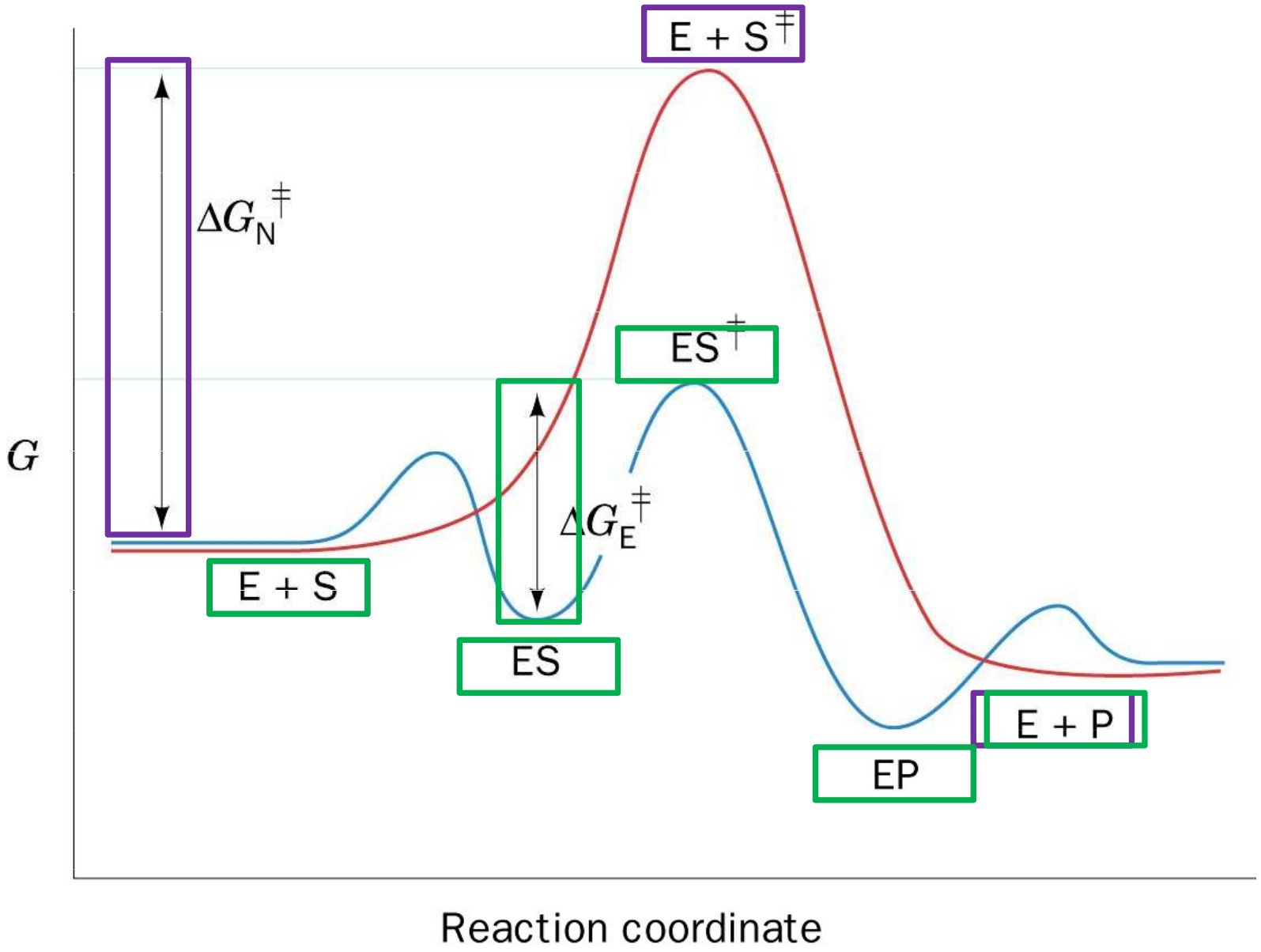
ENZYMOLOGIE

Katalýza - Berzelius 1838


E + S‡
ΔG‡N
ES‡
G
ΔG‡E
E + S
ES
E + P
EP
Reaction coordinate

Požadavky na biokatalyzátory :

A. Reakce musí probíhat cíleně. → konstitutivní
A. Reakce musí probíhat cíleně. → inducibilní

B. Musí probíhat specificky → bez vedlejších produktů

C. Jejich aktivita musí být přesně regulovaná

Bio- versus katalyzátory

- Vyšší reakční rychlostí

- Mírnější podmínky

Citlivost vůči řadě vlivů a menší stabilita

- Vyšší specifita – typ reakce a typ substrátu

- Schopnost regulace

Biokatalyzátory

- Globulární bílkoviny – enzymy
- RNA – ribozymy Cech Altmann NC1986

Enzymy – molekulární stroje

$$2\,H_2O_2 \longrightarrow O_2 + 2H_2O$$

Rychlostní konstanta :

- Bez katalýzy - $0{,}23\ s^{-1}$
- Pt - $1{,}3 \cdot 10^{3}\ s^{-1}$
- Enzym - katalasa - $3{,}7 \cdot 10^{7}\ s^{-1}$

Enzymy – molekulární stroje

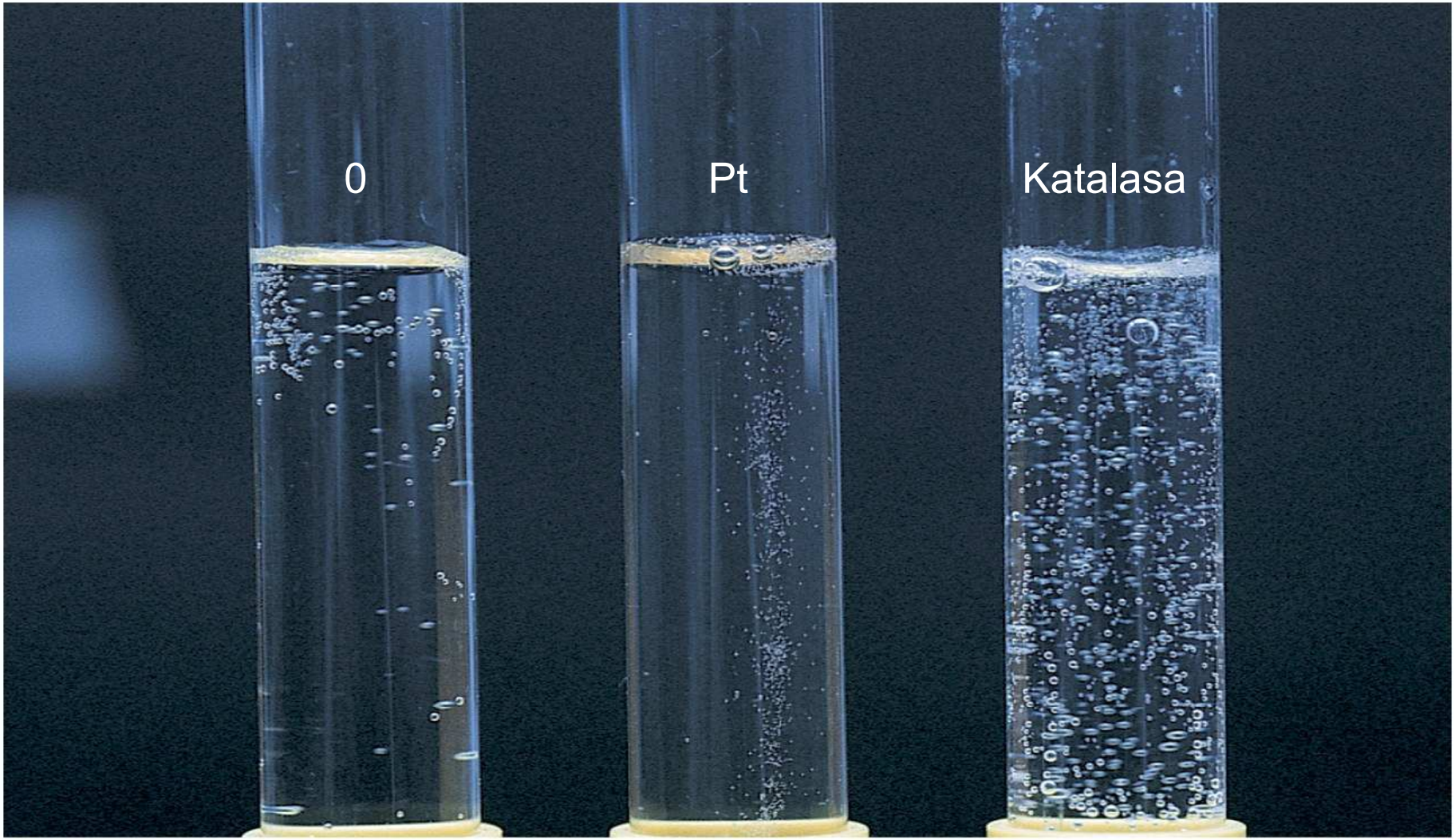
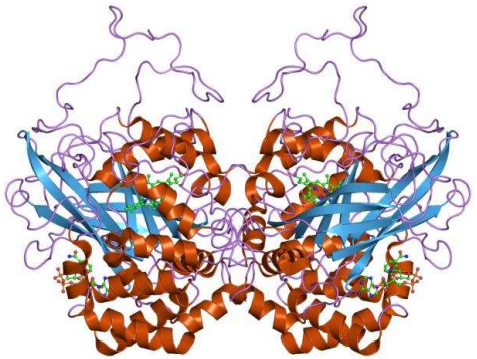


Figure 5-1 Concepts in Biochemistry, 3/e

Enzymy – molekulární stroje

Katalasa



$2\ H_2O_2 \longleftrightarrow O_2 + 2H_2O$

Číslo přeměny 40 000 000

1 molekula enzymu přemění
40 000 000 molekul substrátu za 1 s

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*

$substrát_1$ + $substrát_2$ + 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 (+ H_2O)**
	-	***proteasy***

4. LYASY - **nehydrolytické štěpení (bez H_2O)**

- ***karbonátanhydrasa***

5. IZOMERASY - **přesuny atomů a skupin**

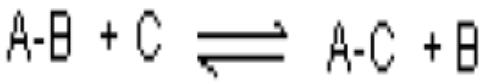
- ***glukosafosfátizomerasa***

6. LIGASY - **vznik vazby za současného rozkladu ATP**

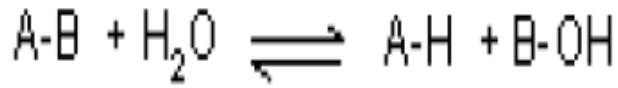
- ***asparaginsynthetasa***

1. Oxidoreduktázy

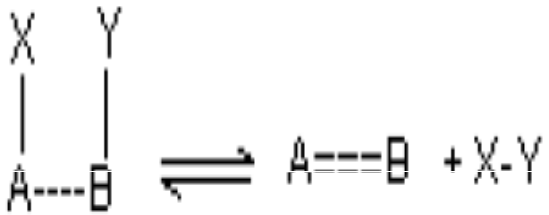
2. Transferázy



3. Hydrolázy

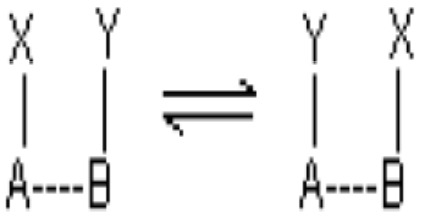


4. Lyázy

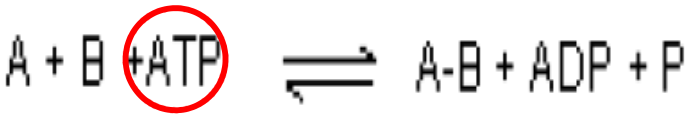


Synthasy

5. Izomerázy



6. Ligázy

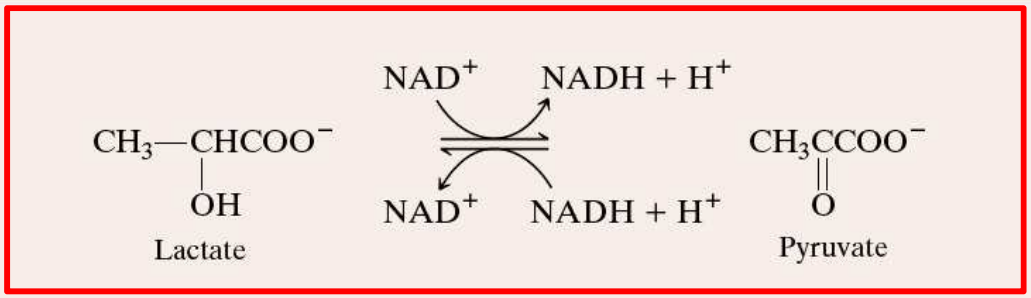


Syntethasy

Table 5.2

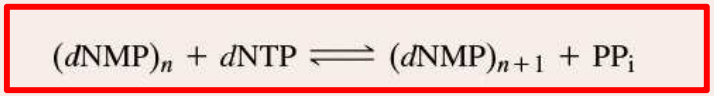
An example of each class of enzyme

1. Oxidoreductases



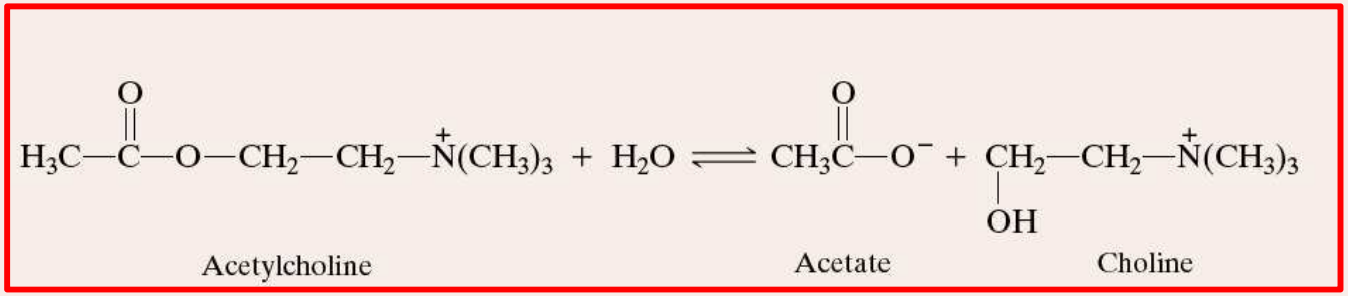
Common name: Lactate dehydrogenase
Official name: L-Lactate:NAD^+ oxidoreductase
Official number: 1.1.2.3

2. Transferases



$(d\text{NMP})_n$ = DNA with n nucleotides
dNTP = deoxynucleoside triphosphate
$(d\text{NMP})_{n+1}$ = 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

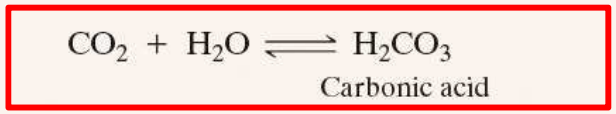


Common name: Acetylcholinesterase
Official name: Acetylcholine acetylhydrolase
Official number: 3.1.1.7

Table 5-2 part 1 Concepts in Biochemistry, 3/e

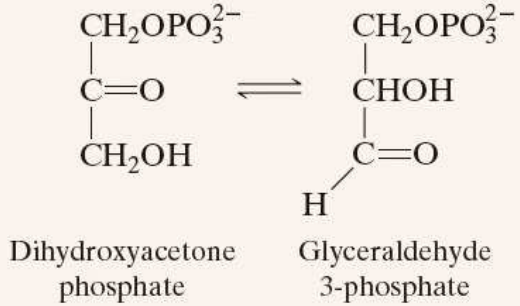
Table 5.2
An example of each class of enzyme

4. Lyases



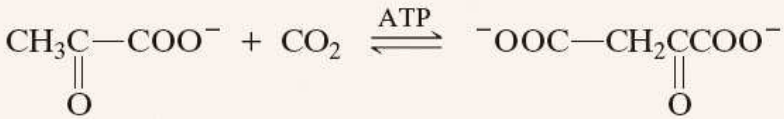
Common name: Carbonic anhydrase
Official name: Carbonate hydrolyase
Official number: 4.2.1.1

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 CO_2 ligase (ADP-forming)
Official number: 6.4.1.1

Table 5-2 part 2 Concepts in Biochemistry, 3/e

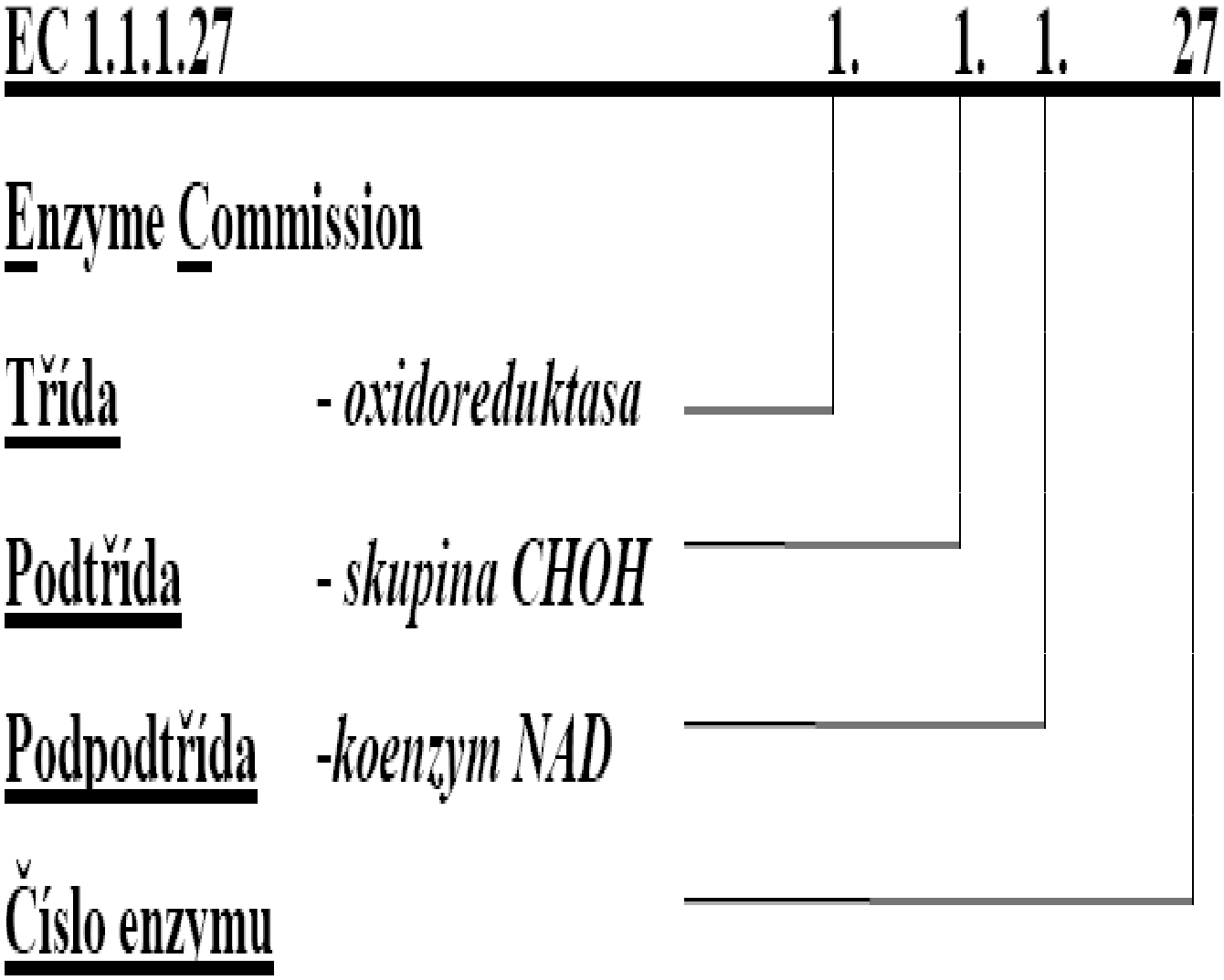
Typy reakcí

jednoduché

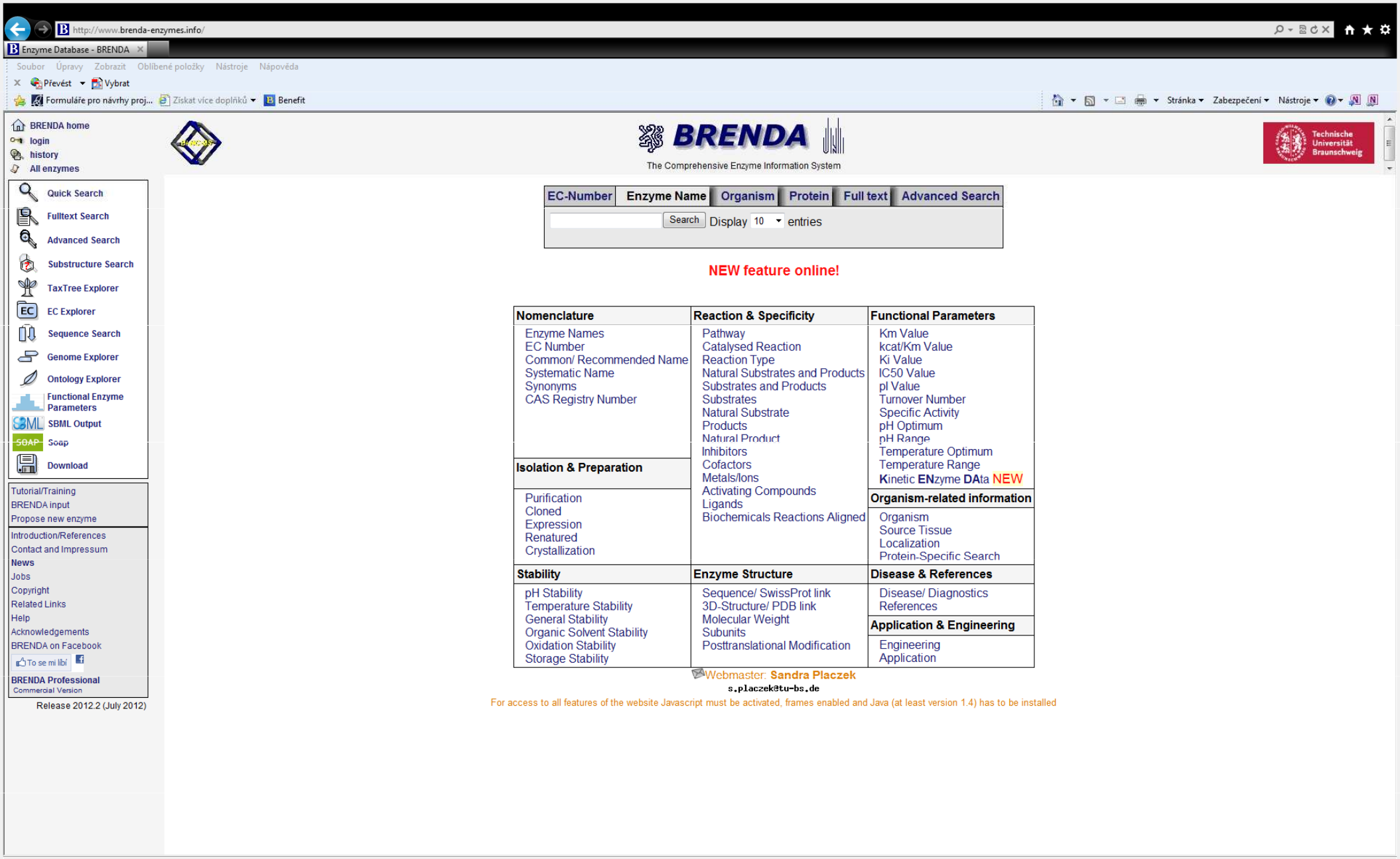
- Katalasa

složité

- DNA polymerasa

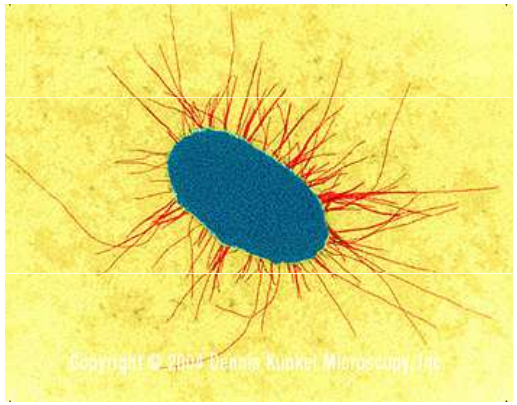

EC 1.1.1.27
1. 1. 1. 27
Enzyme Commission
Třída - oxidoreduktasa
Podtřída - skupina CHOH
Podpodtřída -koenzym NAD
Číslo enzymu

http://www.brenda-enzymes.info/



Enzymy – stanovení koncentrace

Escherichia coli



3 000 bílkovin

Homo sapiens

25 000 bílkovin

Koncentrace ↔ Katalytická aktivita

⇩

Substrát ↔ Produkt

Stanovení aktivity enzymů

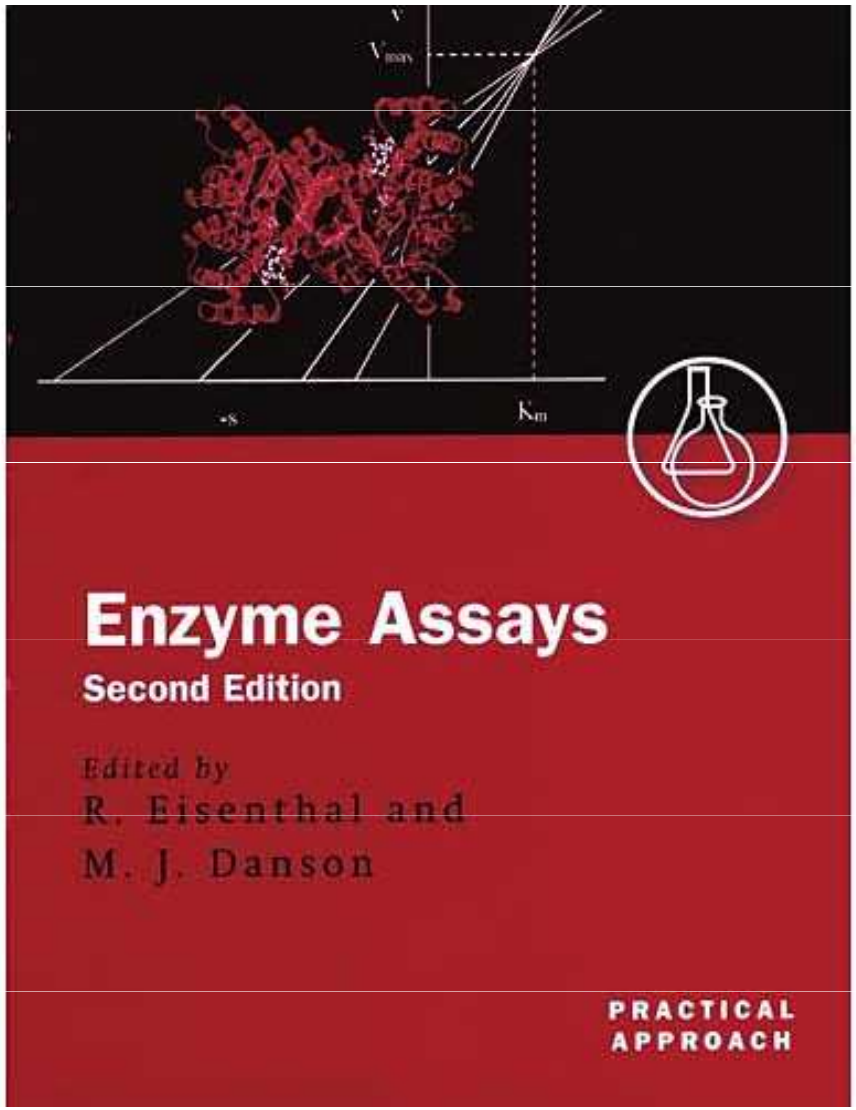
- *Biochemie*
- *Molekulární biologie*

- *Klinická diagnostika*
- *Farmakologie – vývoj léčiv*
- *Biotechnologické procesy*
- *Bioanalytická chemie*

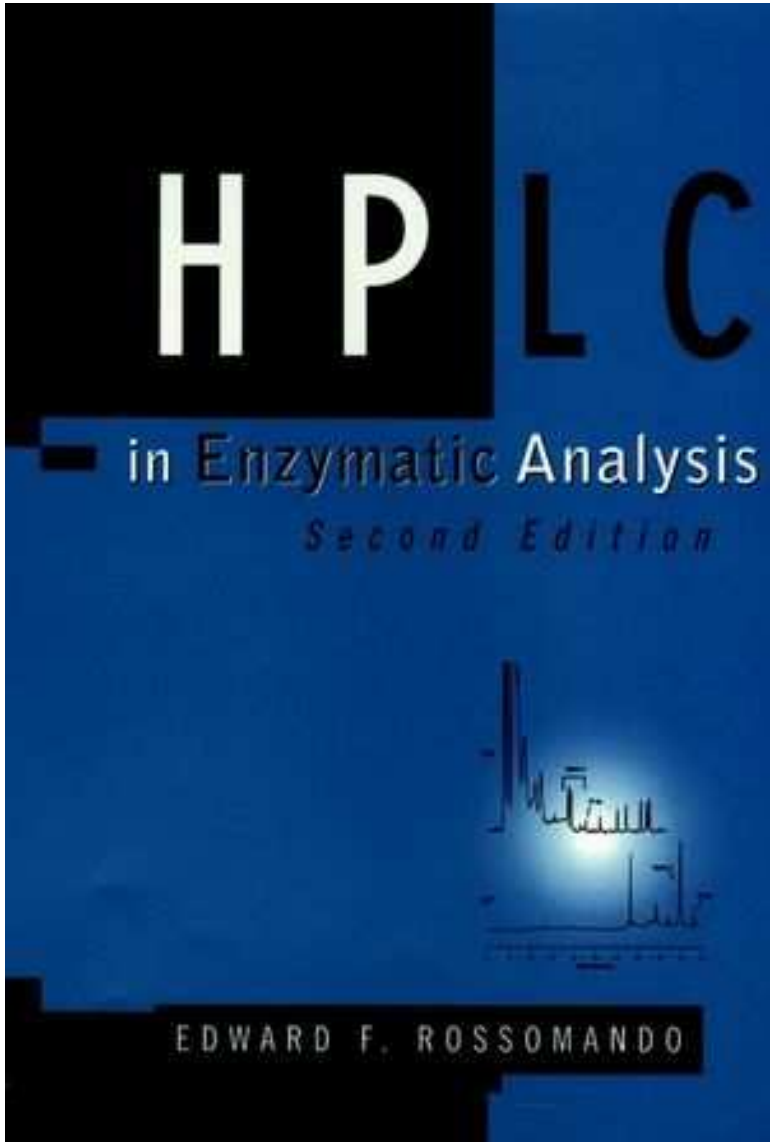
Metody používané pro stanovení aktivity enzymů

- Spektrofotometrické
- Spektrofluorimetrické
- Elektrochemické
- Radiochemické
- Separační – HPLC, GC, CE

Enzyme Assays
R.Eisenthal and M.J. Danson



HPLC in Enzymatic Analysis
E.F. Rossomando



Vyjadřování 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

mikrokatal (μkat) = 10^{-6} kat
nanokatal (nkat) = 10^{-9} kat
pikokatal (pkat) = 10^{-12} kat

1 IU = 1 μmol/min = 1/60 μmol/s = 1/60 μkat = 16,67 nkat

Specifická aktivita - aktivita vztažená na mg bílkoviny

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

STRUKTURA ENZYMŮ

ENZYMY
- **jednoduché**
- **složené** 60 %

KOFAKTOR + APOENZYM → HOLOENZYM

ORGANICKÁ LÁTKA
- **prostetická skupina** - kovalentní vazba
- **koenzym** - disociabilní

Kofaktor - kovový ion nebo organická látka

METALOENZYMY

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

Table 6.2

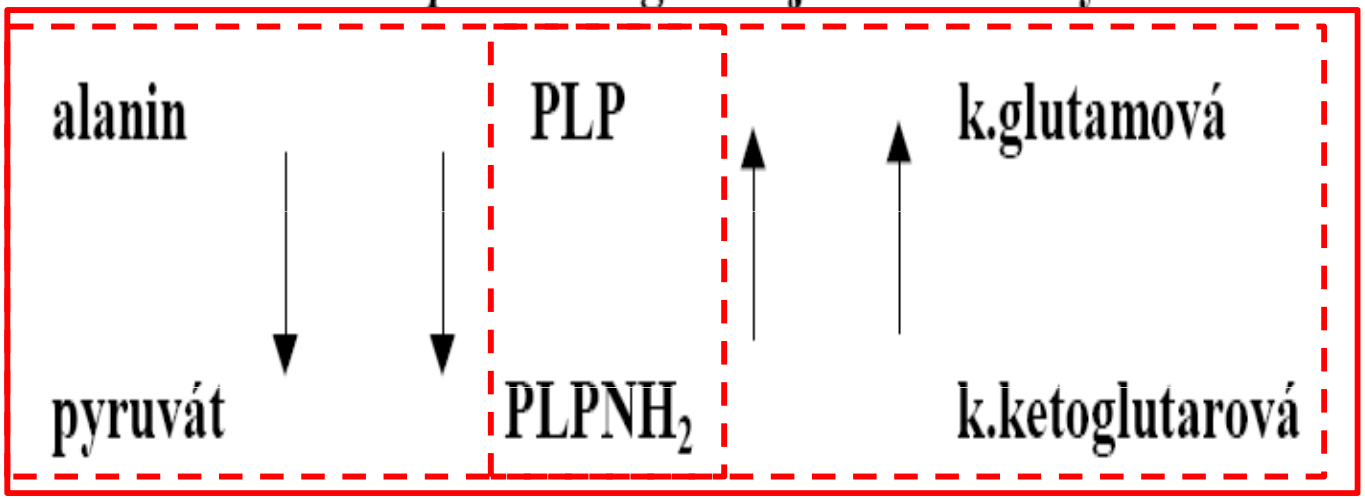
Enzymes requiring metal ions as cofactors

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

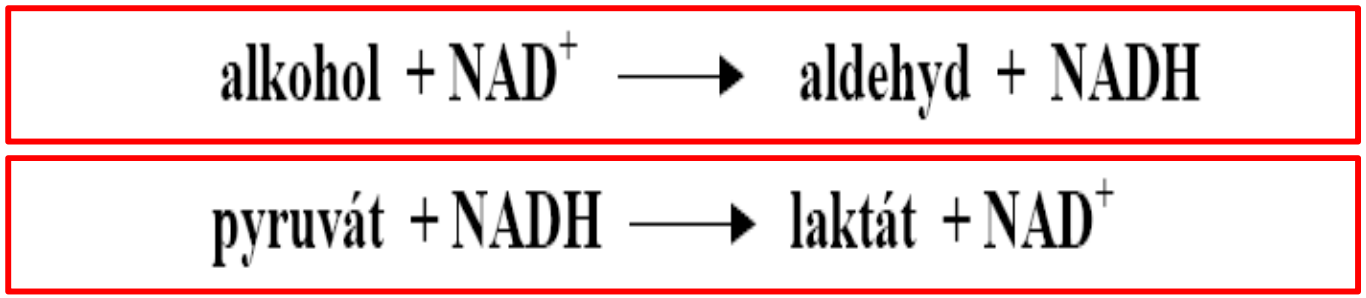
Regenerace kofaktorů

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 :

ADH



LDH

KOFAKTORY A VITAMINY

VITAMIN – FUNK – "amin potřebný pro život"

Vitamin	Kofaktor	Funkce
rozpustné ve vodě		**přenos (reakce)**
thiamin - B_1	thiamindifosfát TPP	aldehydické s.
riboflavin - B_2	FMN, FAD	H
k.nikotinová(nikotinamid)	NAD^+, NADP	H
k.pantothenová	CoA	acylové s.
k.listová	k.listová	C_1 skupin
pyridoxin - B_6	pyridoxalfosfát	aminoskupiny
kobalamin - B_{12}	kobalamin	izomerace
k.askorbová - C	k.askorbová	hydroxylace
biotin - H	biotin	COOH
k. lipoová	k. lipoová	H
rozpustné v tucích		
karotenoidy - A		proces vidění
kalciferoly - D		metabolismus Ca
tokoferoly - E		antioxidans
maftochinony - A		srážení krve

Table 6.1
Characteristics of vitamins and coenzymes

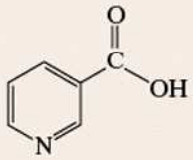
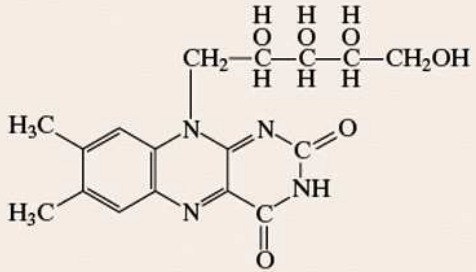
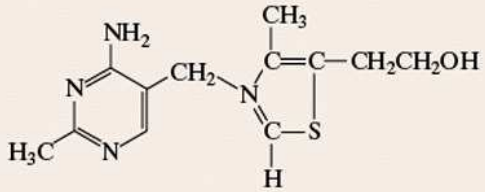
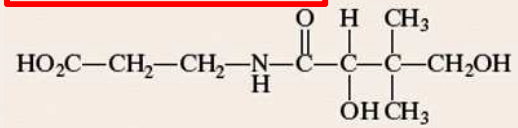
Name/Structure of Vitamin	Related Coenzyme	Reaction type (page numbers[a])	Deficiency Disease
Water-Soluble Vitamins			
Niacin Kyselina nikotinová B_3 	NAD^+, $NADP^+$	Oxidation–reduction (pp. 485-494, 505-508, 515-524)	Pellagra
Riboflavin (vitamin B_2) 	FAD, FMN	Oxidation–reduction (pp. 485-494, 515-524)	Growth retardation
Thiamine (vitamin B_1) 	Thiamine pyrophosphate	Decarboxylation (pp. 461, 463, 487-494)	Beriberi
Pantothenic acid (vitamin B_3) 	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

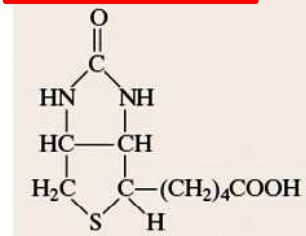
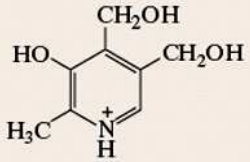
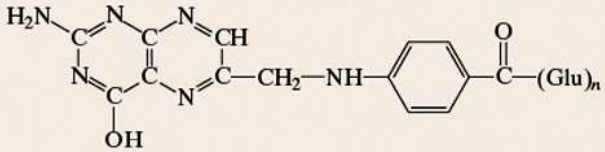
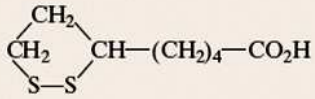
Biotin 	Biotinylated enzymes	CO_2 activation and transfer (pp. 465-466)	Dermatitis (humans)
Pyridoxine (vitamin B_6) 	Pyridoxal phosphate	Amino group transfer (pp. 605-606)	Dermatitis (rats): neurological symptoms
Folic acid 	Tetrahydrofolate	Transfer of one carbon unit (pp. 600-601)	Anemias
Lipoic acid (may not be a vitamin) 	Attached to ε-NH_2 group of Lys in protein	Acyl group activation and transfer (pp. 485-493)	Growth deficiencies

Table 6-1 part 2 Concepts in Biochemistry, 3/e

Table 6.1 *(continued)*
Characteristics of vitamins and coenzymes

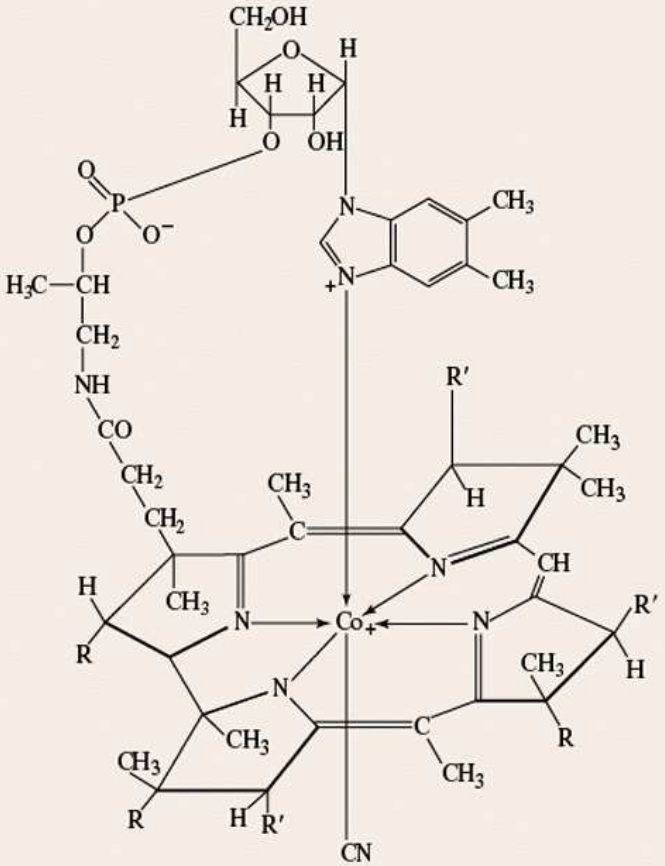
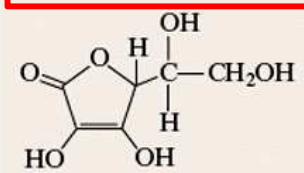
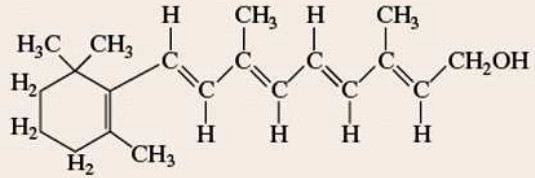
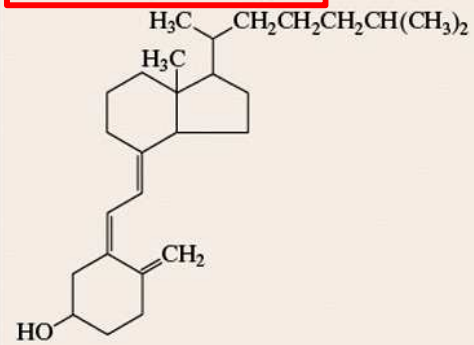
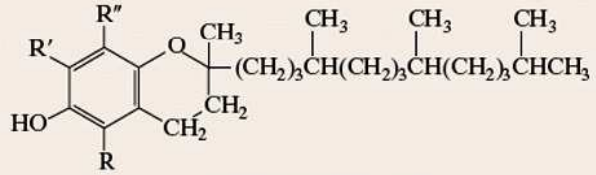
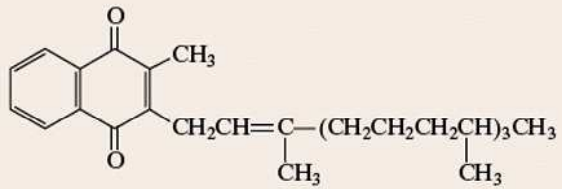
Name/Structure of Vitamin	Related Coenzyme	Reaction type (page numbers[a])	Deficiency Disease
Water-Soluble Vitamins *(continued)*			
Cobalamin (vitamin B_{12}) 	5′-Deoxyadenosylcobalamin	Methyl group transfer (pp. 570-571)	Pernicious anemia
L-Absorbic acid (vitamin C) 	L-Absorbic acid	Hydroxylation (pp. 105-107, 493)	Scurvy

Table 6-1 part 3 Concepts in Biochemistry, 3/e

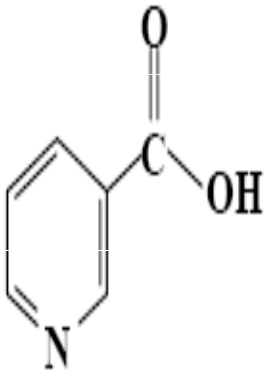
Fat-Soluble Vitamins			
trans-Retinol (vitamin A) 	Associated with visual pigment	(pp. 123-125, 252)	Night blindness, other effects
Cholecalciferol (vitamin D_3) 	None	(pp. 252, 582-584)	Rickets
Tocopherol (vitamin E)  (several variants, with R, R′, R″=H or CH_3)	None	(p. 252)	Reproductive and other problems in rats; uncertain in humans
Phylloquinone (vitamin K_1) 	None	(pp. 252, 539-544)	Problems in blood clotting

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

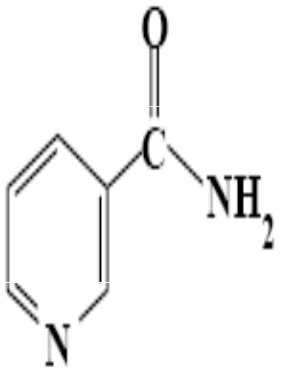
Table 6-1 part 4 Concepts in Biochemistry, 3/e

NIKOTINAMIDOVÉ KOENZYMY

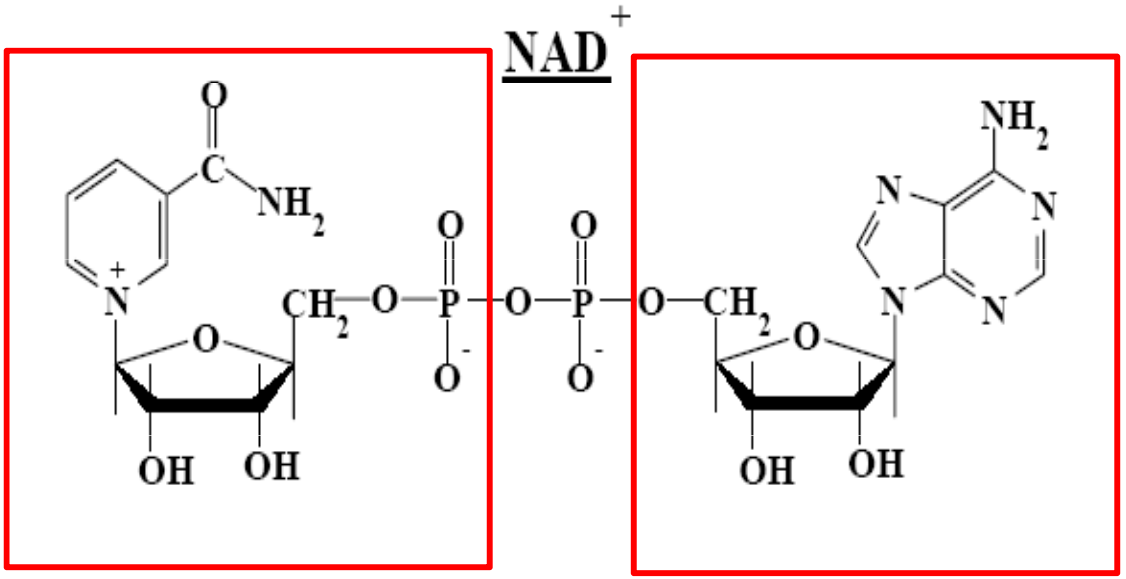
k. nikotinová



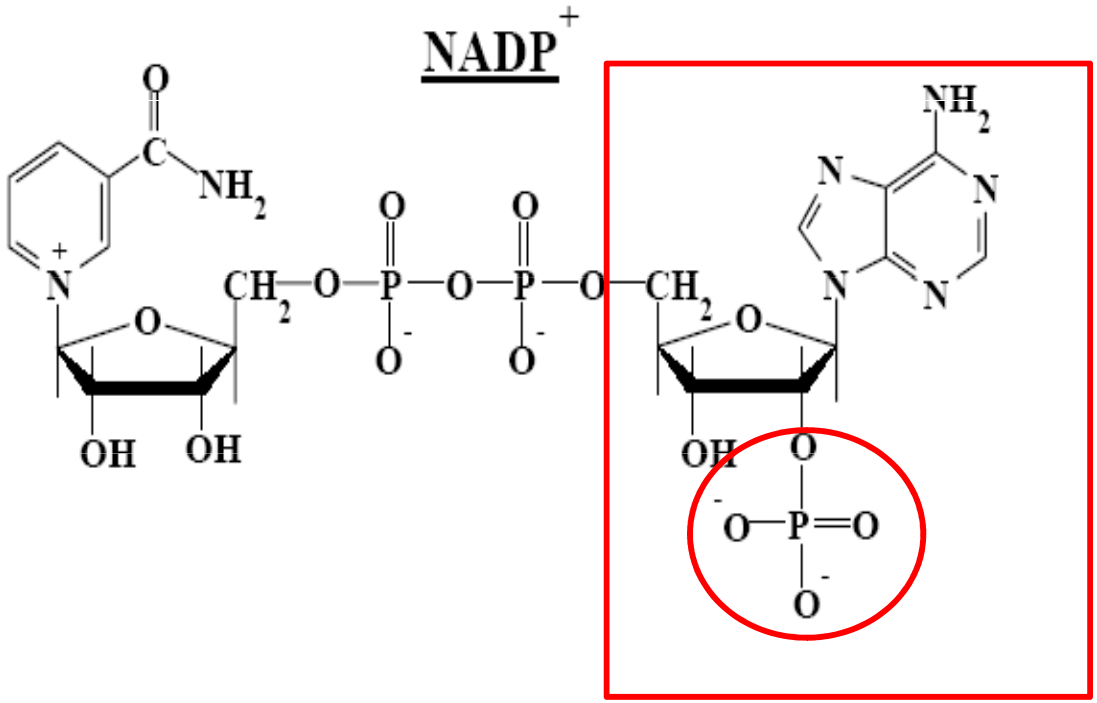
nikotinamid



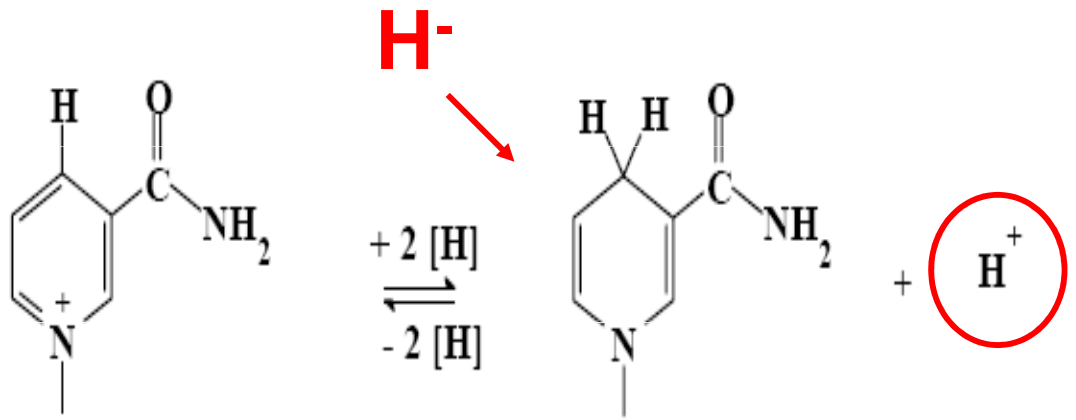
Katalytická část



Vazebná část

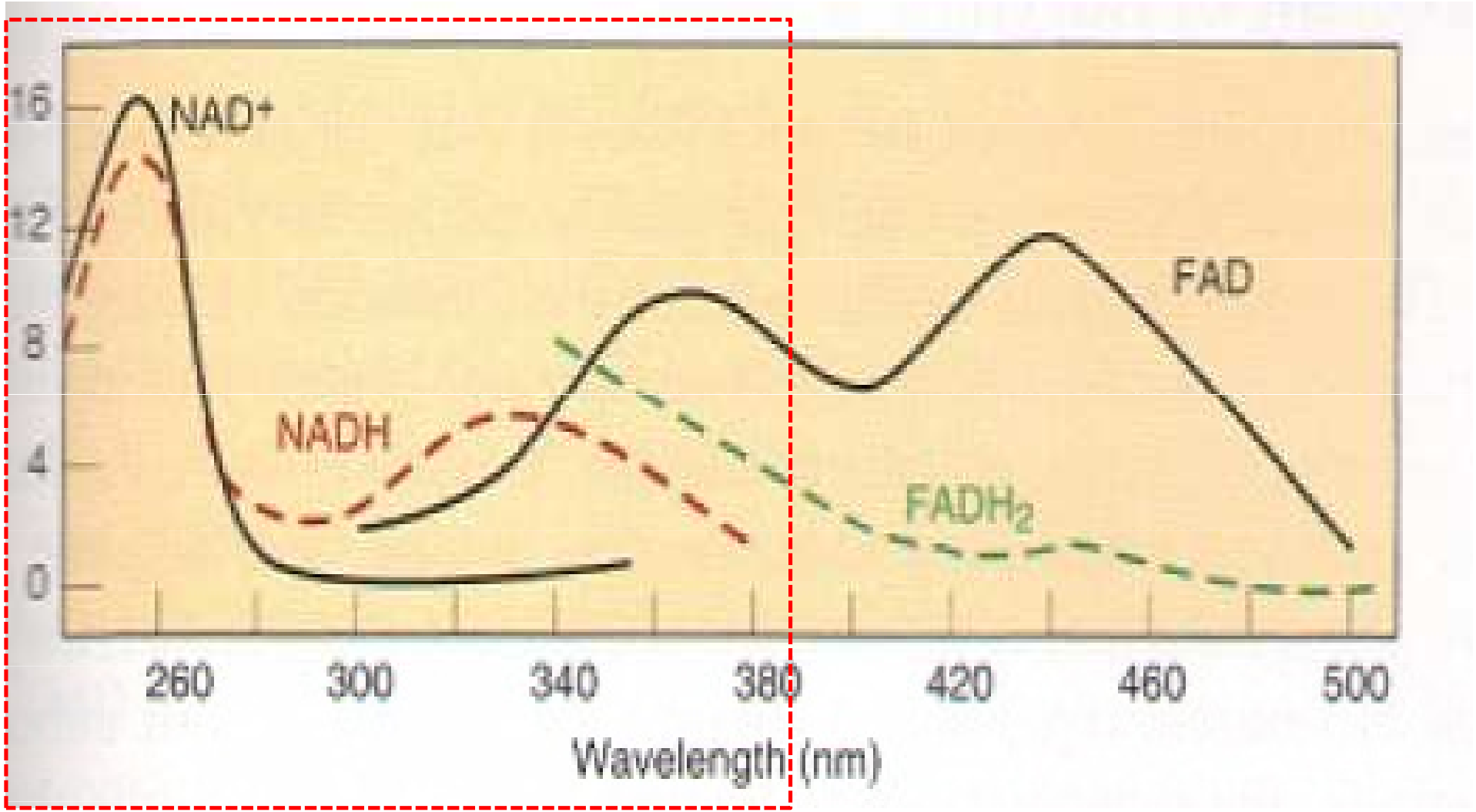


Vazebná část

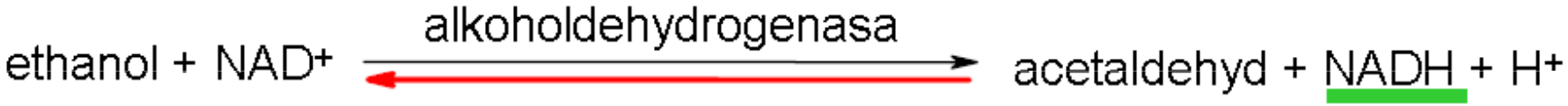


$$CH_3CH_2OH + NAD(P)^+ \rightleftharpoons CH_3CHO + NAD(P)H + H^+$$

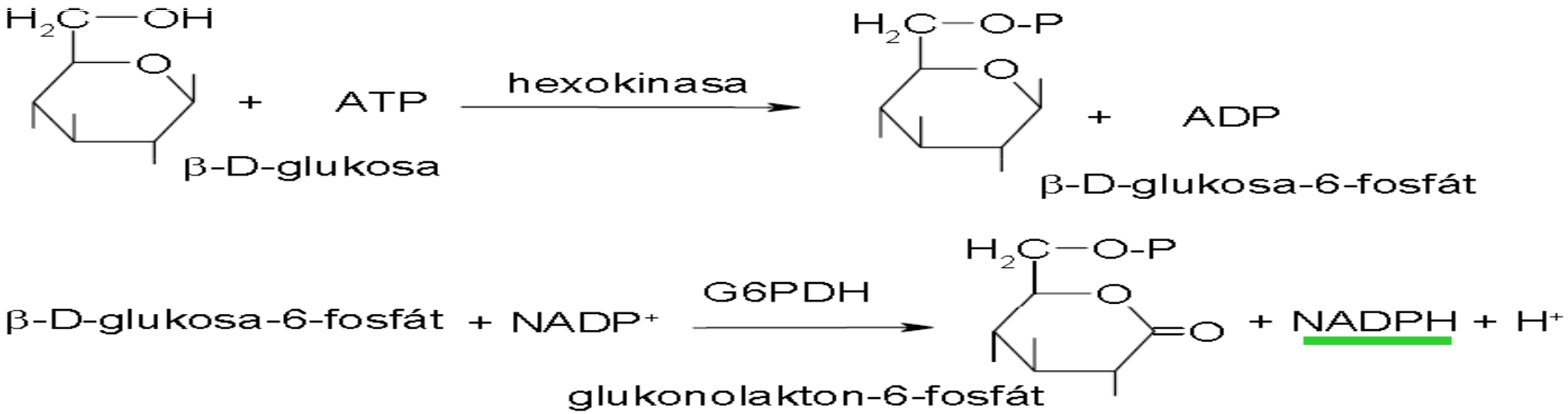
Warburgův optický test



- Přímé stanovení

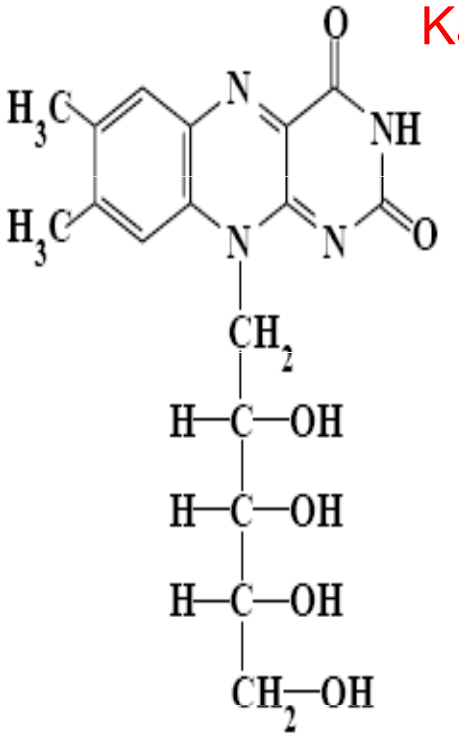


- Pomocná reakce



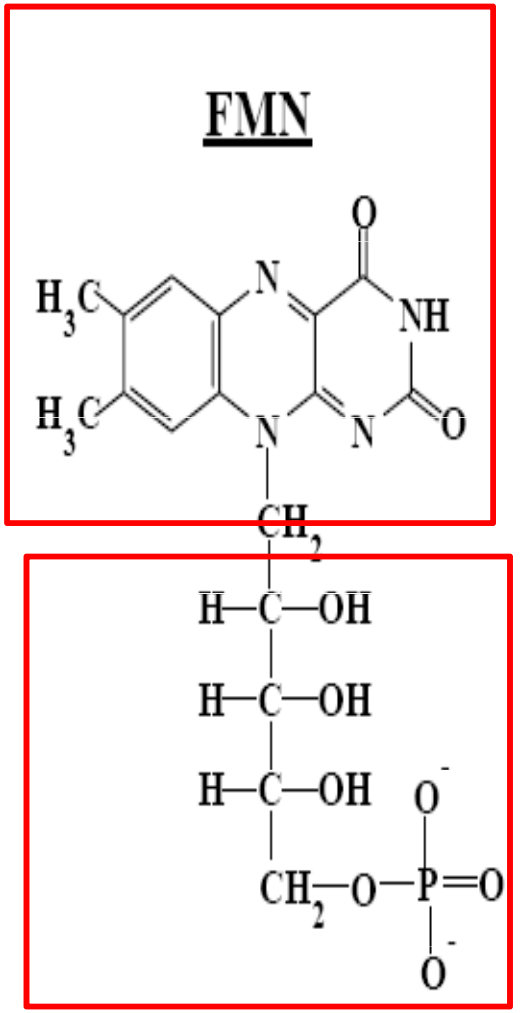
FLAVINOVÉ KOENZYMY

riboflavin



Katalytická část

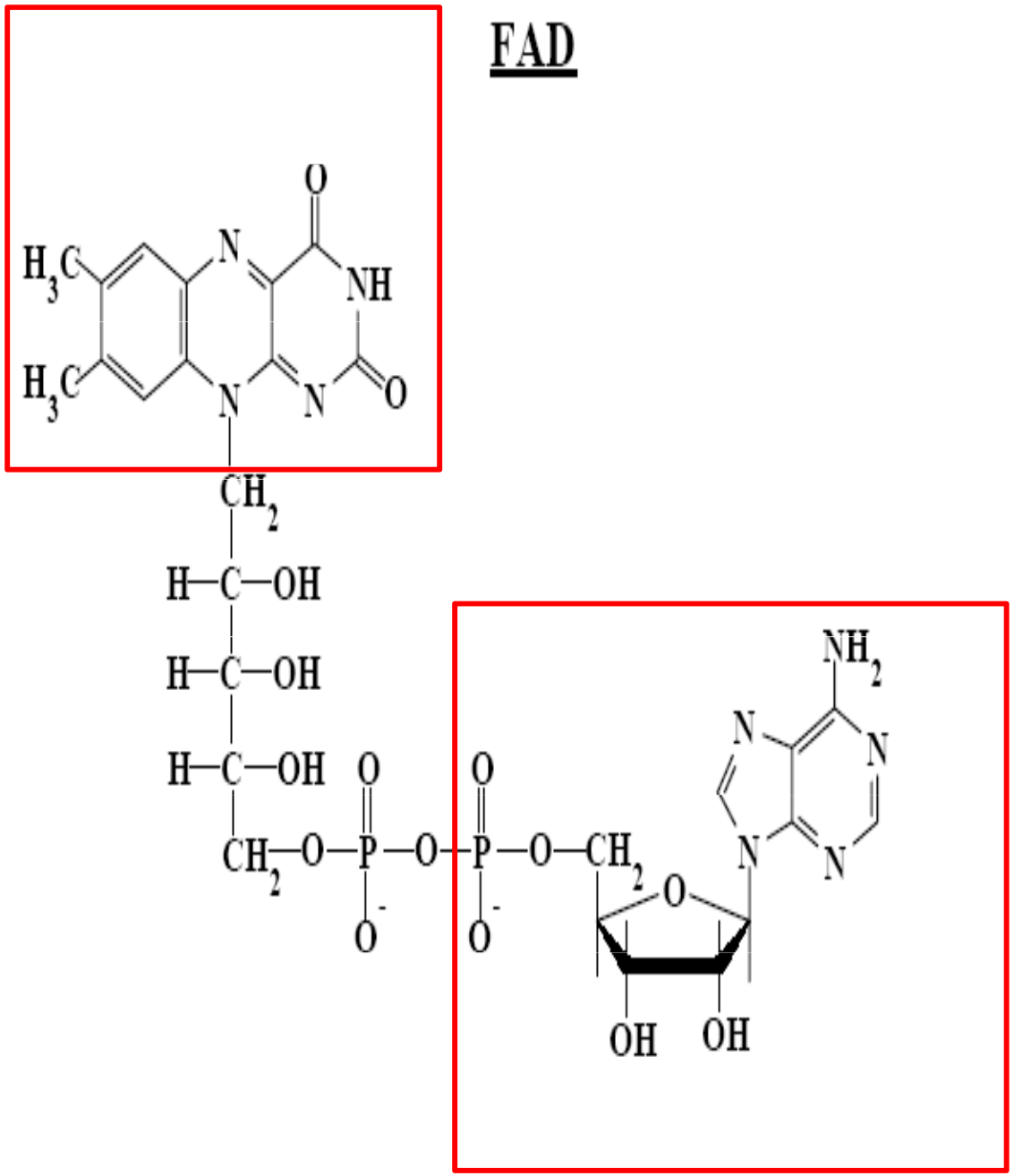
FMN



Vazebná část

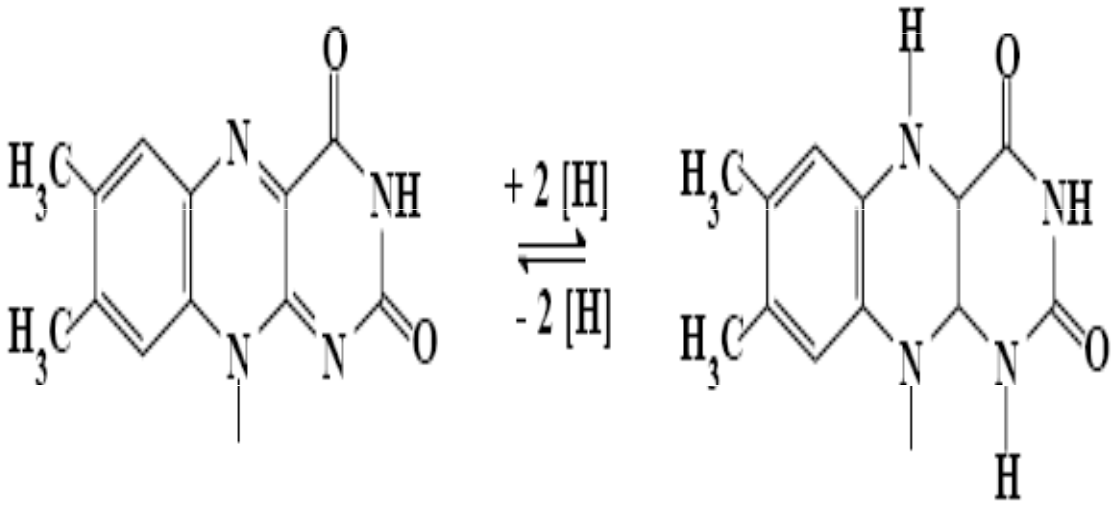
FAD

Katalytická část

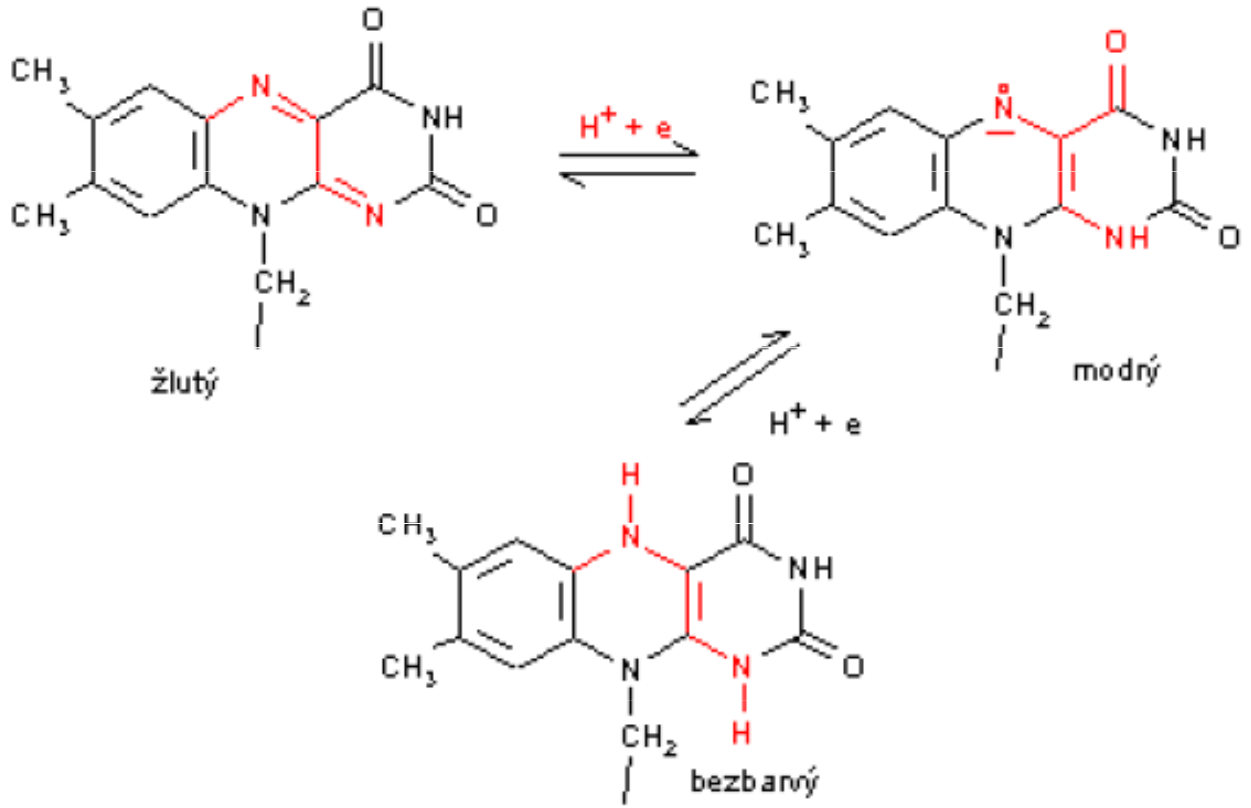


Vazebná část

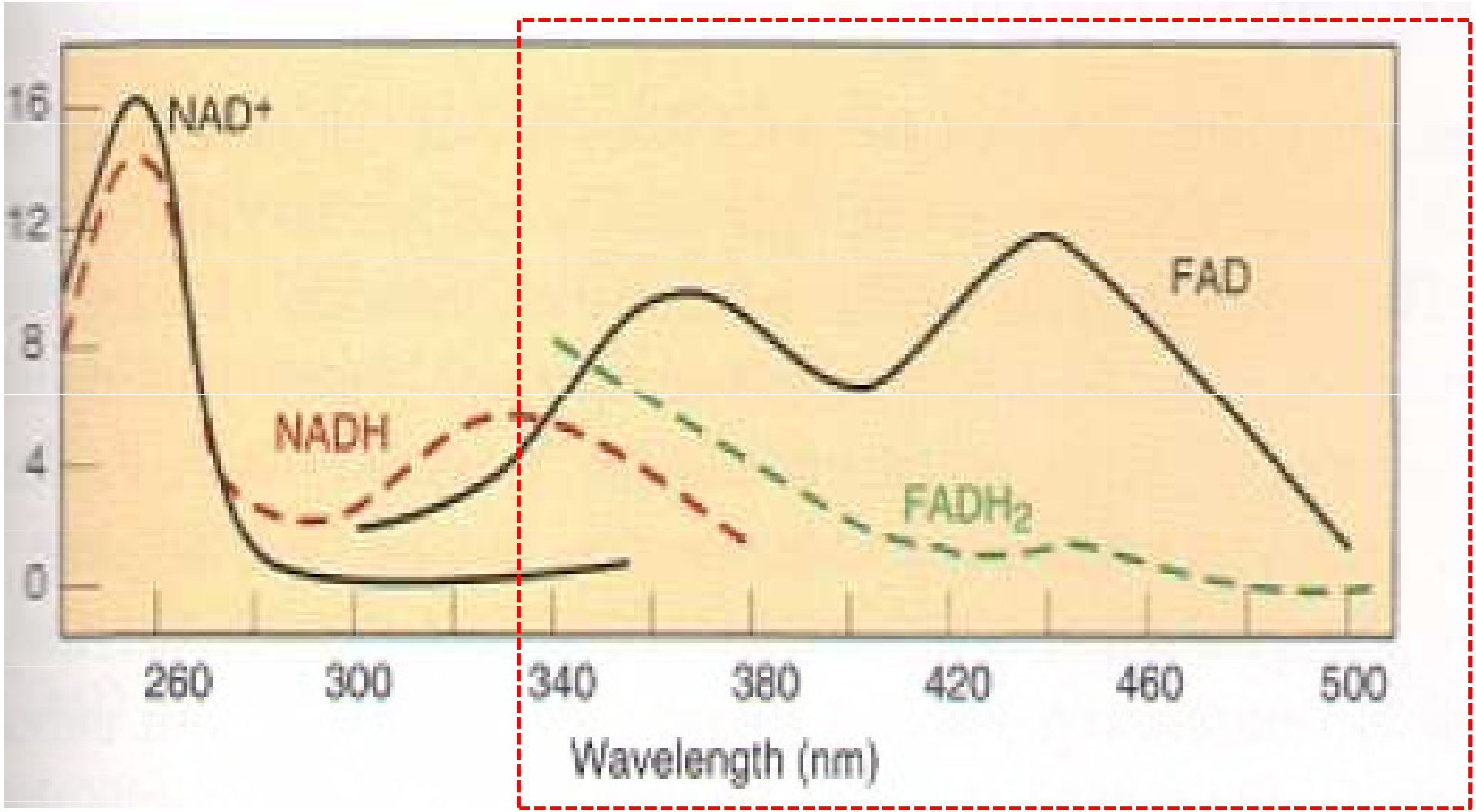
FAD (FMN) $\leftrightarrow$ $FADH_2$ ($FMNH_2$)



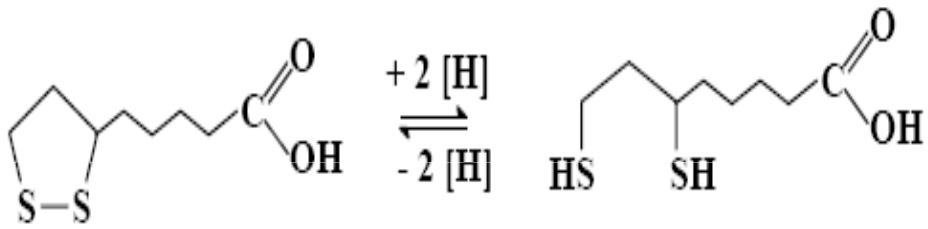
FAD (FMN) ↔ FADH· ↔ $FADH_2$ ($FMNH_2$)



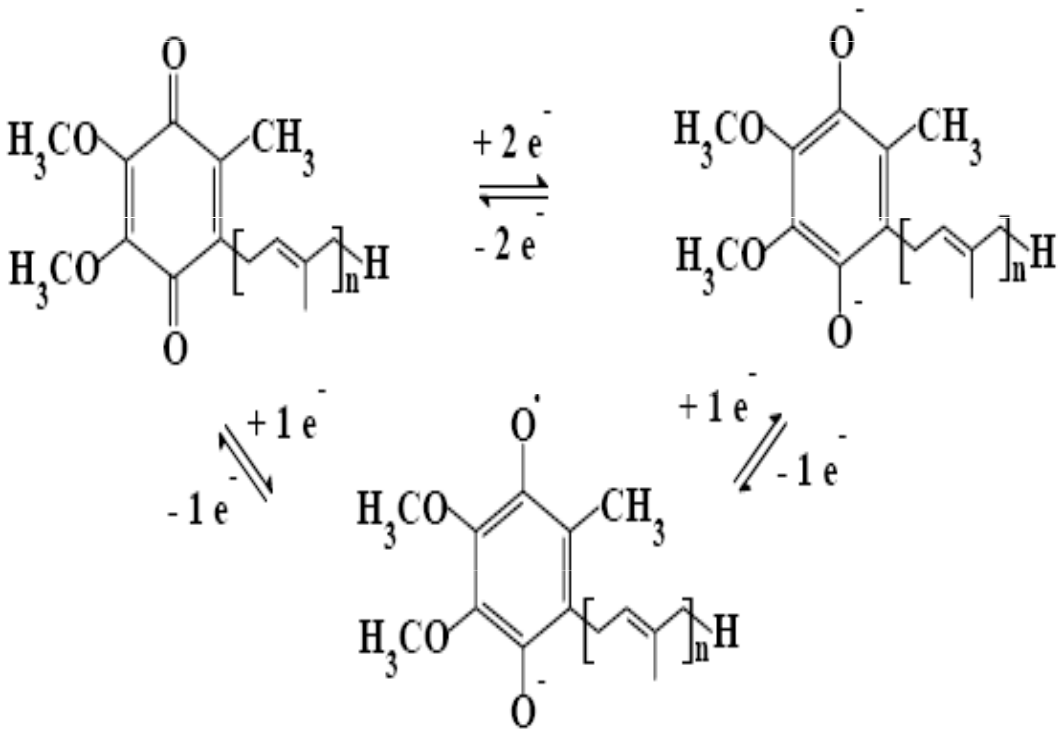
Warburgův optický test



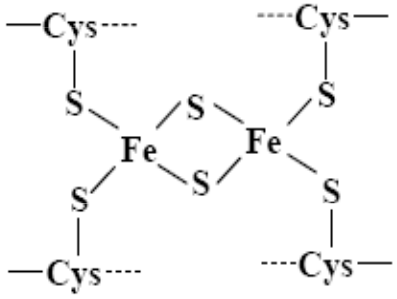
k.lipoová



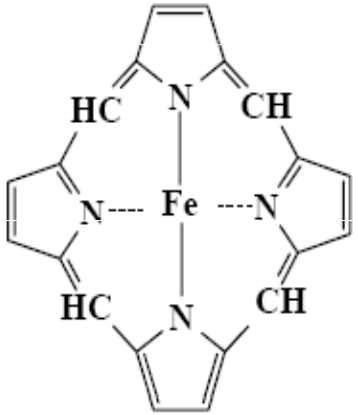
ubichinon



ferredoxiny

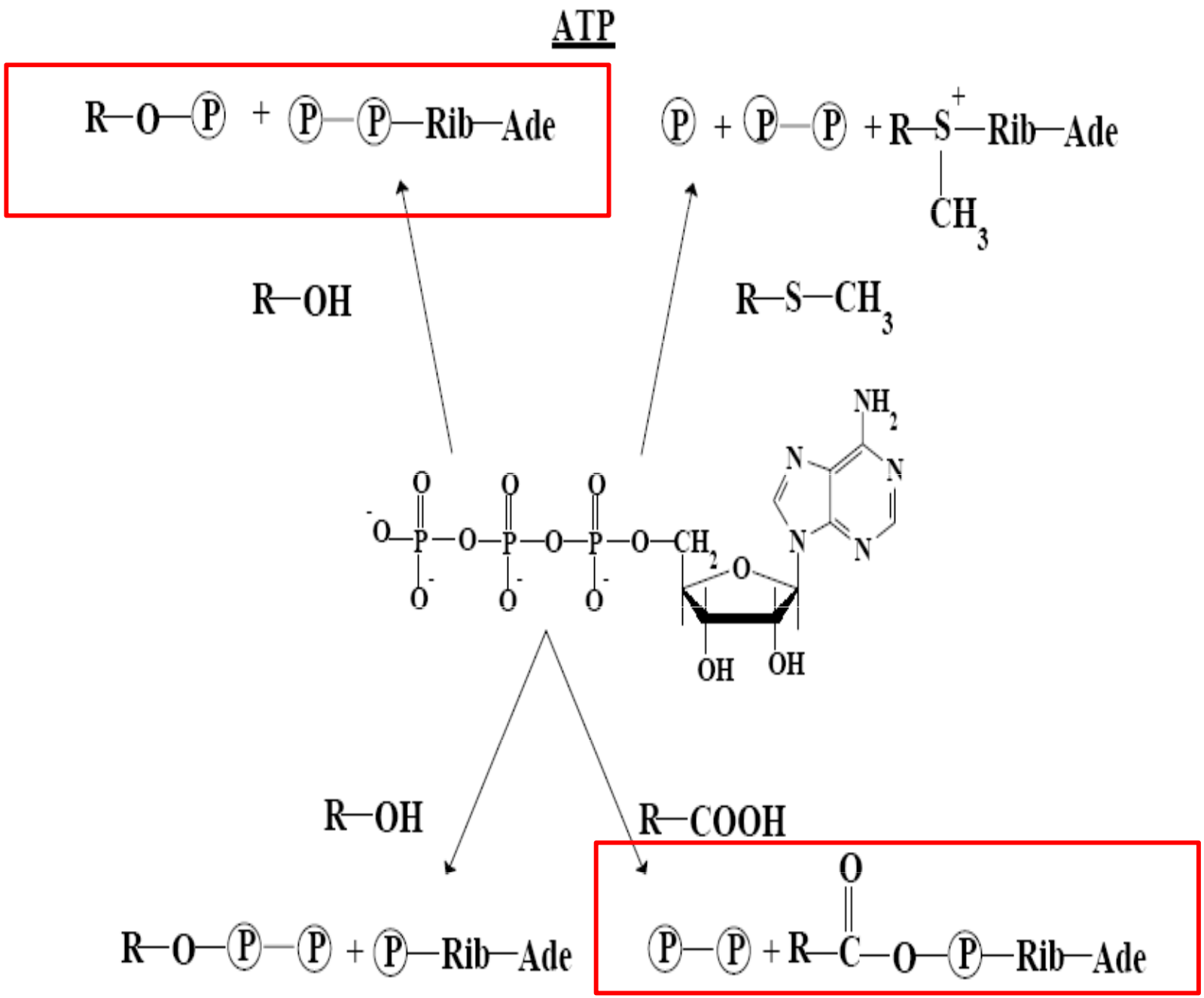


hem

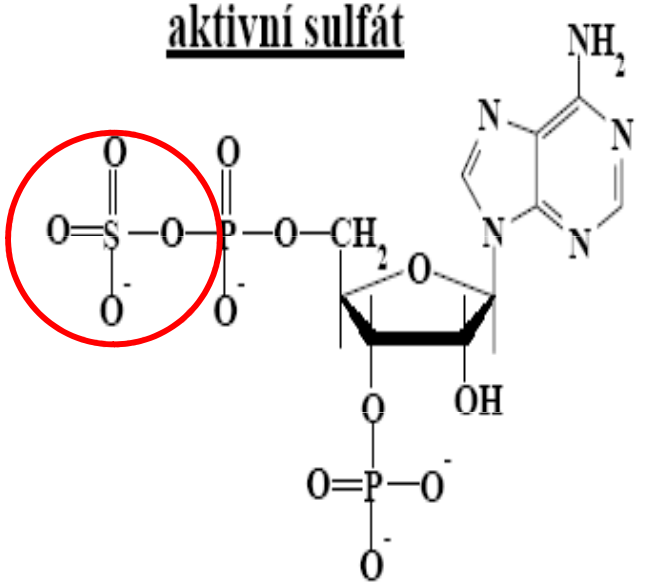


glutathion

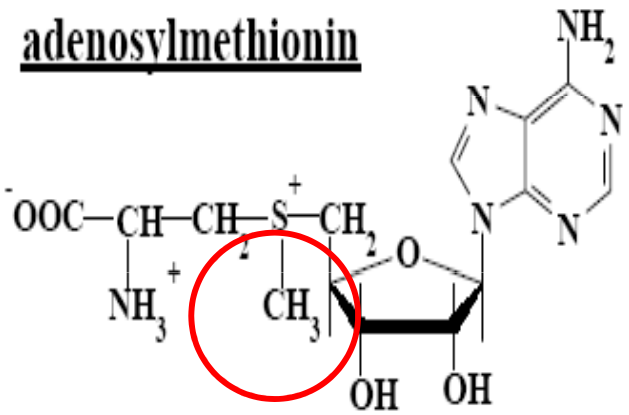
$$2\ GSH \underset{+\ 2\ [H]}{\overset{-\ 2\ [H]}{\rightleftharpoons}} GSSG$$


ATP
R—O—P + P—P—Rib—Ade
P + P—P + R—S+(CH3)—Rib—Ade
R—OH
R—S—CH3
NH2
N
N
N
N
O
O
O
O
O
O
O
O
O
O
O
O
CH2
OH
OH
R—OH
R—COOH
R—O—P—P + P—Rib—Ade
P—P + R—C(=O)—O—P—Rib—Ade

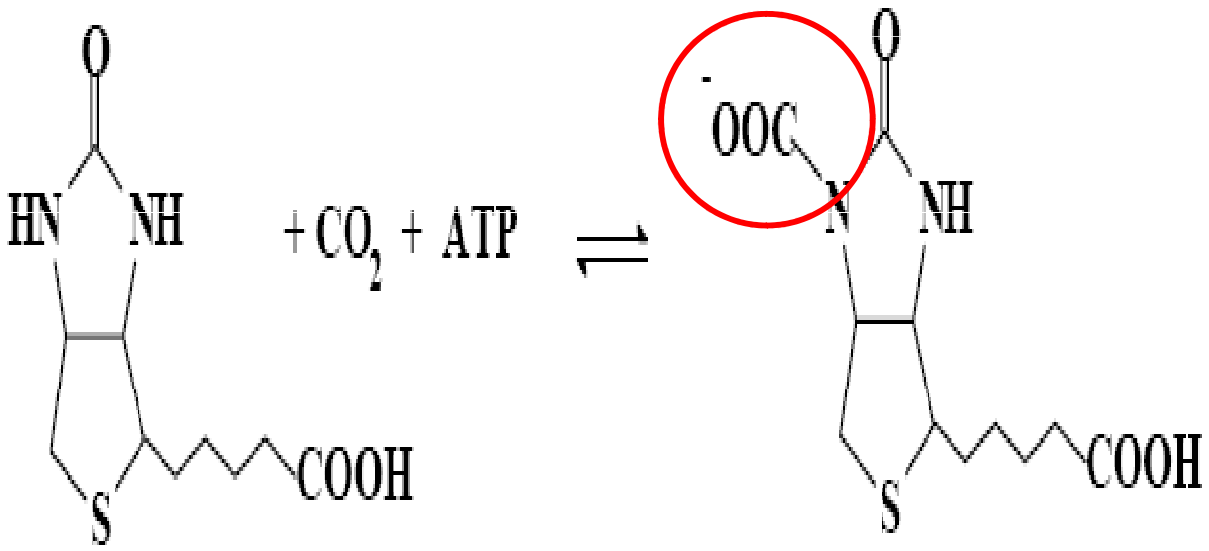
aktivní sulfát



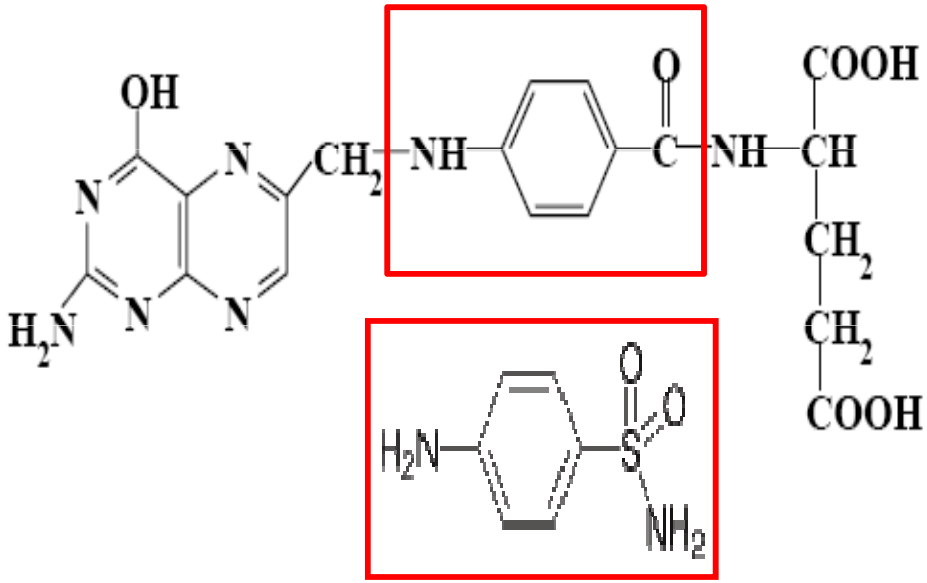
adenosylmethionin



biotin

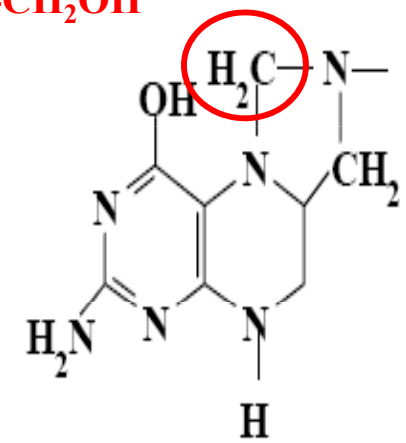


tetrahydrolistová k.



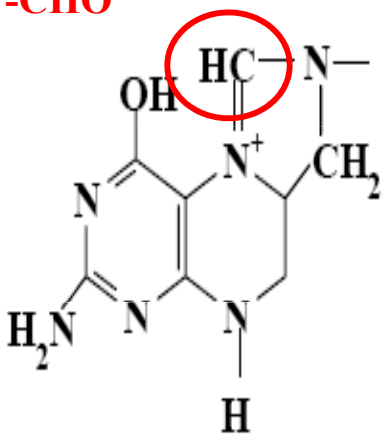
methylentetrahydrolistová k.

-CH_2OH

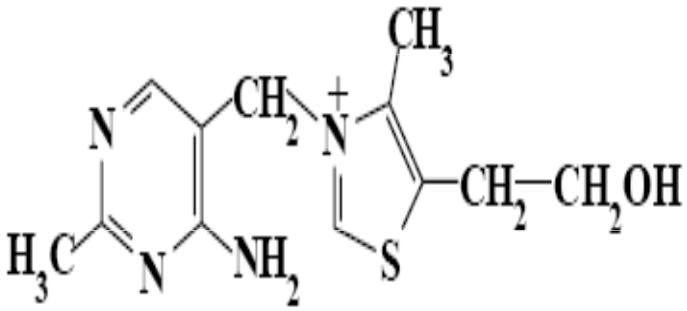


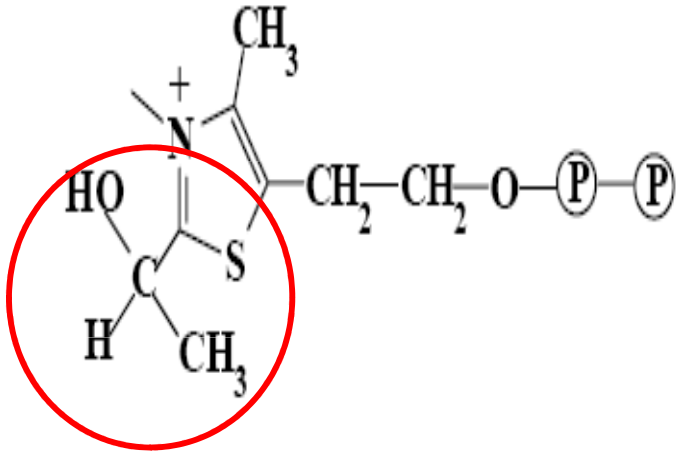
methenyltetrahydrolistová k.

-CHO

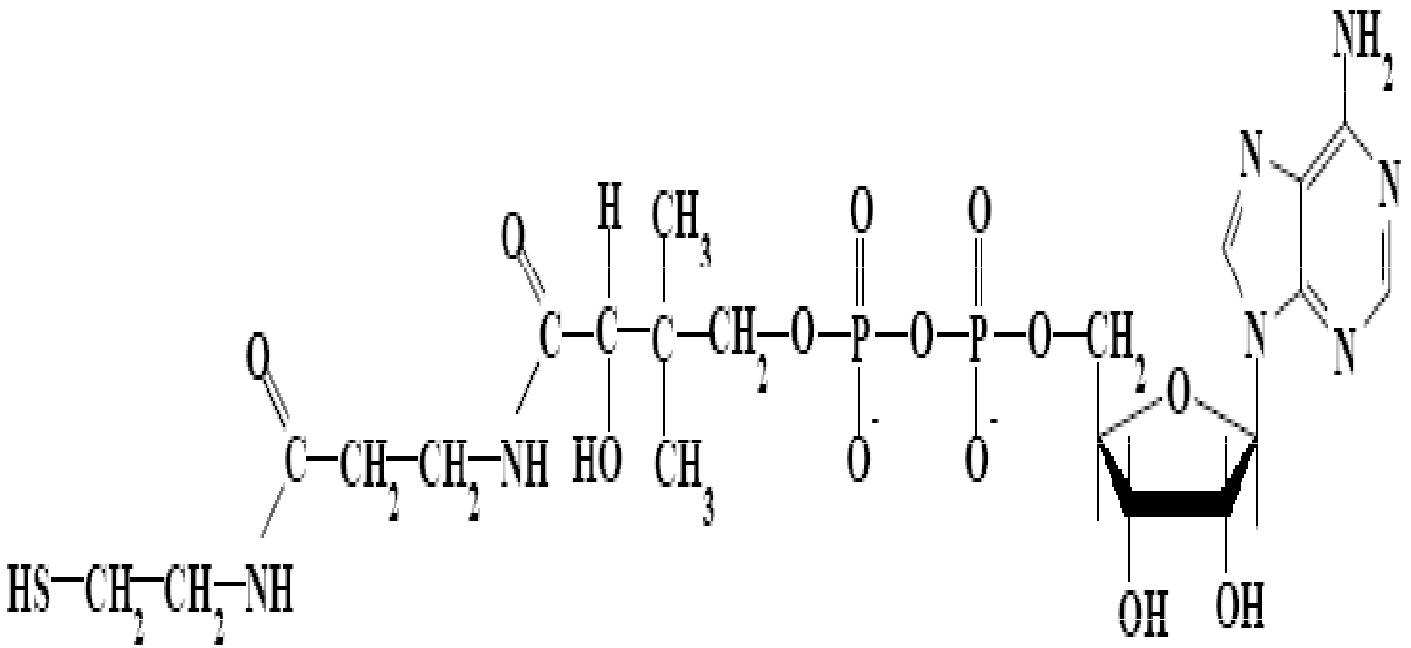


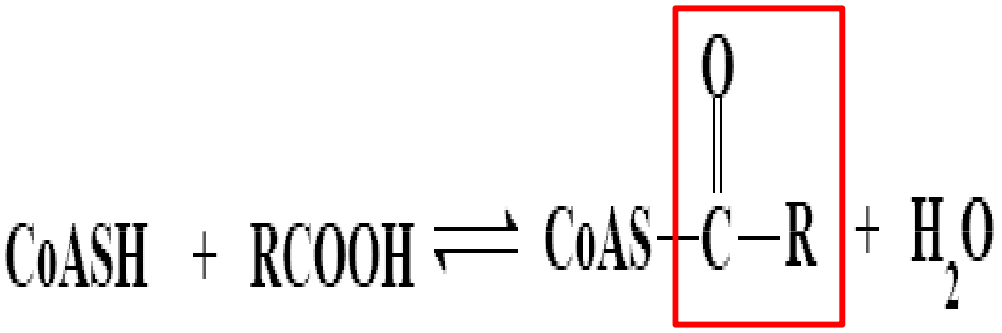
thiamin



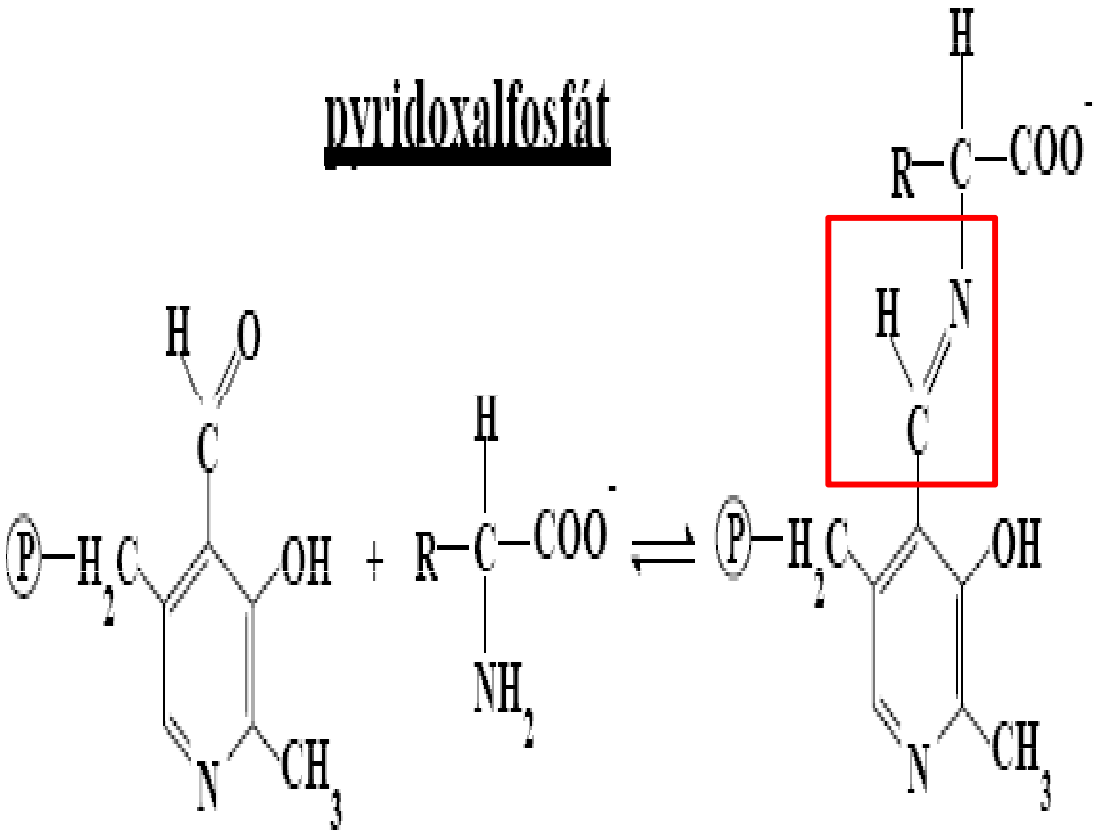


koenzym A - CoA - CoASH

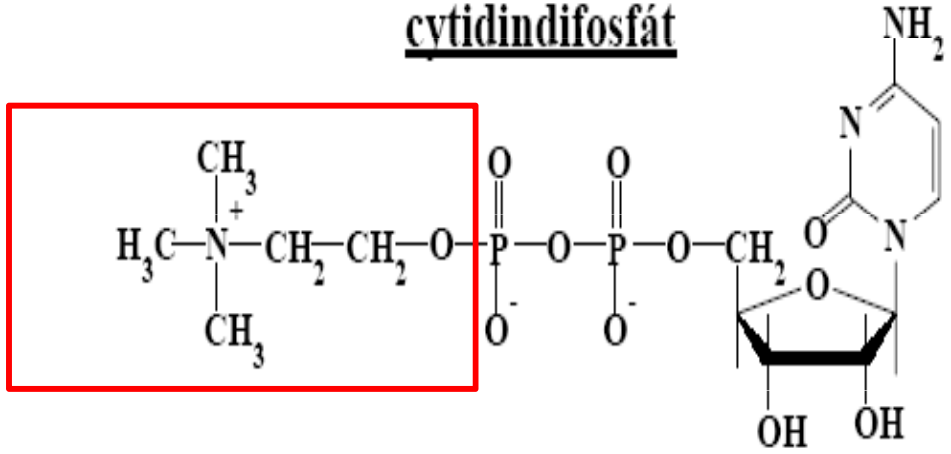




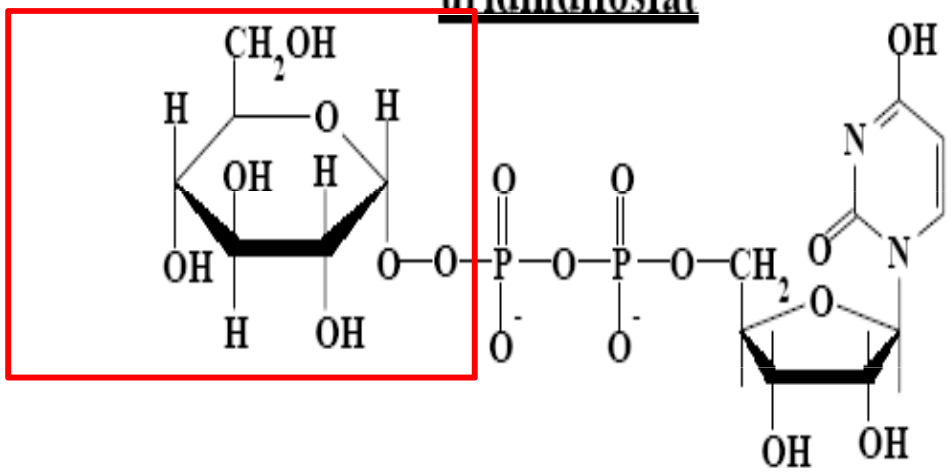
pyridoxalfosfát



cytidindifosfát



uridindifosfát



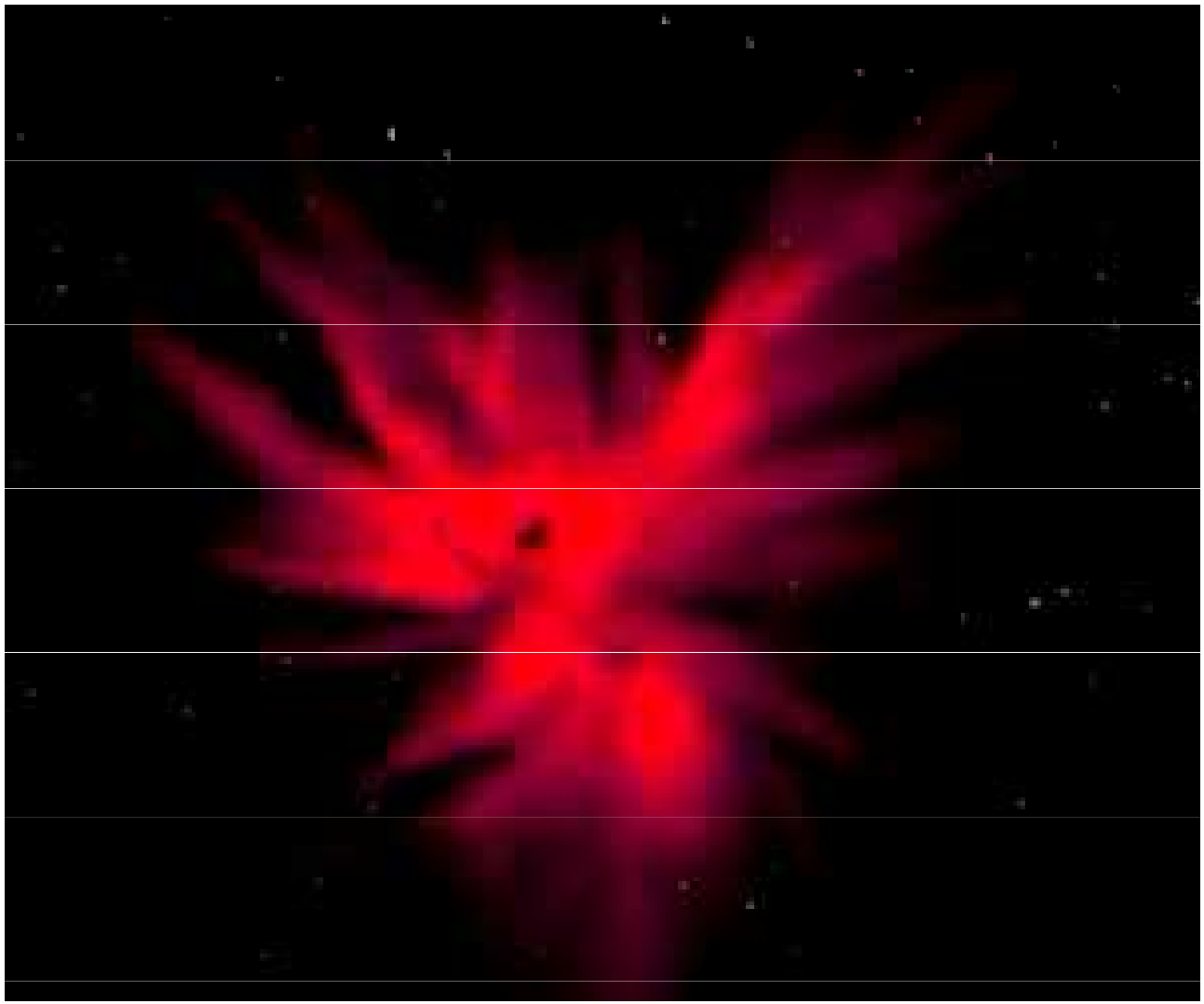
Lyasy a ligasy - bez kofaktoru nebo již popsaným kofaktorem TPP

Hydrolasy - bez kofaktoru

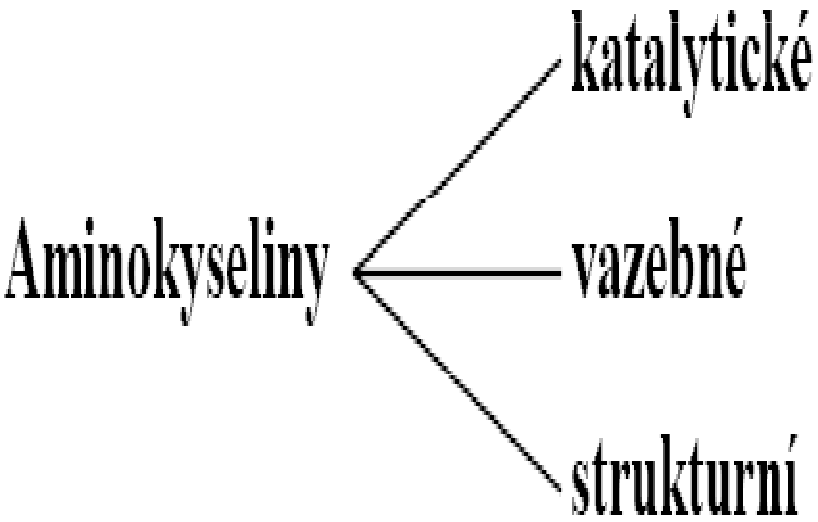
Izomerasy - většinou bez kofaktoru nebo kobalamin,

Enzymové bílkoviny

- monomerní
- oligomerní
- multienzymové komplexy

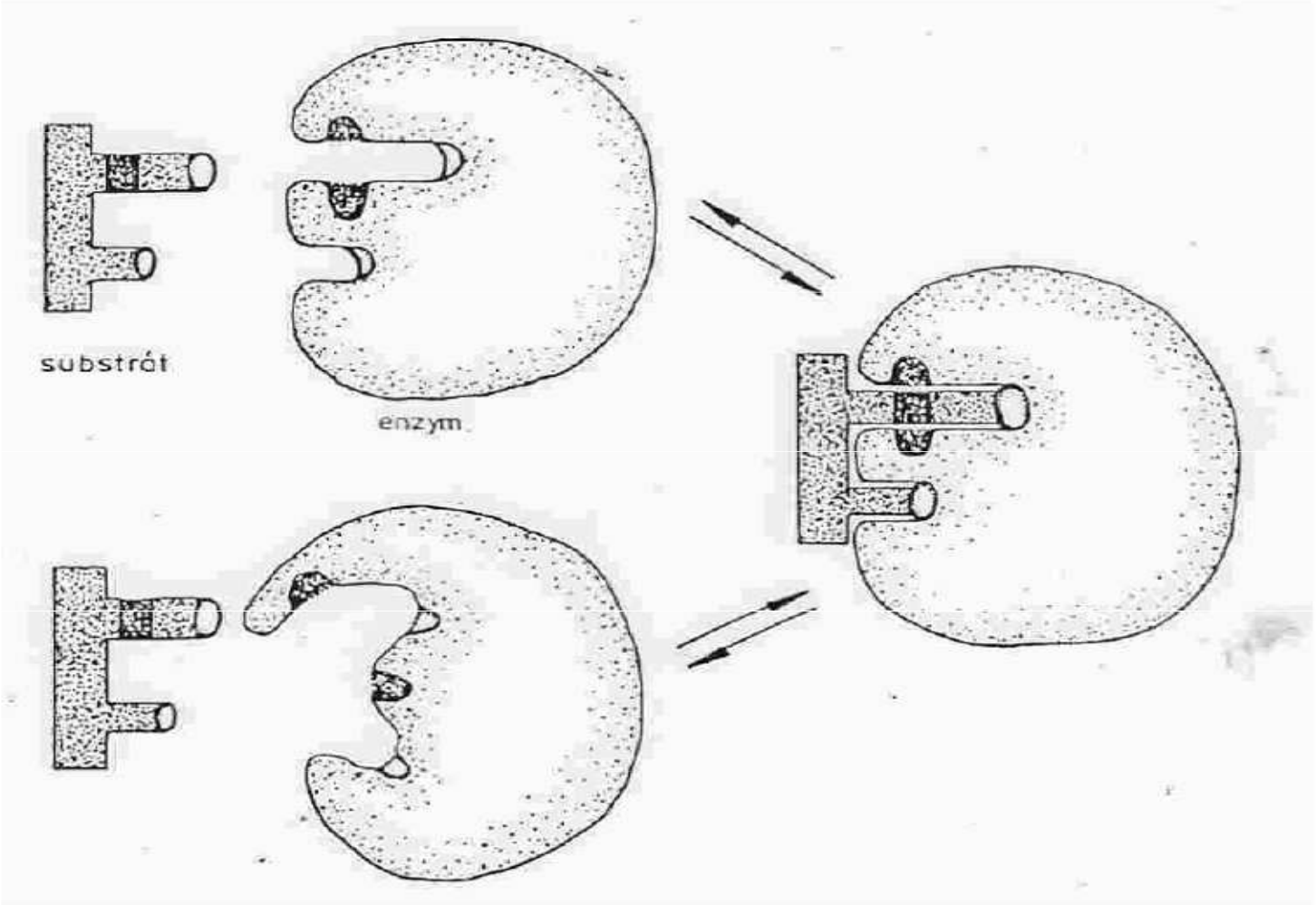


Aktivní místo enzymů



Katalytická triáda

Fischer - 1894 - *teorie o zámku a klíči*



Koshland - 1959 - *teorie indukovaného přizpůsobení*

„Lock and key“ model

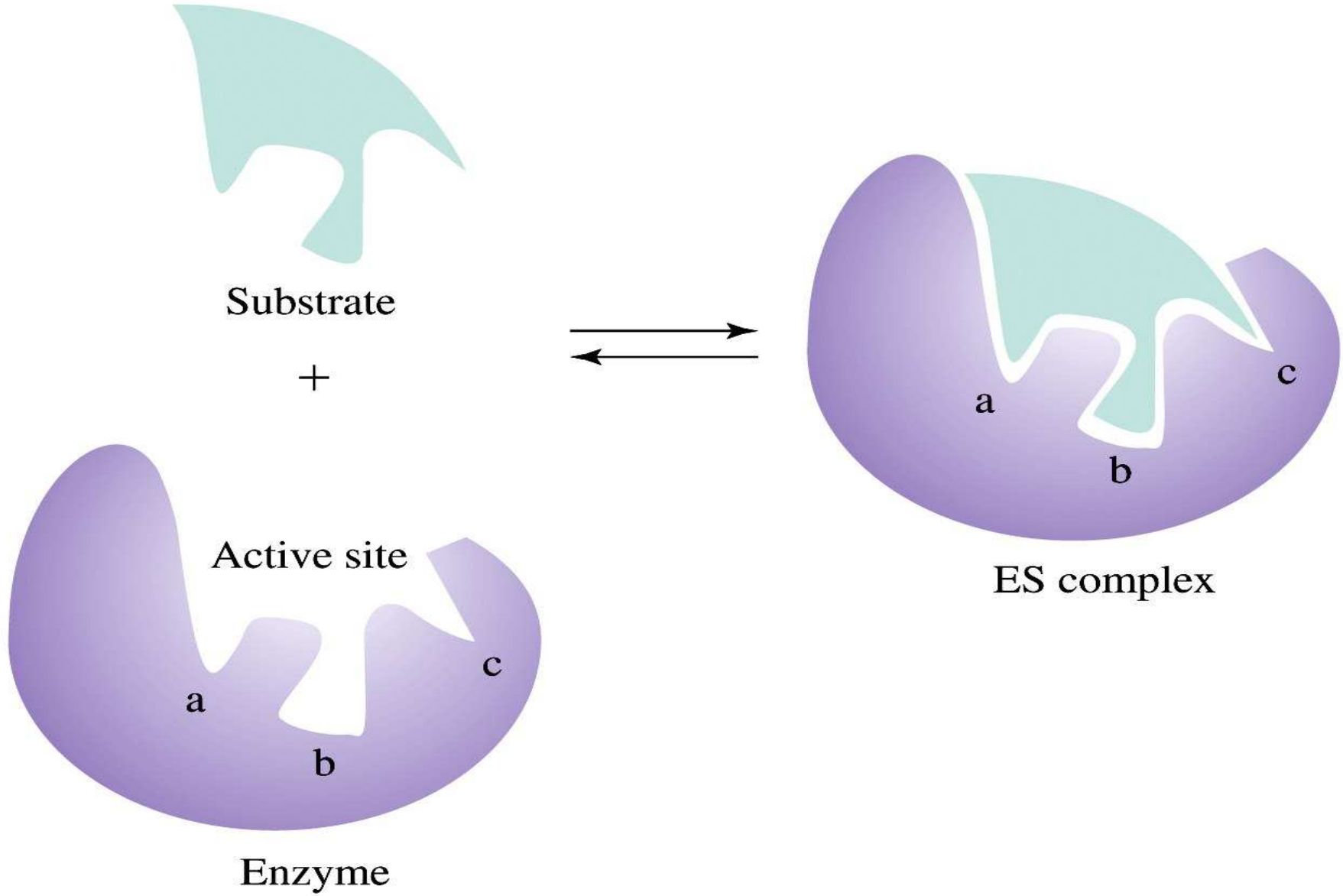


Figure 5-9 Concepts in Biochemistry, 3/e

„Induced fit“ model

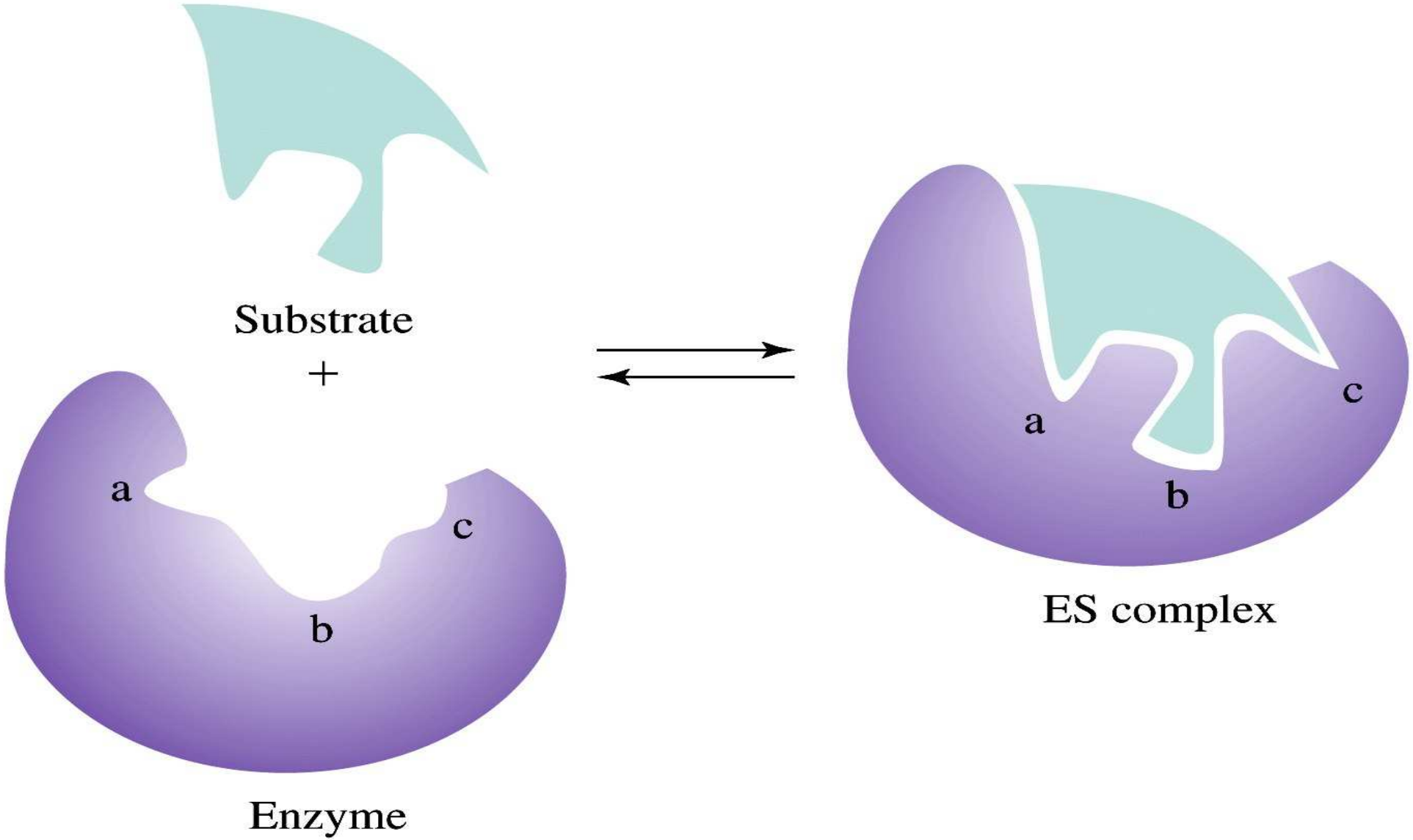


Figure 5-10 Concepts in Biochemistry, 3/e

„Transition state“ model

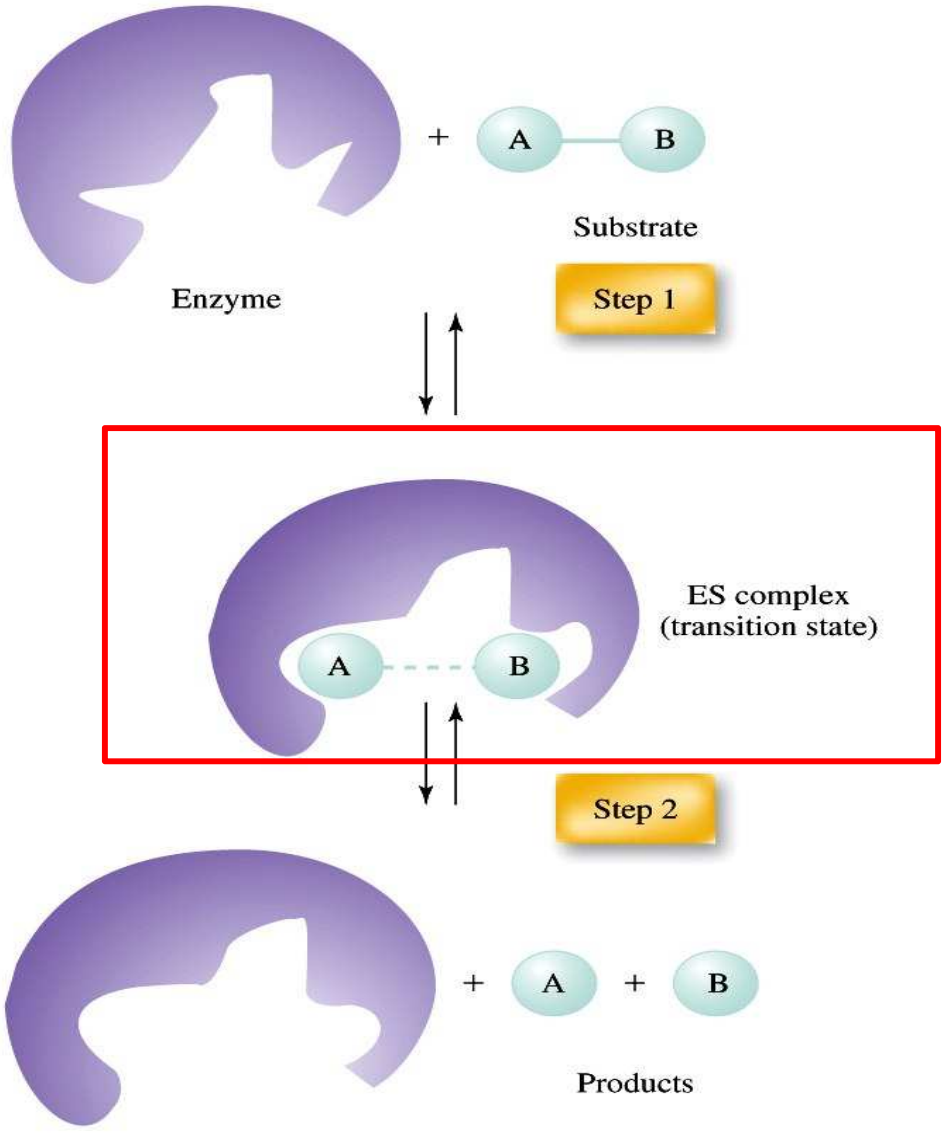


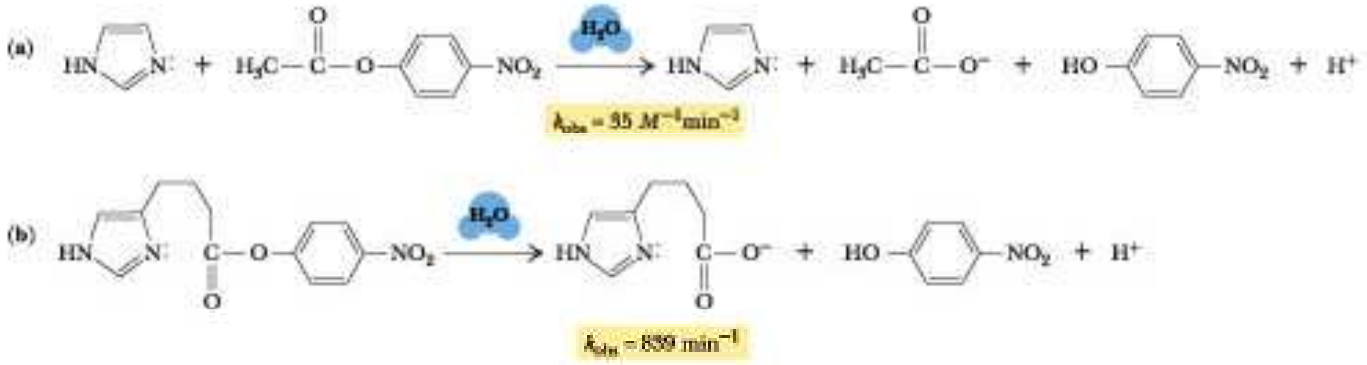
Figure 5-11 Concepts in Biochemistry, 3/e

Aktivní místo

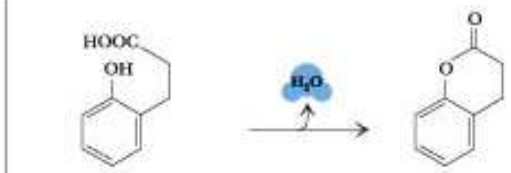
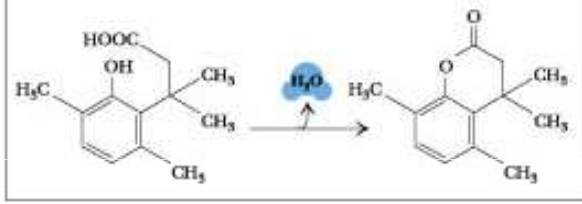
- Efekt přiblížení – překryv orbitalů
- Specifické mikroprostředí – pH, I, hydrofobita atd
- Dehydratace
- Koncentrační efekt - 10^5
- Vhodná orientace

Proximitní a orientační

INTRAMOLEKULÁRNÍ REAKCE



INTERMOLEKULÁRNÍ REAKCE

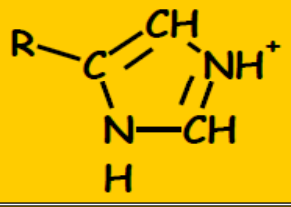
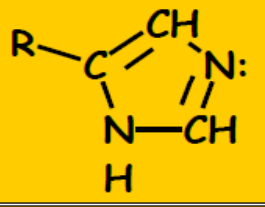
Reaction	Rate const. ($M^{-1}sec^{-1}$)	Ratio
	$5.9x10^{-6}$	
	$1.5x10^{6}$	$2.5x10^{11}$

Aktivační energie

Uvolněna při vazbě substrátu na enzym

Mechanismus katalýzy

- Acidobazická – Asp, Glu, His, Lys, Arg, Cys, Ser, Tyr

Aminokyselina	Kyselá forma (donor protonů)	Zásaditá forma (akceptor protonů)
Glu, Asp	R – COOH	R – COO^-
Lys, Arg	R – NH_3^+	R – NH_2
Cys	R – SH	R – S^-
His		
Ser	R – OH	R – O^-
Tyr		

Acidobazická kyselé proteázy

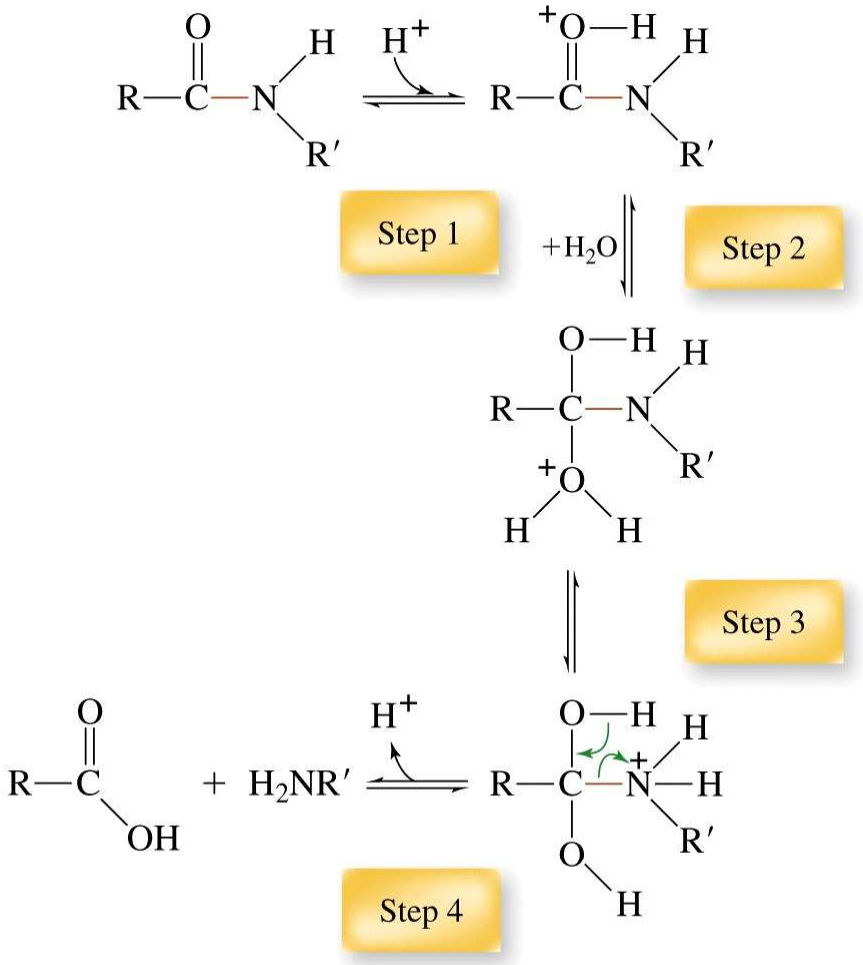
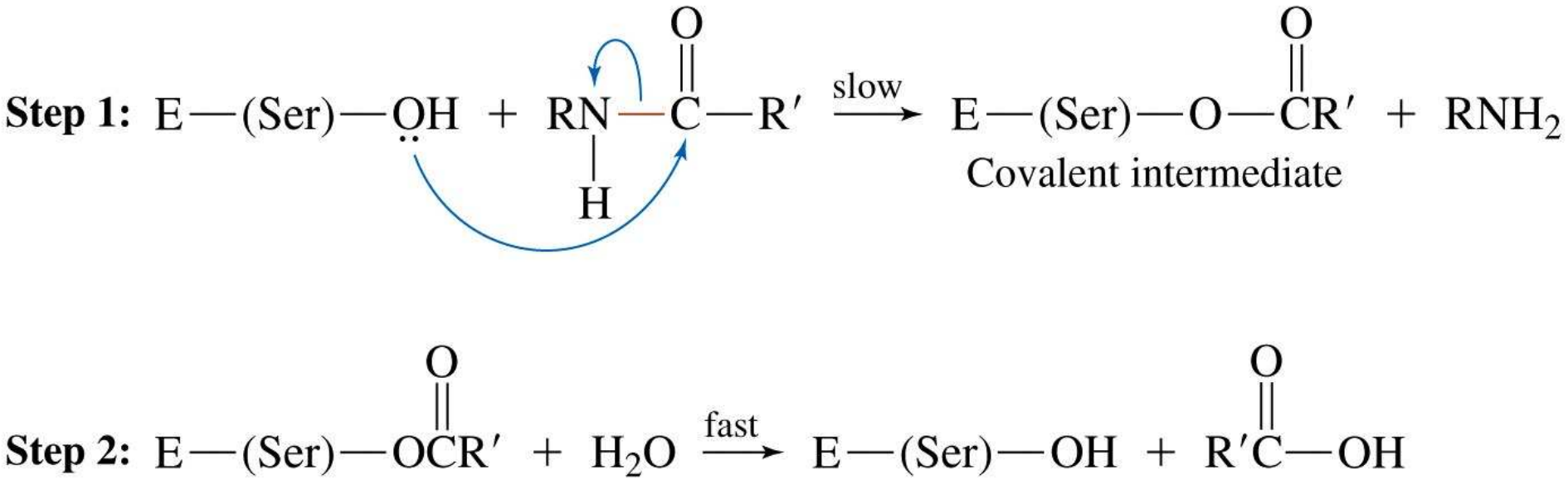


Figure 5-12 Concepts in Biochemistry, 3/e

Kovalentní serinové proteázy



Unnumbered figure pg150 Concepts in Biochemistry, 3/e

Kovovými ionty

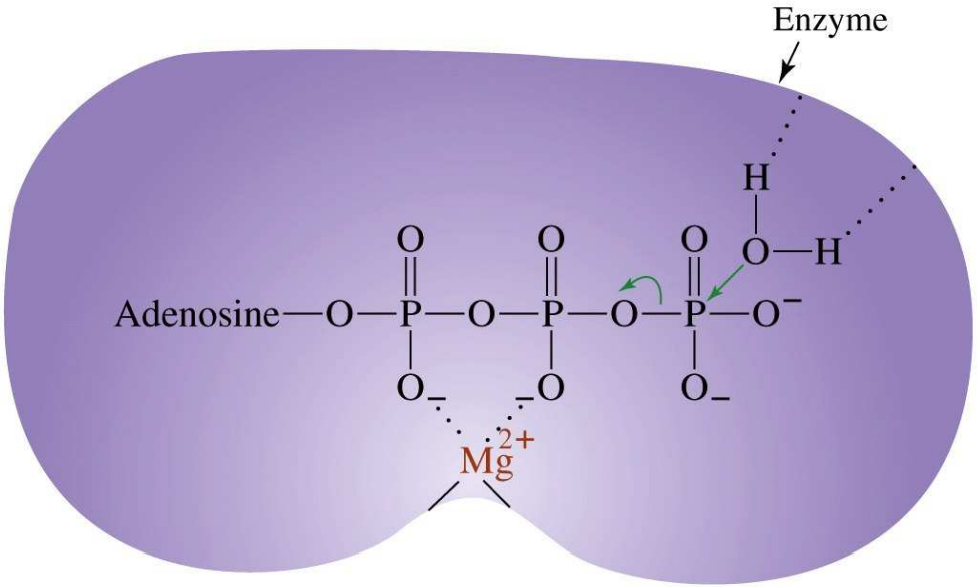


Figure 5-13a Concepts in Biochemistry, 3/e

Kovovými ionty

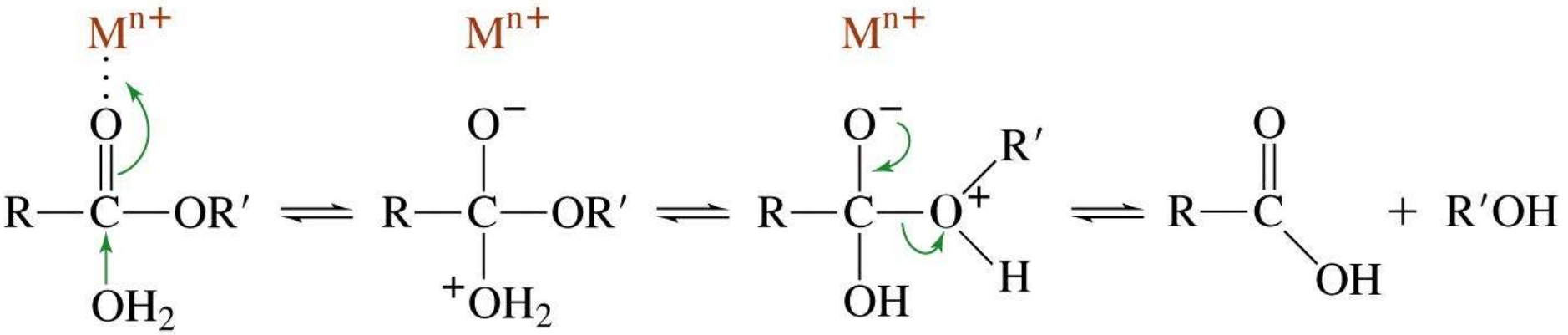


Figure 5-13b Concepts in Biochemistry, 3/e

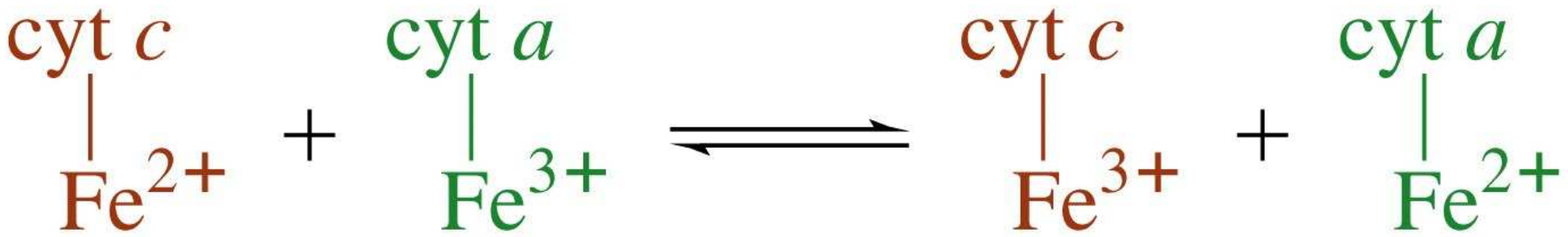
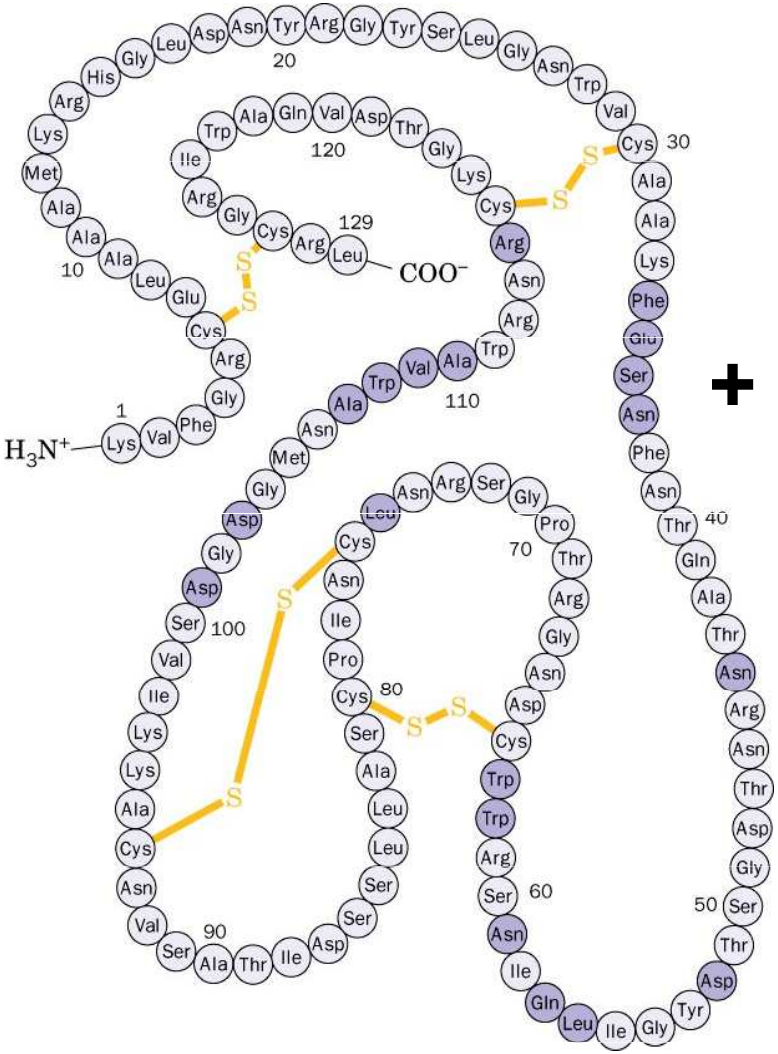
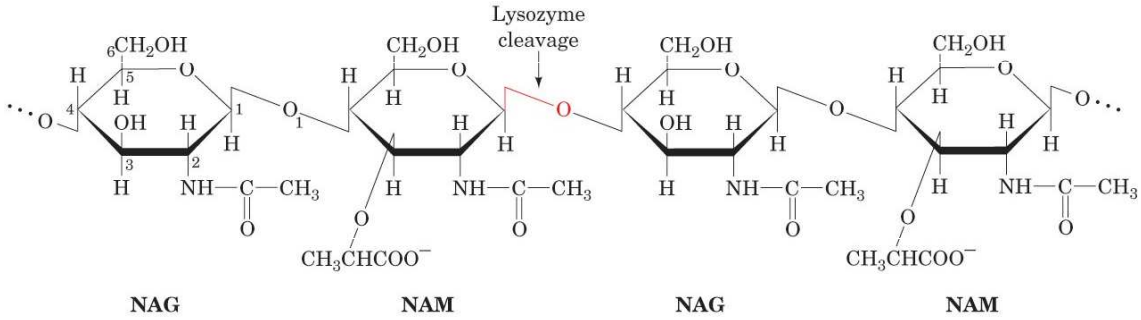


Figure 5-13c Concepts in Biochemistry, 3/e

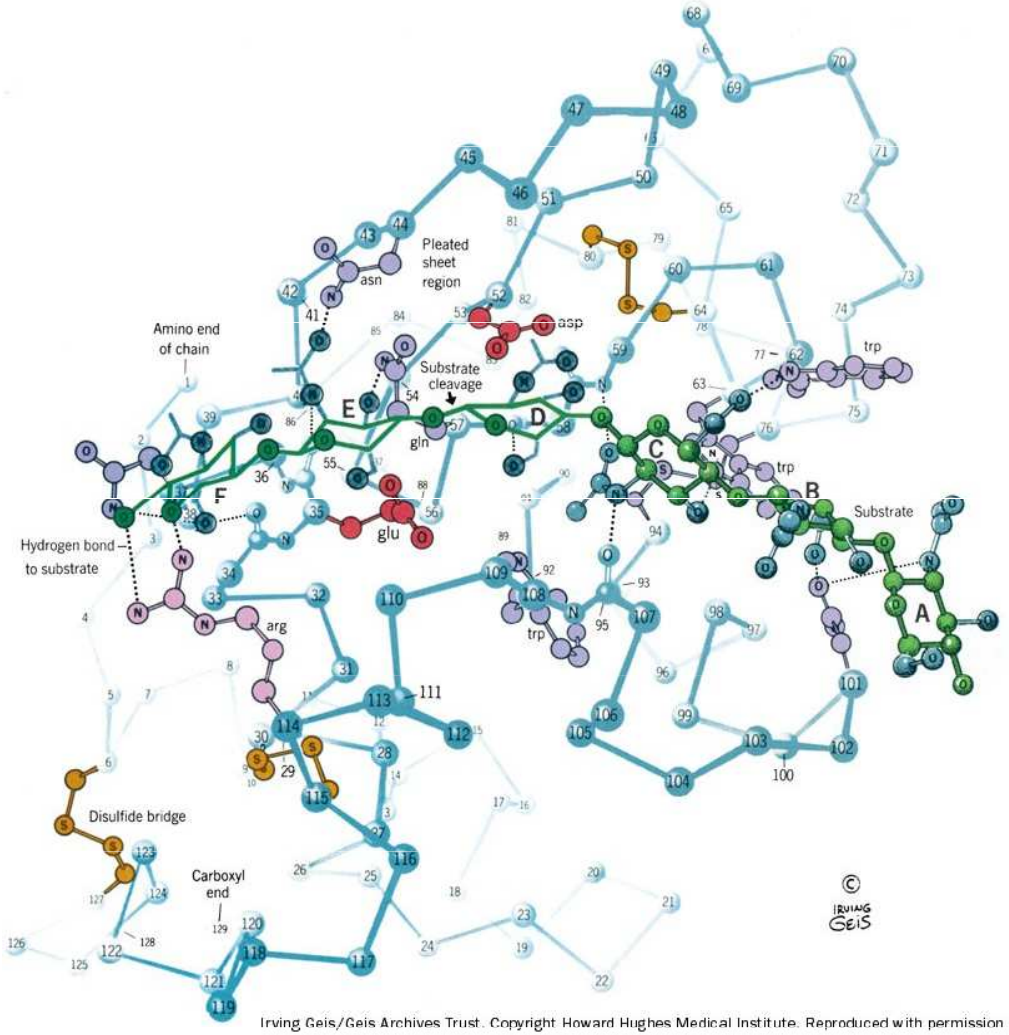
Lysozym

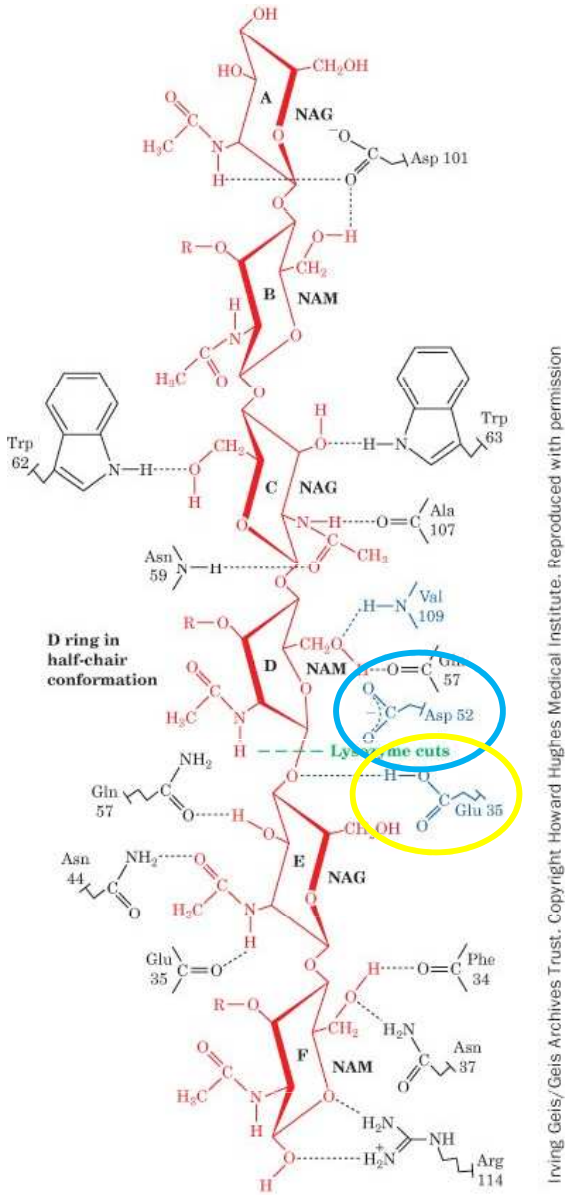

H3N+
COO−
1
10
20
30
40
50
60
70
80
90
100
110
120
129

+

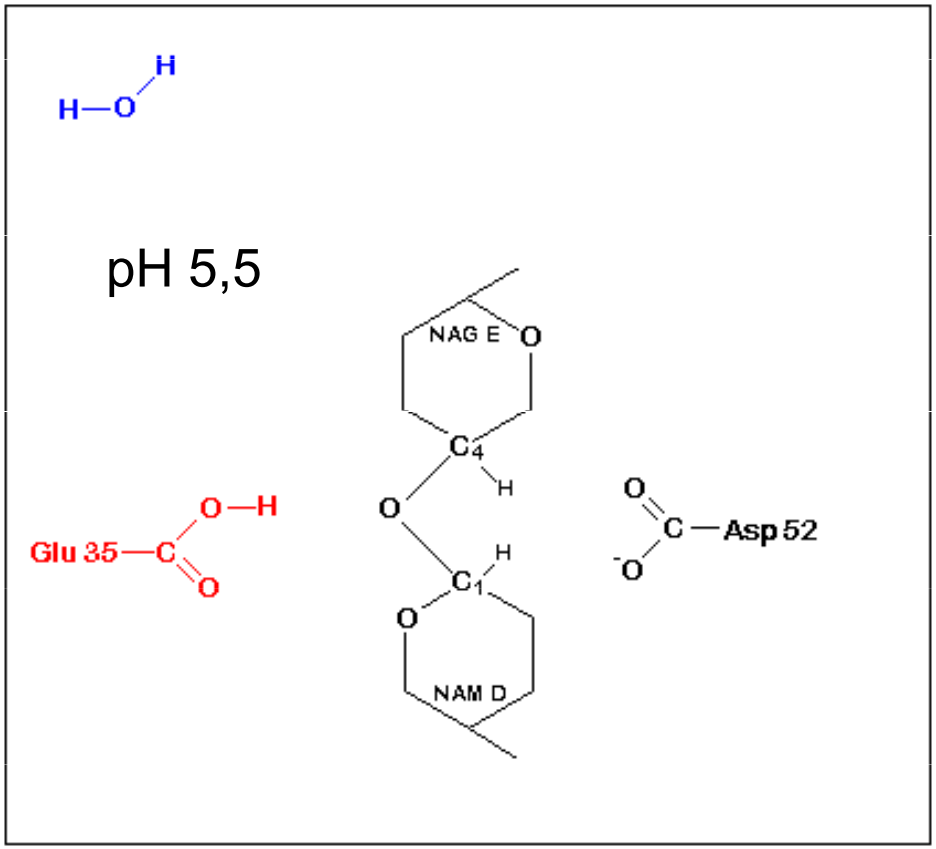

Lysozyme cleavage
NAG
NAM
NAG
NAM

Lysozym



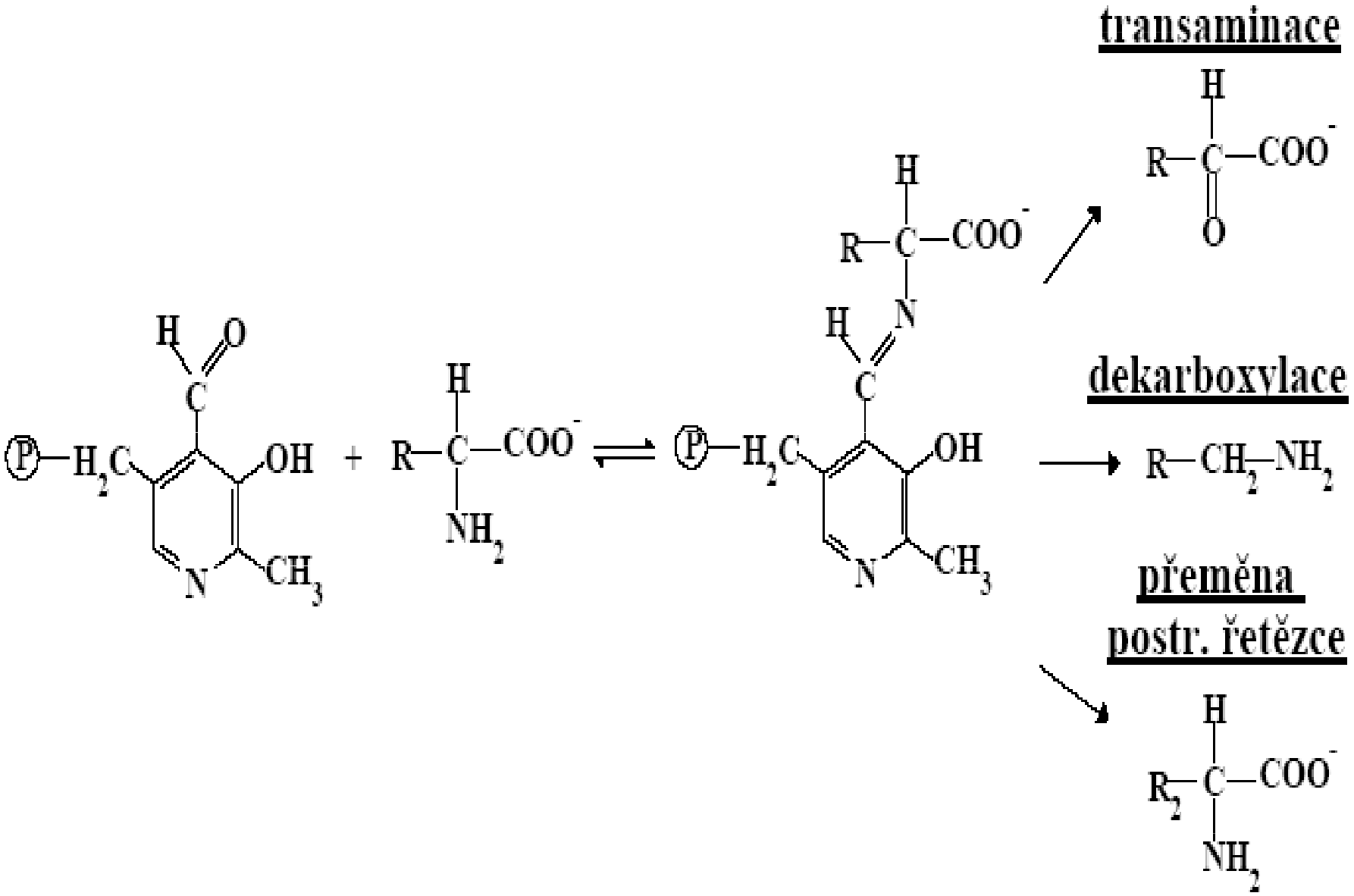


Lysozym



Specifita enzymové reakce

specifita reakční - účinku - jaká reakce proběhne


transaminace
dekarboxylace
přeměna
postr. řetězce

savci

$$\text{glutamát} + NAD^+ + H_2O \leftrightarrow \text{2-oxoglutarát} + NH_3 + NADH + H^+$$

vejcorodí

$$\text{aminokyselina} + FAD + H_2O \leftrightarrow \alpha\text{-oxokyselina} + NH_3 + FADH_2$$

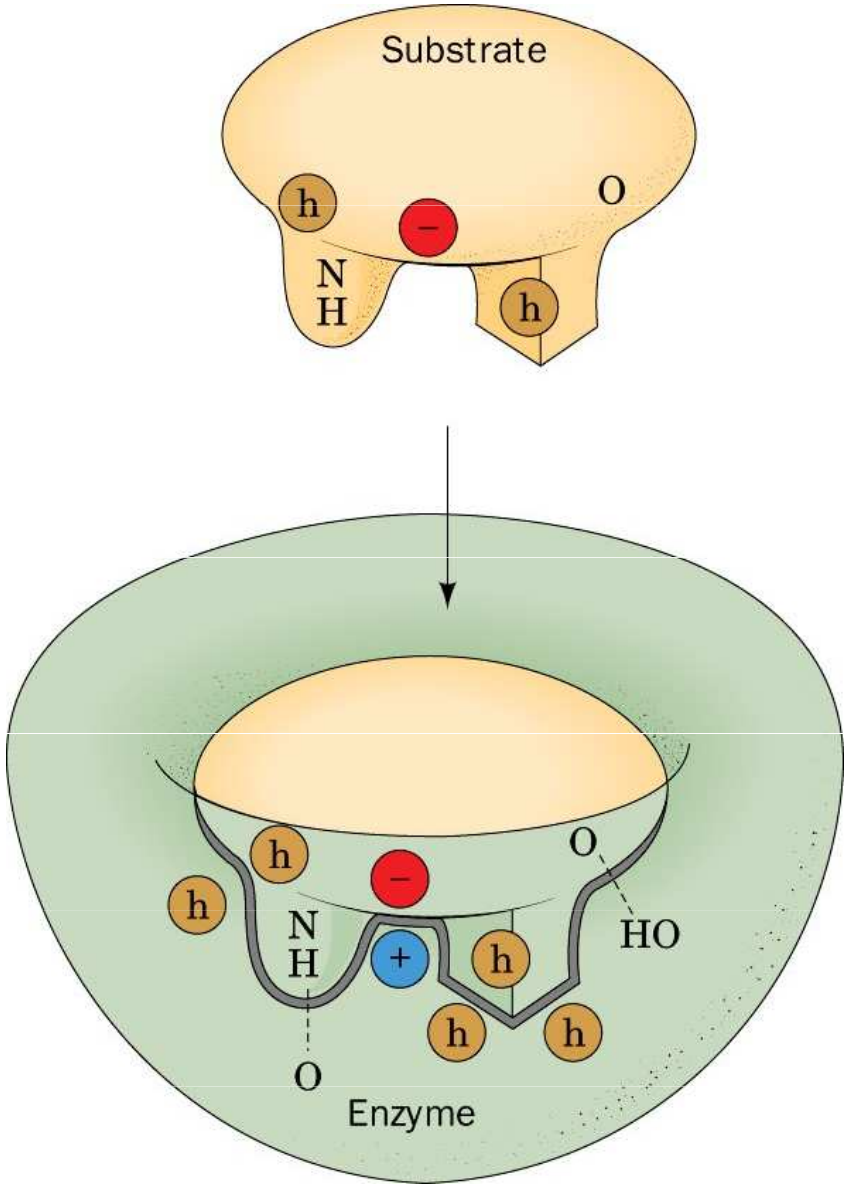
$$FADH_2 + O_2 \leftrightarrow FAD + H_2O_2$$

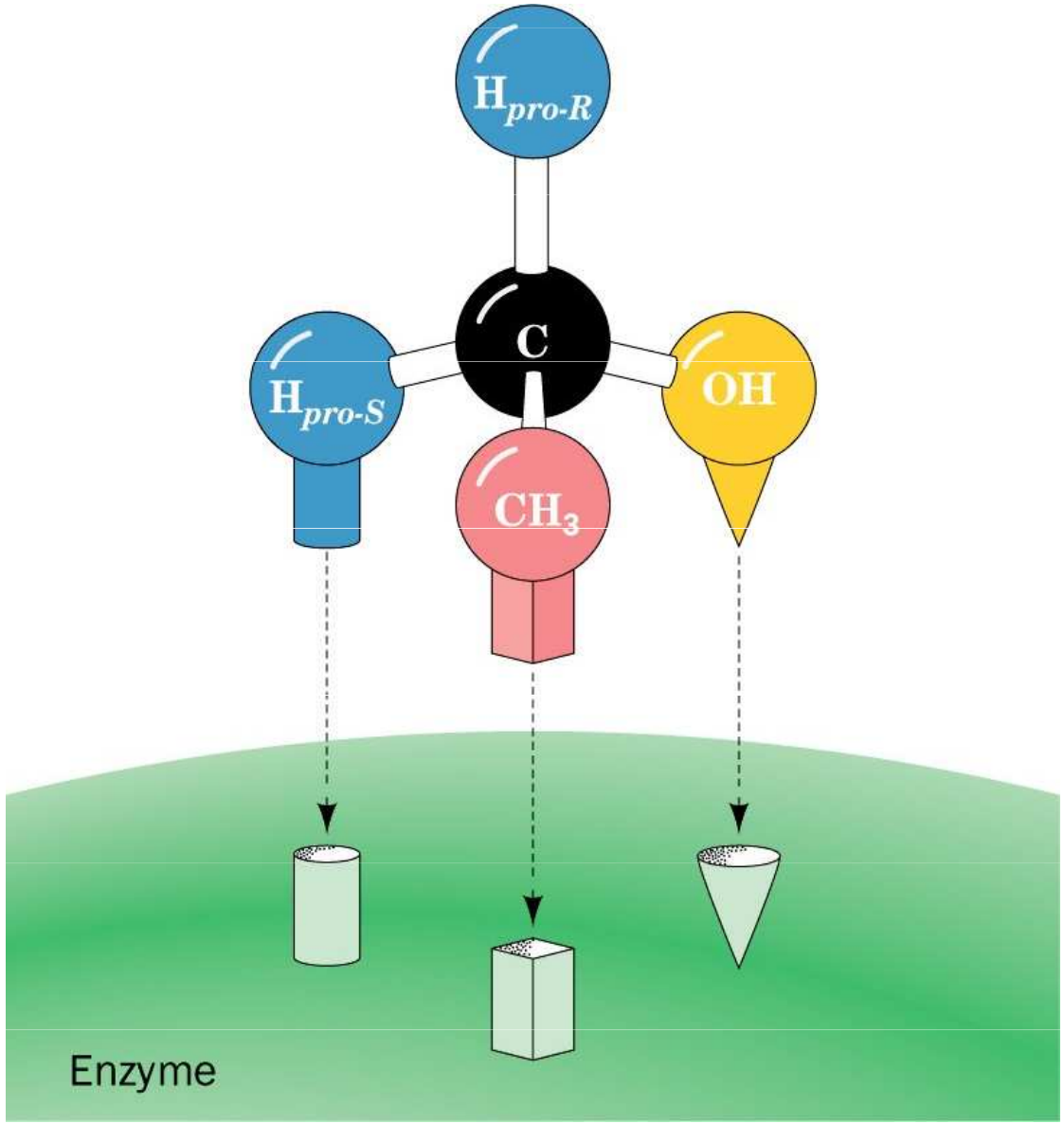
Specifita enzymové reakce

specifita substrátová - absolutní

- skupinová

- stereospecifita


Substrate
h
O
–
N
H
h
h
h
O
–
h
N
H
+
h
O
HO
h
h
h
O
Enzyme


$H_{pro\text{-}R}$
C
$H_{pro\text{-}S}$
OH
CH_3
Enzyme

ENZYMOVÁ KINETIKA

Reakce s jedním substrátem

BROWN 1902

MICHAELIS MENTENOVÁ 1913

Leonor Michaelis
1875–1949

Maud Menten
1879–1960

b) závislost počáteční rychlosti na koncentraci enzymu

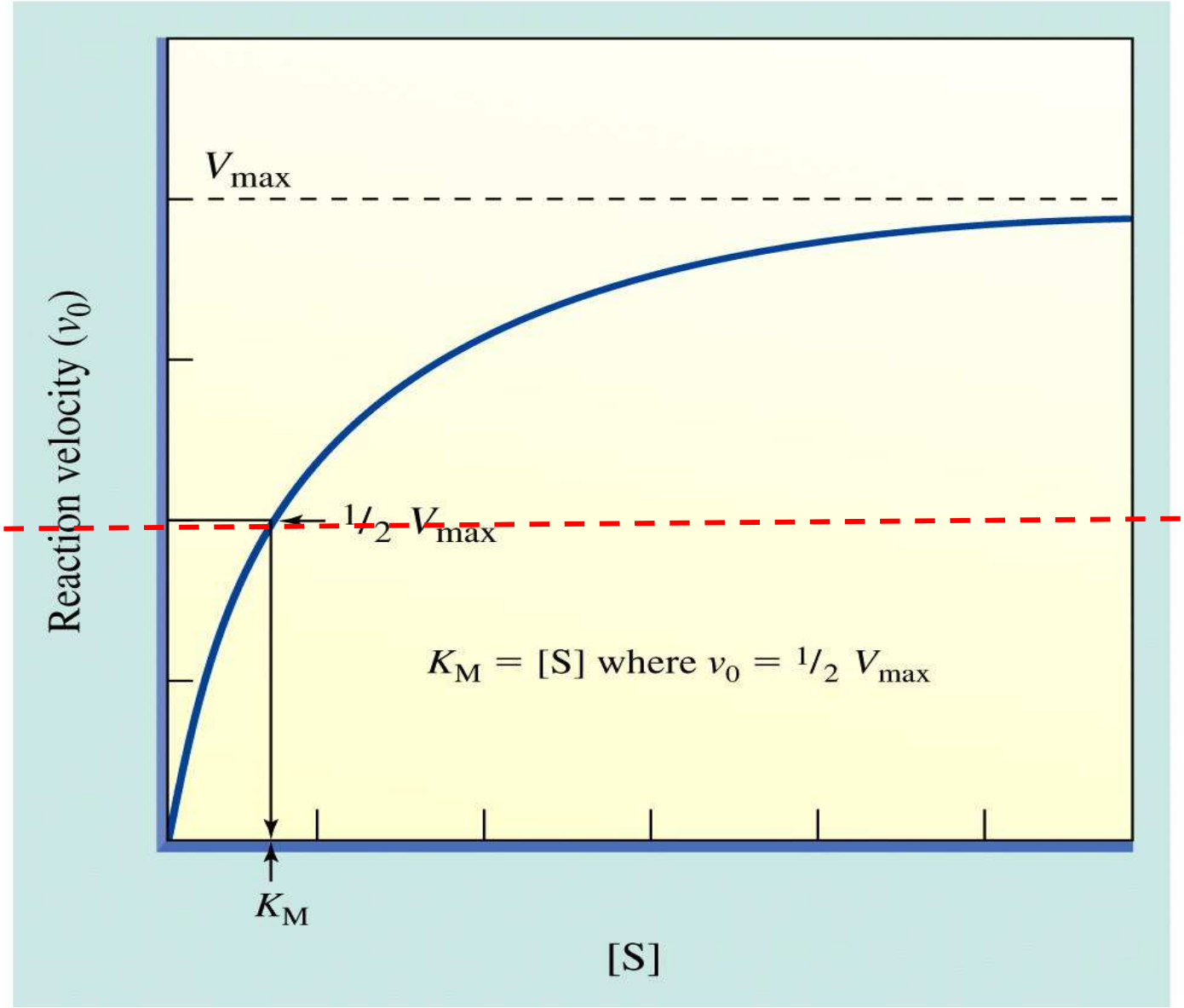
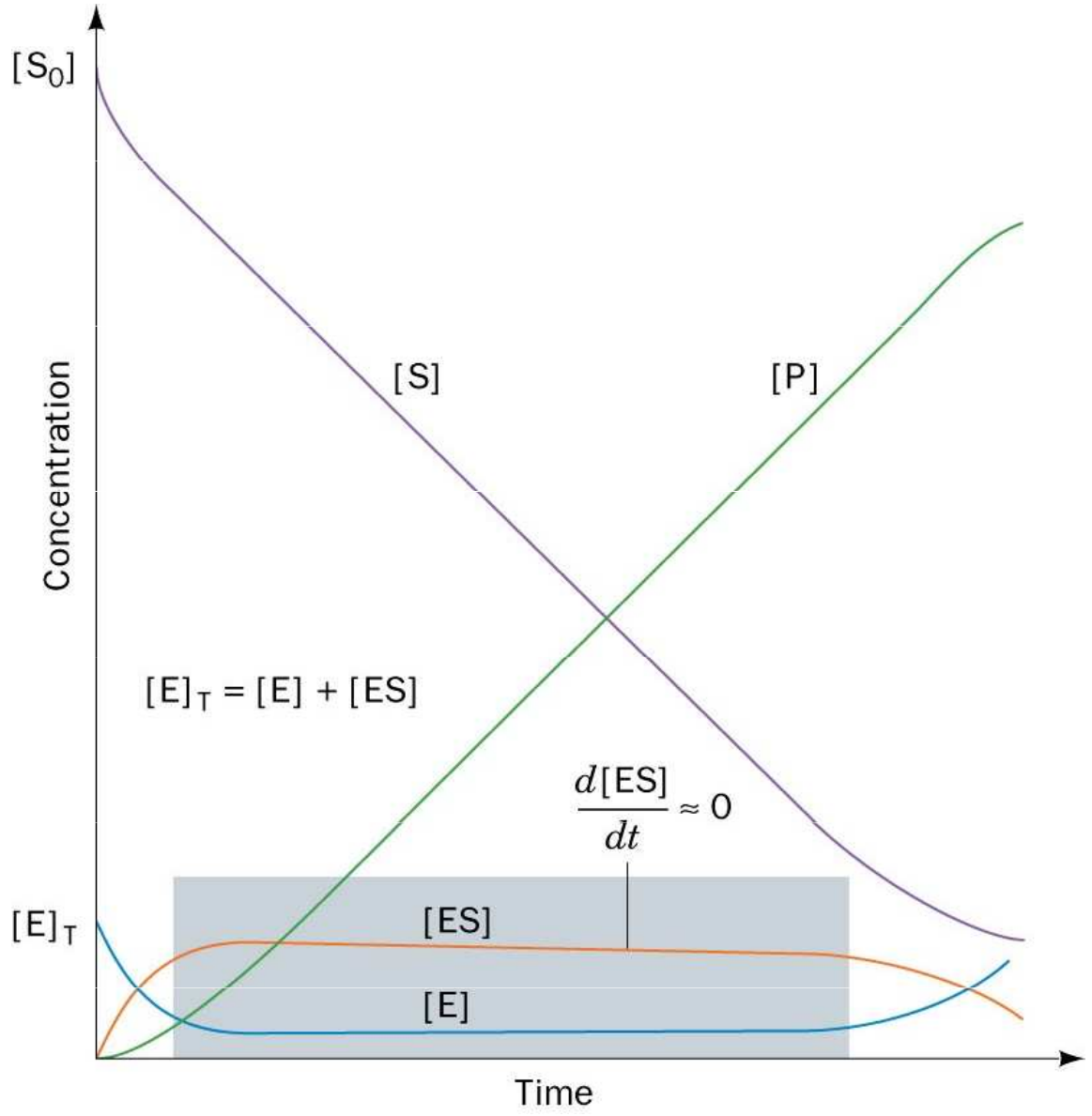


Figure 5-4 Concepts in Biochemistry, 3/e

Ustálený stav

$$E + S \rightleftharpoons ES \leftrightarrow EP \dashrightarrow E + P$$

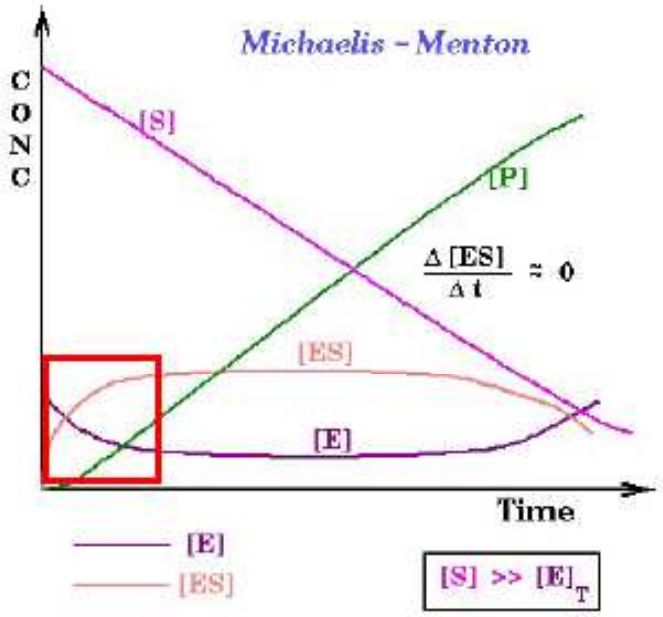


Základní model - Michaelis & Mentenová

$$E + S \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} ES \xrightarrow[(k_{-2})]{k_{+2}} E + P$$

Předpoklady:

1. Koncentrace [ES] je v ustáleném stavu
2. Tvorba produktu P je přímo úměrná koncetraci [ES], tudíž $v_0 = k_2$ [ES]
3. Koncentrace [S] je mnohem vyšší než [E]
4. Zpětnou reakci lze zanedbat?



Odvození rovnice Michaelis Mentenové

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

1.Předpoklad – koncentrace [ES] se v ustáleném, stavu nemění $\frac{d[ES]}{dt} = 0$

$v_1 = v_{-1} + v$

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

$[E]_{tot} = [E] + [ES]$

$[E] = [E]_{tot} - [ES]$

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

$([E]_{tot} - [ES])[S] = (k_{-1} + k_2 / k_1) [ES]$

$K_m = (k_{-1} + k_2 / k_1)$

$([E]_{tot} - [ES])[S] = K_m [ES]$

$[E]_{tot} [S] - [ES][S] = K_m [ES]$

$[E]_{tot} [S] = K_m [ES] + [ES][S]$

$[E]_{tot} [S] = [ES] (K_m + [S])$

$[ES] = [E]_{tot} [S] / (K_m + [S])$

Odvození rovnice Michaelis Mentenové

$$E + S \underset{k_{-1}}{\overset{k_1}{<===>}} ES \overset{k_2}{===>} P + E$$

2.Předpoklad – tvorba produktu je přímo úměrná koncentraci [ES] $v_0 = k_2.[ES]$

$$[ES] = [E]_{tot}\,[S] /(K_m + [S])$$

$[ES] = \boxed{v_0/k_2}$

$$v_0/k_2 = [E]_{tot}\,[S] /(K_m + [S])$$

$$v_0 = k_2\,[E]_{tot}\,[S] /(K_m + [S])$$

$\boxed{V_{max}} = k_2\,[E]_{tot}$

$$v_0 = V_{max}\,[S] /(K_m + [S])$$

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

Rovnice Michaelis Mentenové

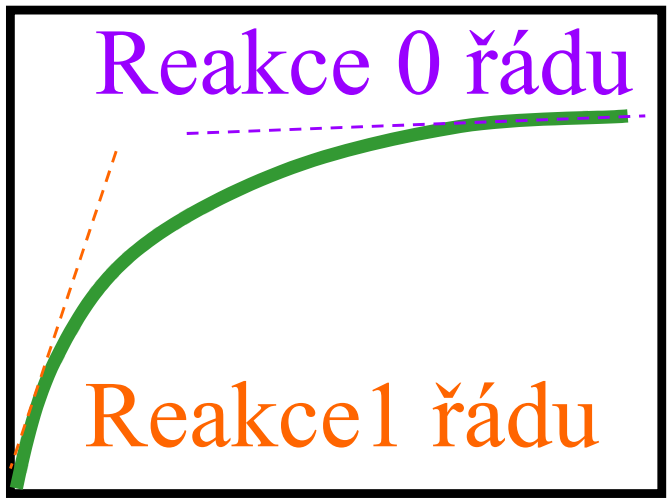
$$v = \frac{V \cdot [S]}{Km + [S]}$$

v - počáteční reakční rychlost

V - maximální (limitní) reakční rychlost

Km - Michaelisova konstanta

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



$[S] \ll Km$

$$v = \frac{V \cdot [S]}{Km} = konst.[S]$$

[S] = nízká → vysoká

Stanovení Km a Vmax

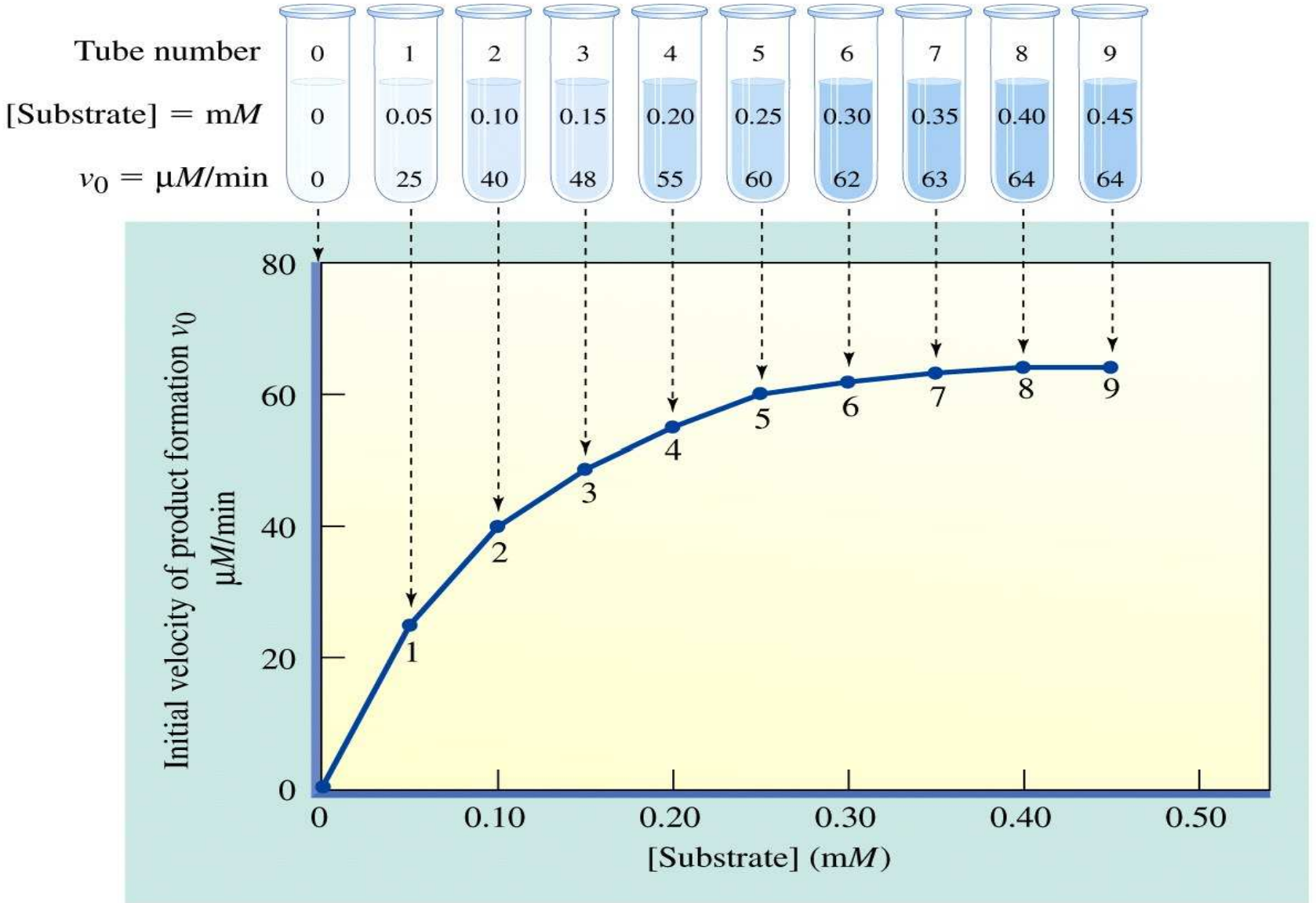
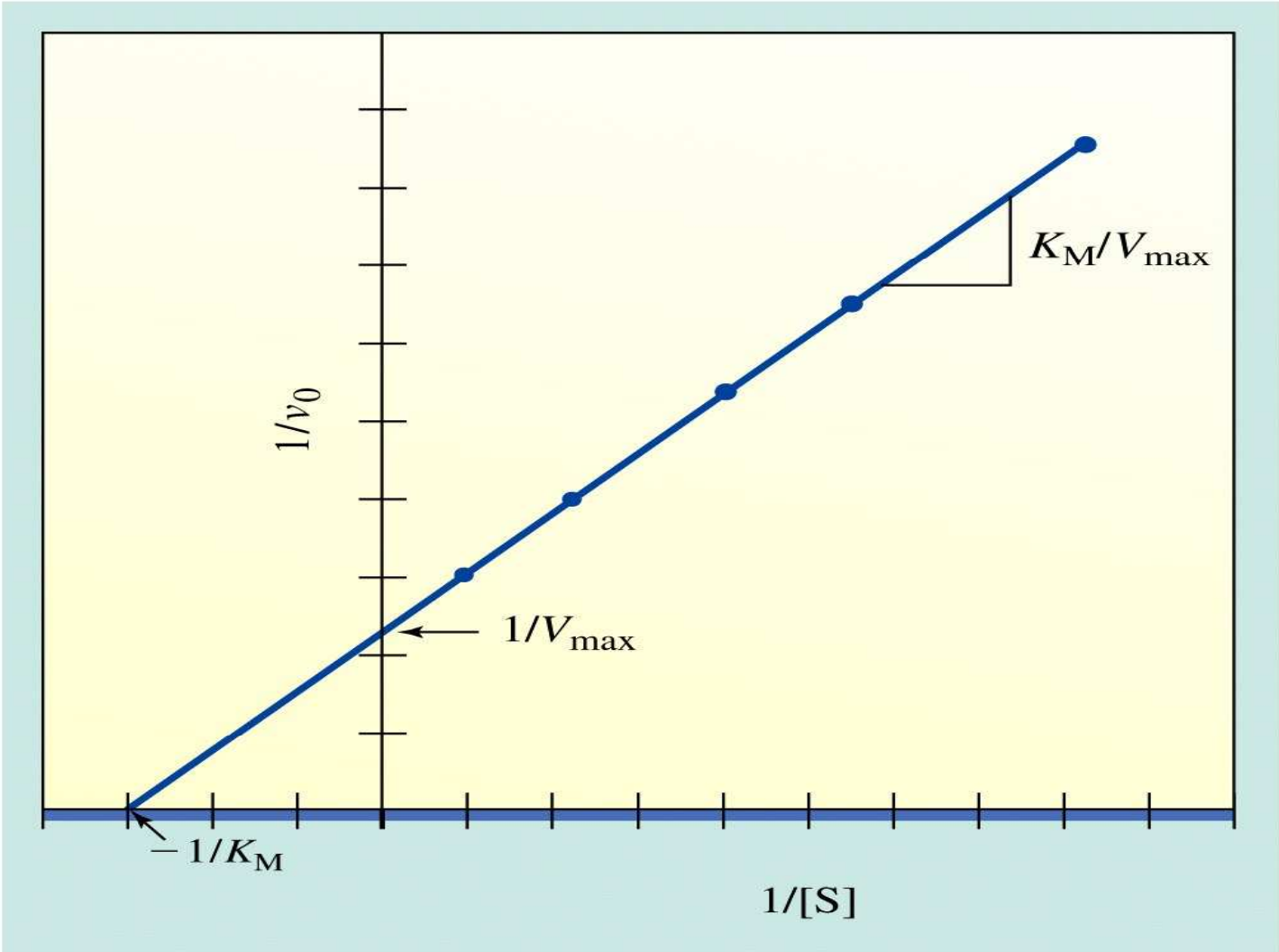


Figure 5-3 Concepts in Biochemistry, 3/e

Stanovení Km :

LINEWEAVER BURKE



Unnumbered figure pg142 Concepts in Biochemistry, 3/e

Table 5.3

K_M values for some enzyme–substrate systems

Enzyme	Substrate	K_M (mM)
Catalase	H_2O_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
	N-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 5-3 Concepts in Biochemistry, 3/e

Table 5.4

Turnover numbers, k_3, for some enzymes

Enzyme	Substrate	k_3 (sec^{-1})
Catalase	H_2O_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

Km ??

- Taková koncentrace substrátu, že reakce poběží polovinou Vmax
- Je mírou afinity substrátu k enzymu
- Nezávisí na koncentraci enzymu, závisí na prostředí T, I, pH, efektory atd.

Proč Km ??

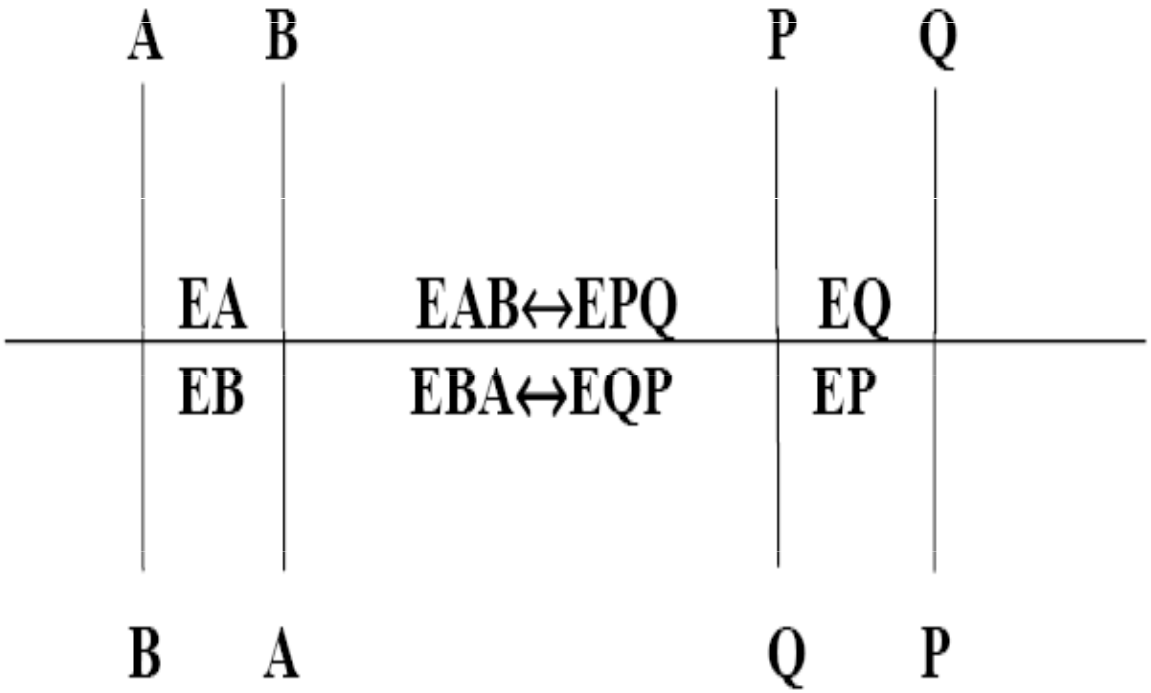
- Přibližná hodnota intracelulární koncentrace substrátu
- Substrát s nižší hodnotou Km je pravděpodobně fyziologický
- Hodnotu lze ovlivňovat – možnost regulace
- Srovnání enzymů
- Stanovení enzymové aktivity

Reakce se dvěma substráty

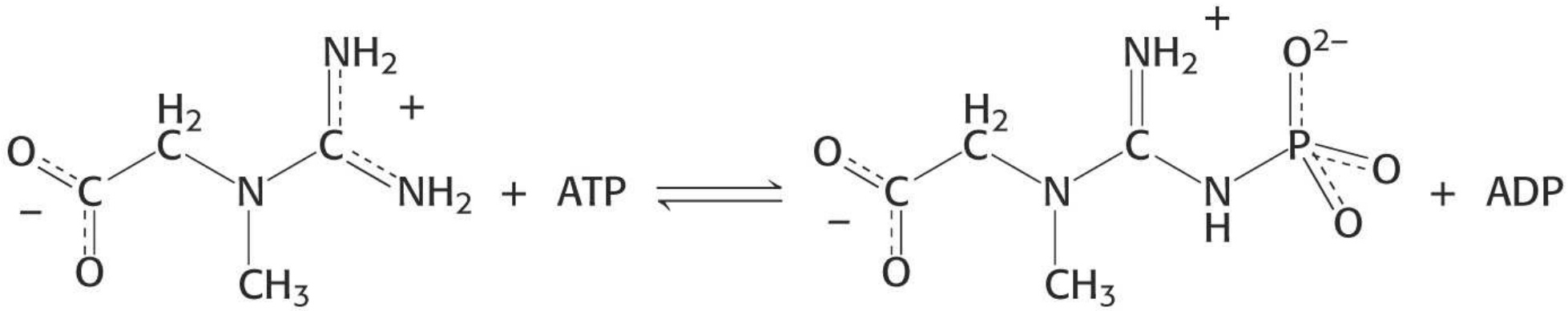
Mechanismy - CLELAND

Sekvenční :

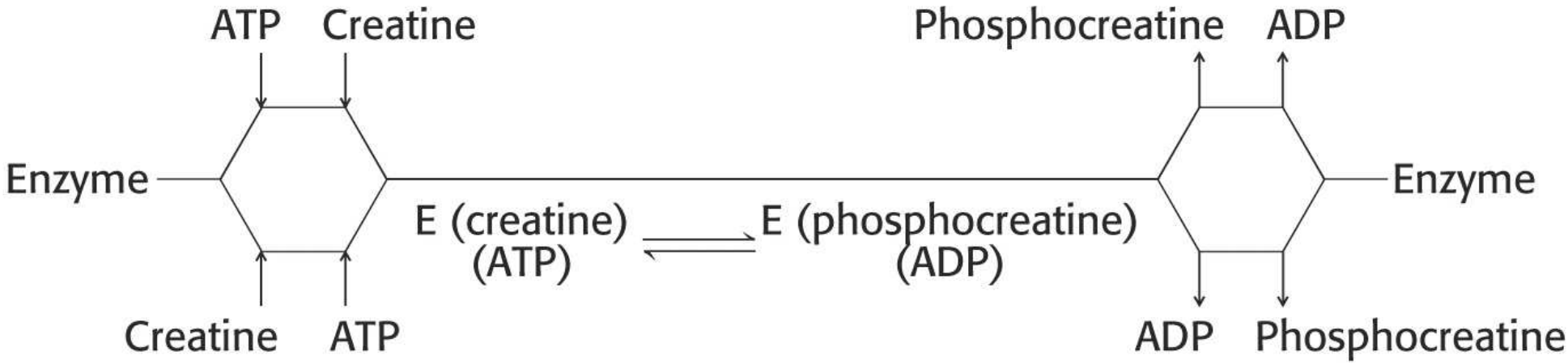
a) *náhodný*



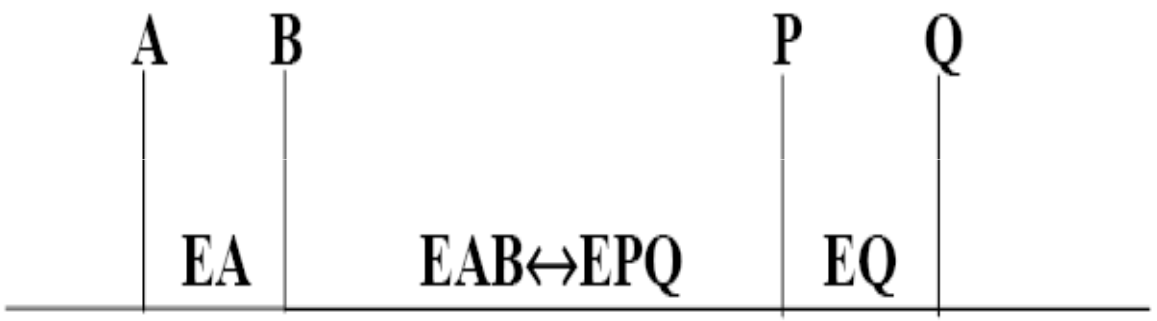
fosforyláza
kreatinkináza



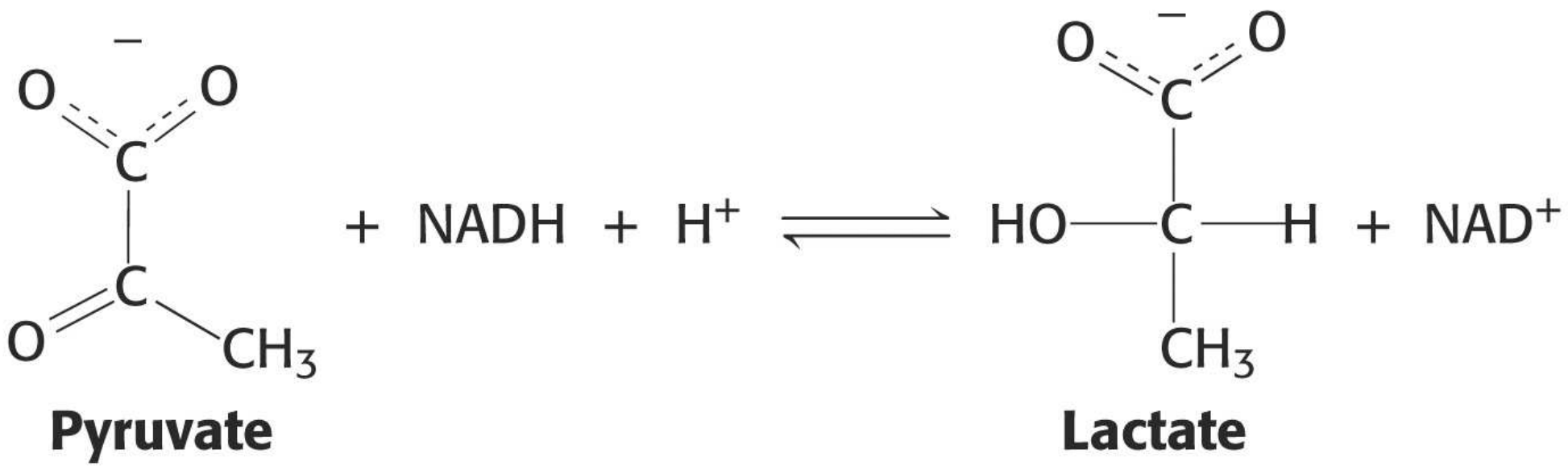
Creatine **Phosphocreatine**

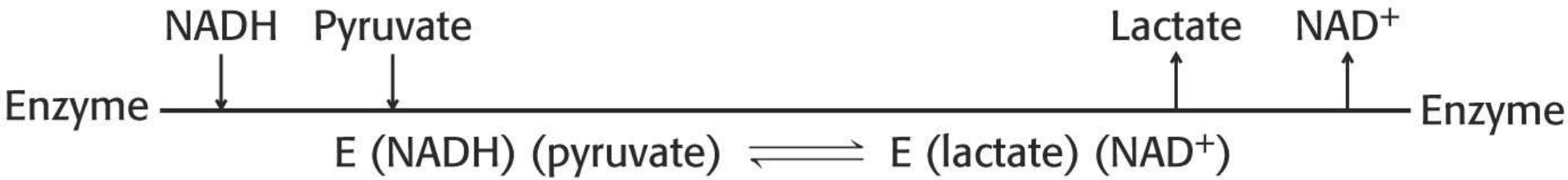

ATP
Creatine
Phosphocreatine
ADP
Enzyme
Enzyme
E (creatine)
(ATP)
E (phosphocreatine)
(ADP)
Creatine
ATP
ADP
Phosphocreatine

b) *uspořádaný*

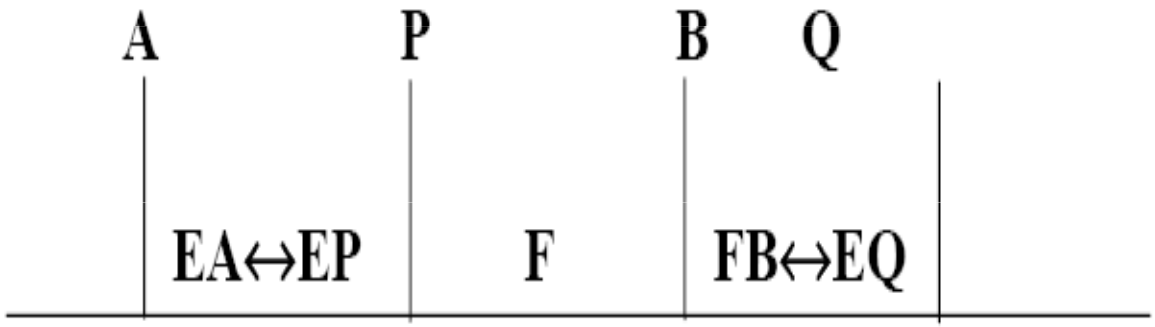


Laktáthydrogenáza
A – NADH, B – Pyr)

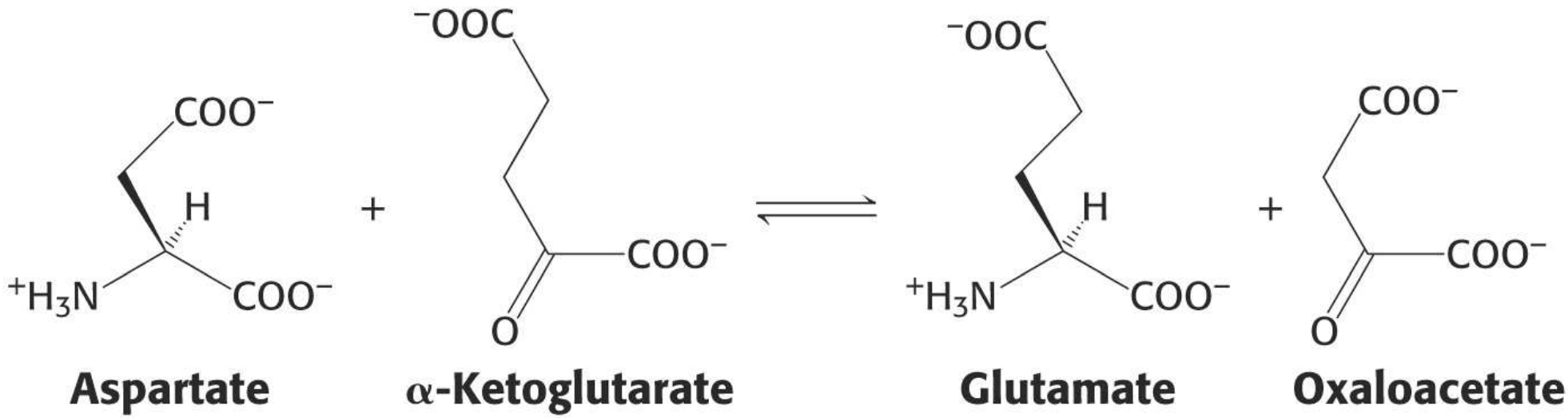

O
O
C
C
O
CH3
+ NADH + H+
HO
C
H
+ NAD+
Pyruvate
Lactate


NADH
Pyruvate
Lactate
NAD+
Enzyme
Enzyme
E (NADH) (pyruvate)
E (lactate) (NAD+)

Pingpongový

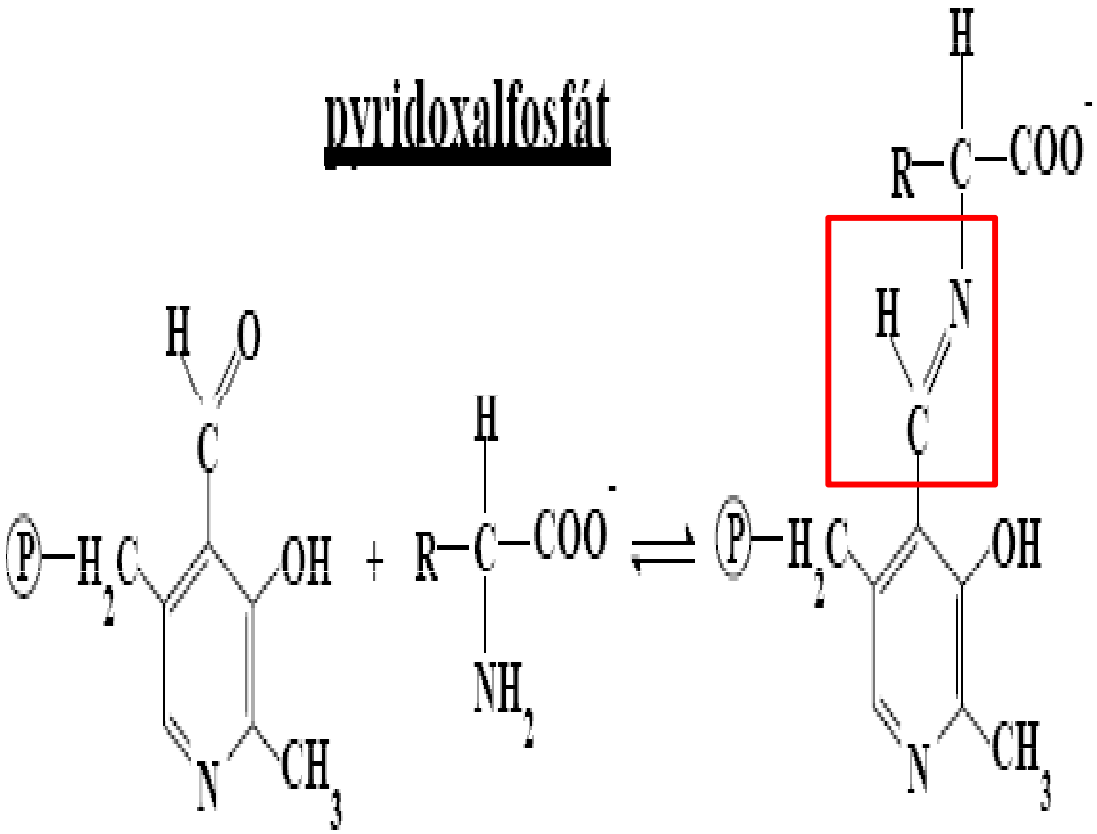


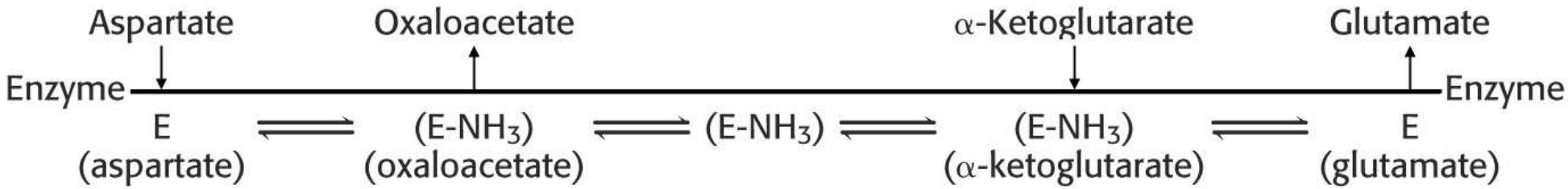
Transaminázy
A-AMK, B-OxoK



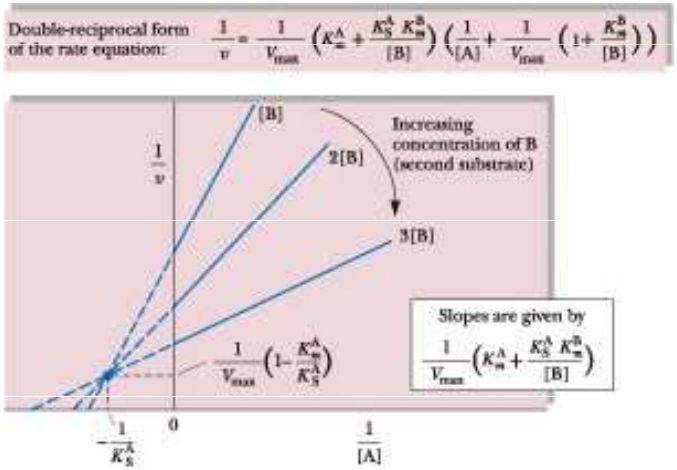
Aspartate + **α-Ketoglutarate** ⇌ **Glutamate** + **Oxaloacetate**

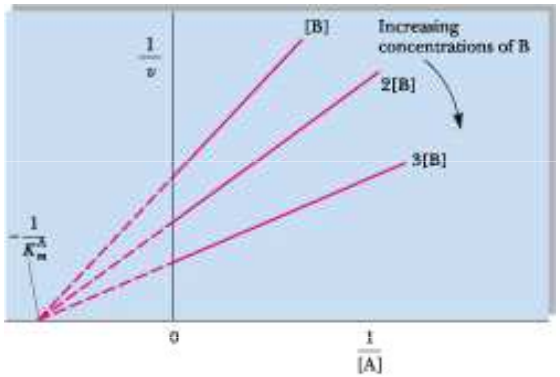
pyridoxalfosfát



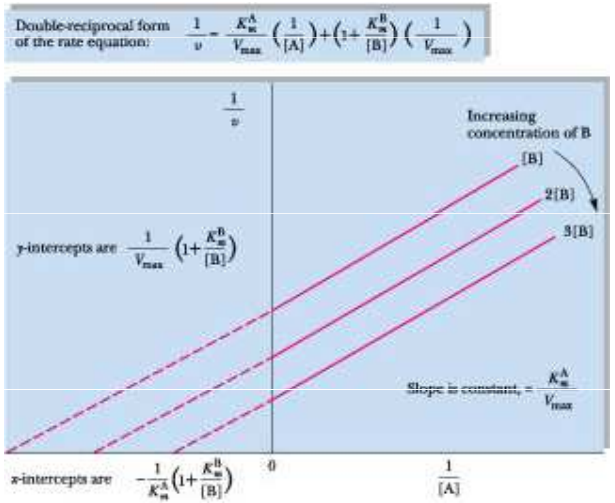

Aspartate
Oxaloacetate
α-Ketoglutarate
Glutamate
Enzyme
Enzyme
E
(aspartate)
(E-NH3)
(oxaloacetate)
(E-NH3)
(E-NH3)
(α-ketoglutarate)
E
(glutamate)

Sekvenční



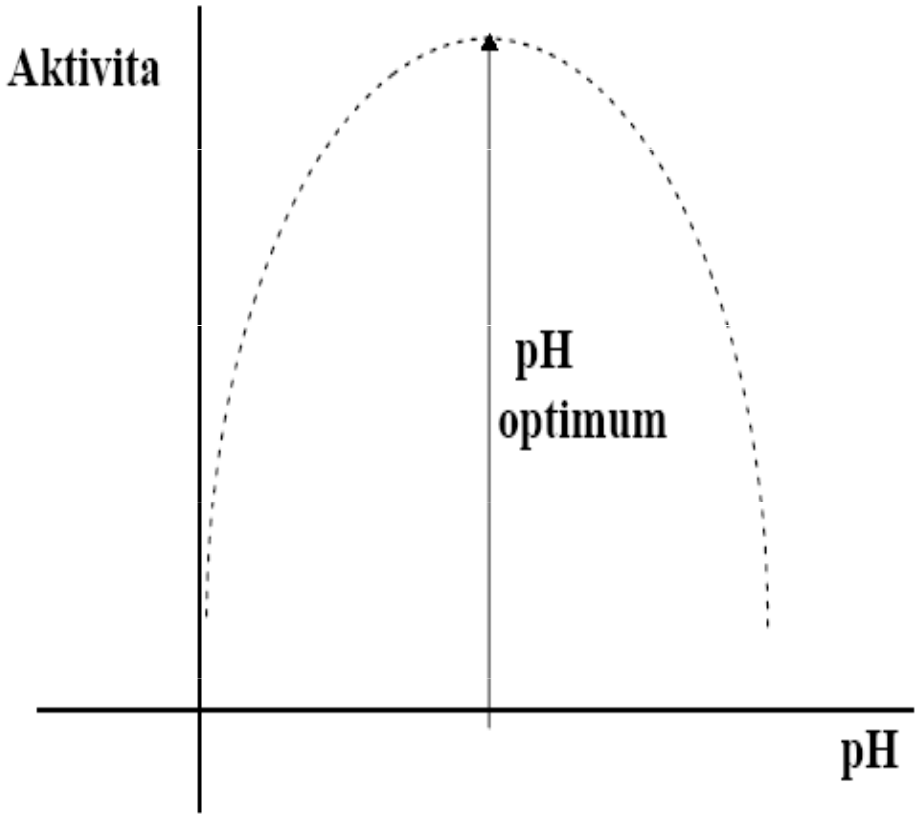


Ping-pongový



Fyzikálně chemické faktory ovlivňující rychlost enzymové reakce

Vliv pH



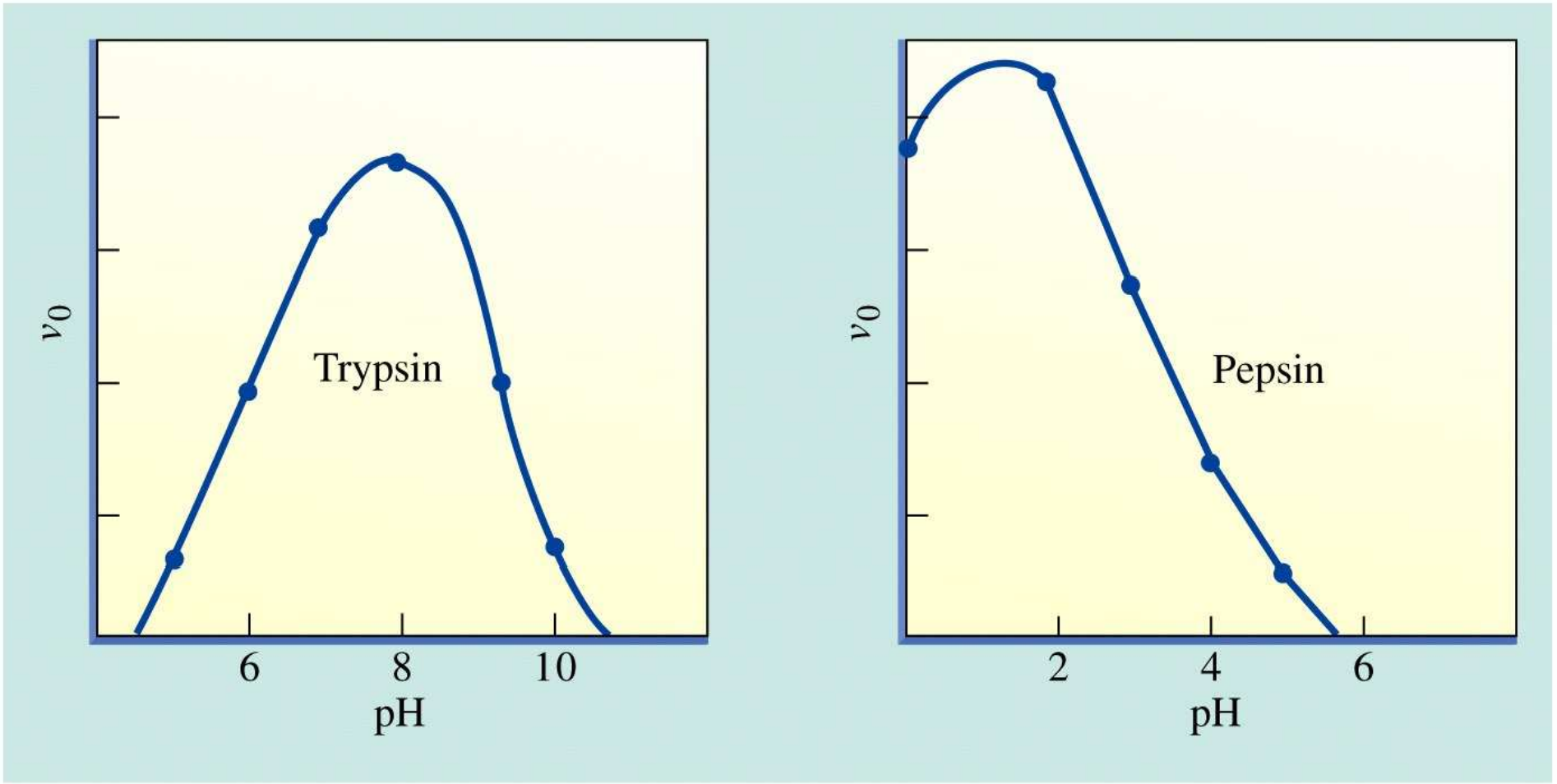
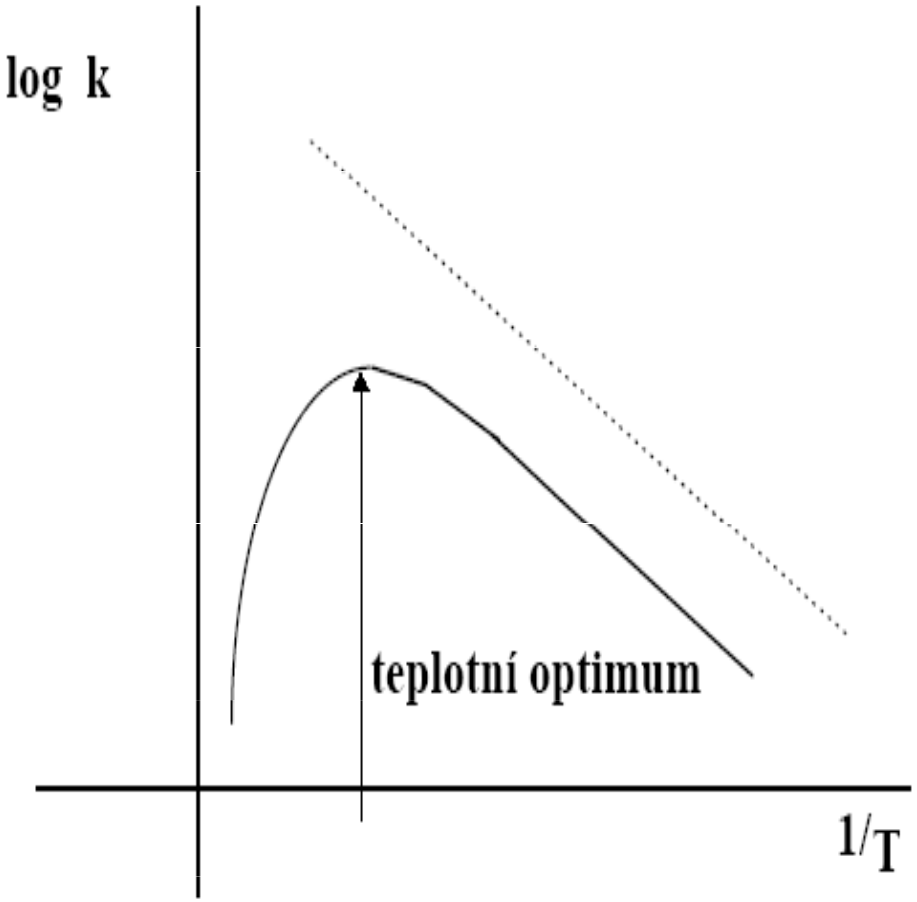


Figure 5-6 Concepts in Biochemistry, 3/e

Vliv teploty



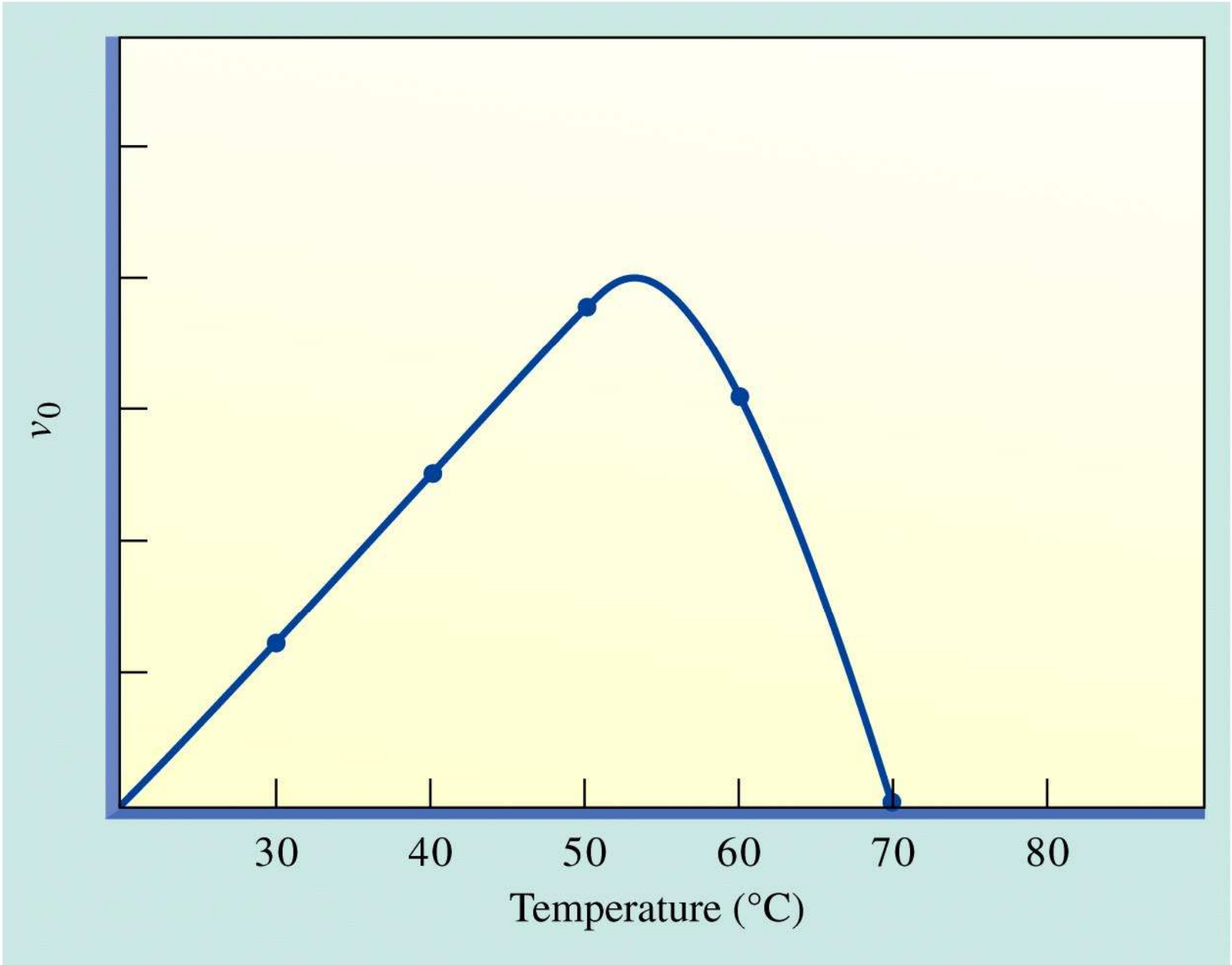
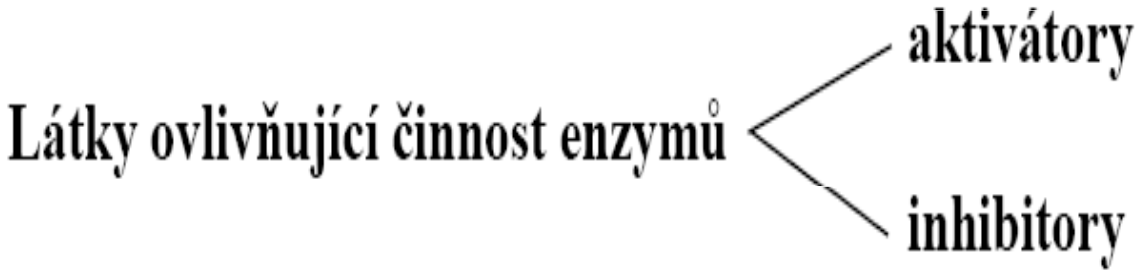


Figure 5-7 Concepts in Biochemistry, 3/e

Látky ovlivňující činnost enzymů



Aktivátory - zvyšují rychlost enzymové reakce

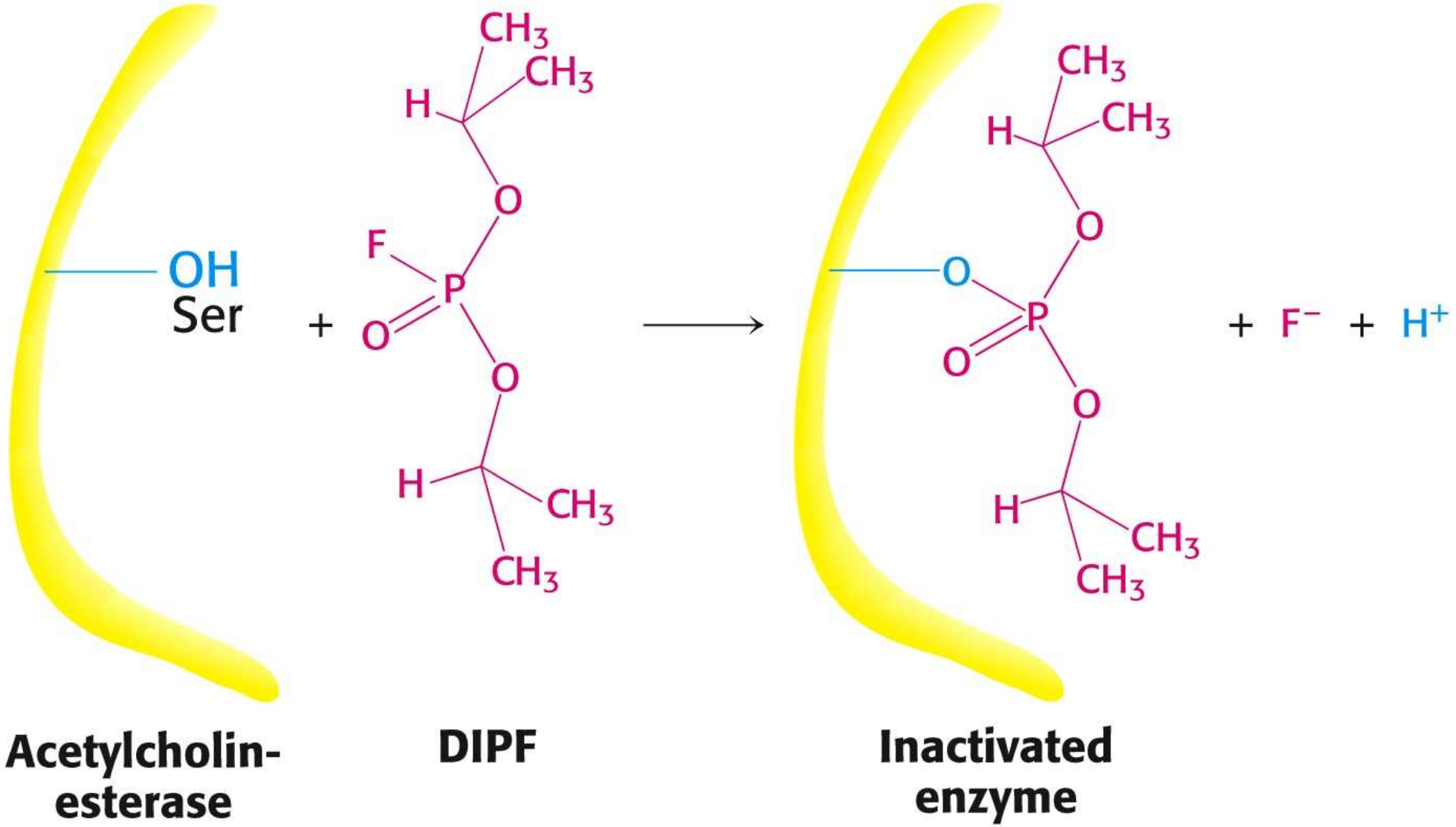
Inhibitory - snižují rychlost enzymové reakce

Inhibice

- Ireverzibilní inhibice

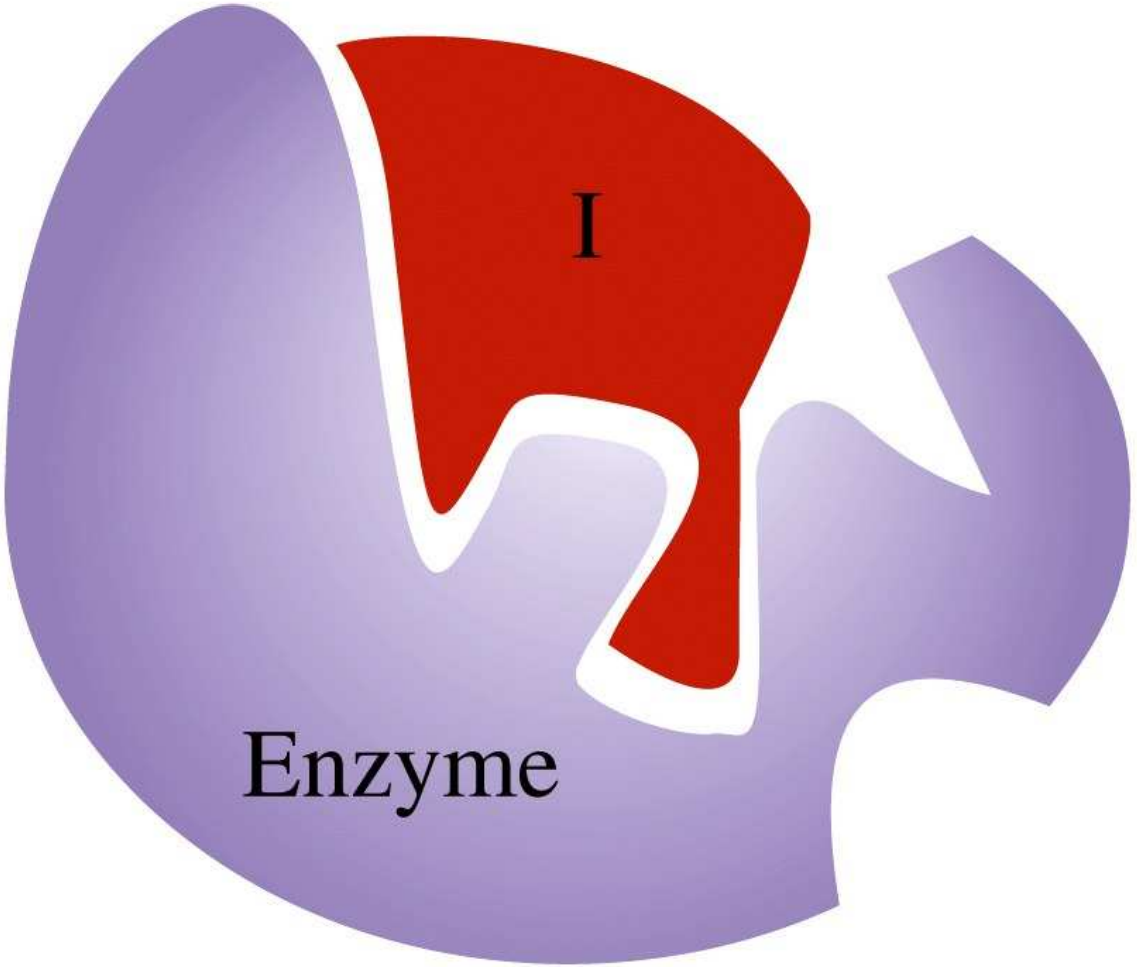
$$E + I \rightarrow EI$$

Inaktivace Ser diisopropylfosfofluoridem **(DIPF)**



- **Reverzibilní inhibice**

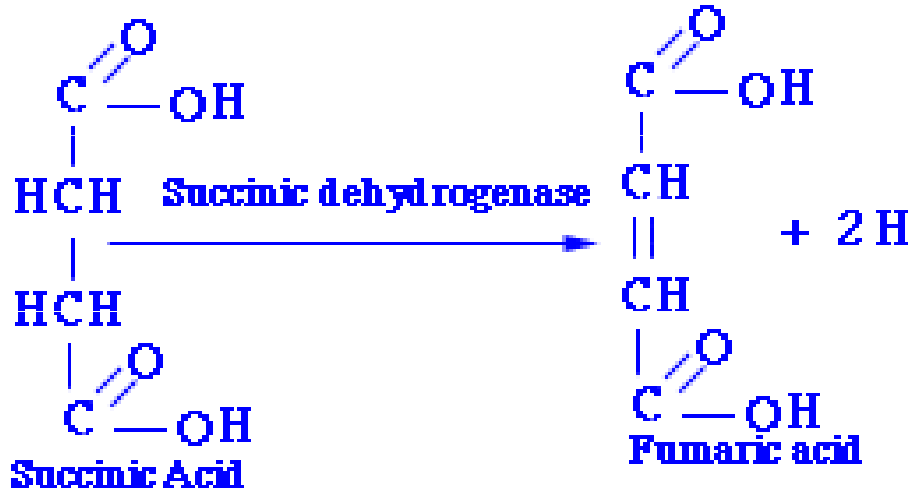
Kompetitivní inhibice

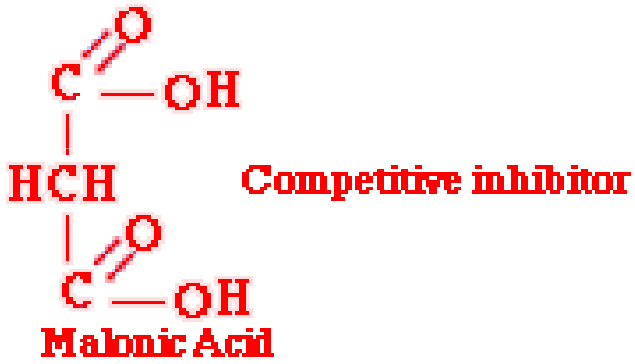


Competitive (inhibitor binds in active site)

Figure 5-15b Concepts in Biochemistry, 3/e

Inhibice SDH malátem





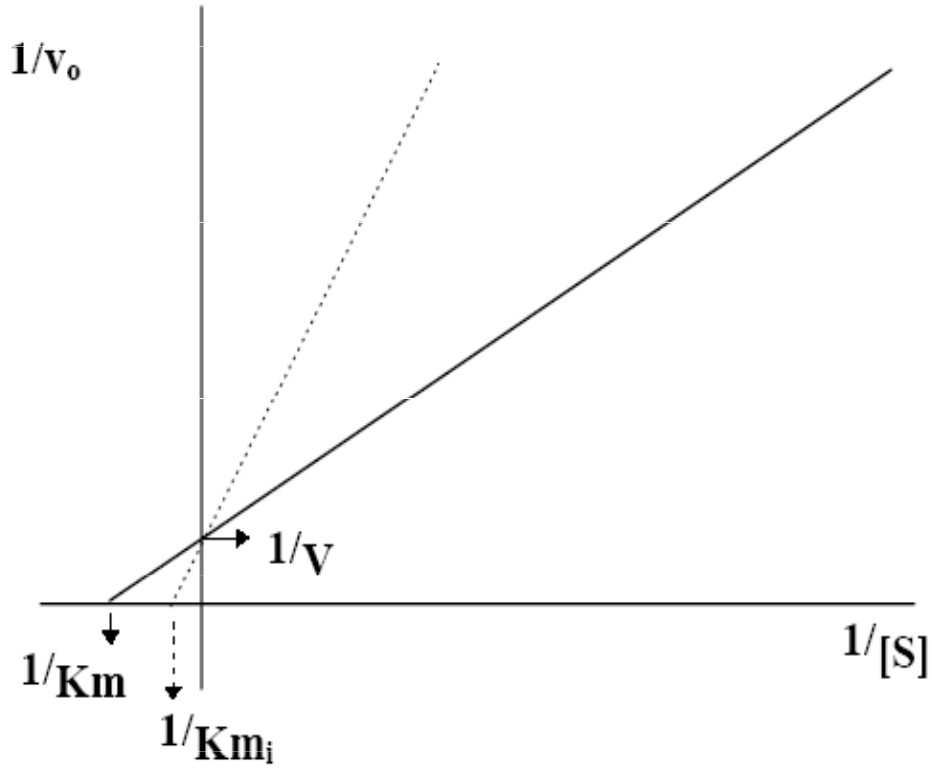
$$E + I \longrightarrow EI$$

$$K_i = [E][I]/[EI]$$

- **Reverzibilní inhibice**

Kompetitivní inhibice

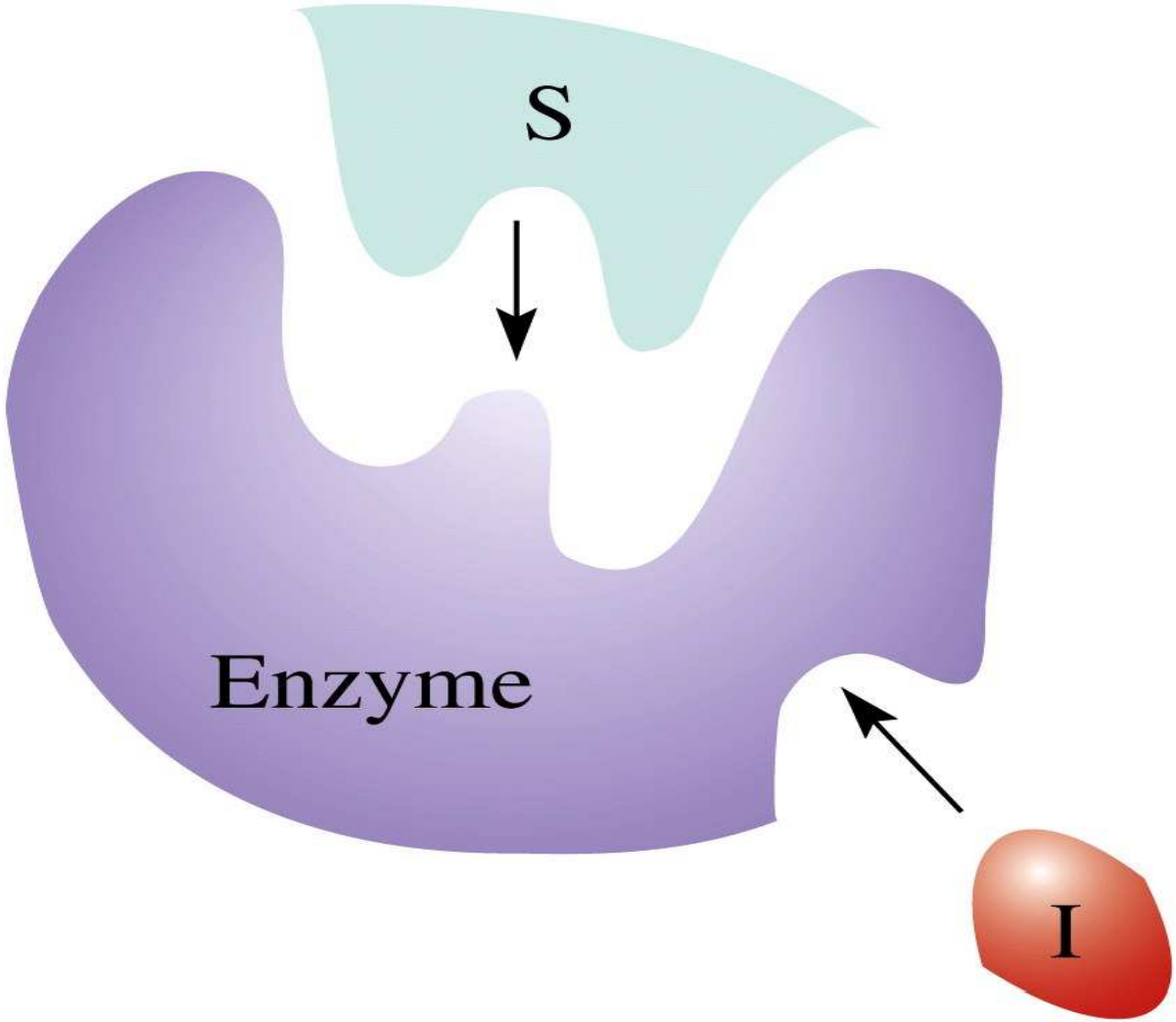
$$E + I \leftrightarrow EI$$



$$Km_i > Km \quad V_i = V$$

Nekompetitivní inhibice

$$E + I \leftrightarrow EI \qquad EA + I \leftrightarrow EAI$$

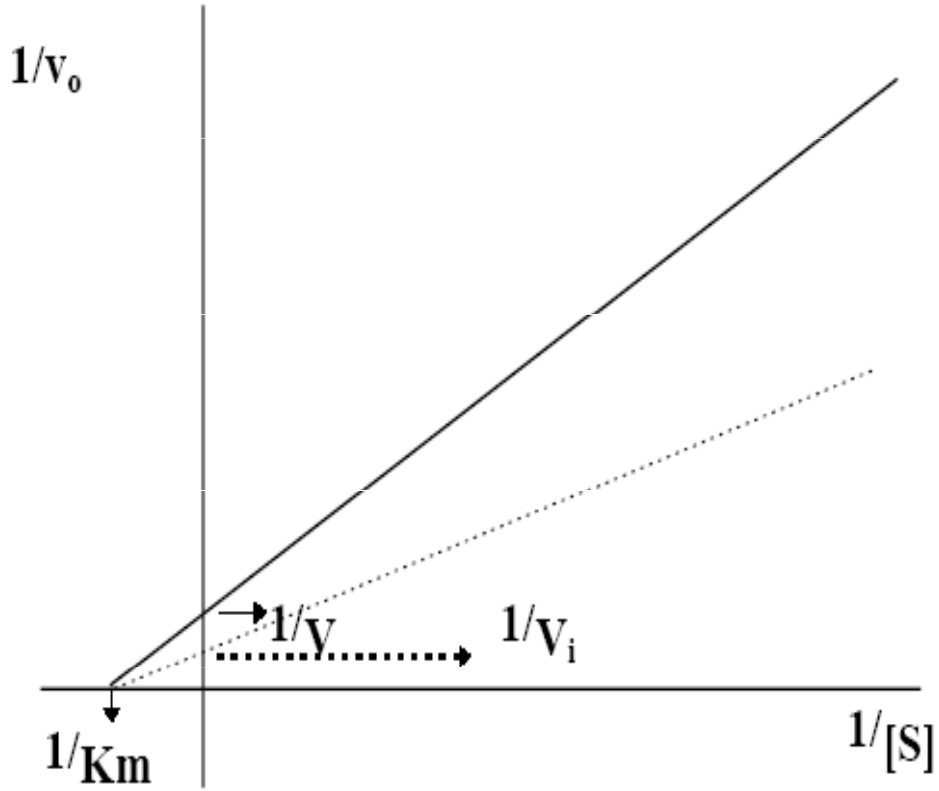


Noncompetitive (inhibitor binds at another site)

Figure 5-15c Concepts in Biochemistry, 3/e

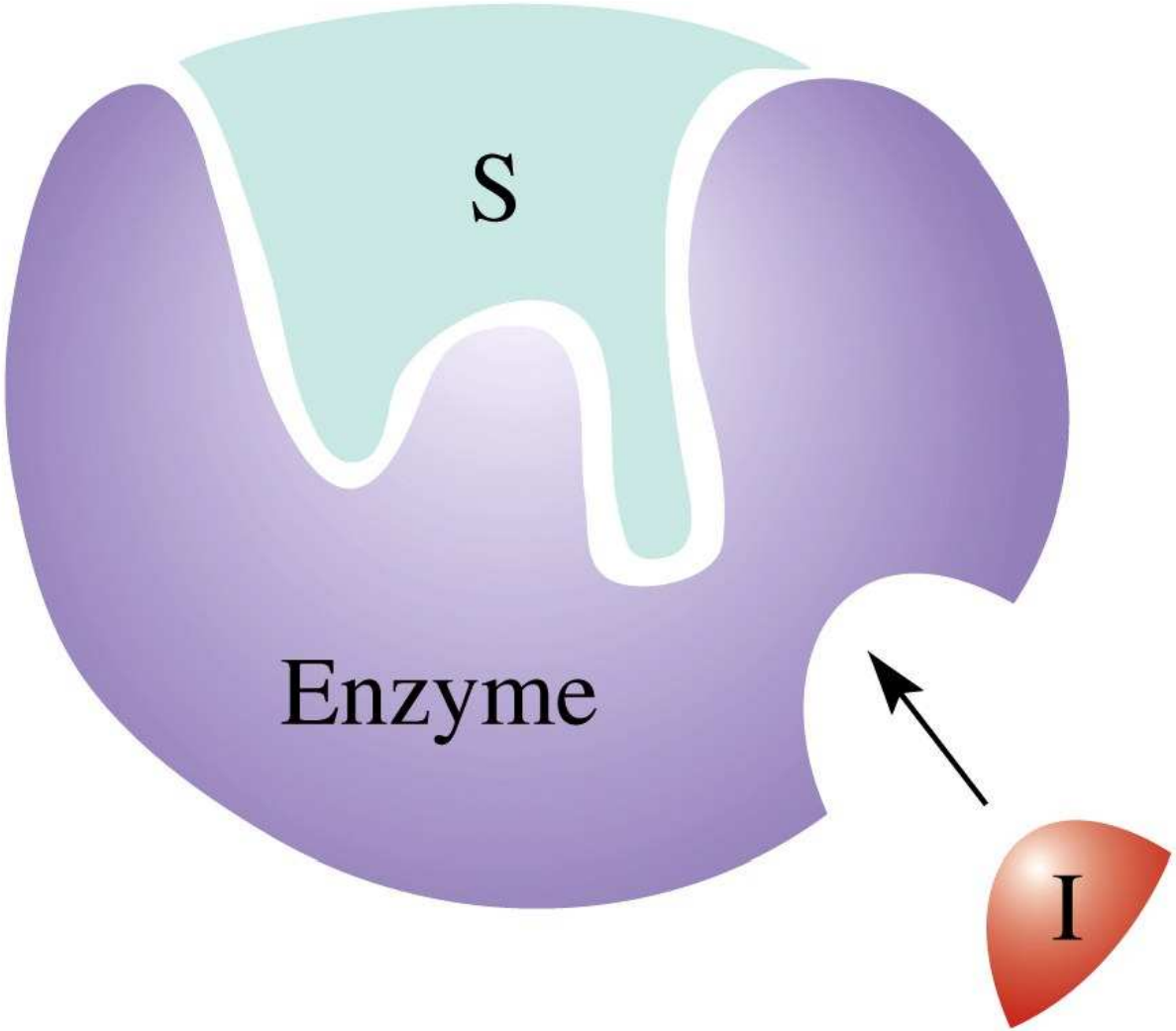
Nekompetitivní inhibice

$$E + I \leftrightarrow EI \qquad EA + I \leftrightarrow EAI$$



$$Km_i = Km \qquad V_i < V$$

Akompetetivní inhibice



Uncompetitive (inhibitor binds after S binding)

Figure 5-15d Concepts in Biochemistry, 3/e

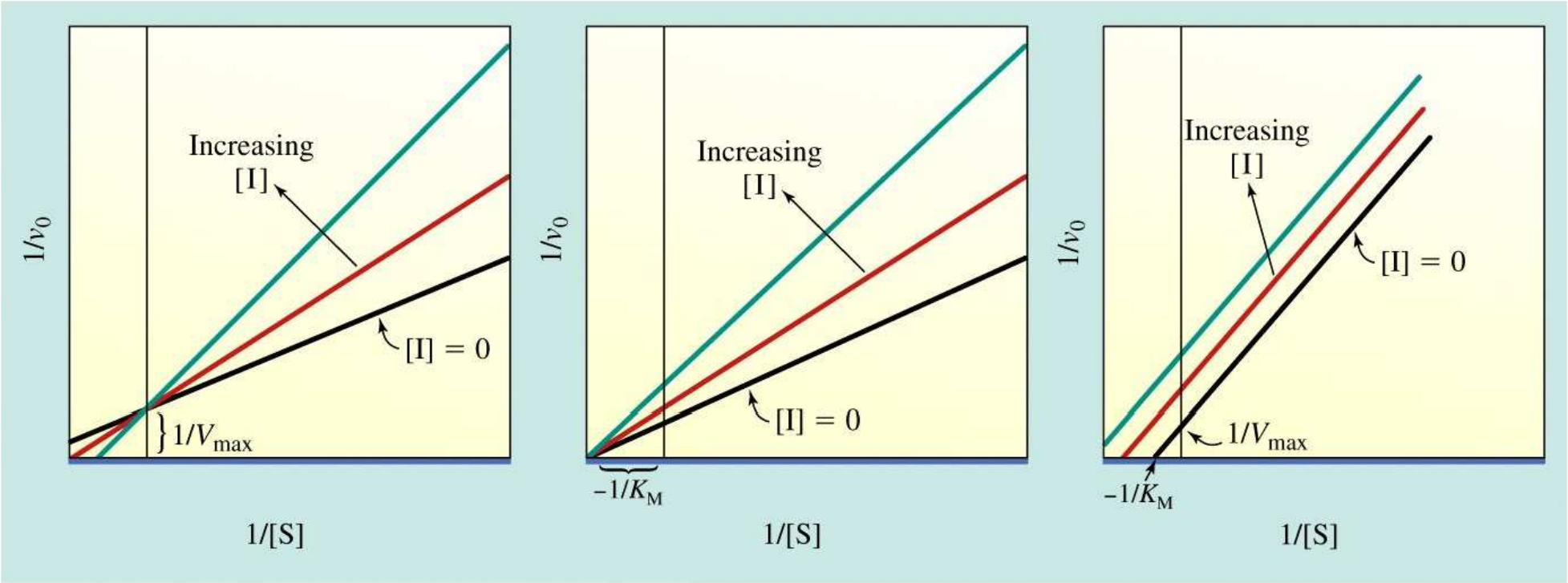
Table 5.5

Kinetic characteristics of reversible inhibition

	Effect of Inhibition[a]		
Type of Inhibition	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.

Table 5-5 Concepts in Biochemistry, 3/e



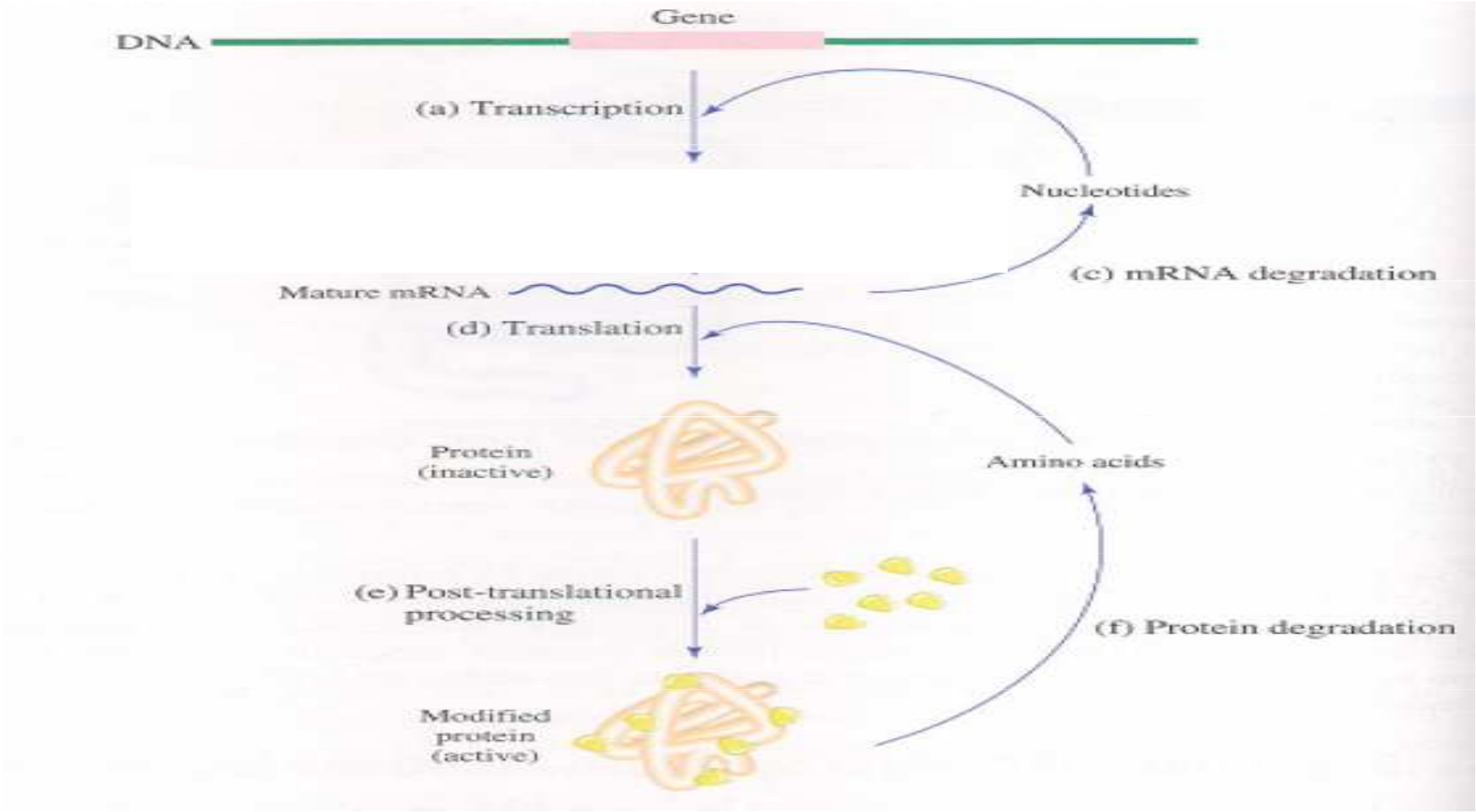
(a) Competitive inhibition (b) Noncompetitive inhibition (c) Uncompetitive inhibition

Figure 5-18 Concepts in Biochemistry, 3/e

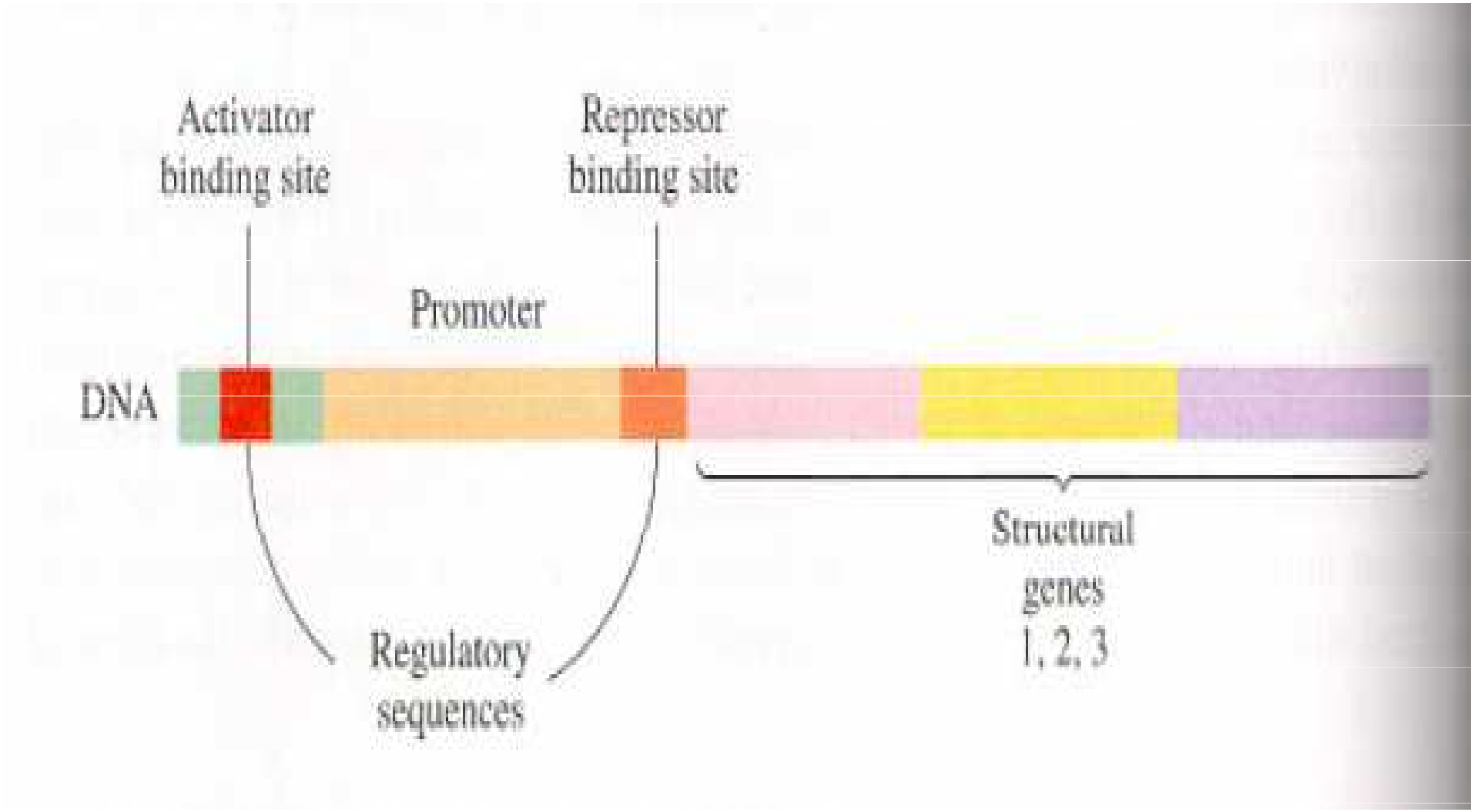
Regulace činnosti enzymu

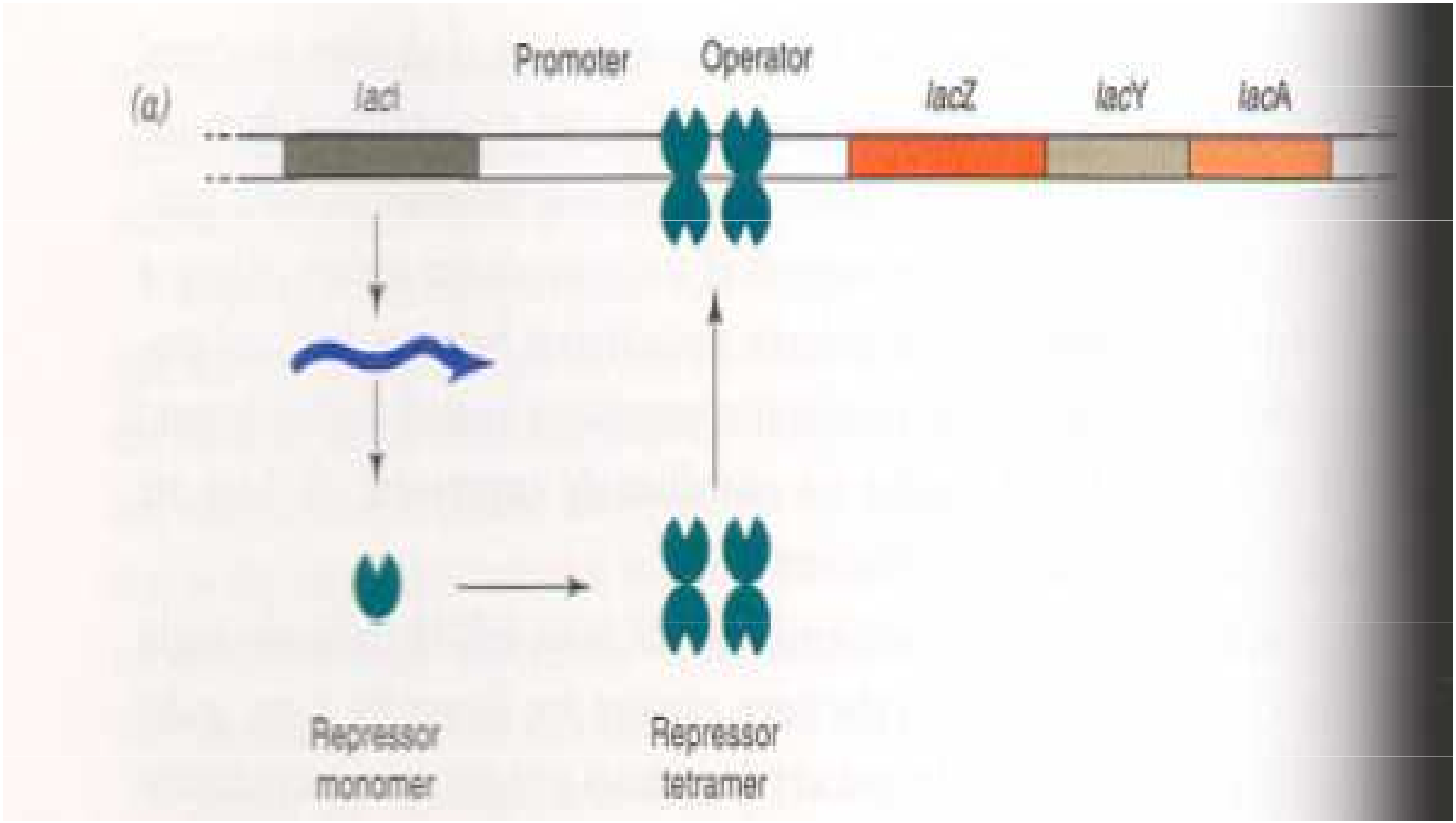
- Regulace koncentrace enzymu
- Allosterická regulace MONOD 1963
- Regulace zpětnou vazbou
- Regulace kovalentní modifikací
- Kompartmentace

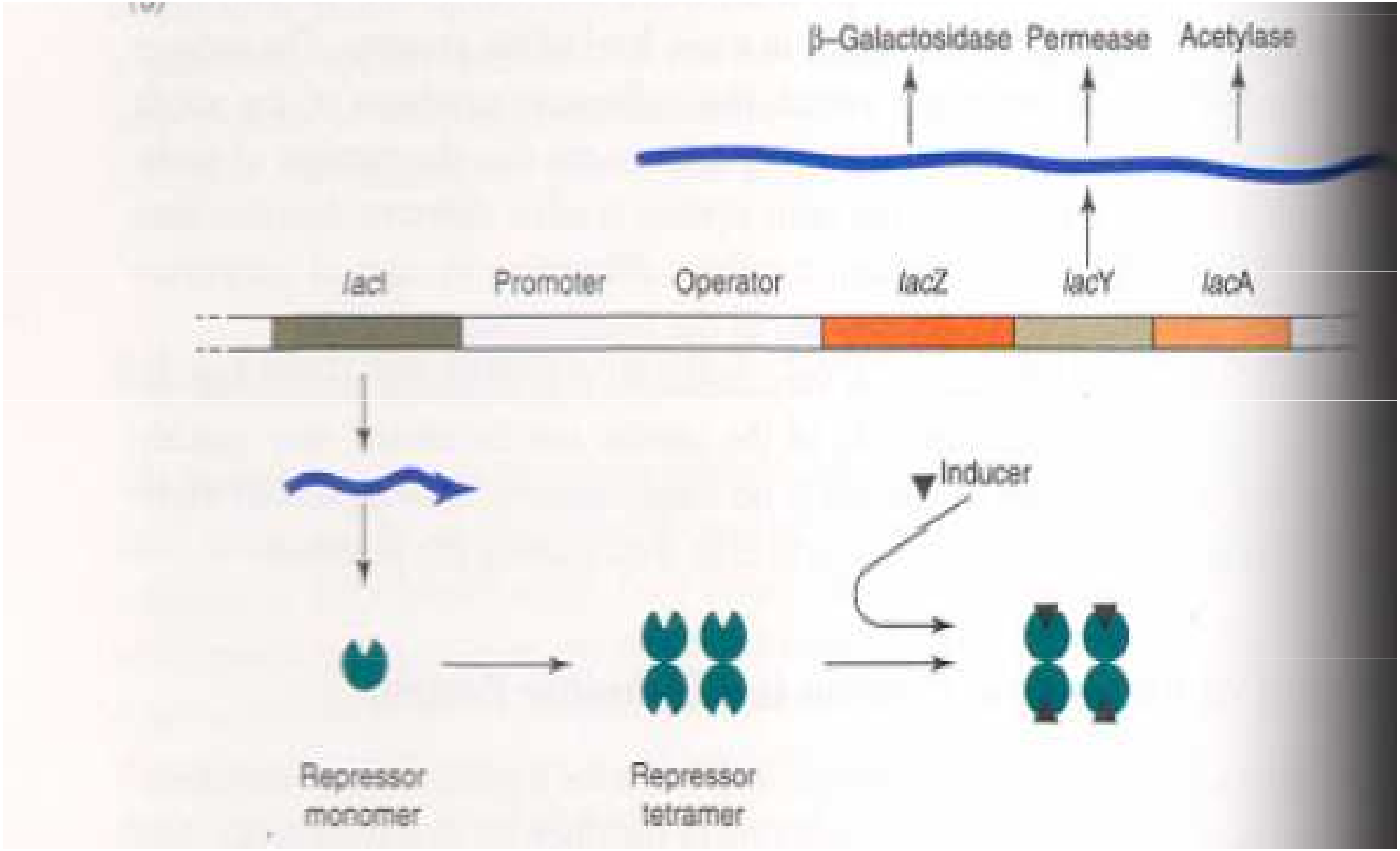
Regulace koncentrací enzymu



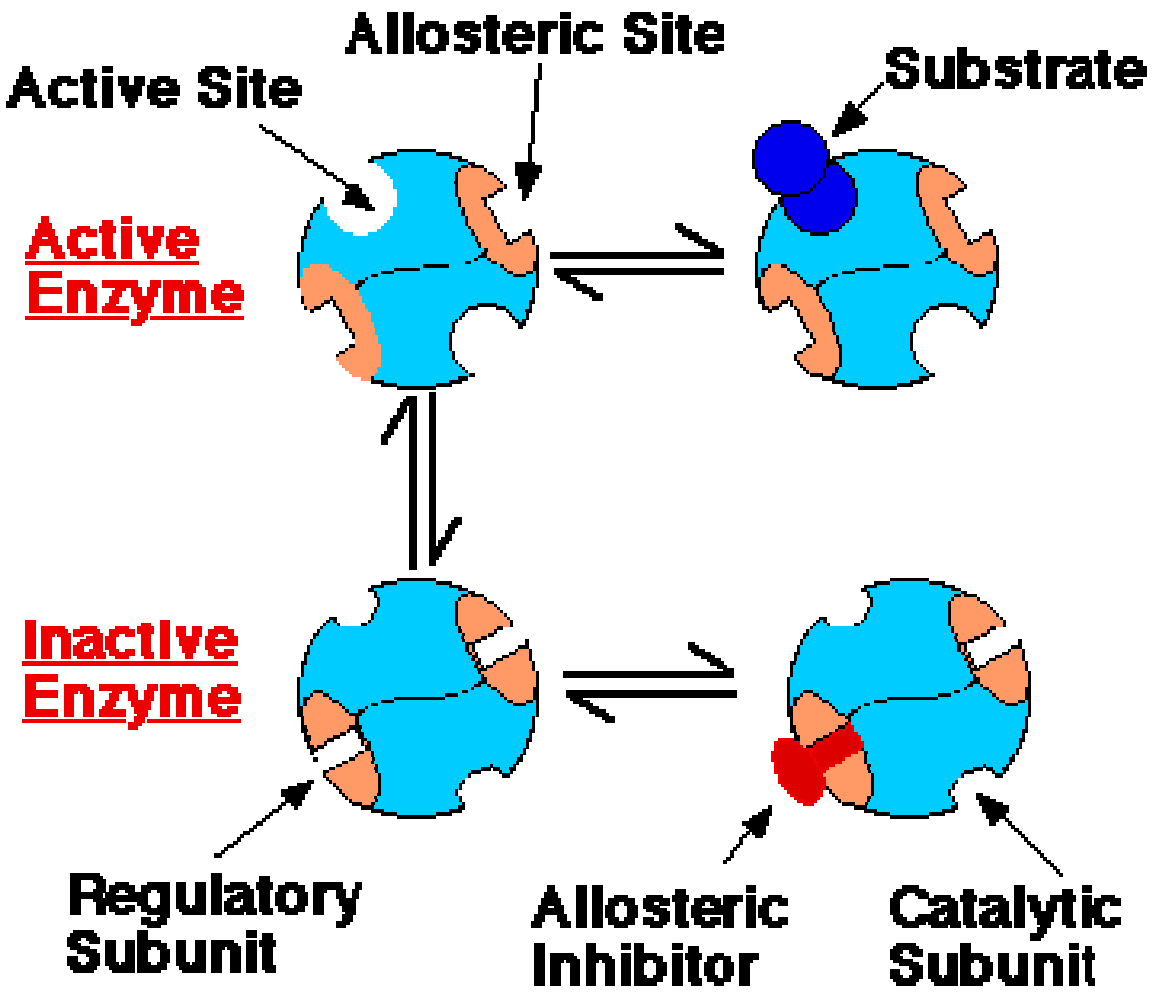
Operonový model



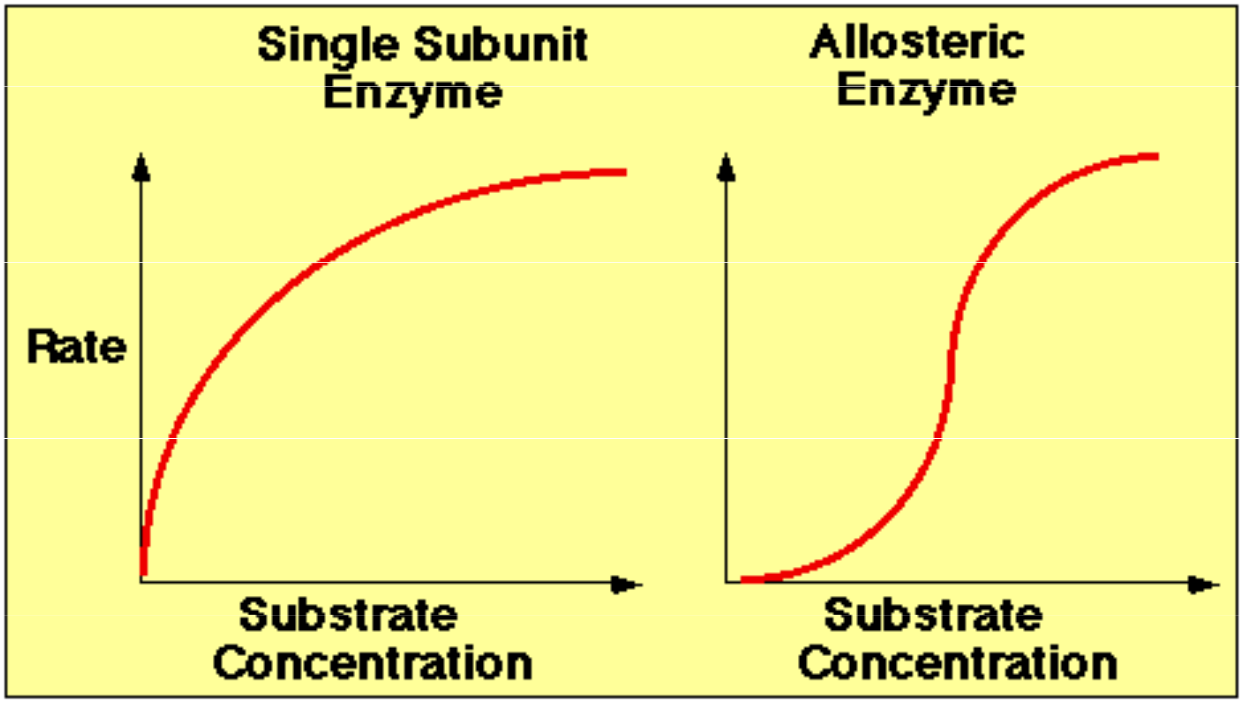

(a)
lacI
Promoter
Operator
lacZ
lacY
lacA
Repressor monomer
Repressor tetramer


β–Galactosidase
Permease
Acetylase
lacI
Promoter
Operator
lacZ
lacY
lacA
Inducer
Repressor monomer
Repressor tetramer

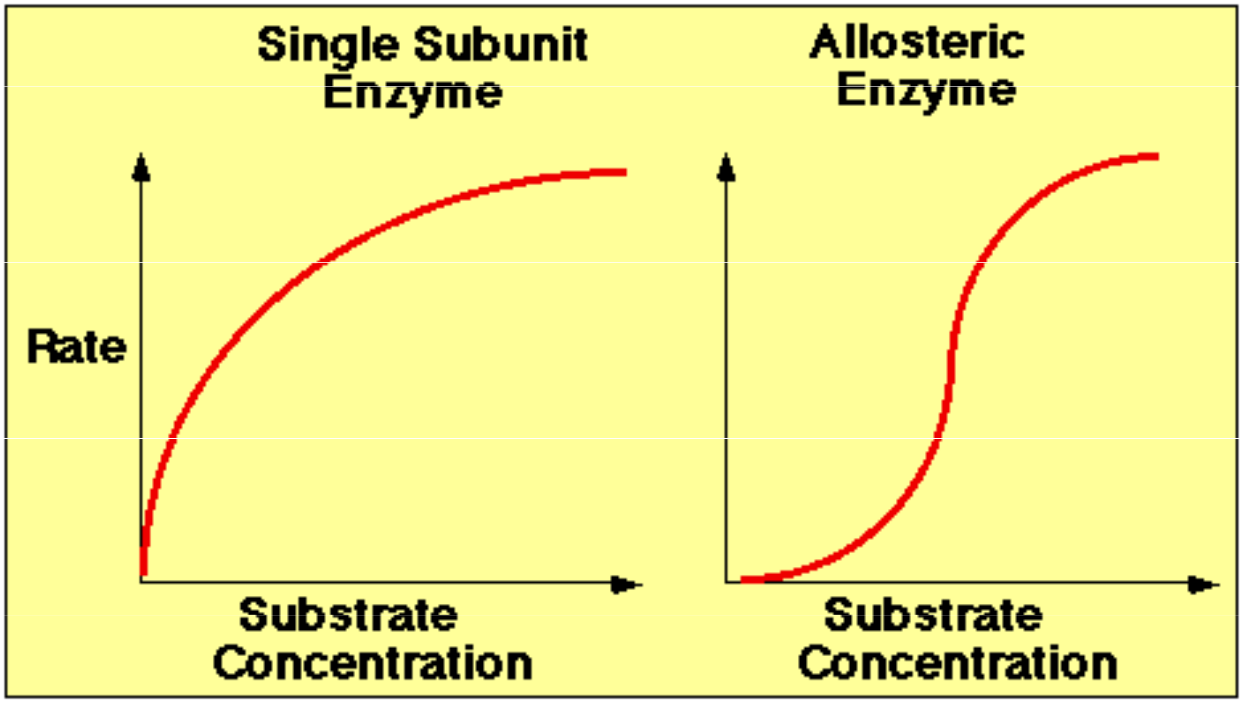
Allosterie


Allosteric Site
Active Site
Substrate
Active Enzyme
Inactive Enzyme
Regulatory Subunit
Allosteric Inhibitor
Catalytic Subunit

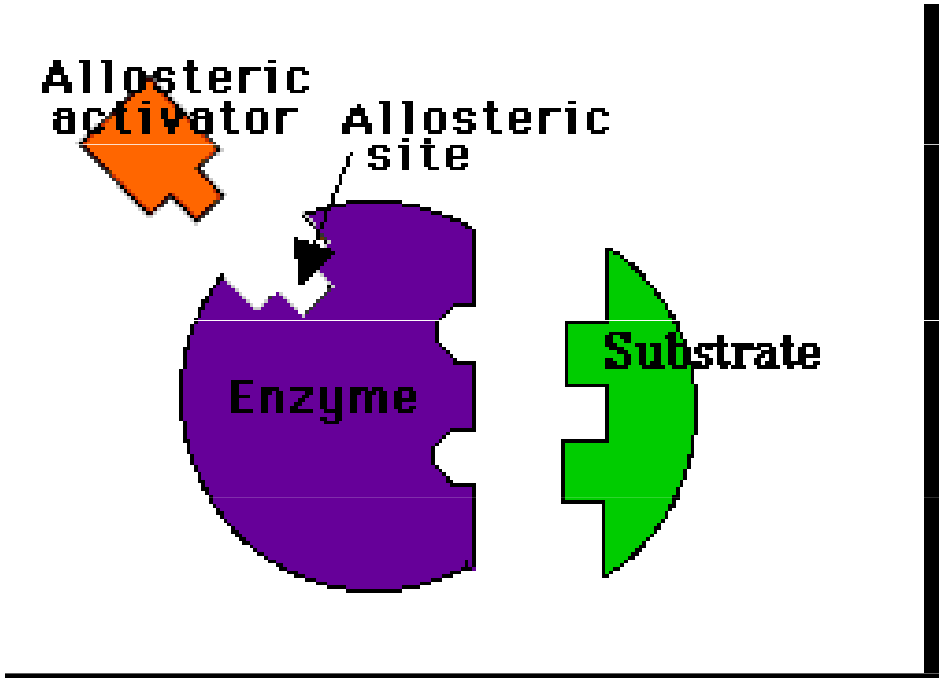
Allosterie


Single Subunit Enzyme
Allosteric Enzyme
Rate
Substrate Concentration
Substrate Concentration

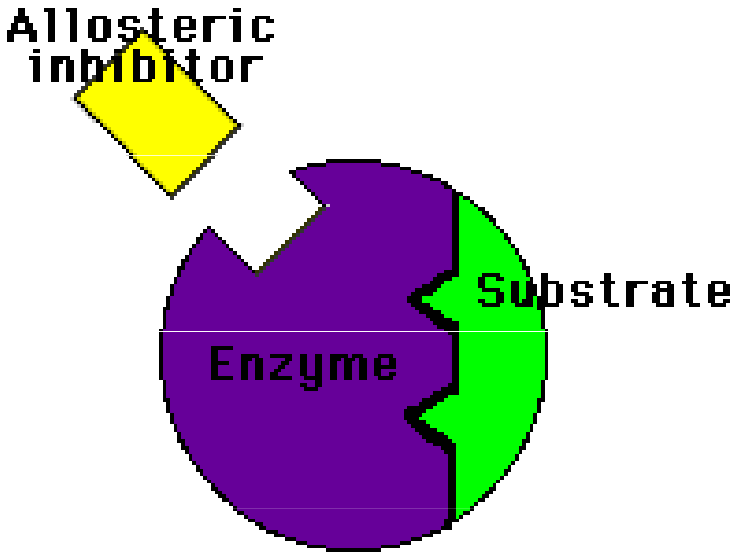
Allosterie


Single Subunit Enzyme
Allosteric Enzyme
Rate
Substrate Concentration
Substrate Concentration

Allosterický aktivátor



Allosterický inhibitor



Symetrický model

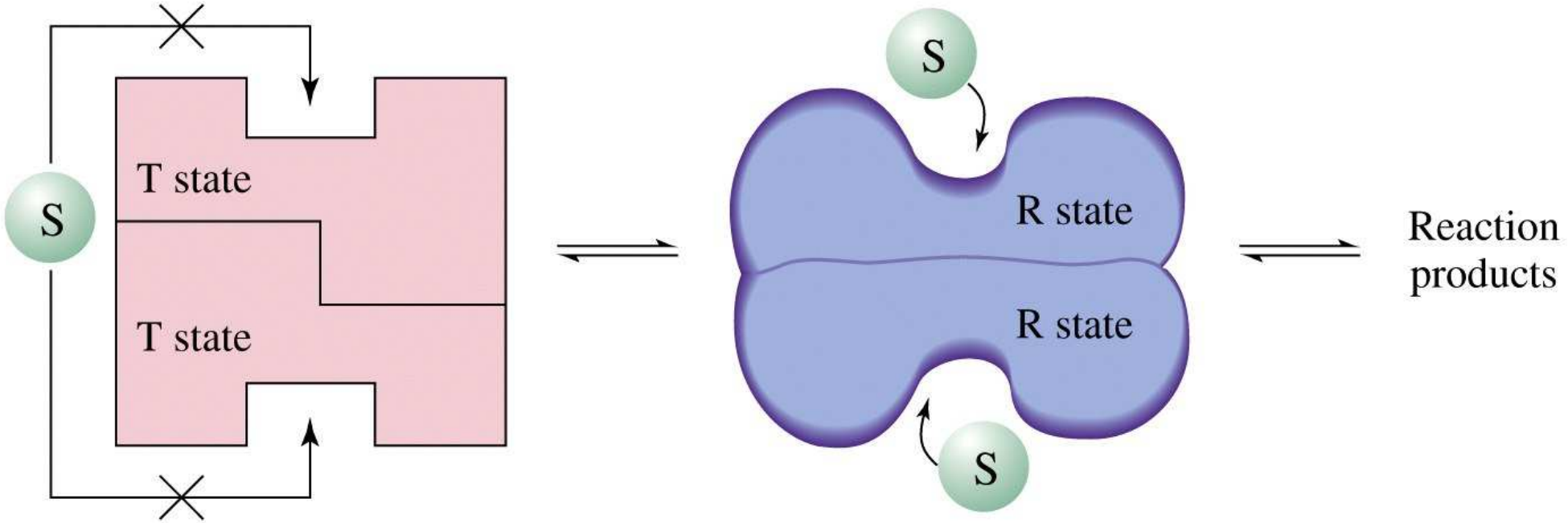


Figure 6-5 Concepts in Biochemistry, 3/e

Sekvenční model

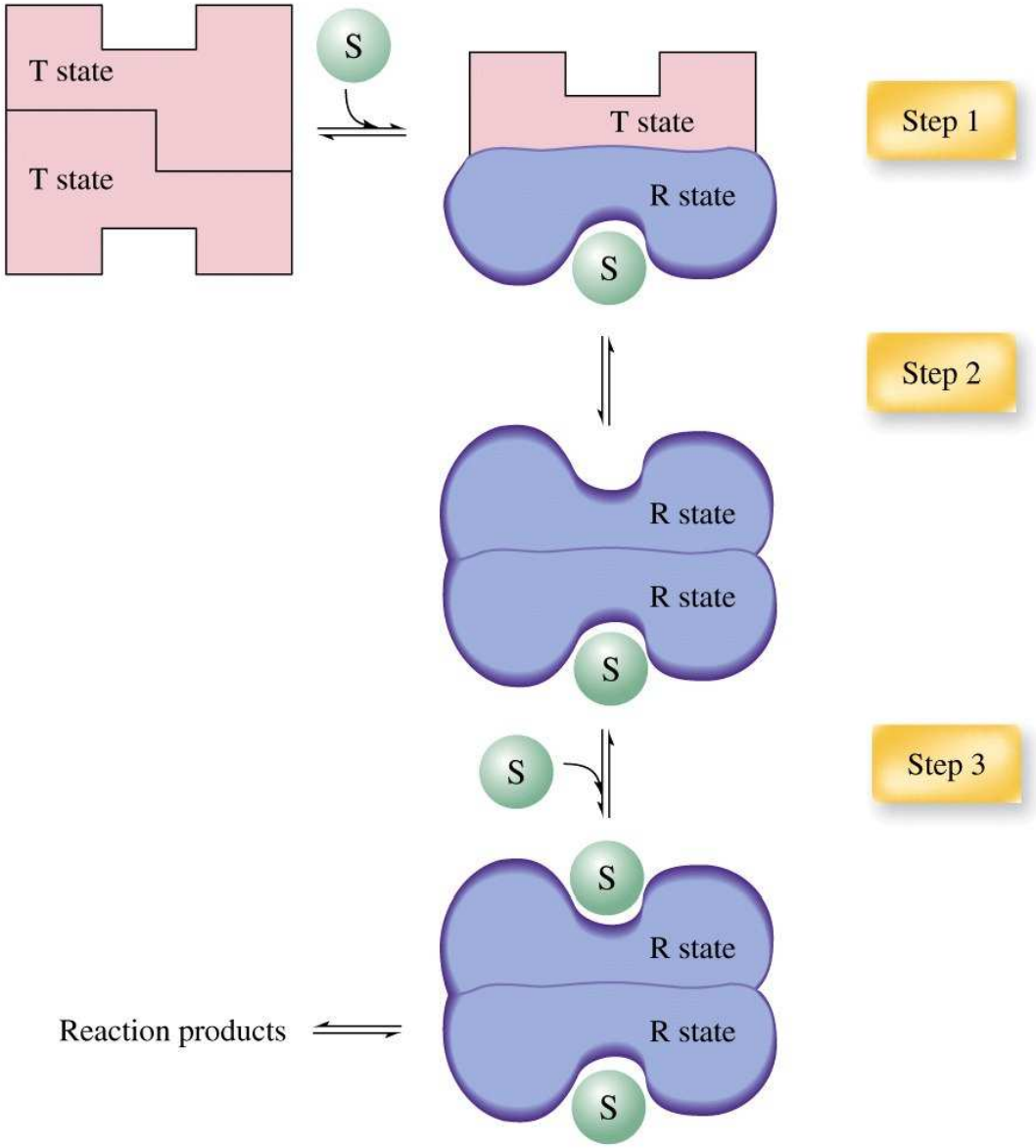
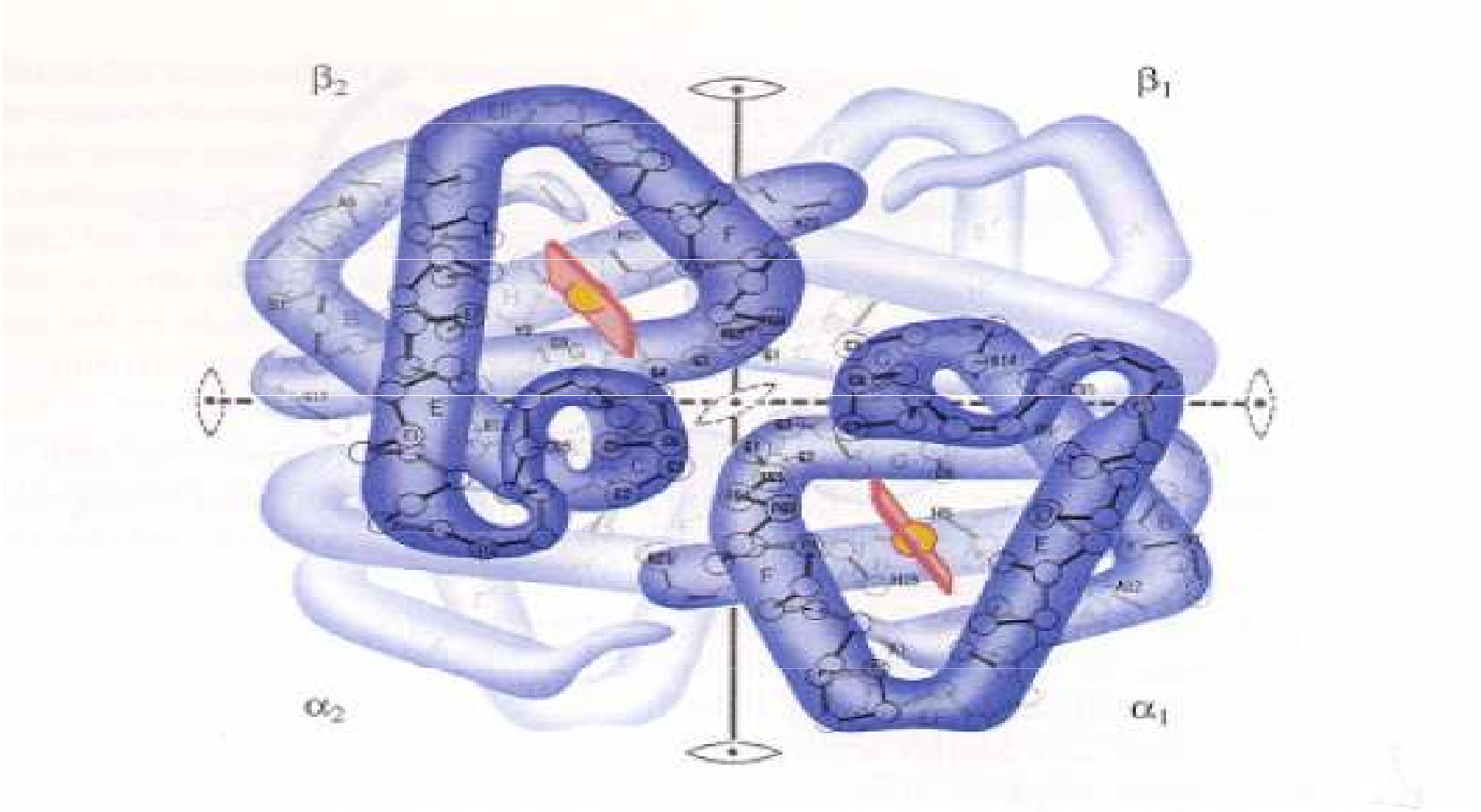
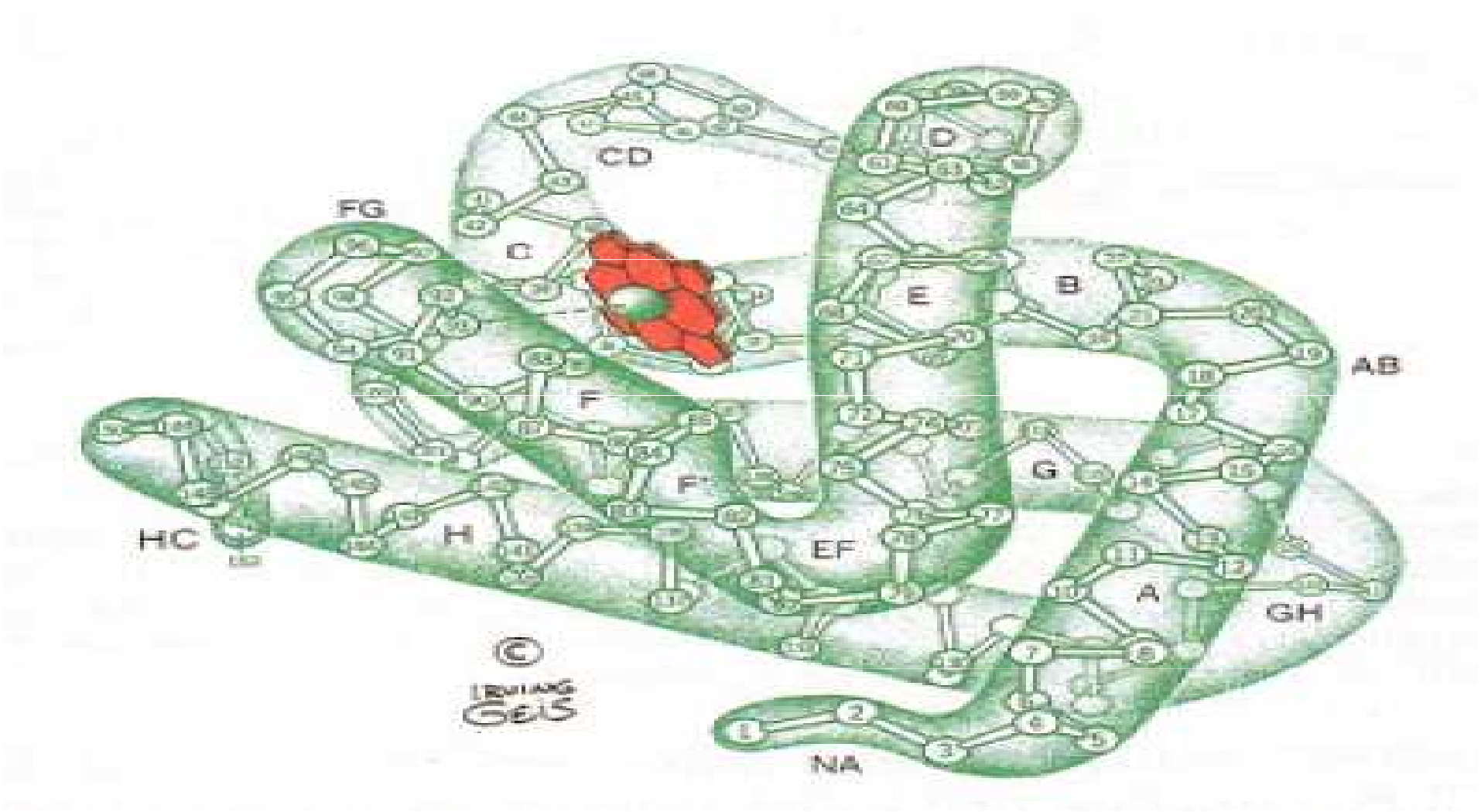


Figure 6-6 Concepts in Biochemistry, 3/e

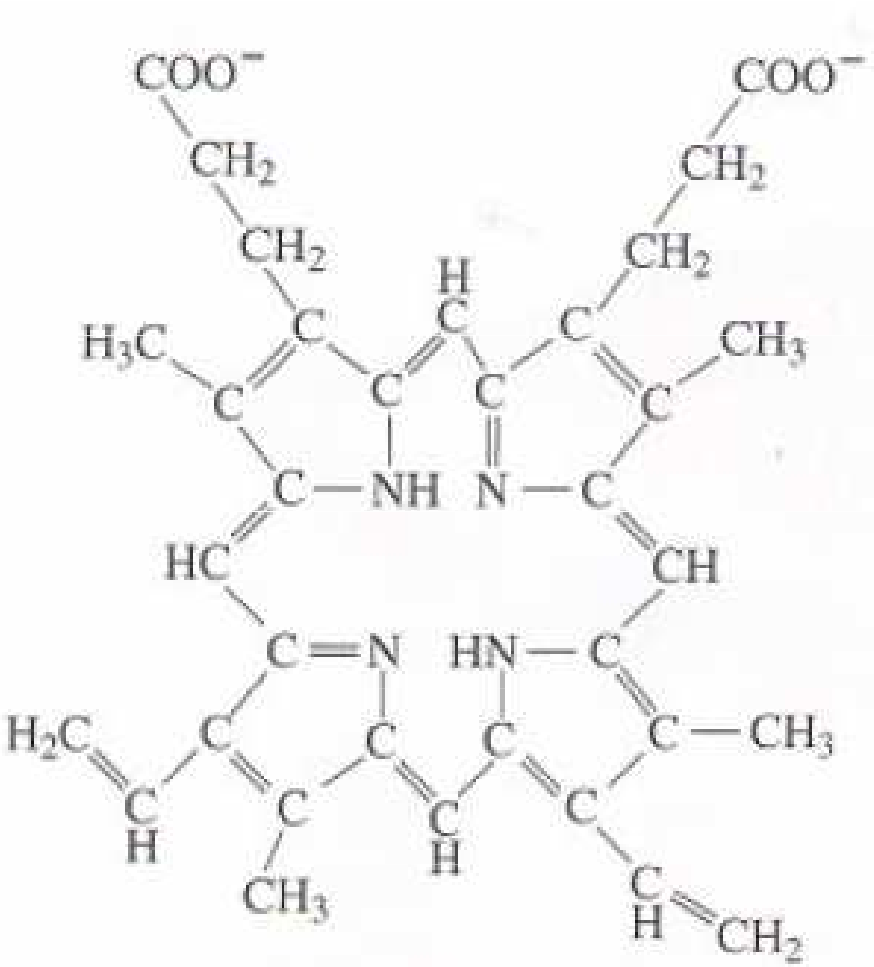
Allosterie hemoglobinu



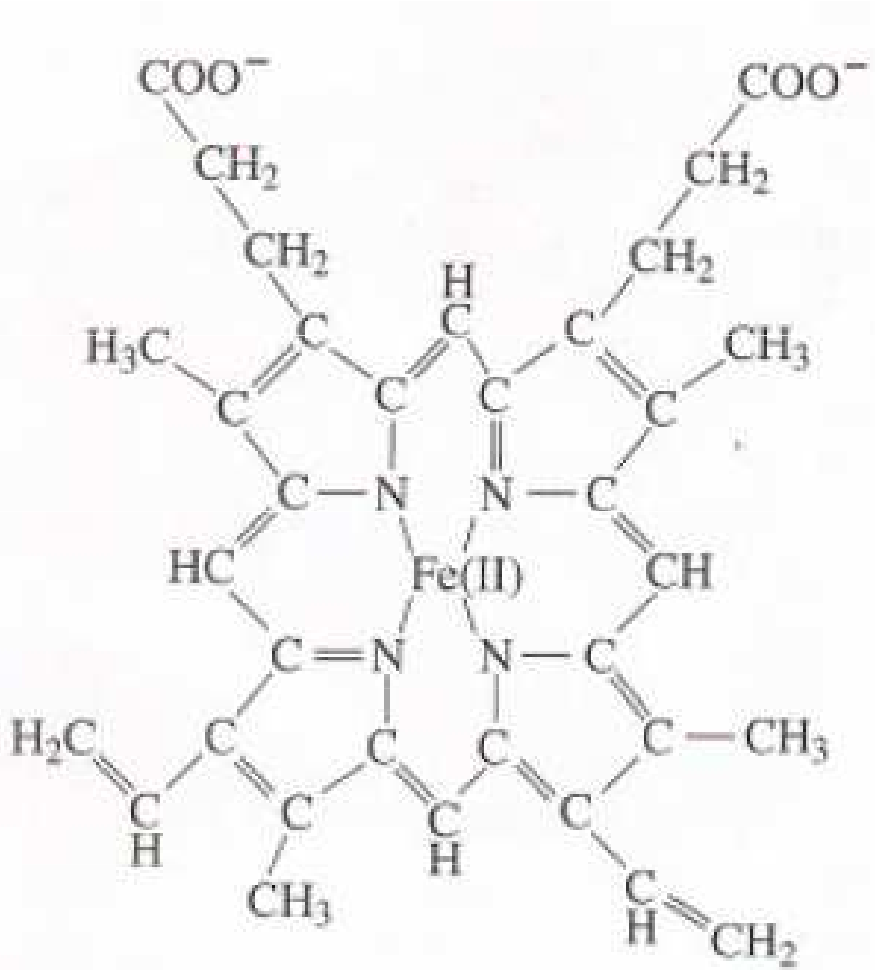
Myoglobin



Hem

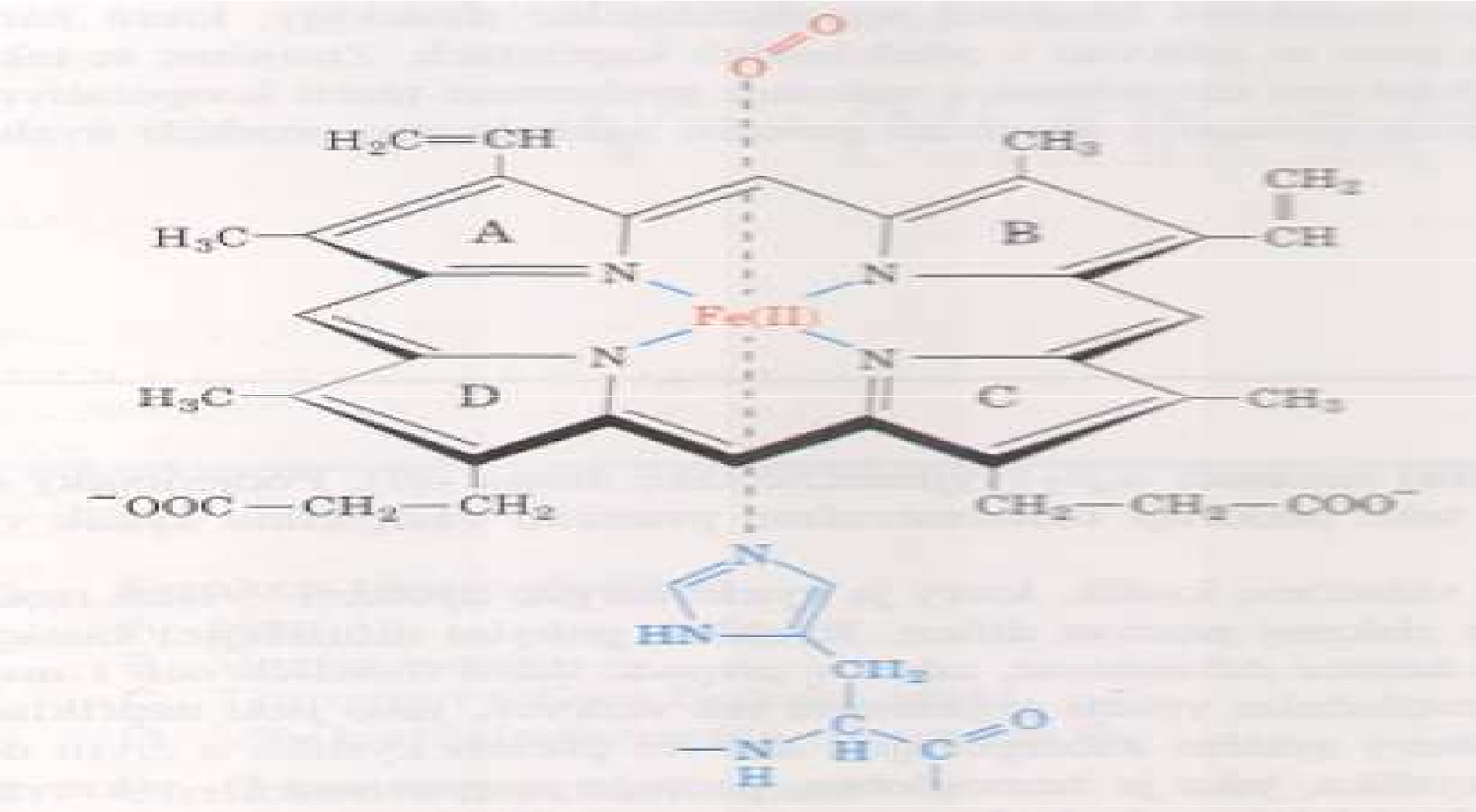


(a) Protoporphyrin IX

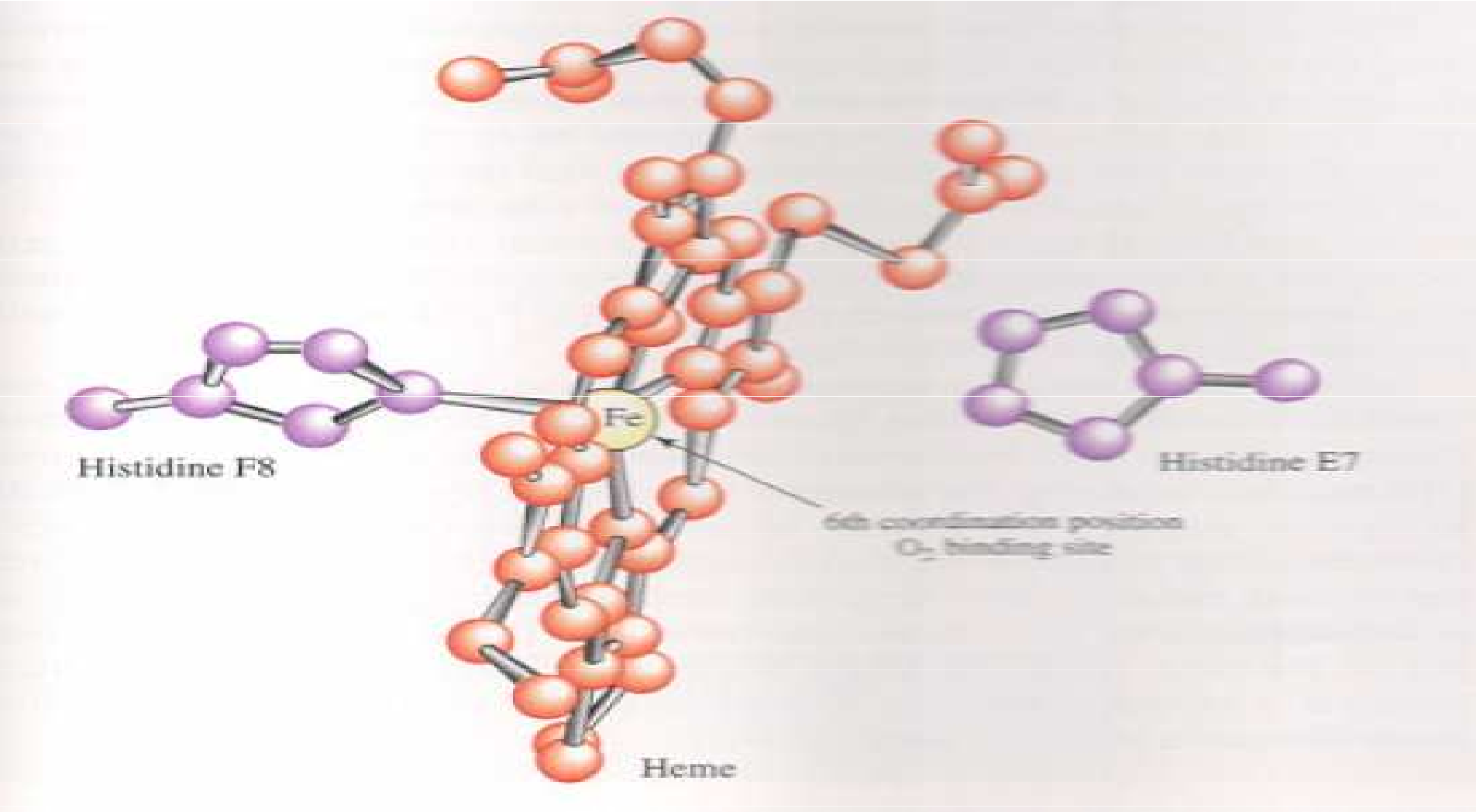


(b) Heme (Fe-protoporphyrin IX)

Vazba O_2 na Hb



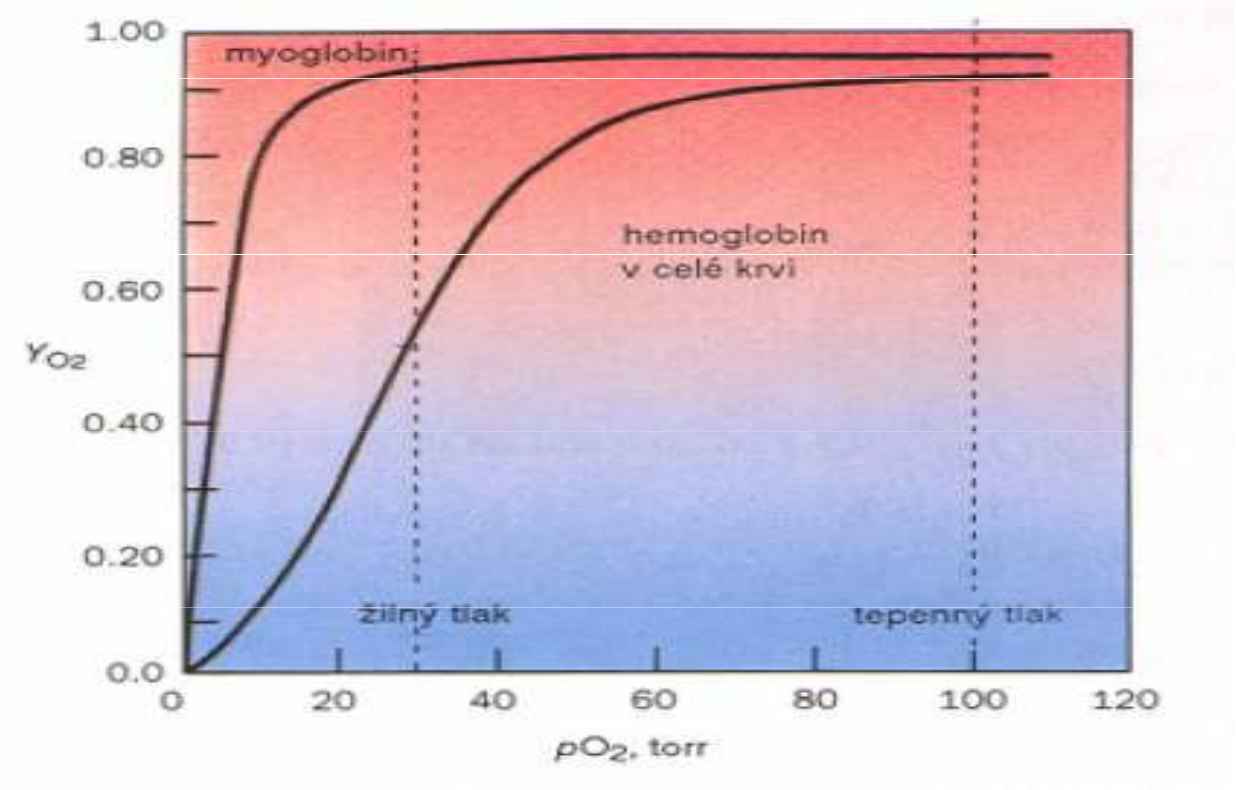
Vazba O_2 na Hb



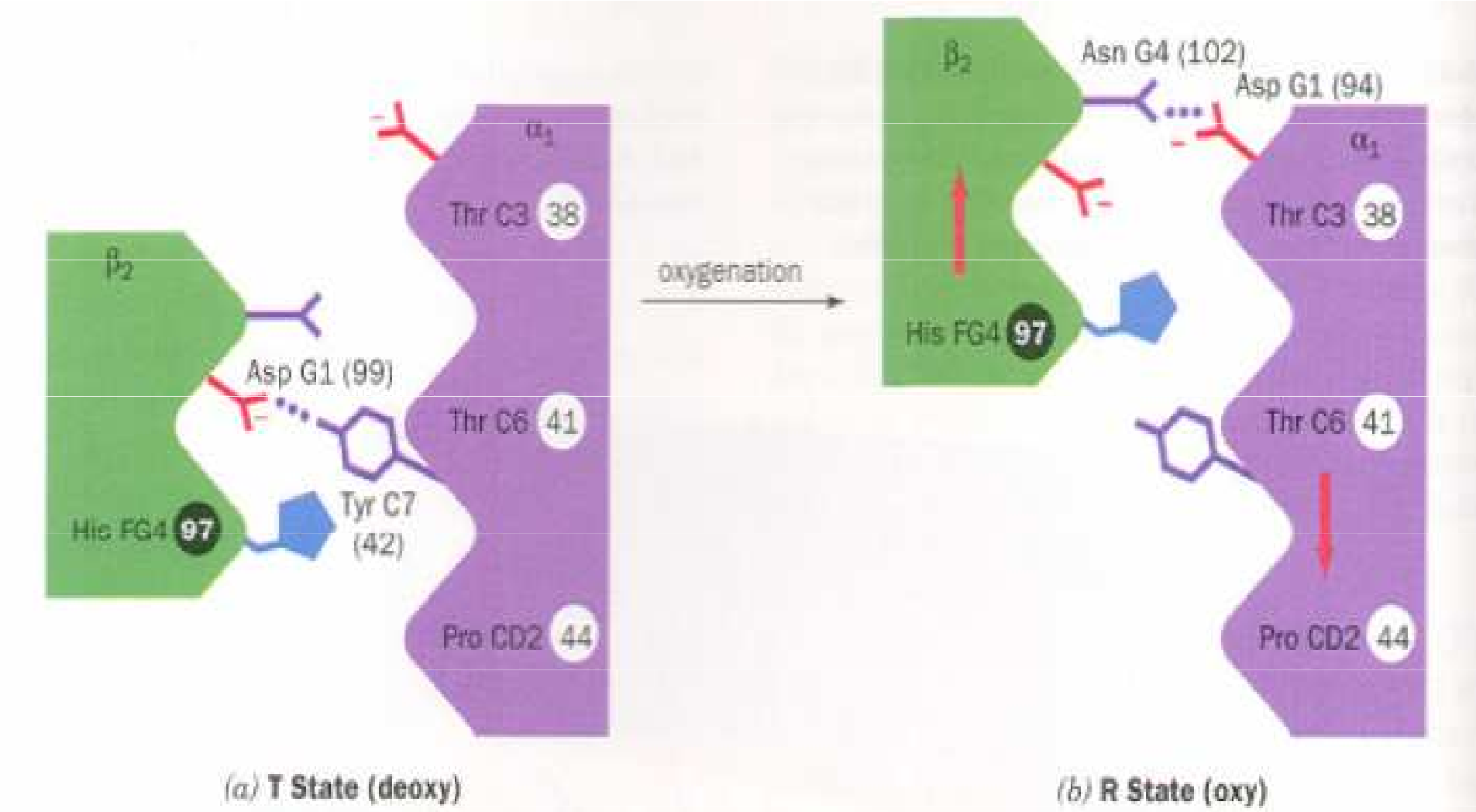
Hb versus Mb

$$Mb + O_2 \rightleftharpoons MbO_2$$

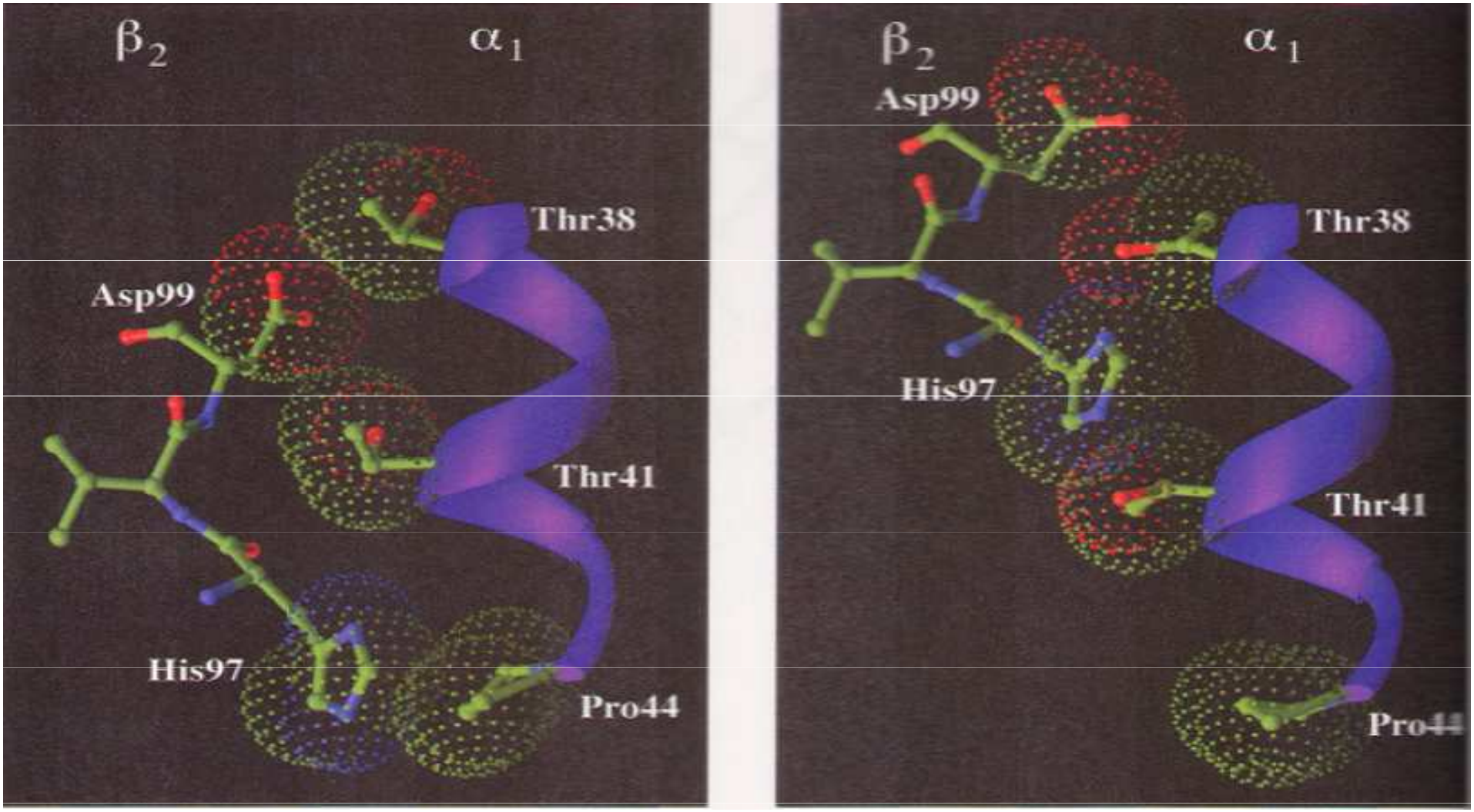
$$Hb + O_2 \rightleftharpoons Hb(O_2)$$
$$Hb(O_2) + O_2 \rightleftharpoons Hb(O_2)_2$$
$$Hb(O_2)_2 + O_2 \rightleftharpoons Hb(O_2)_3$$
$$Hb(O_2)_3 + O_2 \rightleftharpoons Hb(O_2)_4$$



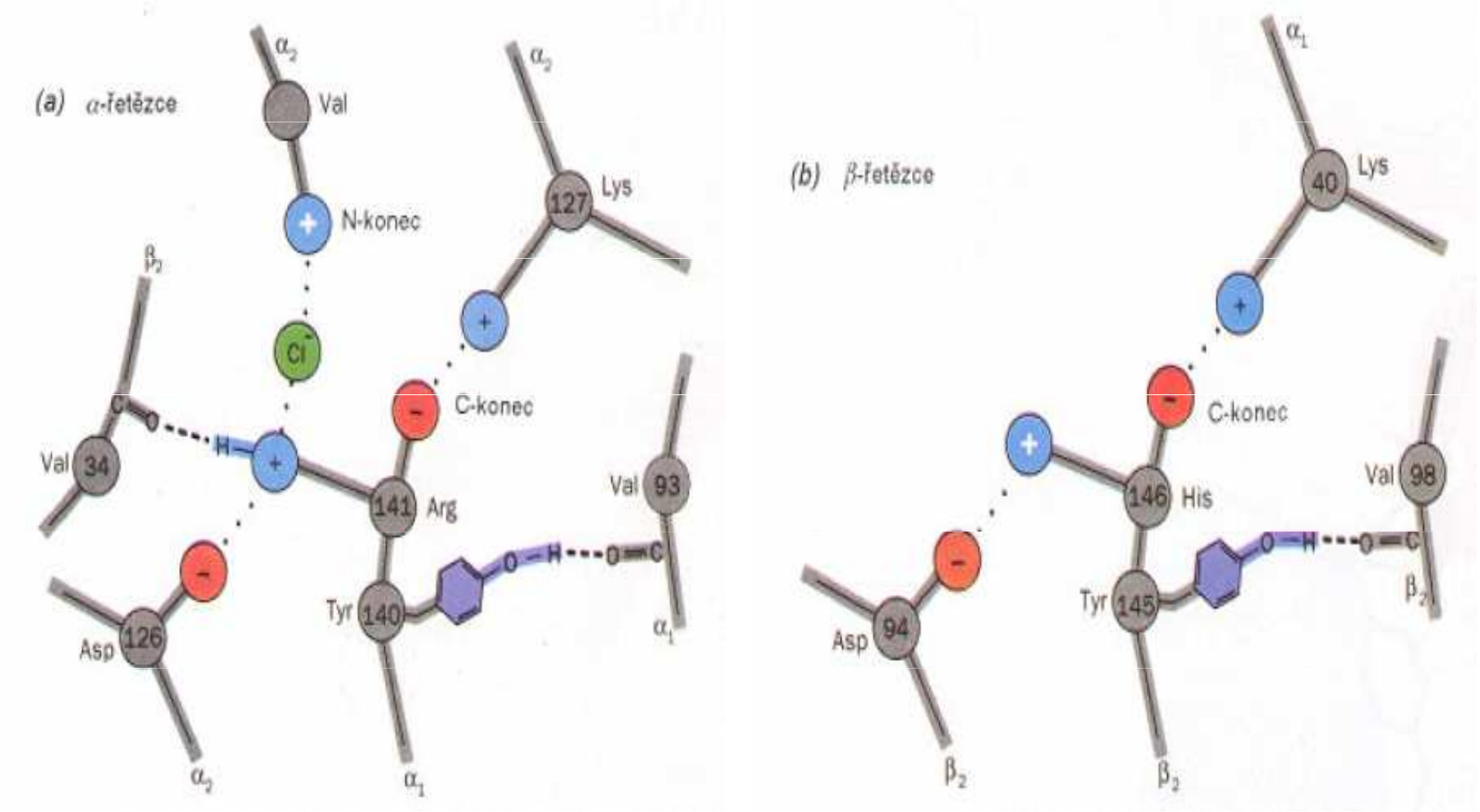
Solné můstky



Solné můstky



Solné můstky

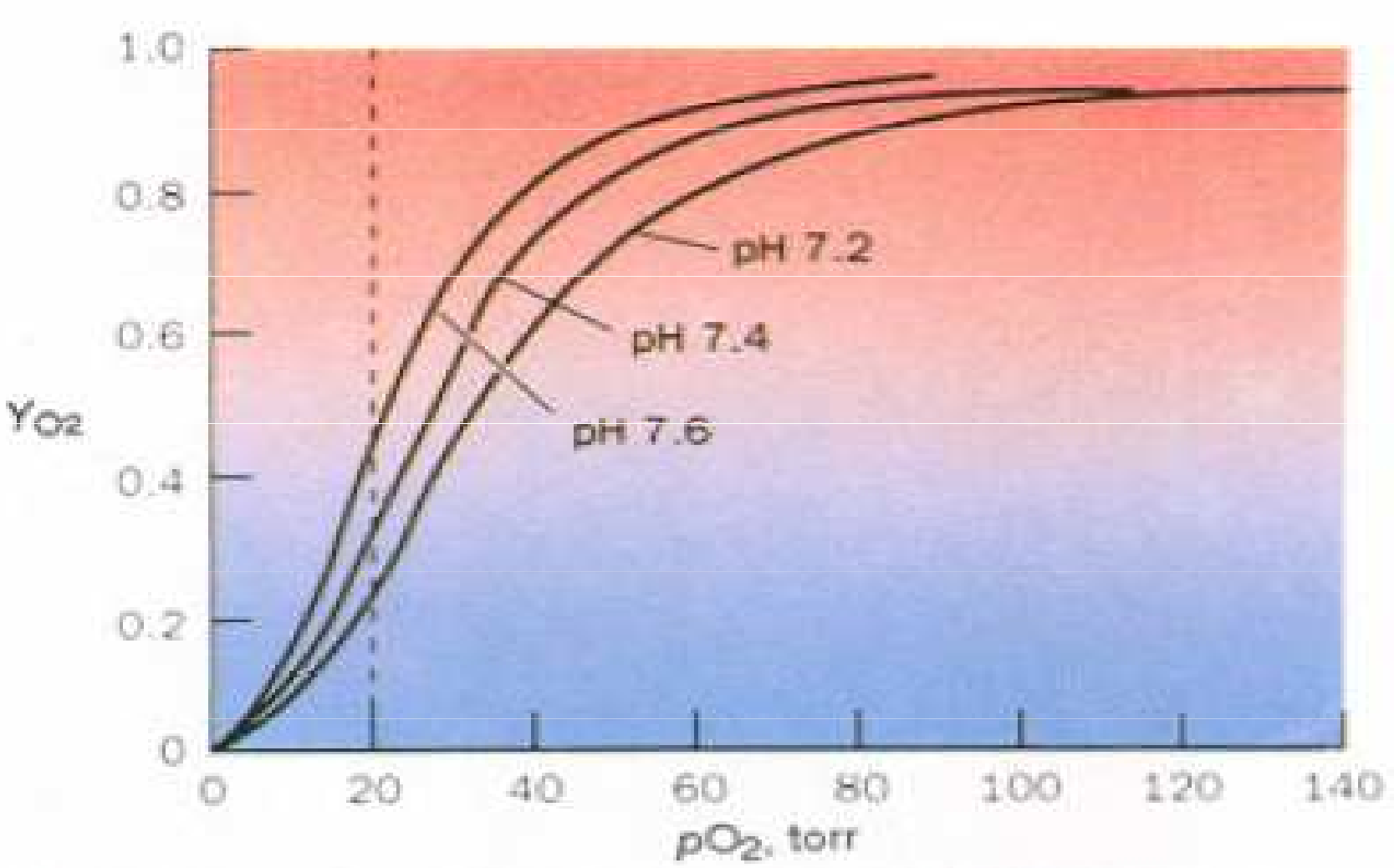


Bohrův effekt – vliv H^+ a CO_2

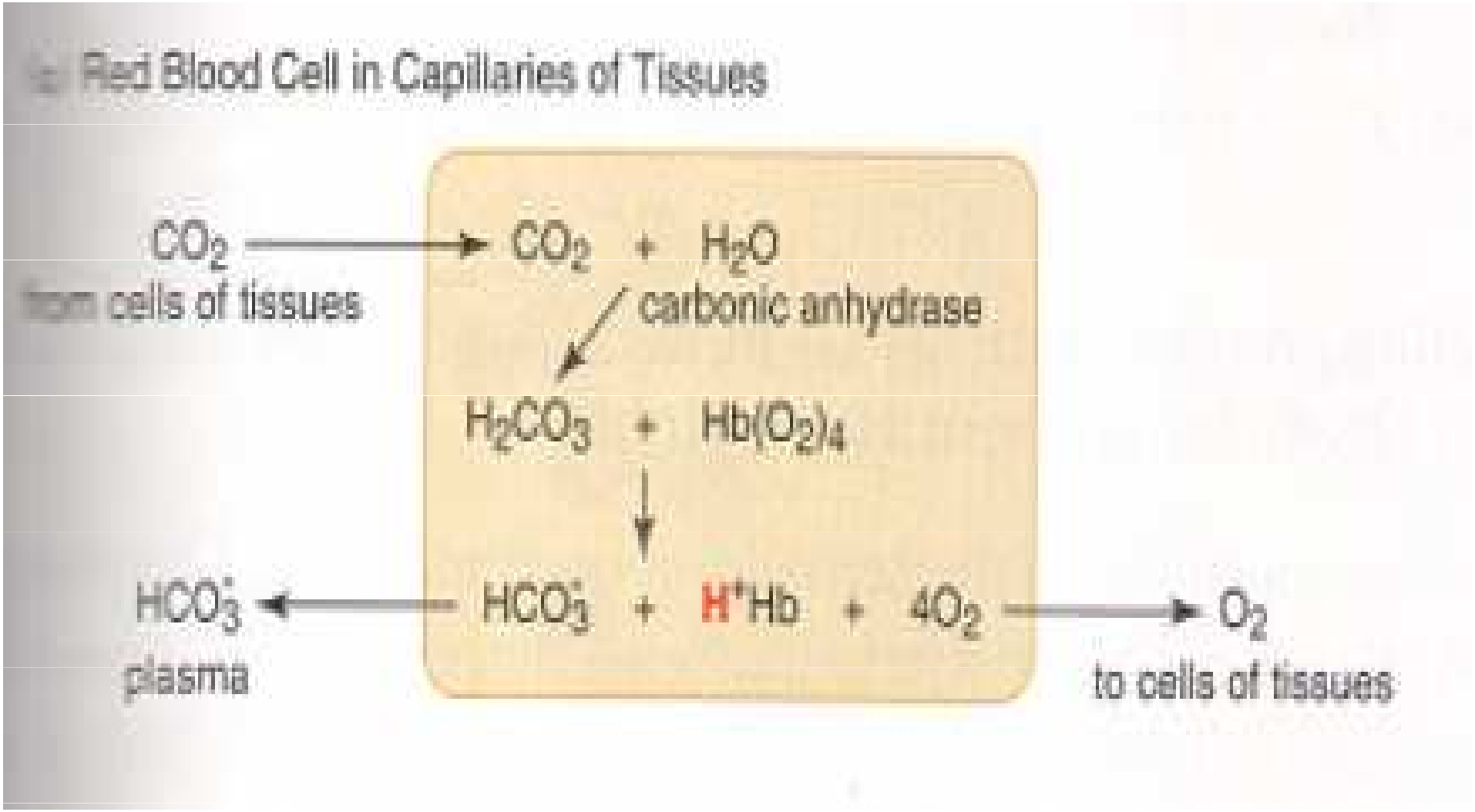
$$Hb(O_2)_nH_x + O_2 \rightleftharpoons Hb(O_2)_{n+1} + xH^+$$

$$CO_2 + H_2O \rightleftharpoons H^+ + HCO_3^-$$

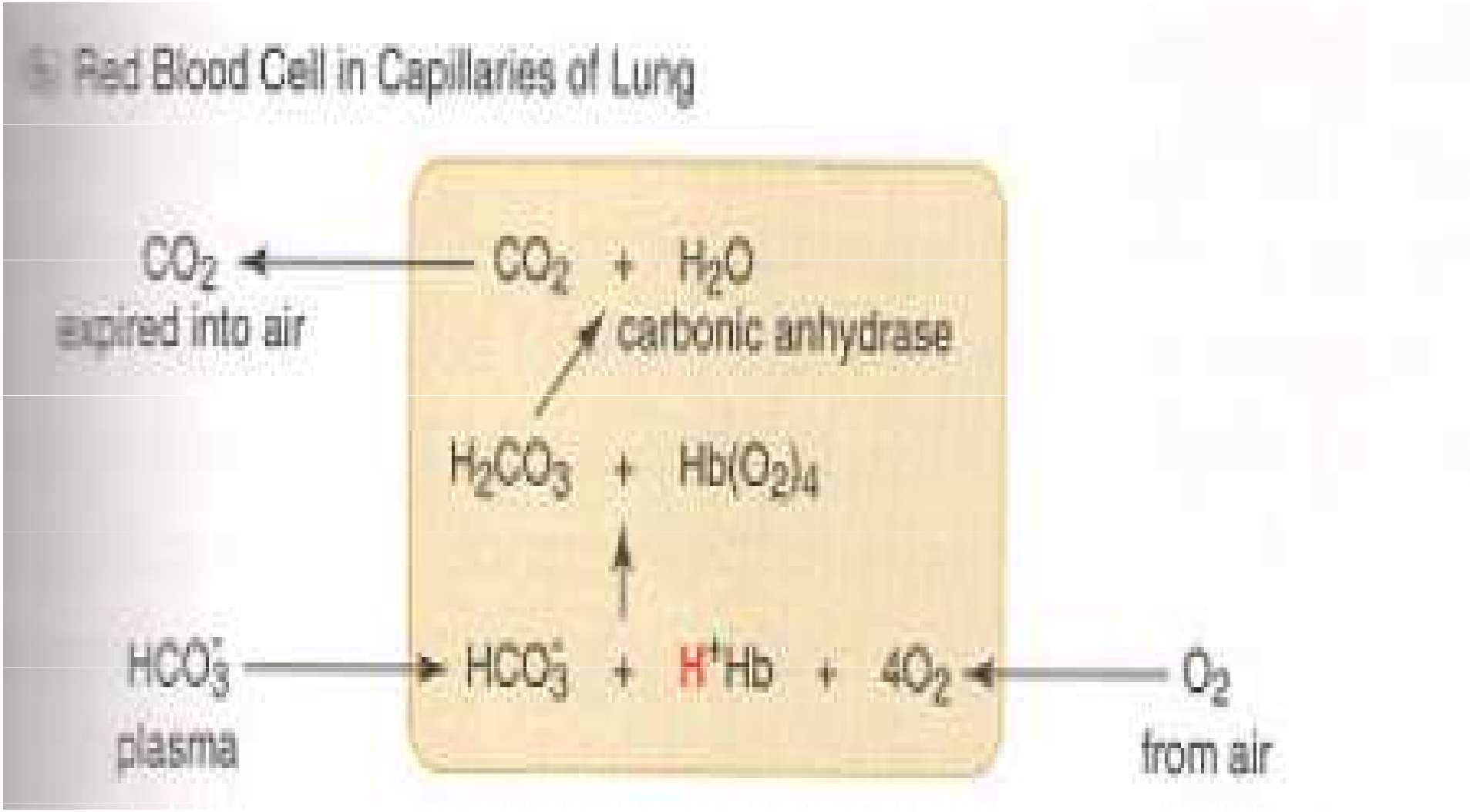
Bohrův effekt – vliv H^+ a CO_2



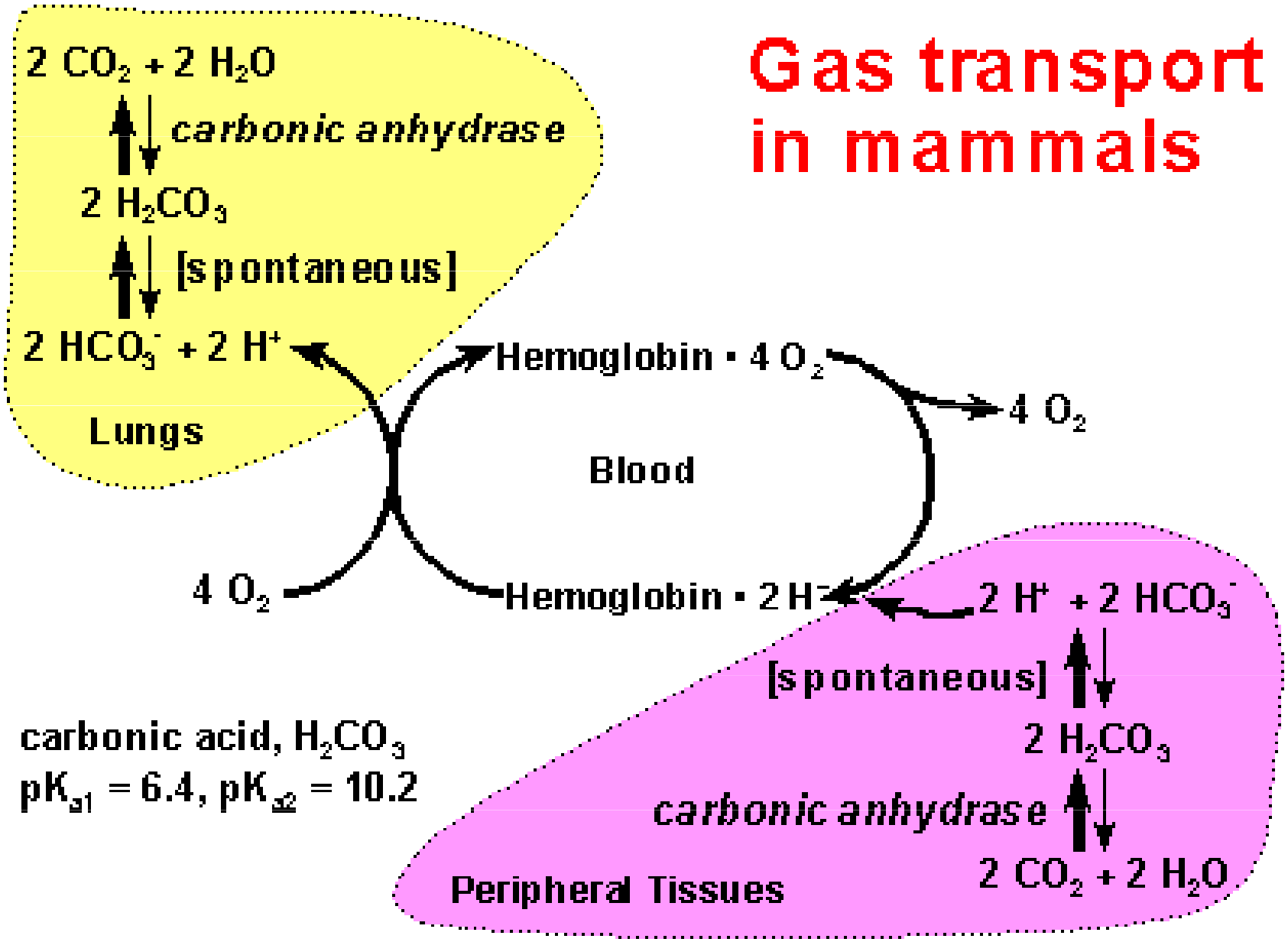
Bohrův effekt – vliv H^+ a CO_2



Bohrův effekt – vliv H^+ a CO_2



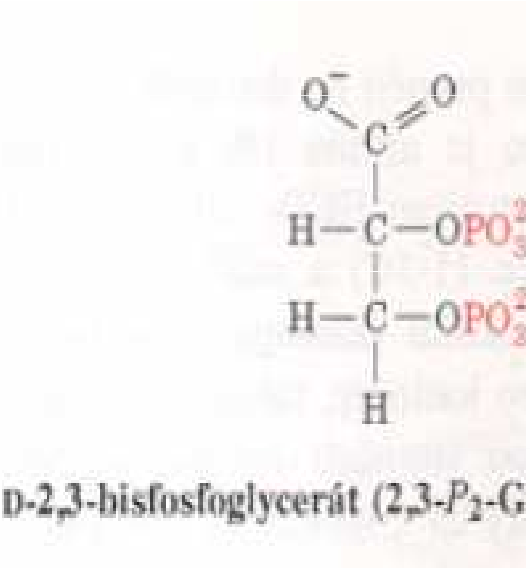
Gas transport in mammals

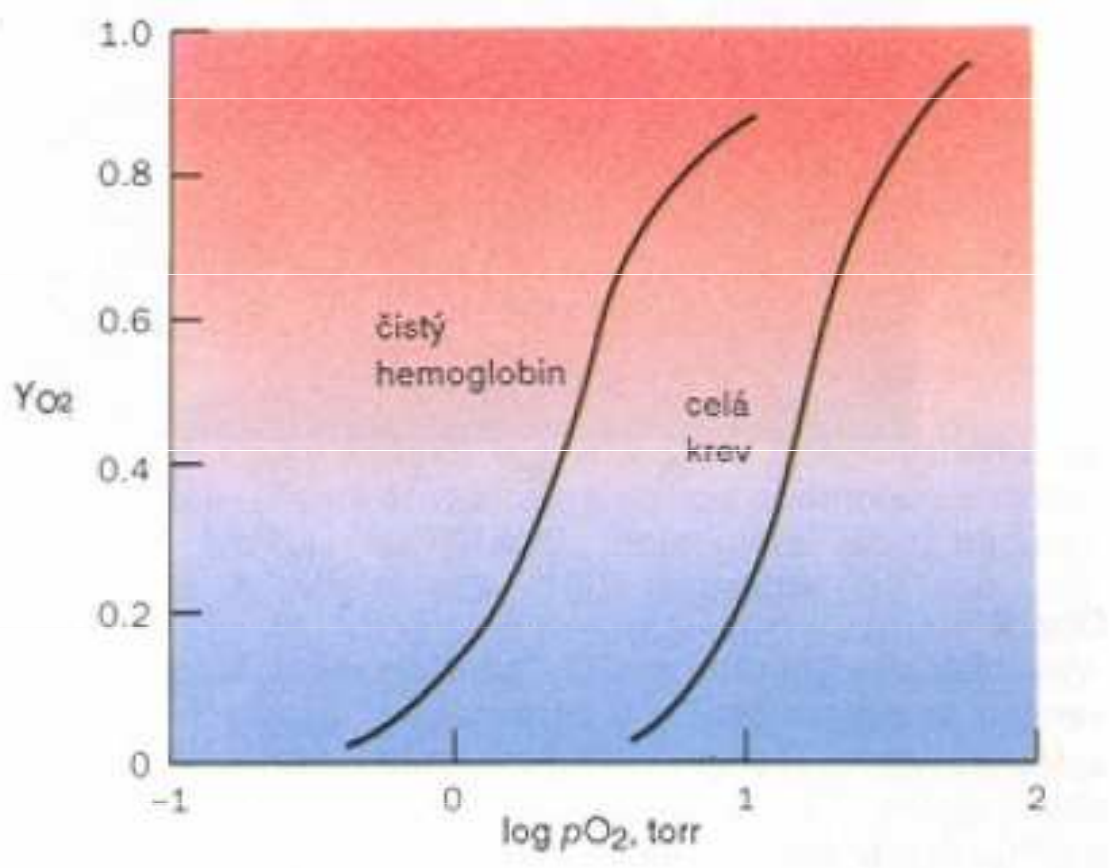


carbonic acid, H_2CO_3

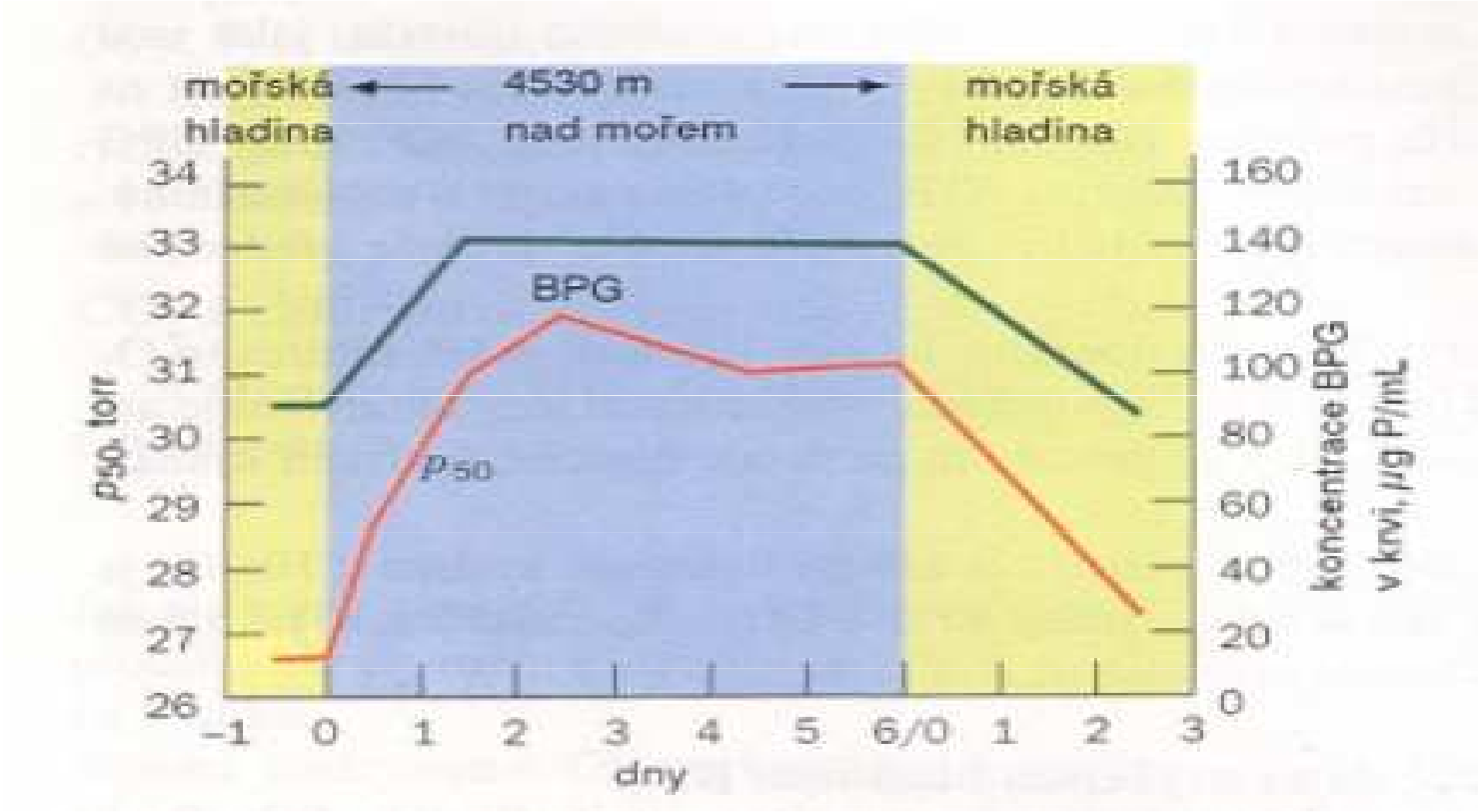
$pK_{a1} = 6.4$, $pK_{a2} = 10.2$

Vliv BPG

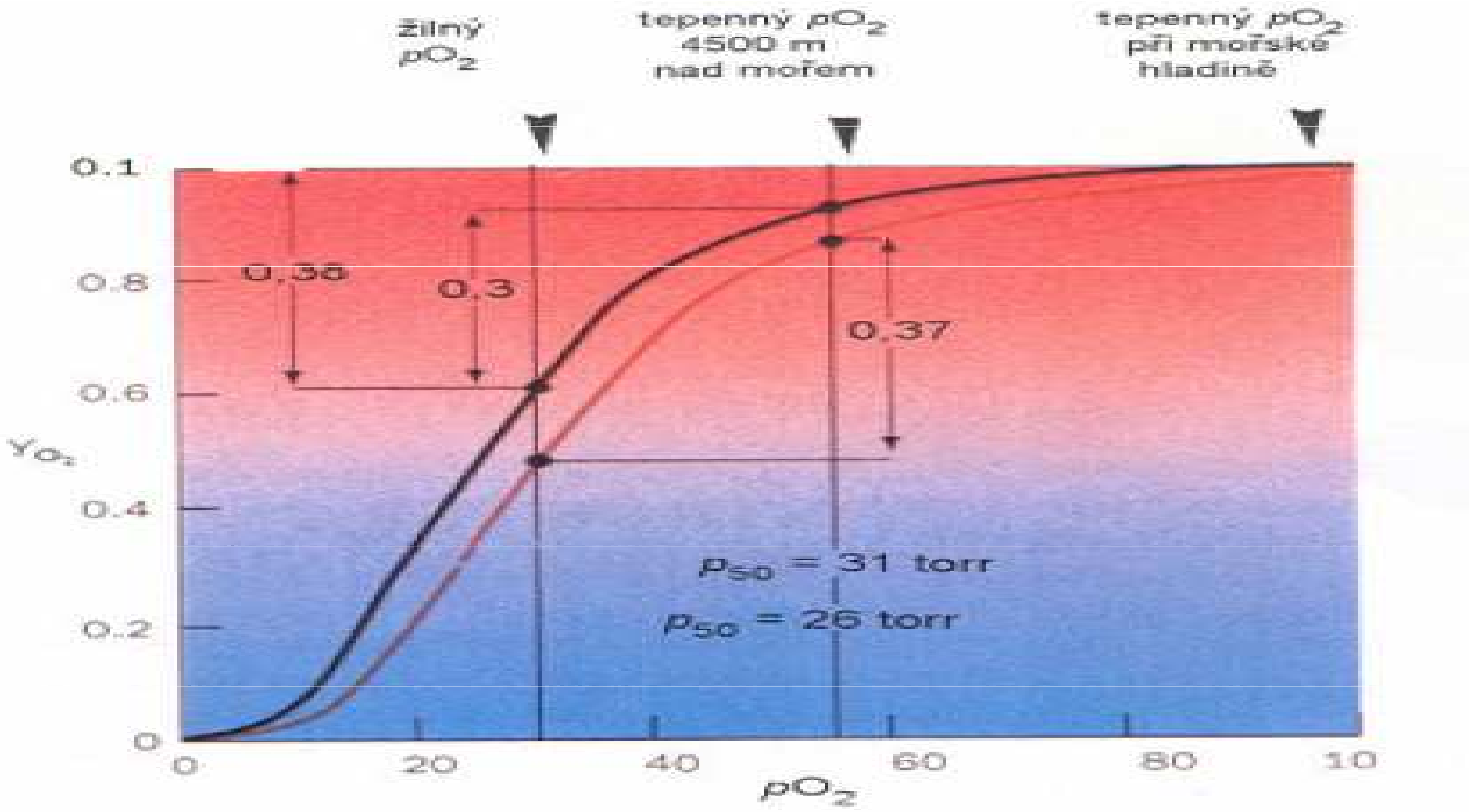

D-2,3-bisfosfoglycerát (2,3-P_2-G


1.0
0.8
0.6
0.4
0.2
0
Y_{O2}
čistý hemoglobin
celá krev
−1
0
1
2
log pO_2, torr

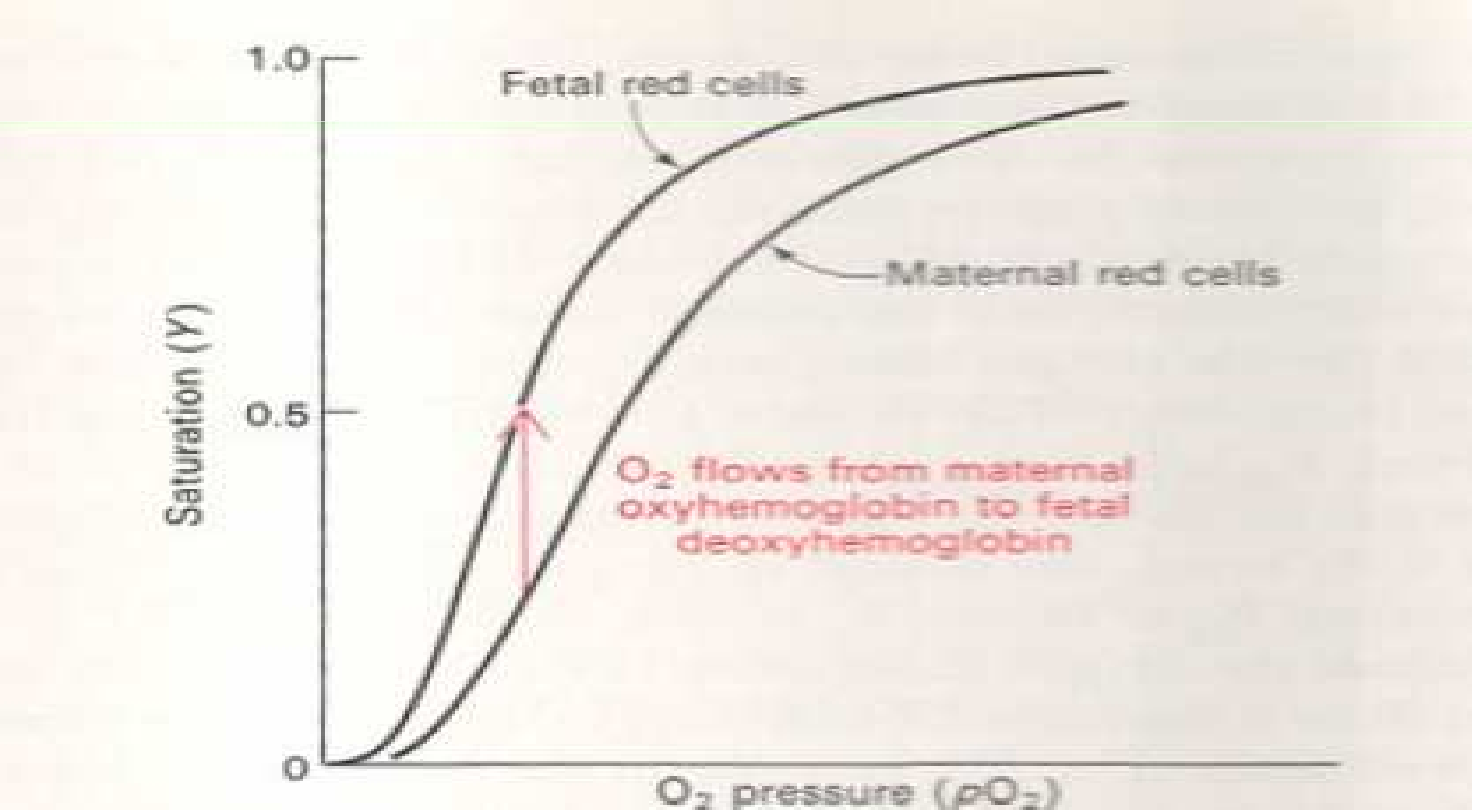
Vliv BPG a nadmořská výška



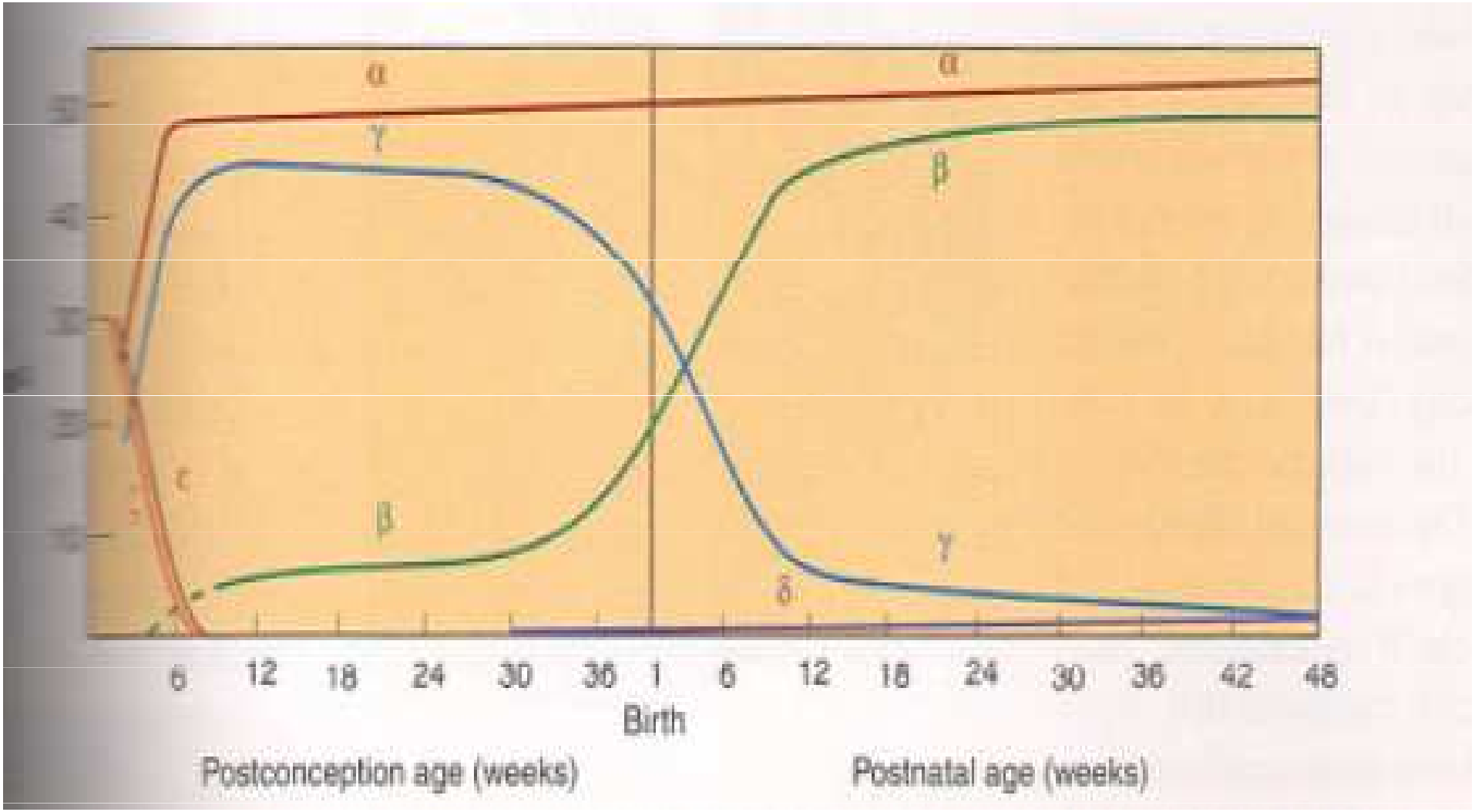
Vliv BPG a nadmořská výška



Fetální versus normální Hb

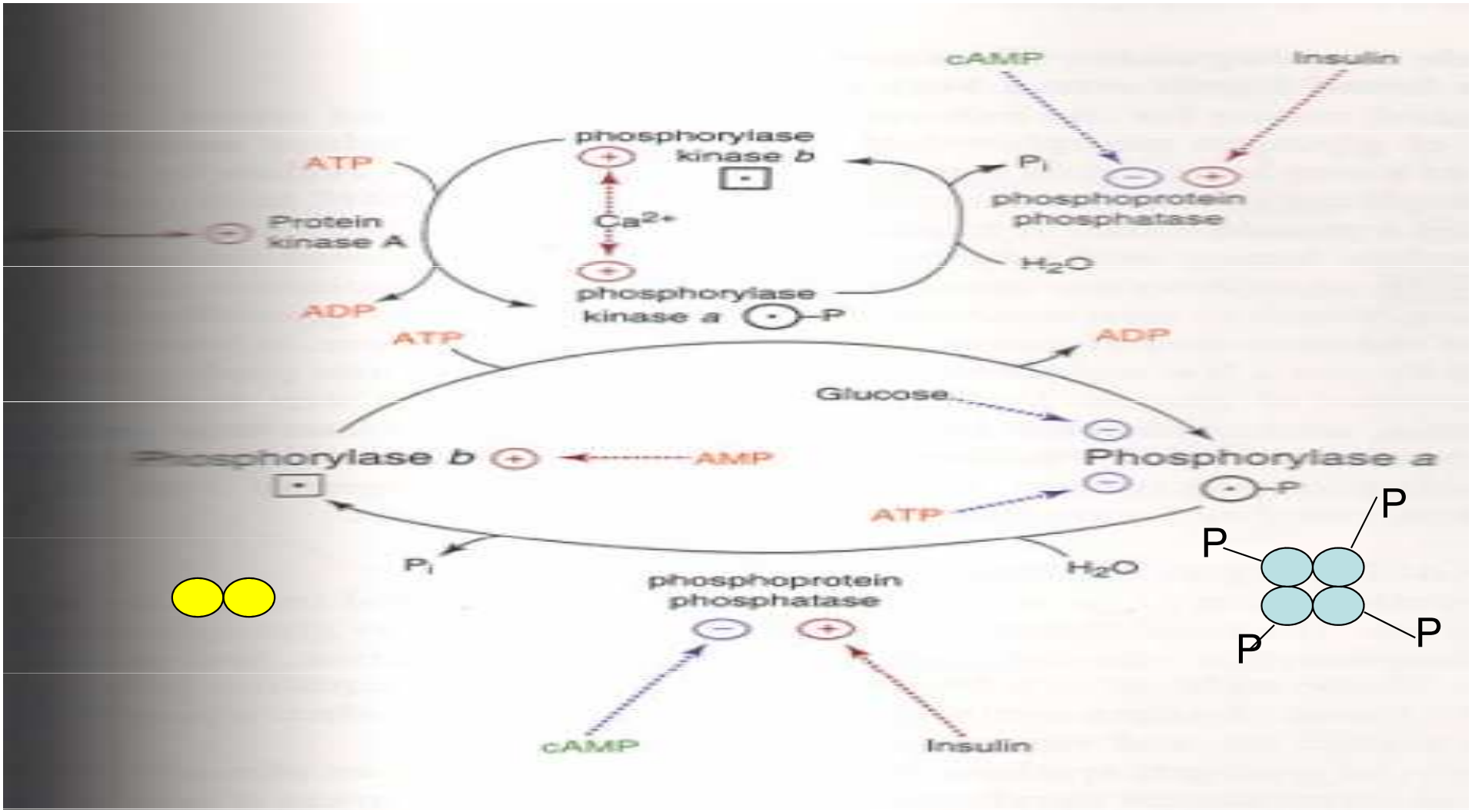


Fetální versus normální Hb



Regulace kovalentní modifikací

glykogenfosforylasa



Regulace kovalentní modifikací

glykogenfosforylasa

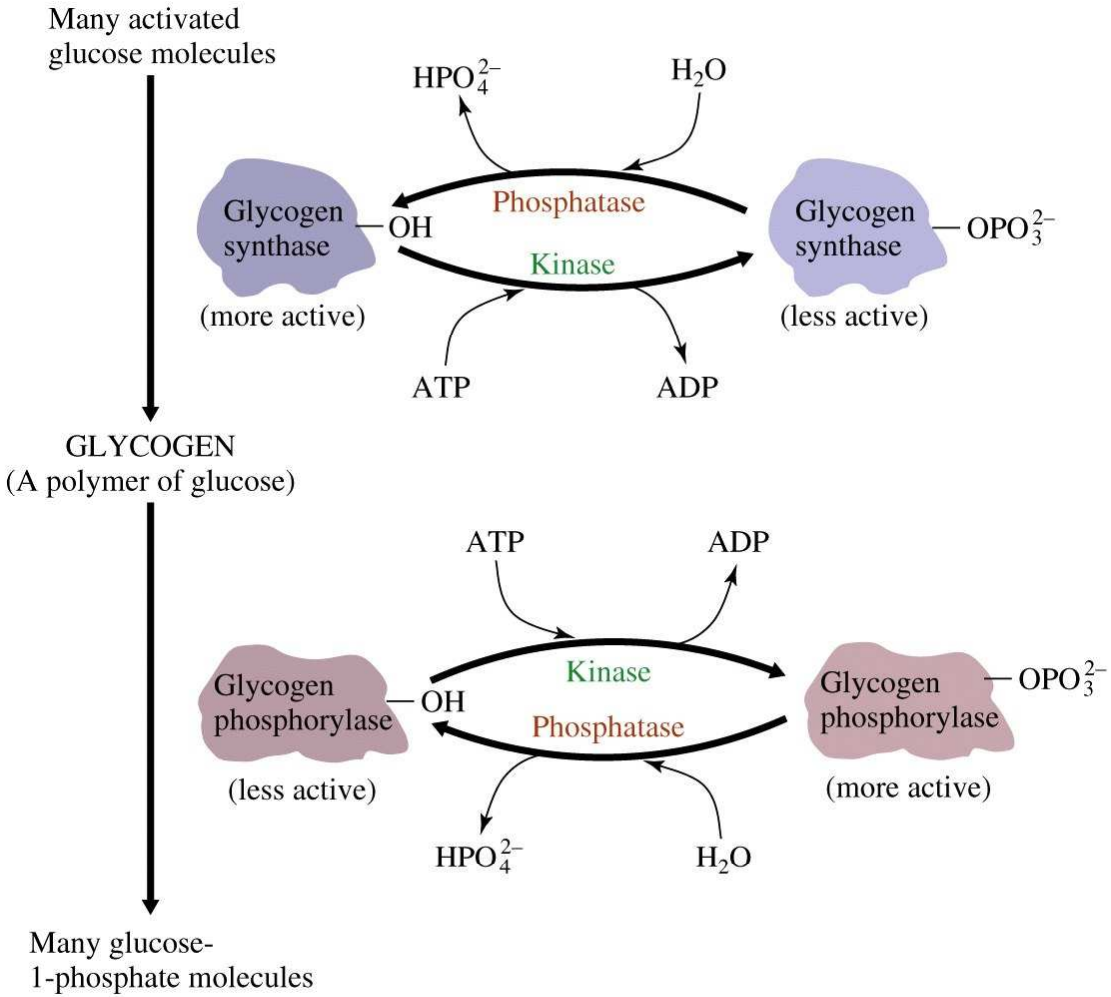


Figure 6-7 Concepts in Biochemistry, 3/e

Aktivace zymogenů

žaludek

pepsinogen → pepsin

pH

slinivka břišní

enterokinasa

trypsinogen → trypsin

chymotrypsinogen → chymotrypsin

proelastasa → elastasa

Regulace kovalentní modifikací

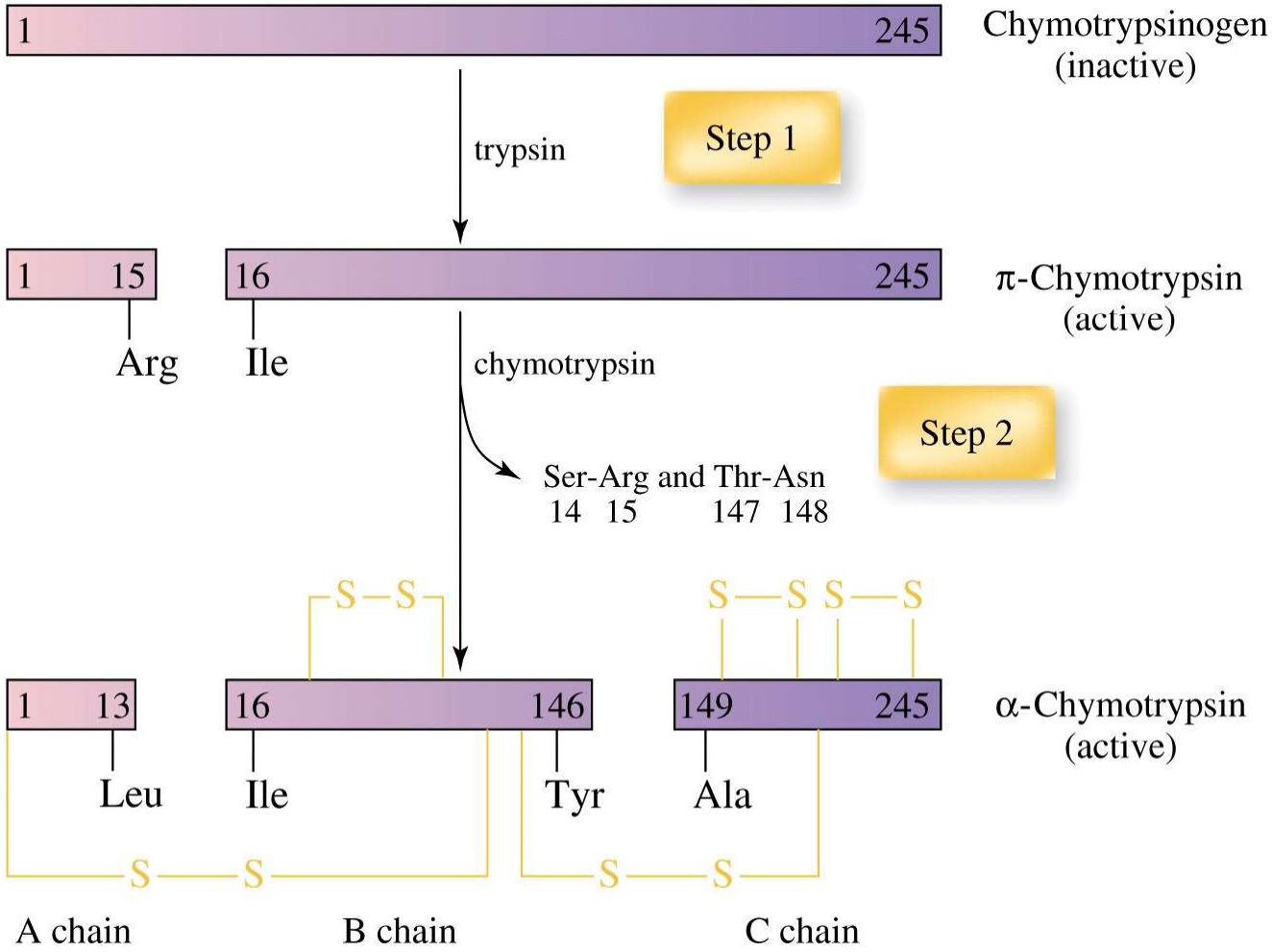
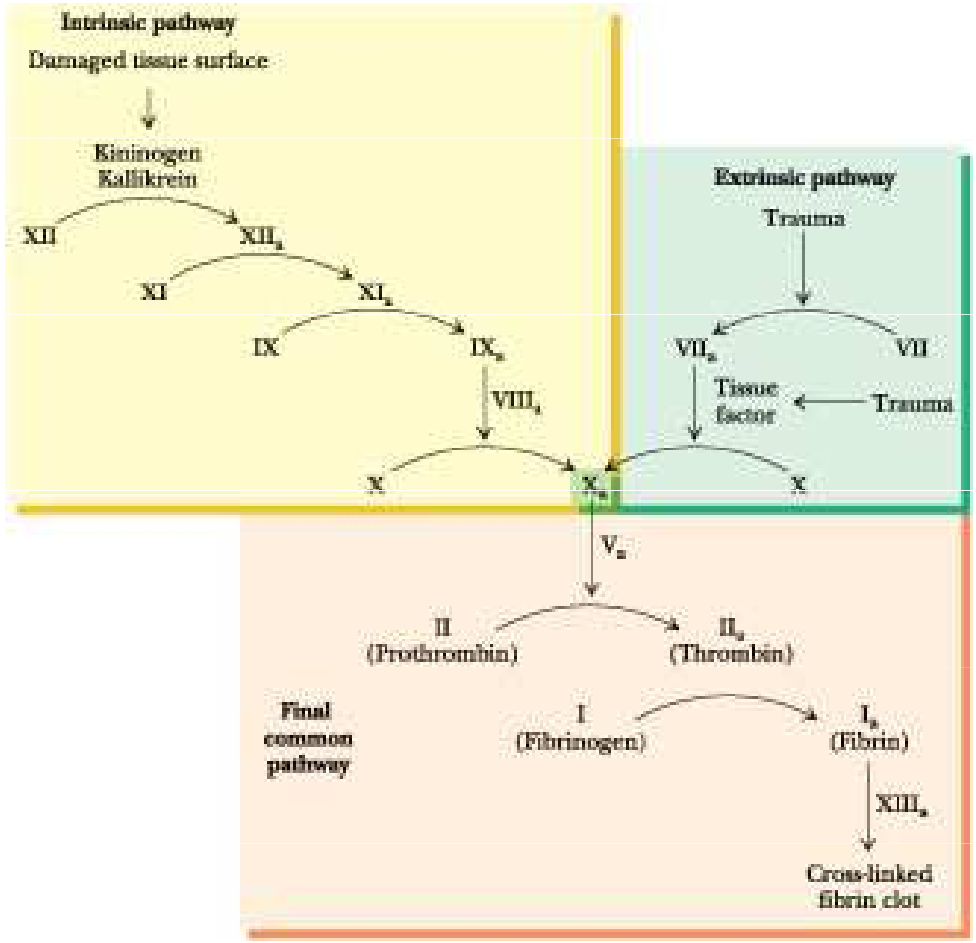
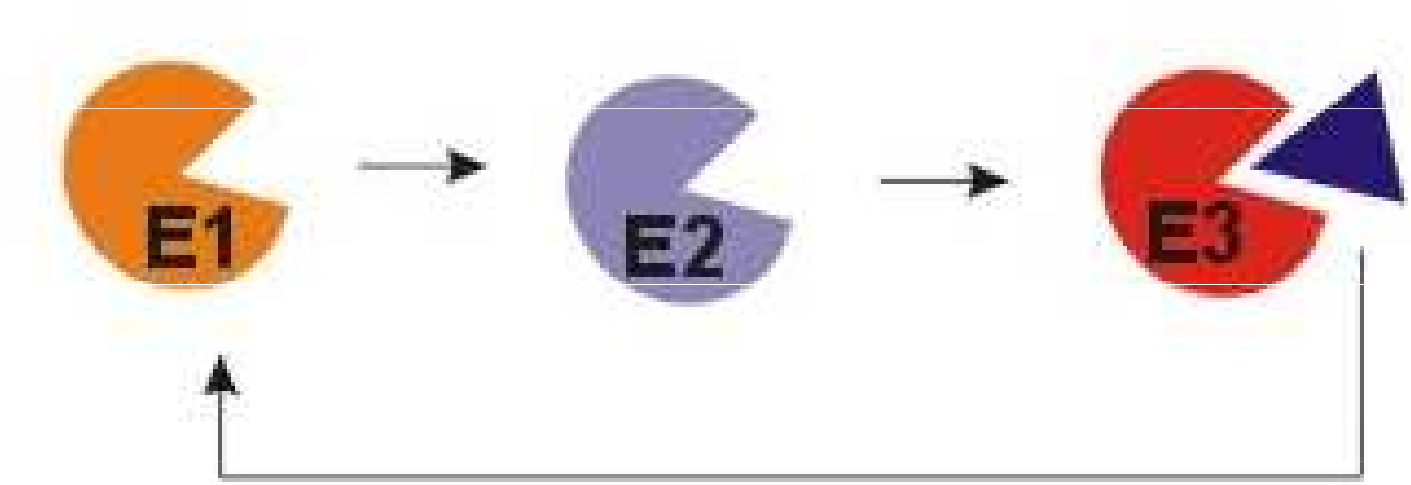


Figure 6-8 Concepts in Biochemistry, 3/e

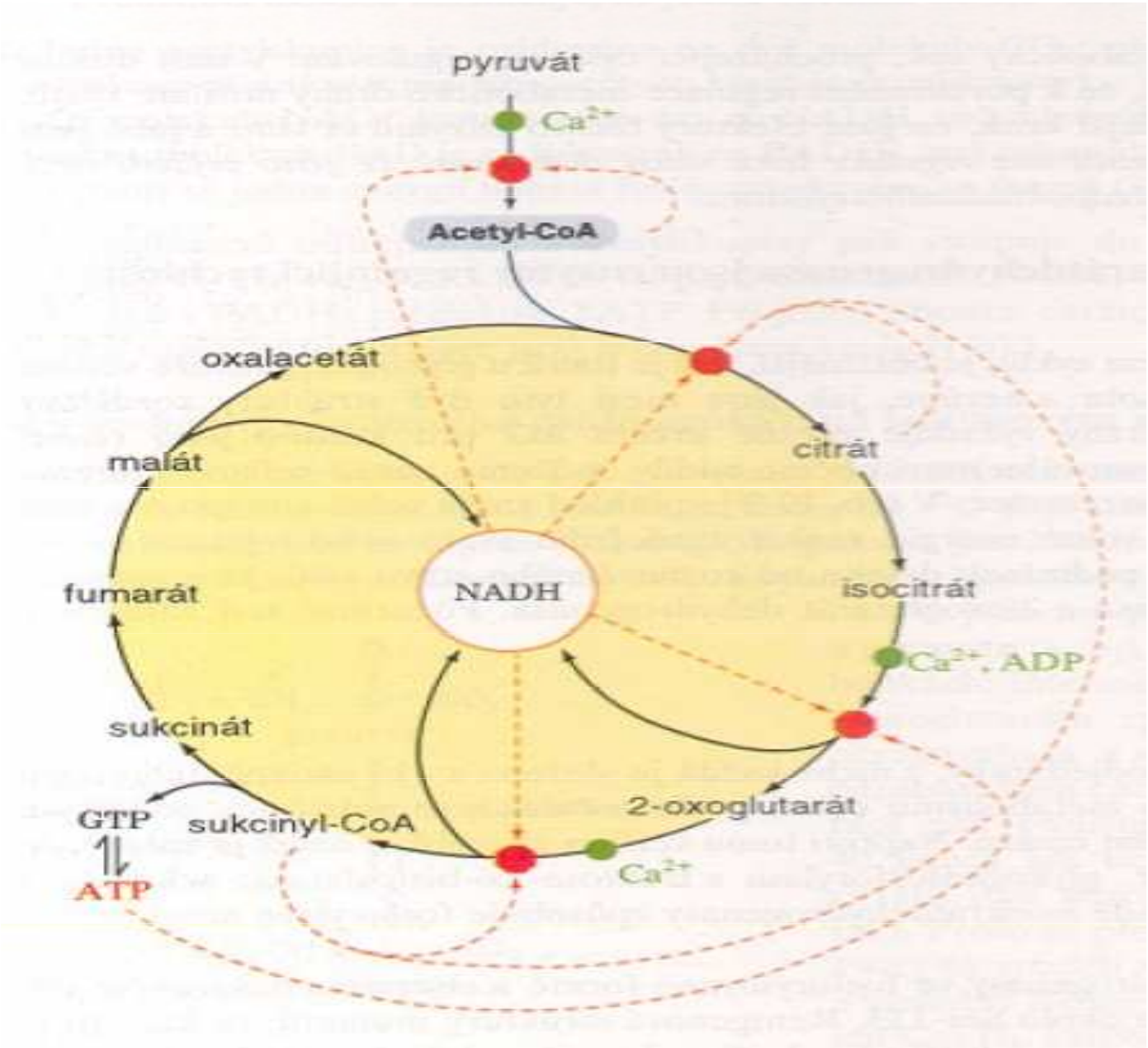
Regulace kovalentní modifikací



Regulace zpětnou vazbou



Regulace


pyruvát
Ca2+
Acetyl-CoA
oxalacetát
malát
citrát
fumarát
NADH
isocitrát
Ca2+, ADP
sukcinát
GTP
ATP
sukcinyl-CoA
2-oxoglutarát
Ca2+

Regulace činnosti enzymu

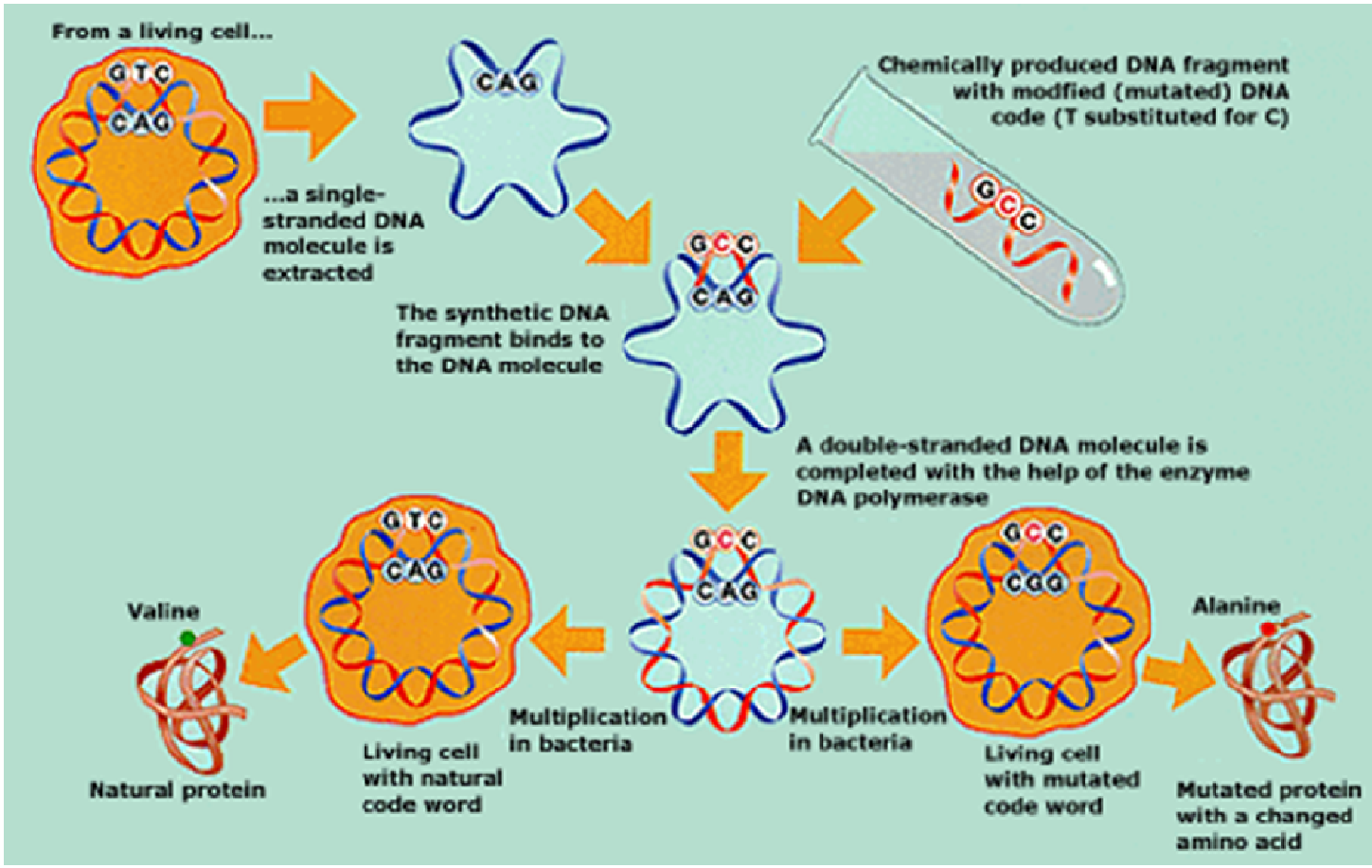
- Kompartmentace

Umělé enzymy

↓

Úprava přírodních enzymů

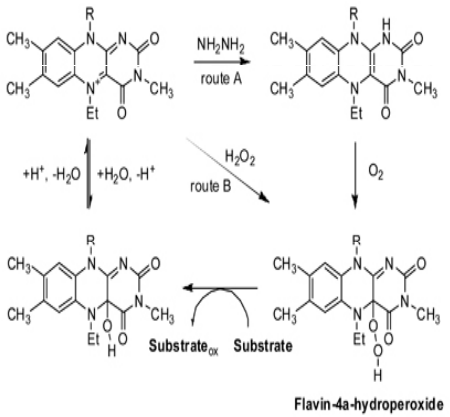
Řízena evoluce *versus* Místně cílená mutageneze

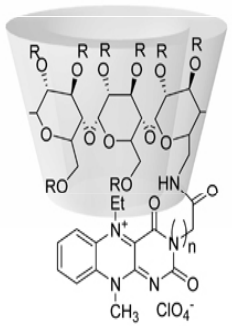


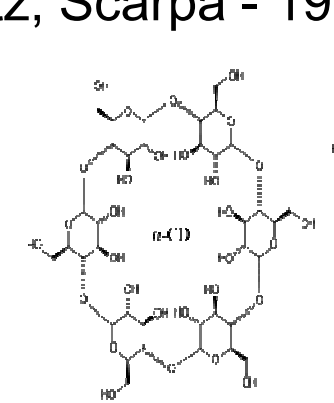
Umělé enzymy

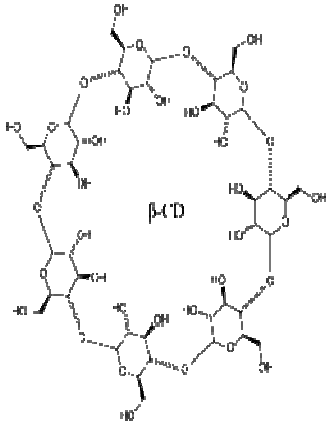
Klotz, Scarpa - 1971

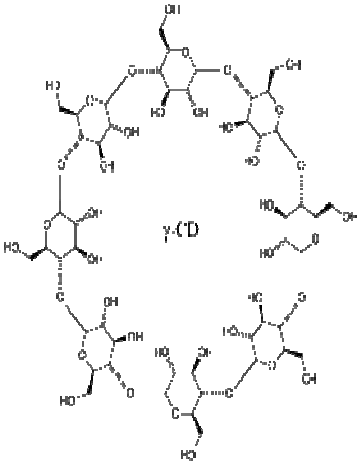
- Synzymy











Abzymy

Schultz, Lerner - 1986

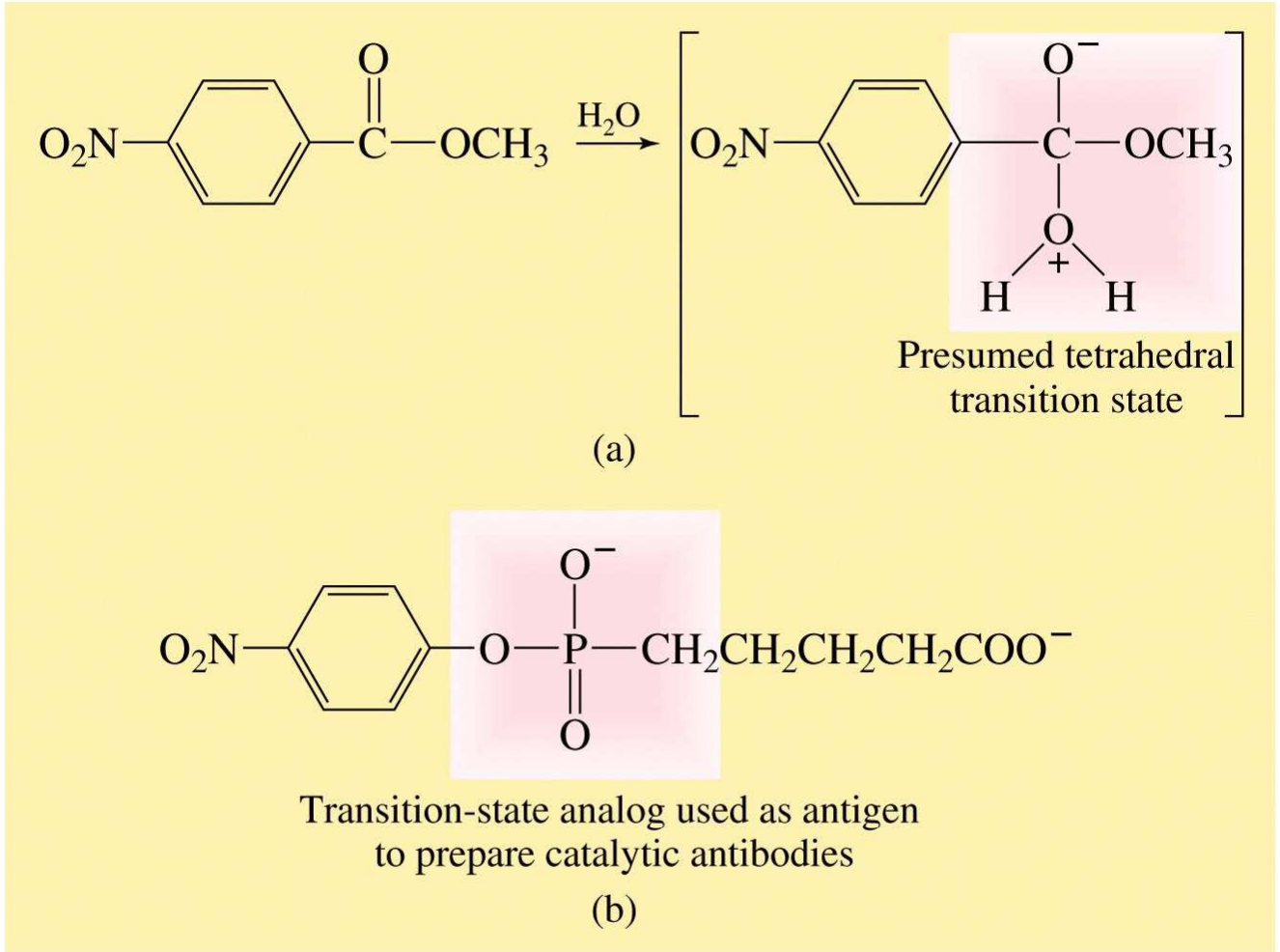


Figure 6-11 Concepts in Biochemistry, 3/e

Ribozymy – katalytická RNA

1989 Nobelova cena

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

Ribonukleasa P (Altman)

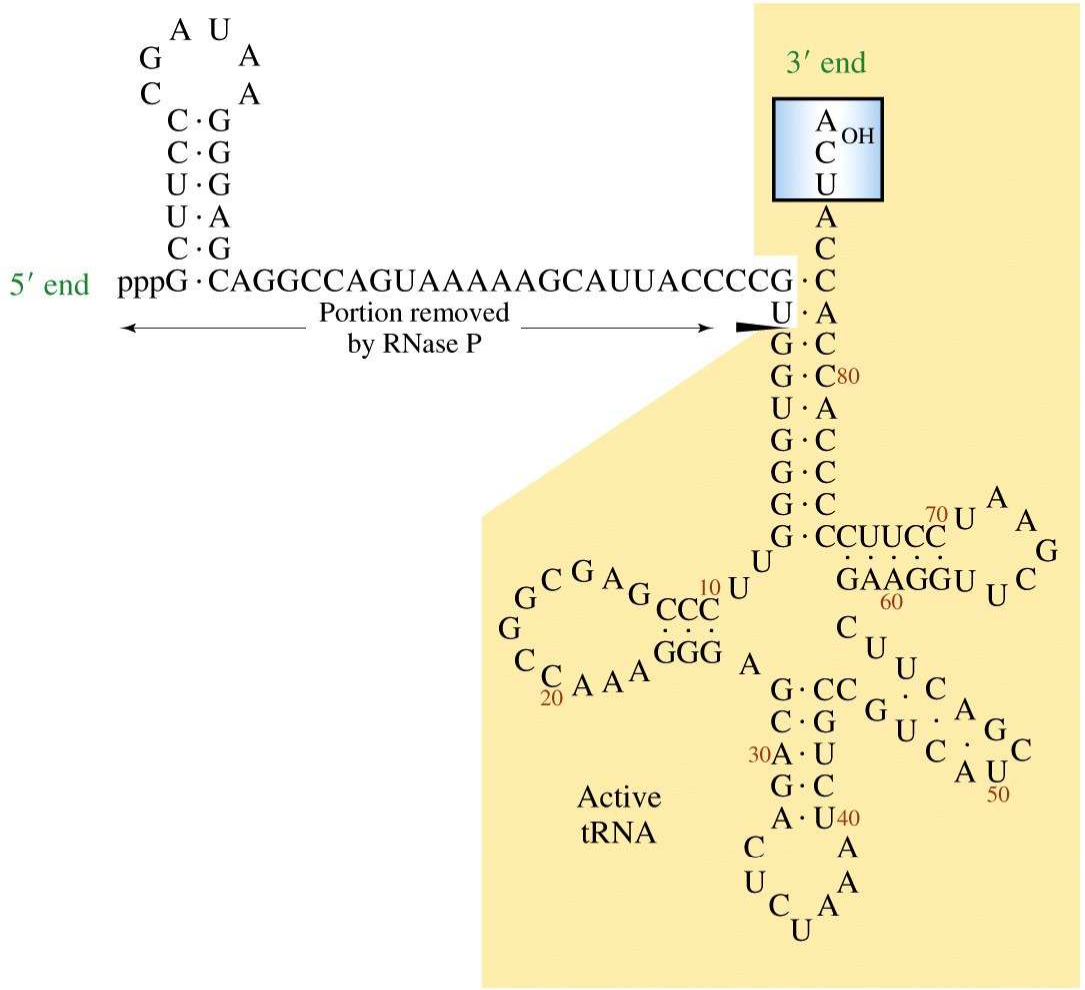


Figure 6-12 Concepts in Biochemistry, 3/e

Autokatalytická mRNA (Cech)

Tetrahymena thermophila

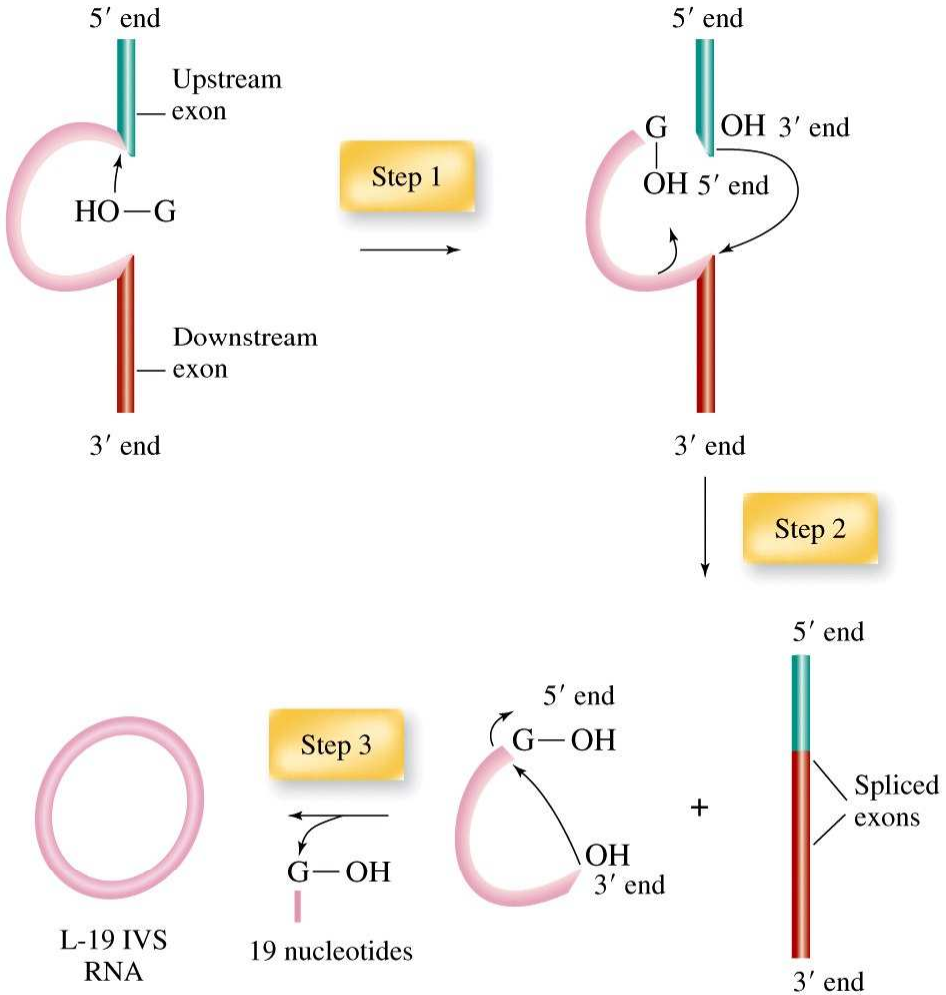


Figure 6-13 Concepts in Biochemistry, 3/e

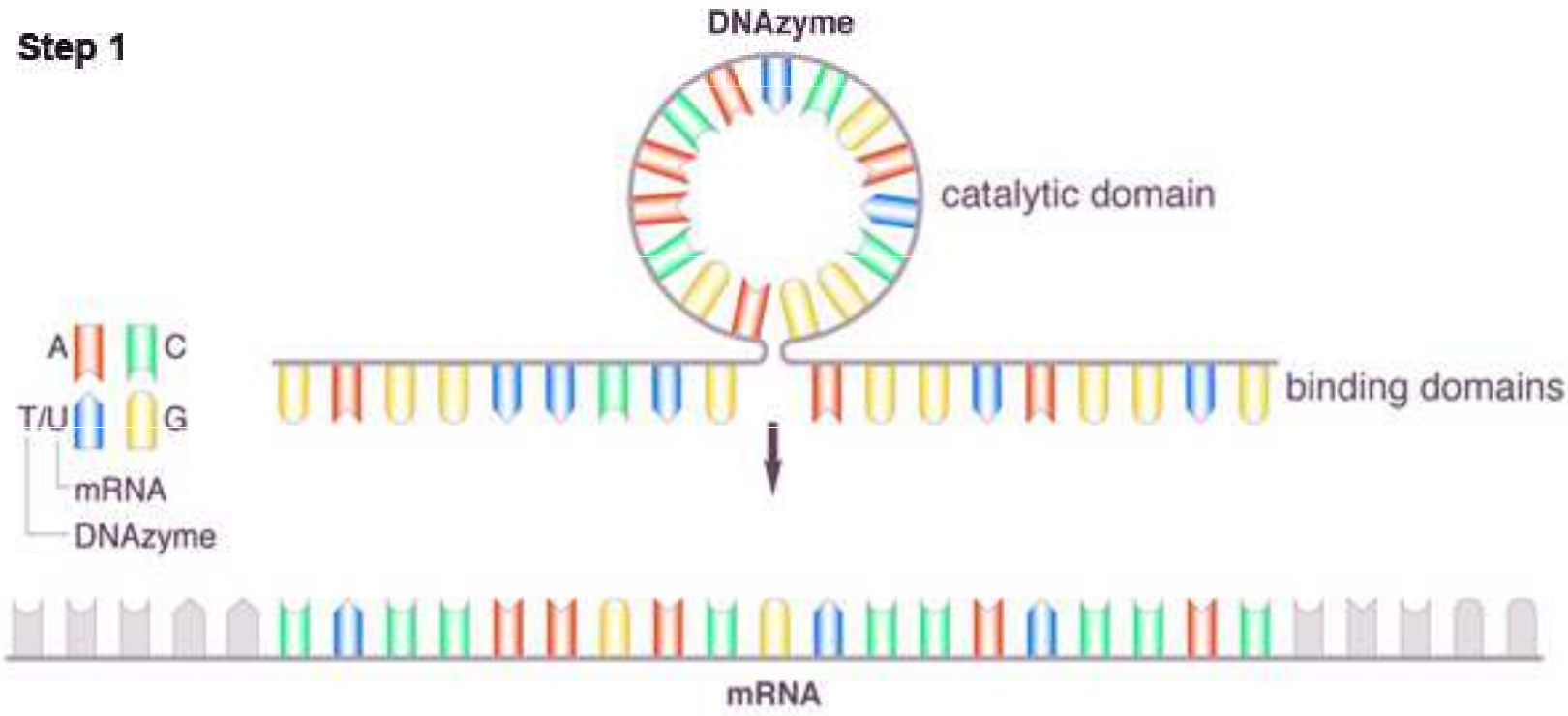
DNAzymy (1994)

- Ronald R. Breaker (Yale University)
- Štěpení RNA v přítomnosti Pb^{2+}

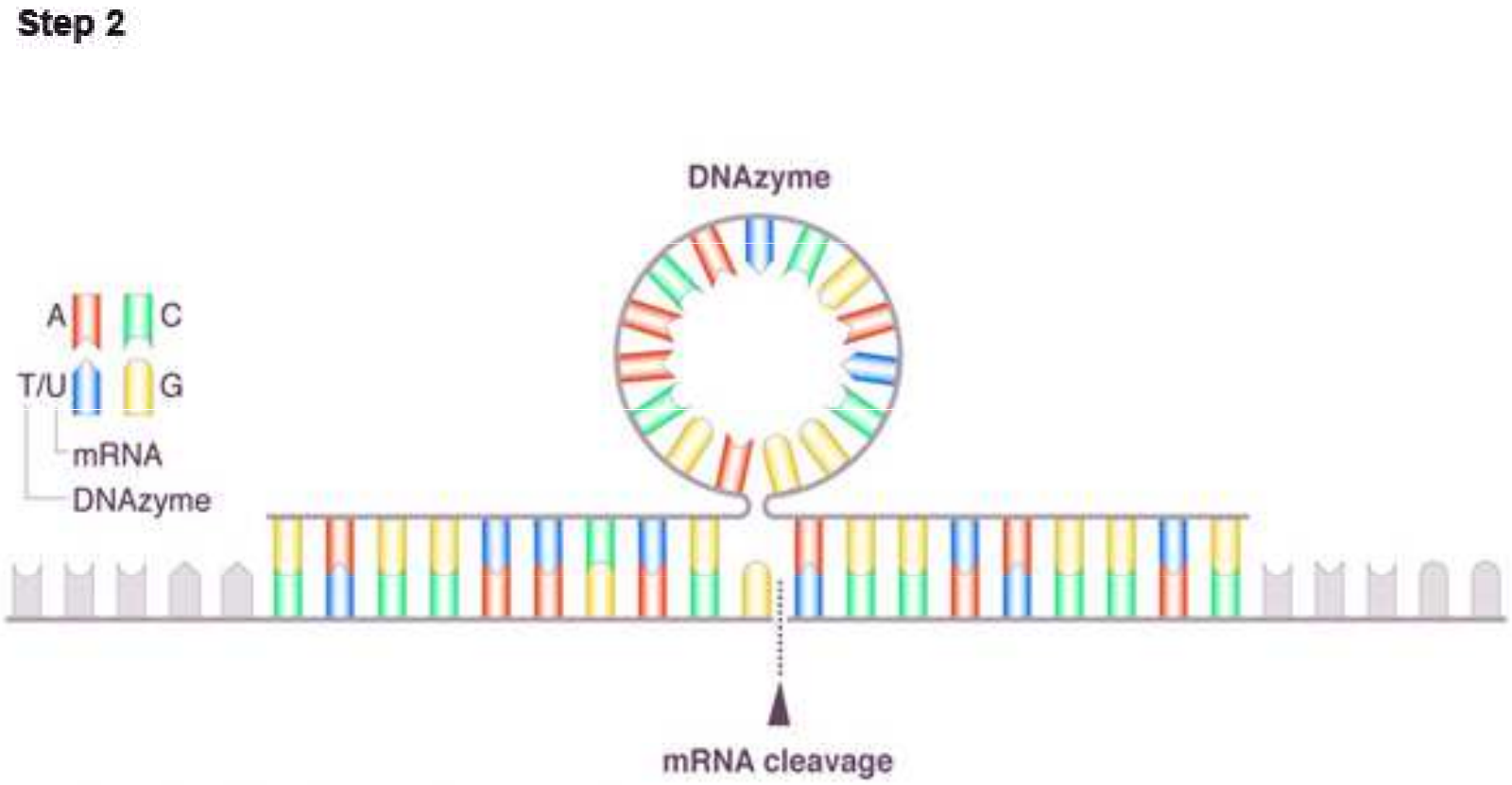
DNAzymy

- Katalyzují např. :
  - DNA fospforylaci
  - DNA adenylaci
  - DNA deglykosylaci
  - DNA štěpení

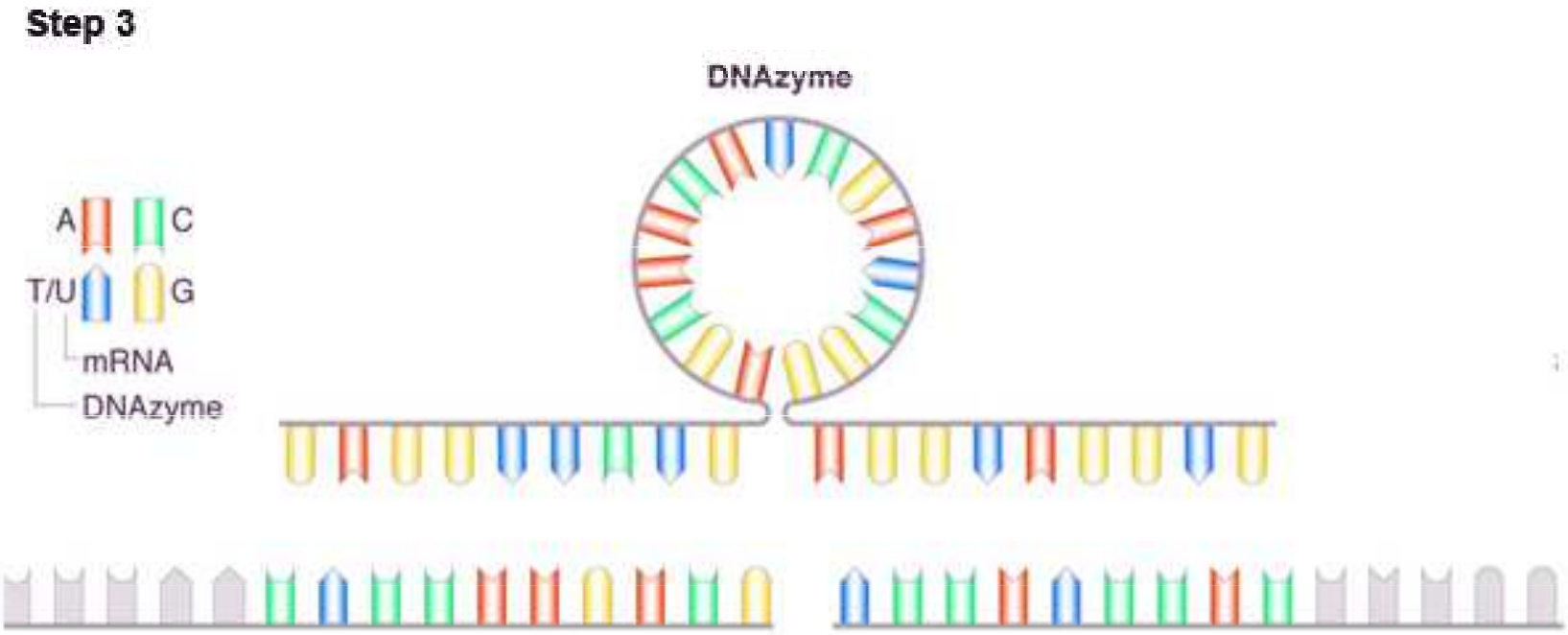
10-23 DNAzym



10-23 DNAzym



10-23 DNAzym



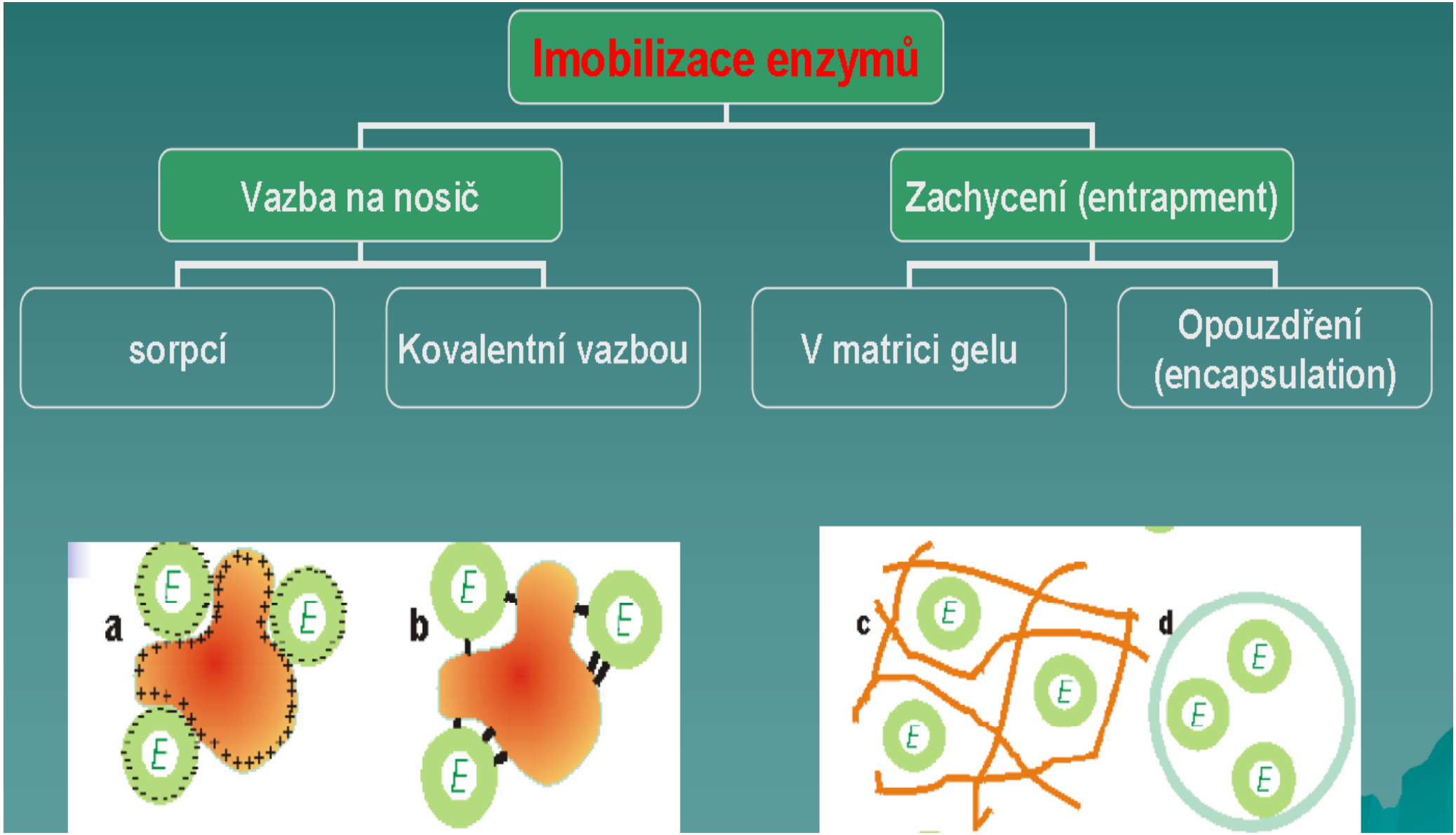
Využití enzymů

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

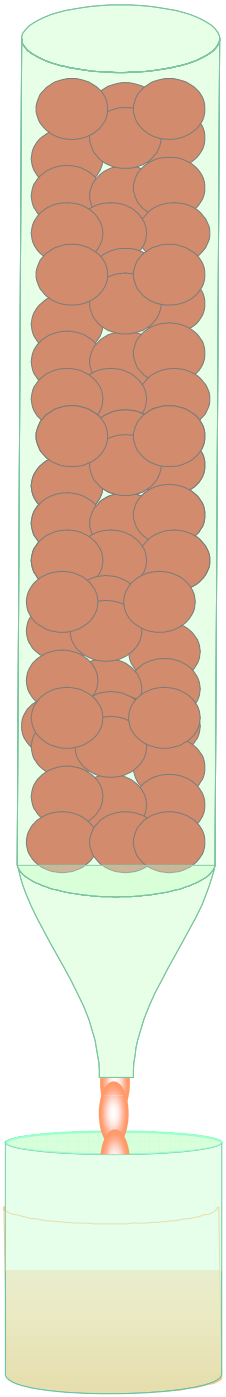
Využití enzymů

- Celé buňky
- Extrakty z buněk
- Enzymy volné *versus* imobilizované

Volné *versus* imobilizované enzymy



Výhody imobilizovaných enzymů



Stabilita enzymů vzrůstá v imobilizovaném stavu

Imobilizované enzymy mohou být používány opakovaně → pokles nákladů

Produkt reakce není kontaminován enzymem → odpadá potřeba purifikace

Imobilizované enzymy mohou být použity v kontinuálních procesech

Využití enzymů – celé buňky

- Nejstarší metody
- Potravinářství
  - Výroba sýrů a jogurtů (*Lactobacillus*)
  - Výroba piva a vína (*Saccharomyces cerevisiae*)
  - Výroba octa (*Saccharomyces cerevisiae*)
- Chemické výroby
  - Výroba kyseliny citronové (*Aspergillus niger*)
  - Výroba antibiotik (plísně)
  - Výroba vitaminů, steroidů a aminokyselin
- Těžké technologie
  - Čištění odpadních vod
  - Zpracování rud

Rhodanasa (EC 2.8.1.1)

$$CN^- + S_2O_3^{2-} \rightarrow SCN^- + SO_3^{2-}$$

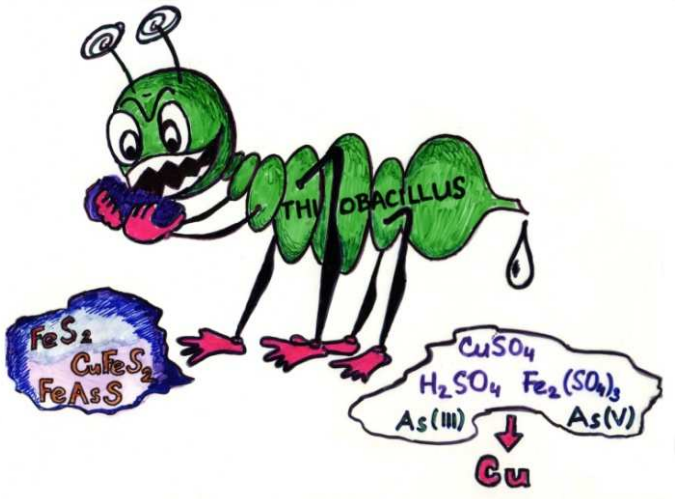
Homo sapiens

Detoxikace kyanidu - otravy
- cigaretový kouř
- glykosinoláty *Brassicacceae*

Acidithiobacillus ferrooxidans

Biohydrometalurgie

Životní prostředí



Využití enzymů – isolované enzymy

- Široká paleta enzymových preparátů

- Invertasa – výroba invertovaného cukru
- Proteasy, lipasy – prací prostředky
- DNA-polymerasy, restrikční endonukleasy, ligasy – genové technologie
- β-galaktosidasa – odstraňování laktosy z mléka

- Další možná využití:
  - chemické synthesy

Organické syntézy

+ - specifita (stereospecifita)
- neextrémní podmínky (ekonomika, ŽP)

- - malá stabilita
- nevodná prostředí
- omezená dostupnost (cena)
- regenerace

Nejvýznamnější technické aplikace enzymů

Proteolytické enzymy

- Biodetergenty (termostabilní, alkalické bakteriální proteasy)
- Mlékárenský průmysl (chymosin z telecích žaludků → specifická proteolýza kapa-kaseinu → tvorba sýřeniny)
- Krmivářský průmysl → výroba technických hydrolyzátů bílkovin
- Masný průmysl → tenderizace (změkčení) masa (rostlinná proteasa papain)
- Pivovarnictví → enzymové stabilizátory piva (odstraňování chladových zákalů)

Nejvýznamnější technické aplikace enzymů

Amylasy

- α-amylasy → hydrolýza 1,4-α-glukosidických vazeb uvnitř polysacharidové molekuly
- Ztekucování škrobu (nezbytné při následné výrobě glukosových syrupů a glukosy)
- Součást biodetergentů (odstraňování škrobového pojidla z textilních vláken)

Nejvýznamnější technické aplikace enzymů (glykosidasy)

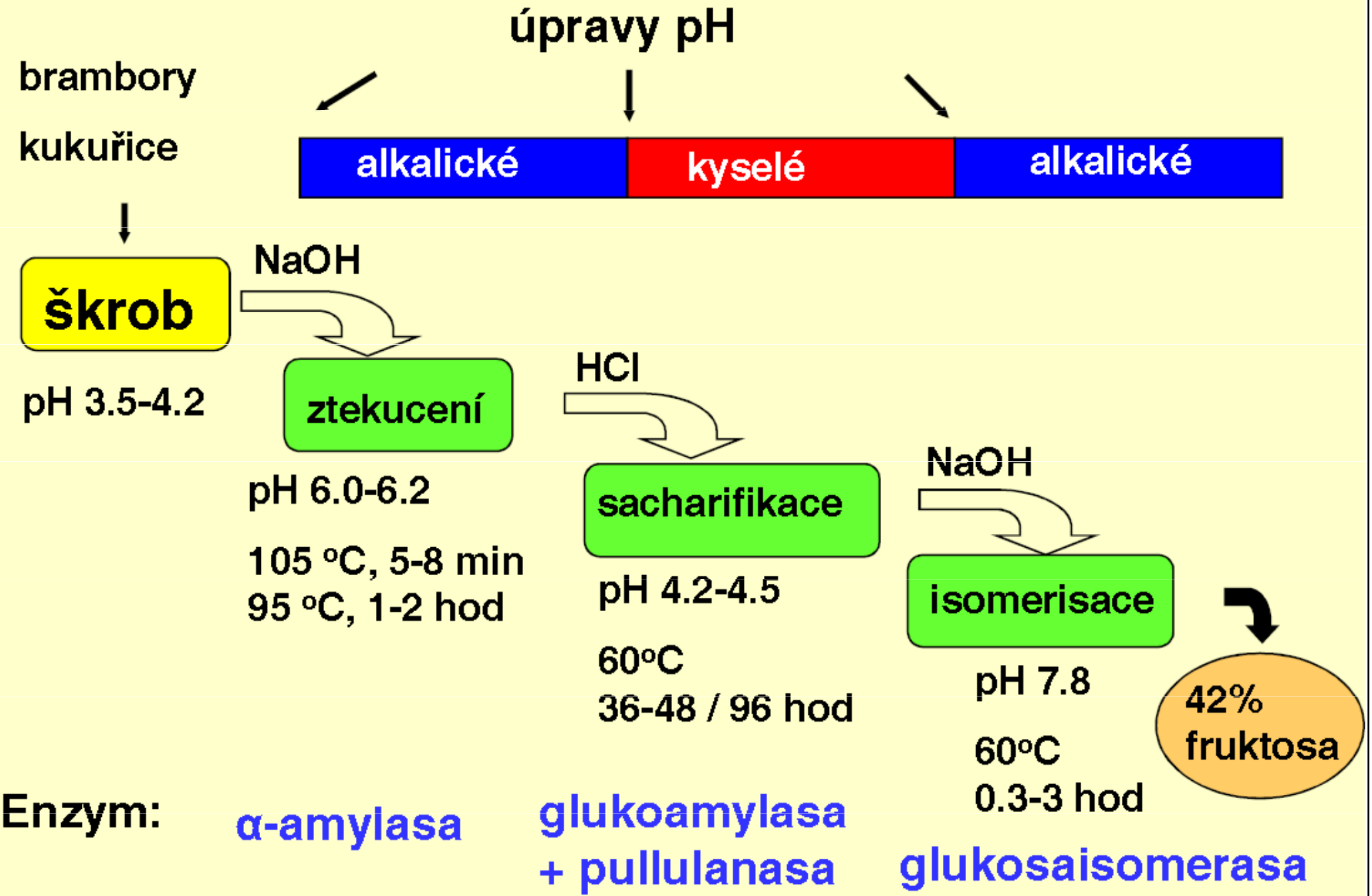
- β-amylasy → odštěpují maltosové jednotky z neredukujícího konce polysacharidového řetězce
- Glukoamylasa → odštěpuje glukosové jednotky od neredukujícího konce (zpracování škrobu na škrobové sirupy; odbourávání zbytkových dextrinů v pivu → vyšší stupeň prokvašení, diabetické pivo)
- Invertasa → hydrolýza sacharosy na glukosu a fruktosu, výroba invertního cukru
- β-galaktosidasa → hydrolýza laktosy na glukosu a galaktosu (výroba delaktosovaného mléka, mléko pro výrobu zmrzliny (zabránění krystalizace laktosy)

Nejvýznamnější technické aplikace enzymů

Glukosaisomerasa (xylosaisomerasa)

- Isomerace glukosy na fruktosu
- Výroba fruktosových sirupů (42 % - 55 % fruktosy) z glukosových sirupů (zejména z kukuřičných a obilních škrobů) → vyšší sladivost

Sacharidy ze škrobu



Nejvýznamnější technické aplikace enzymů

Celulasy

- Komplexní enzymový systém katalyzující hydrolýzu celulosy
- Celulasa z *Trichoderma viridae* → odbourání nativní celulosy
- Zpracování celulosové suroviny (dřevěné odpady, odpadní papír)
- Výroba instatních potravin (káva, čaj), digestiva v krmných směsích, zvýšení účinnosti extrakce šťav z rostlinných materiálů

Nejvýznamnější technické aplikace enzymů

Lipasy

- Biodetergenty
- Ovlivnění chuti a vůně potravinářských výrobků (sýrařství !!!)
- Součást digestivních přípravků

Příklady lékařských aplikací enzymů

- Fibrinolýza (cílené rozpouštění krevních sraženin) → plasmin, streptokinasa, urokinasa (aktivátory plasminogenu)
- Cílená tvorba krevních sraženin → thrombin
- Trávicí enzymy
- Trypsin → čištění ran od hnisu
- Lysozym → oční kapky

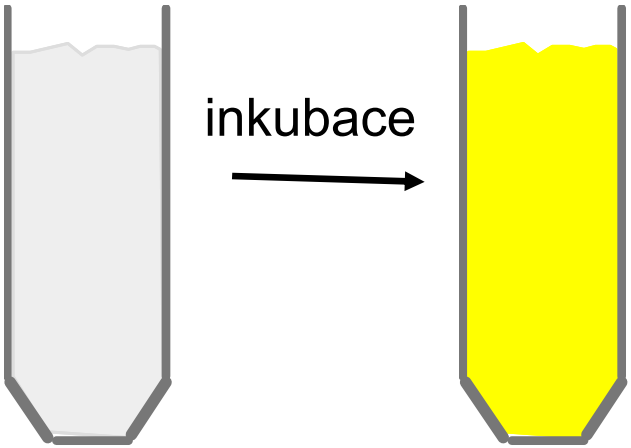
Enzymy jako analytická čidla

- Specifita – reagují pouze s daným substrátem
- Citlivost
- Analýza nepřečištěným vzorků – tělní tekutiny

Analytická biochemie

- Stanovení substrátů
- Stanovení inhibitorů
- Stanovení aktivity enzymů

End-point versus kinetické stanovení

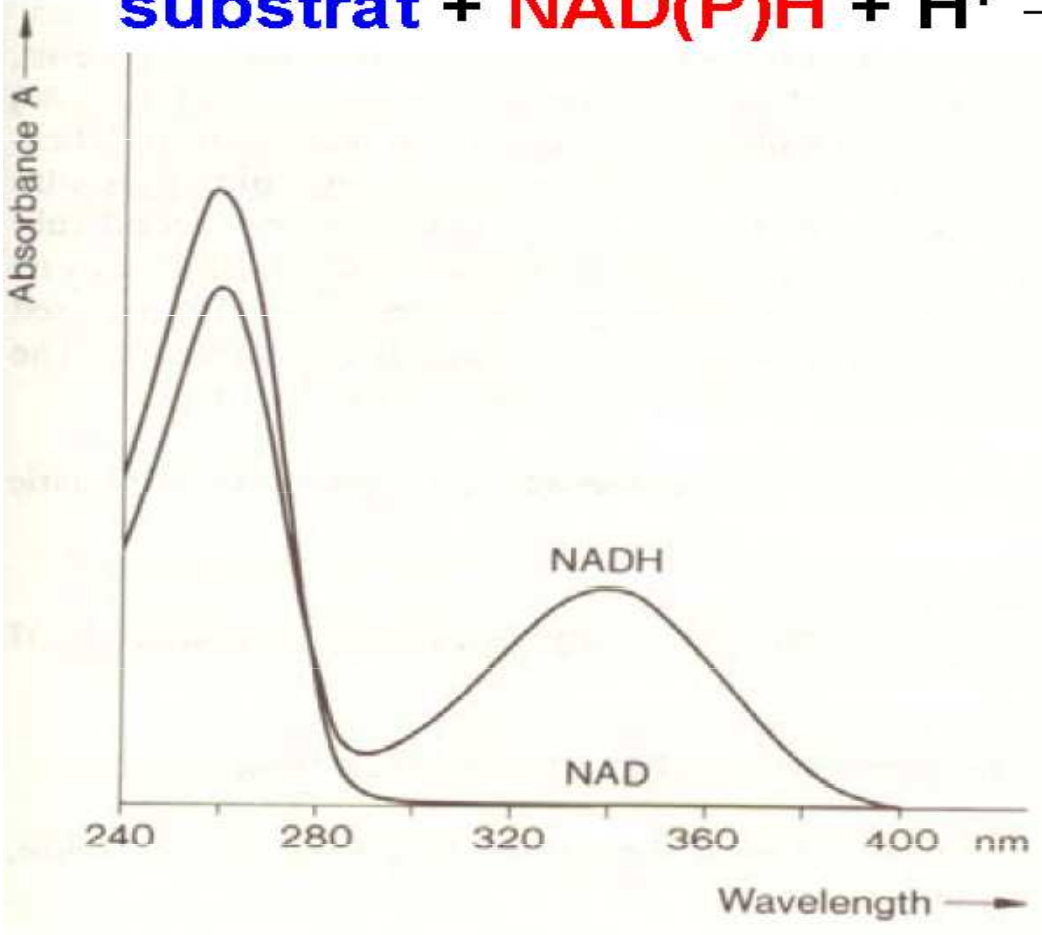


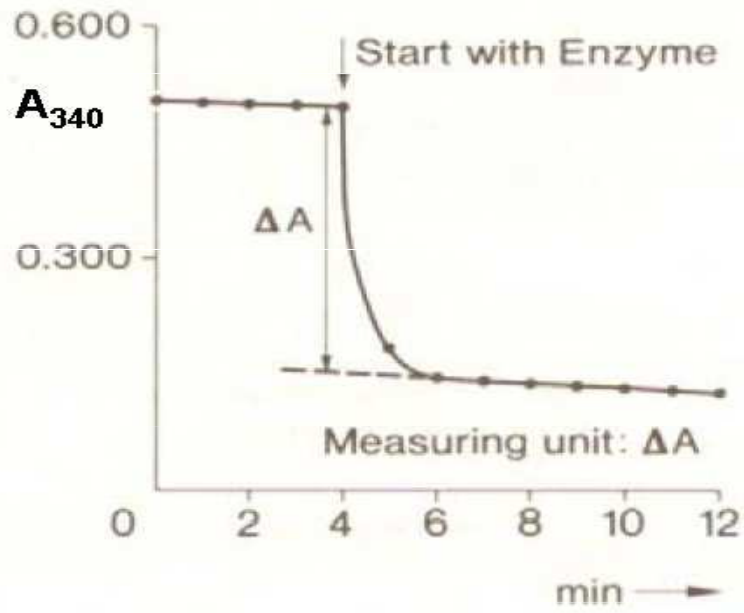
$A \rightarrow P$

Reakce v roztoku

substrát + NAD(P)$^+$ → produkt + **NAD(P)H** + H$^+$

substrát + **NAD(P)H** + H$^+$ → produkt + NAD(P)$^+$

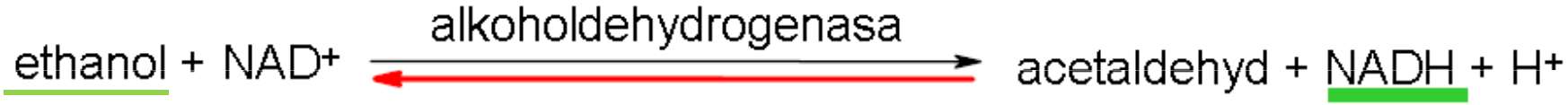




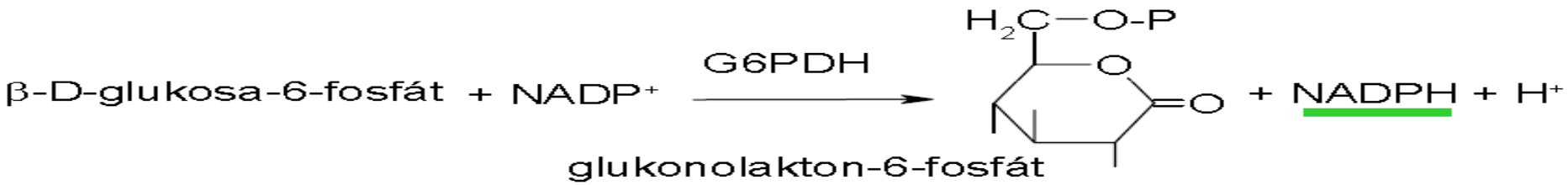
Výpočet látkového množství substrátu:

$$n = \frac{V \cdot \Delta A}{\varepsilon_{NADH} \cdot l}$$

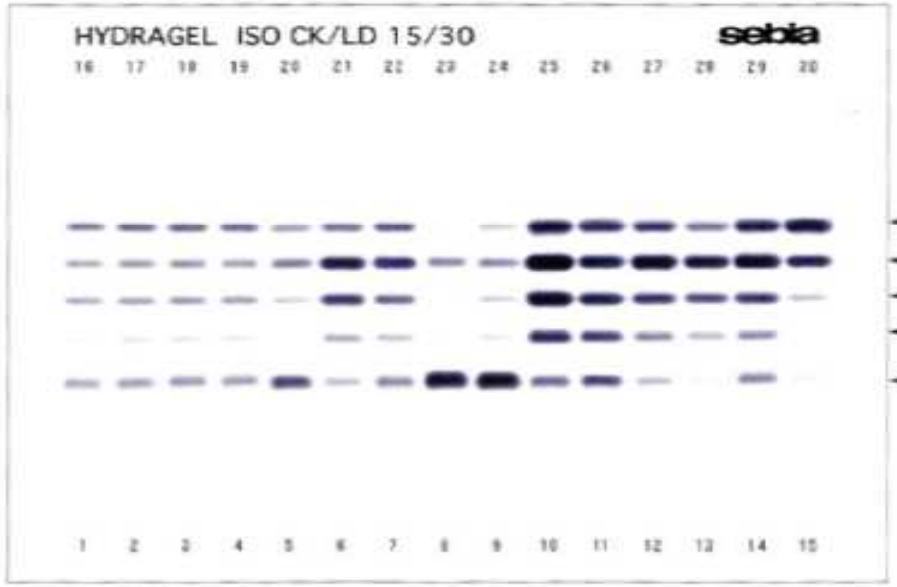
- Přímé stanovení

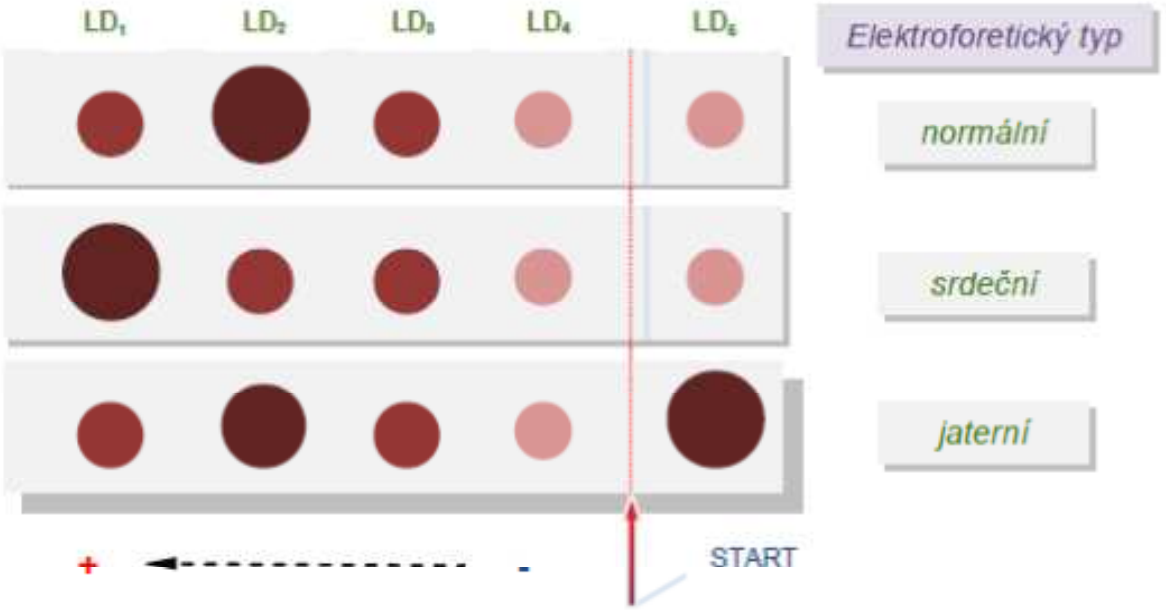


- Pomocná reakce



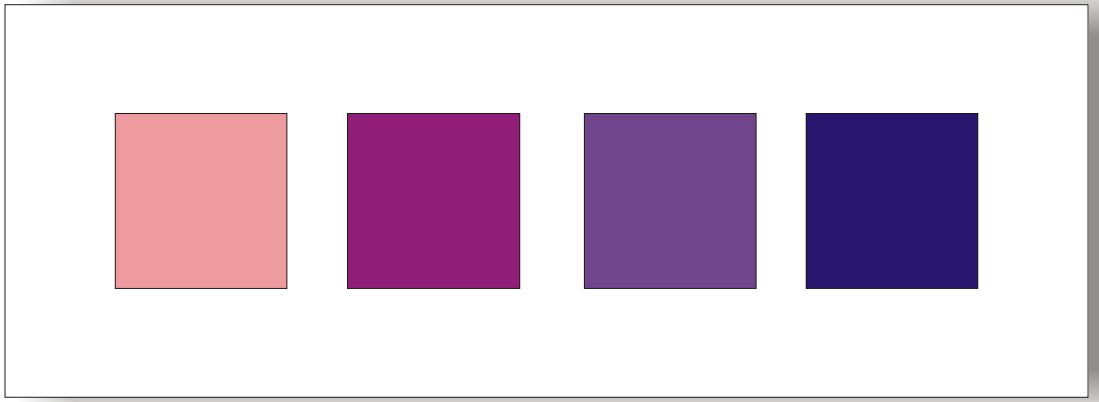
Izoenzymy LHD





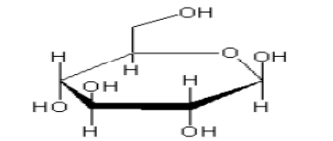
Automatické analyzátory

Diagnostické proužky

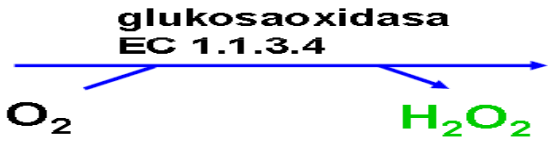


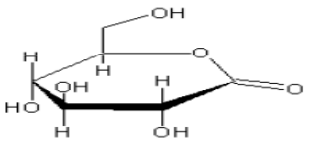
žádná glukosa

Zvyšující se množství glukosy →

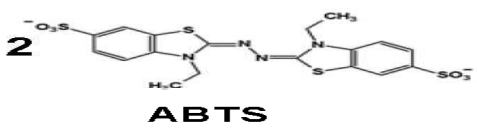


β-D-glukosa

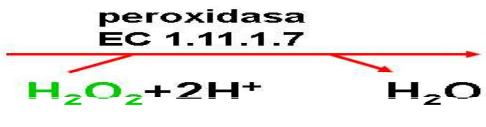


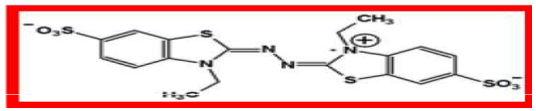


D-glukono-1,5-lakton



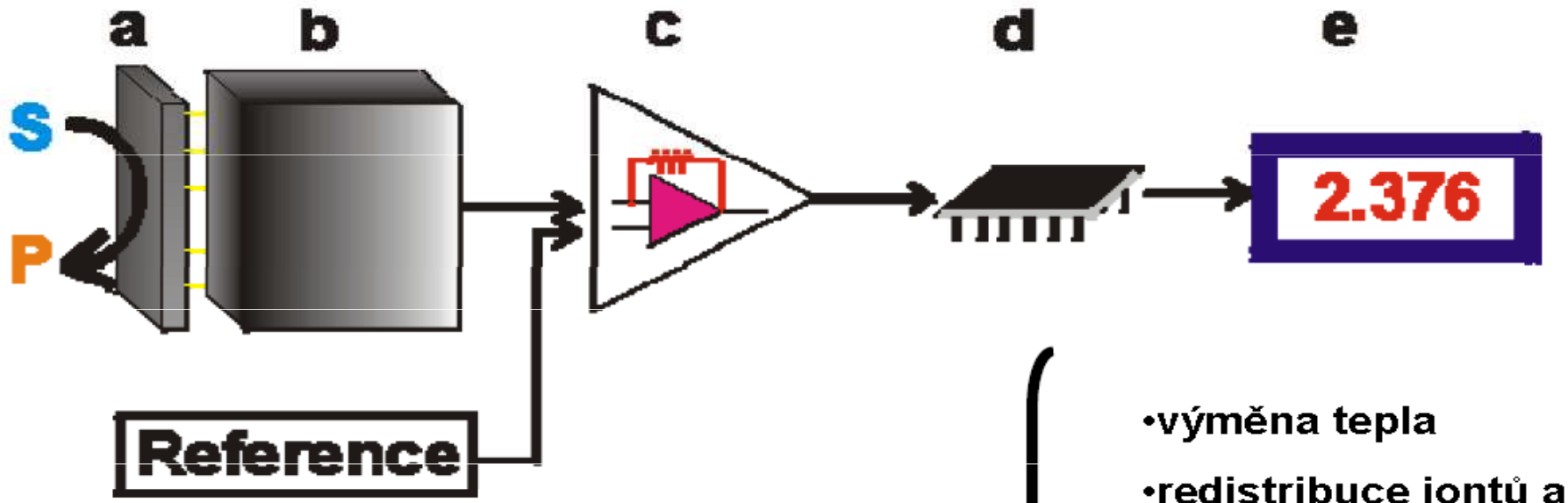
ABTS





λ_{max} = 417, 645, 728 nm

Biosenzory



a) Biokatalyzátor (přeměna substrátu na produkt)

b) Převodník (generování elektrického signálu)

c) Zesilovač (zesílení signálu)

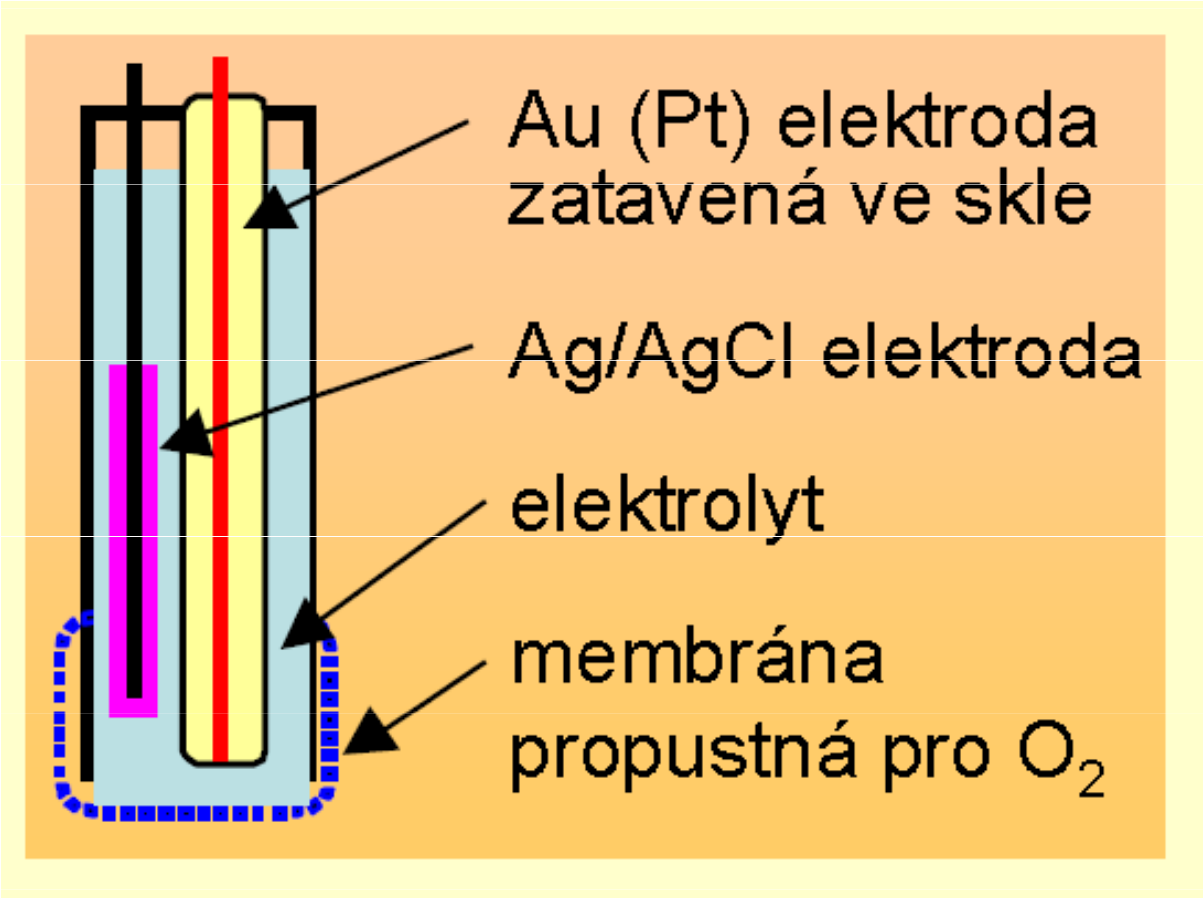
d) Procesor (vyhodnocení signálu)

e) Výstup výsledku

- výměna tepla
- redistribuce iontů a změna elektrického potenciálu
- výměna elektronů
- změna vodivosti
- změna optických vlastnosti
- změna hmotnosti

Amperometrické biosenzory

Leland C. Clark Jr.
1956



glucose oxidase

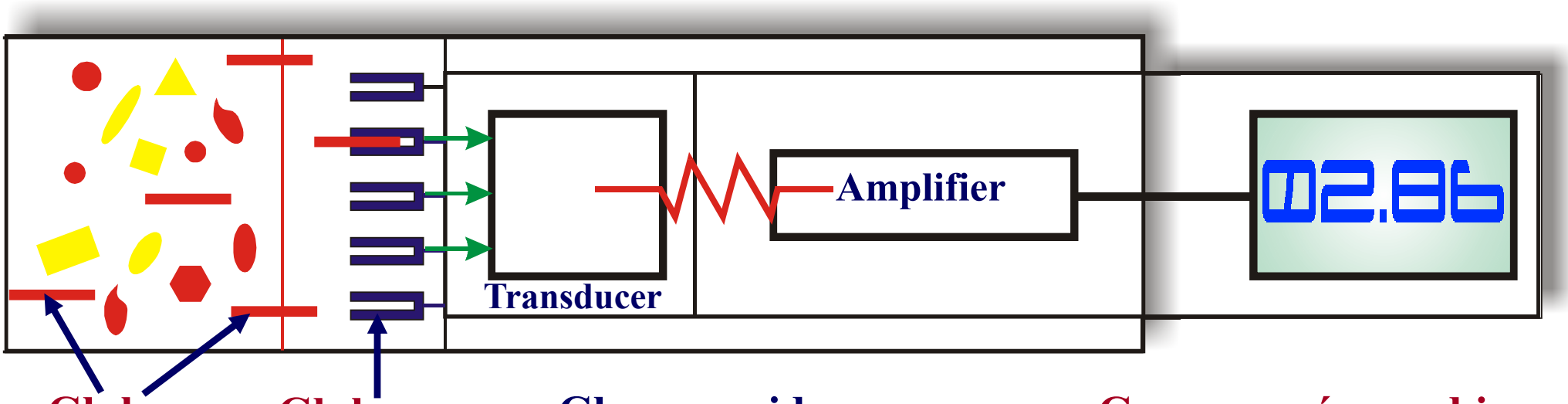
$$\text{D-glucose} + O_2 \longrightarrow \text{D-glucono-1,5-lactone} + H_2O_2$$

Biosensor pro glukosu

Enzymová elektroda na jedno použití

Měřící jednotka

Biosensor na glukosu



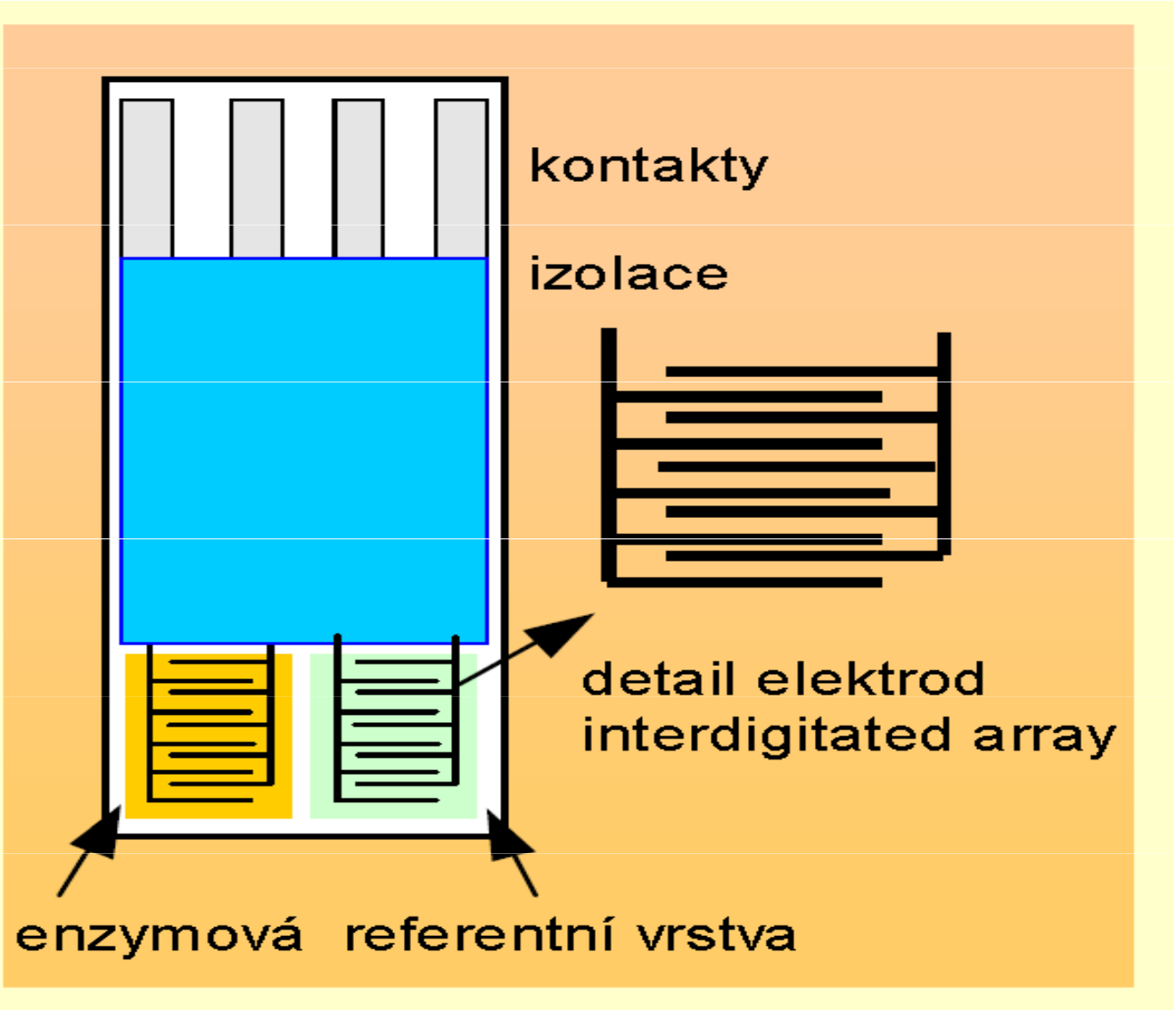
Glukoza
V krvi

Glukoza
oxidáze

Glucose oxidase
oxiduje glukozu
za uvolnění
elektronů – které
jsou detekovány převodníkem
A převedeny na elektrický proud

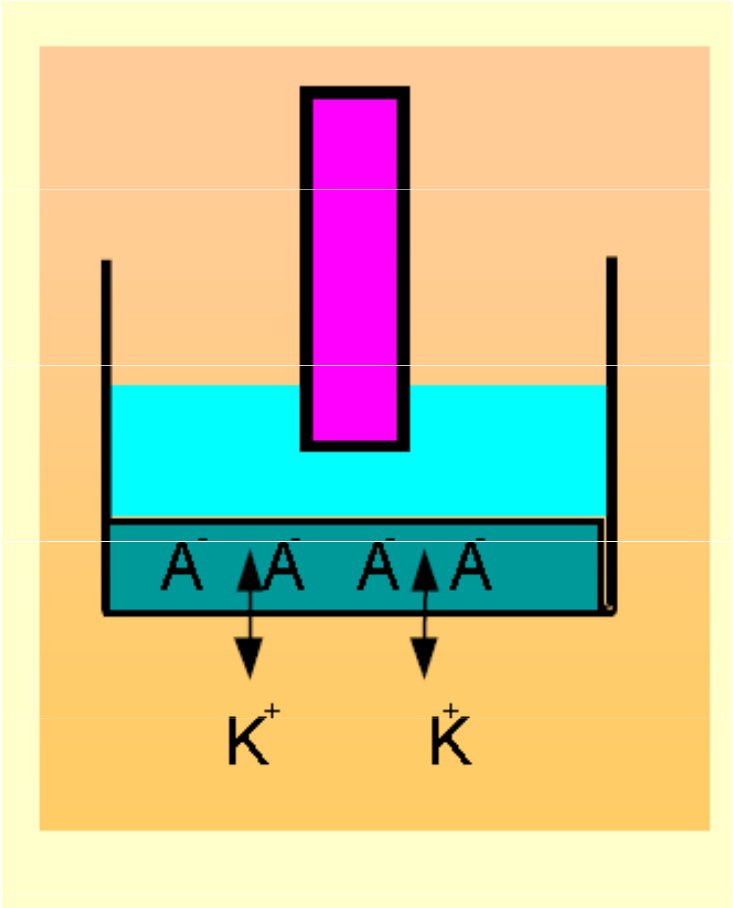
Generovaný proud je
proporcionální
množství glukózy
znázorněnému na display

Konduktometrické biosenzory



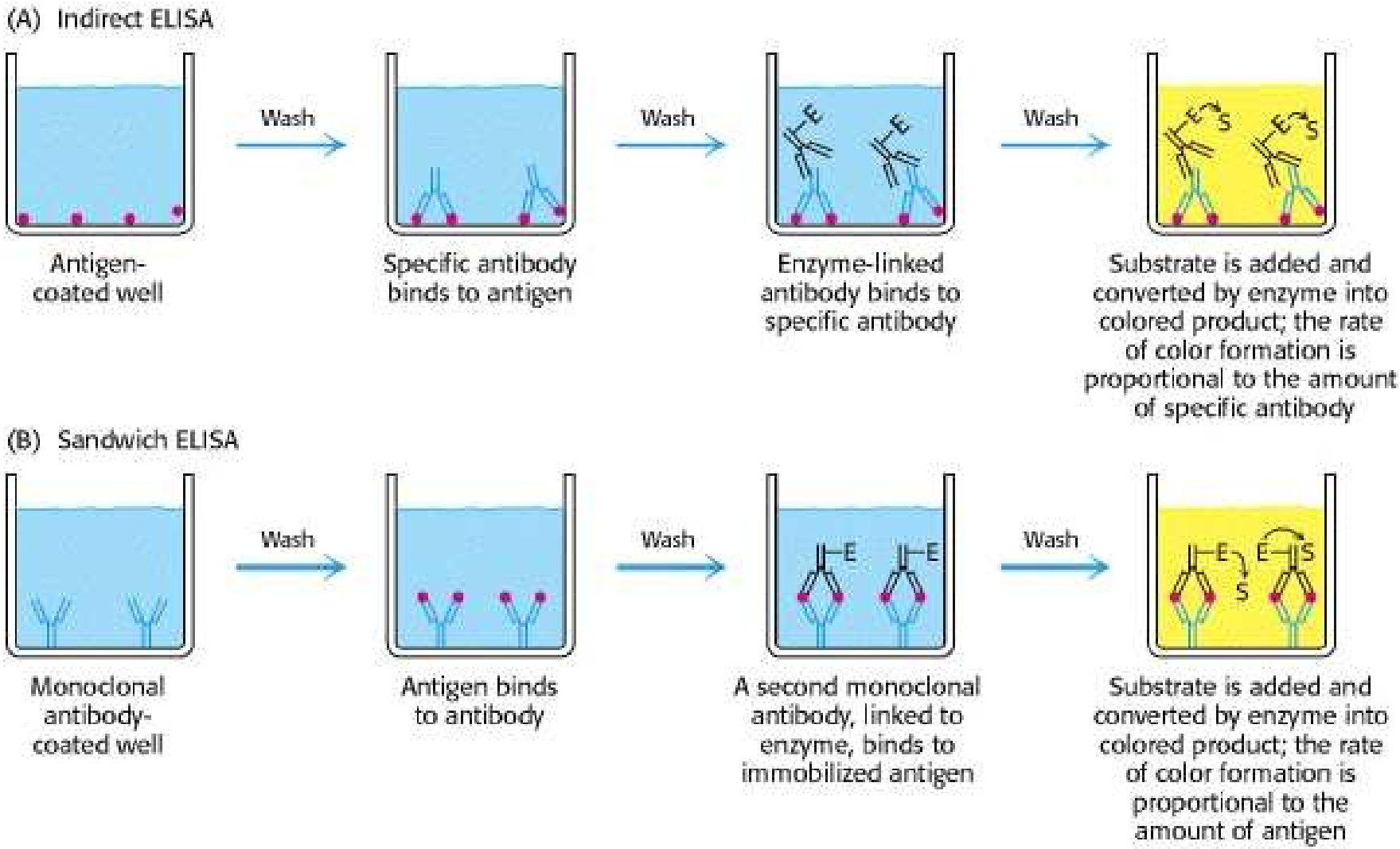
$$NH_2CONH_2 + 3\,H_2O \longrightarrow 2\,NH_4^+ + HCO_3^- + OH^-$$

Potenciometrické biosenzory

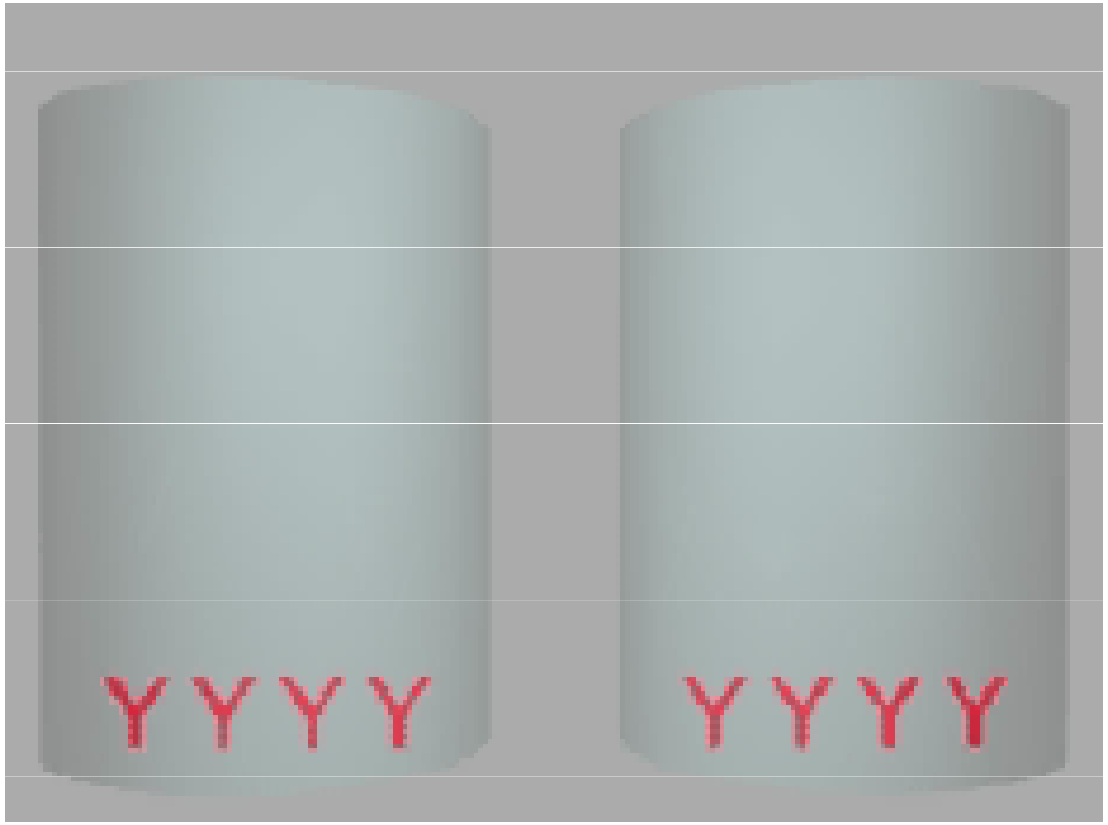


$$NH_2CONH_2 + 3\,H_2O \longrightarrow 2\,NH_4^+ + HCO_3^- + OH^-$$

ELISA

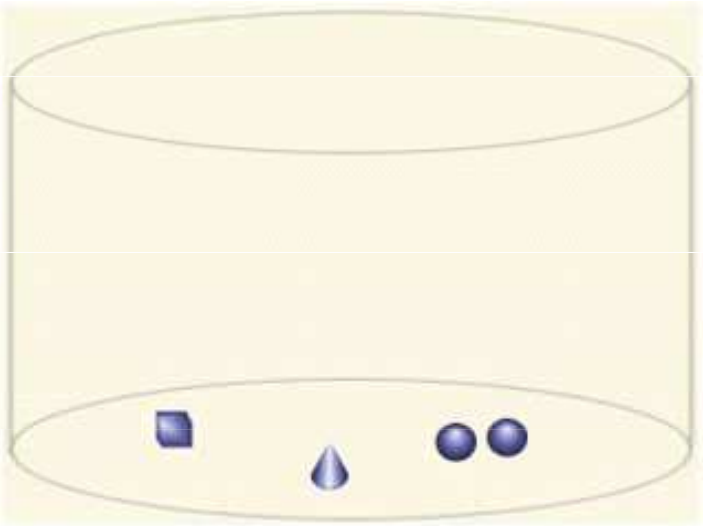

(A) Indirect ELISA
Wash
Wash
Wash
Antigen-coated well
Specific antibody binds to antigen
Enzyme-linked antibody binds to specific antibody
Substrate is added and converted by enzyme into colored product; the rate of color formation is proportional to the amount of specific antibody
(B) Sandwich ELISA
Wash
Wash
Wash
Monoclonal antibody-coated well
Antigen binds to antibody
A second monoclonal antibody, linked to enzyme, binds to immobilized antigen
Substrate is added and converted by enzyme into colored product; the rate of color formation is proportional to the amount of antigen

ELISA – stanovení antigenu

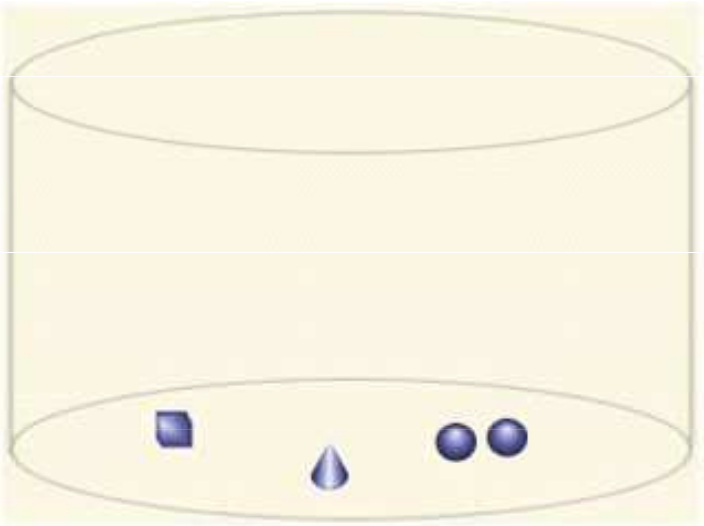


ELISA – stanovení protilátky

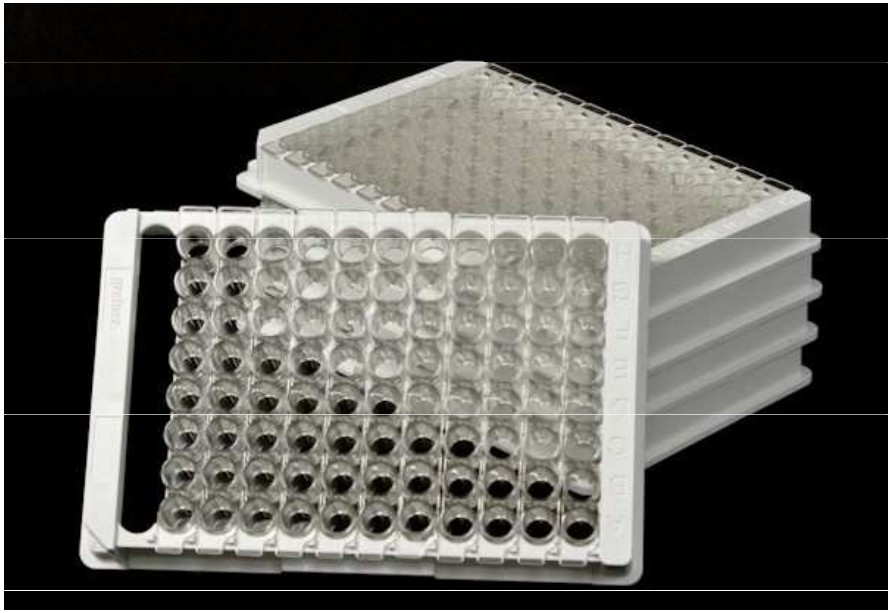
negativní



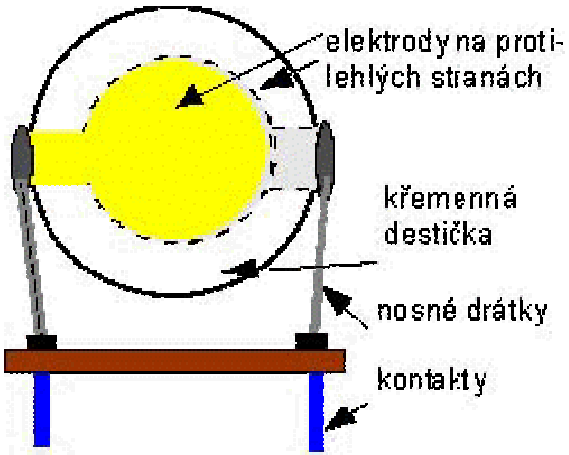
pozitivní

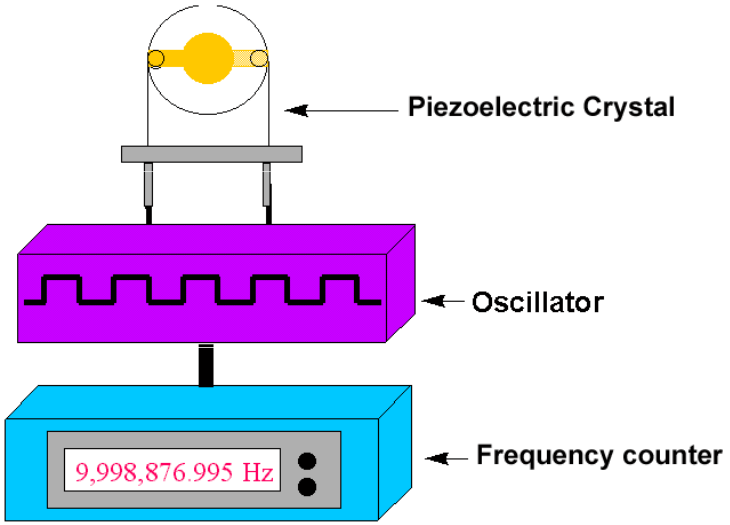


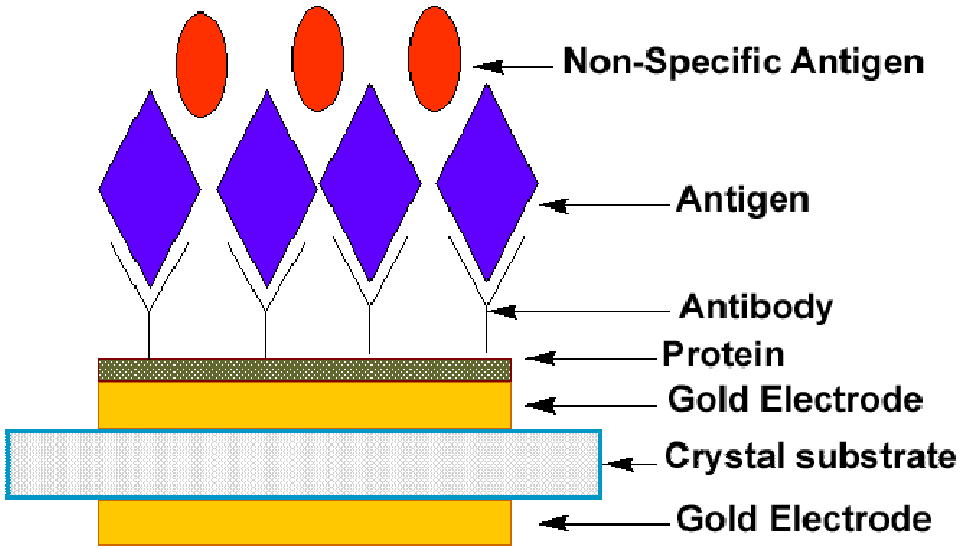
ELISA - vybavení



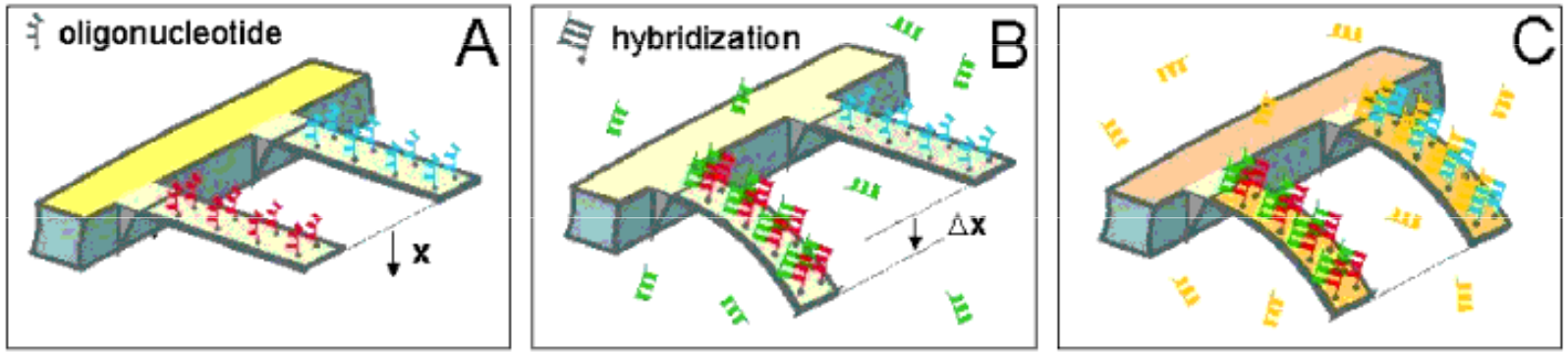
Piezoelektrický biosenzor

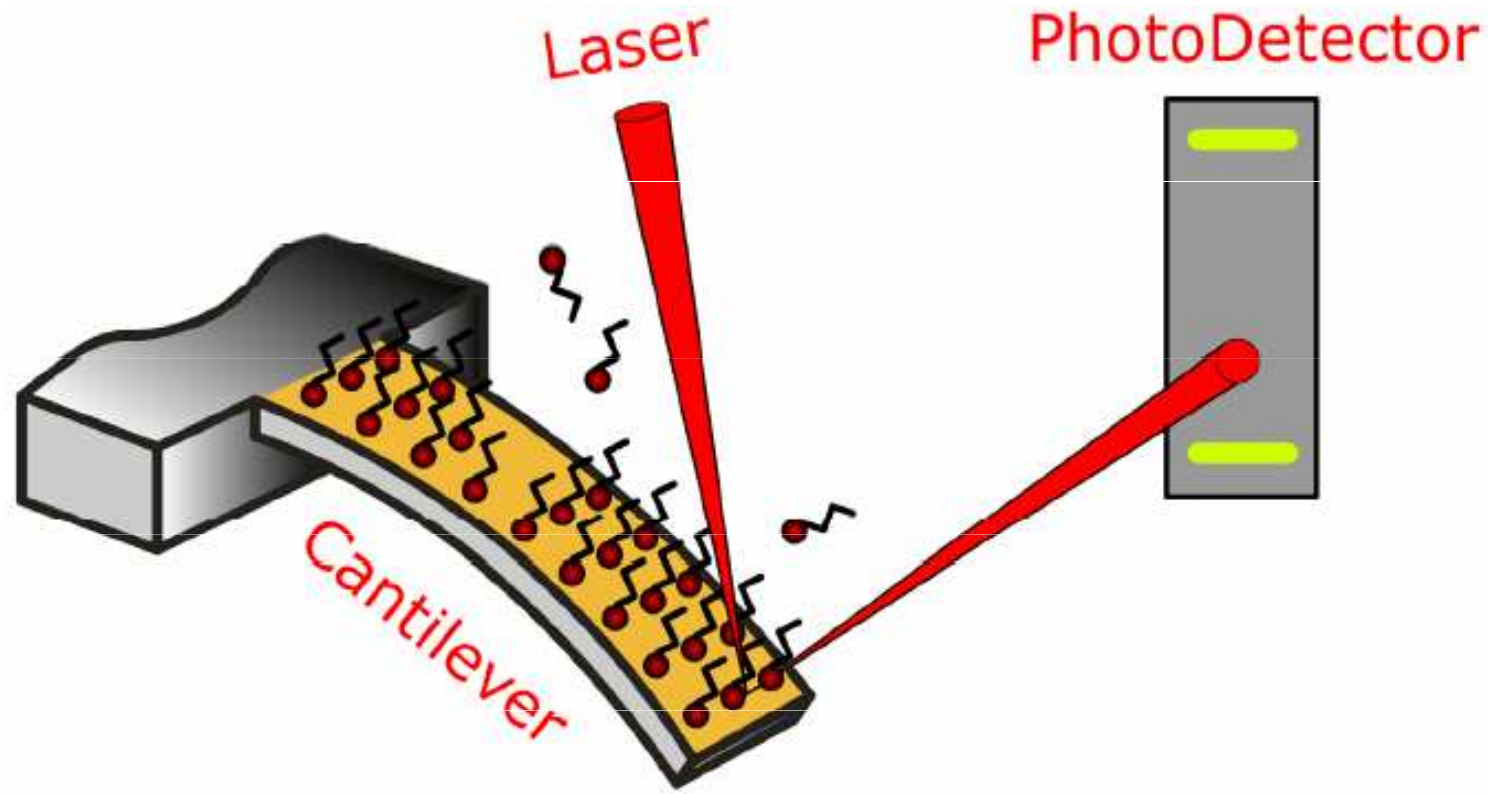






Kantilever


oligonucleotide
A
x
hybridization
B
Δx
C


Laser
PhotoDetector
Cantilever