

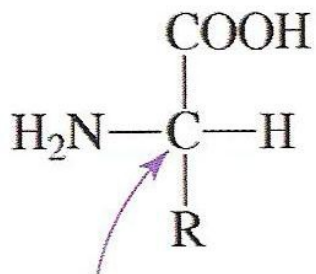
Biološke makromolekule

Aminokisljine

Peptidi in proteini

Encimi

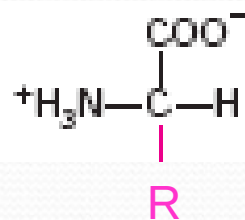
Aminokisljine



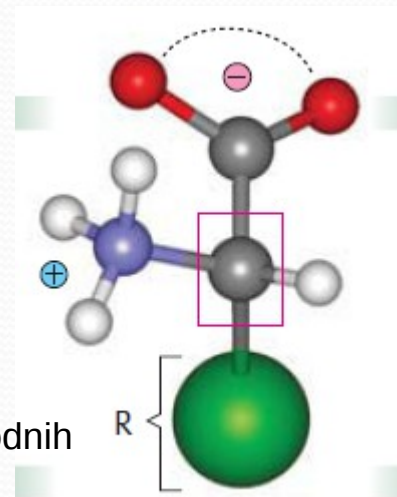
α -ogljik

Asimetrični α -ogljikov atom

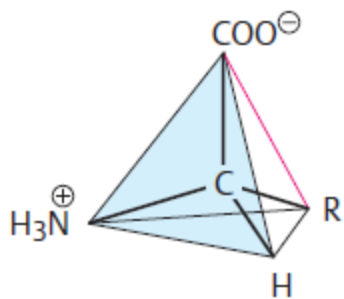
ion dvojček oz. zwitterion



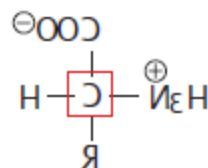
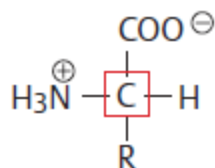
Pri fiziološkem pH (7,4) so AK v vodnih raztopinah v dipolarni ionski obliki



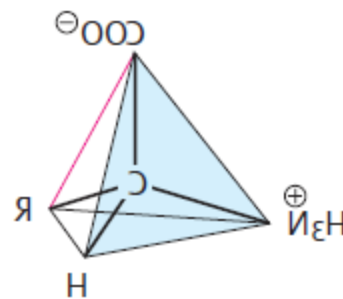
2 enantiomeri: D in L



L-Amino acid



Fischer projections



D-Amino acid
(mirror image)

Referenčna spojina za D,L konfiguracijo: gliceraldehid, referenčni atom v AK: C v COOH

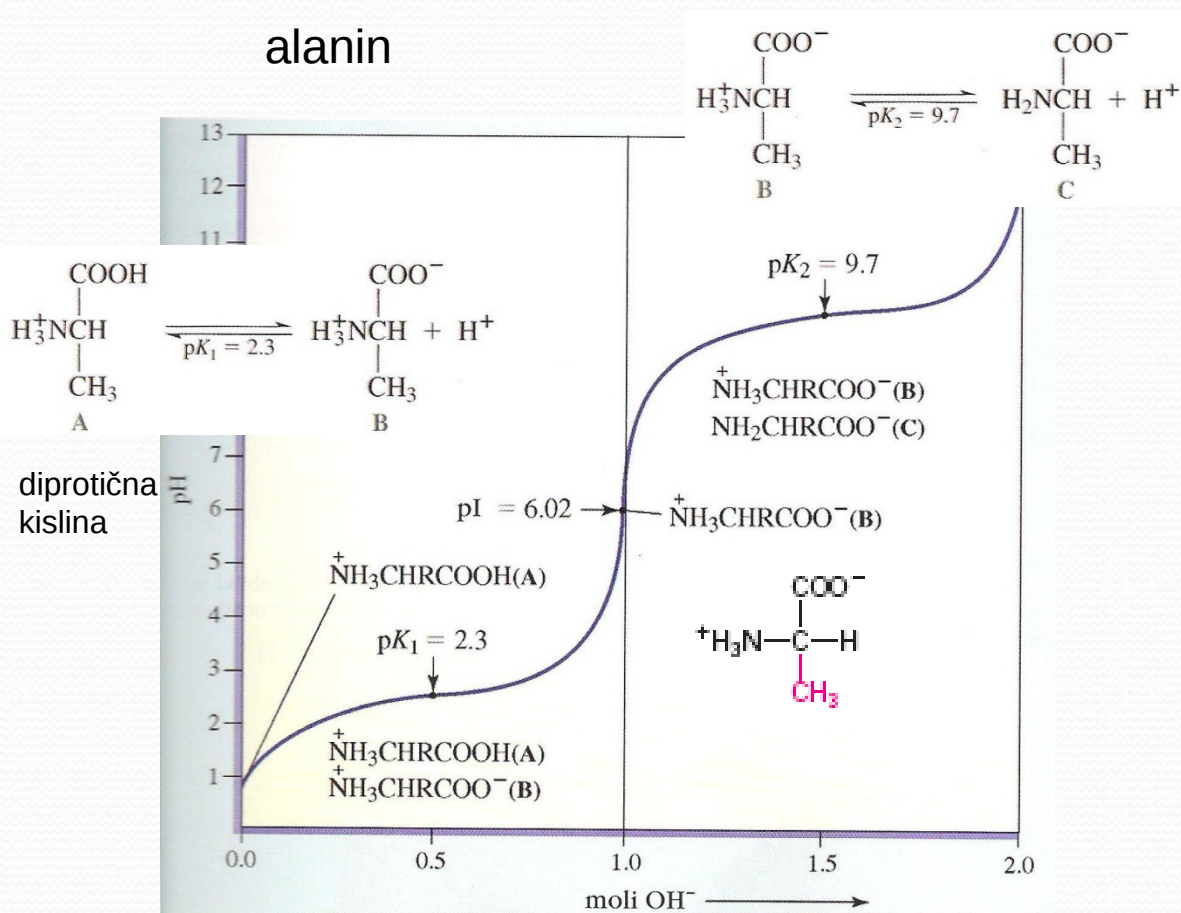
Aminokisljine

- 20 AK v proteinih
- V proteinih le L-izomere, v naravi tudi D-enantiomere
- Lastnosti AK:
 - Bele kristalinične snovi z visokim tališčem
 - Topne v vodi; v vodi ionizirane
 - Netopne v organskih topilih (aceton, eter, kloroform)
 - Vodne raztopine AK prevajajo električni tok
 - Kislinsko-bazične lastnosti (amfoterne spojine)

Kislinsko-bazične lastnosti

AK

alanin



ime	pK_1	pK_2	pK_R
glicin	2,4	9,8	
alanin	2,3	9,9	
valin	2,3	9,6	
levcin	2,4	9,6	
izolevcin	2,4	9,7	
metionin	2,3	9,2	
fenilalanin	1,8	9,1	
prolin	2,0	10,6	
serin	2,1	9,2	
treonin	2,6	10,4	
cistein	1,8	10,8	8,3
asparagin	2,0	8,8	
glutamin	2,2	9,1	
tirozin	2,2	9,1	10,9
triptofan	2,4	9,4	
aspartat	2,0	10,0	3,9
glutamat	2,2	9,7	4,3
histidin	1,8	9,2	6,0
lizin	2,2	9,2	10,8
arginin	1,8	9,0	12,5

* Vrednosti pK_1 se nanašajo na α -karboksilno skupino, vrednosti pK_2 na α -aminsko skupino in vrednosti pK_R na skupine, ki lahko ionizirajo v stranski verigi (R).

titracijska krivulja

Izoelektrična točka

- **izoelektrična točka pI**

- pH vrednost, pri kateri je aminokislina v obliki zwitteriona (iona dvojčka),
- molekula je električno nevtralna,
- v električnem polju se ne giblje
- pI AK brez ionizirajočih stranskih skupin

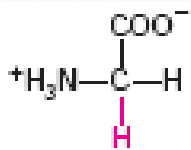
$$pI = \frac{1}{2}(pK_1 + pK_2)$$

Amino Acid	pI	pK ₁ (α-COOH)	pK ₂ (α-NH ₃ ⁺)
Alanine	6.01	2.35	9.87
Cysteine	5.05	1.92	10.70
Aspartic acid	2.85	1.99	9.90
Glutamic acid	3.15	2.10	9.47
Phenylalanine	5.49	2.20	9.31
Glycine	6.06	2.35	9.78
Histidine	7.60	1.80	9.33
Isoleucine	6.05	2.32	9.76
Lysine	9.60	2.16	9.06
Leucine	6.01	2.33	9.74
Methionine	5.74	2.13	9.28
Asparagine	5.41	2.14	8.72
Proline	6.30	1.95	10.64
Glutamine	5.65	2.17	9.13
Arginine	10.76	1.82	8.99
Serine	5.68	2.19	9.21
Threonine	5.60	2.09	9.10
Valine	6.00	2.39	9.74
Tryptophan	5.89	2.46	9.41
Tyrosine	5.64	2.20	9.21

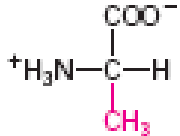
- Nad pI: skupen naboj je negativen (pomik proti anodi)
- Pod pI: skupen naboj je pozitiven (pomik proti katodi)

Aminokisljine

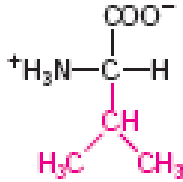
nepolarne aminokisljine



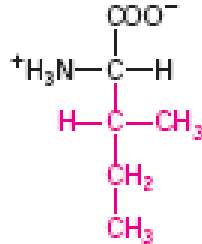
glicin
(Gly ali G)



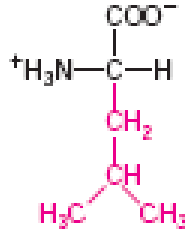
alanin
(Ala ali A)



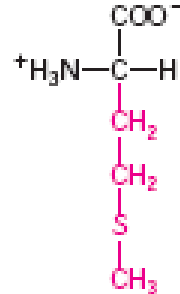
valin
(Val ali V)



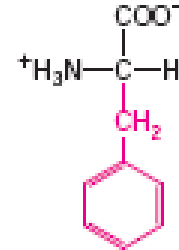
izolevcin
(Ile ali I)



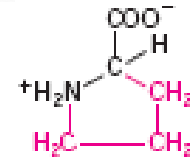
levcin
(Leu ali L)



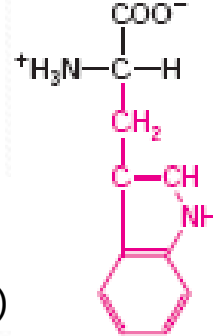
metionin
(Met ali M)



fenilalanin
(Phe ali F)



prolin
(Pro ali P)

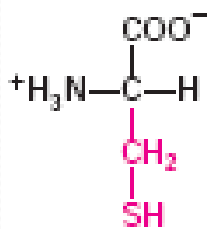


triptofan
(Trp ali W)

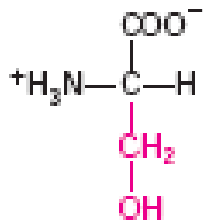
alifatske oz. aromatske stranske skupine, hidrofoben značaj,
običajno v notranjosti proteinov

Aminokisljine

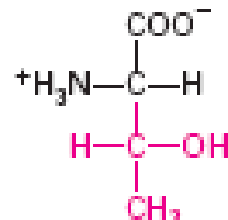
polarne, nenabite aminokisljine



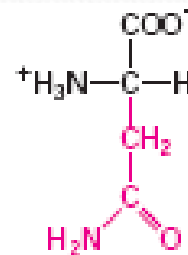
cistein
(Cys ali C)



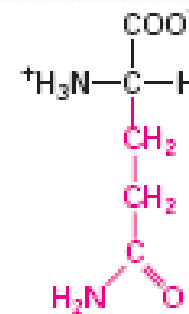
serin
(Ser ali S)



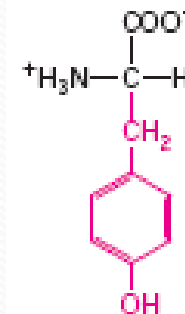
treonin
(Thr ali T)



asparagin
(Asn ali N)



glutamin
(Gln ali Q)



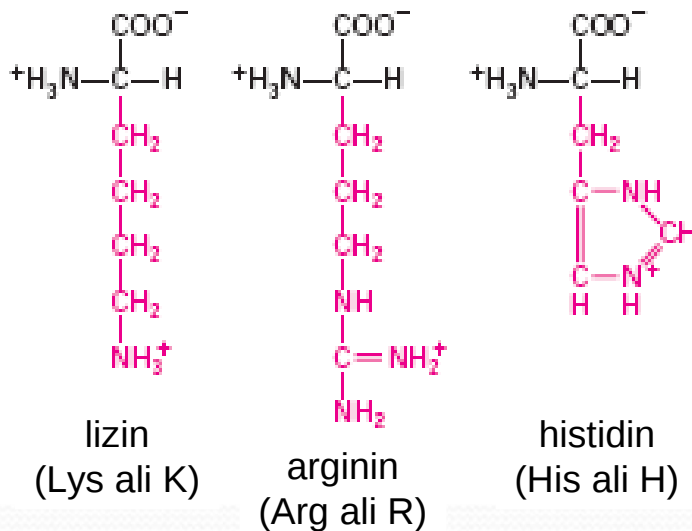
tirozin
(Tyr ali Y)

prisotnost heteroatoma (N, O ali S), ki omogoča nastanek vodikovih vezi

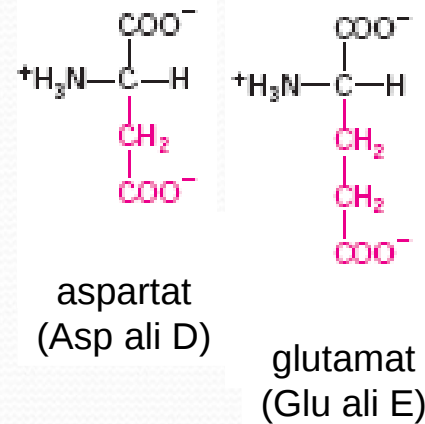
Aminokisljine

polarne, nabite aminokisljine pri fiziološkem pH

bazične



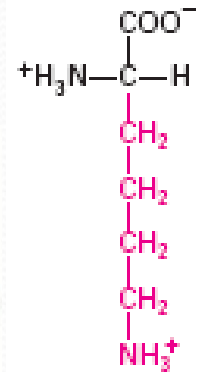
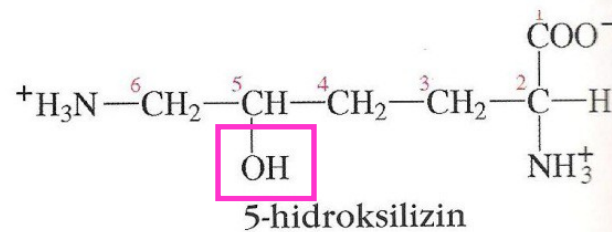
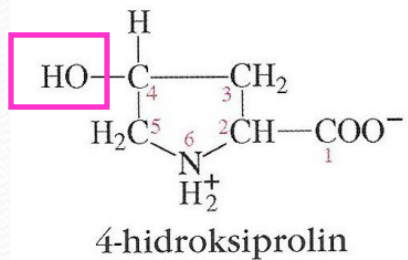
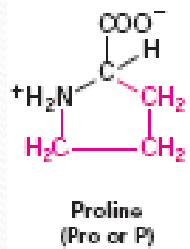
kisle



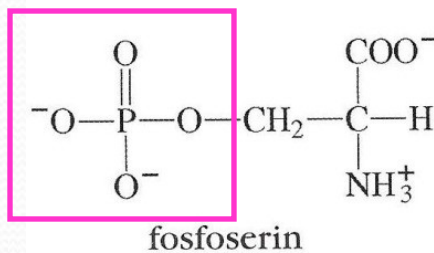
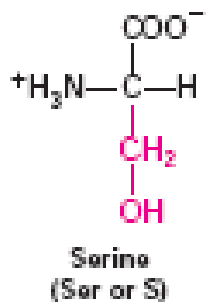
pogosto na površini proteinov

Neto naboj Asp in Glu pri pH 7.4 je -1 , neto naboj Lys, Arg in His pa $+1$.

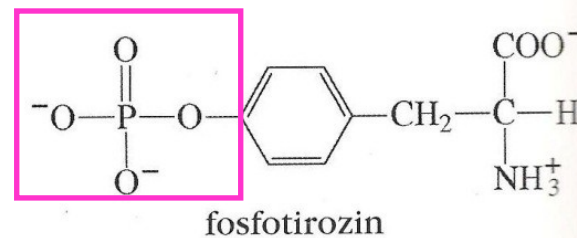
Modificirane AK v proteinih



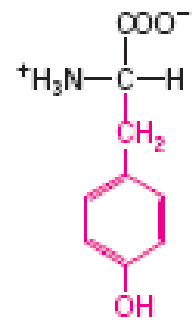
Lysine
(Lys or K)



fosfoserin



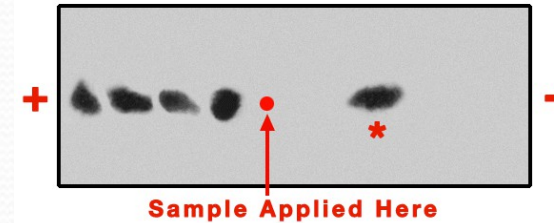
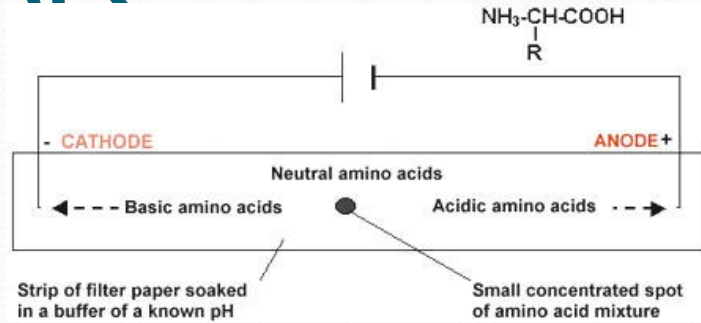
fosfotirozin



Tyrosine
(Tyr or Y)

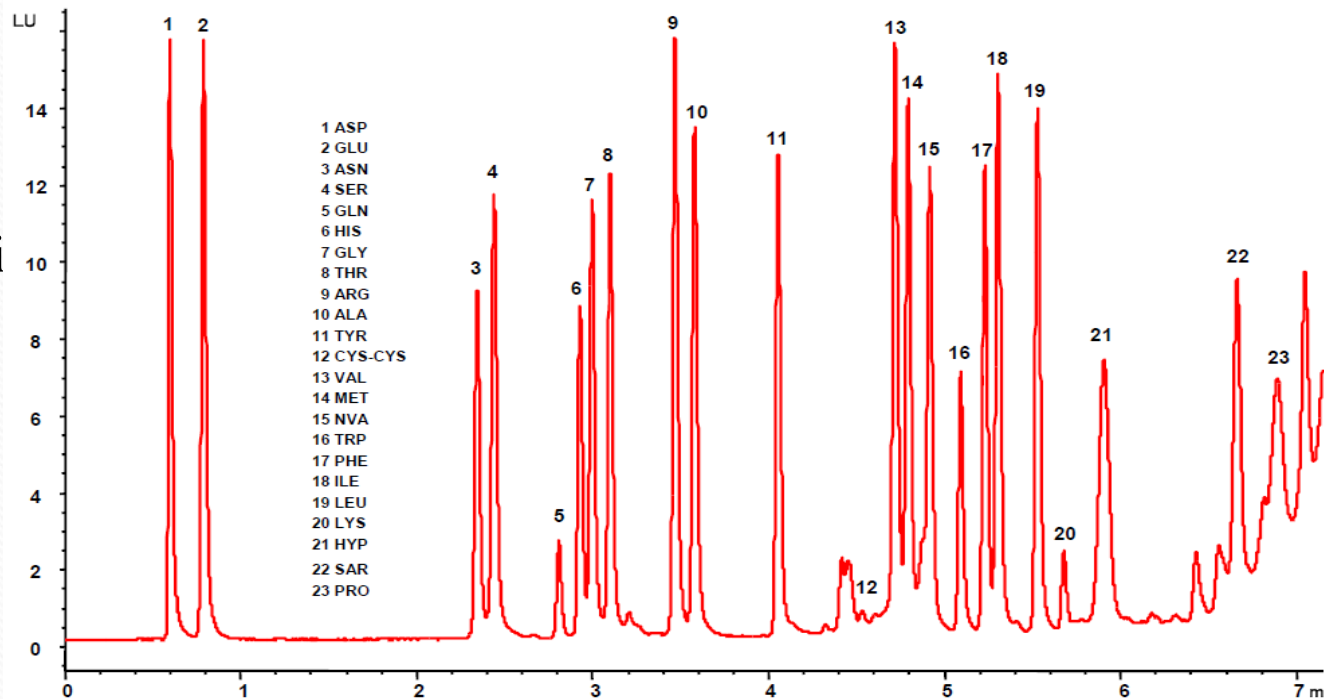
Analiza AK

- Papirna elektroforeza (električni naboj)



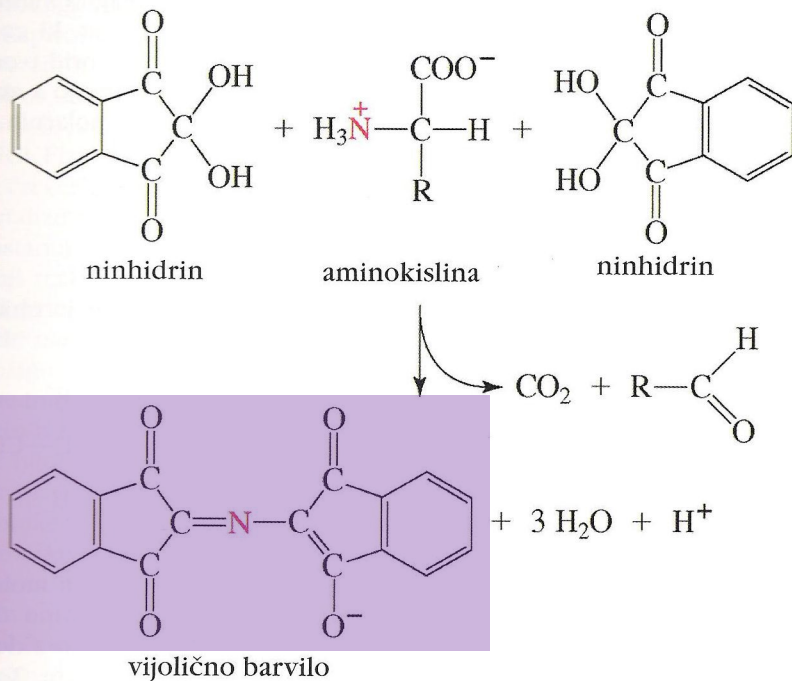
- Ionska izmenjevalna kromatografija (stopnja ionizacije)

- Pretvorba v derivate, ki jih lahko detektiramo z občutljivimi tehnikami (UV-VIS ter fluorescenčna spektrofotometrija)

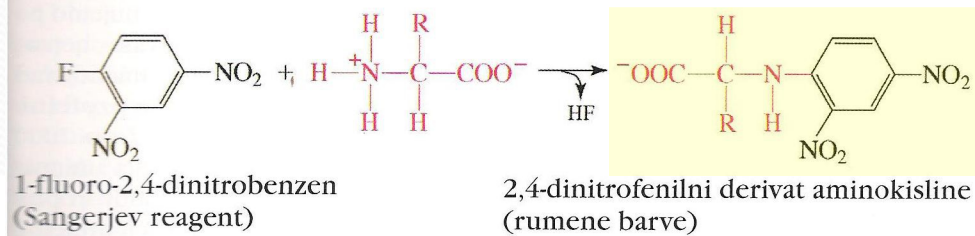


Reakcije AK

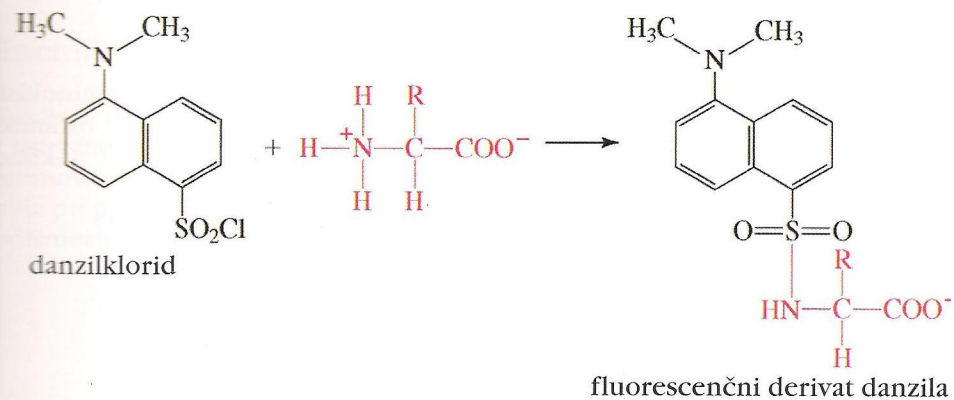
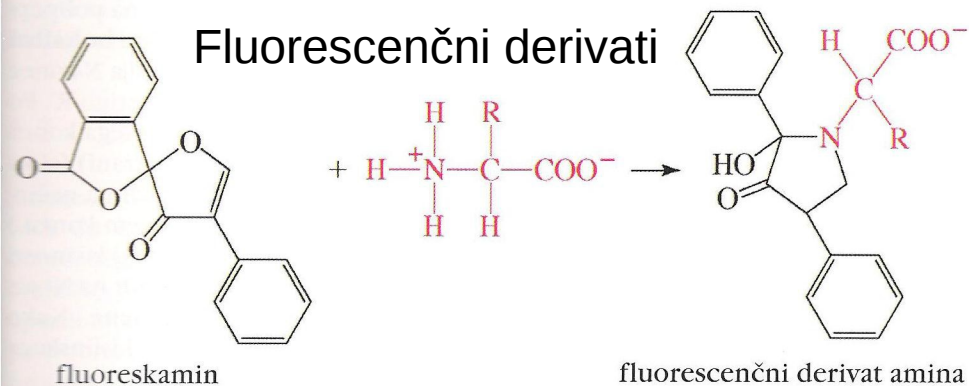
Ninhidrinska



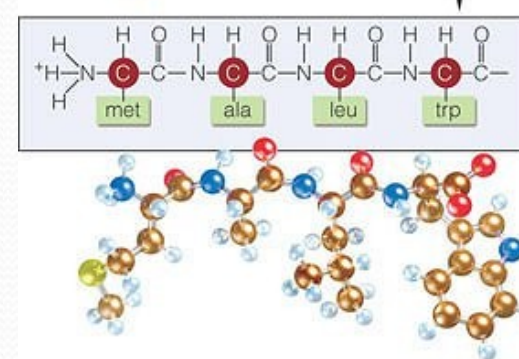
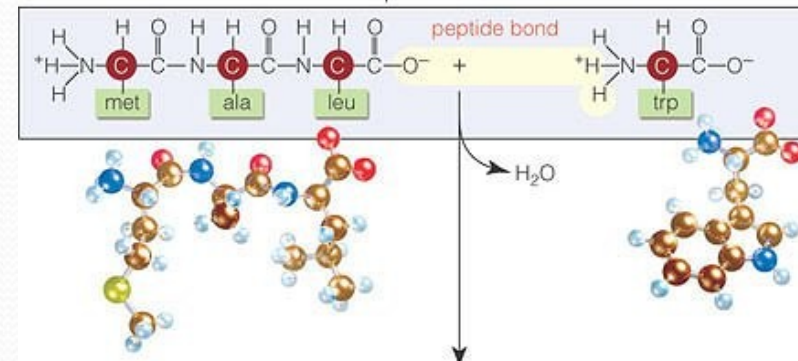
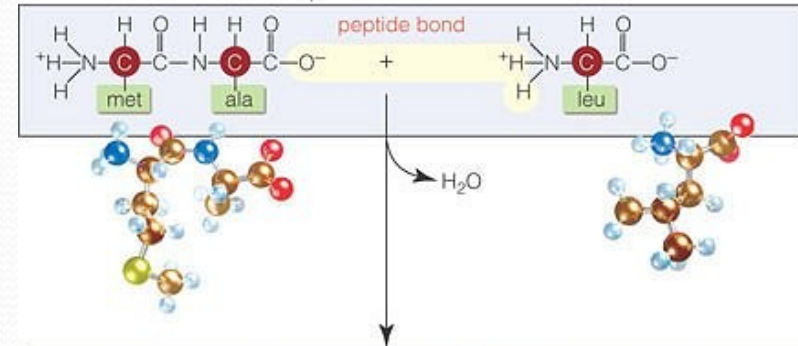
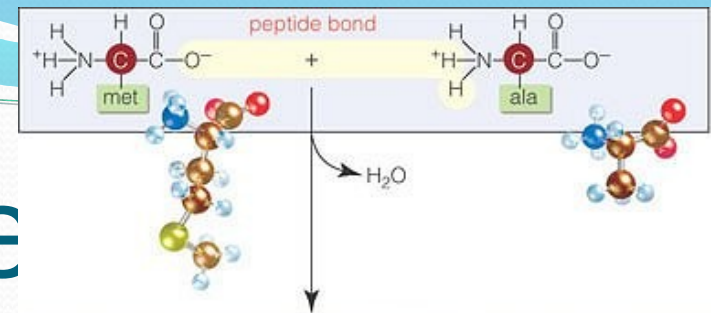
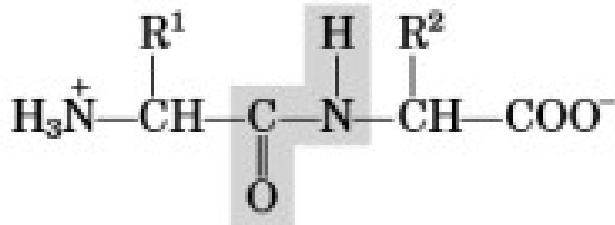
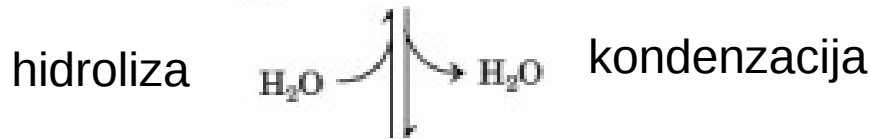
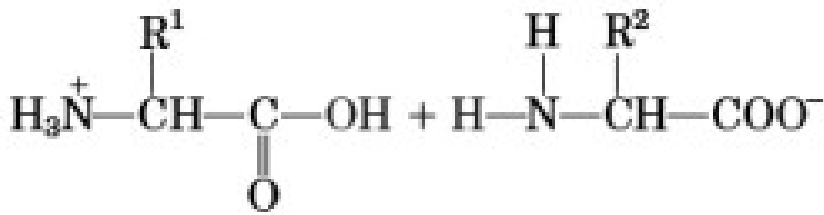
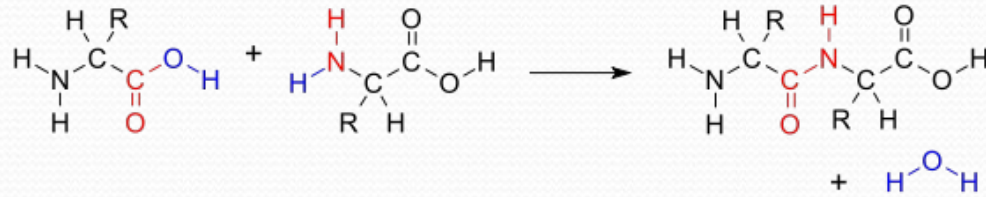
Sangerjev reagent



Fluorescenčni derivati



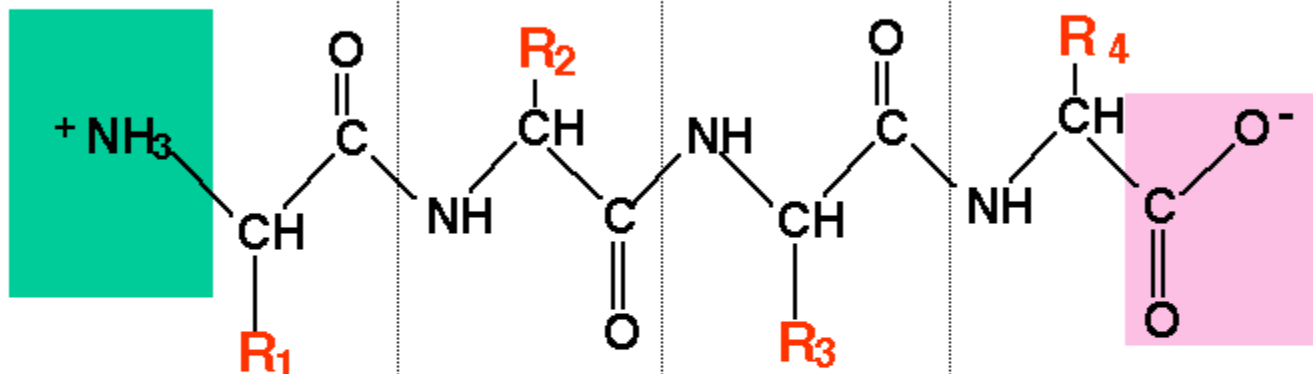
Tvorba peptidne ve



Peptidna veriga

aminski
konec

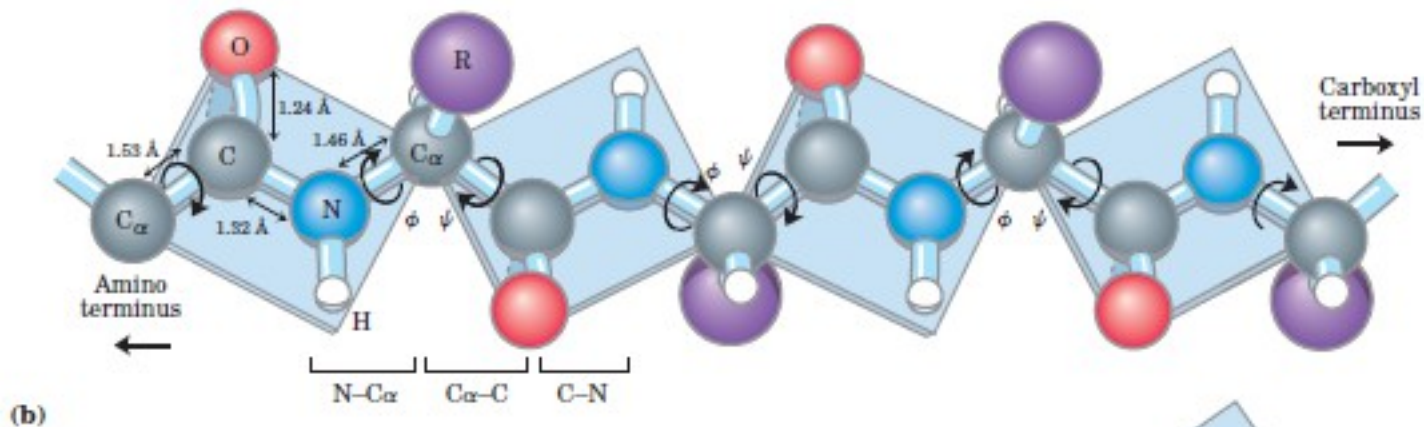
N - konec



karboksilni
konec

C - konec

peptidna veriga – zaporedje aminokislinskih ostankov,
povezanih s peptidno vezjo

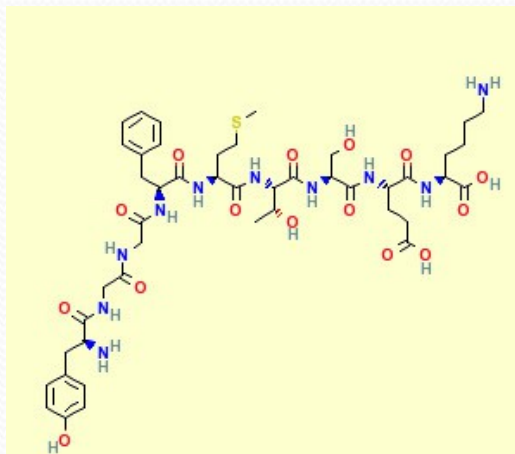


Peptidi

- **glede na dolžino verige:**

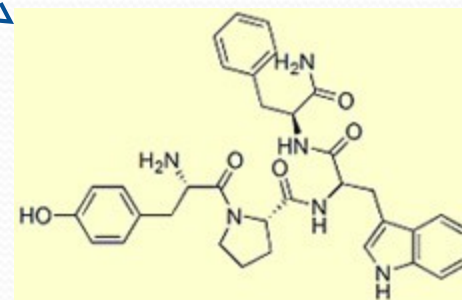
- dipeptidi
- tripeptidi
- tetrapeptidi
- pentapeptidi
- ... oligopeptidi ($n = 2 - \sim 20$)
- polipeptidi = proteini

Beta-endorfin: Tyr Gly Gly Phe Met
Thr Ser Glu Lys Ser Gln Thr Pro
Leu Val Thr Leu Phe Lys Asn Ala
Ile Ile Lys Asn Ala Tyr Lys Lys Gly
Glu



- **glede na funkcijo:**

- peptidni antibiotiki
- intermediati razgradnje proteinov
- signalne molekule: vplivajo na krvni tlak, strjevanje krvi, delovanje možganov (vazoaktivni, opioidni peptidi,...)



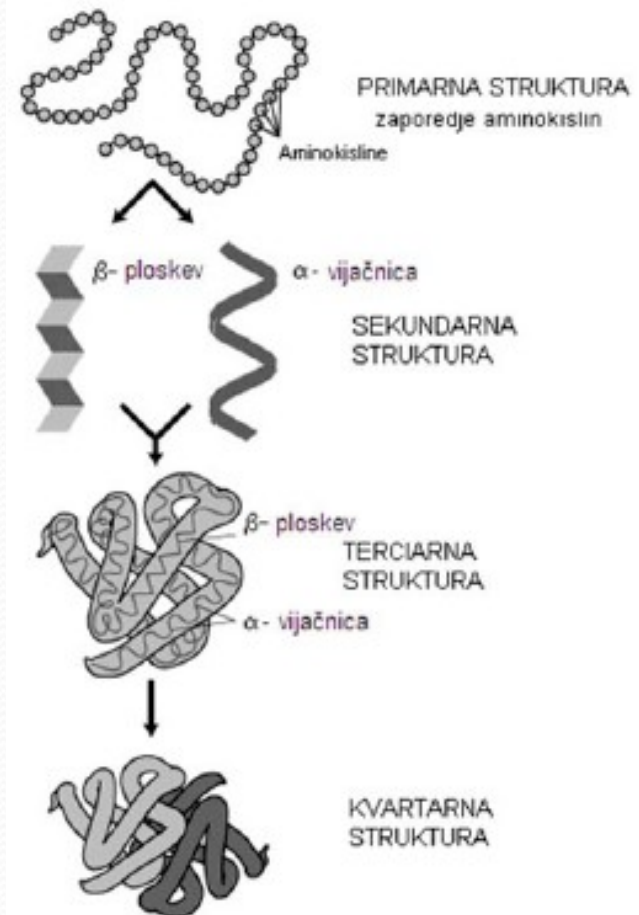
Endomorphin 1: Tyr-Pro-Trp-Phe-NH₂

Endomorphin 2: Tyr-Pro-Phe-Phe-NH

Proteini – ravni strukture

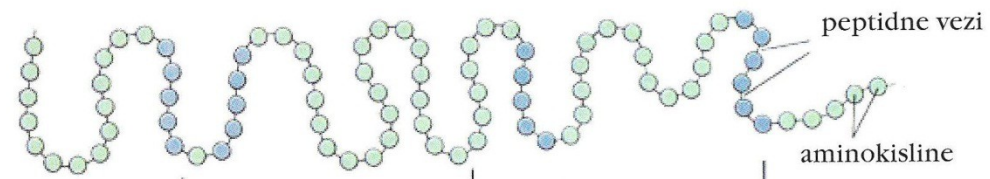
4 ravni strukture:

1. primarna – zaporedje AK
2. sekundarna – ponavljajoči se elementi:
 - α - vijačnica
 - β – struktura (ploskev)
3. terciarna – 3D ureditev sekundarnih struktur
4. kvartarna – ureditev več podenot

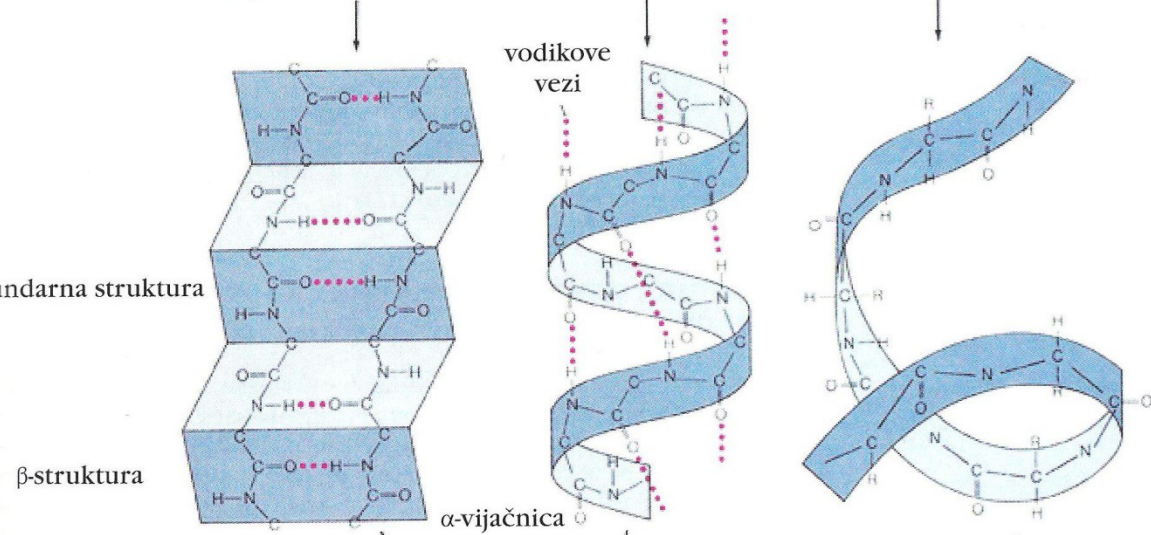


Proteini – ravni strukture

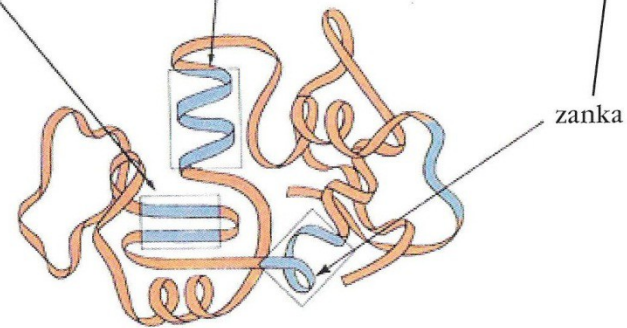
a) primarna struktura



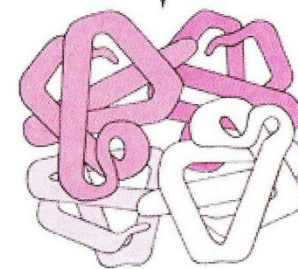
b) sekundarna struktura



(c) terciarna struktura



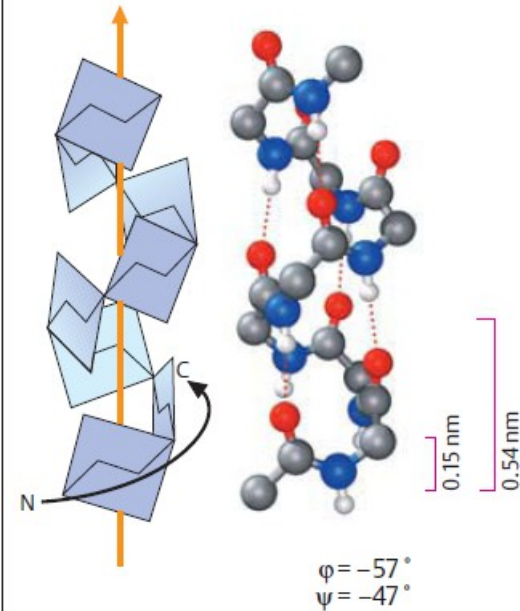
(d) kvartarna struktura



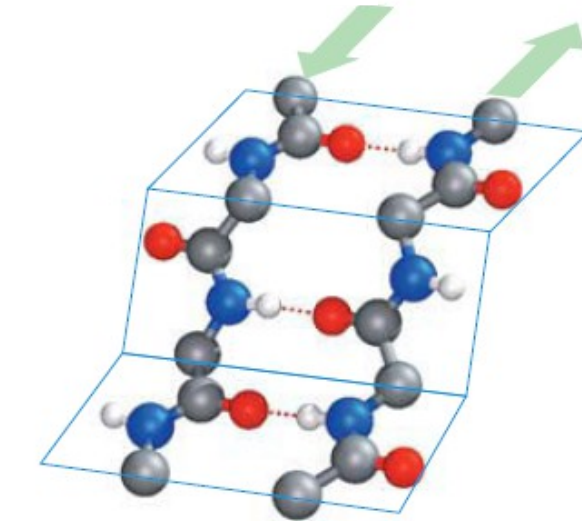
Sekundarna struktura

Krajši strukturni elementi, ki se ponavljajo

α -vijačnice



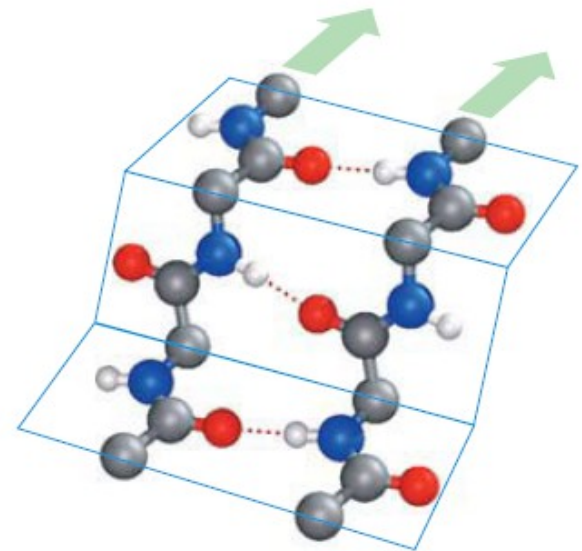
β -strukture



antiparalelne

$$\varphi = -139^\circ$$

$$\psi = +135^\circ$$

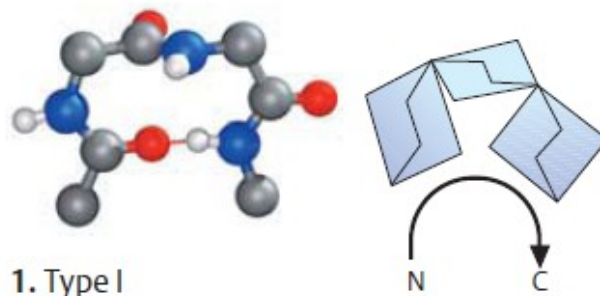


paralelne

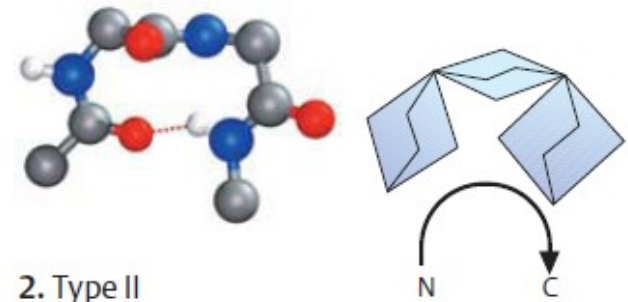
$$\varphi = -119^\circ$$

$$\psi = +113^\circ$$

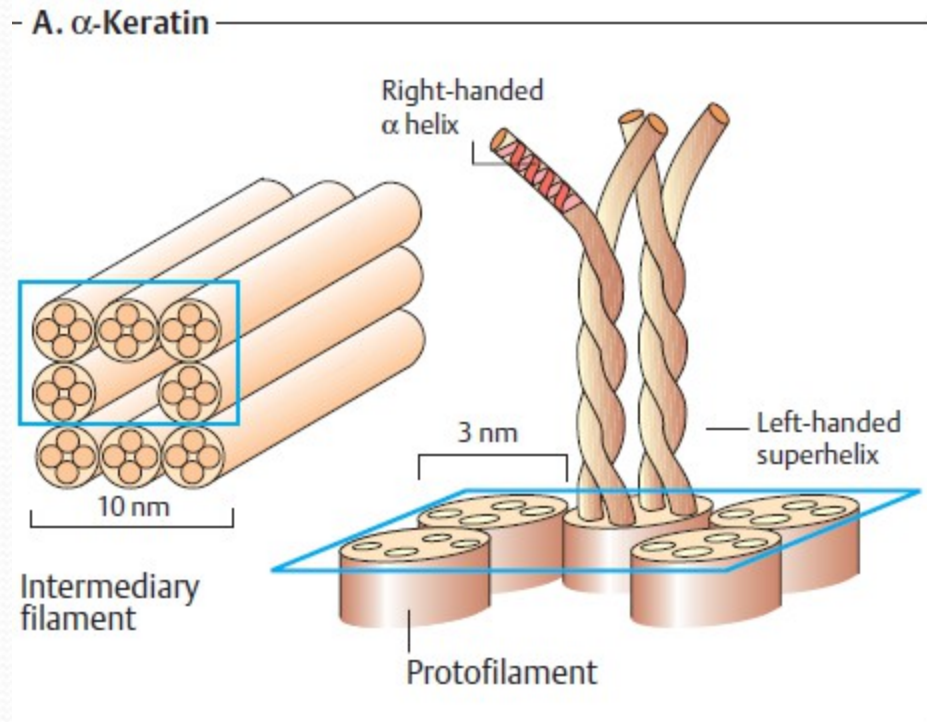
β -zavoji



Koolman, Color Atlas of Biochemistry, 2nd edition © 2005 Thieme



Sekundarne strukture proteinov



α -vijačnice manj stabilne

- B. Collagen



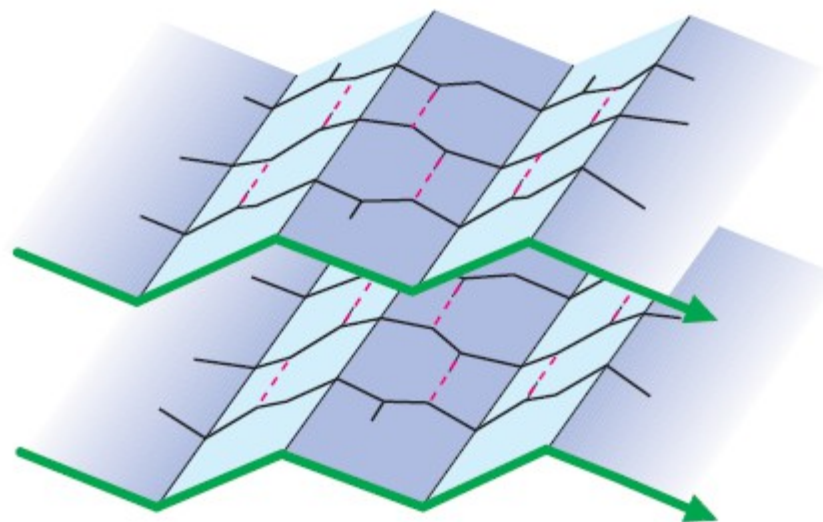
Trojne vijačnice
bolj stabilne

1. Triple helix (section)

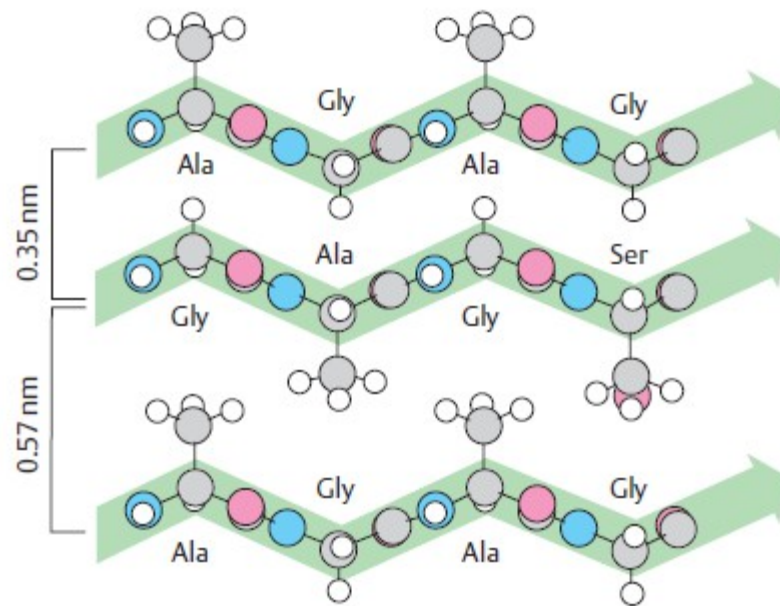
Sekundarne strukture proteinov

- β -strukture

H vezi med ploskvami, bolj stabilne kot α -vijačnice

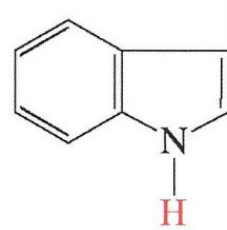


1. Spatial illustration

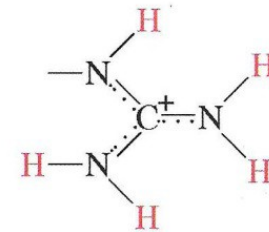


2. Front view

Stranske verige AK, ki v proteinih tvorijo vodikove

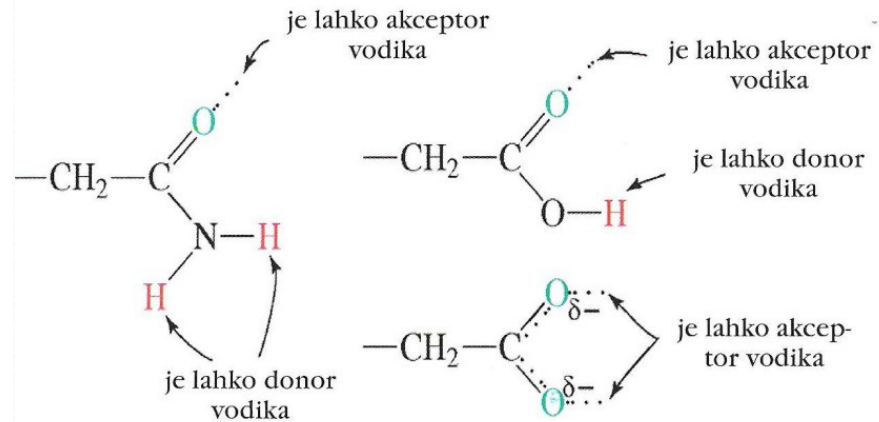


donor vodika na triptofanu



donor vodika na argininu

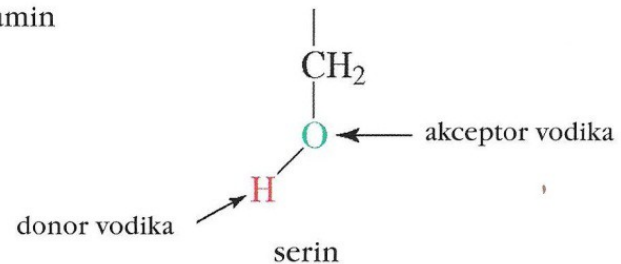
Amino acid residues	General acid form (proton donor)	General base form (proton acceptor)
Glu, Asp	$R-COOH$	$R-COO^-$
Lys, Arg	$R-\overset{H}{\underset{H}{\overset{+}{N}}}$	$R-\ddot{N}H_2$
Cys	$R-SH$	$R-S^-$
His	$R-C=CH$ $HN-C=NH$	$R-C=CH$ $HN-C=N:$
Ser	$R-OH$	$R-O^-$
Tyr	$R-C_6H_4-OH$	$R-C_6H_4-O^-$



asparagin ali glutamin

protonirana oblika asparaginske ali glutaminske kisline

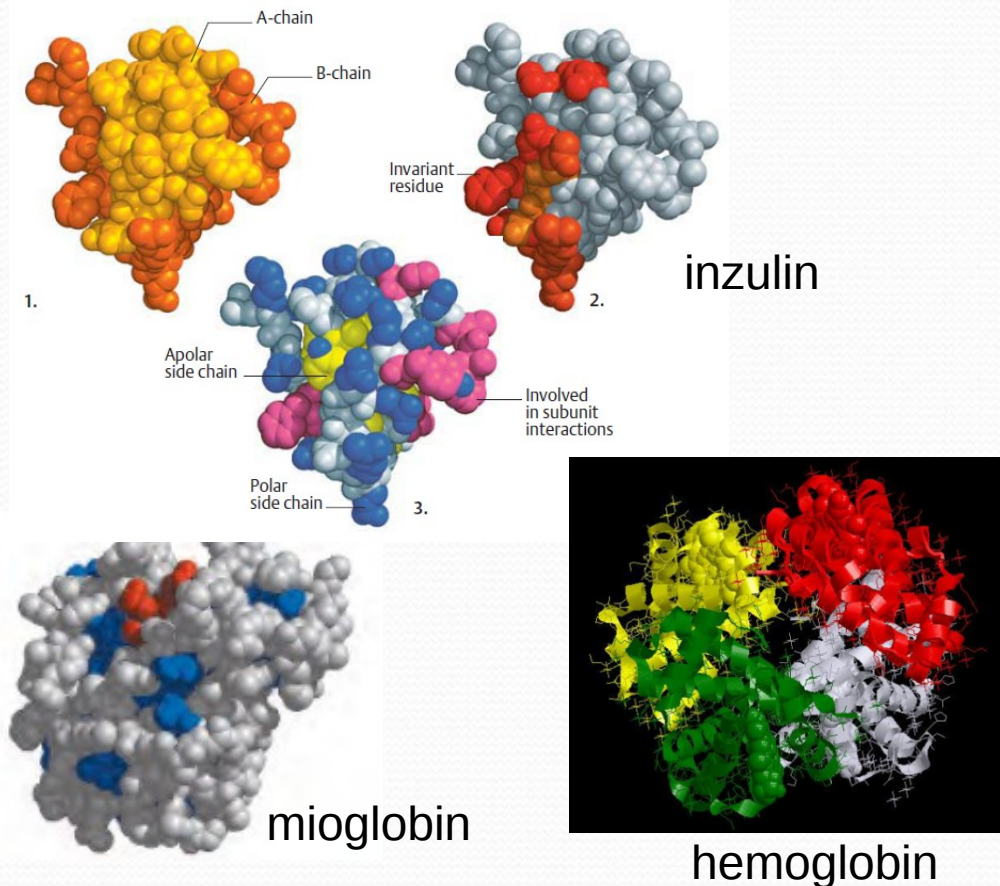
ionizirana oblika asparaginske ali glutaminske kisline



serin

Proteini glede na obliko in topnost

globularni (večinoma topni)

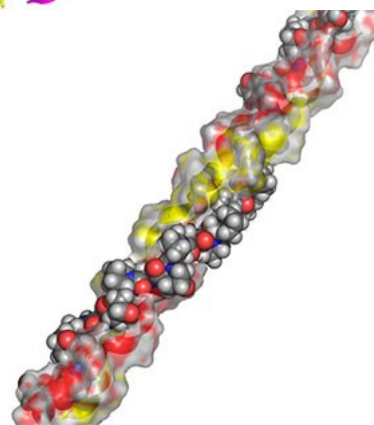
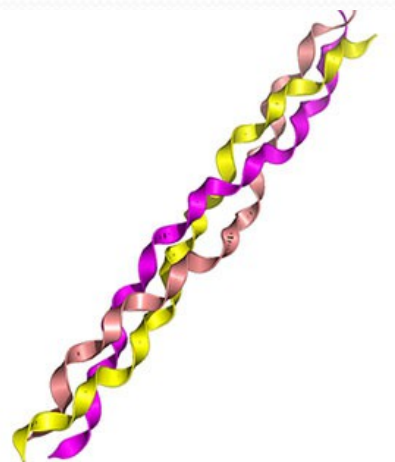


Vrsta proteina	vloga
Globularni proteini	
hemoglobin	transport kisika
mioglobin	skladičenje kisika
ribonukleaza	hidroliza RNA
lizocim	hidroliza bakterijske stene
citokrom c	transport elektronov
imunoglobulin	obramba (protitelesa)
aktin	gibanje (mišični protein)
inzulin	hormon

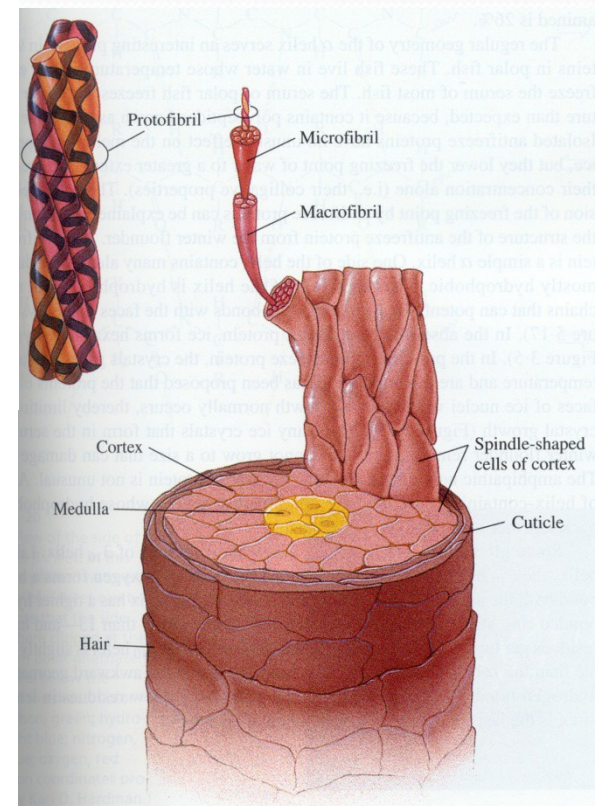
Proteini glede na obliko in topnost

- fibrilarni (netopni)

Vrsta proteina	vloga
Fibrilarni proteini	
kolagen	Strukturni protein
keratin	Strukturni protein
miozin	Gibanje (mišični protein)
elastin	prožnost



kolagen v koži



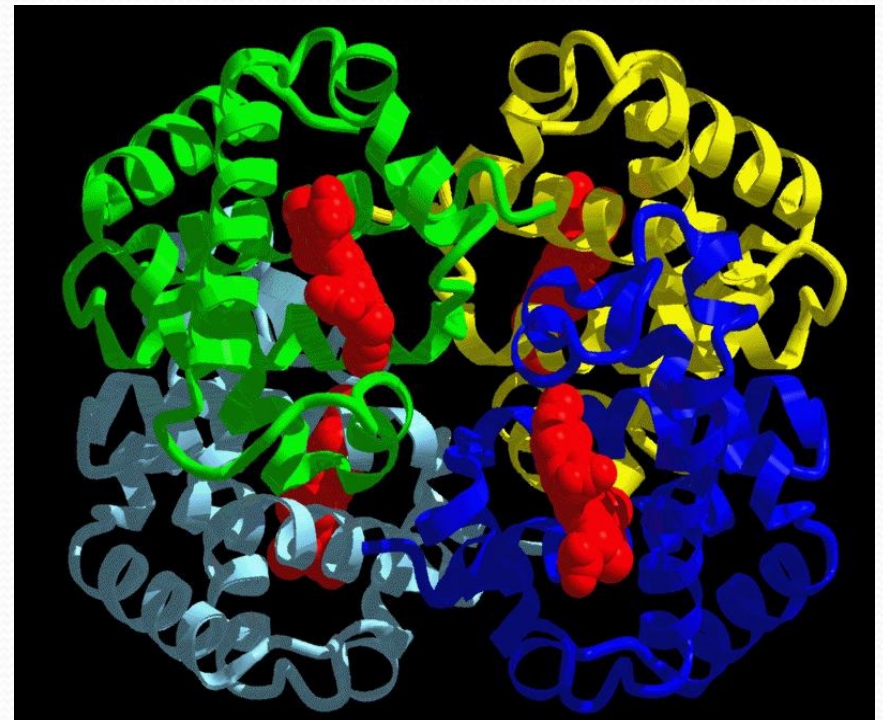
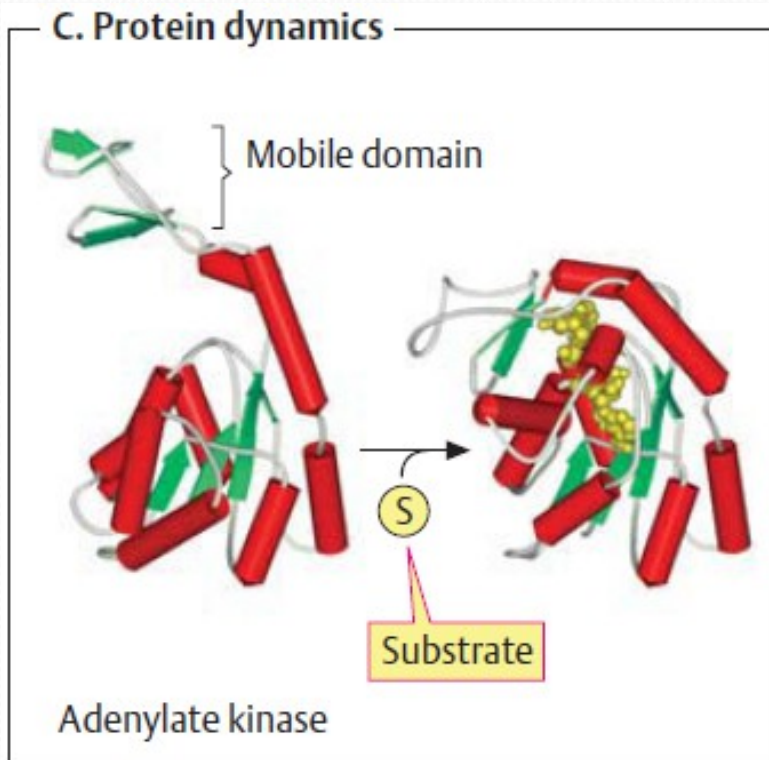
keratin v lasu

Proteini – domene, podenote

Proteinska **domena**: del proteina, ki se zvije neodvisno in lahko opravlja neko posebno funkcijo, lahko tudi ločeno od ostalega proteina.

Proteinska **podenota**: pri proteinih s kvartarno strukturo.

primer: hemoglobin



Velikost proteinov, število verig

Monomerni proteini: ena sama veriga.

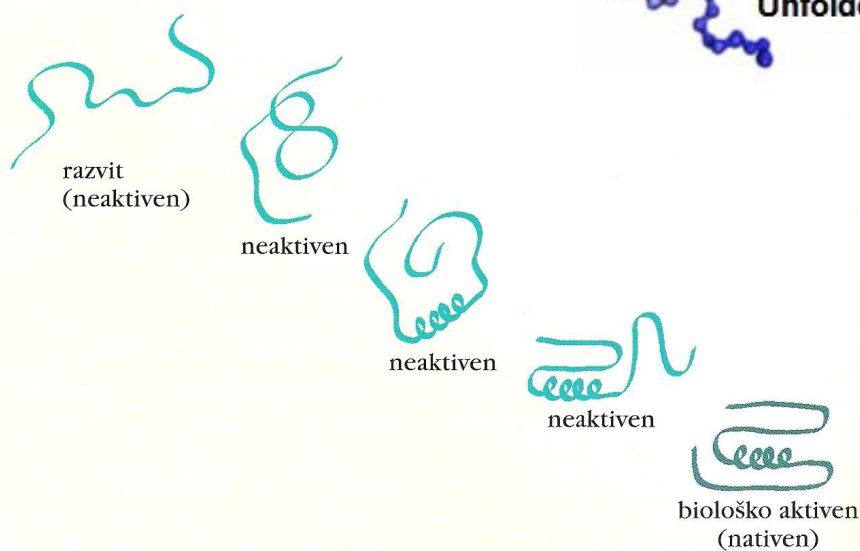
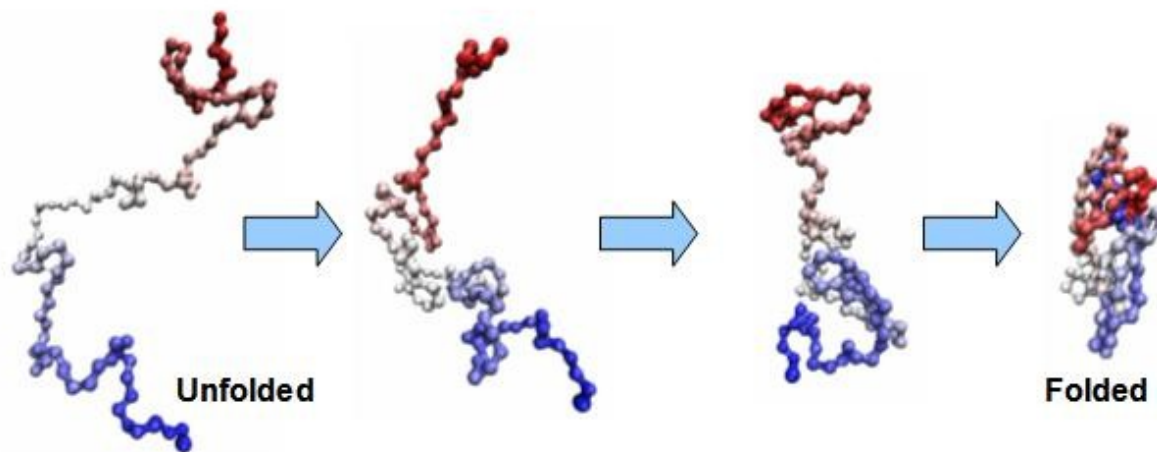
Oligomerni proteini: več verig, vsaka veriga svoja **podenota**.

protein	molekulska masa (Da)	število aminokislinskih ostankov	število polipeptidnih verig
inzulin (goveji)	5733	51	2
citokrom <i>c</i> (človeški)	13 000	104	1
ribonukleaza A (goveji pankreas)	13 700	124	1
lizocim (jajčni beljak)	13 930	129	1
mioglobin (konjsko srce)	16 890	153	1
kimotripsin (goveji pankreas)	21 600	241	3
hemoglobin (človeški)	64 500	574	4
serumski albumin (človeški)	68 500	550	1
imunoglobulin G (človeški)	145 000	1320	4
RNA-polimeraza (<i>E. coli</i>)	450 000	4100	5
feritin	450 000	4100	24
glutamat-dehidrogenaza (goveja jetra)	1 000 000	8300	40

Biološko aktivna (nativna) konformacija proteinov

- zvitje proteinov (sekundarna in terciarna struktura) določa zaporedje AK (primarna struktura)
- izjemno hitro zvitje (angl. folding) – v nekaj sekundah
- **denaturacija:** kovalentna veriga ostane intaktna, polipeptidna veriga se razvije v ravno, nepravilno in spreminjajočo konformacijo v prostoru
 - segrevanje
 - ekstremni pH
 - obdelava s sečnino

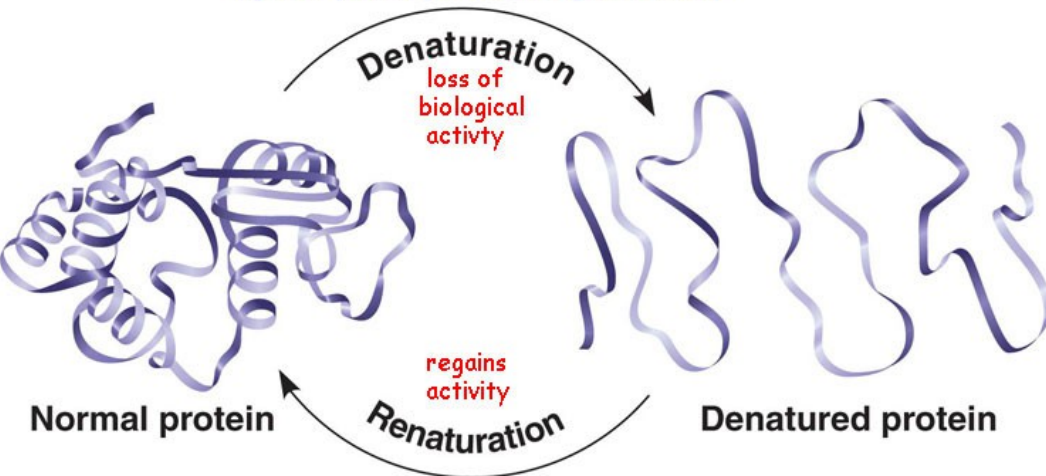
Proteini - zvitje



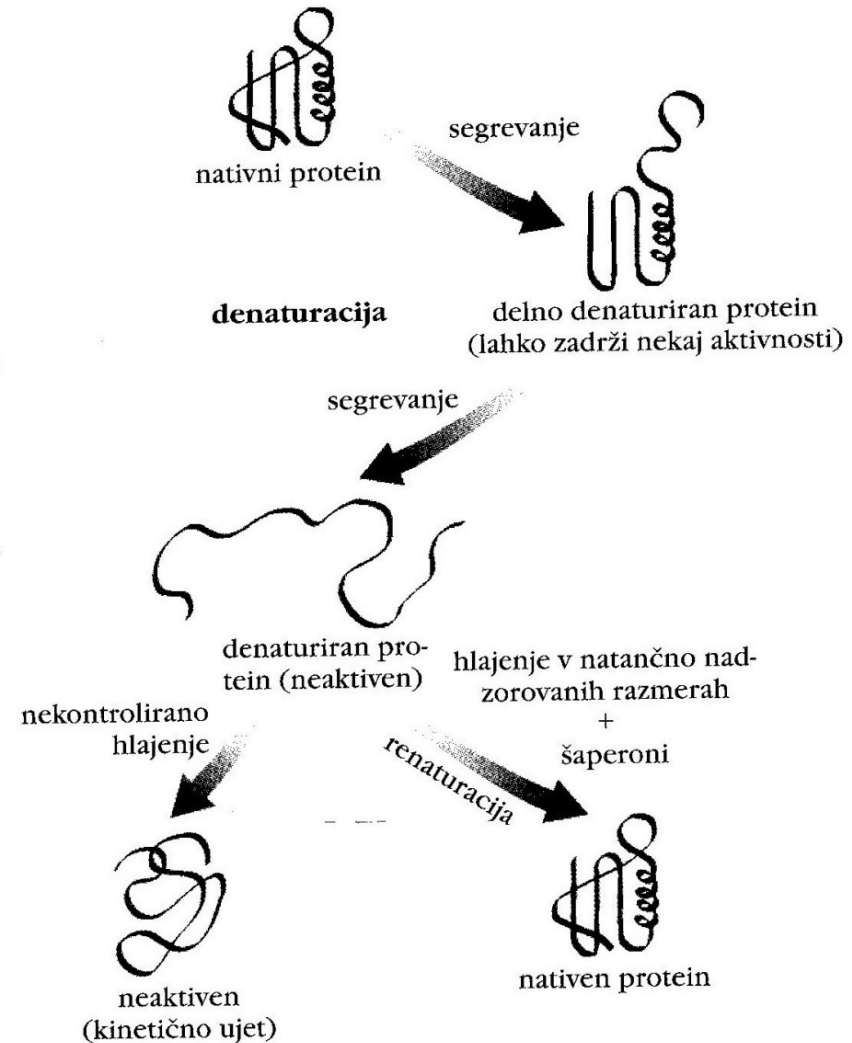
postopno, ko sinteza na ribosomih še poteka

Denaturacija in renaturacija

agents: pH, temp, ionic strength, solubility

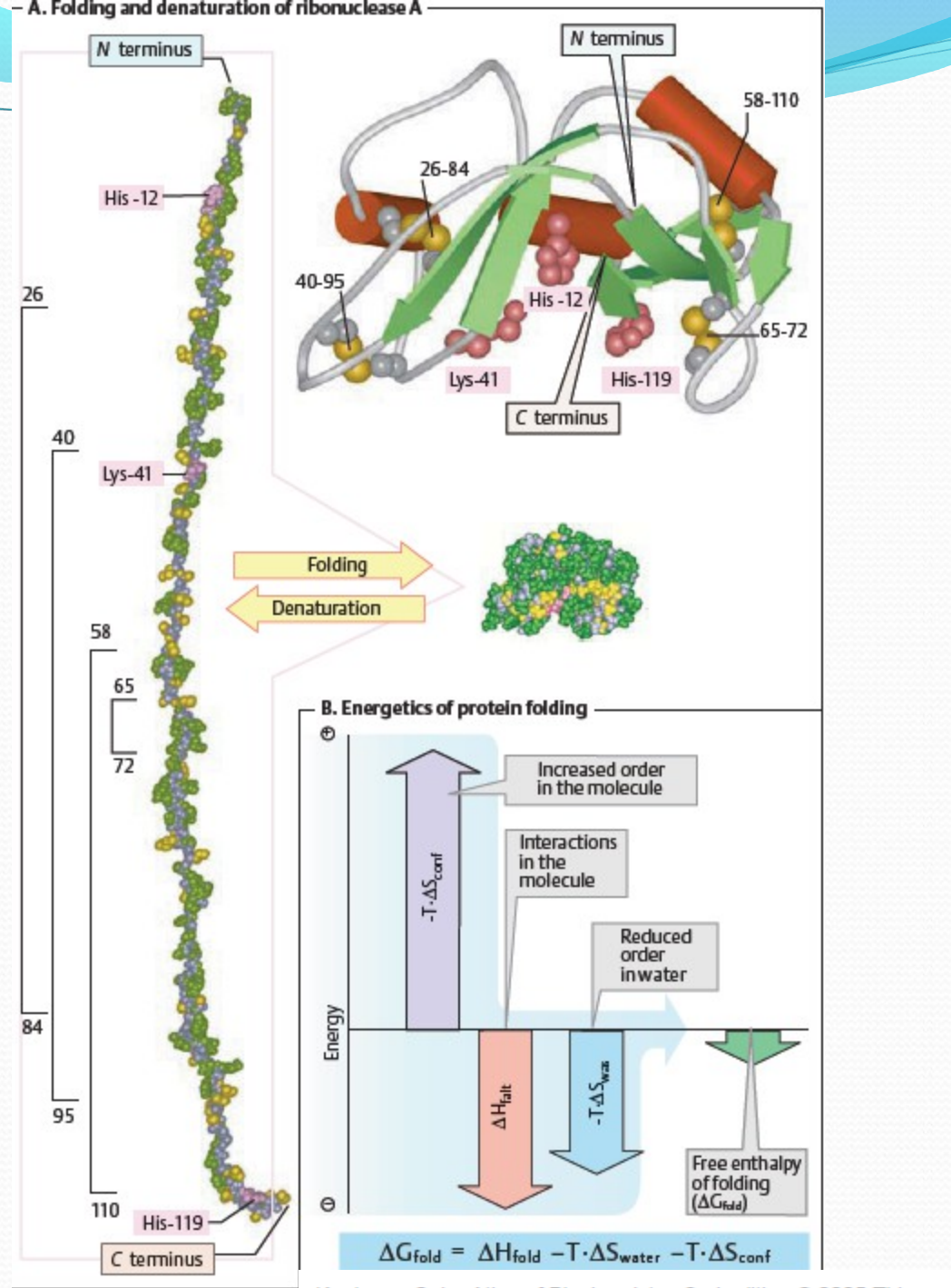


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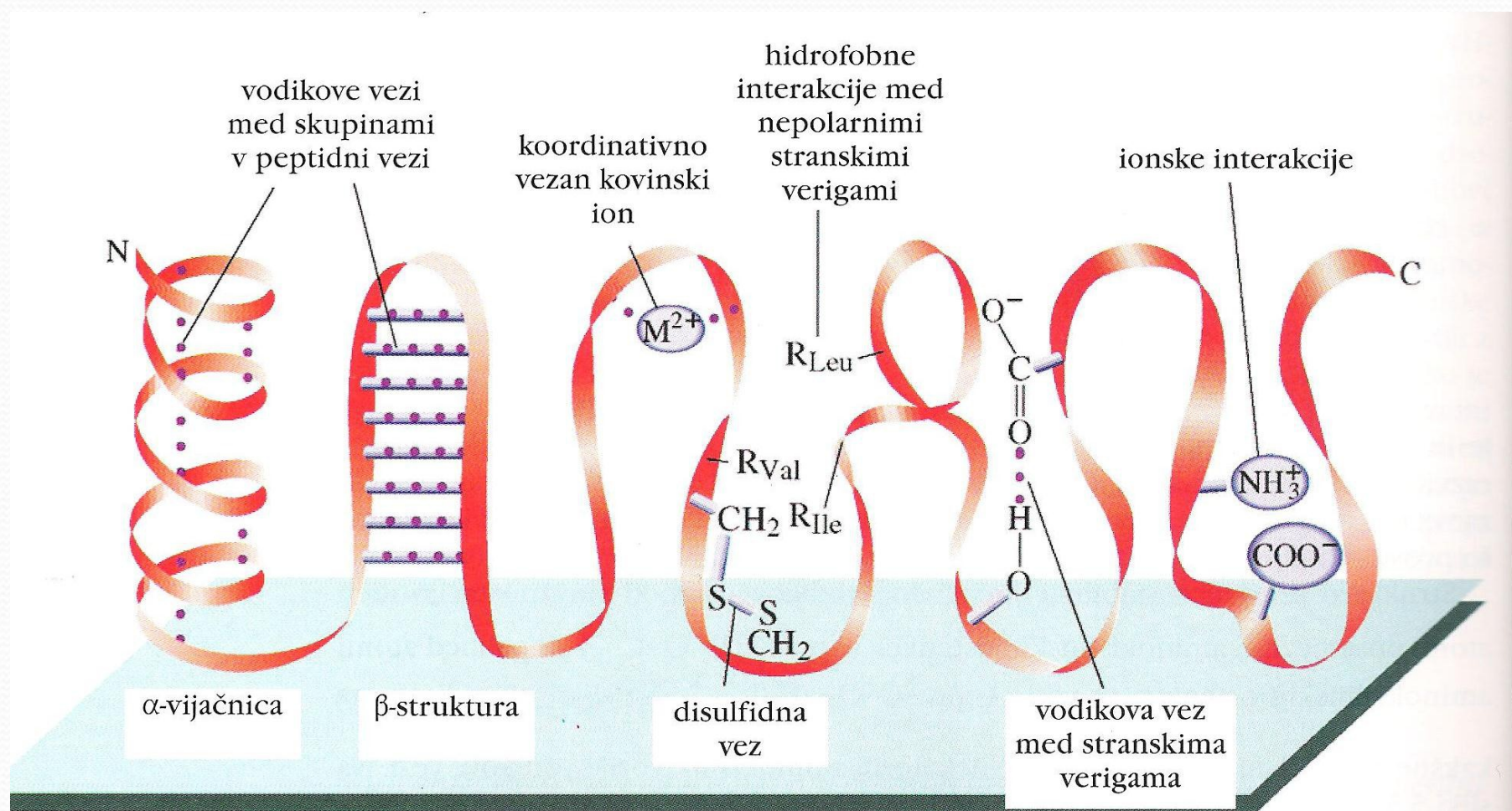


Proteini

- zvitje in denaturacija encima ribonukleaza A
- His-12, Lys-41 in His-119 so v aktivnem centru encima
- energetika zvitja:
 $\Delta G < 0$, spontana reakcija

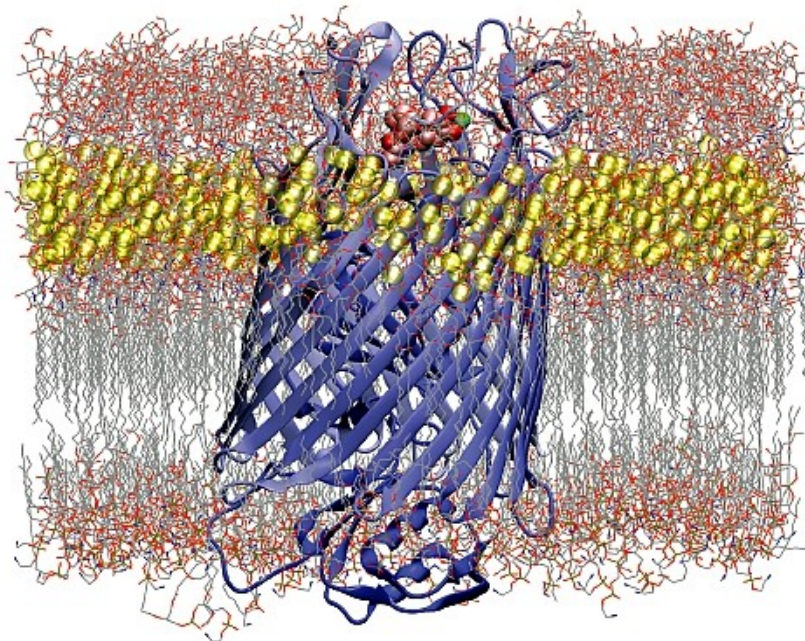


Intramolekularne interakcije v proteinih

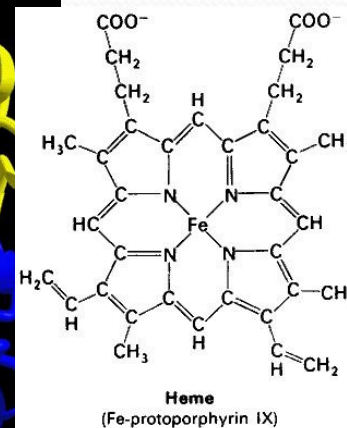
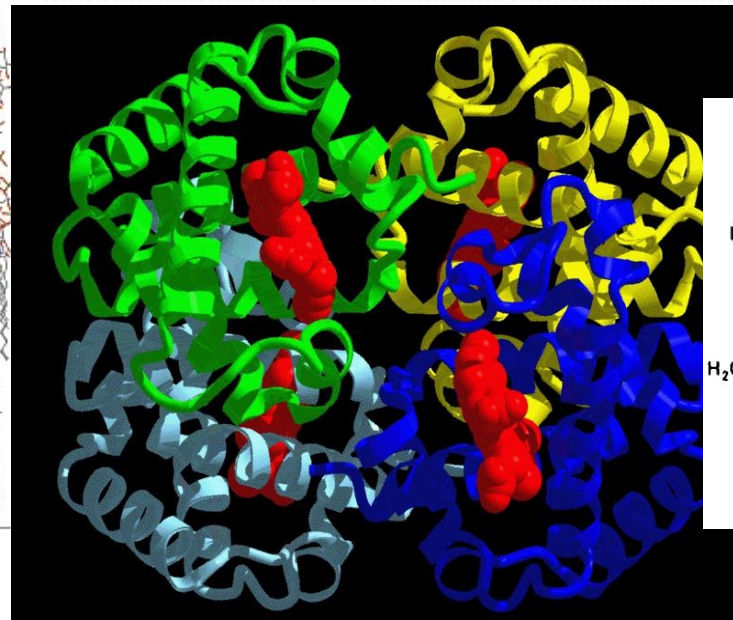


Biološka vloga proteinov

- encimi
- transportni proteini (hemoglobin, lipoproteini)
 - v krvni plazmi vežejo in nosijo specifične molekule ali ione od enega organa k drugemu
 - sodelujejo pri prenosu snovi skozi membrane



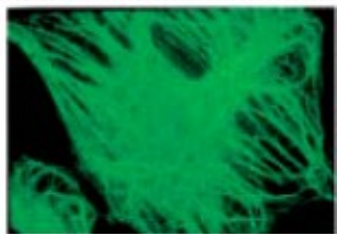
FpvA : signalni in transportni protein
Pseudomonas aeruginosa za Fe



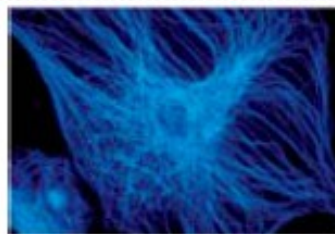
Hemoglobin: hem je označen z rdečo, vsaka podenota s svojo barvo

Biološka vloga proteinov

- **kontraktilni proteini** (aktin, miozin, tubulin - citoskelet)
dajejo celicam in organizmom sposobnost krčenja, spreminjanja oblike oz. gibanja



srednje veliki filamenti

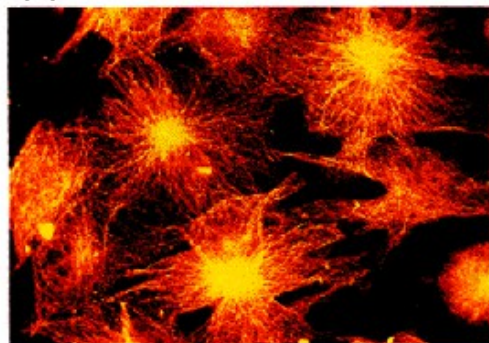


mikrotubuli

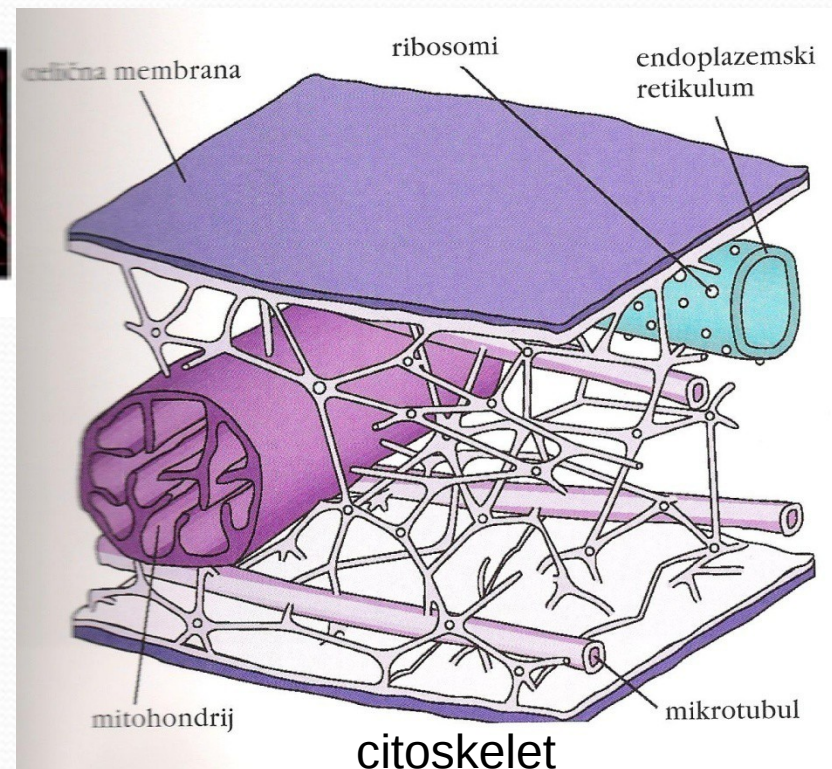
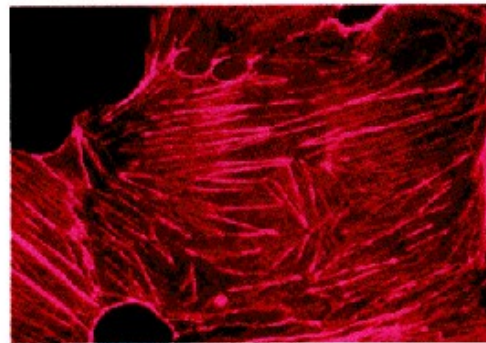


mikrofilamenti

(a) tubulin



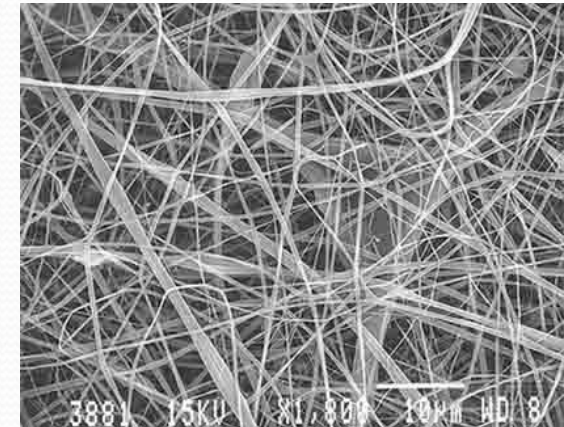
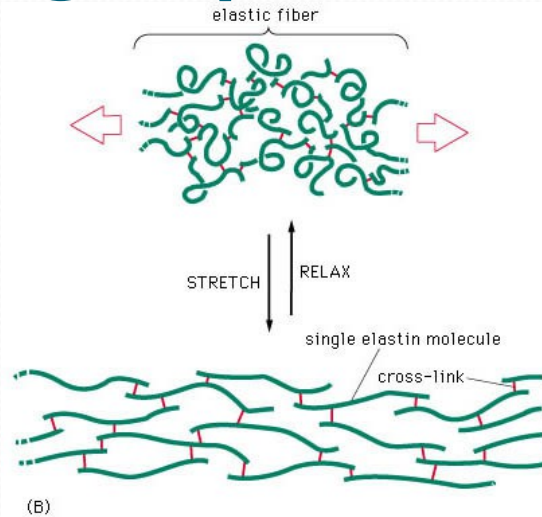
(b) aktin



Biološka vloga proteinov

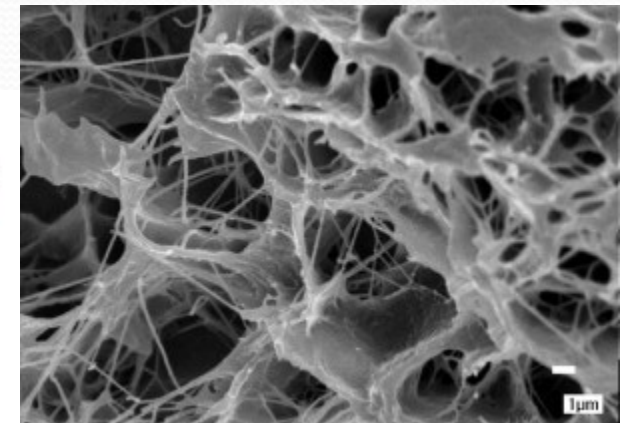
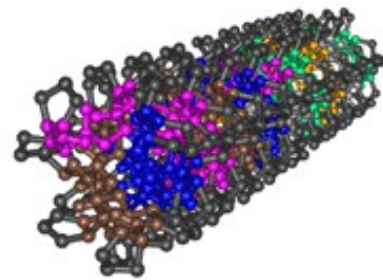
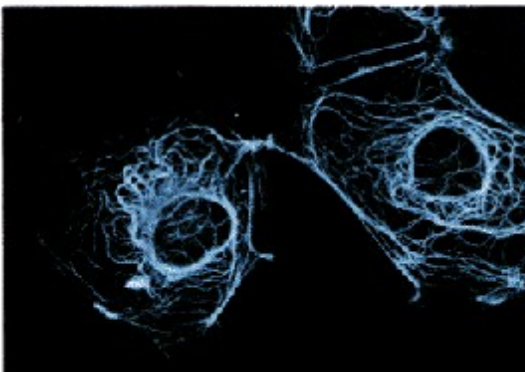
- **strukturni proteini**
(α -keratin, kolagen, elastin...)

služijo kot podporni filamenti in dajejo trdnost ali zaščito



elastin

(c) keratin

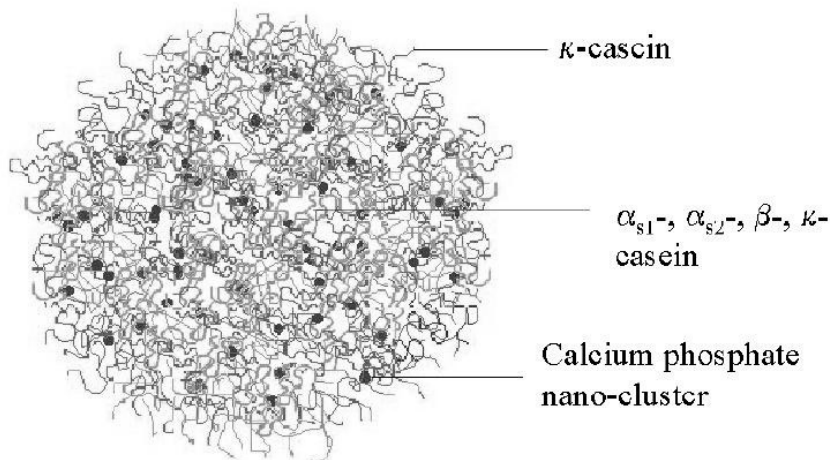


kolagen: 25-35 % vseh proteinov v telesu sesalcev

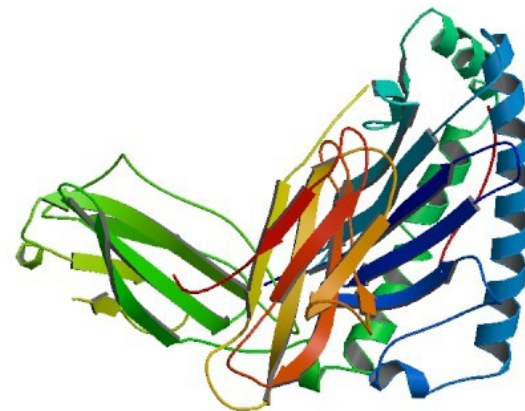
Biološka vloga proteinov

- **hranilni in skladiščni proteini** (kazein, ovalbumin, feritin, glutenin)

v semenih rastlin, v mleku, beljaku – potrebni za rast



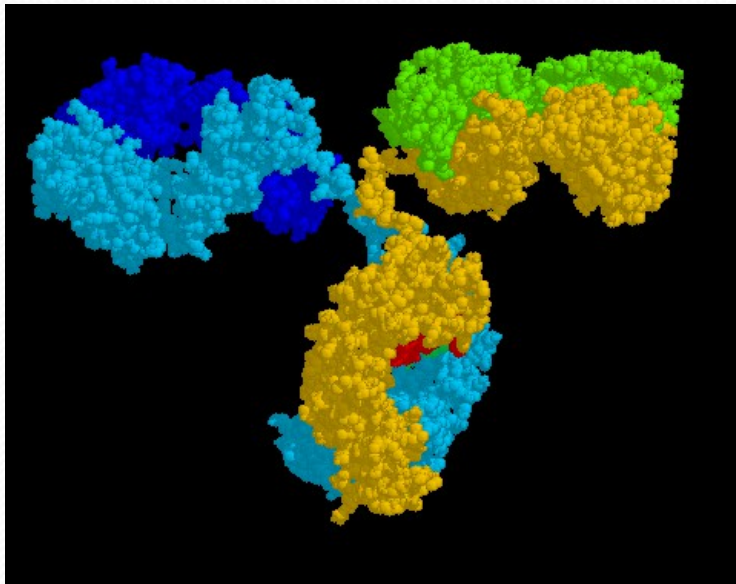
miceli kazeina v mleku
 α S1, α S2, β , in κ -kazein



ovalbumin (60-65 % beljaka)

Biološka vloga proteinov

- **obrambni proteini** (protitelesa, fibrinogen, trombin)
branijo organizme pred vdorom drugih vrst oz. jih ščitijo pred poškodbami (imunoglobulini,...)
- toksini (kačji strup, amanitin – v gobah,...)



IgG2a (miš)



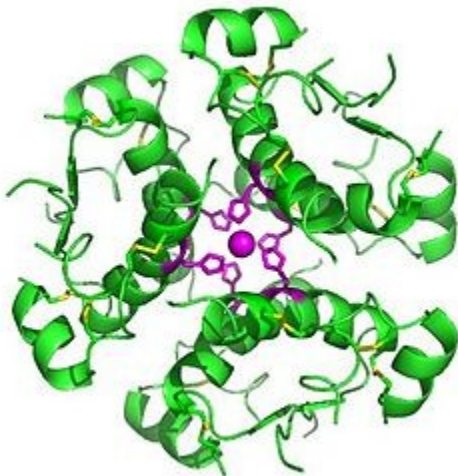
α -amanitin

Biološka vloga proteinov

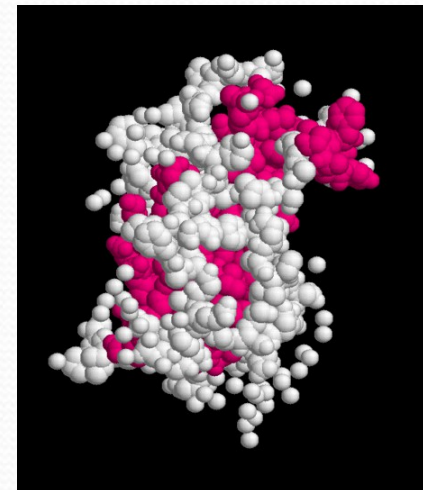
- **regulatorni proteini**

regulirajo celične in fiziološke aktivnosti

- hormoni (inzulin, glukagon, kortikotropin, rastni hormon)
- represorji (regulacija biosinteze encimov)



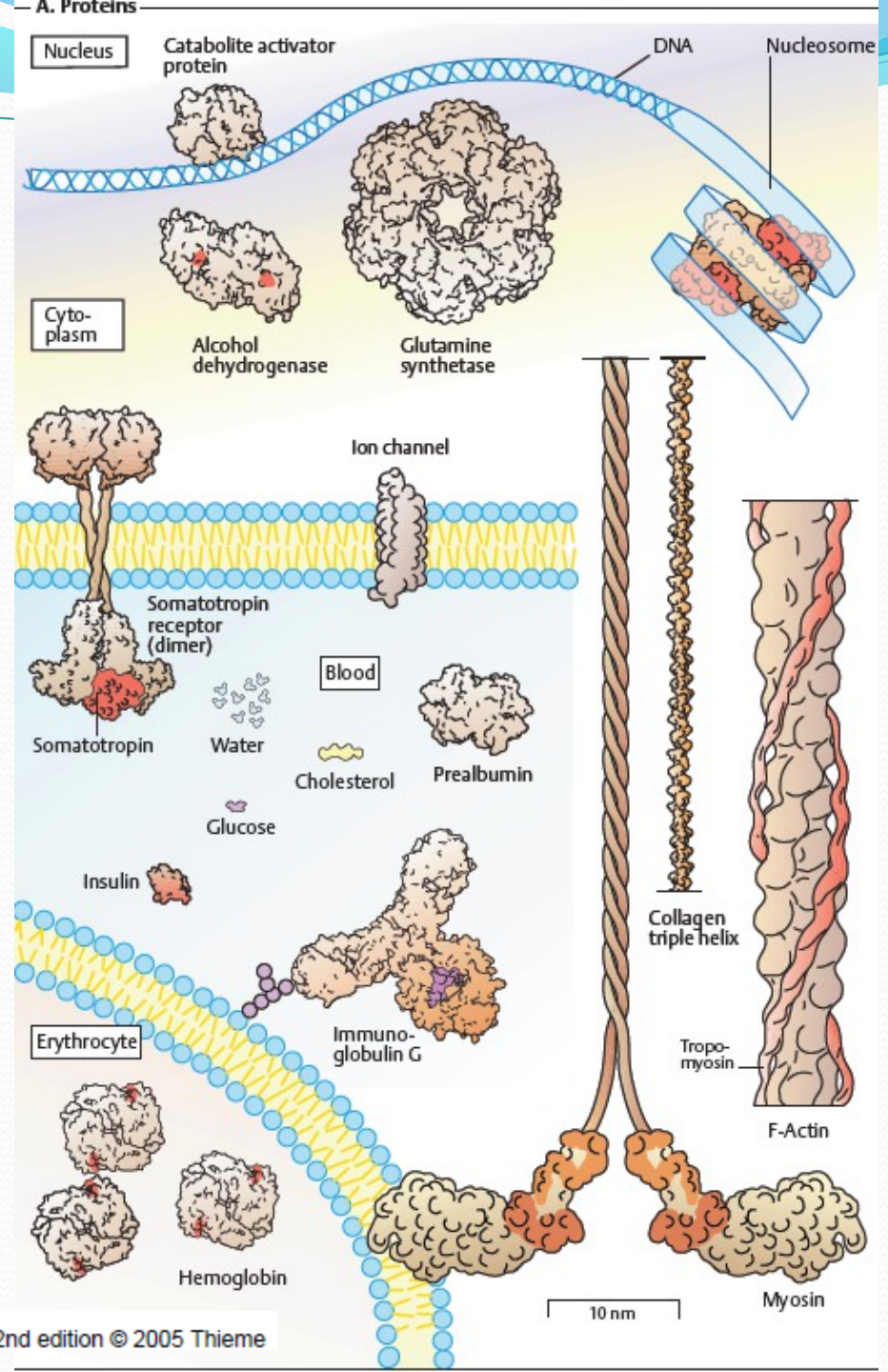
heksamer inzulina (51 AK, M = 5808 Da),
vijolični: His ostanki, ki sodelujejo v vezavi Zn



rastni hormon

Vsebnost proteinov

- virusi 50–90 % (ut.)
- bakterije 50–70 %
- kvasovke 35–45 %
- nitaste glive 25–40 %

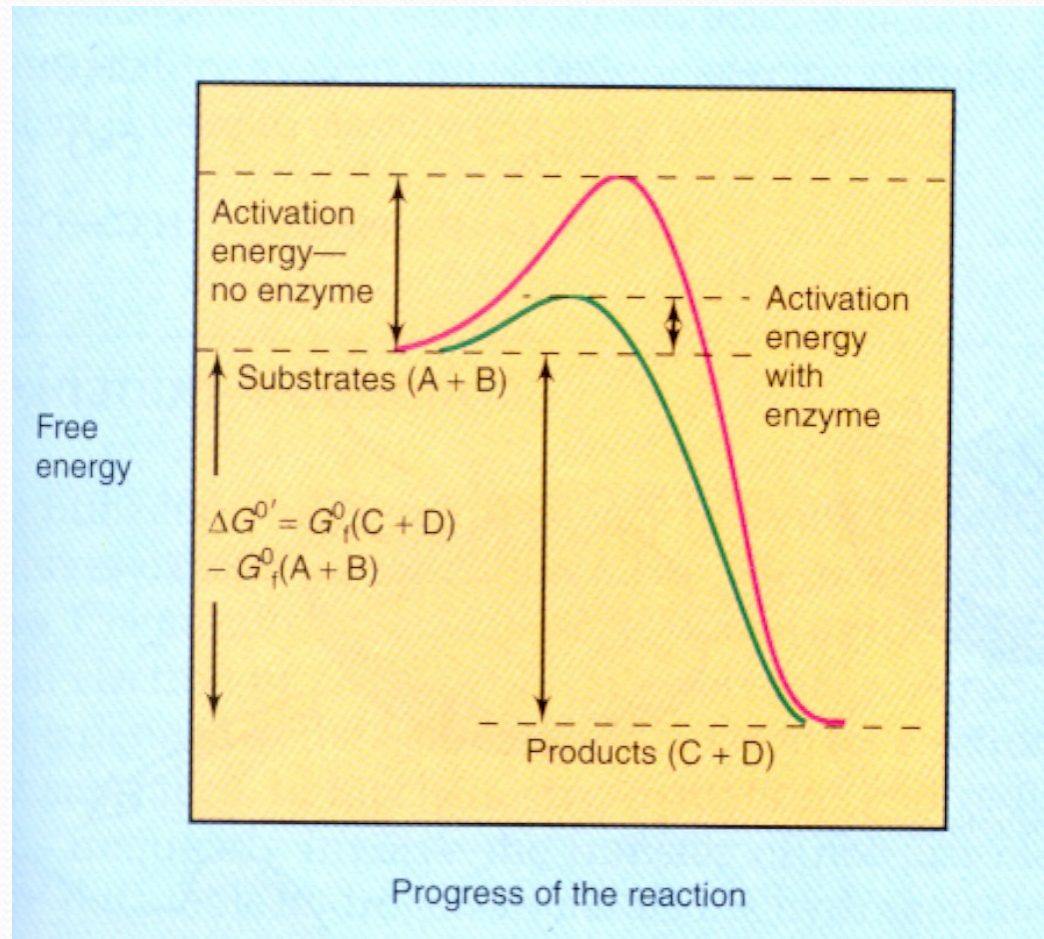


Encimi -biokatalizatorji

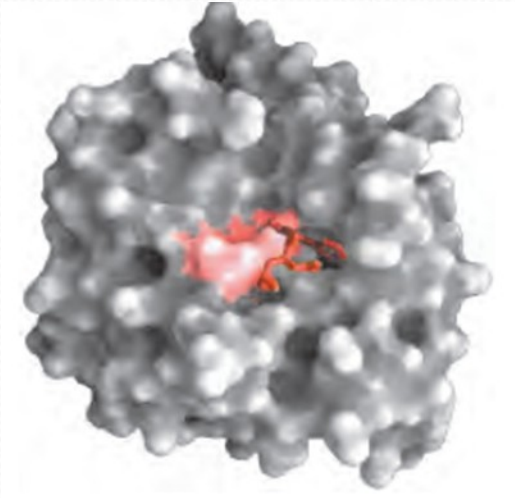
povečanje hitrosti reakcij
za faktor 10^8 do 10^{12}

velikost: 12.000 – 1.000.000 Da

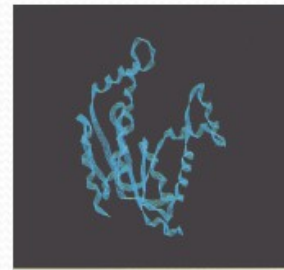
Ne spremenijo
ravnotežja reakcije



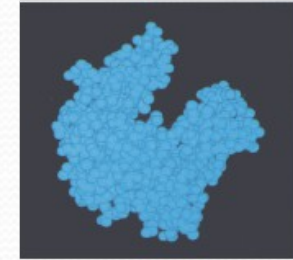
Encimi - struktura



ektivno mesto encima



adenilat kinaza



cito krom b₅₆₂



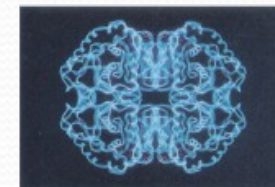
nukleaza iz bakterij
Staphylococcus



lizocim



fosfoglukom utaza



laktat
dehidrogenaza

IMENOVANJE ENCIMOV

- po **substratih**, na katere delujejo:
SUBSTRAT + AZA
ureaza, amilaza, celulaza, hitinaza, glukanaza
- po **reakcijah**, ki jih katalizirajo:
 - alkohol-dehidrogenaza (odcep vodika z alkohola)
 - piruvat-karboksilaza (karboksilacija piruvata)
 - DNA-polimeraza III (podaljševanje verige DNA)
- **trivialna** (zgodovinska) imena:
kimotripsin, tripsin, katepsin, pepsin, renin

KLASIFIKACIJA ENCIMOV

na osnovi reakcije, ki jo katalizirajo – 6 RAZREDOV: **EC i.i.i.i.**

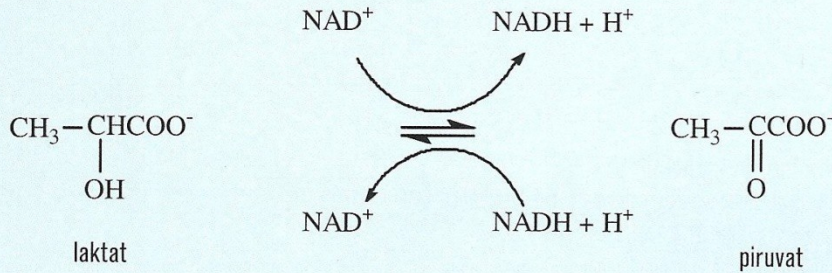
- EC 1. **OKSIDOREKTUKTAZE**: oksidacije-redukcije oz. prenos elektronov (večinoma dehidrogenaze, tudi oksidaze, peroksidaze, reduktaze...)
- EC 2. **TRANSFERAZE**: prenos skupin (kinaze)
- EC 3. **HIDROLAZE**: hidrolitske reakcije (proteaze)
- EC 4. **LIAZE**:
 - nehidrolitske in neoksidativne reakcije eliminacij oz. lize substrata, kar vodi v nastanek dvojne vezi
 - adicija na dvojno vez (sintaze)
- EC 5. **IZOMERAZE**: izomerizacije (1 substrat – 1 produkt)
- EC 6. **LIGAZE**: kondenzacije, povezane z vnosom energije (npr. ATP)

Razredi encimo v

Class	Reaction type	Important subclasses
1 Oxidoreductases	<p>○ = Reduction equivalent</p> <p>$A_{red} + B_{ox} \rightleftharpoons A_{ox} + B_{red}$</p>	Dehydrogenases Oxidases, peroxidases Reductases Monooxygenases Dioxygenases
2 Transferases	<p>$A-B + C \rightleftharpoons A + B-C$</p>	C ₁ -Transferases Glycosyltransferases Aminotransferases Phosphotransferases
3 Hydrolases	<p>$A-B + H_2O \rightleftharpoons A-H + B-OH$</p>	Esterases Glycosidases Peptidases Amidases
4 Lyases ("synthases")	<p>$A + B \rightleftharpoons A-B$</p>	C-C-Lyases C-O-Lyases C-N-Lyases C-S-Lyases
5 Isomerases	<p>$A \rightleftharpoons \text{Iso-A}$</p>	Epimerases <i>cis trans</i> Isomerases Intramolecular transferases
6 Ligases ("synthetases")	<p>$A + B + XTP \rightleftharpoons A-B + XDP + P_i$ $X = A, G, U, C$</p>	C-C-Ligases C-O-Ligases C-N-Ligases C-S-Ligases

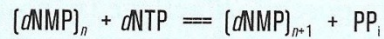
Klasifikacija encimov - primeri

oksidoreduktaza



trivialno ime: laktat-dehidrogenaza
 sistematično ime: L-laktat: NAD⁺-oksidoreduktaza
 klasifikacijska številka: 1.1.2.3

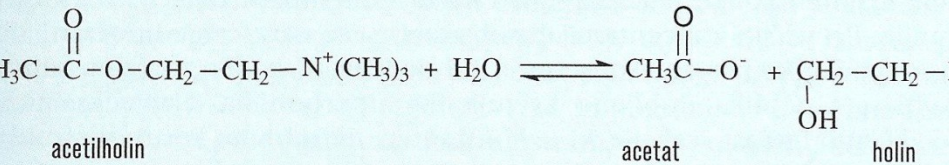
transferaza



[d-NMP]_n = DNA z n nukleotidi
 [d-NMP]_{n+1} = DNA z n+1 nukleotidi
 PP_i = pirofosfat

trivialno ime: DNA-polimeraza
 sistematično ime: deoksinukleozidtrifosfat: DNA-deoksinukleotidiltransferaza, ki jo usmerja DNA
 klasifikacijska številka: 2.7.7.7

hidrolaza



trivialno ime: acetilholin-esteraza
 sistematično ime: acetilholin-acetilhidrolaza
 klasifikacijska številka: 3.1.1.7

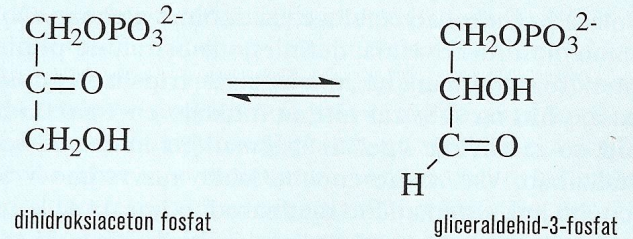
liaza



ogljikova kislina

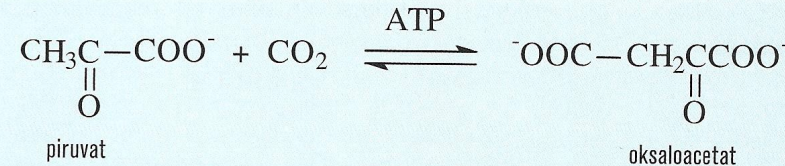
trivialno ime: karboanhidraza
 sistematično ime: karbonat-hidroliza
 klasifikacijska številka: 4.2.1.1

izomeraza



trivialno ime: triozafosfat-izomeraza
 sistematično ime: D-gliceraldehid-3-fosfat-ketoizomeraza
 klasifikacijska številka: 5.3.1.1

ligaza



trivialno ime: piruvat-karboksilaza
 sistematično ime: piruvat: CO₂-ligaza [ob sproščanju ADP]
 klasifikacijska številka: 6.4.1.1



BRENDA



The Comprehensive Enzyme Information System

Nomenclature	Reaction & Specificity	Functional Parameters			
Enzyme Names	Pathway	Km Value			
EC Number	Catalysed Reaction	kcat/Km Value			
Common/ Recommended Name	Reaction Type	Ki Value			
Systematic Name	Natural Substrates and Products	IC50 Value			
Synonyms	Substrates and Products	pI Value			
CAS Registry Number	Substrates	Turnover Number			
Isolation & Preparation	Natural Substrate	Specific Activity			
	Products	pH Optimum			
	Natural Product	pH Range			
	Inhibitors	Temperature Optimum			
	Cofactors	Temperature Range			
	Metals/Ions	Kinetic ENzyme DATA			
	Activating Compounds	NEW			
Purification	Ligands	Organism-related information			
	Biochemicals Reactions All		Organism		
	Cloned		Source Tissue		
	Expression		Localization		
	Renatured		Protein-Specific Search		
Crystallization					
Stability	Enzyme Structure	Disease & References			
			pH Stability	Disease/ Diagnostics	
			Temperature Stability	References	
			General Stability	Application & Engineering	
			Organic Solvent Stability		Engineering
			Oxidation Stability		Application
			Storage Stability	Modification	

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

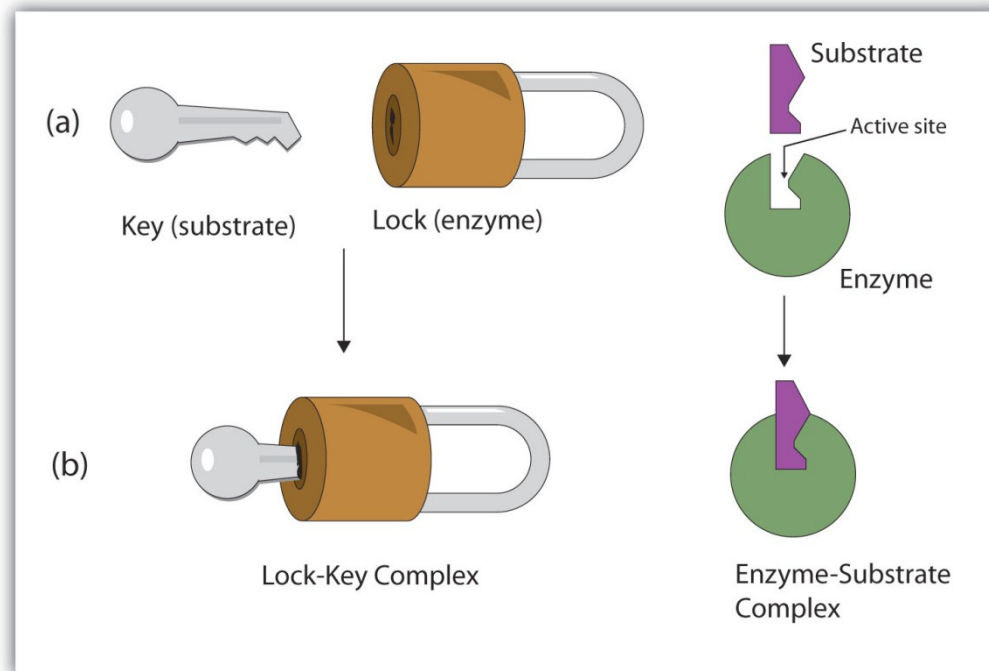
Razširjenost uporabe encimov

(1987 – 1999)

razred encimov	št. uvrščениh	št. razpoložljivih	ocenjen delež uporabe
1. oksidoreduktaze	650	90	25%
2. transferaze	720	90	~ 5%
3. hidrolaze	636	150	65%
4. liaze	255	35	~ 5%
5. izomeraze	120	6	~ 1%
6. ligaze	80	5	~ 1%

Modeli delovanja encimov

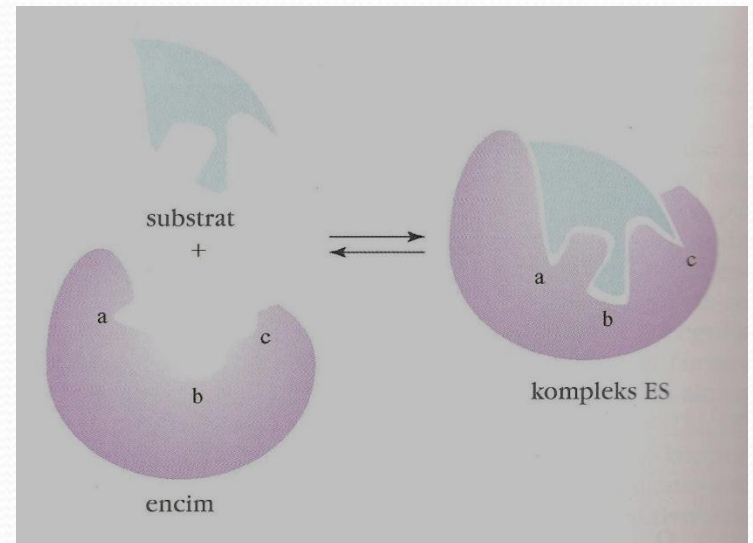
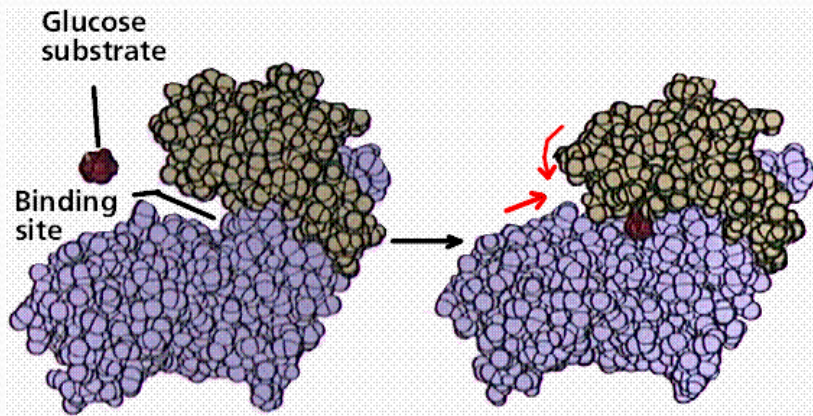
- **Ključ – ključavnica**
(Emil Fischer, 1894)



Modeli delovanja encimov

- Inducirana prilagoditev

(angl. induced fit; Daniel Koshland, 1958)



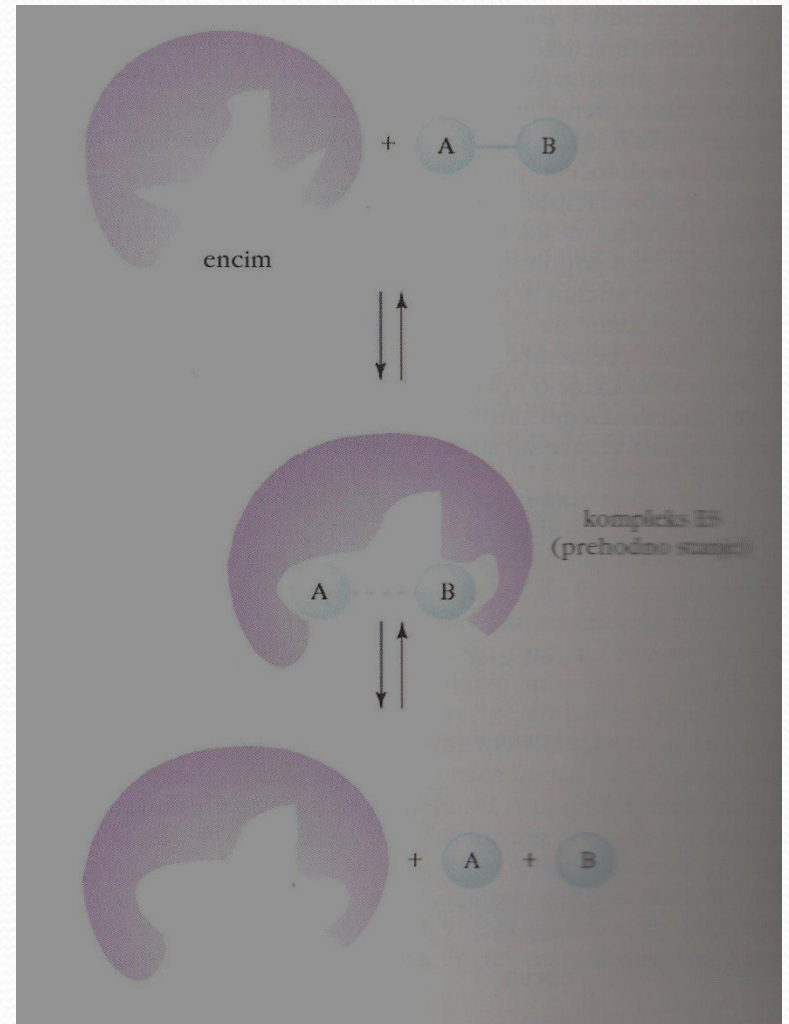
Modeli delovanja encimov

- Model analoga prehodnega stanja

Aktivno mesto

- prepozna in veže substrat
- substrat pravilno usmeri in aktivira
- ko je substrat vezan v aktivnem mestu, prevzame strukturo prehodnega stanja

Ključne: šibke interakcije encim-substrat

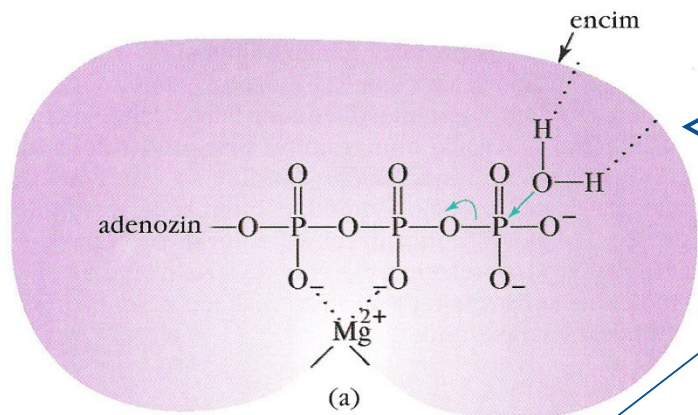


Mehanizem encimskih reakcij

- **Kislinsko-bazična kataliza:** funkcionalne skupine v aktivnem mestu lahko delujejo kot kisline ali baze, ki pomagajo pri prenosu protona
- **Kovalentna kataliza:** substrat se kovalentno veže na encim preko nukleofilne funkcionalne skupine
- **Kataliza s pomočjo kovinskega iona:** sodelujejo kovinski ioni, ki so vezani na encim ali substrat (30 % encimov)
 - Držijo substrat v pravilni usmeritvi
 - Polarizacija vezi, ki se bo razcepila, ali s stabilizacijo intermedata
 - Sodelujejo pri prenosu elektronov (oksidoredukcijske reakcije)

Kovinski ioni kot kofaktorji

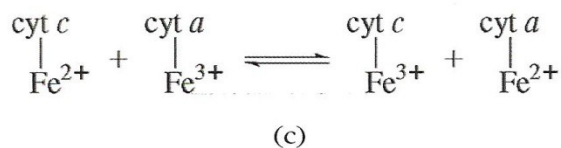
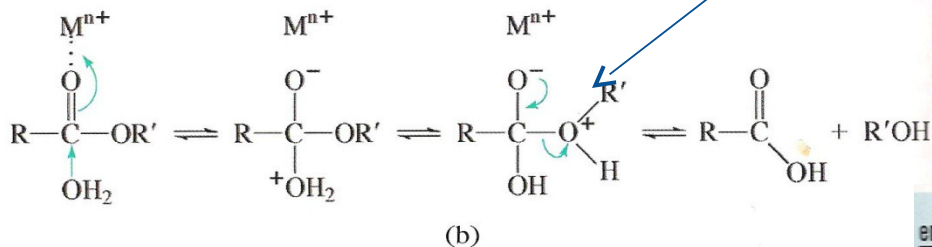
kovinski ioni, vezani na encim ali substrat:
potrebni v mikrokoličinah



a) držijo substrat v pravilni usmeritvi
(koordinacijske kovalentne vezi)

b) polarizacija vezi, ki se bo cepila
ali stabilizacija nabitega intermedjata

c) reverzibilni prenos elektronov med
kovinskimi ioni in substratom



encim	kovinski ion
katalaza, peroksidaza, akonitaza in citokrom-oksidadza	Fe ²⁺ in Fe ³⁺
alkohol-dehidrogenaza, karboksipeptidaza A, karboksipeptidaza B in DNA-polimeraza	Zn ²⁺
citokrom-oksidadza, protein-lizin-6-oksidadza in superoksid-dismutaza	Cu ²⁺
heksokinaza, glukoza-6-fosfataza	Mg ²⁺
arginaza	Mn ²⁺
piruvat-kinaza	K ⁺
ureaza	Ni ²⁺
nitrat-reduktaza	Mo ⁴⁺ in Mo ⁶⁺
karboanhidraza	Zn ²⁺ , Cd ²⁺

Encimi in kofaktorji

- **holoencim**: celoten encim s kofaktorjem
- **apoencim**: samo proteinski del (brez kofaktorja)
- **kofaktorji** - pomagajo encimu (lahko šibko ali močno vezani na encim)
 - **koencimi**: organske spojine
 - kosubstrati
 - prostetične skupine - močno (lahko kovalentno) vezane na apoencim
 - **esencialni kovinski ioni**
 - aktivatorski ioni – šibko vezani
 - kovinski ioni v metaloencimih –močno vezani

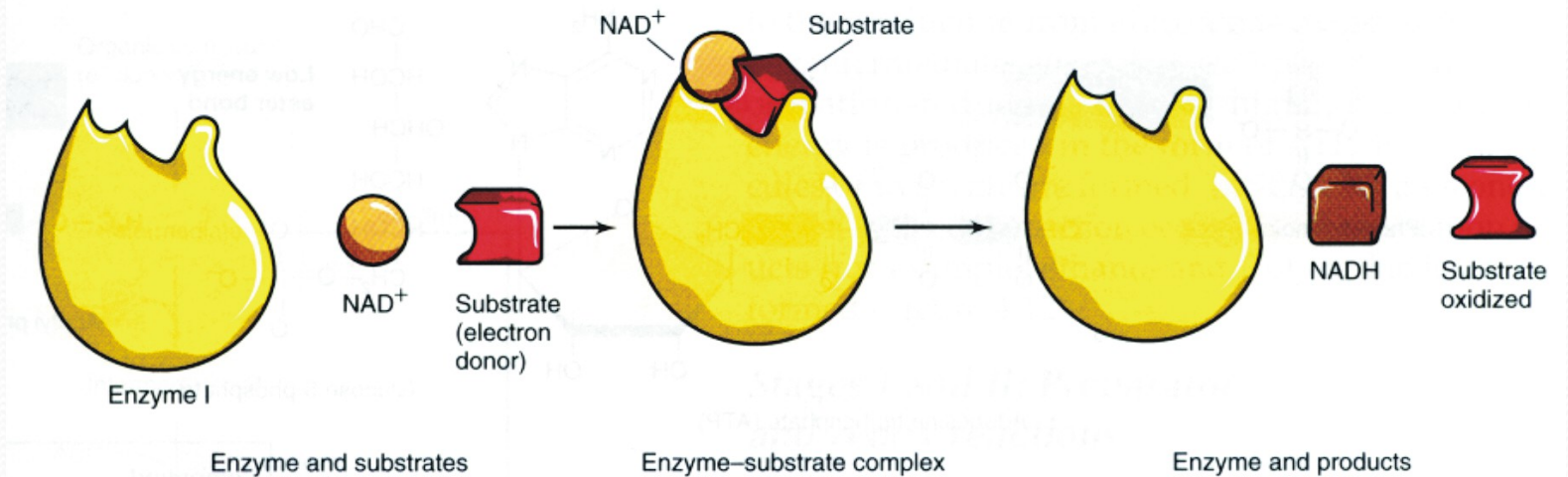
Koencimi

- **Nukleotidi** ali njihovi derivati: ATP, NAD⁺, NADP⁺, FAD, FMN..
- **Derivati vitaminov**: koencimi po strukturi zelo sorodni vitaminom-pri živalih sinteza številnih koencimov iz vitaminov

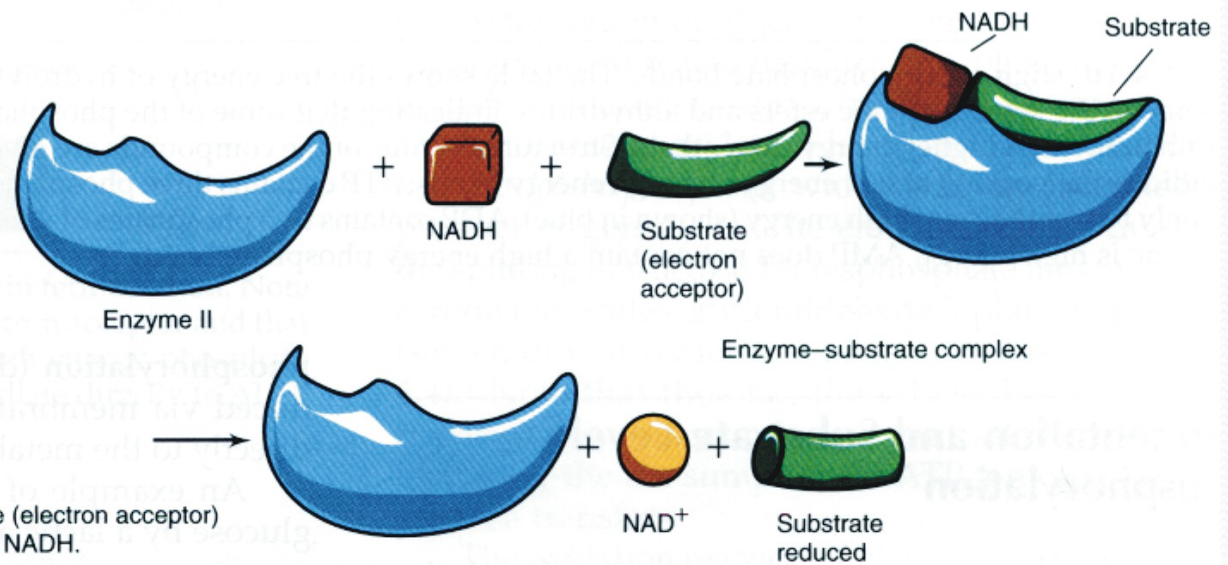
Vitamini: vitalni amini, mikronutrienti (mg ali µg dnevno)

- vodotopni: vitamin B1 – tiamin, vitamin B2-riboflavin, vitamin B12, nikotinska kislina, pantotenska kislina, biotin, folna kislina, vitamin C (askorbinska kislina)
- lipidotopni: vitamin A –retinol, vitamin D – holekalciferol, vitamin E – tokoferol, vitamin K -

Koencimi: oksidoredukcijske reakcije



Enzyme I reacts with substrate (electron donor) and oxidized form of coenzyme, NAD^+ .



Enzyme II reacts with substrate (electron acceptor) and reduced form of coenzyme, NADH.

Encimi: kinetika



Ko je doseženo *dinamično ravnotežje*, sta hitrosti tvorbe in razpada kompleksa ES enaki:

$$k_1 [E][S] = (k_2 + k_{cat}) [ES] \quad \text{oz.} \quad [ES] = \frac{[E][S]}{(k_2 + k_{cat})/k_1}$$

$$[E] = [E_t] - [ES]$$

Michaelisova konstanta: $K_M = \frac{k_2 + k_{cat}}{k_1} \text{ [mM]}$

$$[ES] \cdot K_M = [E_t][S] - [ES][S]$$

$$[ES](K_M + [S]) = [E_t][S]$$

$$[ES] = \frac{[E_t][S]}{K_M + [S]}$$

Najvišja reakcijska hitrost je dosežena, ko so vsa katalitska mesta zasedena s substratom:

$$v = k_{cat} [ES]$$

$$V_{max} = k_{cat} [E_t] \text{ [}\mu\text{M/min]}$$

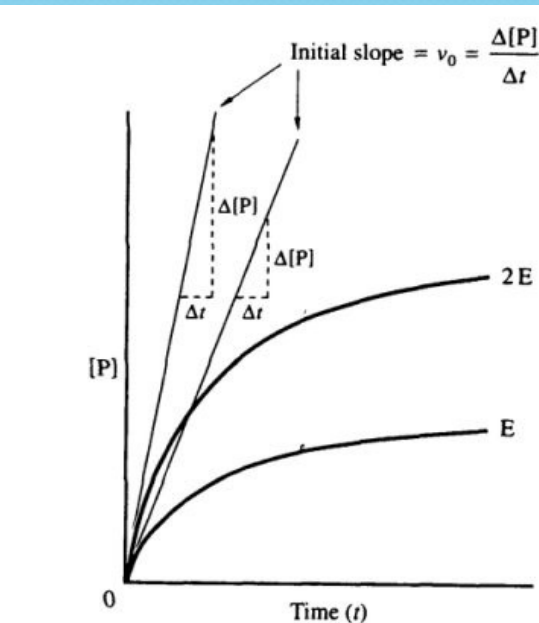
$$v_0 = \frac{k_{cat} [E_t][S]}{K_M + [S]}$$

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

pretvorbena število (turnover number):

$$k_{cat} = \frac{V_{max}}{[E_t]} \text{ [min}^{-1} \text{ ali s}^{-1}\text{]}$$

Enota encimske aktivnosti:
 $U = \mu\text{mol/min}$



$[E_t]$... celotna konc. encima
 v_0 ... začetna hitrost reakcije
 V_{max} ... maksimalna hitrost reakcije

Kinetika

Michaelisa in Mentenove



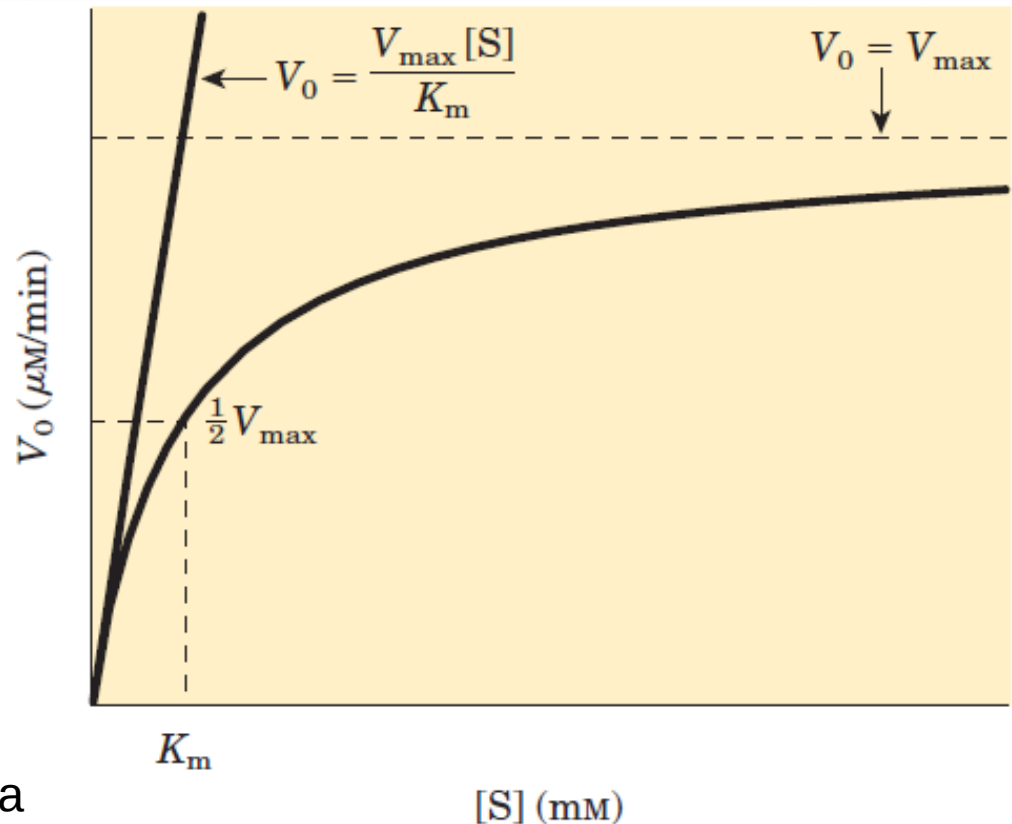
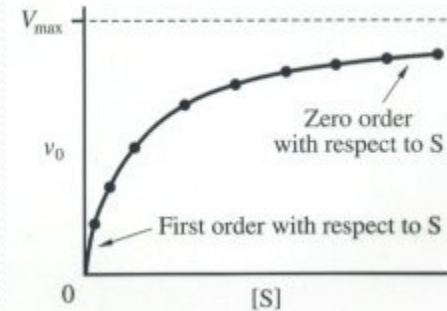
Leonor Michaelis,
1875–1949



Maud Menten,
1879–1960

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

$$K_M = [S], \quad \text{pri kateri je } v_0 = \frac{V_{\max}}{2}$$



nizek K_M , večja afiniteta do substrata

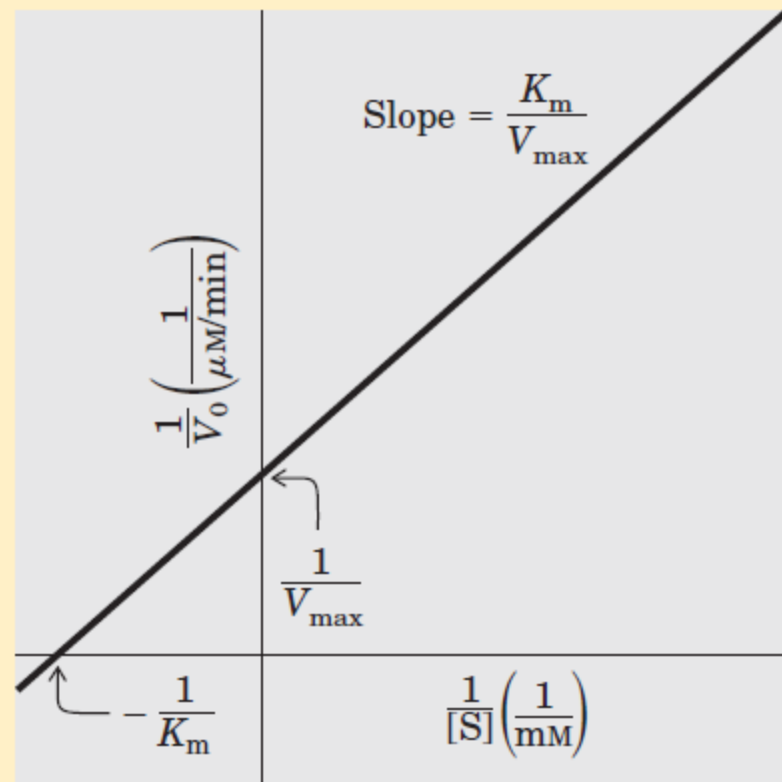
Lineveawer-Burk

linearizacija modela
Michaelisa in Mentenove

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

enostavno določanje kinetičnih
parametrov:

- maksimalne hitrosti V_{\max} in
- Michaelisove konstante K_M



Encimi: kinetične konstante

encim	substrat	K_m (mM)
katalaza	H_2O_2	0,001
heksokinaza (iz možganov)	ATP	0,4
	D-glukoza	0,05
	D-fruktoza	1,5
karboanhidraza	HCO_3^-	9
kimotripsin	gliciltirozinihglicin	108
	N-benzoiltirozinamid	2,5
β -galaktozidaza	laktoza	4,0
penicilinaza	benzilpenicilin	0,050
piruvat-karboksilaza	ATP	0,060
	piruvat	0,40
	HCO_3^-	1,0
ribuloza-1,5-bisfosfat-karboksilaza (rubisco)	ribuloza-1,5-bisfosfat	0,028
	CO_2	0,009
ribuloza-1,5-bisfosfat-oksigenaza (rubisco)	ribuloza-1,5-bisfosfat	0,028
	CO_2	0,535

encim	substrat	k_3 (s^{-1})
katalaza	H_2O_2	40 000 000
karboanhidraza	HCO_3^-	400 000
acetilholin-esteraza	acetilholin	25 000
penicilinaza	benzilpenicilin	2000
laktat-dehidrogenaza	laktat	1000
kimotripsin	gliciltirozinihglicin	100
DNA-polimeraza	DNA	15
ribuloza-1,5-bisfosfat-karboksilaza	ribuloza-1,5-bisfosfat + CO_2	3,3
ribuloza-1,5-bisfosfat-oksigenaza	ribuloza-1,5-bisfosfat + O_2	2,4

$$k_3 = k_{cat} = \frac{V_{max}}{[E_t]}$$

Vpliv pH in T na aktivnost encimov

– A. pH and temperature dependency of enzyme activity

