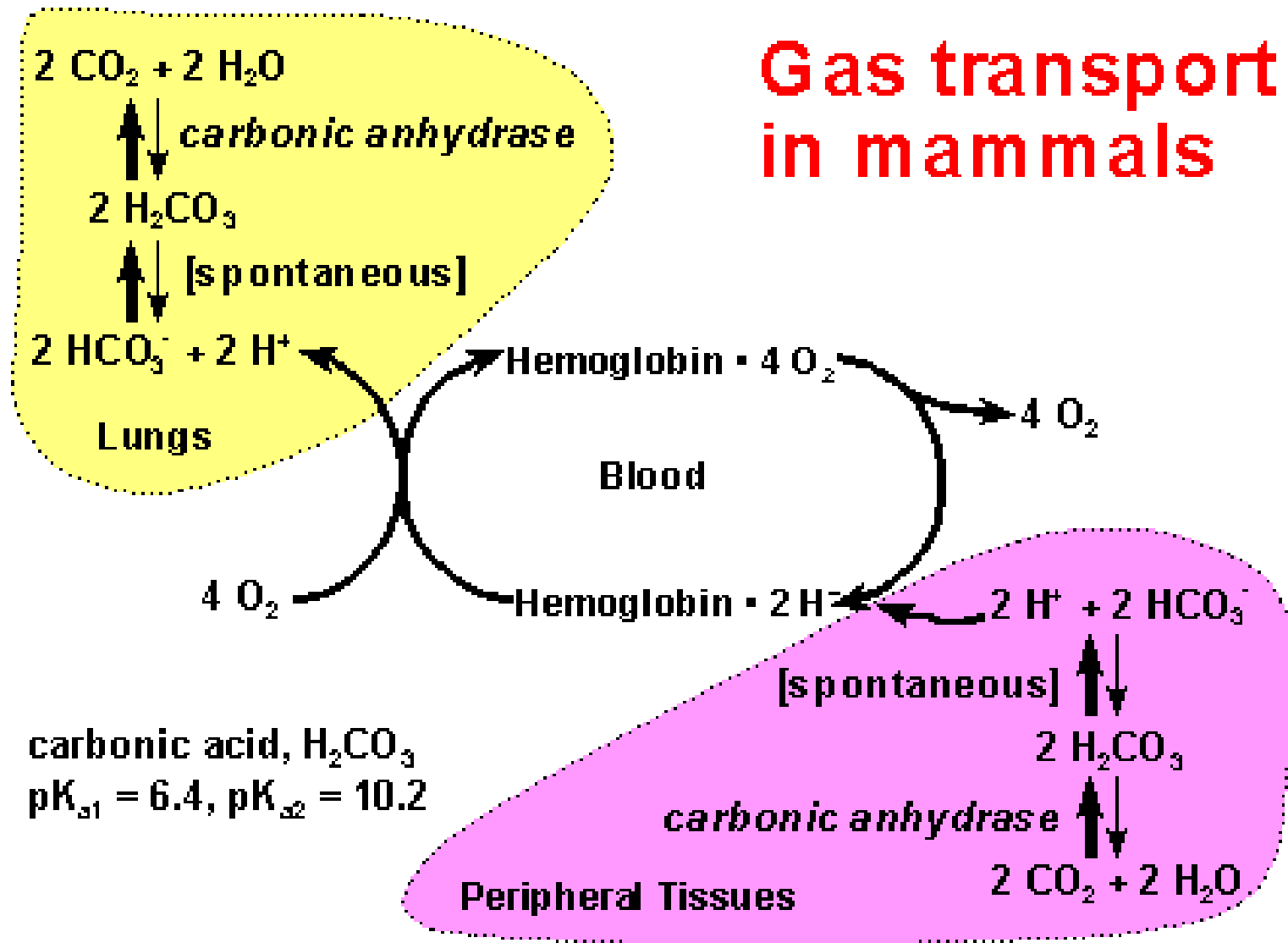
Bohrův efekt – vliv H^+ a CO_2



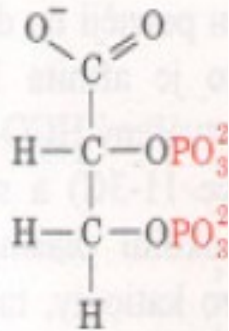
Bohrův efekt – vliv H^+ a CO_2



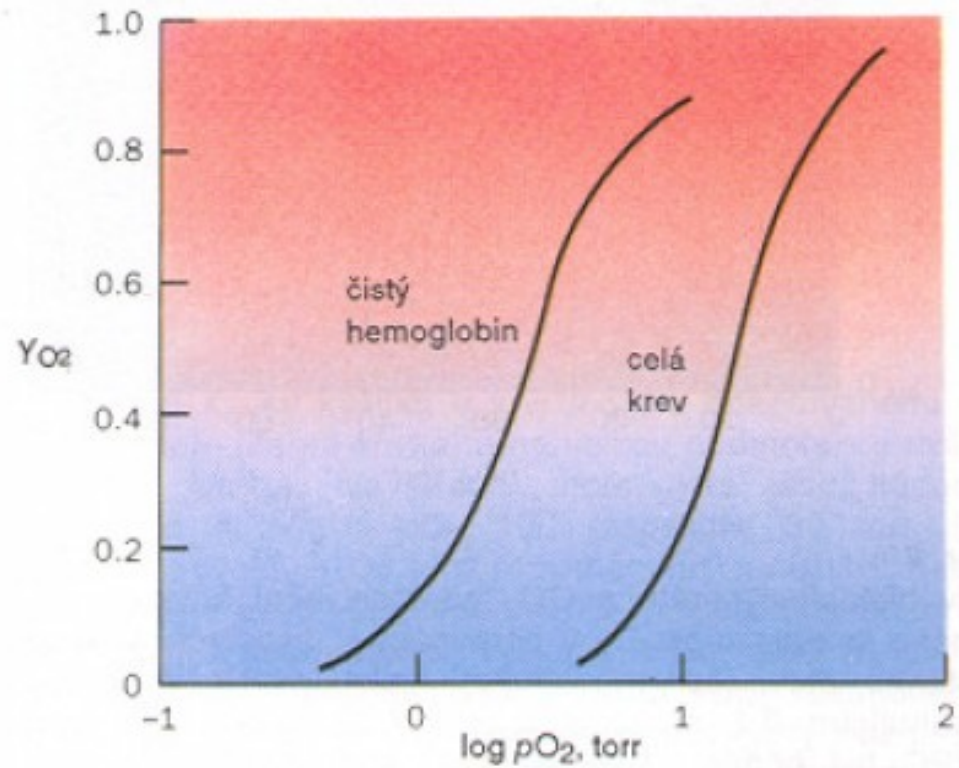
Gas transport in mammals



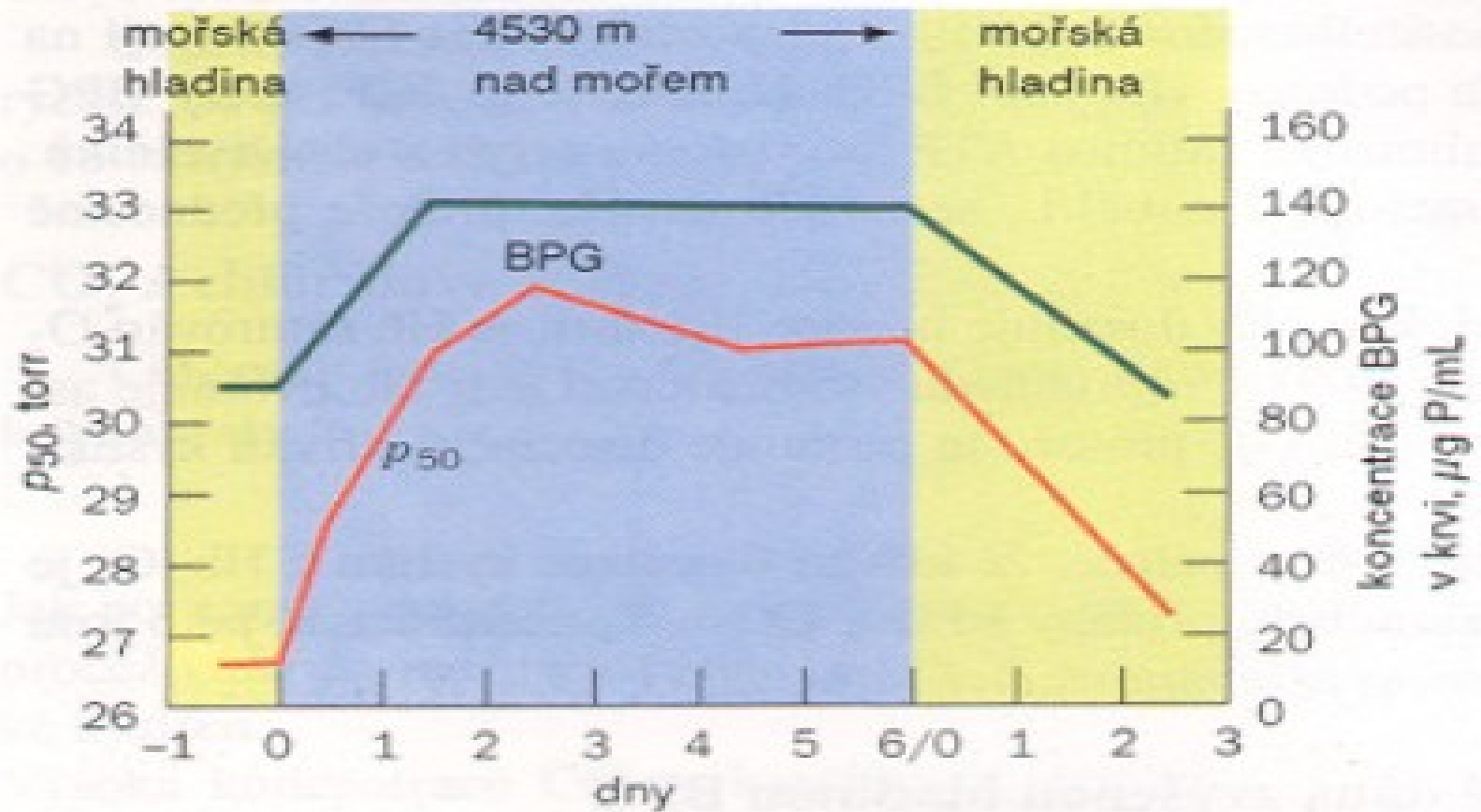
Vliv BPG



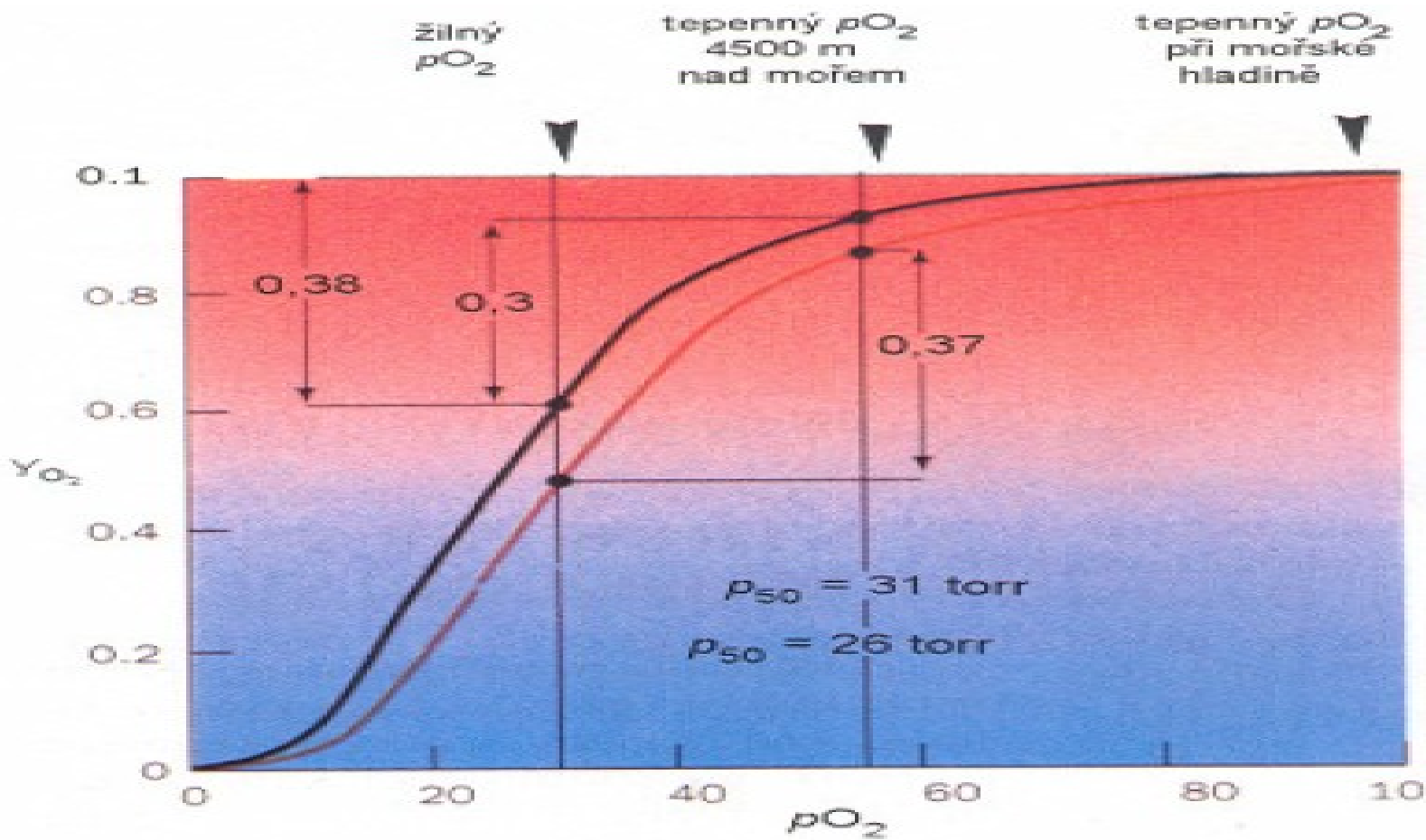
D-2,3-bisfosfoglycerát (2,3-P₂-G



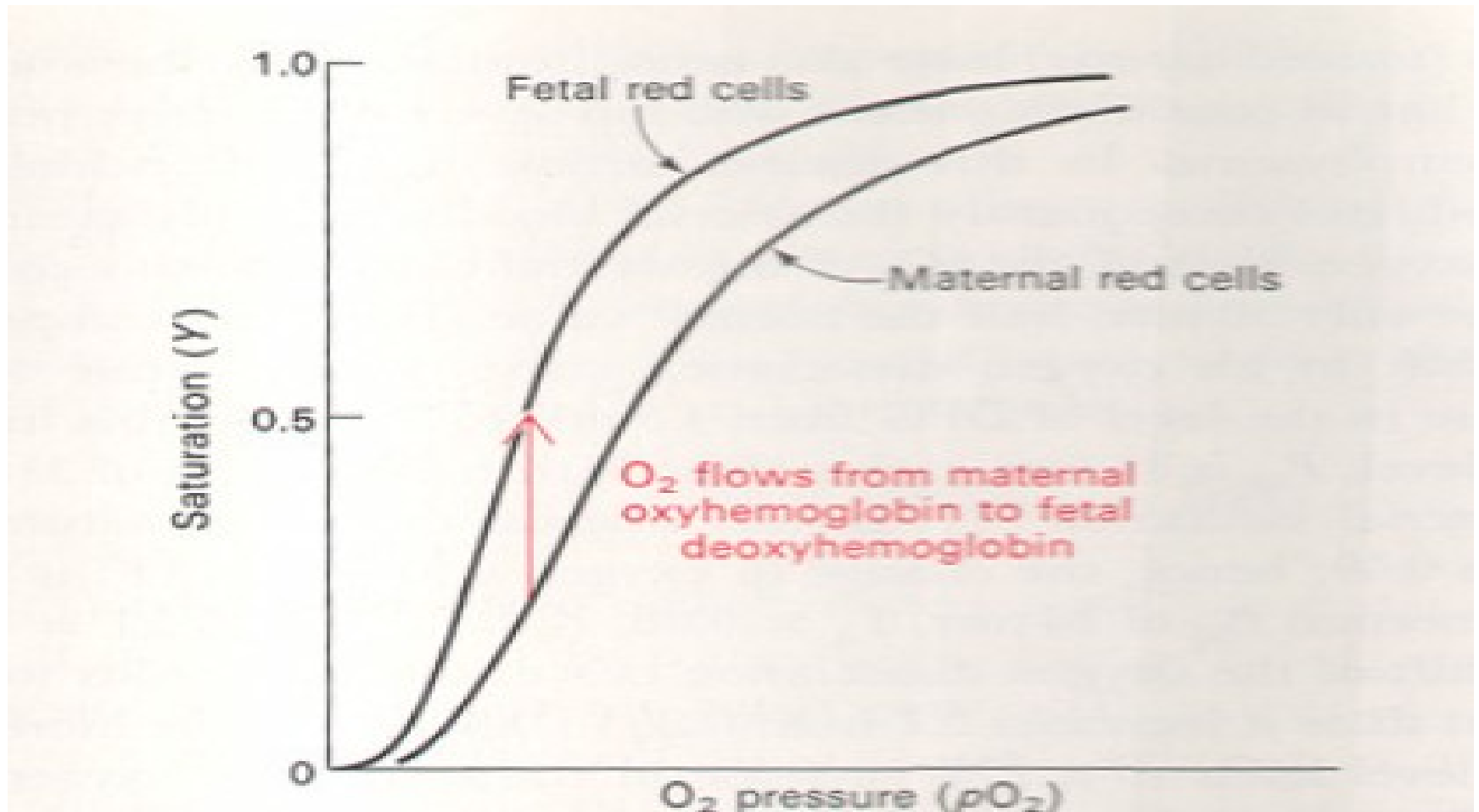
Vliv BPG a nadmořská výška



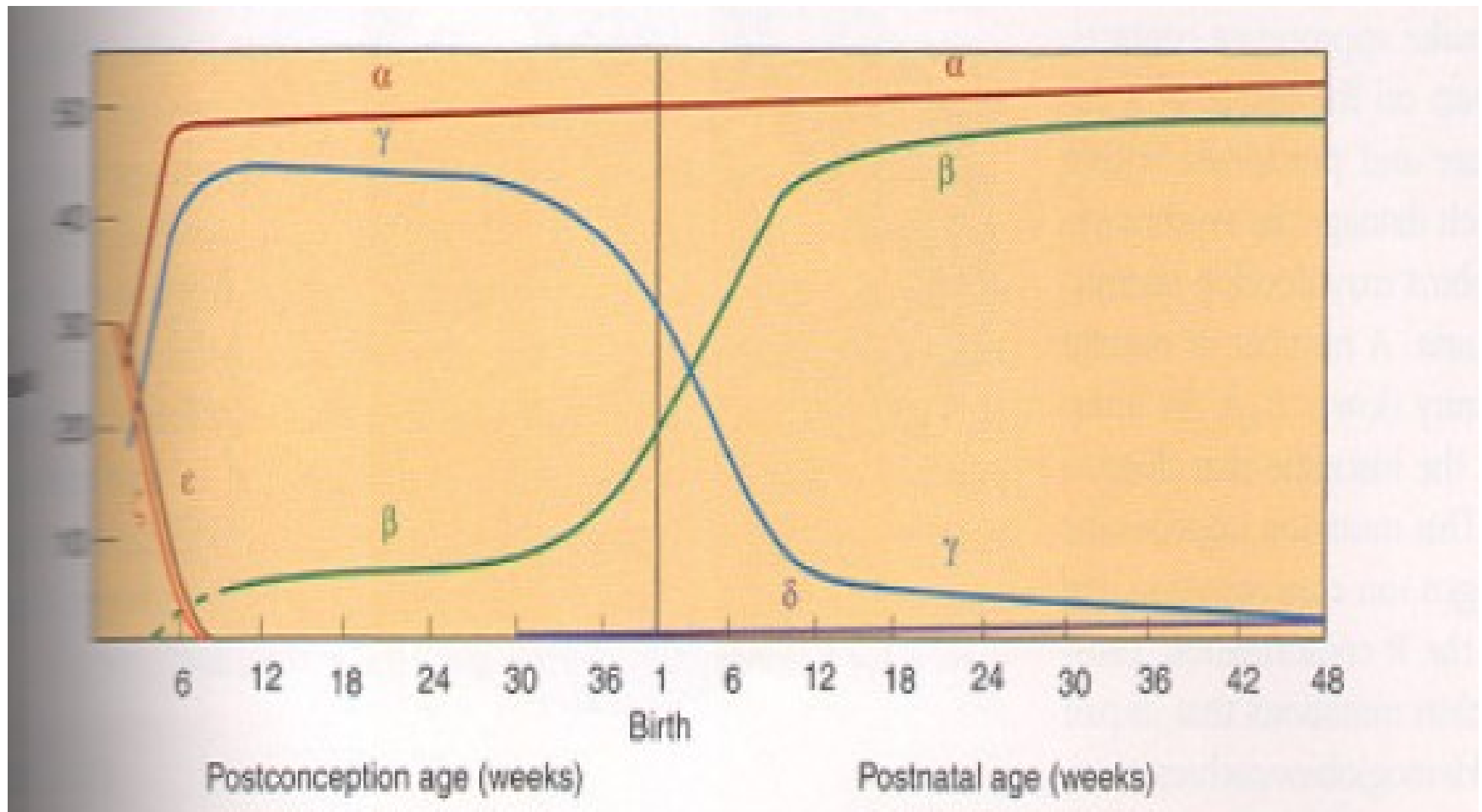
Vliv BPG a nadmořská výška



Fetální versus normální Hb



Fetální versus normální Hb



Regulace kovalentní modifikací glykogenfosforylasa

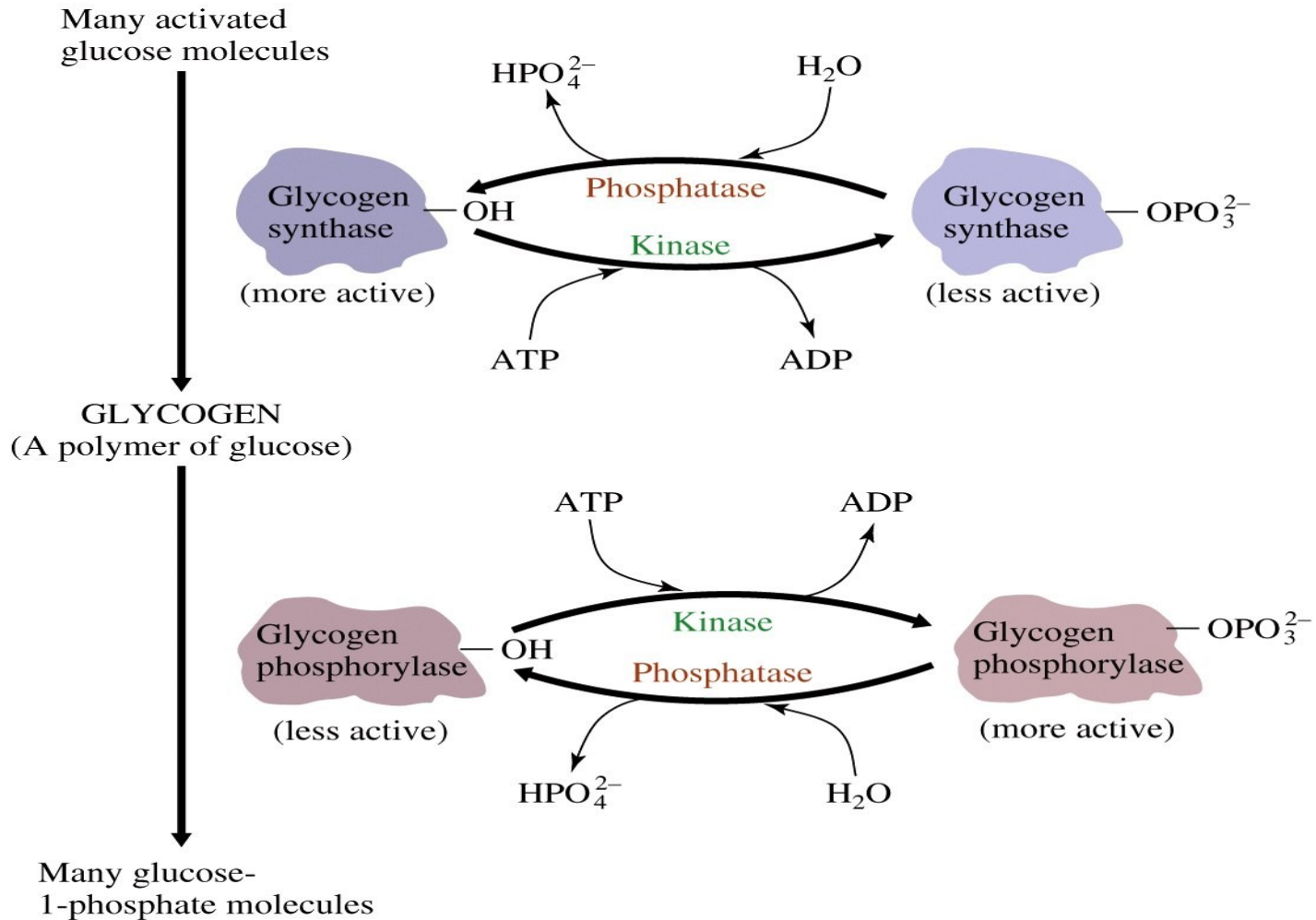
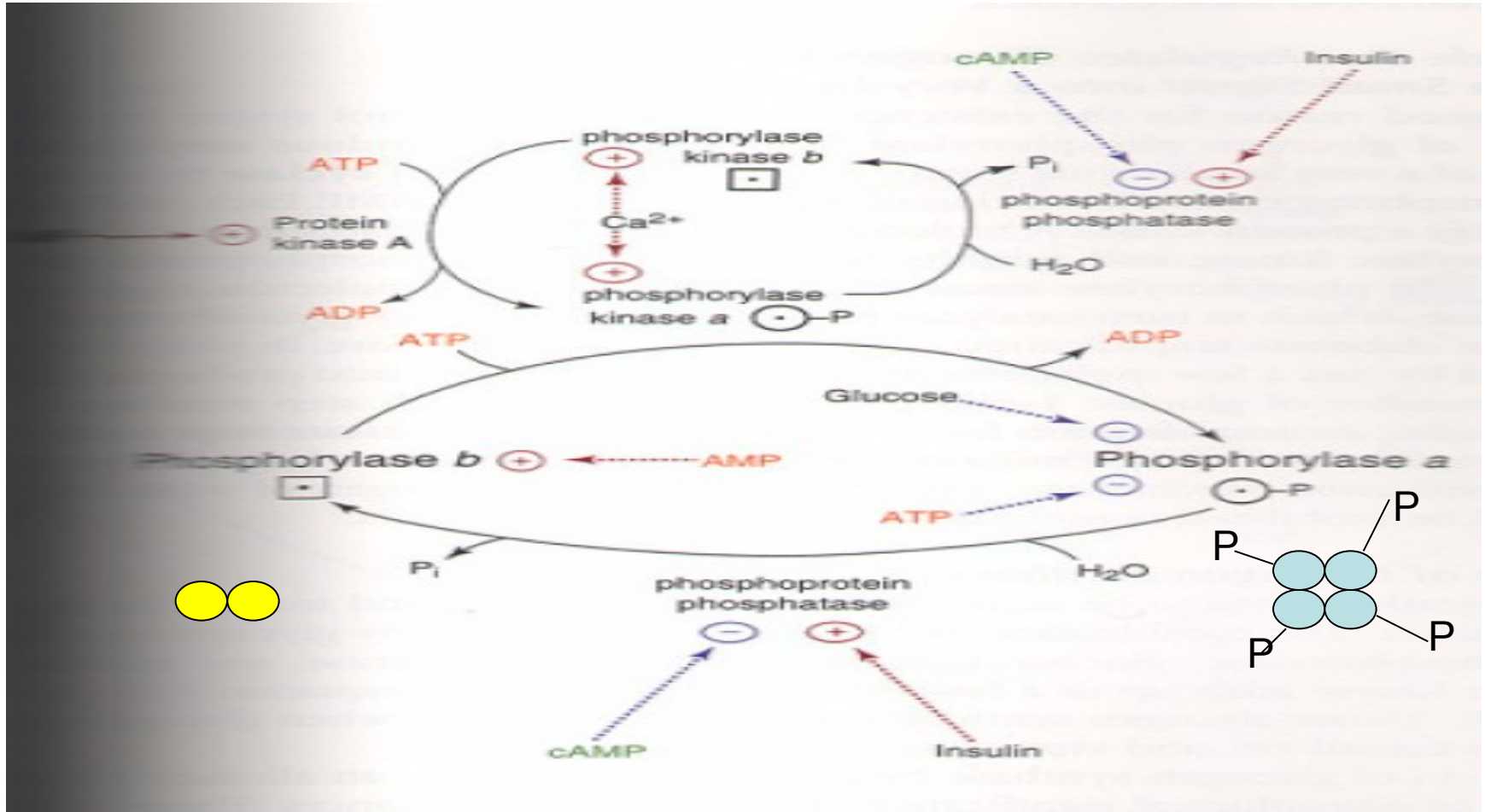


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Regulace kovalentní modifikací glykogenfosforylasa



Aktivace zymogenů

žaludek

pepsinogen → pepsin
↑ ↑
pH

slinivka břišní

enterokinasa



trypsinogen → trypsin



chymotrypsinogen → chymotrypsin

proelastasa → elastasa

Regulace kovalentní modifikací

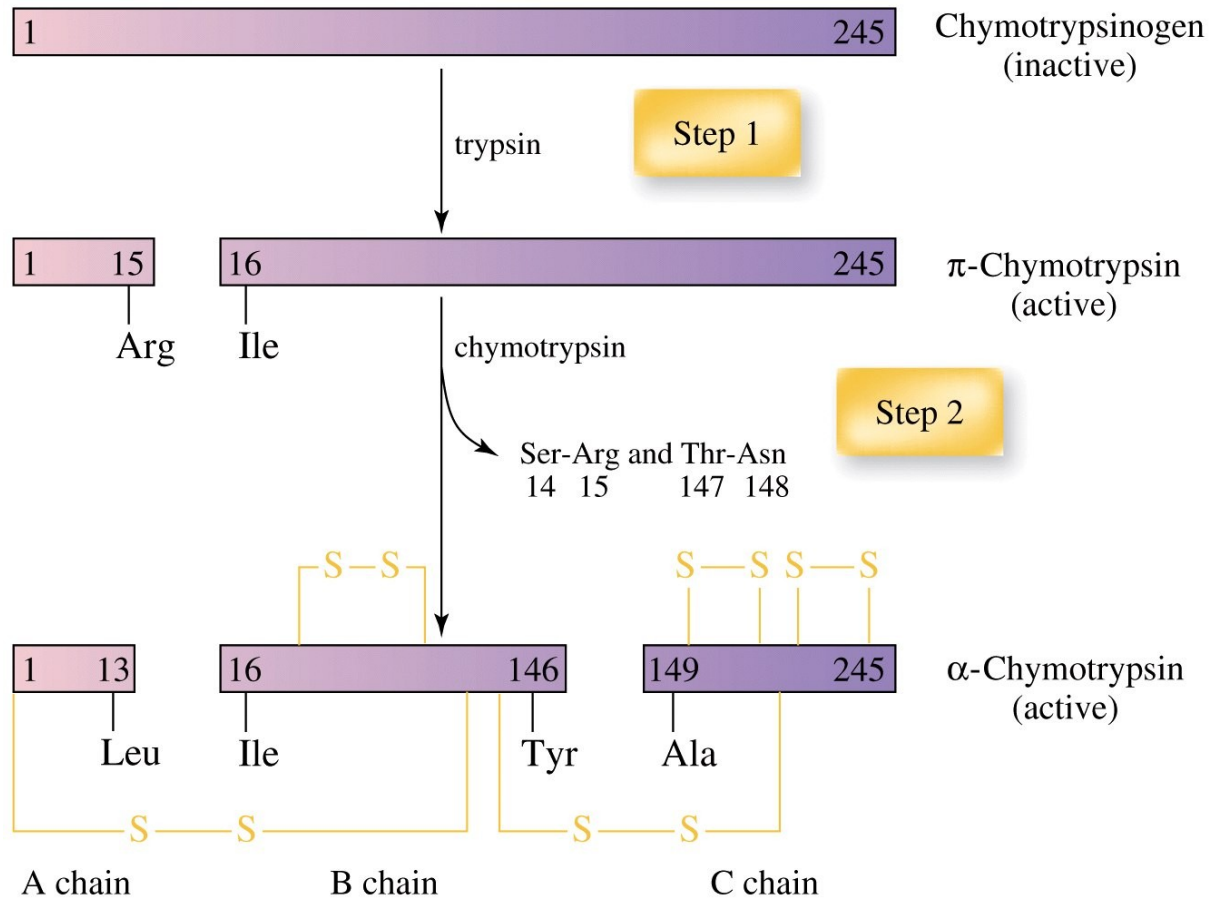
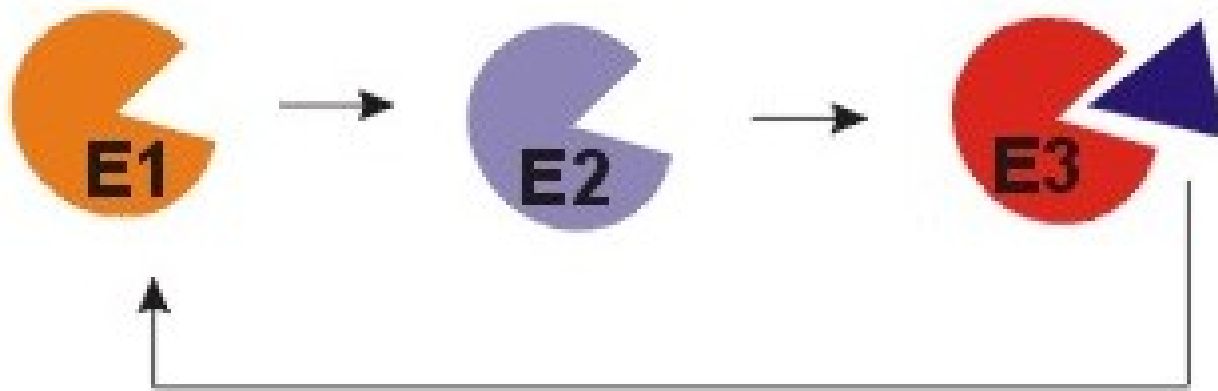
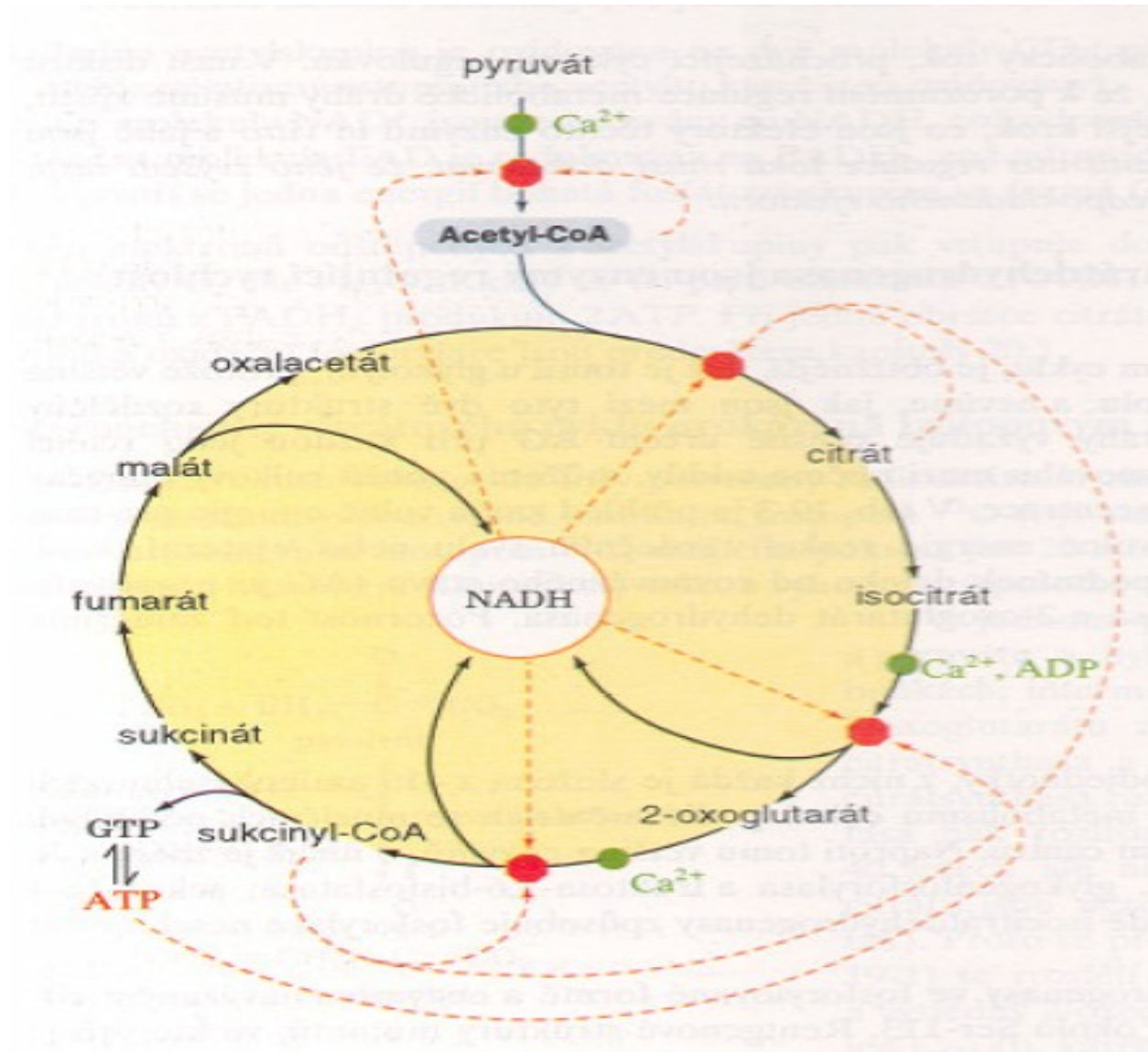


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Regulace zpětnou vazbou



Regulace



Využití enzymů

- bioanalytická chemie
 - stanovení substrátů
 - stanovení inhibitorů
 - nepřímé stanovení
- lékařství
- průmyslové využití
- průmyslové využití
 - prací prostředky
 - krmivářství
 - potravinářství
 - farmacie
- enzymová katalýza v organické chemie

Umělé enzymy

- Synzomy
- Abzomy

Abzymy

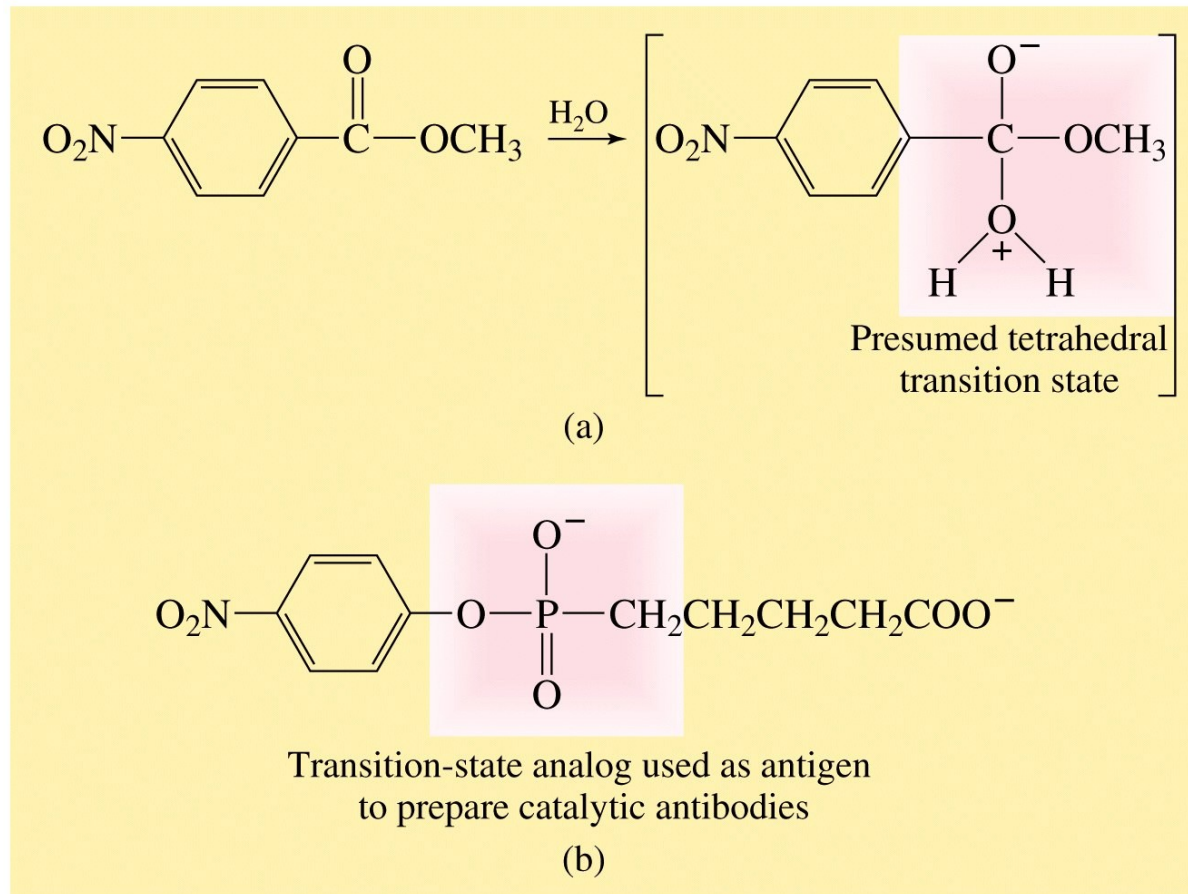


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Ribozymy – katalytická RNA

1989 Nobelova cena

- Altman (Yale University) ribonukleasa P
- Cech (University of Colorado) mRNA

Ribonukleasa P

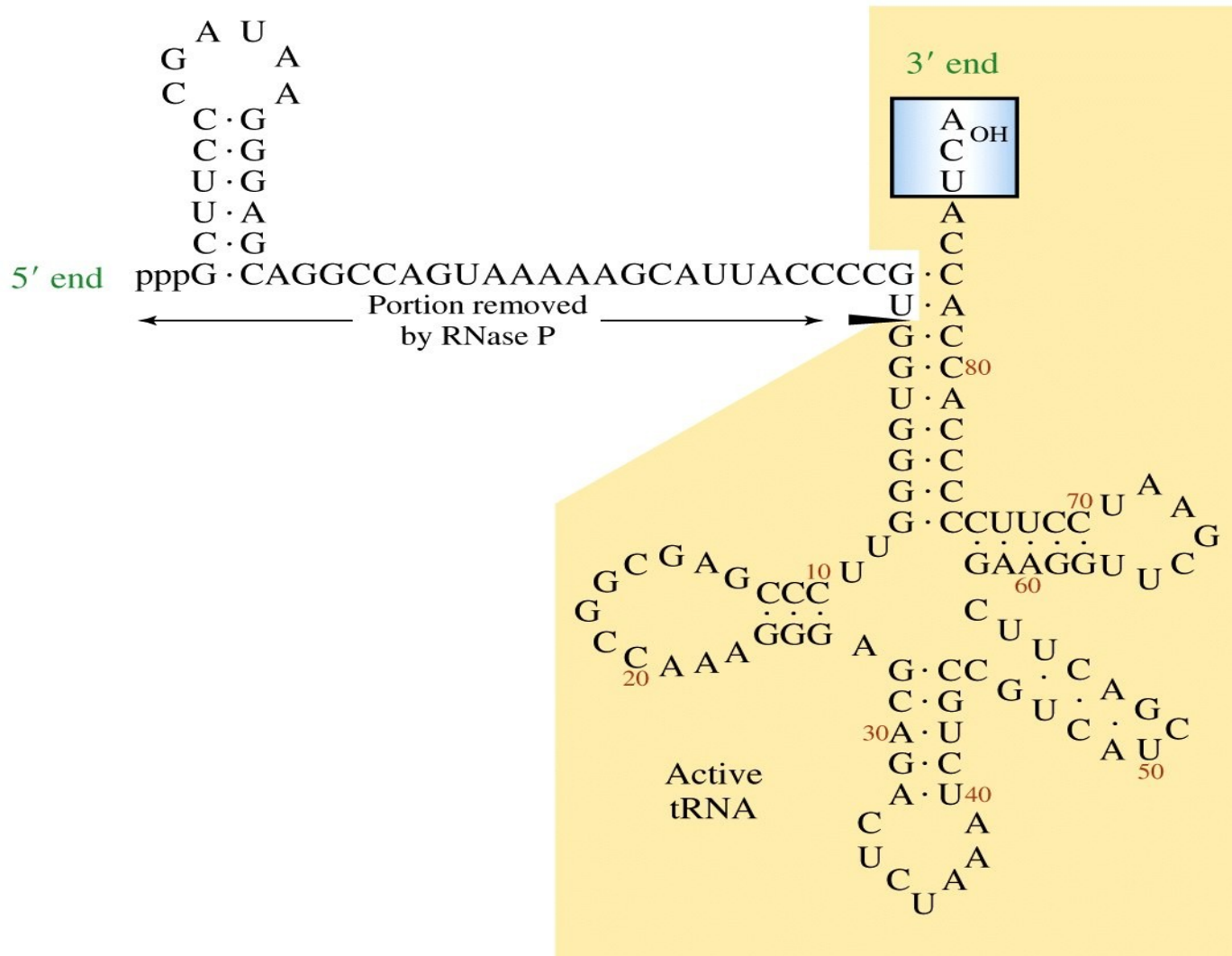


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Autokatalytická mRNA

Tetrahymena thermophila

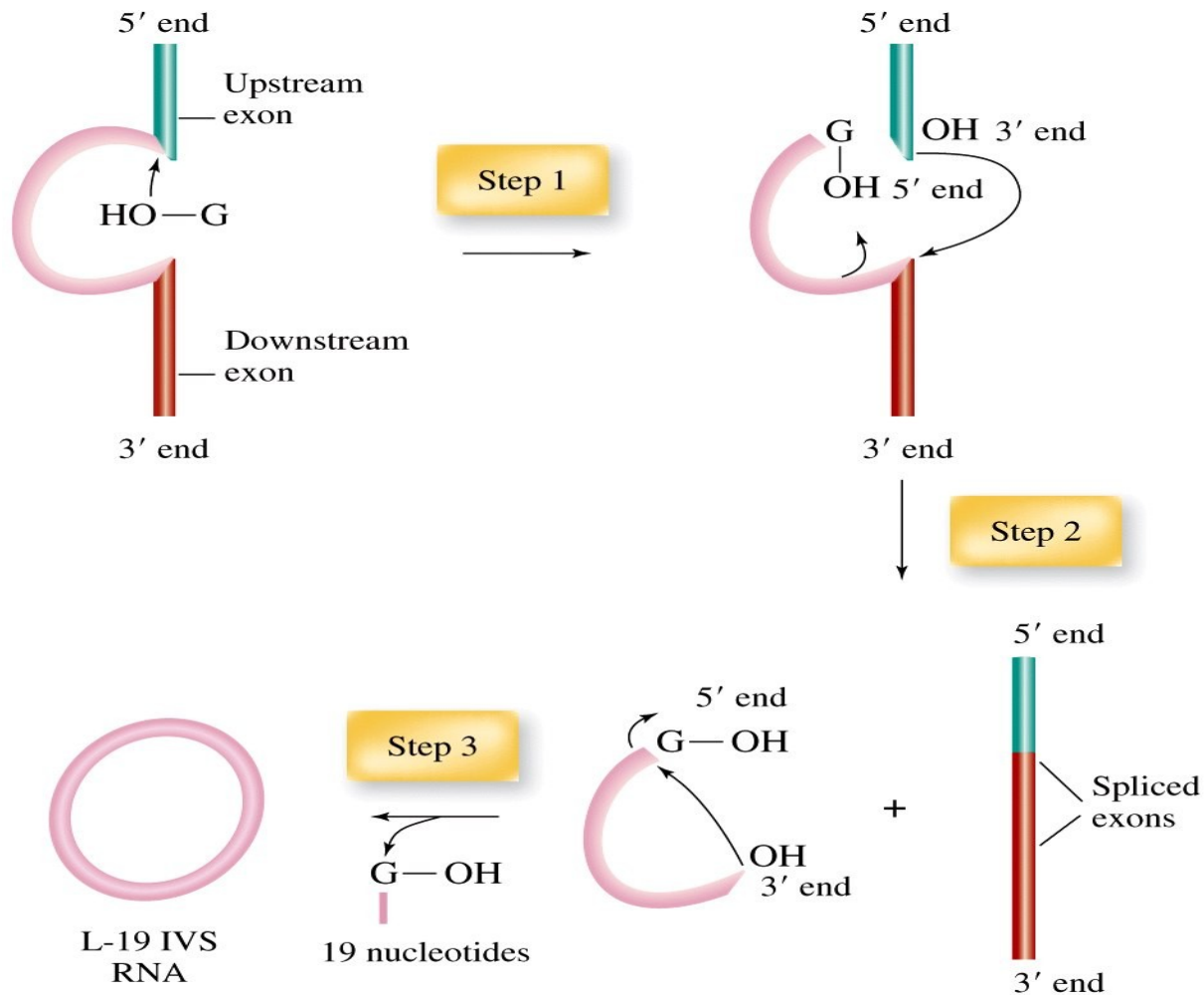


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