Univerza v Ljubljani, Fakulteta za farmacijo, 2009

MOLEKULARNA ENCIMOLOGIJA

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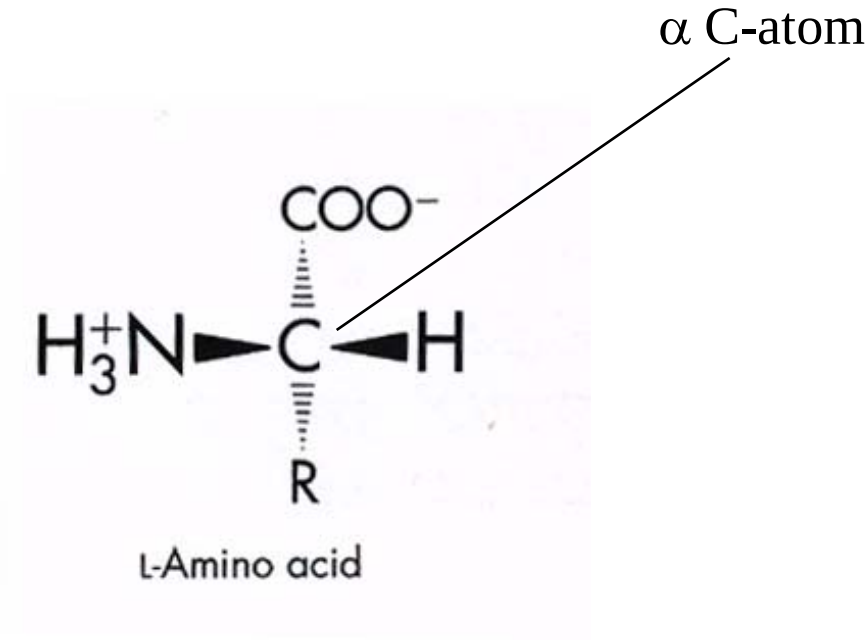
GLEJ: http://ibk.mf.uni-lj.si/teaching/lab_medicina/default.html

I. Glavne lastnosti (20 standardnih) aminokislin

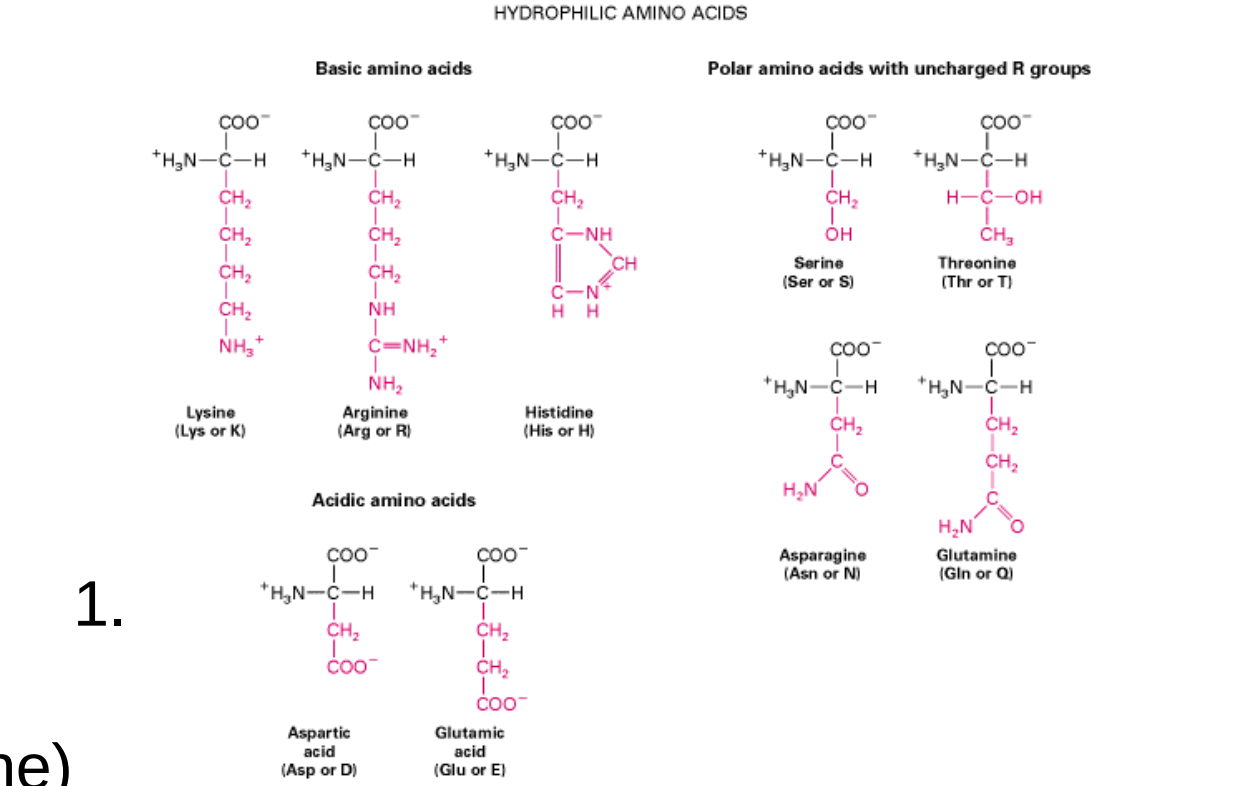
- klasifikacija AK
- velikost
- naboj
- polarnost
- hidrofobnost
- aromatske AK
- optična aktivnost

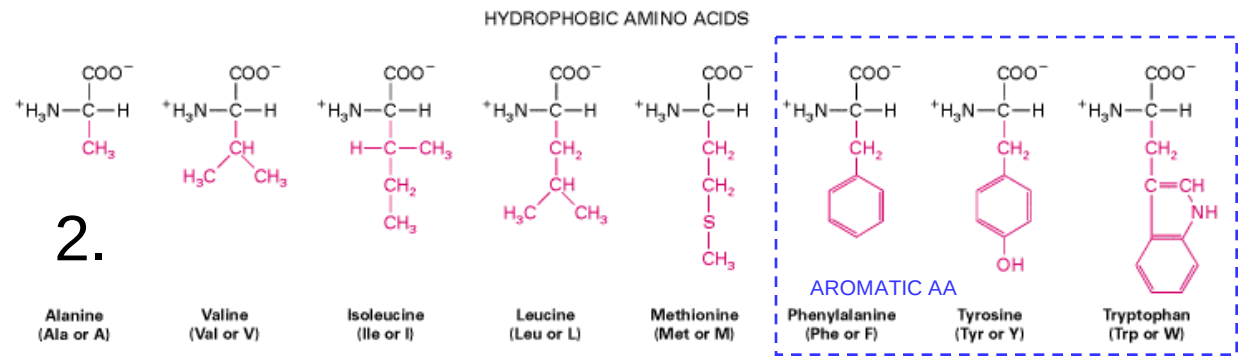


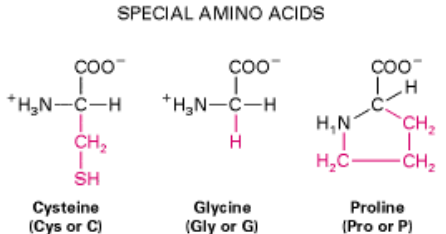
PAZI: več 100 nestandardnih AK (post-transl. modif. & D-AK)!

Klasifikacija (R!):

1. hidrofilne (polarne):
   - bazične
   - kisle
   - nenabit R
2. hidrofobne (nepolarne)
3. ‘posebne’







Velikost AK-ostanka:

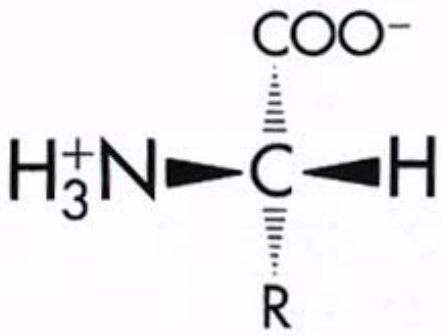
100-130 Da ($\overline{110}$ Da),
a nekaj izjem!

AK ostanek (residue)

AK radikal

dodaj 18 (H_2O) za maso AK!

odštej 56 za maso stranske verige!

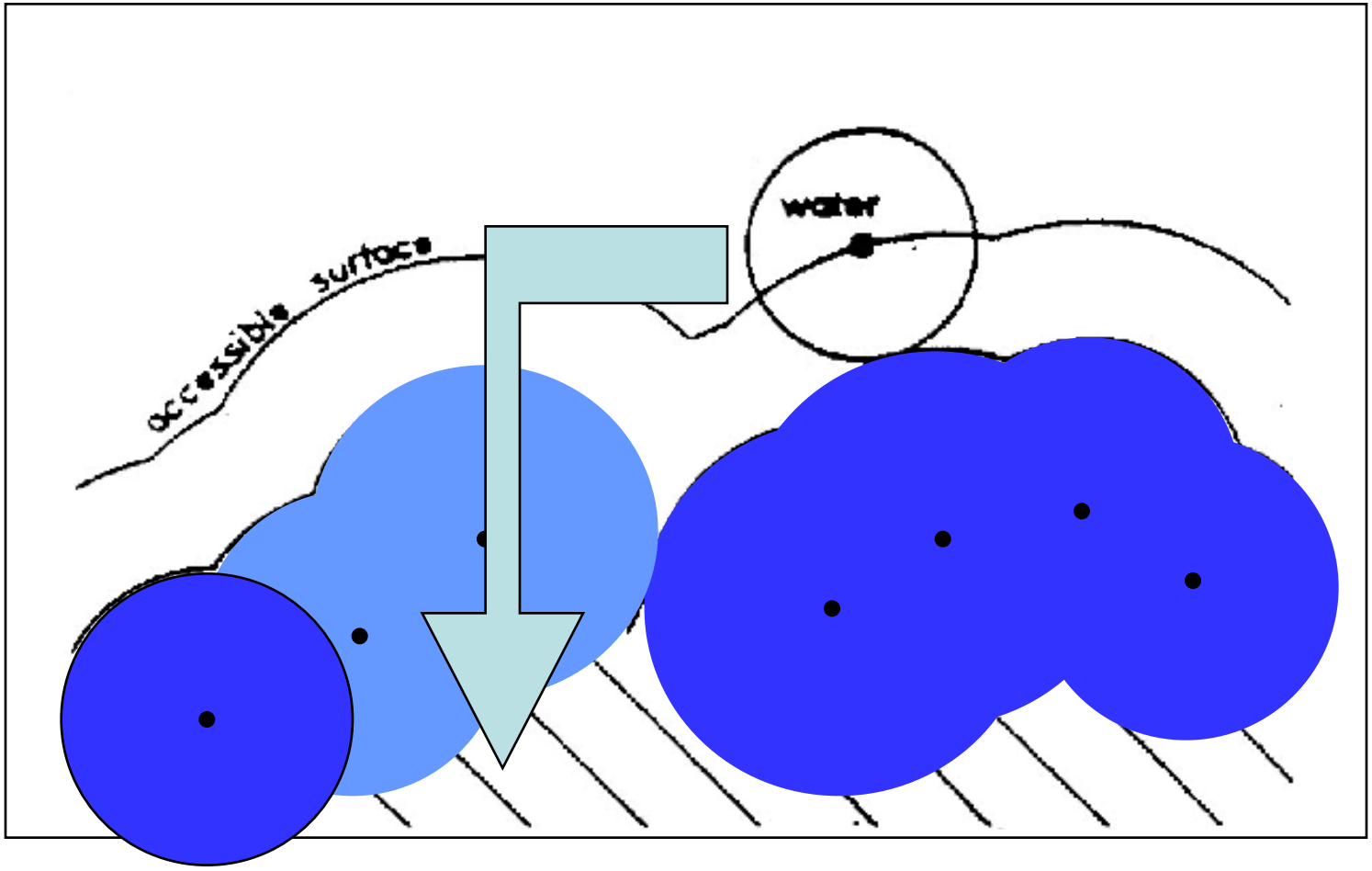


L-Amino acid

Table 1.1 Properties of Individual Amino Acid Residues

Residue	Residue mass[a] (daltons)	Van der Waals volume[b] (Å³)	Frequency in proteins[c] (%)
Ala (A)	71.09	67	8.3
Arg (R)	156.19	148	5.7
Asn (N)	114.11	96	4.4
Asp (D)	115.09	91	5.3
Cys (C)	103.15	86	1.7
Gln (Q)	128.14	114	4.0
Glu (E)	129.12	109	6.2
Gly (G)	57.05	48	7.2
His (H)	137.14	118	2.2
Ile (I)	113.16	124	5.2
Leu (L)	113.16	124	9.0
Lys (K)	128.17	135	5.7
Met (M)	131.19	124	2.4
Phe (F)	147.18	135	3.9
Pro (P)	97.12	90	5.1
Ser (S)	87.08	73	6.9
Thr (T)	101.11	93	5.8
Trp (W)	186.21	163	1.3
Tyr (Y)	163.18	141	3.2
Val (V)	99.14	105	6.6

Velikost je pomembna pri zamenjavah aminokislin!

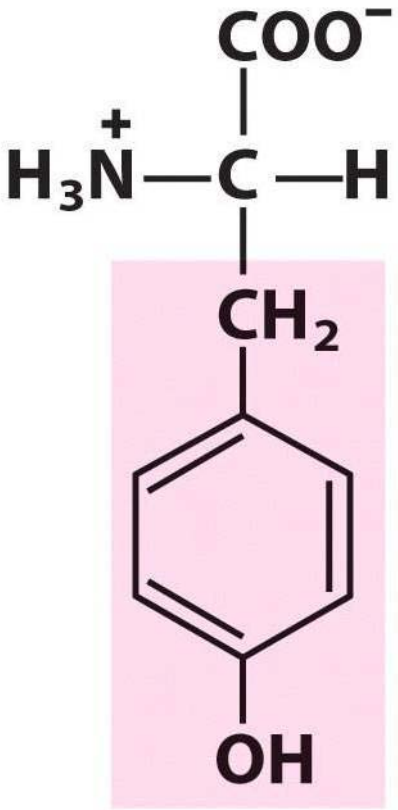


Samo R, N- and C- konci so so pomembni pri proteinih

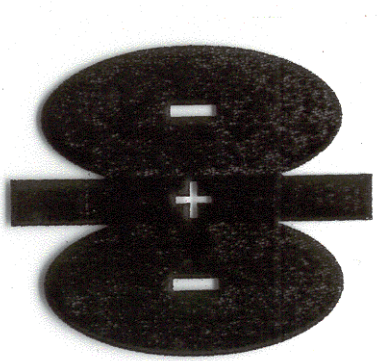
NABOJ:
Odvisen od pK_a vrednosti (25°C)

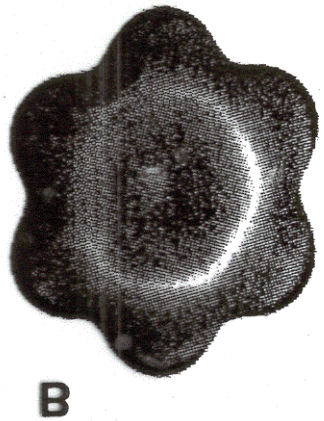
Amino acid	α-COOH group	α-NH_3^+ group	Side chain (R)
Alanine	2.3	9.9	
Glycine	2.4	9.8	
Phenylalanine	1.8	9.1	
Serine	2.1	9.2	
Valine	2.3	9.6	
Aspartic acid	2.0	10.0	3.9
Glutamic acid	2.2	9.7	4.3
Histidine	1.8	9.2	6.0
Cysteine	1.8	10.8	8.3
Tyrosine	2.2	9.1	10.9 !
Lysine	2.2	9.2	10.8
Arginine	1.8	9.0	12.5

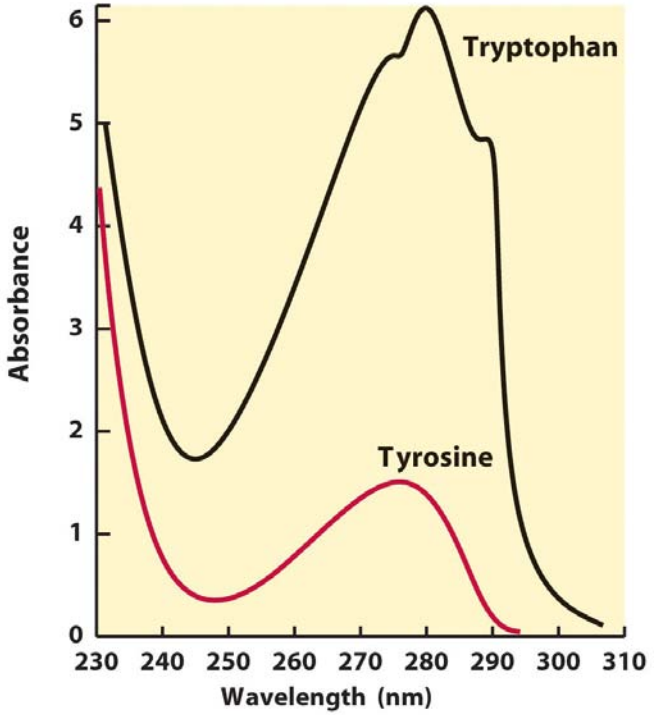
Aromatske AK (Phe, Tyr, Trp)

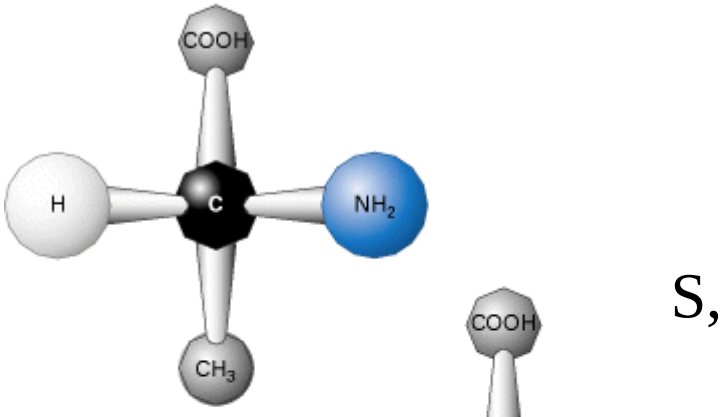


- velike
- resonanca: -OH v Tyr bolj ‘kisla’
- polarnost: posebne interakcije
- absorbirajo UV svetlobo

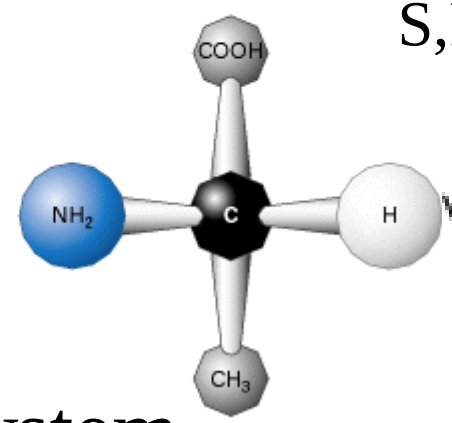






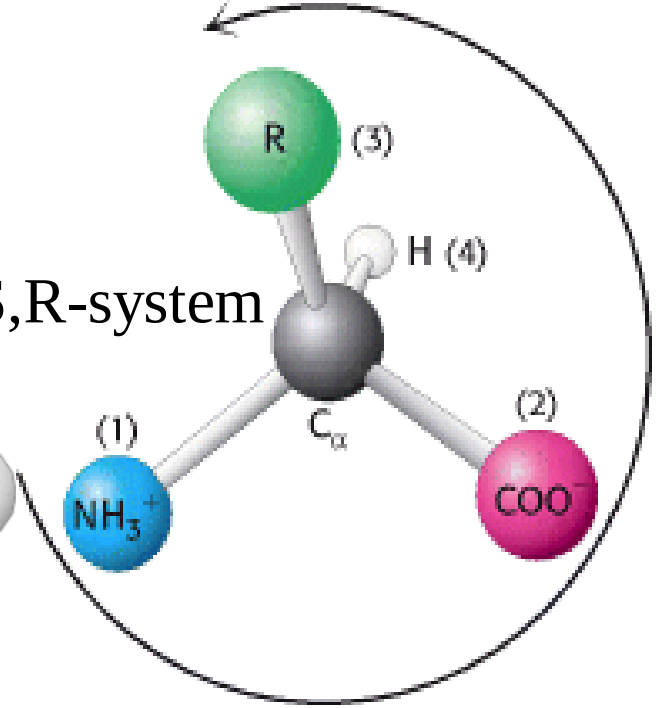


D-Alanine



L-Alanine

D,L-system

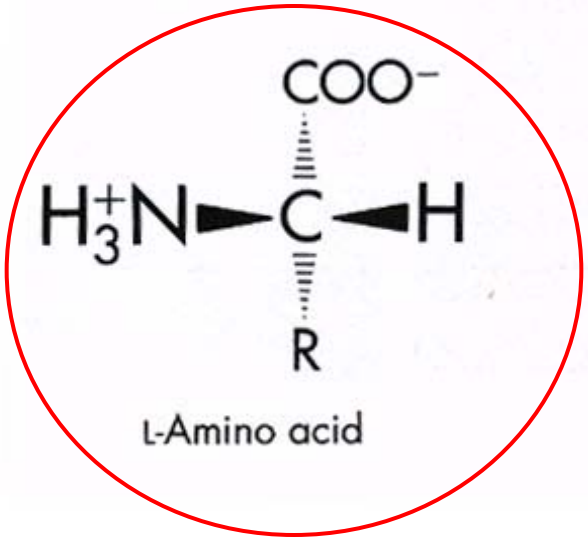


S,R-system

rotacija linearno polarizirane svetlobe

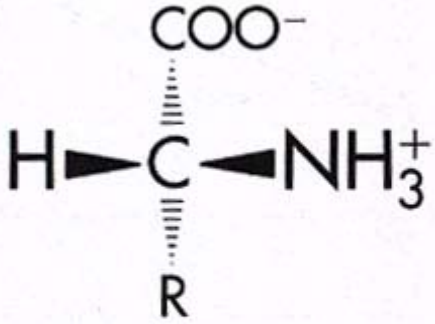
($\alpha = [\alpha].l.c$)

POMEMBNO ZA PREPOZNAVANJE: E-S, R-H, Ag-Ab, etc.



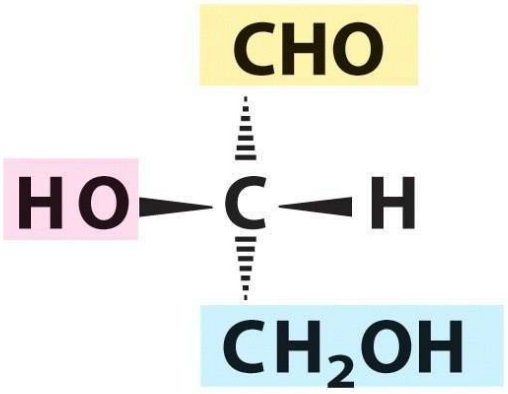
L-Amino acid

or



D-Amino acid

Posebne funkcije!



L-Glyceraldehyde

STANDARD!

Ne-esencialne	Esencialne
Alanine	Histidine
Arginine	Isoleucine
Asparagine	Leucine
Aspartate	Lysine
Cysteine	Methionine
Glutamate	Phenylalanine
Glutamine	Threonine
Glycine	Tryptophan
Proline	Valine
Serine	
Tyrosine	

Bliskovit pregled proteinske sinteze

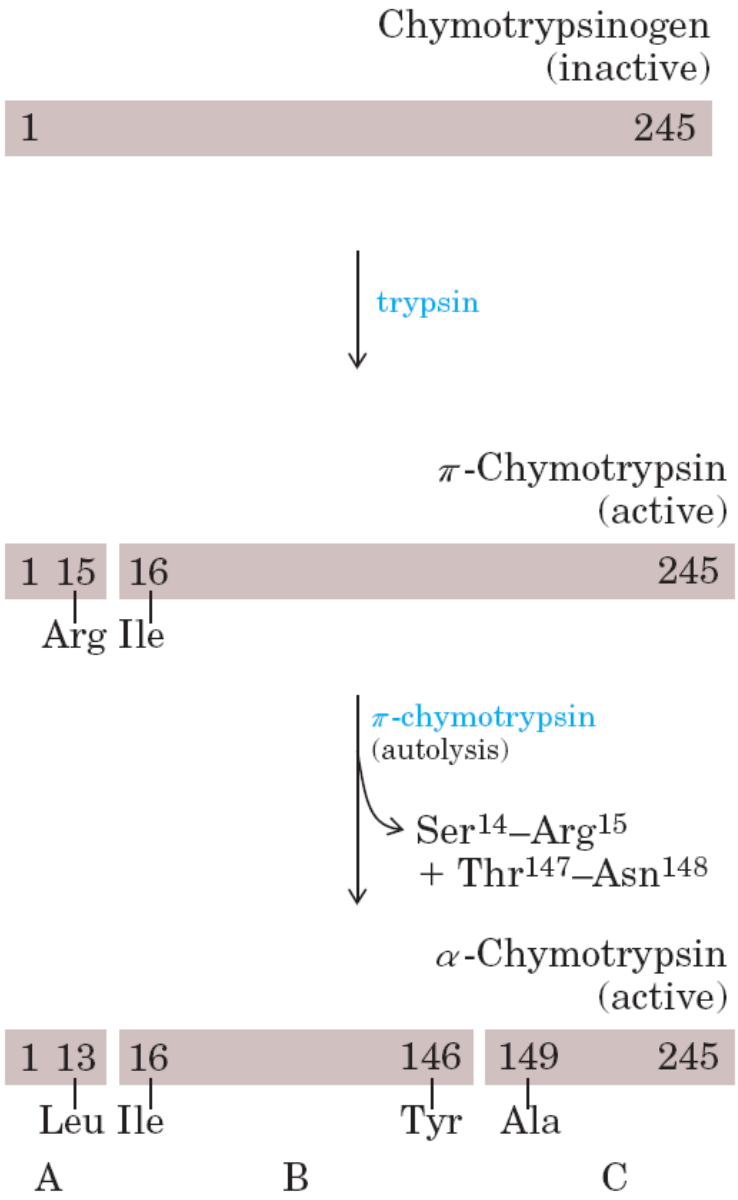
- DNA → primarni transkript (pre-mRNA)
- pre-mRNA se procesira
- mRNA je zapis za I. str. proteinov (I. struktura)
- nekatere AK se modificirajo
- 1D → 3D strukturo
- kontrola kvalitete delovanja

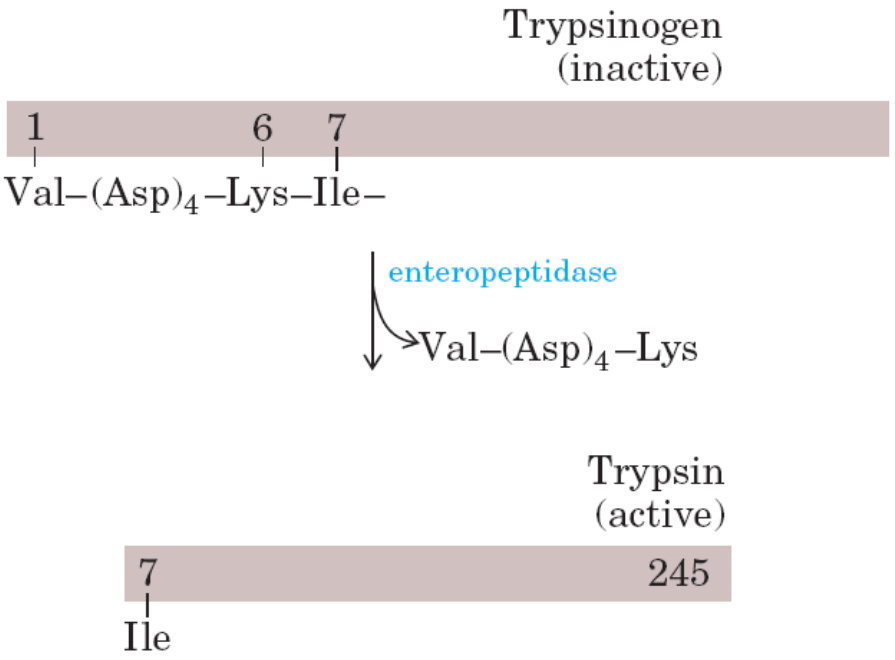
Post-translacijske modifikacije vpeljejo nove funkcionalne skupine in imajo tudi regulatorno vlogo. Znanih je več kot 80.

Pomembne modifikacije:

- proteolitično procesiranje
- spremembe N- in C-koncev
- glikozilacija
- pripenjanje lipidov
- sulfatiranje
- γ-karboksi-glutaminska kislina
- hidroksilacija
- fosforilacija
- ADP-ribozilacija
- disulfidni mostički

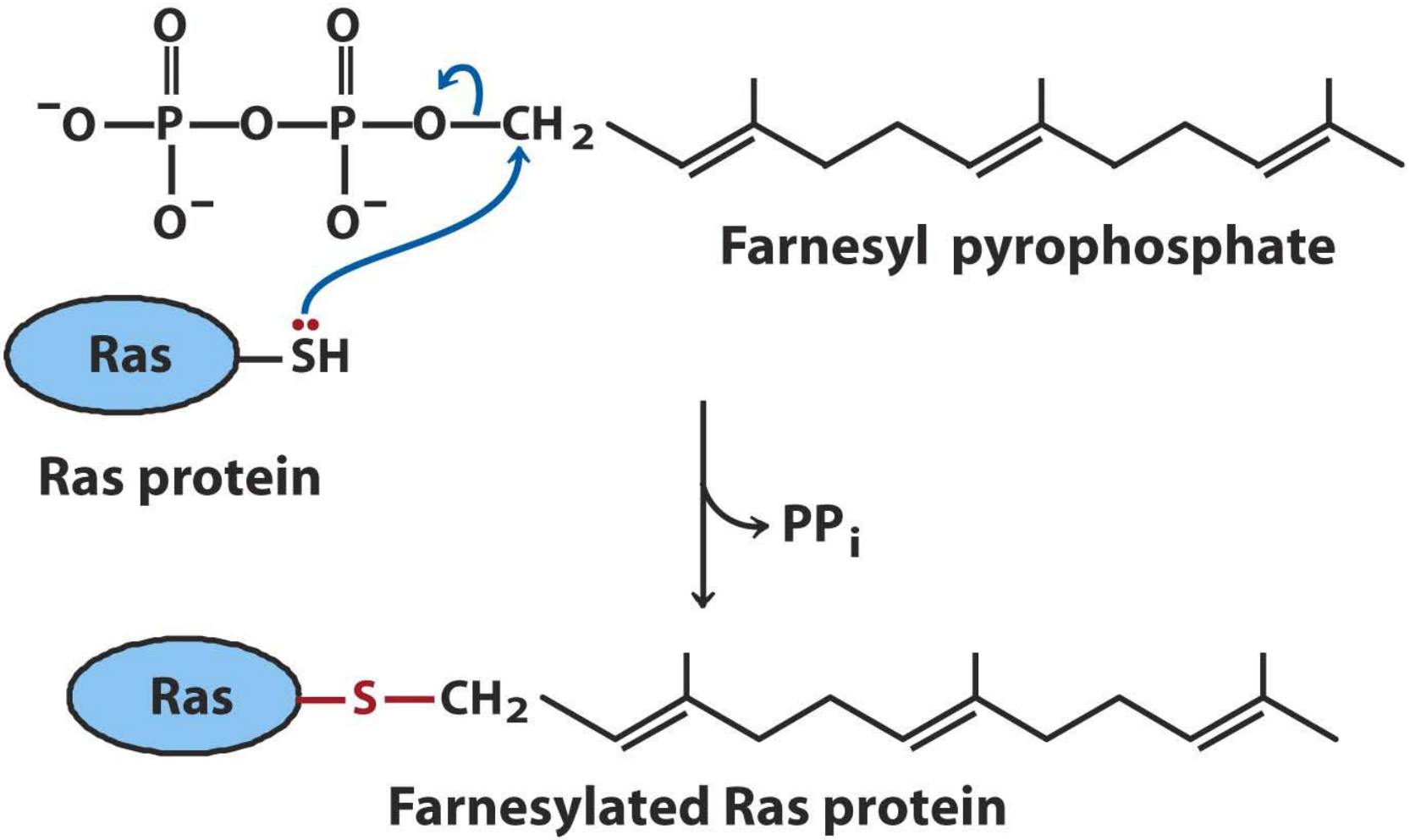
Proteolitično procesiranje: ‘aktivacija’ encimov





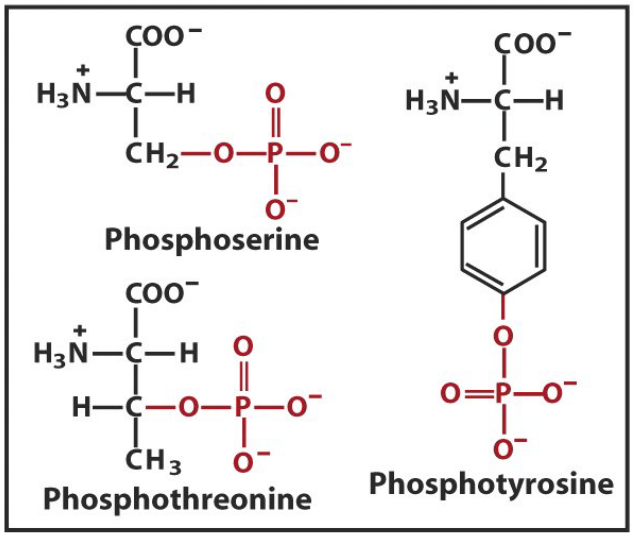
Namen:encim postane aktiven tam kjer deluje in ne na mestu sinteze!

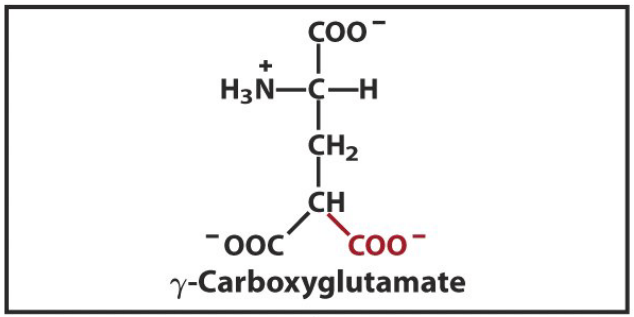
Pripenjanje lipidov: interakcija z membrano, 3D struktura

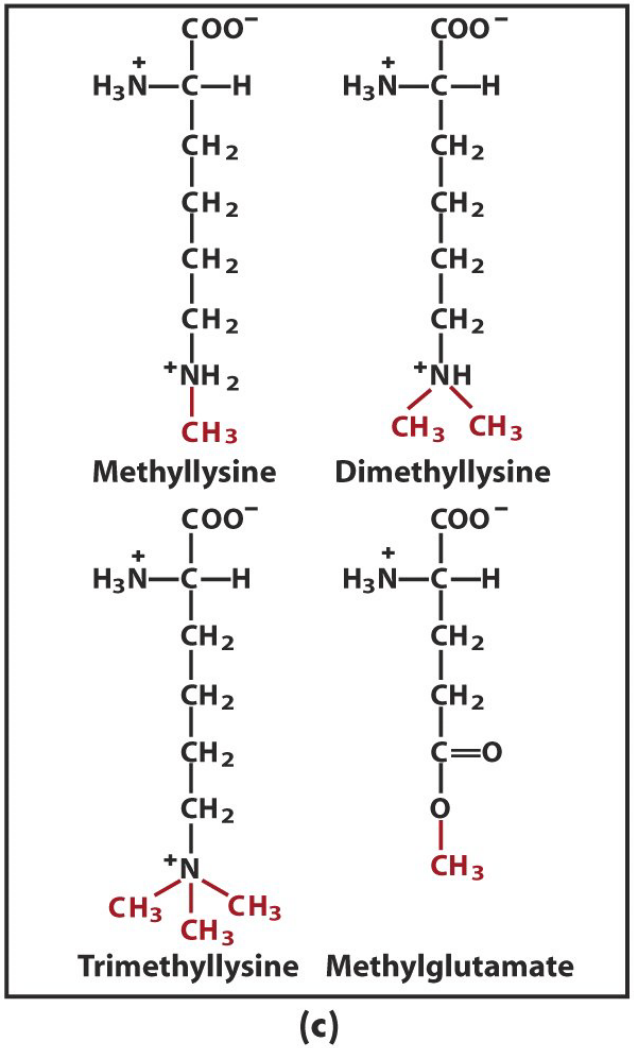


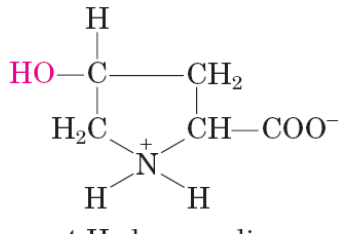
Pripenjanje malih funkcionalnih skupin

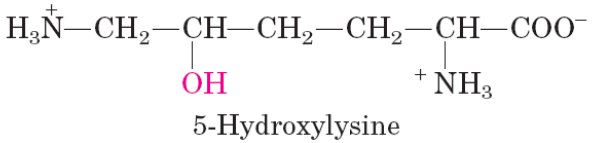
- fosforilacija
- γ-karboksi-glutamat
- metilacija
- sulfatiranje
- hidroksilacija



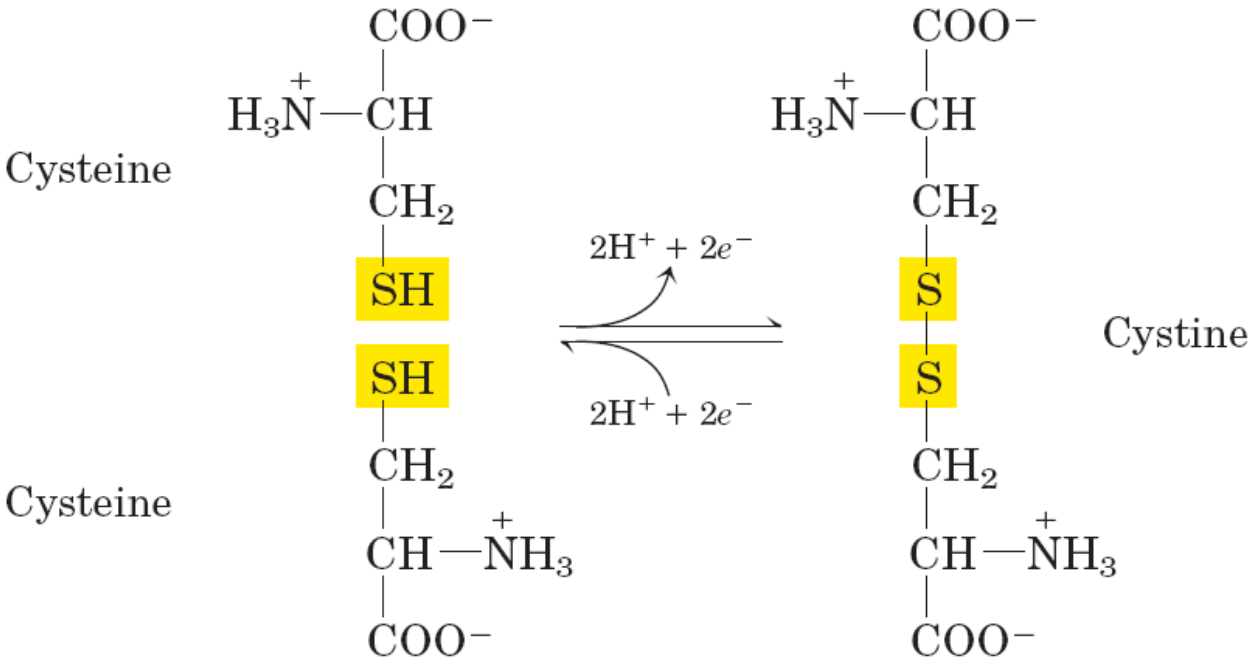


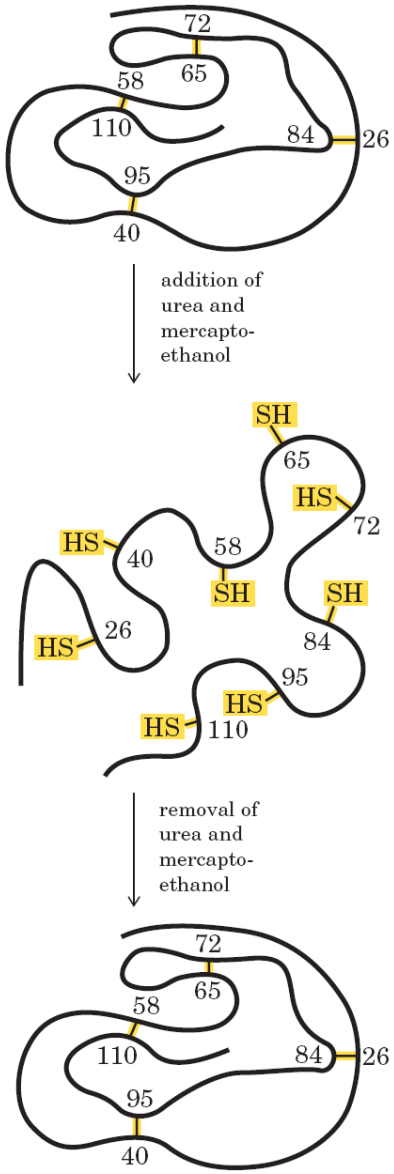




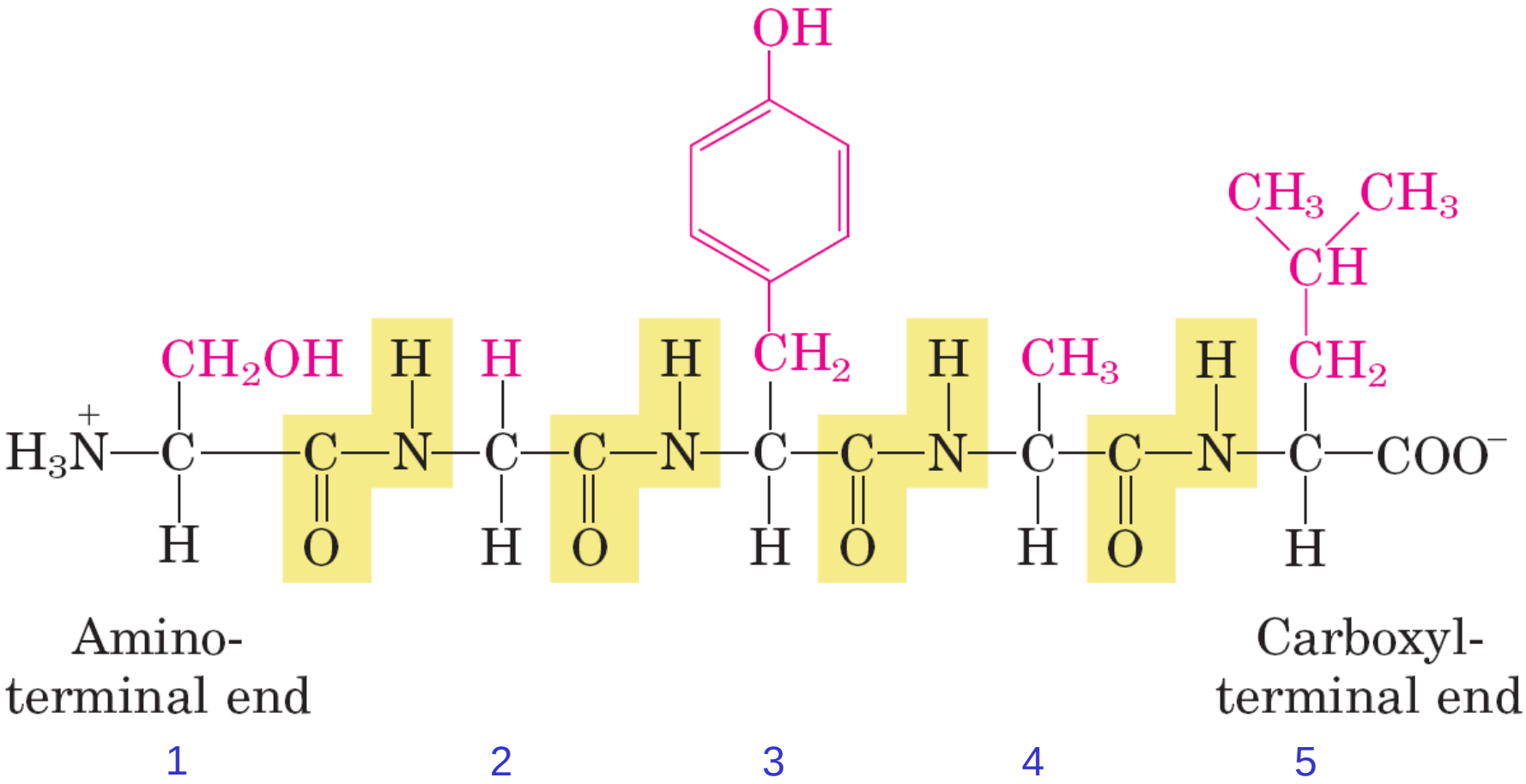


Nastanek disulfidne vezi: stabilizacija 3D-strukture





Primarna struktura: sekvenca aminokislin



Sekvenco lahko določimo neposredno iz proteina ali posredno iz mRNA ali DNA.

S poravnavo proteinskih sekvenc določamo sorodnost med vrstami.

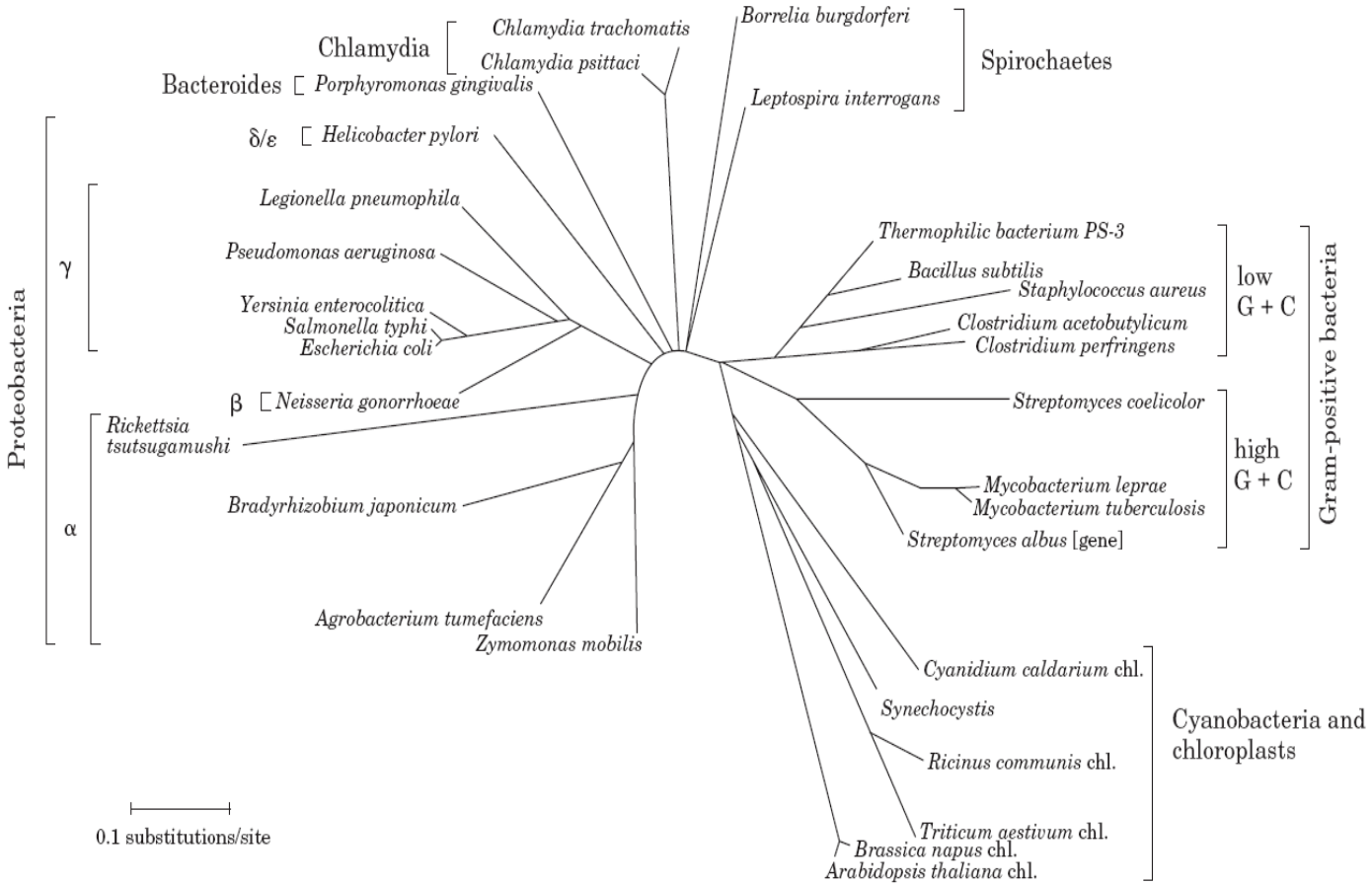
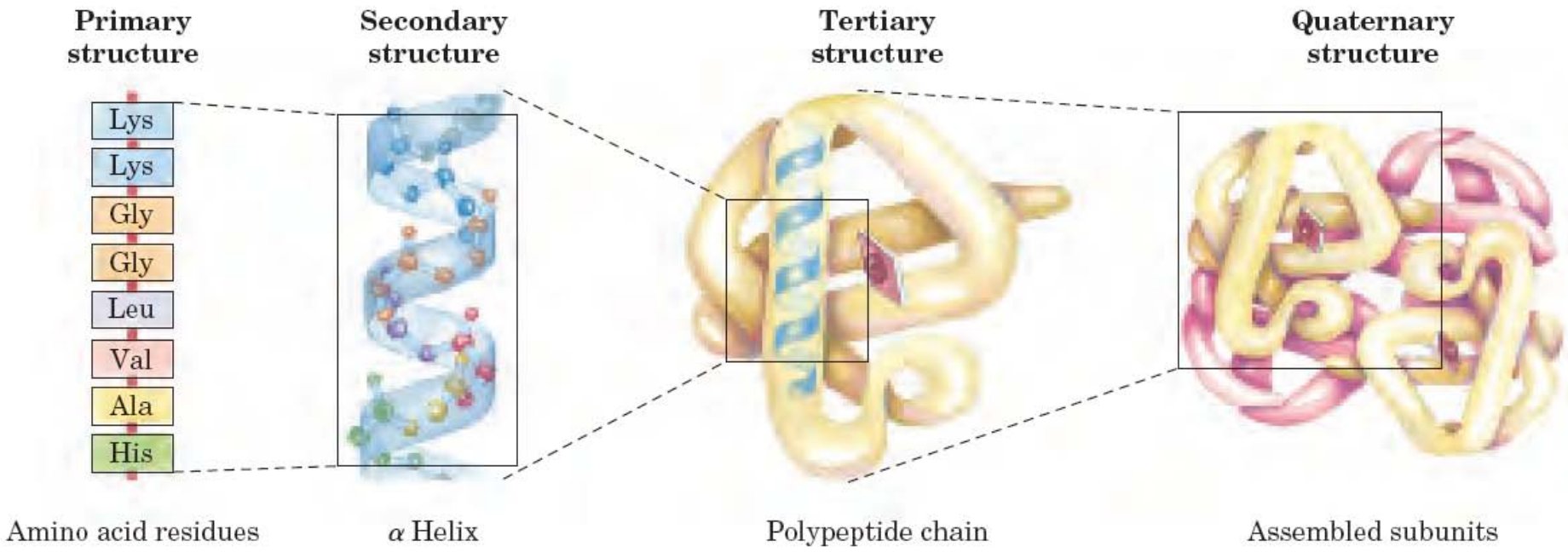


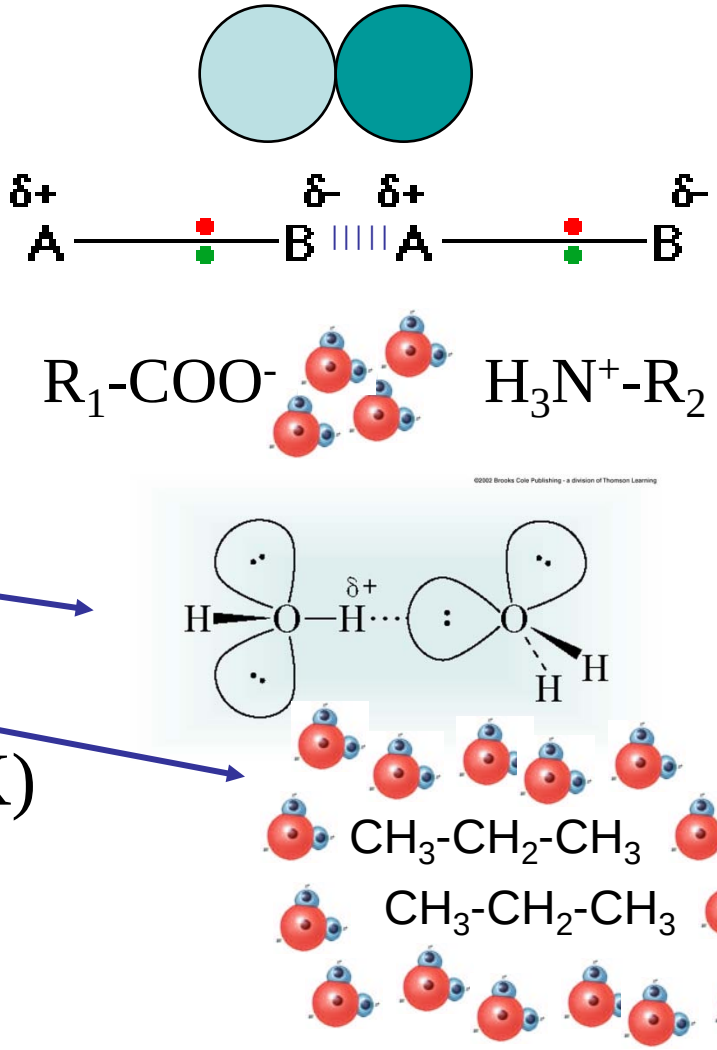
FIGURE 3-33 Evolutionary tree derived from amino acid sequence comparisons. A bacterial evolutionary tree, based on the sequence divergence observed in the GroEL family of proteins. Also included in this tree (lower right) are the chloroplasts (chl.) of some nonbacterial species.

Samosestavljanje polipeptidne verige: od primarne do terciarne (kvartarnarne) strukture

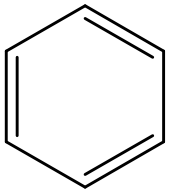


ŠIBKE INTERAKCIJE

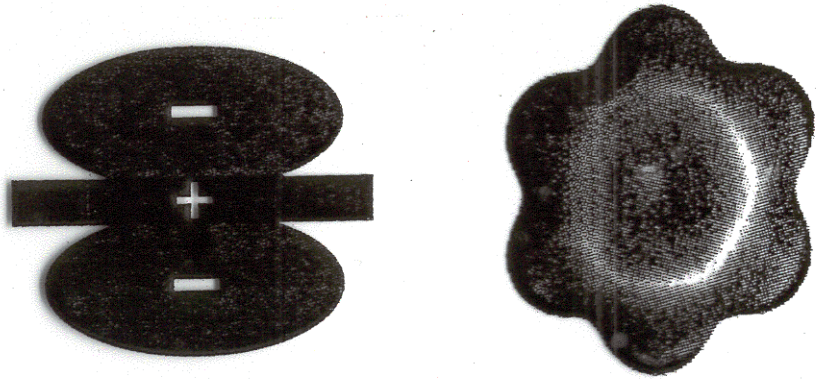
- odboj pri dotiku
- van der Waalsove interakcije
- elektrostatske interakcije
- vodikova vez
- hidrofobne interakcije
- ion - π interakcije (aromatske AK)

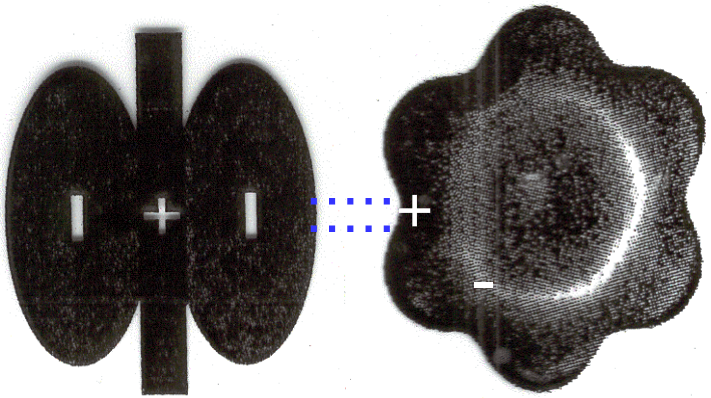


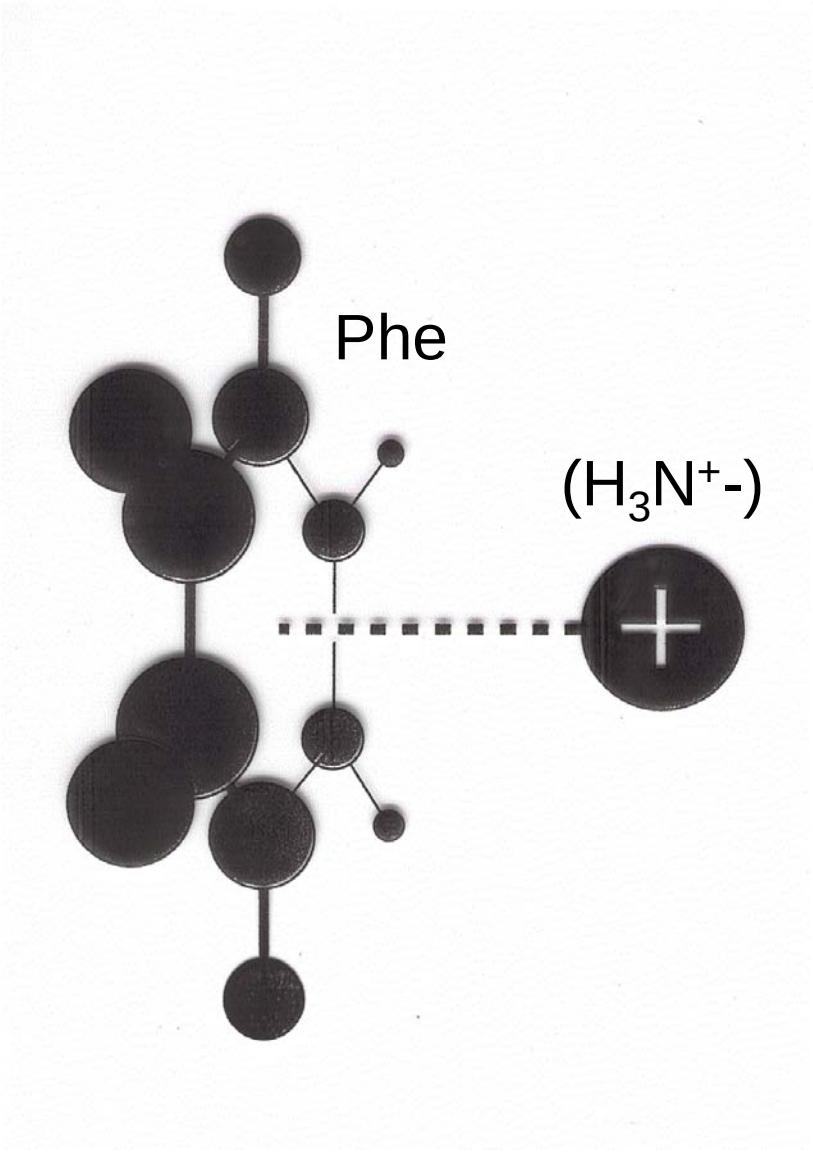
Ion - π interakcije:



$NH_4^{\oplus}$



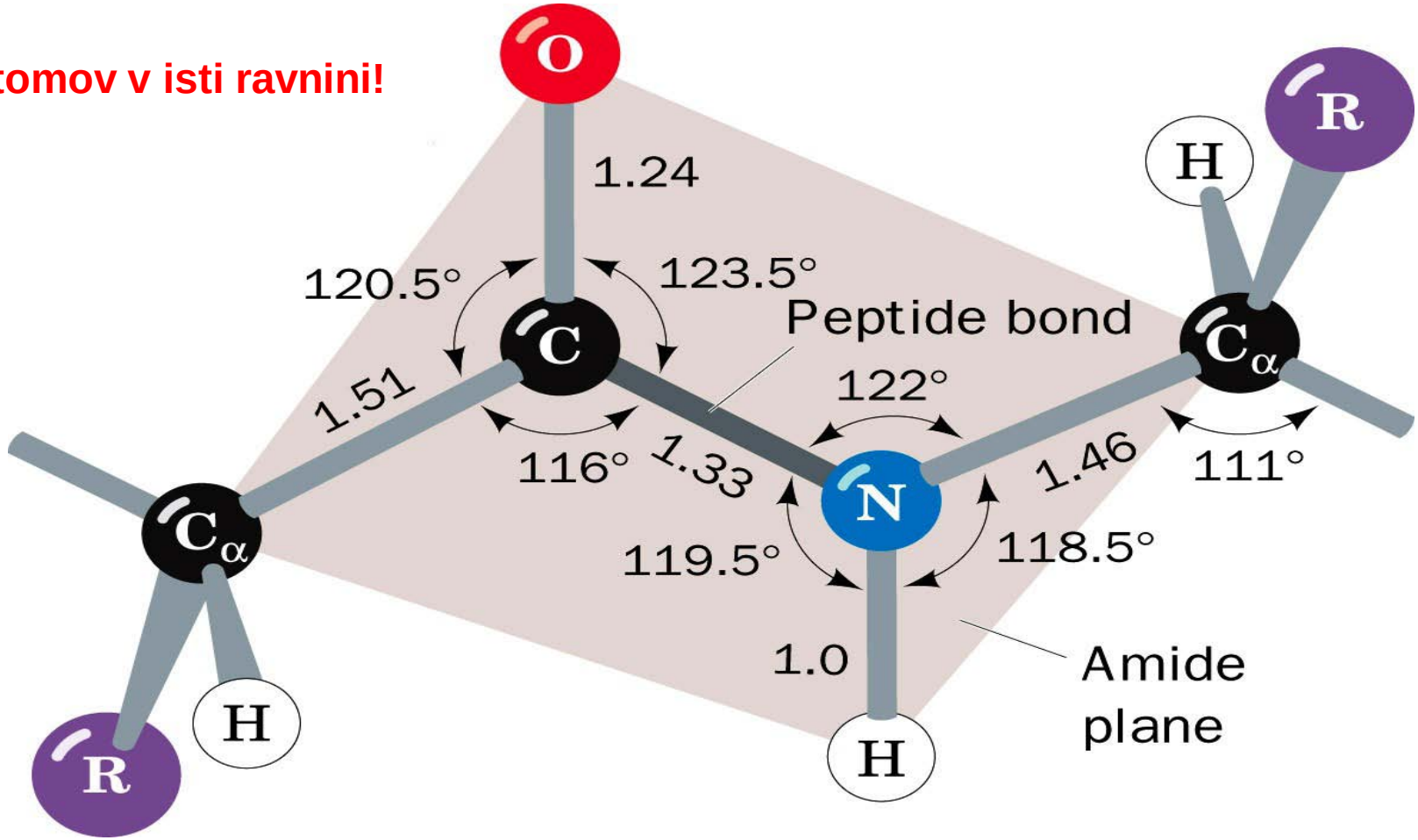




Peptidna vez

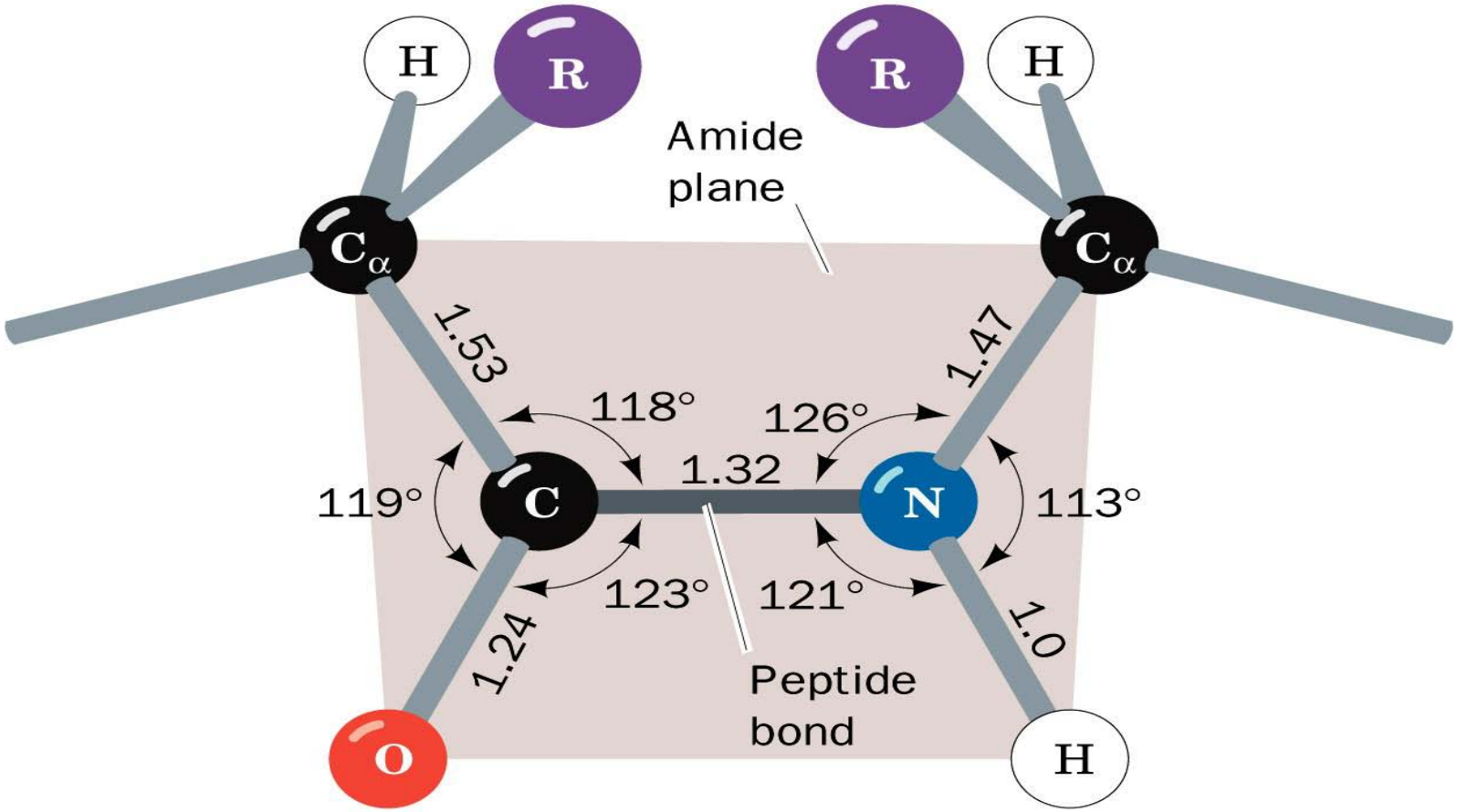
$R_1\text{-}COOH + H_2N\text{-}R_2 \leftrightarrow R_1\text{-}CONH\text{-}R_2 + H_2O$

6 atomov v isti ravnini!



Velika večina peptidnih vezi je v trans konfiguraciji.

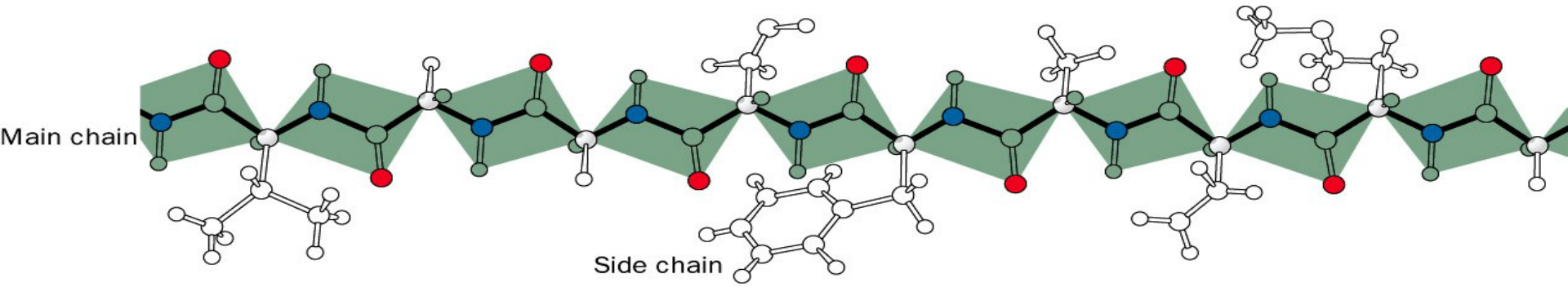
Cis-peptidna vez



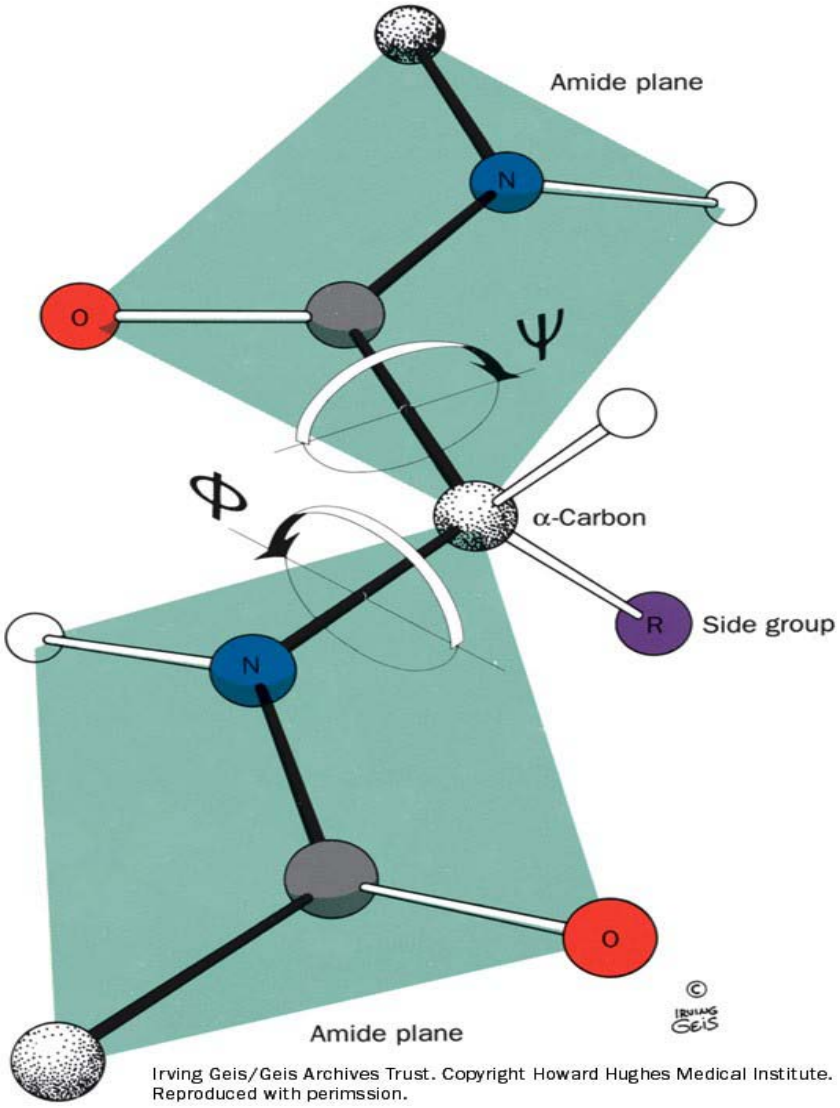
V zavojih ob Pro je približno 6% peptidnih vezi cis

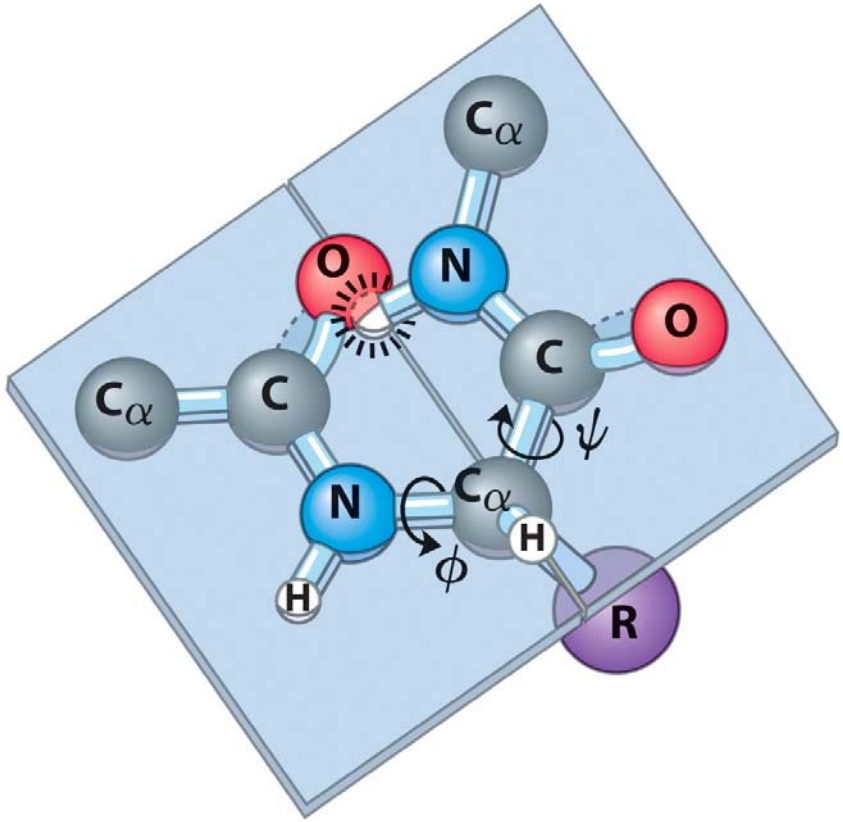
Polipeptidna veriga v iztegnjeni konformaciji

POZOR: OMEJENA GIBLJIVOST ZARADI OMEJITEV V VRTLJIVOSTI OKROG PEPTIDNE VEZI.



Dihedralna kota Φ (fi) in Ψ (psi)

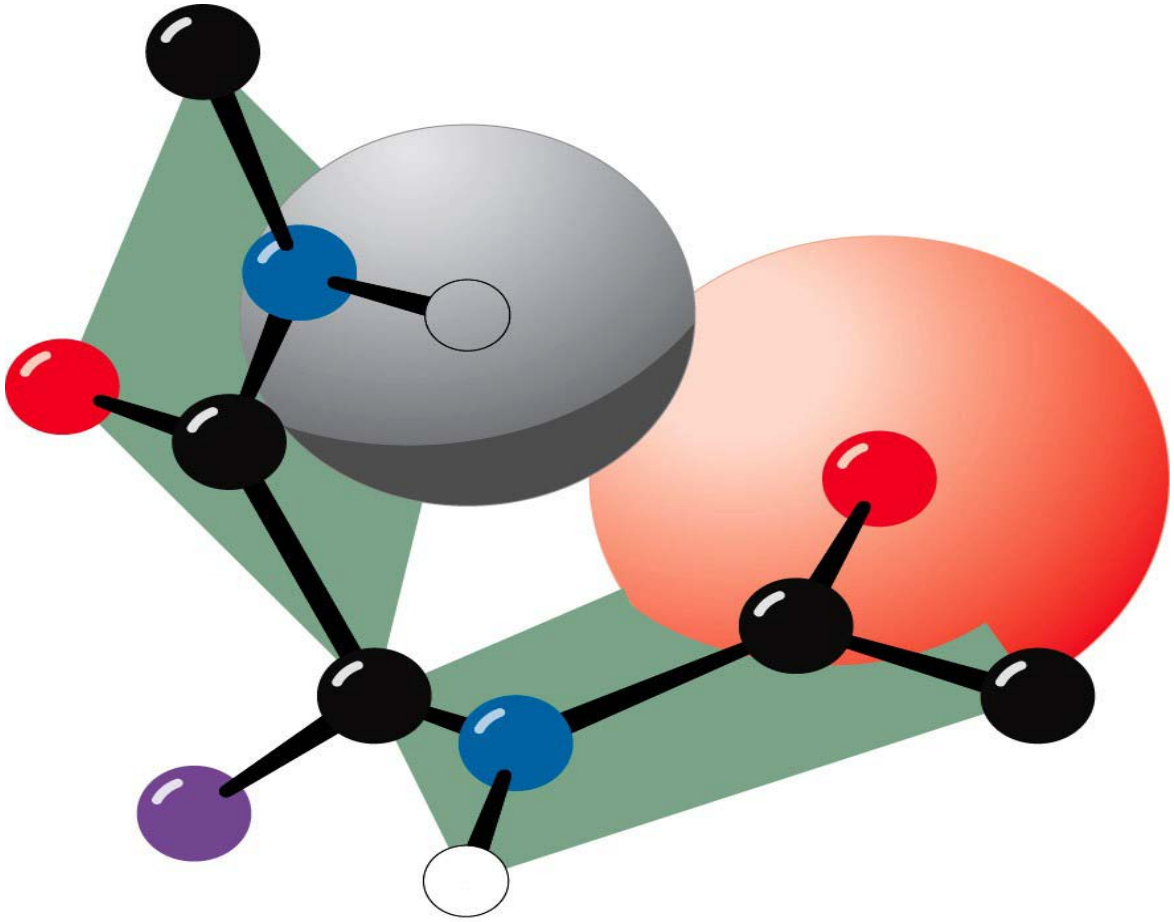




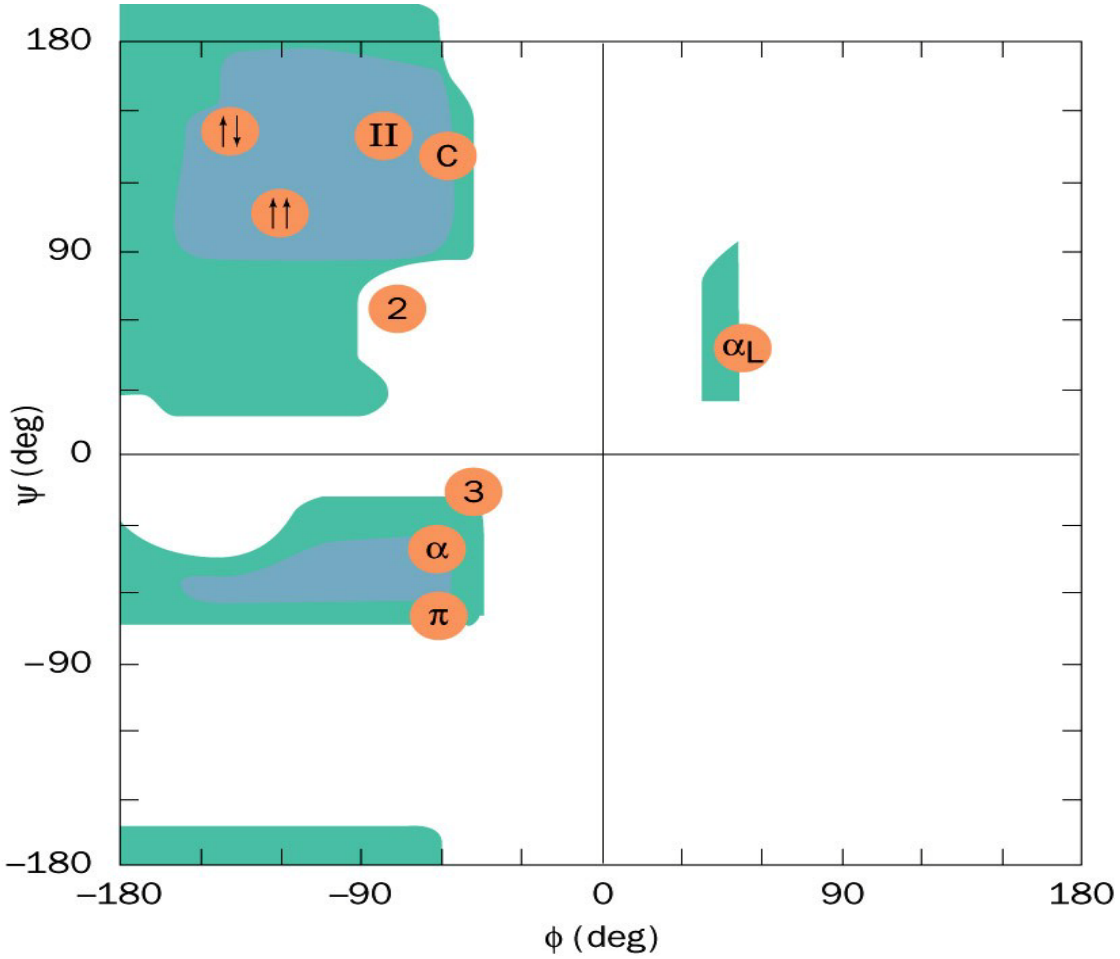
Začetni položaj:

Φ = Ψ = 0 deg

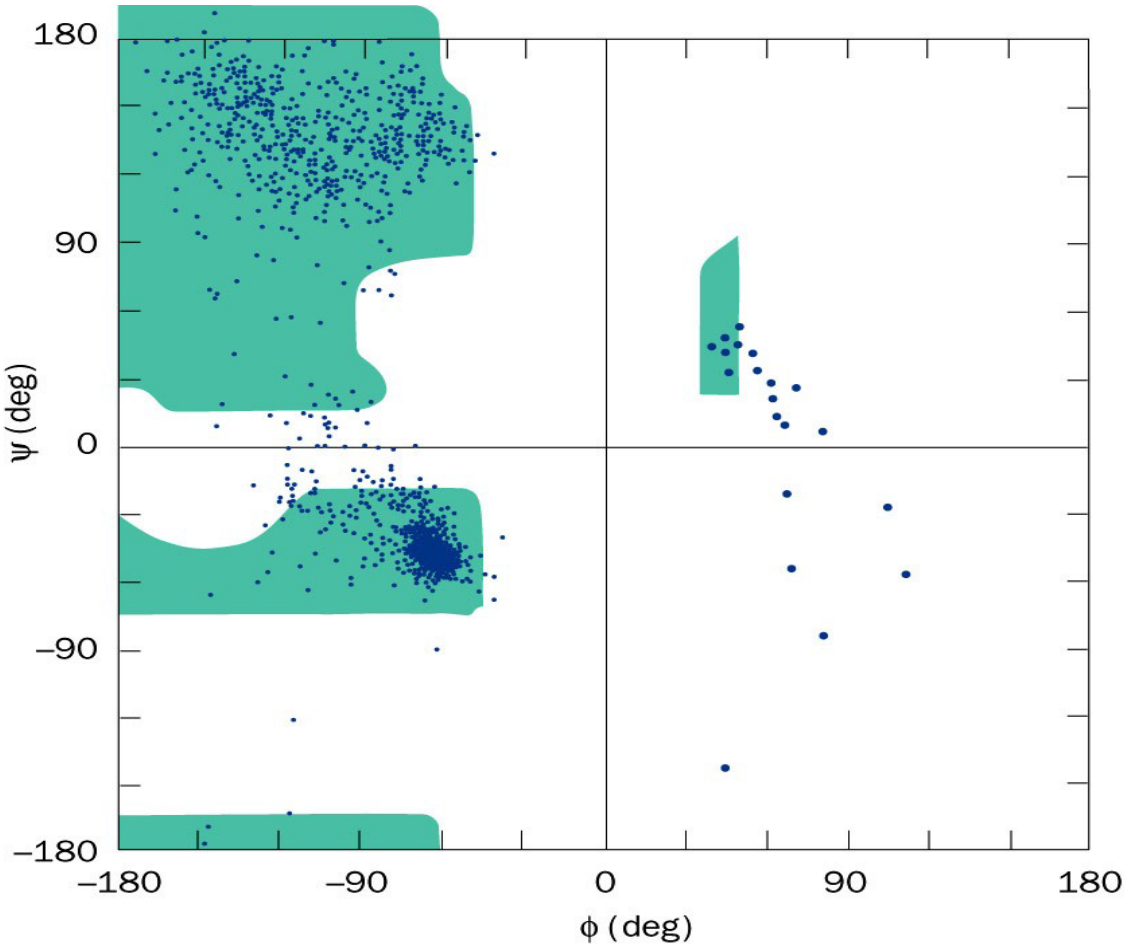
Sterično oviranje pri vrtenju sosednjih AK ostankov



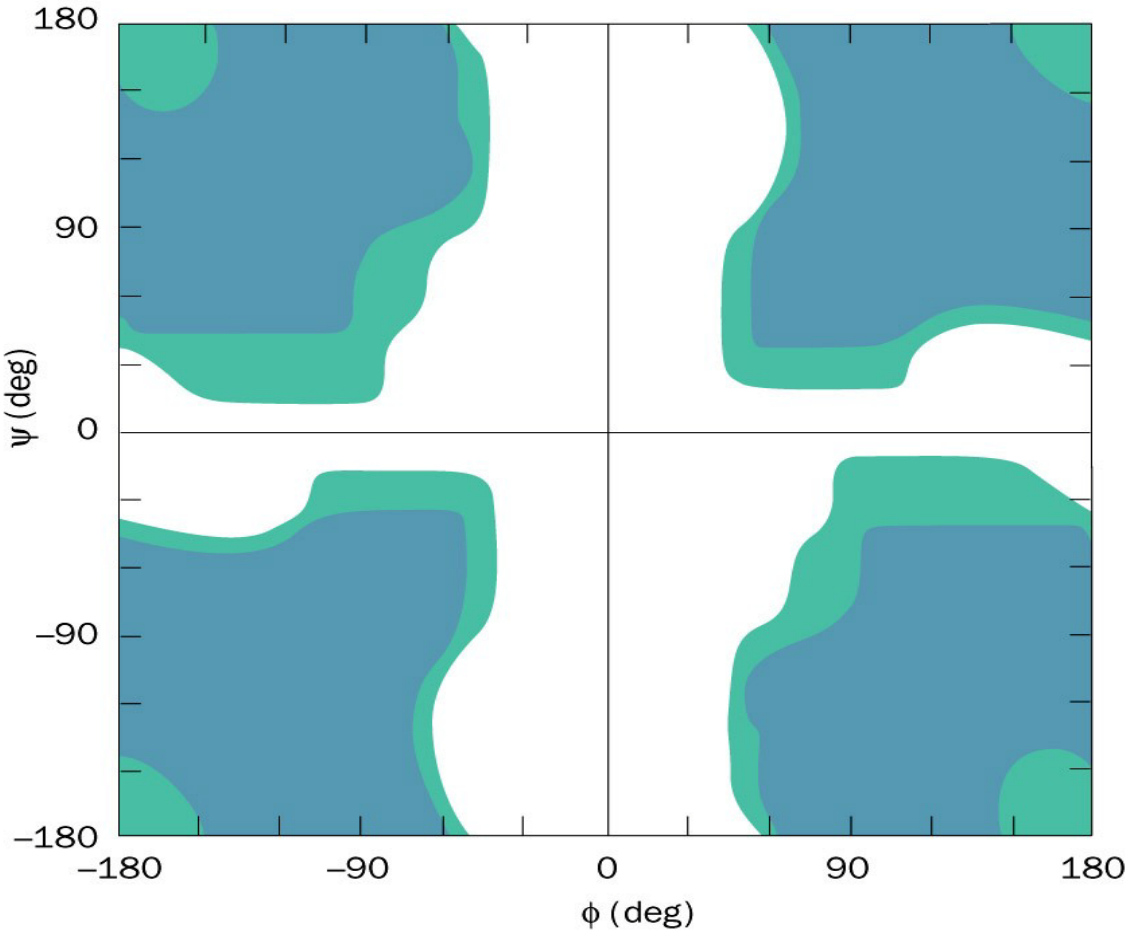
Ramachandranov diagram kaže ‘dovoljena’ področja v 2D prostoru Φ - Ψ



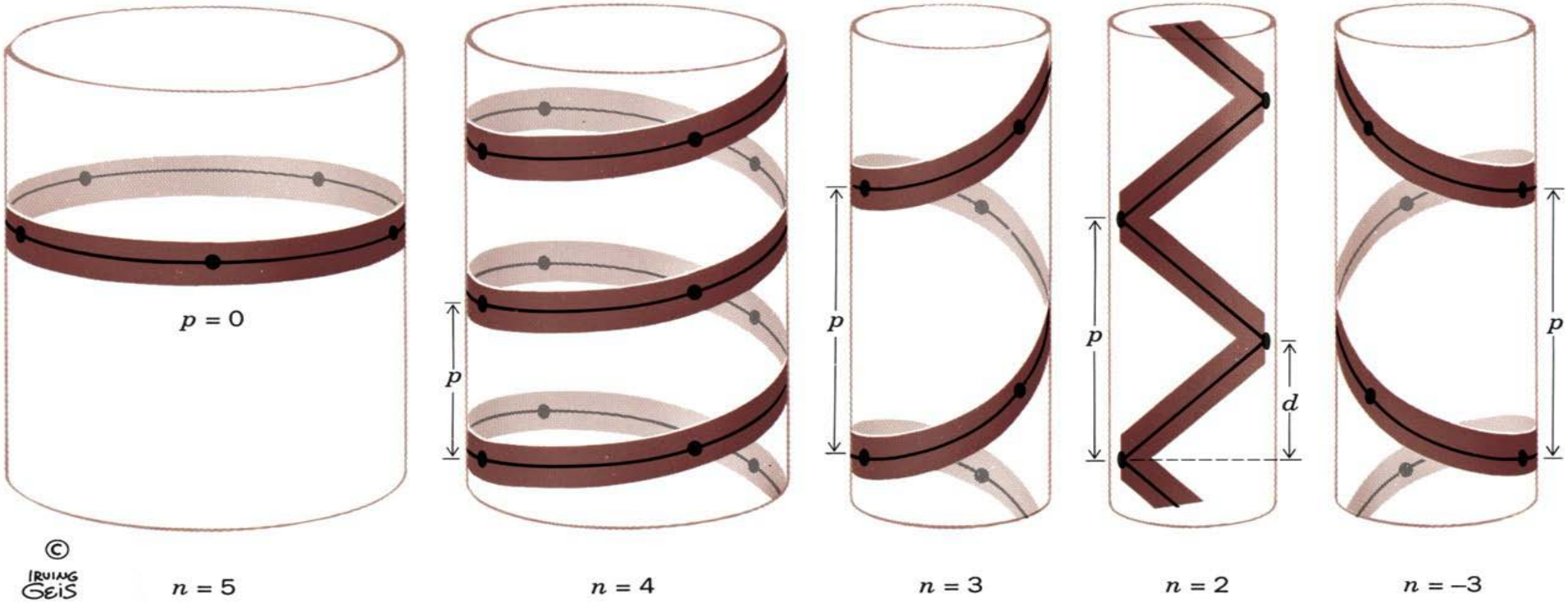
Izmerjene vrednosti Φ in Ψ pri proteinih



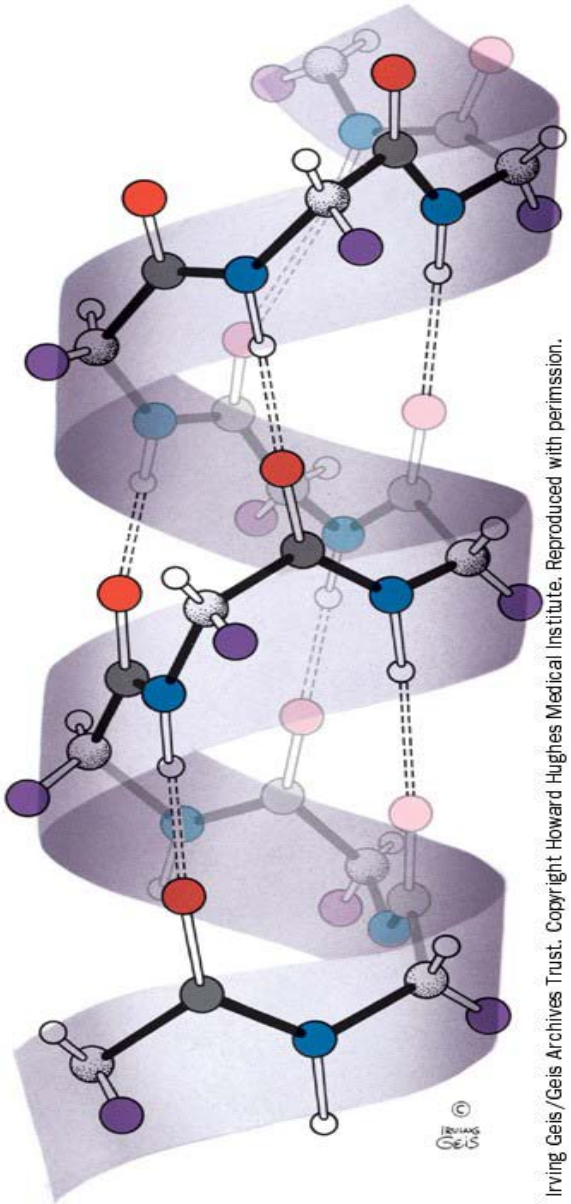
Ramachandranov diagram za poli-Gly



Različni heliksi (vijačnice)

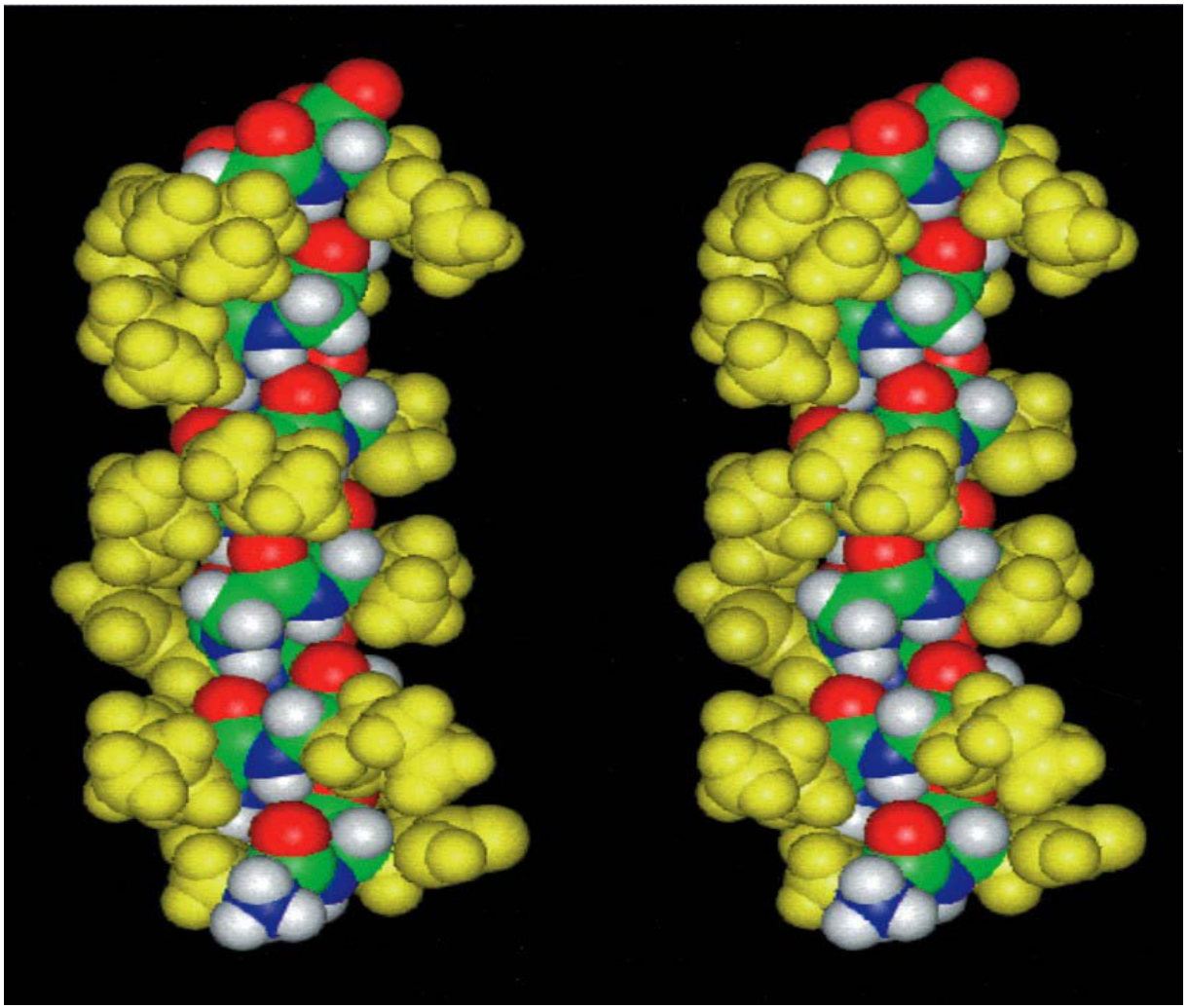


desnosučna α-vijačnica

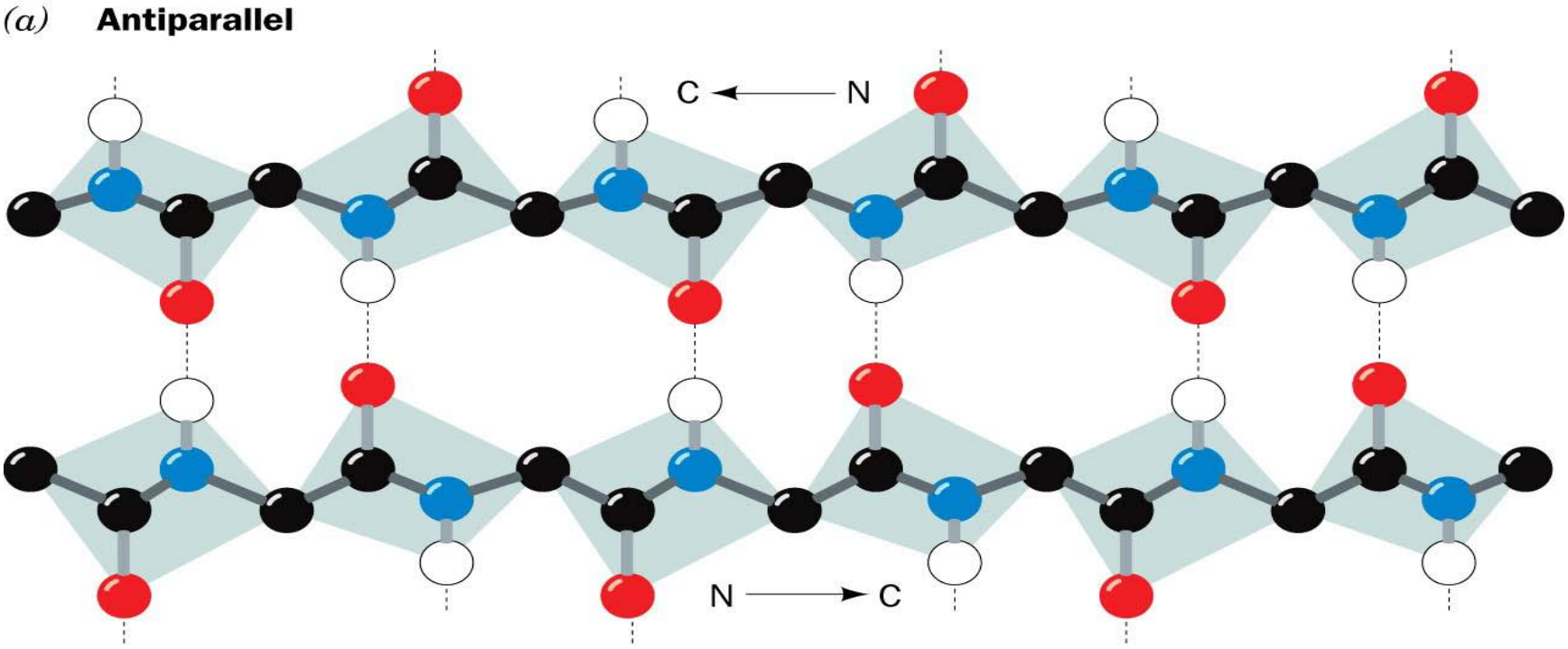


Stereoskopski prikaz α-vijačnice (H_E Mb)

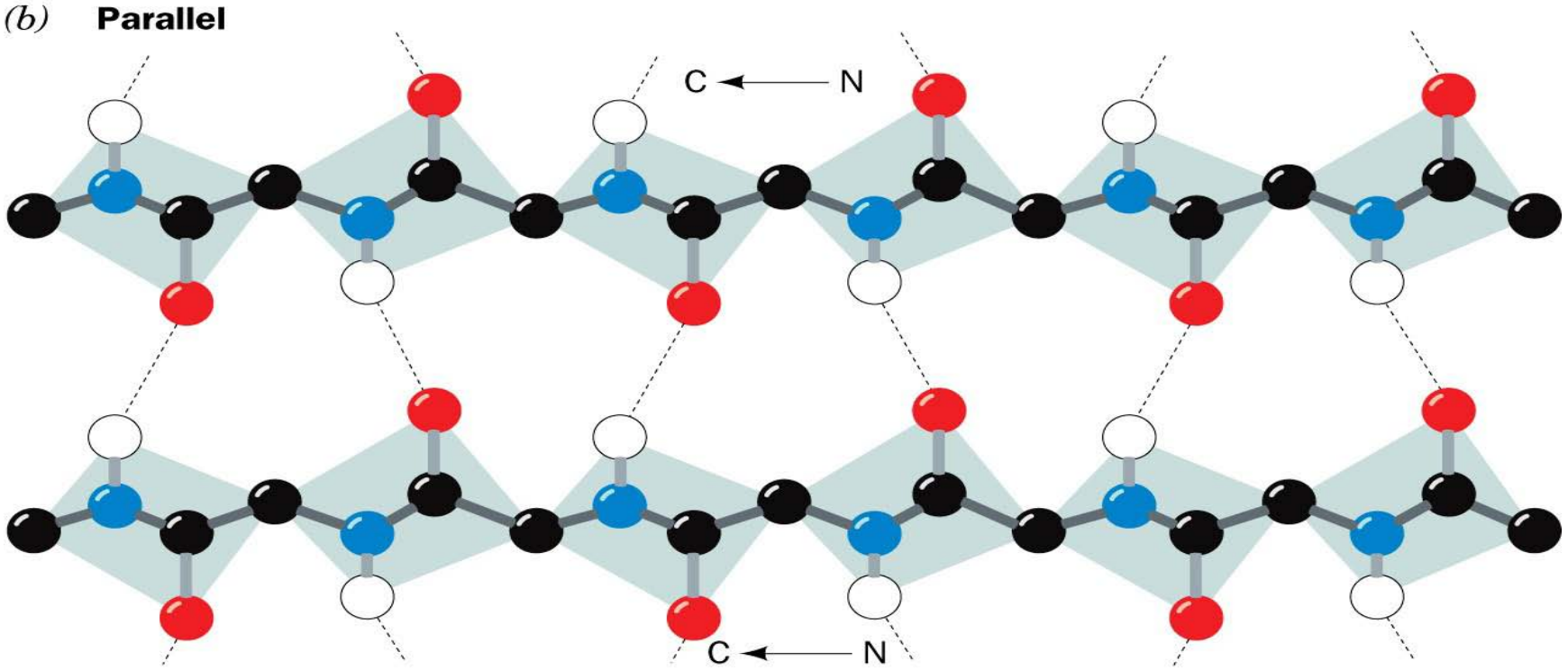
POZOR: AK RADIKALI MOLIJO NAVZVEN; ČE SO VELIKI SKUPAJ, SE OVIRAJO; V NOTRANJOSTI NI VRZELI (NITI ZA MOLEKULE VODE).



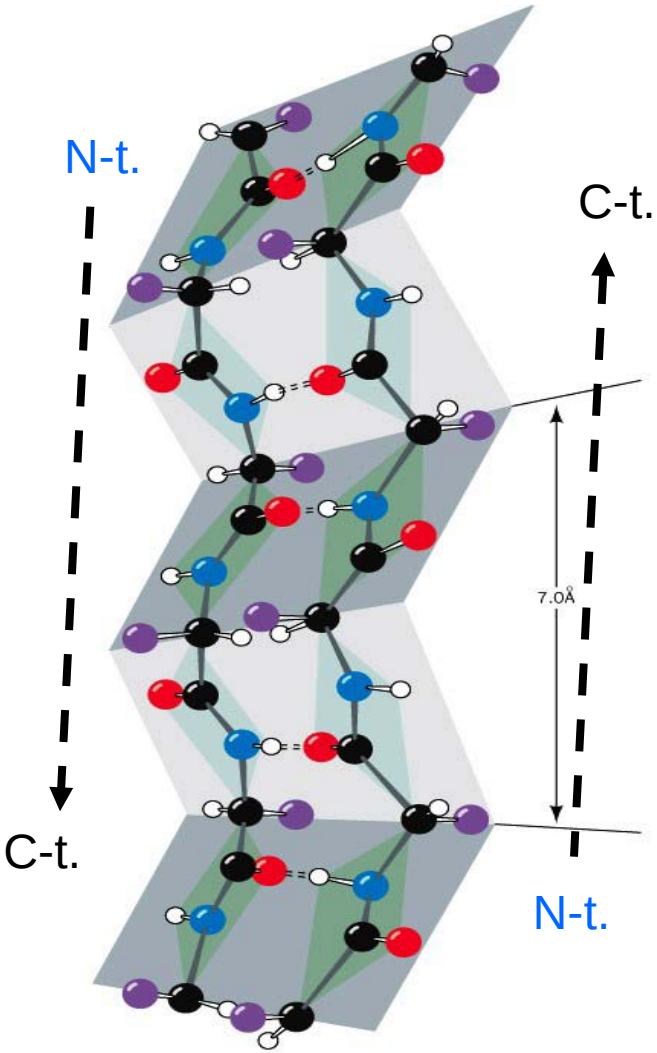
β struktura (antiparalelna)



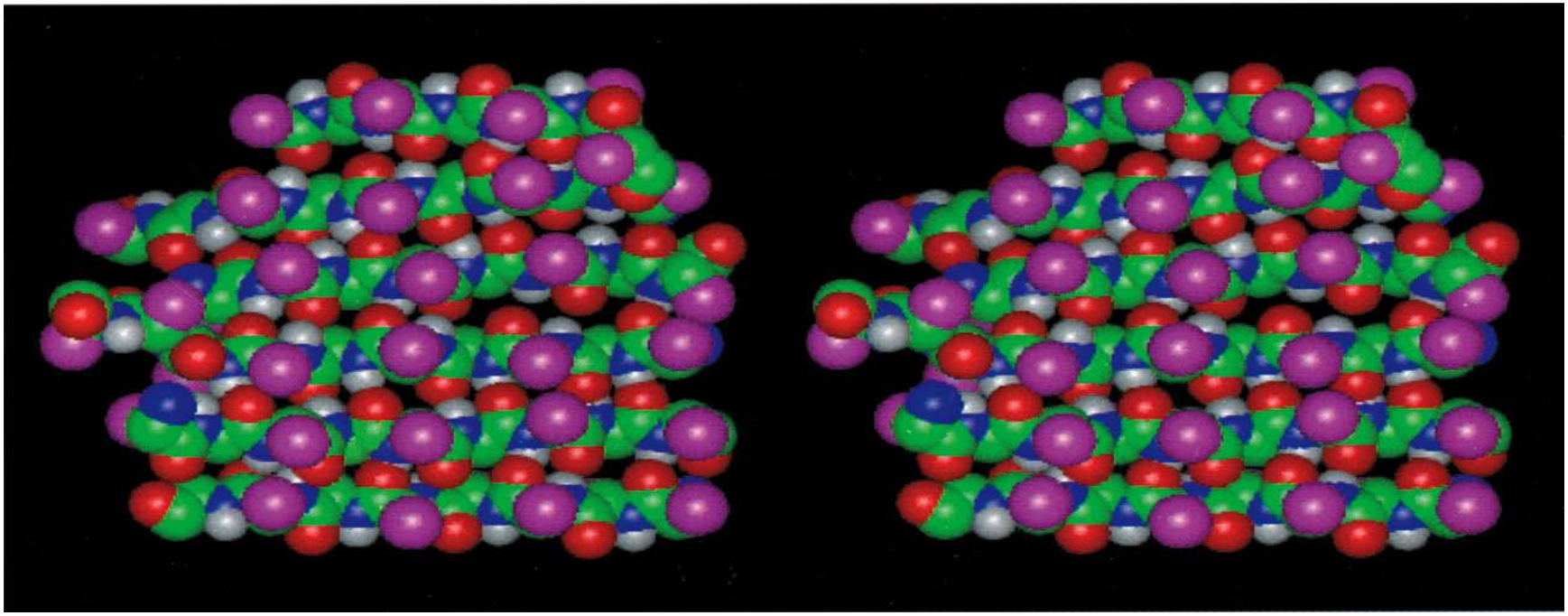
β struktura (paralelna)



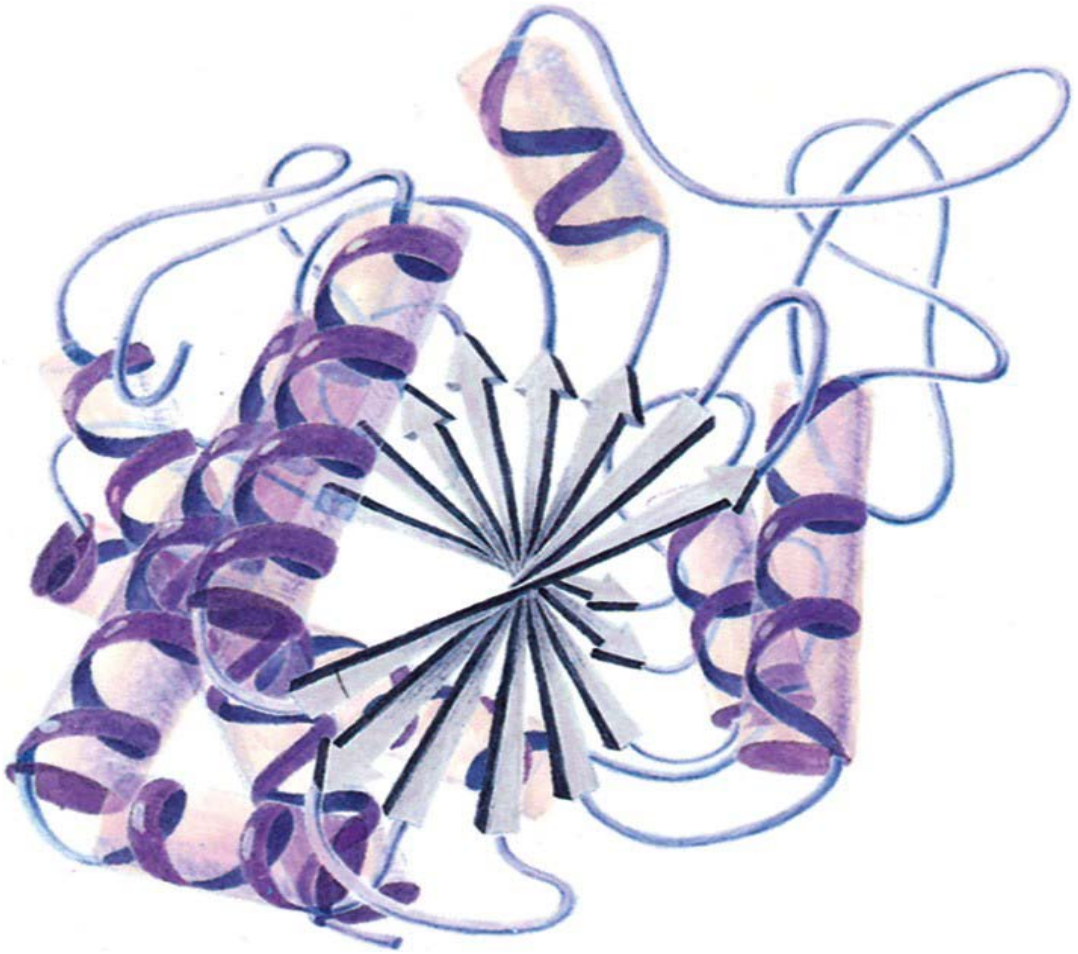
Nagubani list



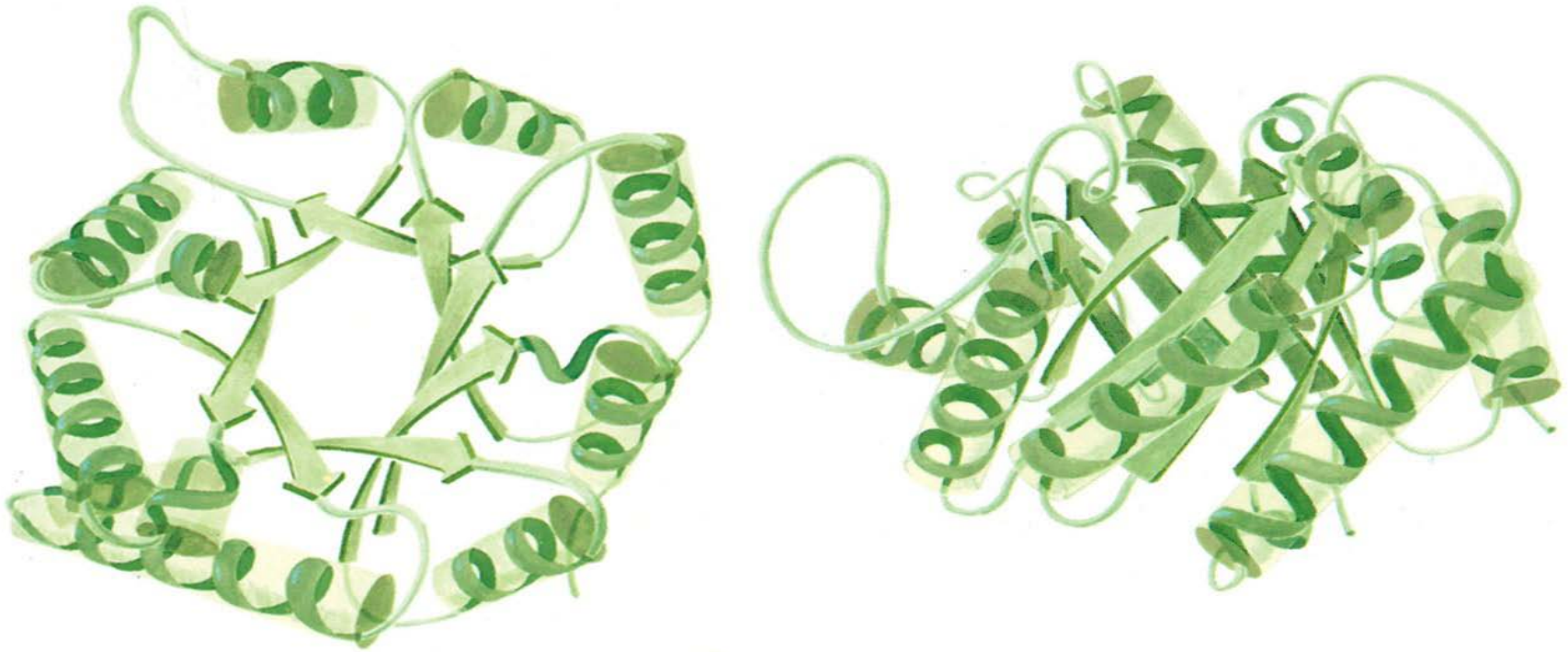
Stereoskopski prikaz nagubanega lista iz 6 verig



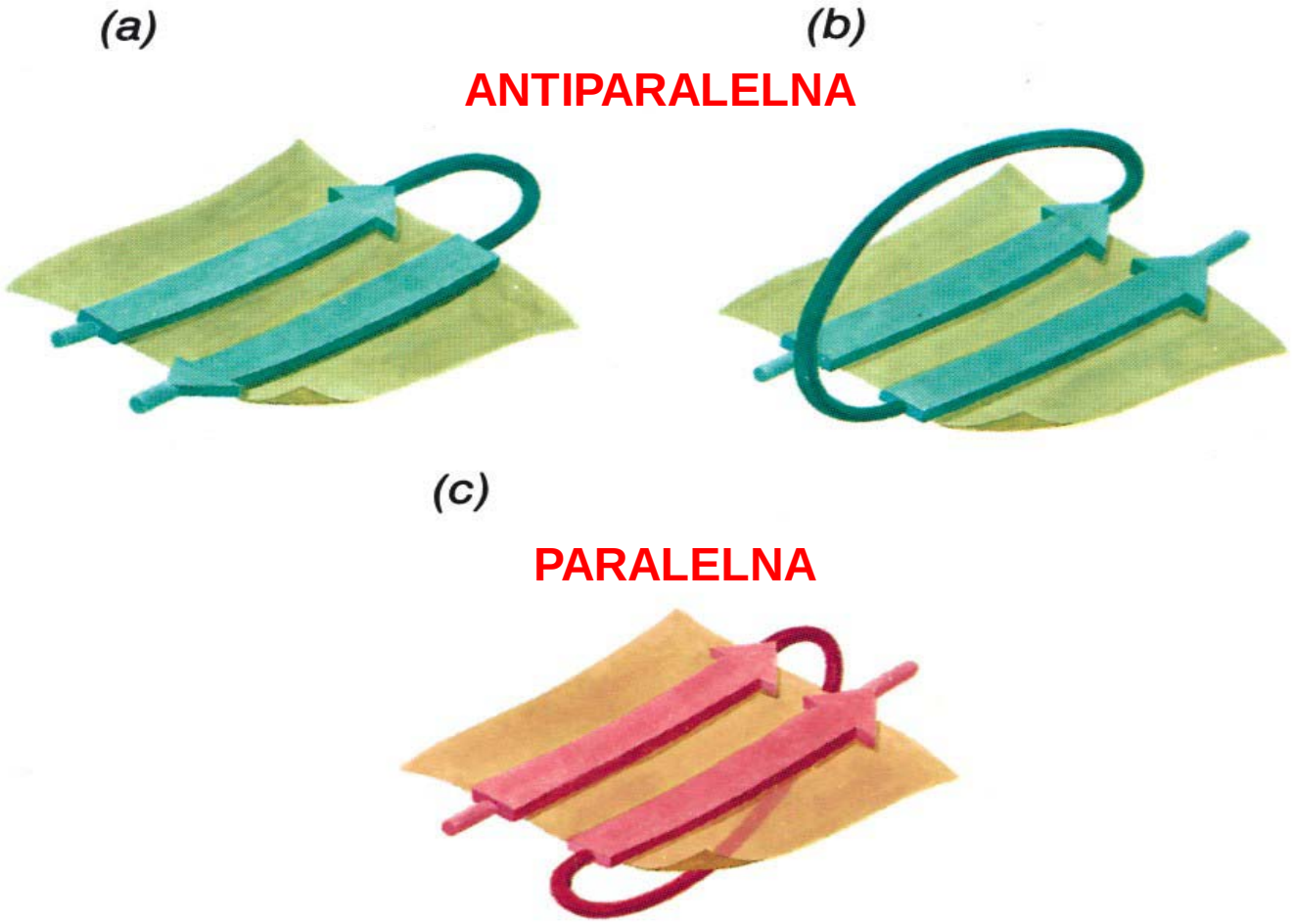
Nagubani list je pogosto zvit in podprt z vijačnicami.



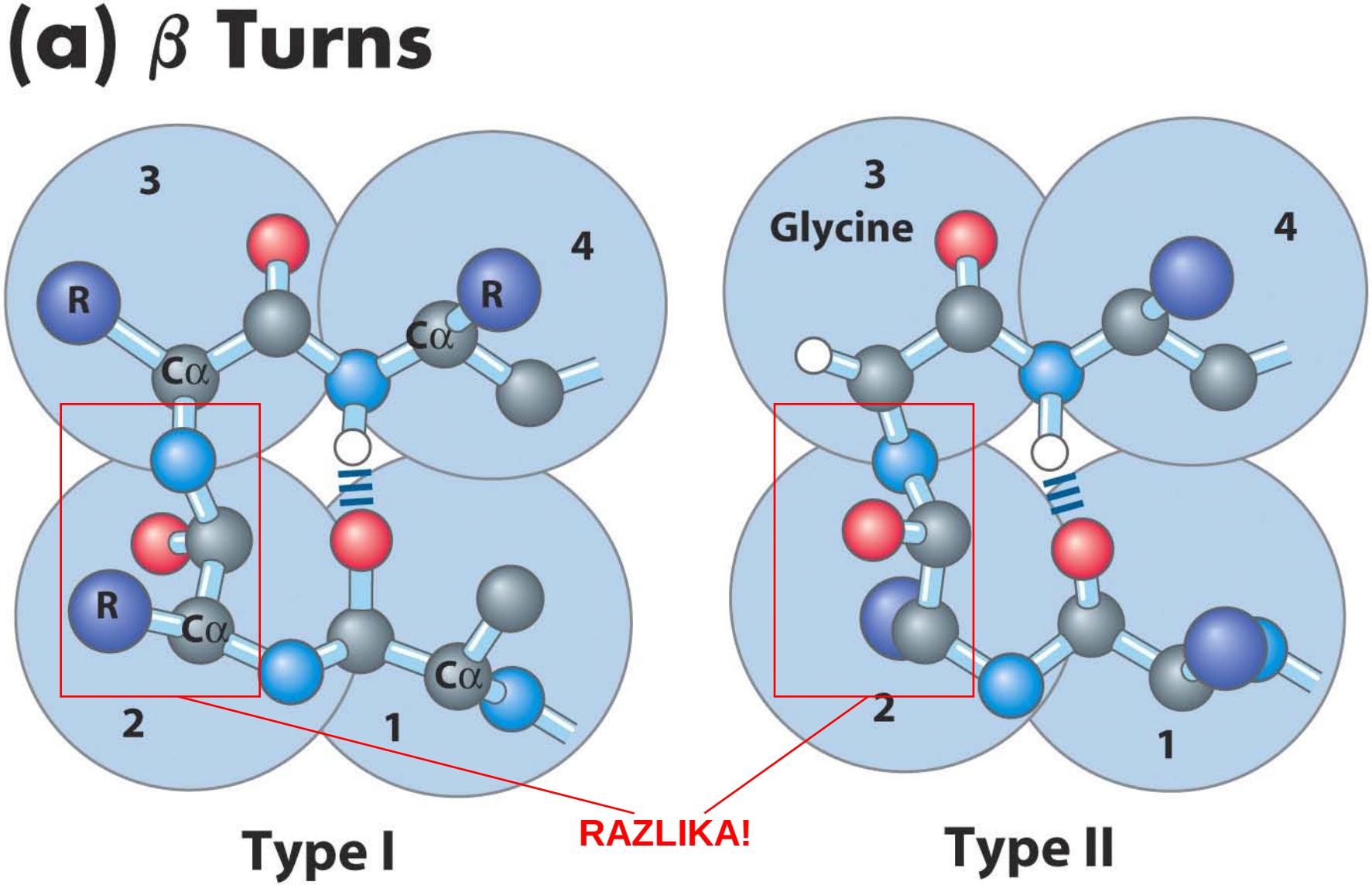
Nagubani list je pogosto zvit in podprt z vijačnicami (trioza-fosfat izomeraza)



Povezave verig v nagubanem listu

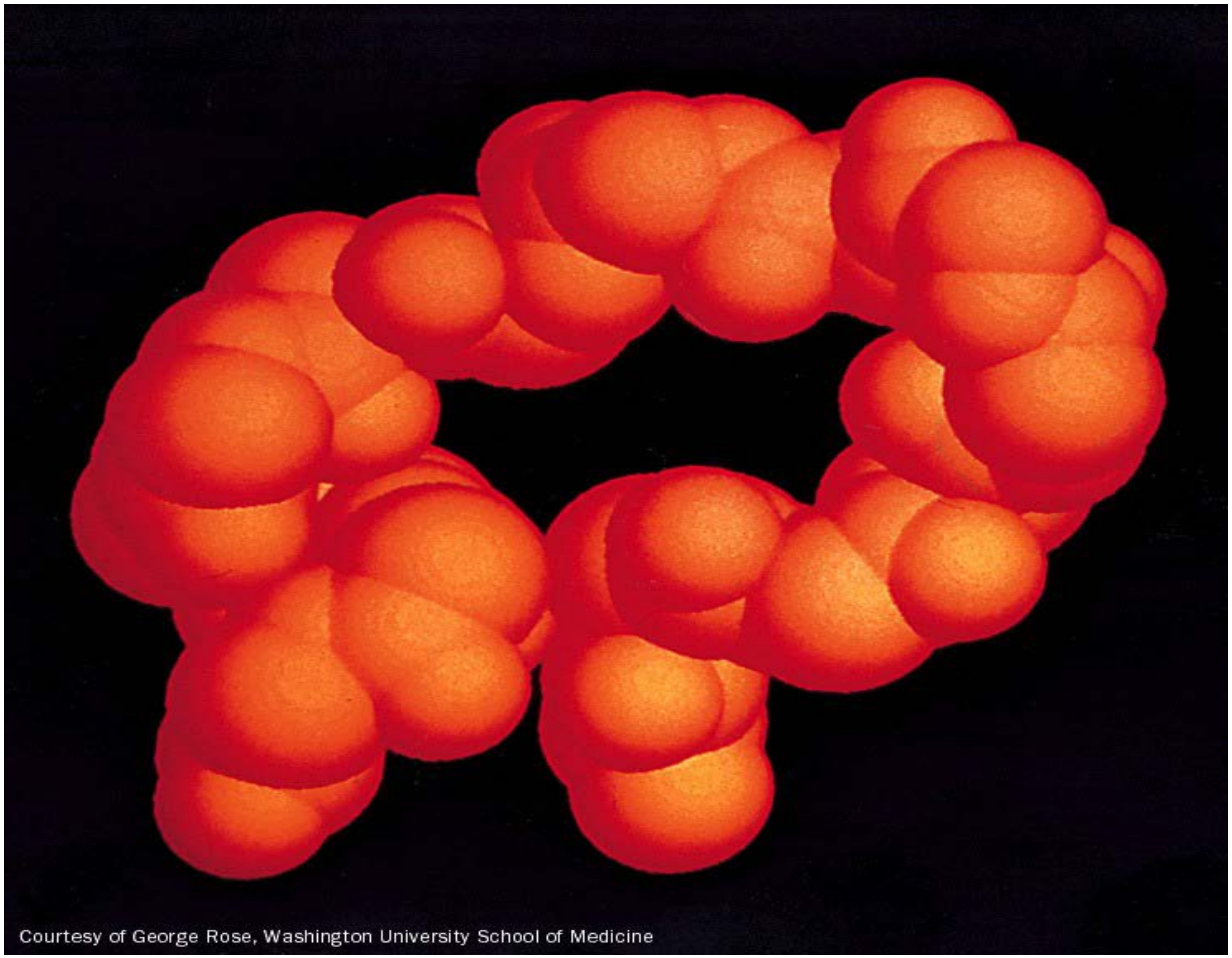


β-zavoji

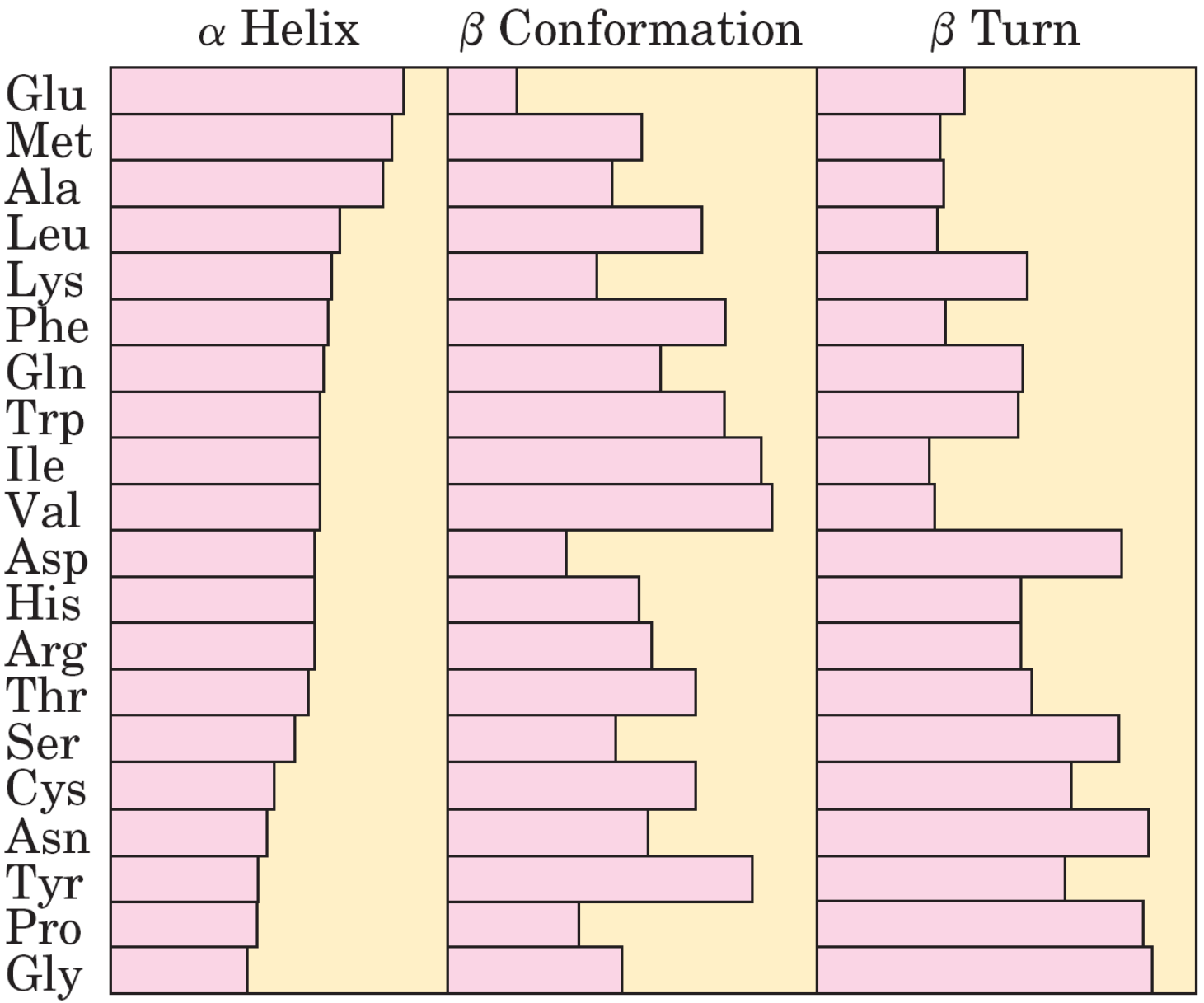

(a) β Turns
3
4
R
Cα
R
Cα
R
Cα
Cα
2
1
Type I
RAZLIKA!
3
Glycine
4
2
1
Type II

Zanke: Ω zanka je navadno iz 40 do 54 AK (citokrom *c*)

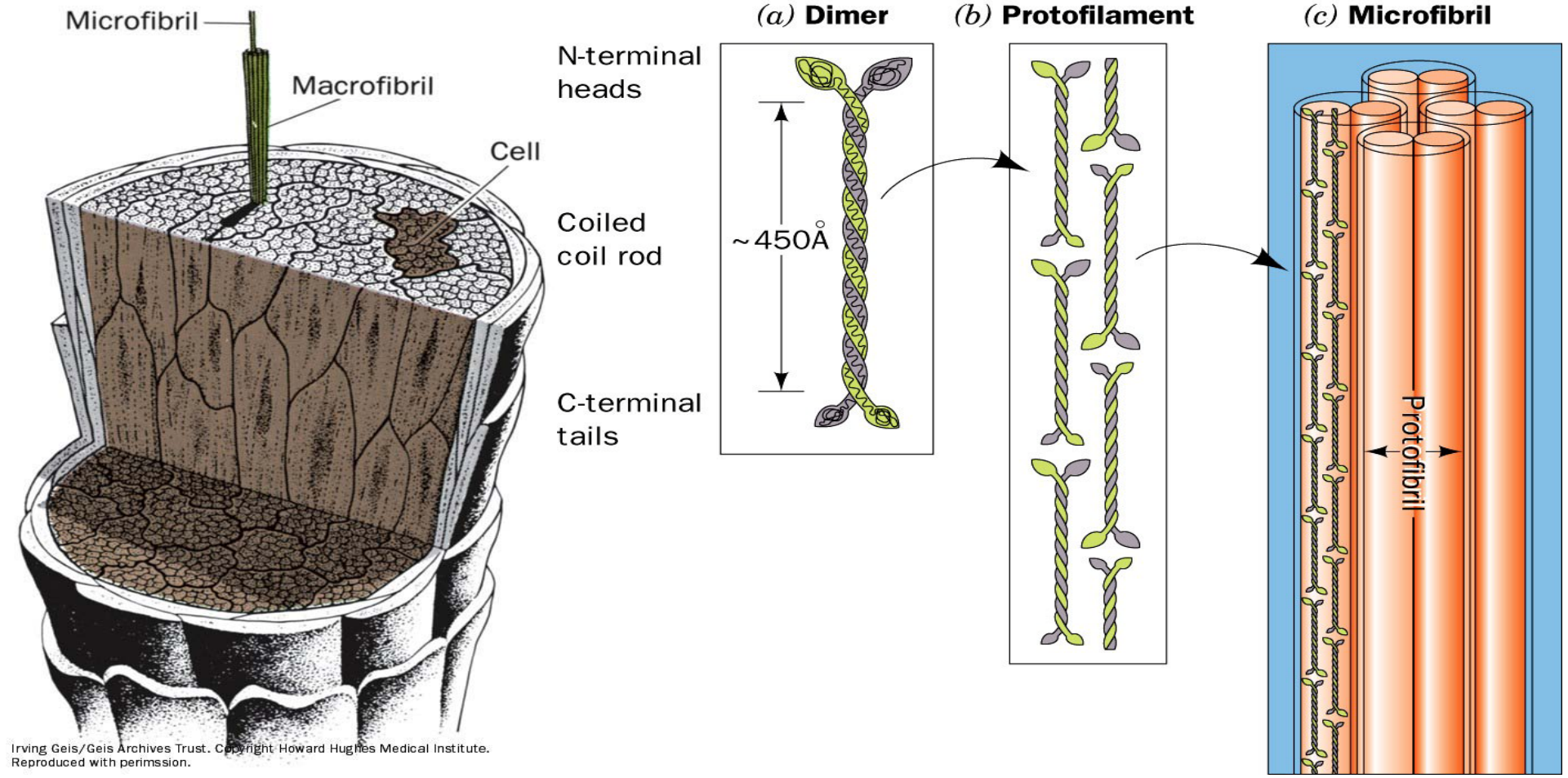
Ω



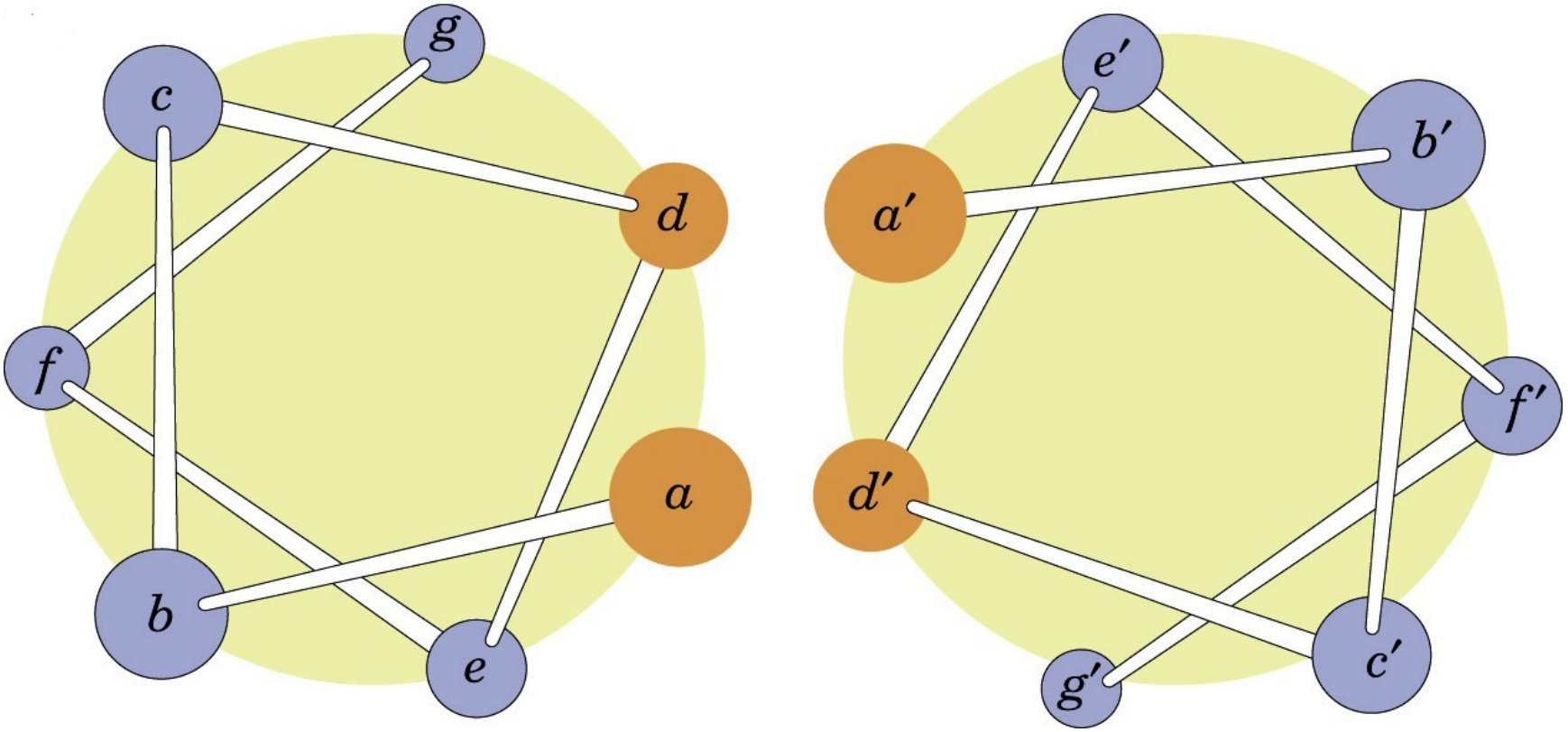
Verjetnost za nahajanje AK v določeni sekundarni strukturi



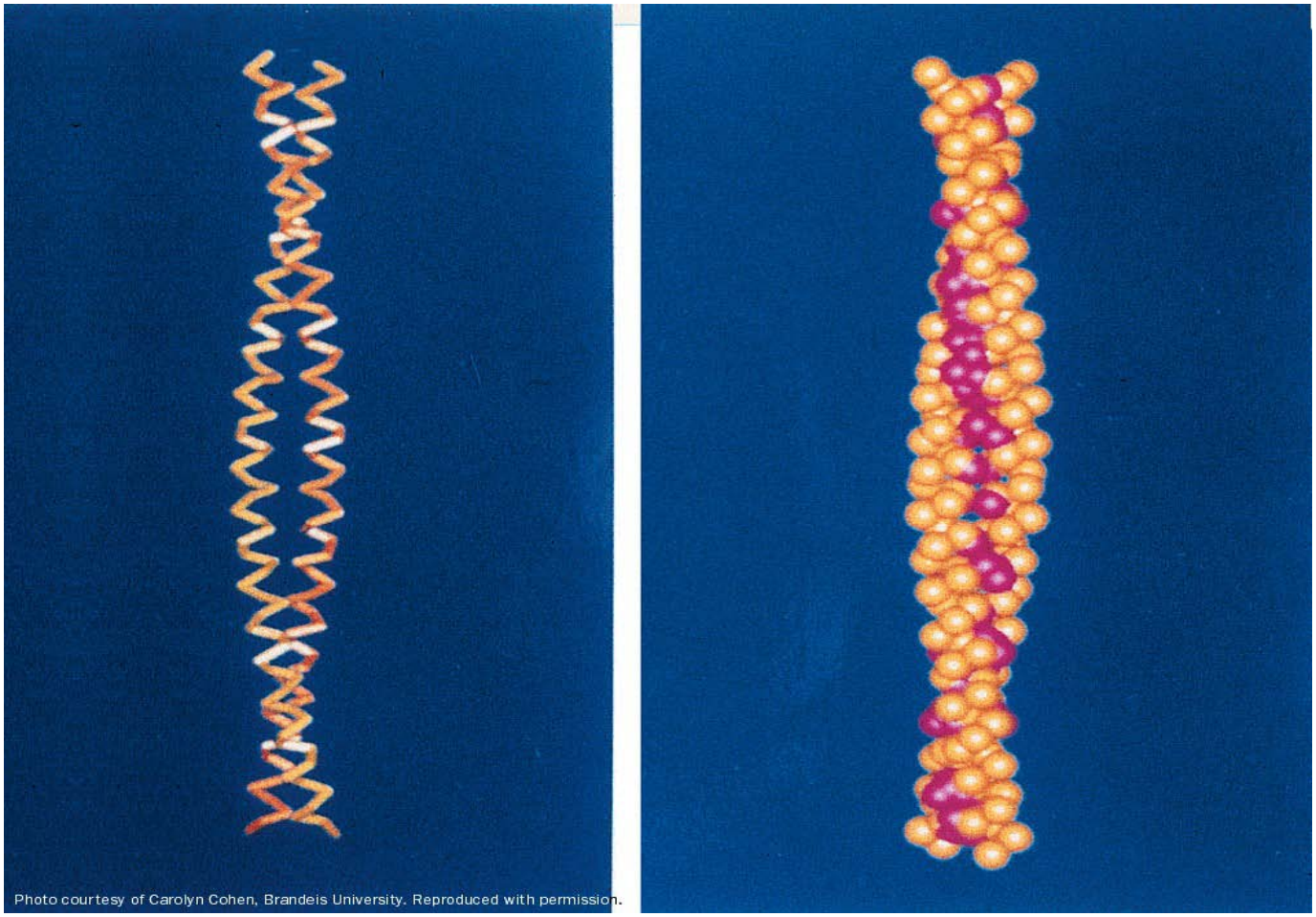
FIBRILARNI PROTEINI
Lasje so iz α-keratina



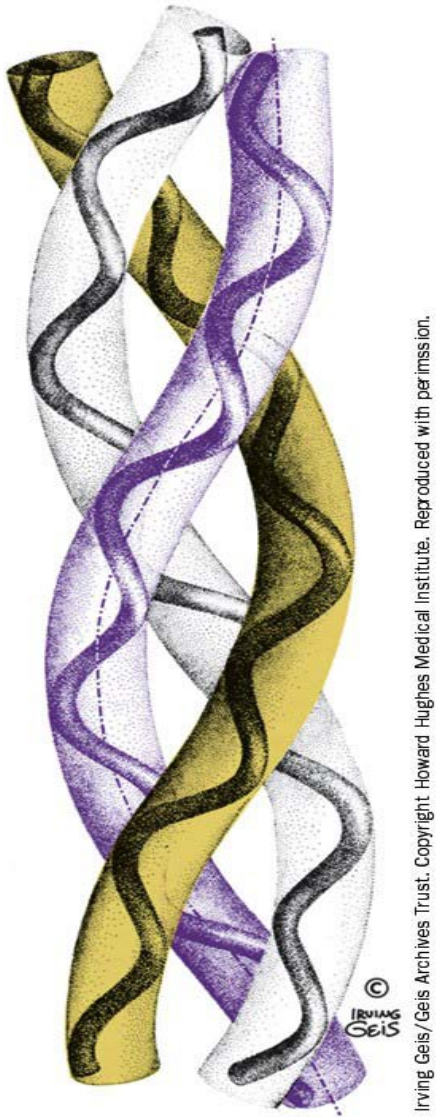
Nepolarna področja α heliksov držijo skupaj hidrofobne interakcije



Za optimalno vzpostavitev hidrofobnih vezi se morata vijačnici oviti ena okrog druge.

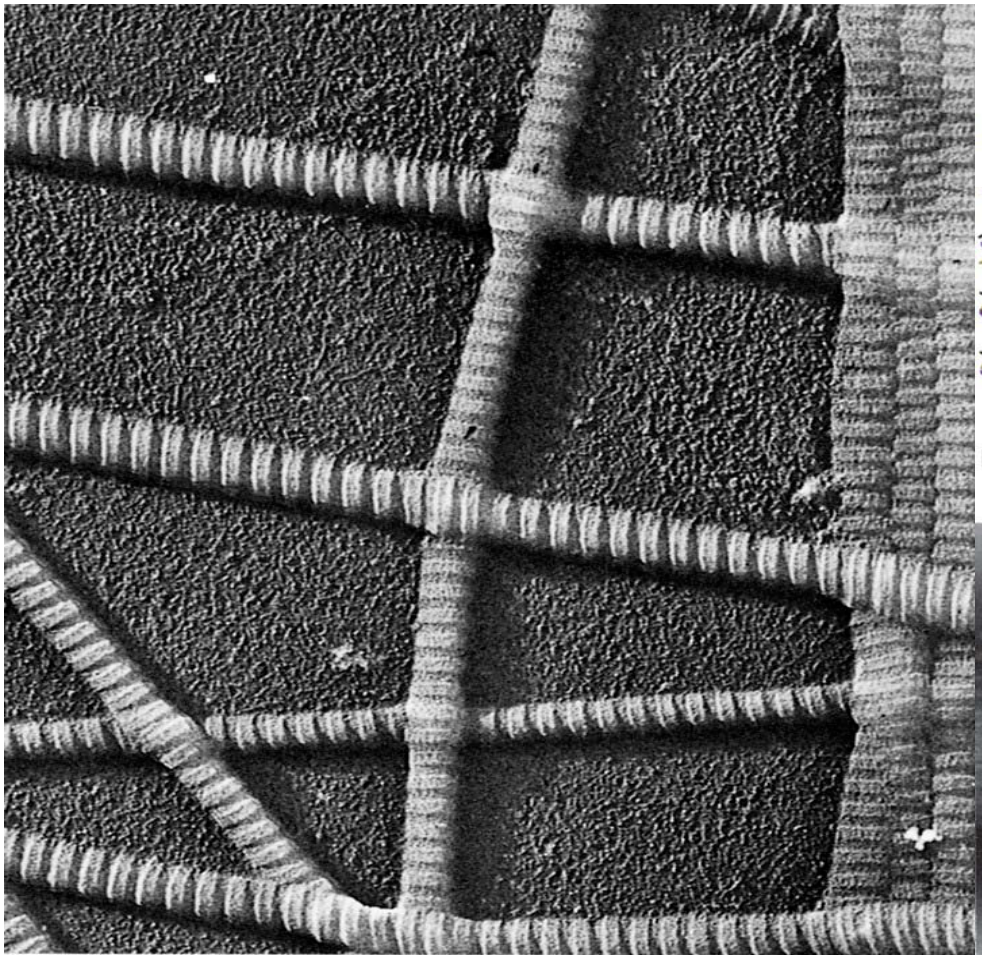


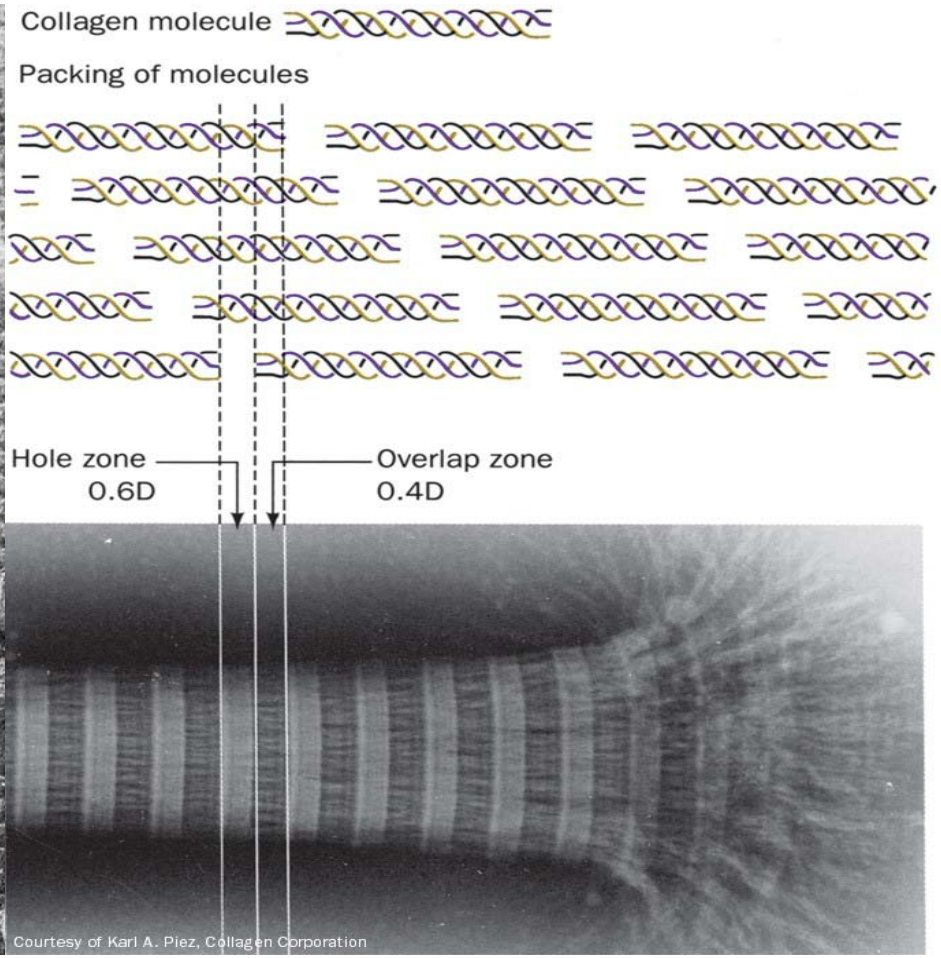
Trojni heliks v kolagenu (niso α-heliksi!)



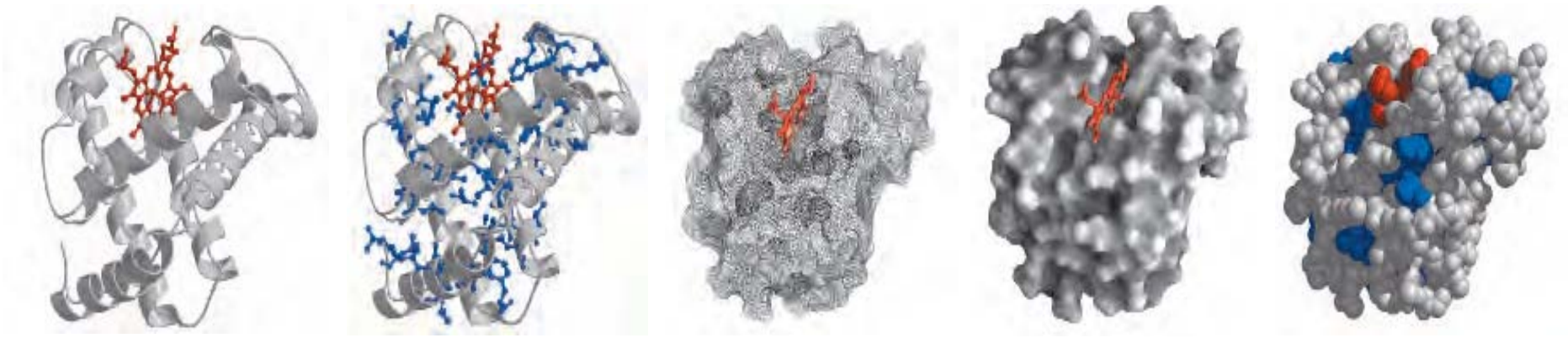
POZOR: ČE VERIGE LOČIMO, VIJAČNICE RAZPADEJO

Struktura kolagenskih vlaken





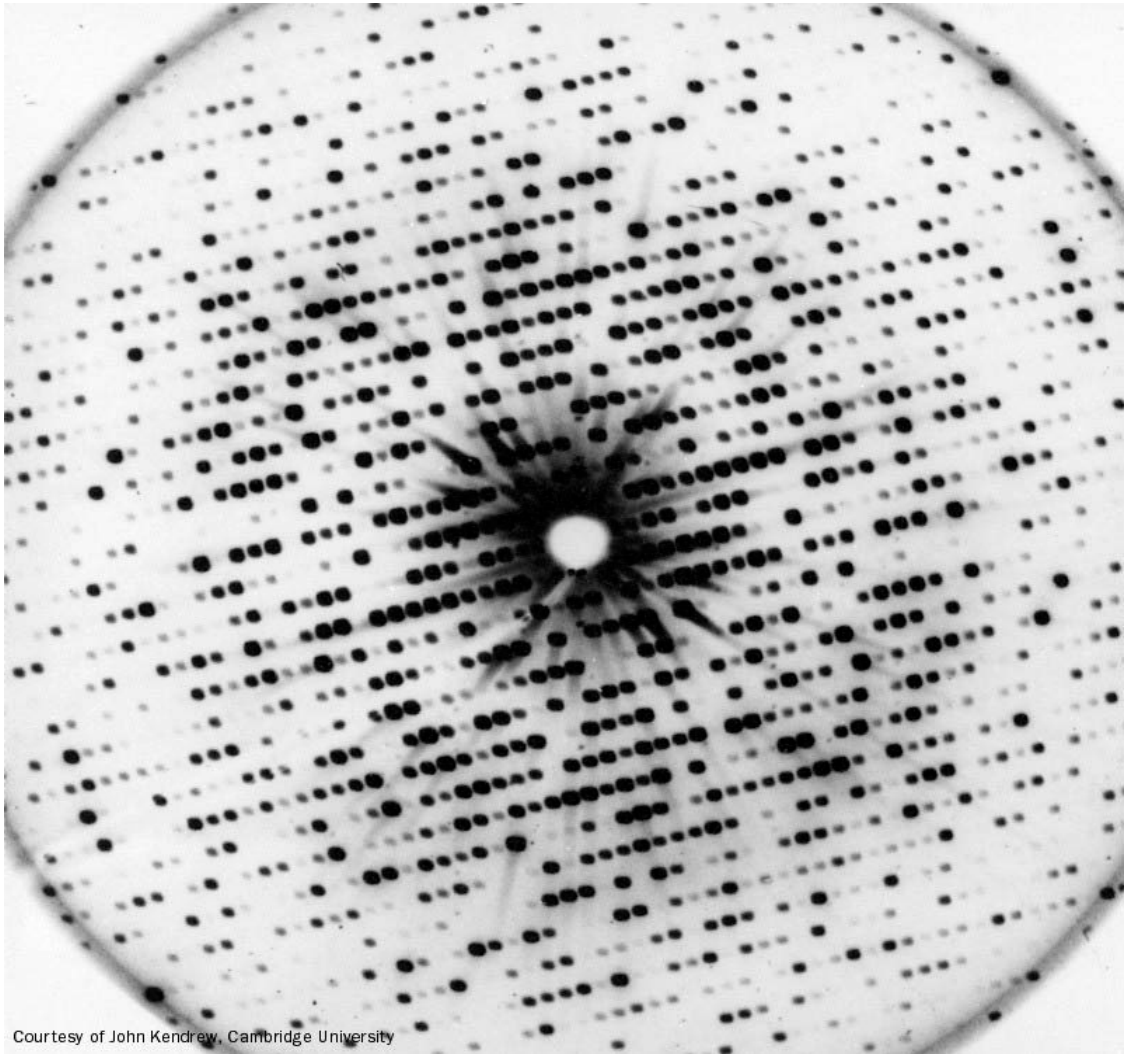
TERCIARNA STRUKTURA GLOBULARNIH PROTEINOV



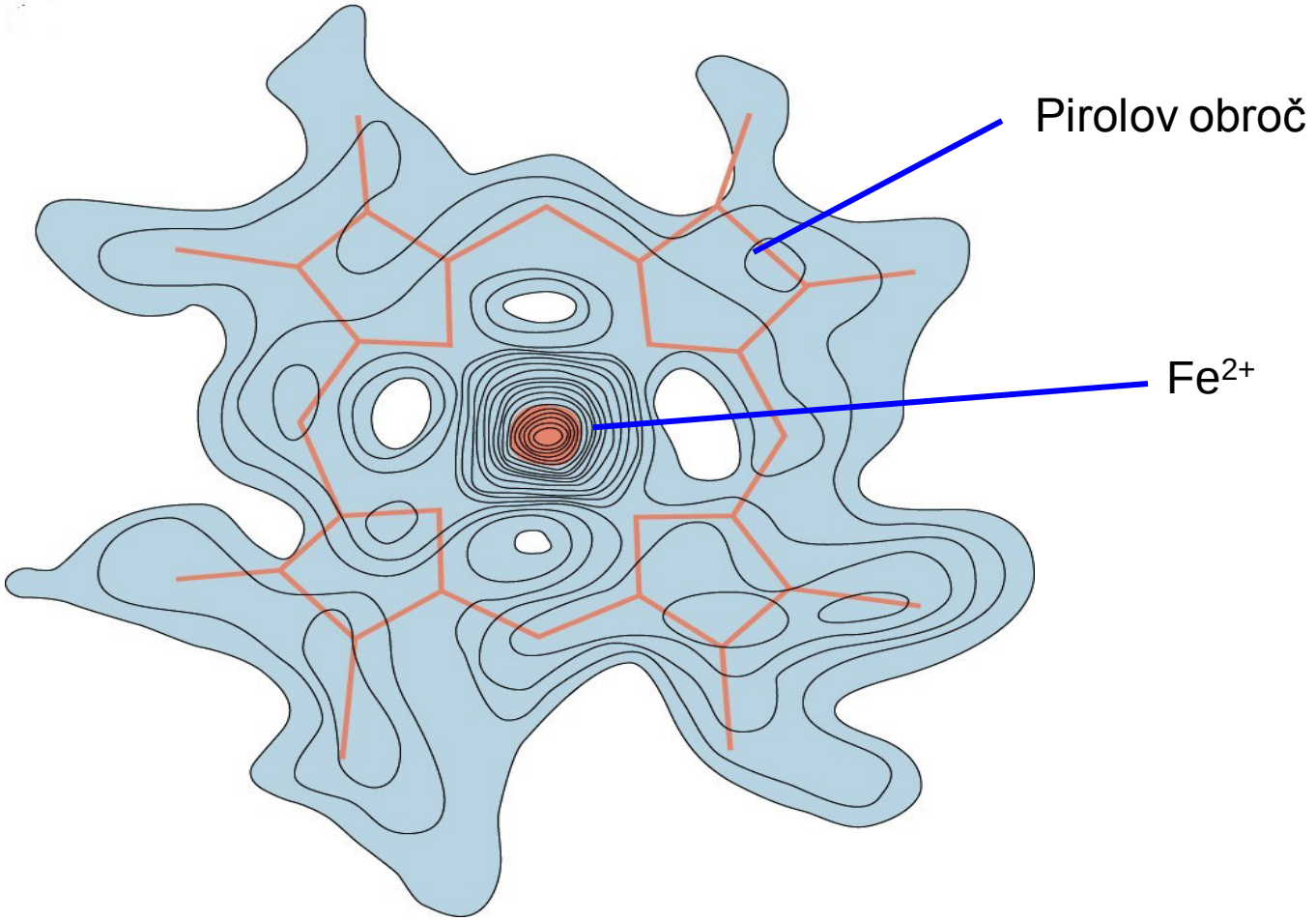
Pet različnih prikazov terciarne strukture mioglobina.

- III. struktura: zvitje v nativno konformacijo
- vse vrste II. strukture so običajno vključene (tudi neurejena struktura)
- III. strukturo vzdržujejo številne šibke interakcije med AK ostanki
- III. struktura je stabilna a fleksibilna
- poznati III. strukturo pomeni poznati položaj vseh atomov v prostoru (3D)

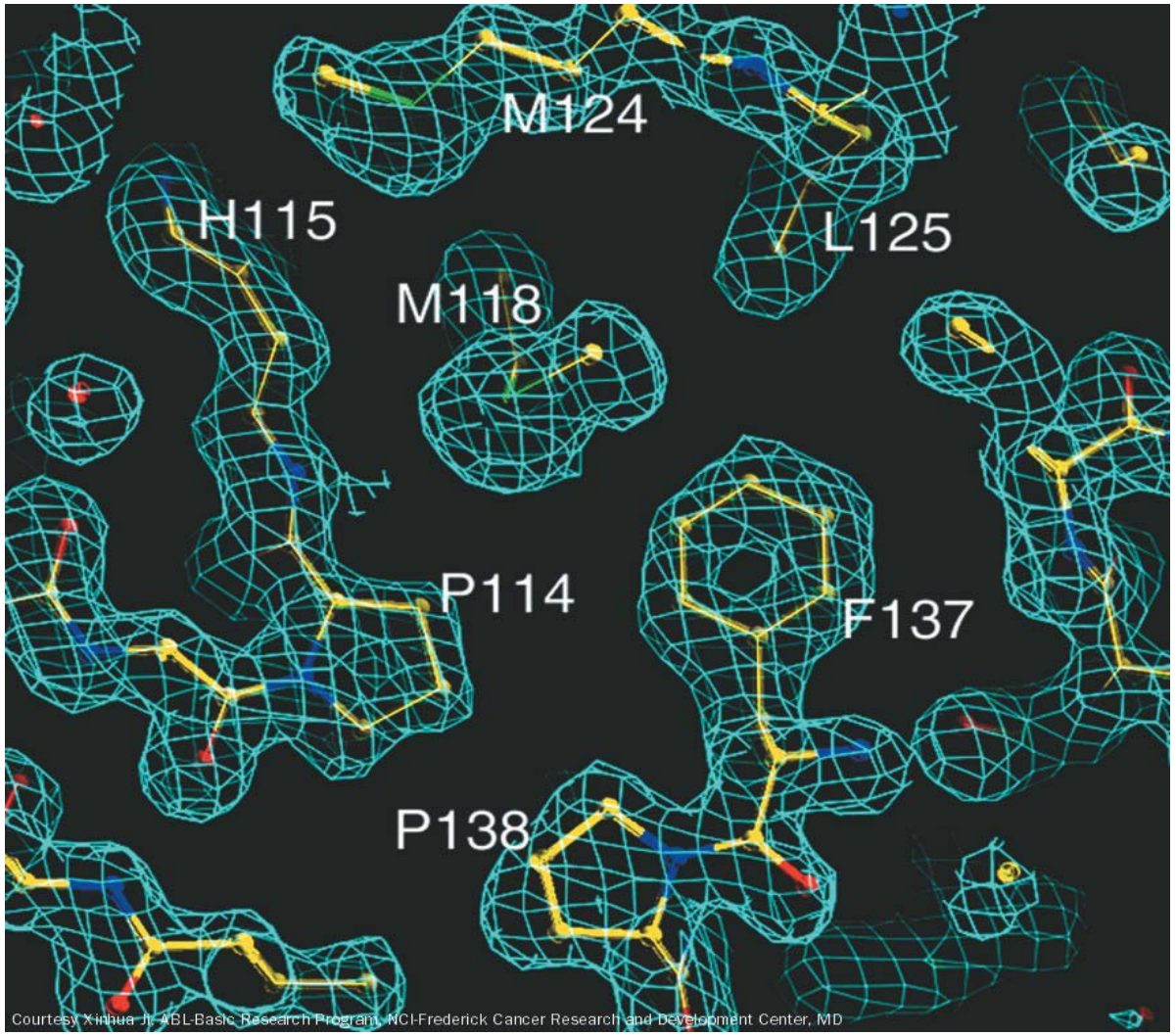
Fotografija lis na filmu po uklonu rentgenskih žarkov skozi kristal Mb.



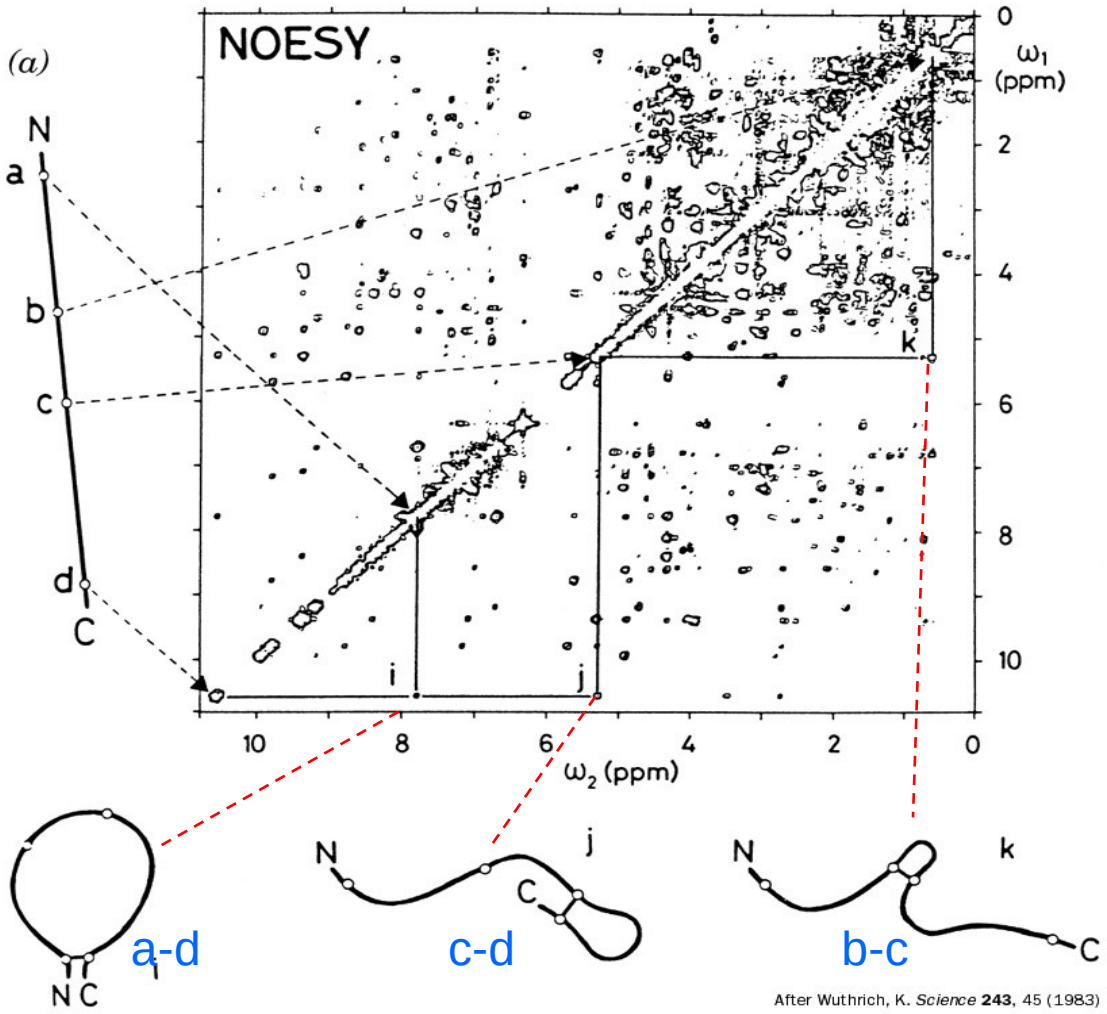
‘Zemljevid’ elektronske gostote hema.



‘Zemljevid’ elektronske gostote proteina.

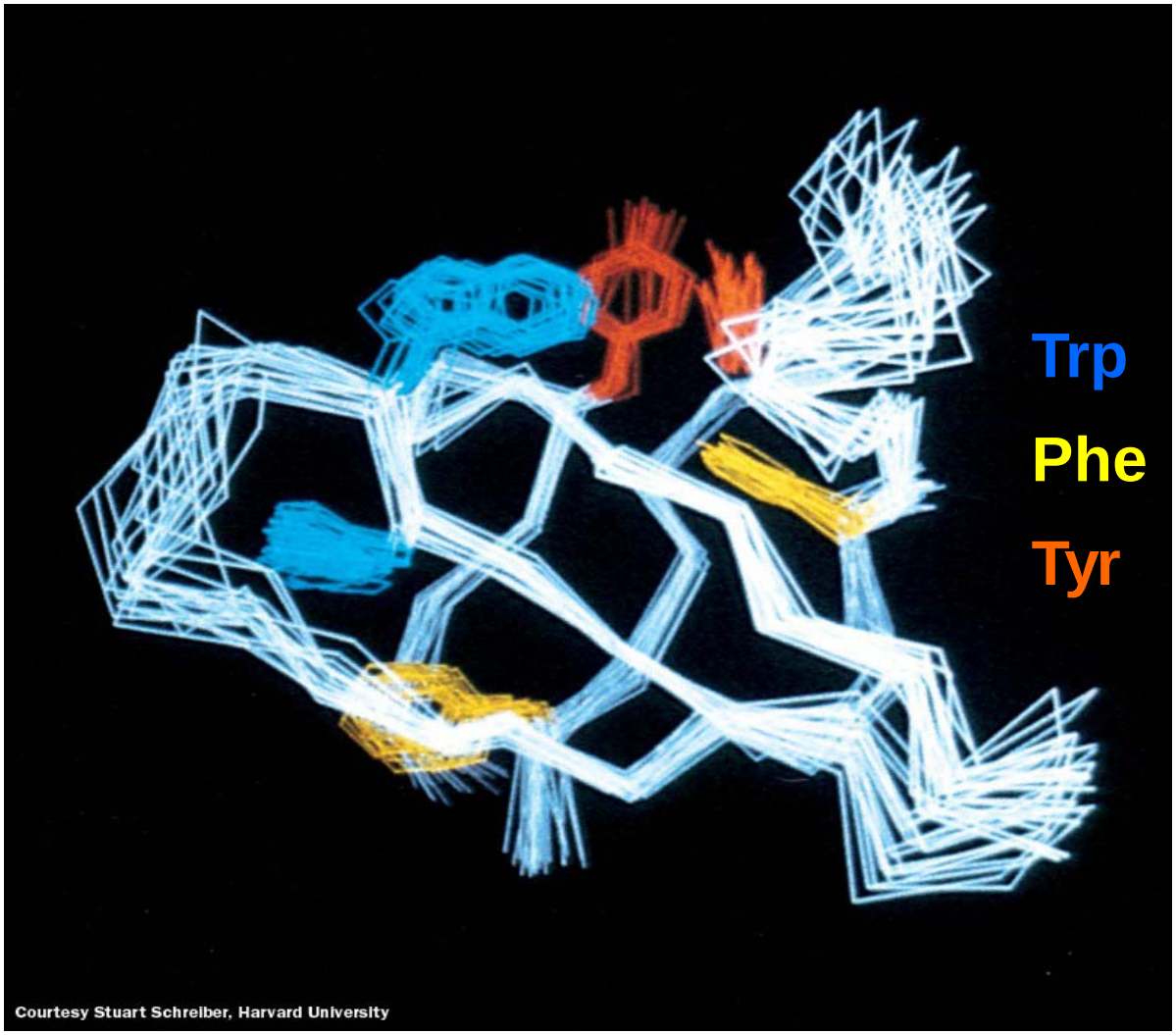


Spektri 2D protonske NMR tudi omogočajo razrešitev strukture proteinov (v raztopini!).



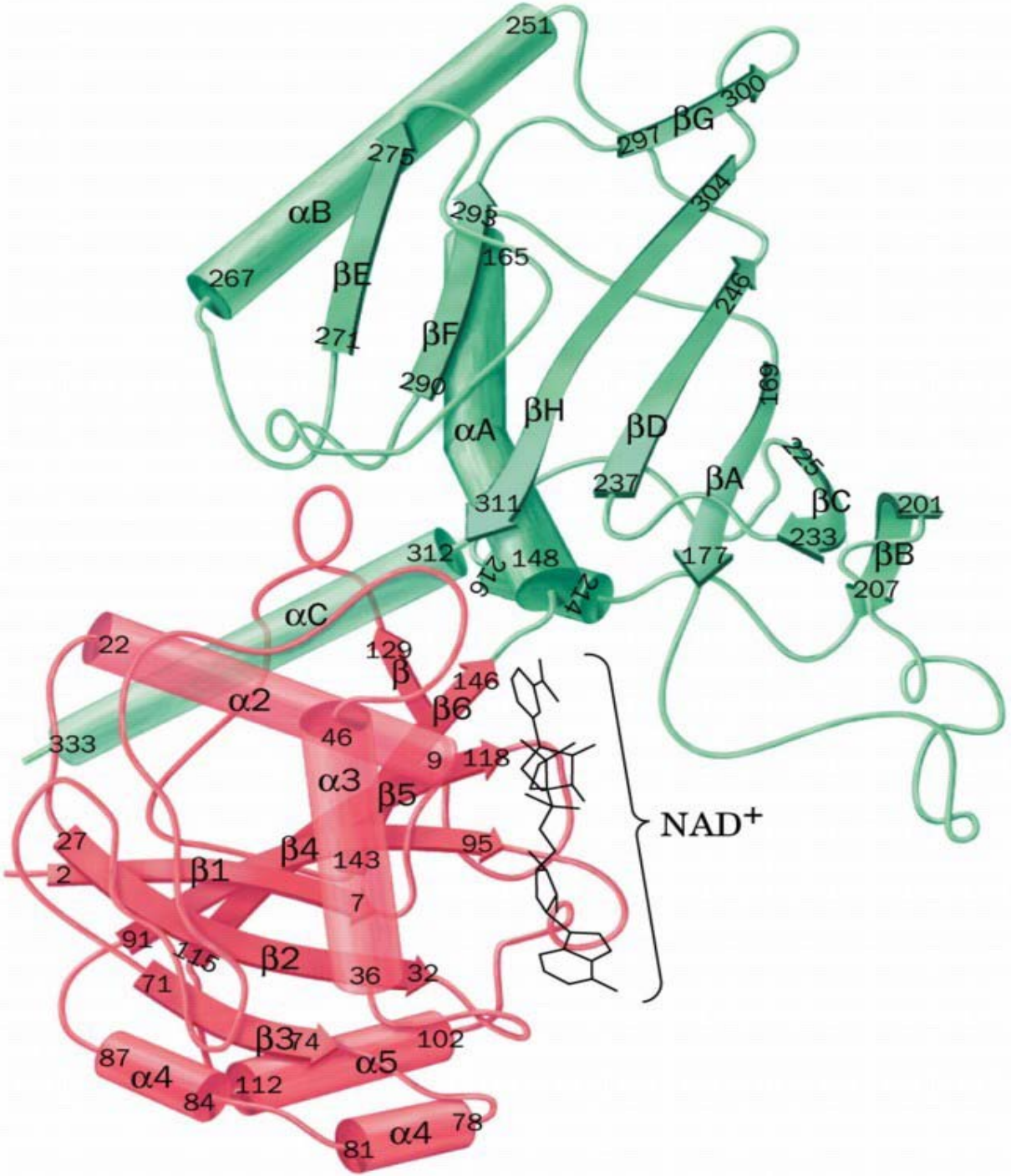
After Wuthrich, K. *Science* **243**, 45 (1983)

Struktura proteina (**Src protein SH3 domena)** dobljena z 2D protonsko NMR; vidijo se področja večje fleksibilnosti.

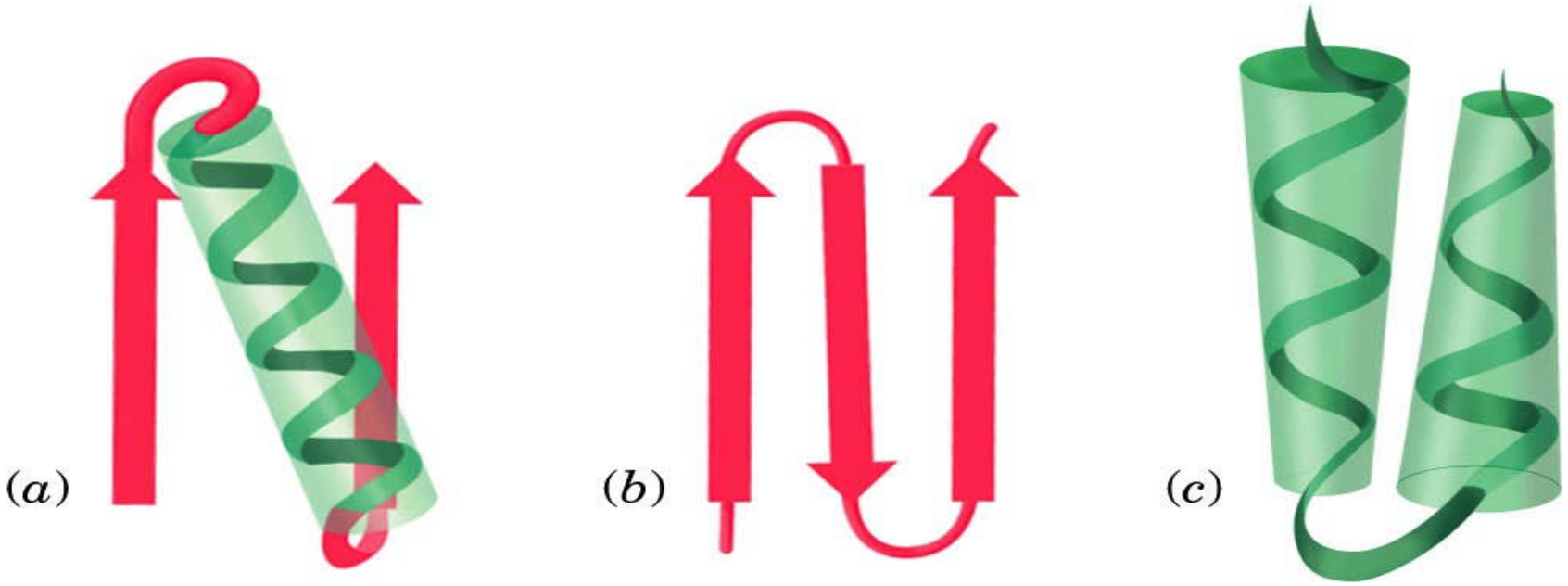


Ena podenota encima gliceraldehid-3-fosfate dehidrogenase iz *Bacillusa stearothermophilusa*.

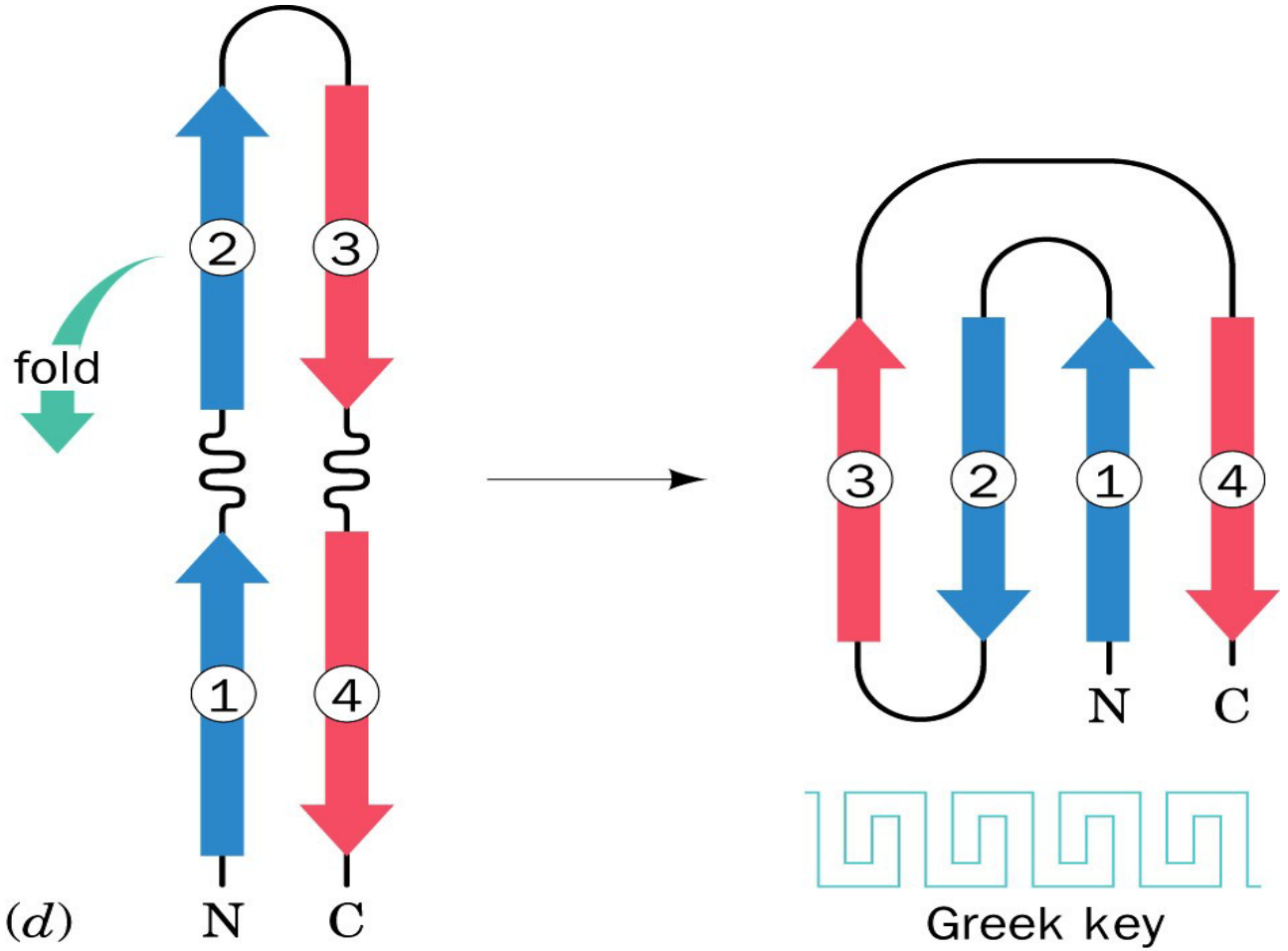
POZOR: Kombinacija različnih II. struktur. Jasno se vidita dve domeni (ena rdeče in druga zelene barve.



Različne supersekundarne strukture (motivi).

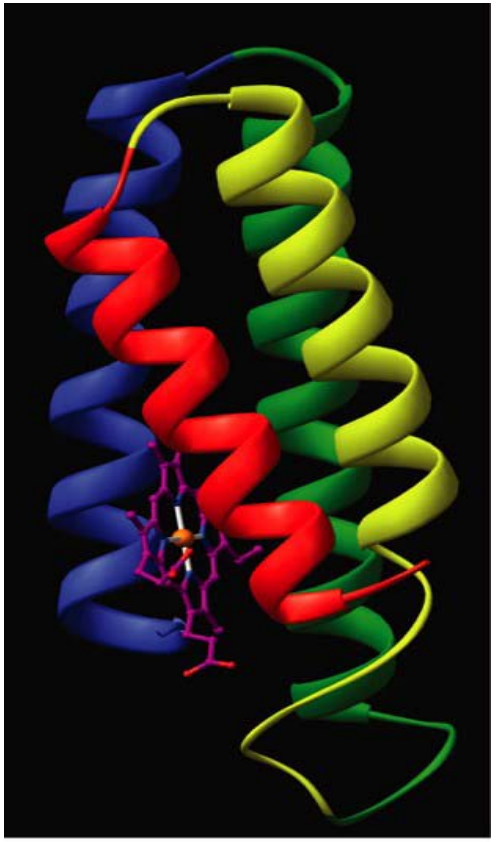


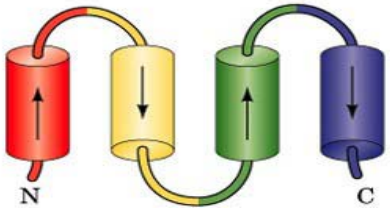
Različne supersekundarne strukture (motivi).

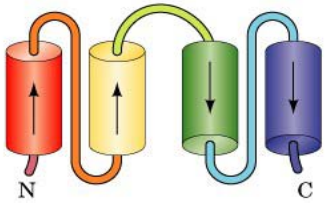


meander

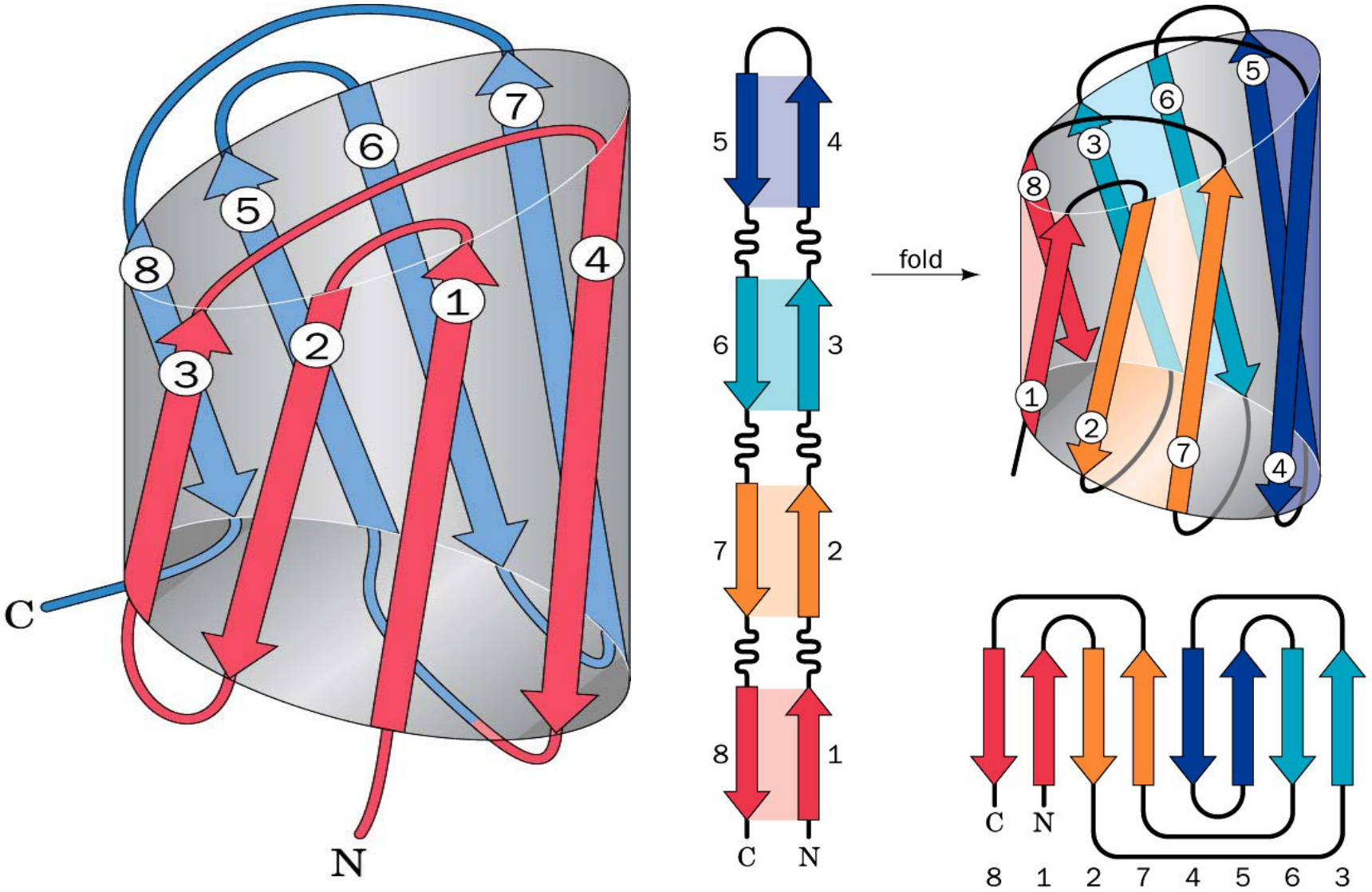
4-helix bundle proteins:
(*a*) *E. coli* cytochrome b_{562} and (*b*) Human growth hormone.



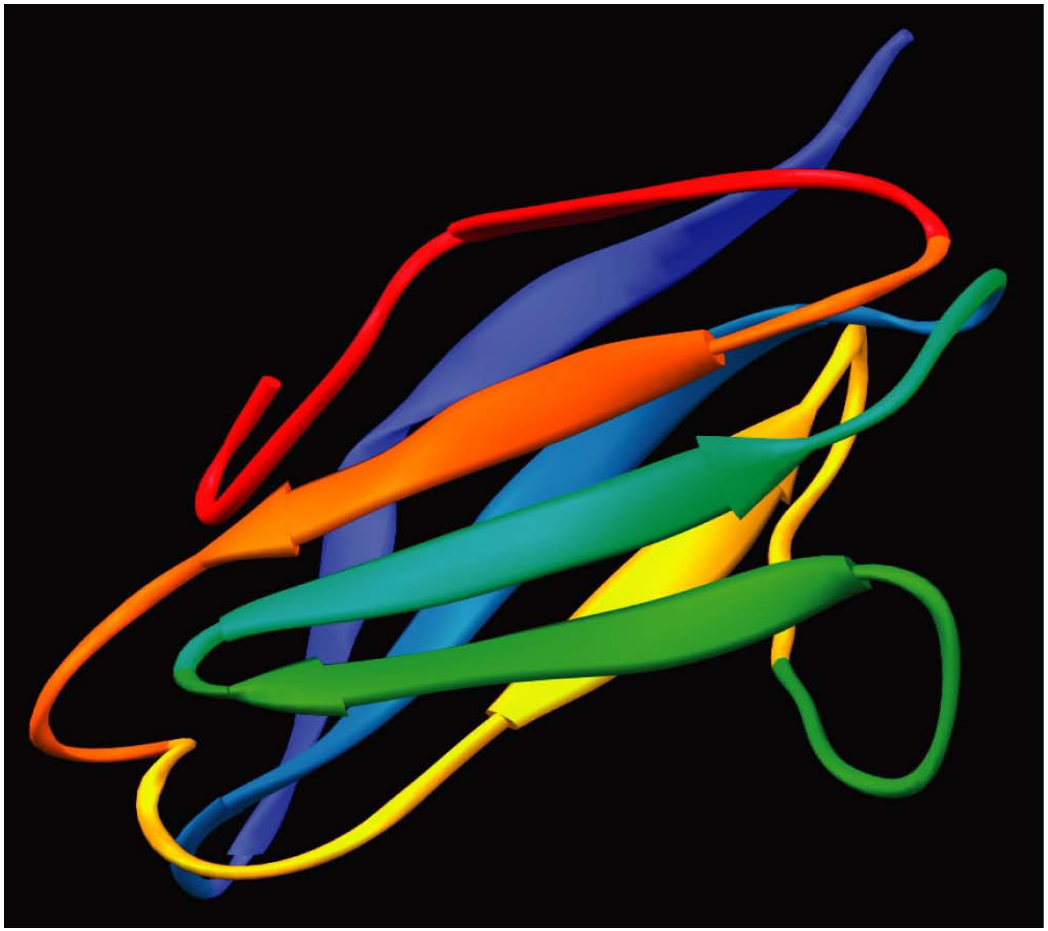


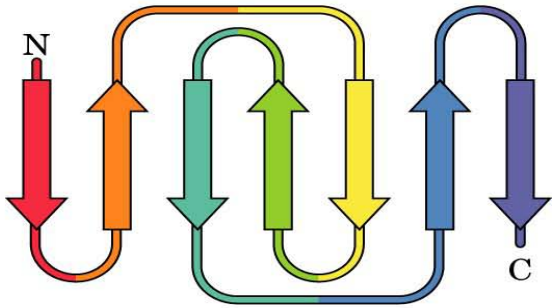


Struktura C-terminalne domene γ-β kristalina. (*a*) Organizacija meandrov (Greek key) v β sodček.

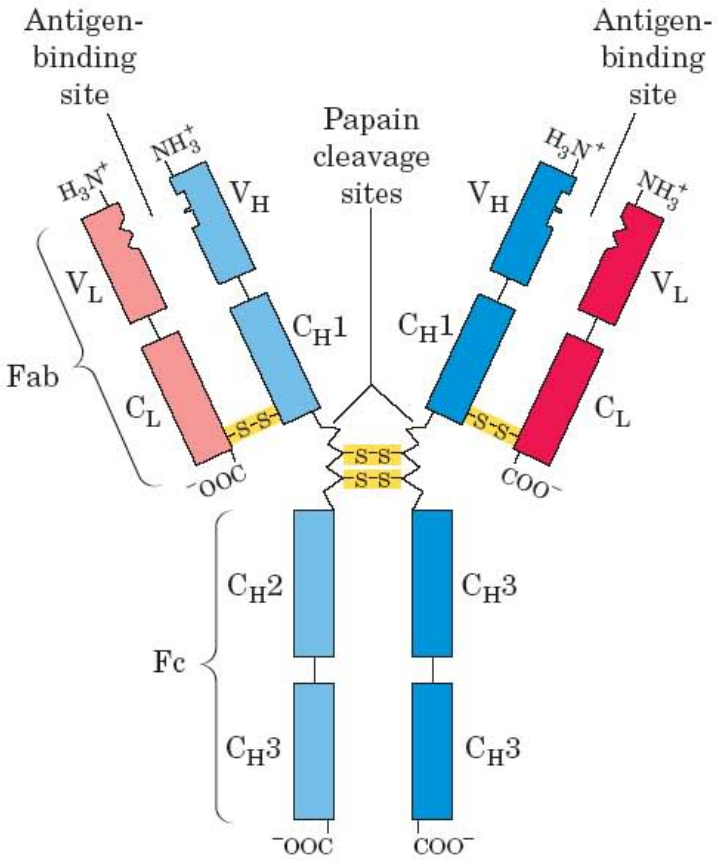


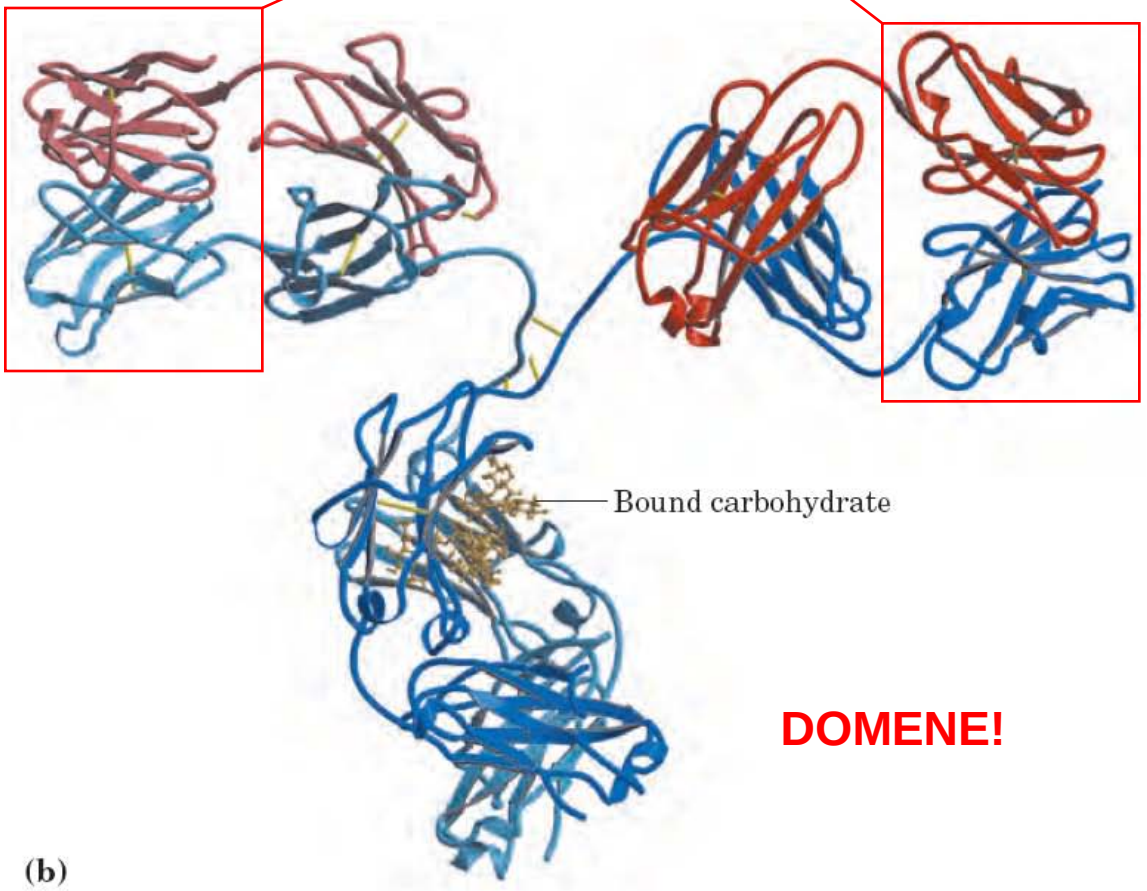
Imunoglobulinsko zvitje.





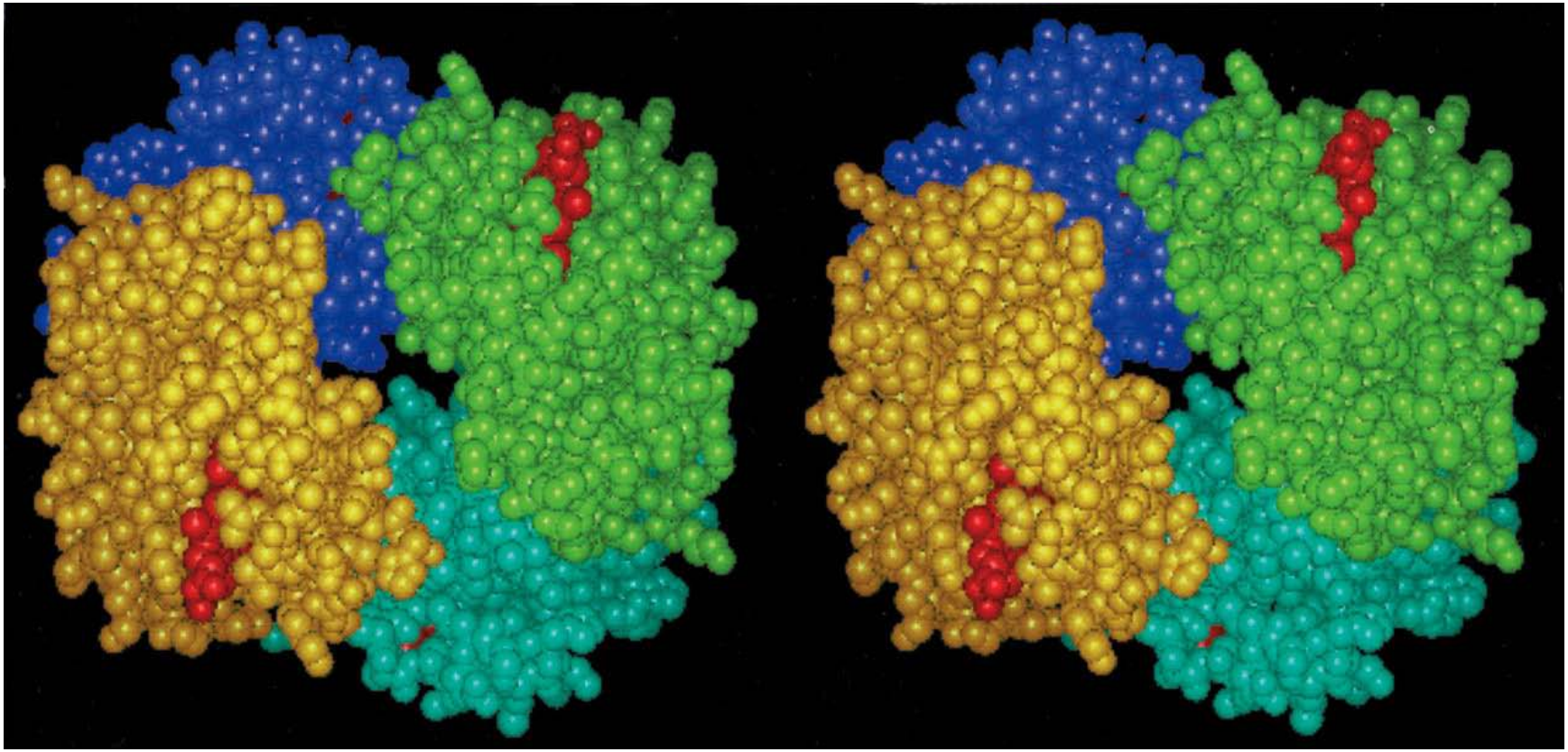
Imunoglobulinsko zvitje





DOMENE!

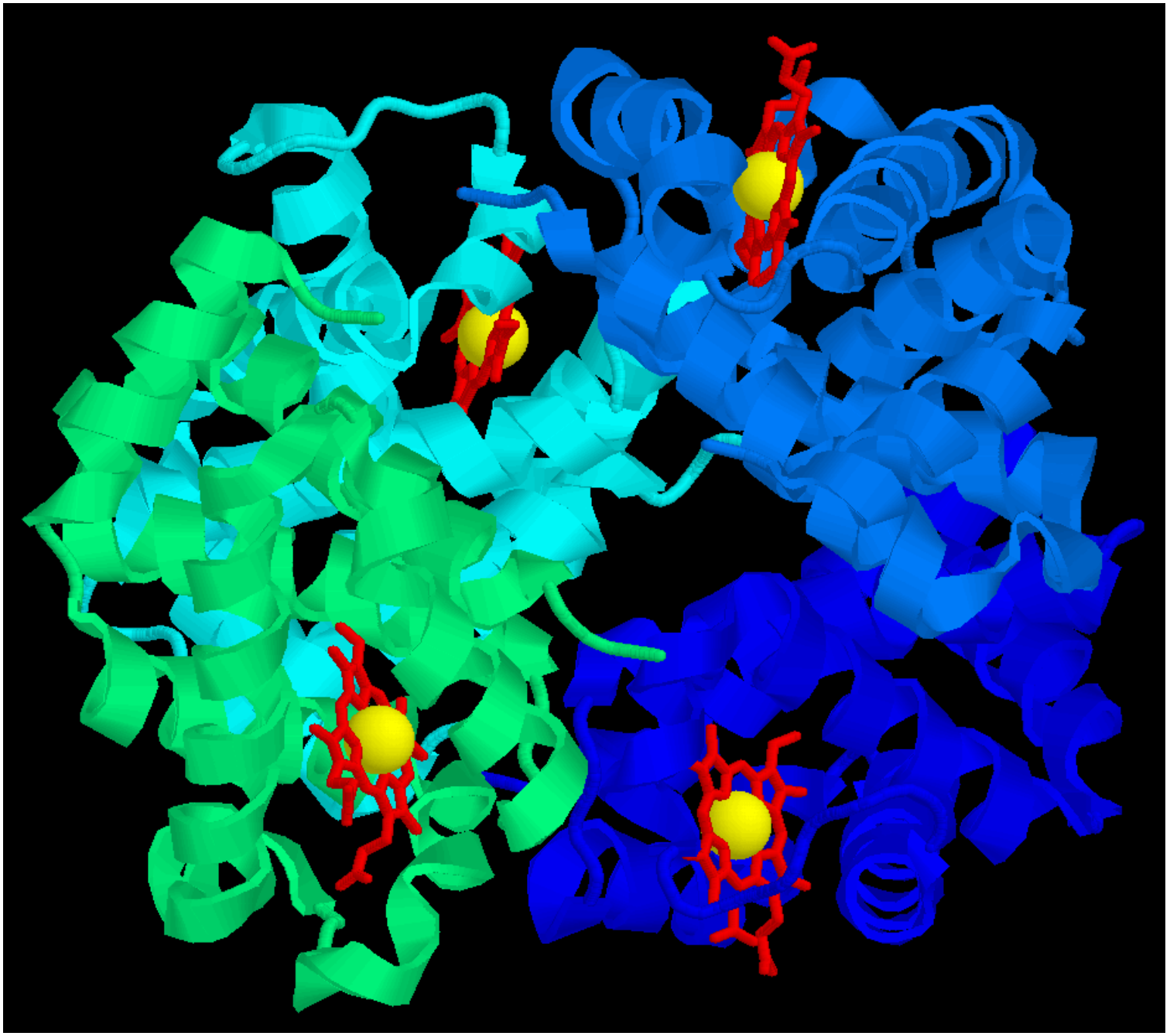
Kvartarna struktura hemoglobina.



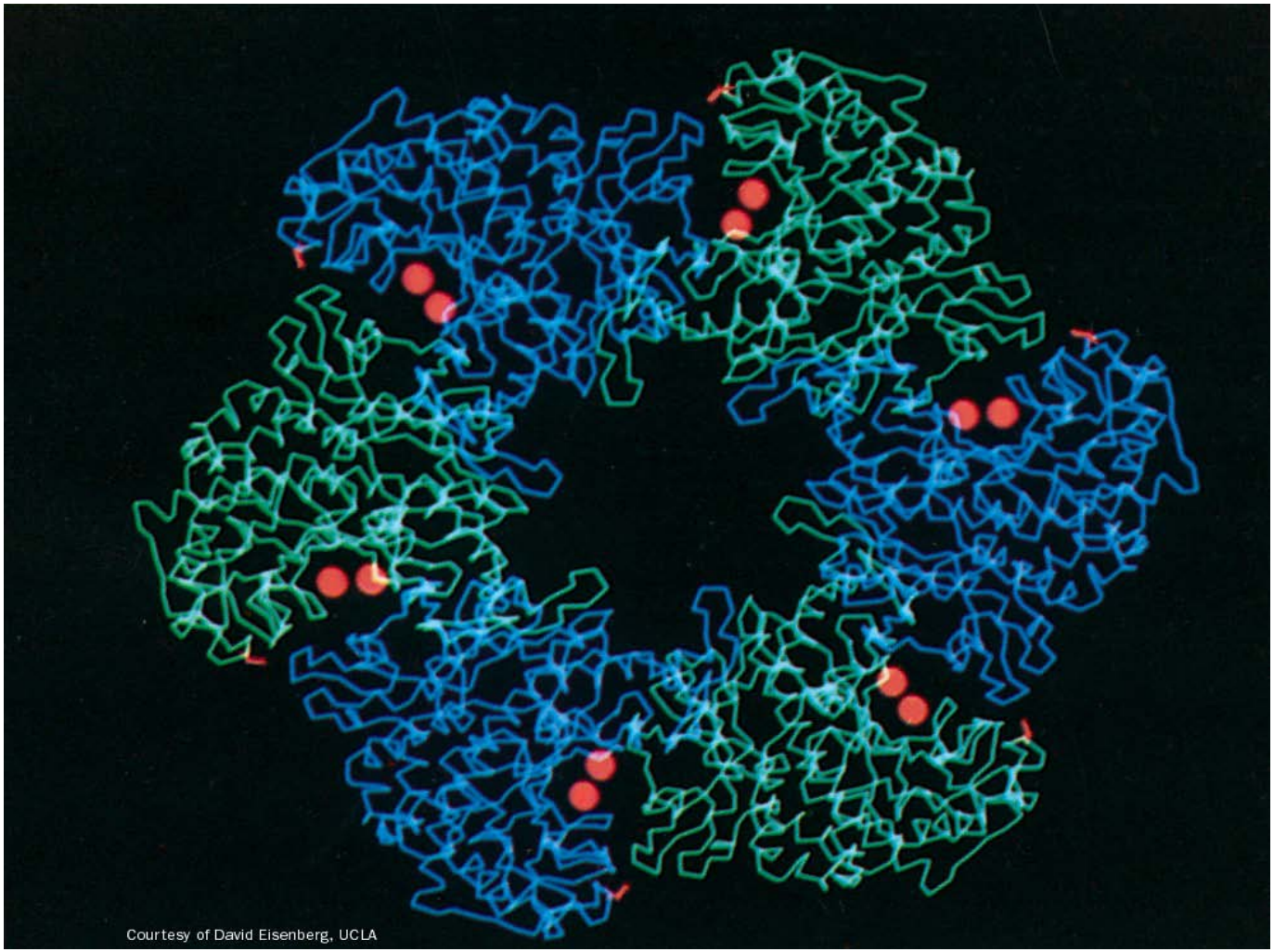
Hb

(2D5Z.pdb)

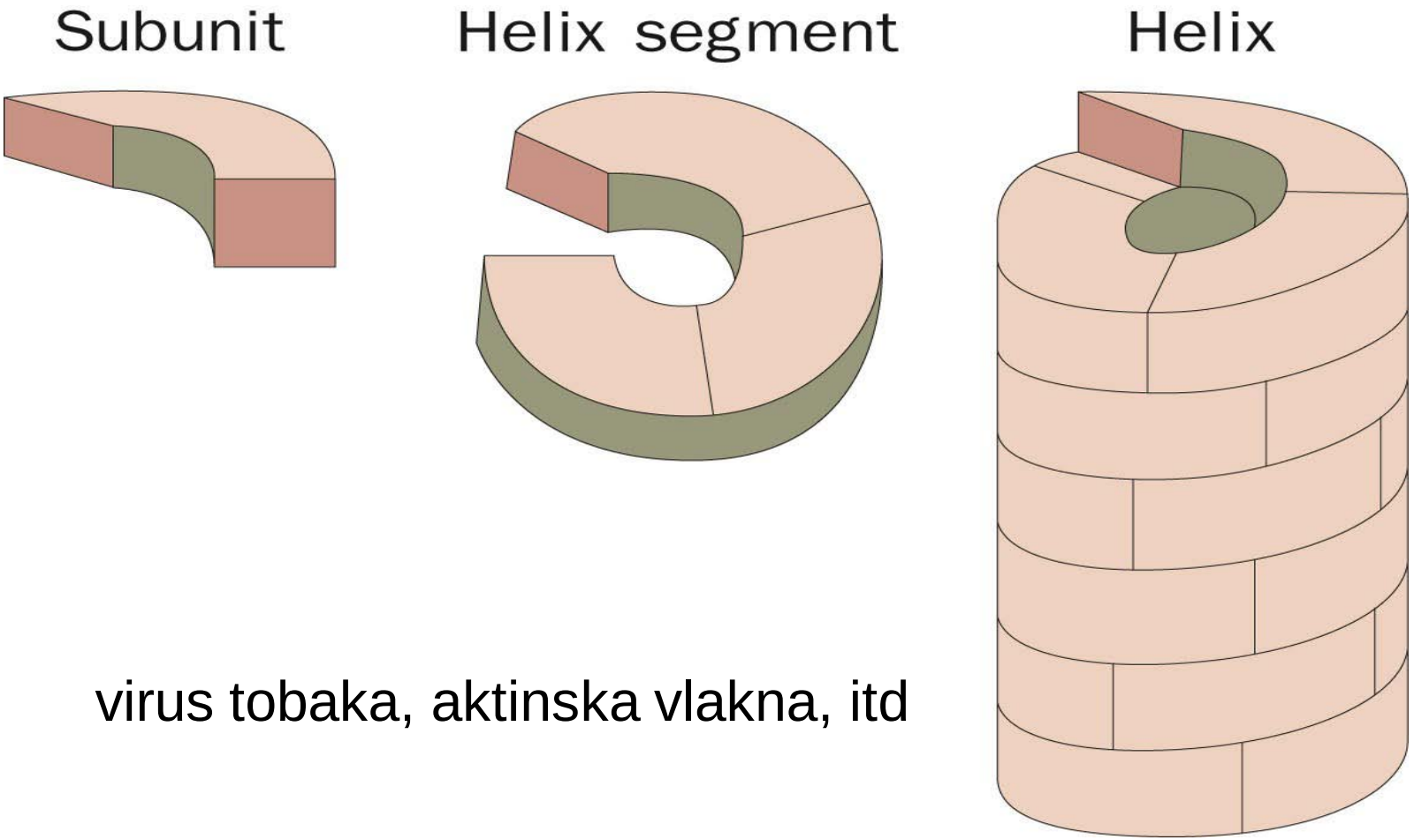
POKAŽI!



Glutamin-sintetaza (*Salmonella typhimurium)*.

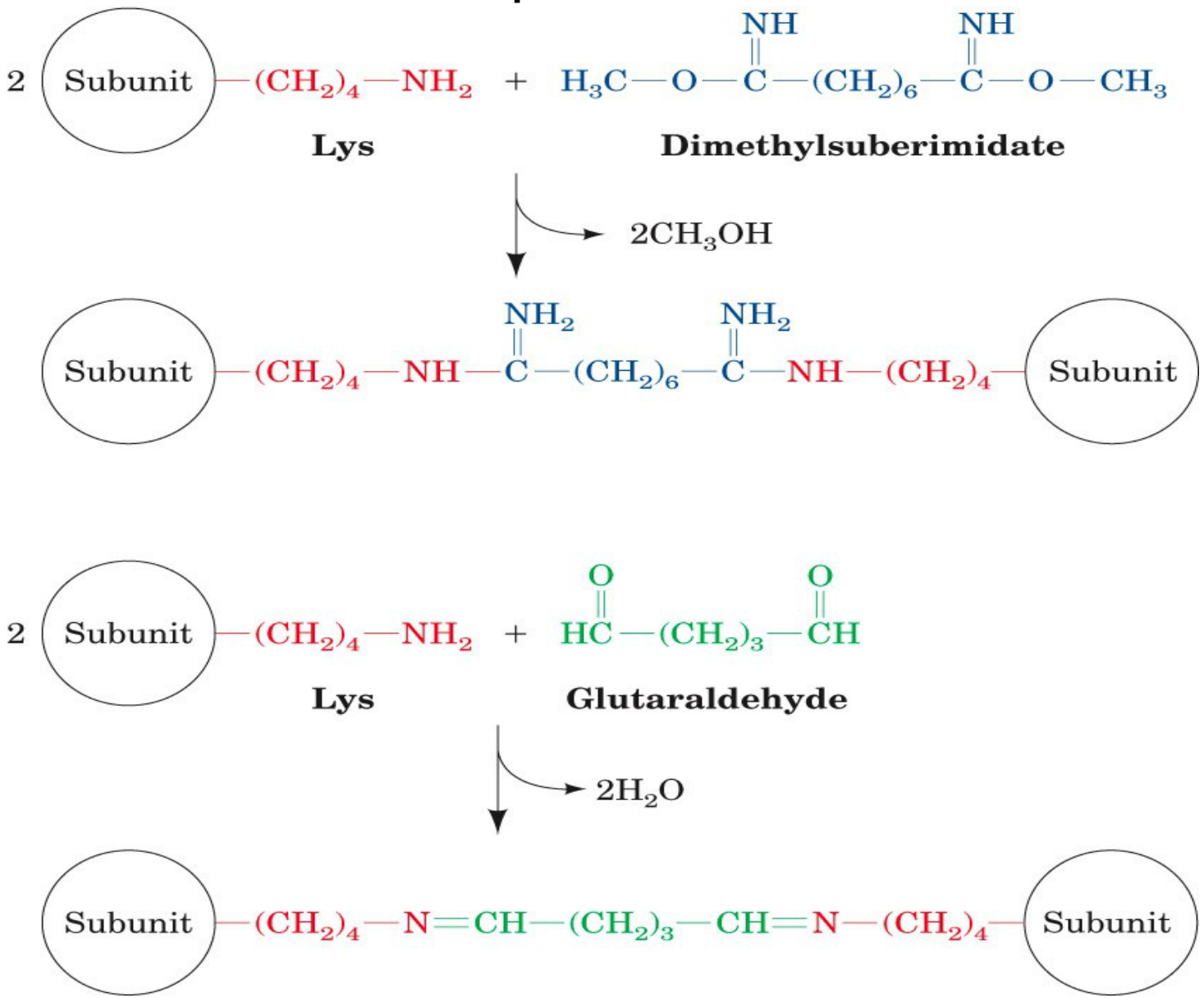


Kvartarna struktura iz ene same podenote.



virus tobaka, aktinska vlakna, itd

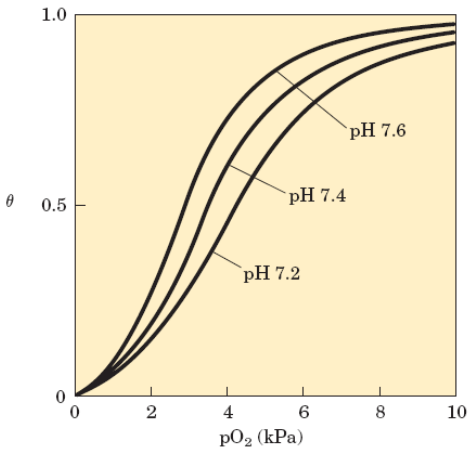
Premreženje omogoča identifikacijo stičnih mest med podenotami

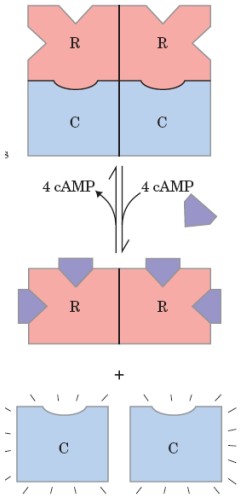


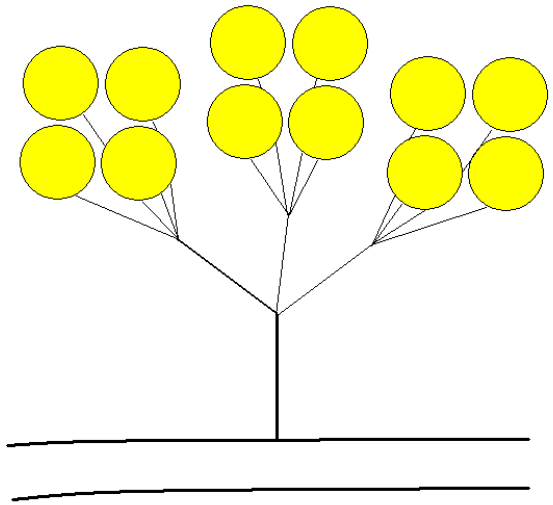
ZAKAJ KVARTARNA STRUKTURA?

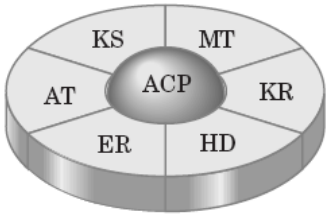
- VARNOST (Hb: 2α+2β, 2 različna gena)
- ALOSTERIČNI EFEKTI (regulacija – Hb, alosterični encimi)
- POVEČANA & LOKALIZIRANA KONCENTRACIJA AKTIVNIH MEST (AChE)
- MULTIFUNKCIONALNI PROTEINI:

  maščobnokislinska sintetaza: 7 aktivnih mest z različnimi funkcijami:

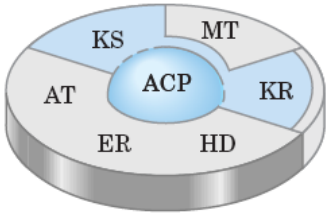




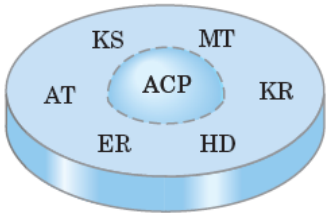




bacteria

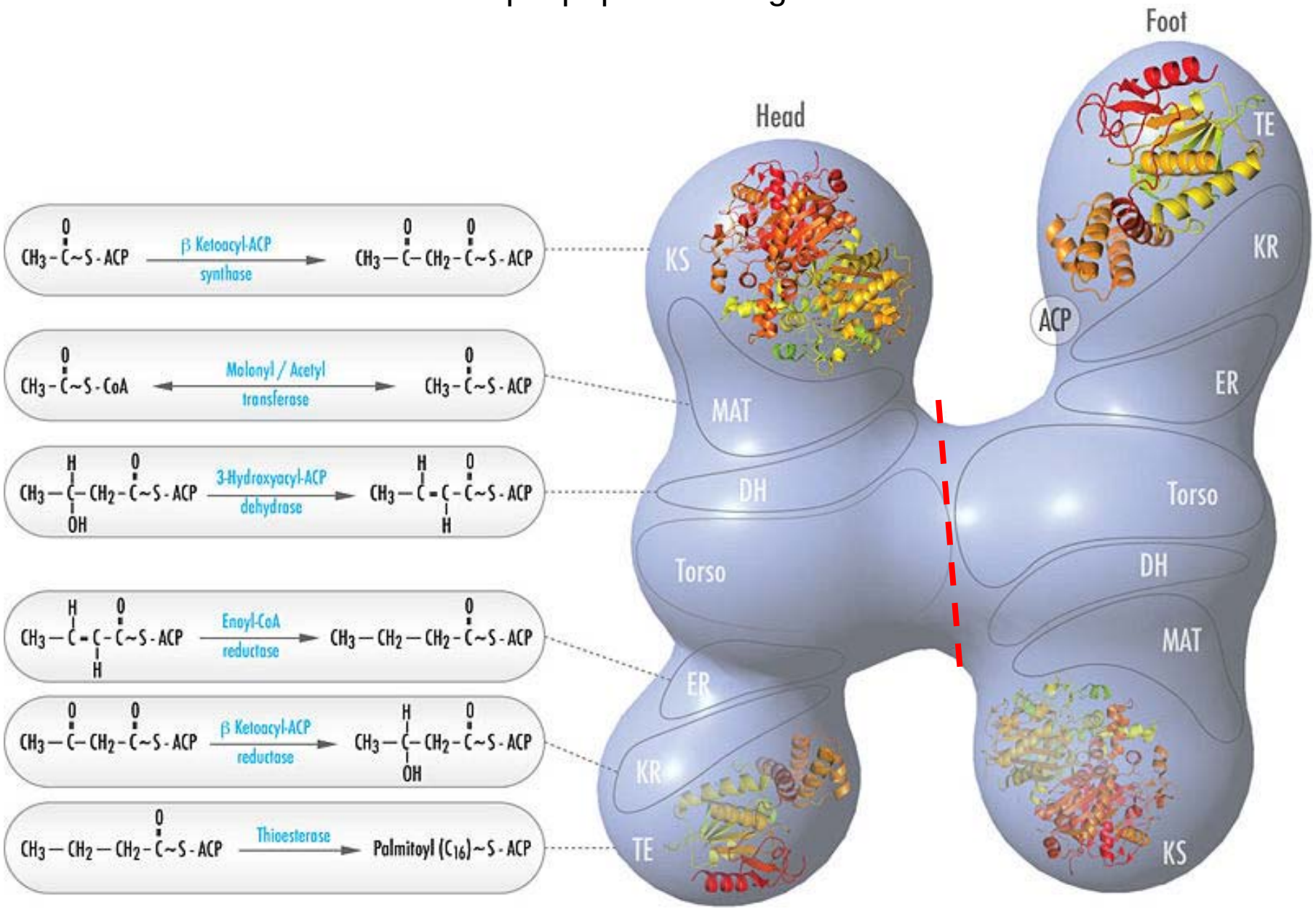


yeast



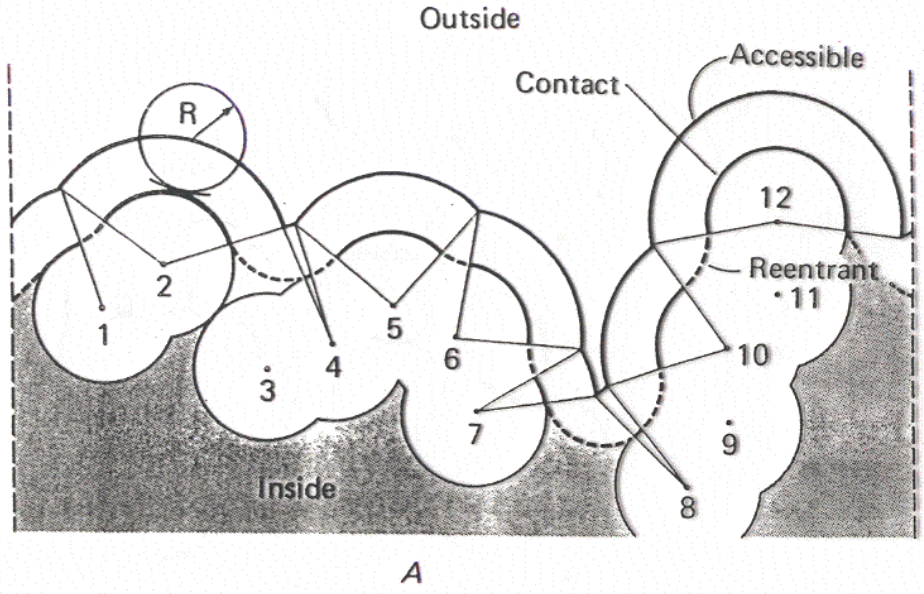
human

Multifunkcionalni proteini: maščobnokislinska sintetaza sesalcev: dimer dveh polipeptidnih verig

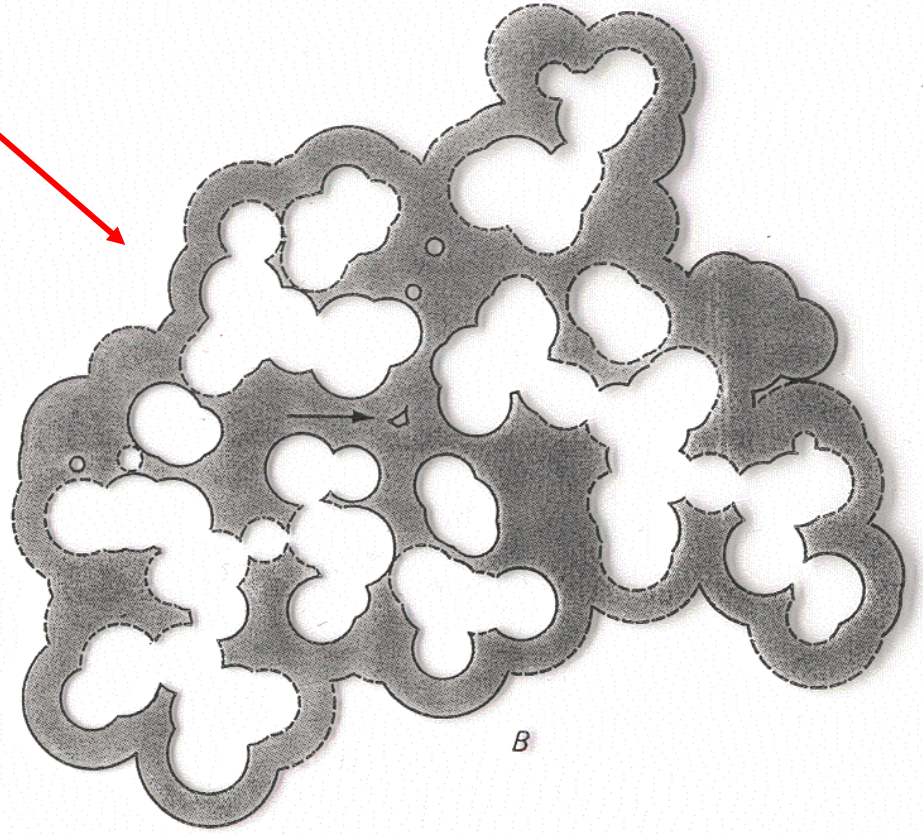


Nativna konformacija je:

- gosta
- kompaktna
- lahko ima vrzeli (luknje)
- voda je njen integralni del

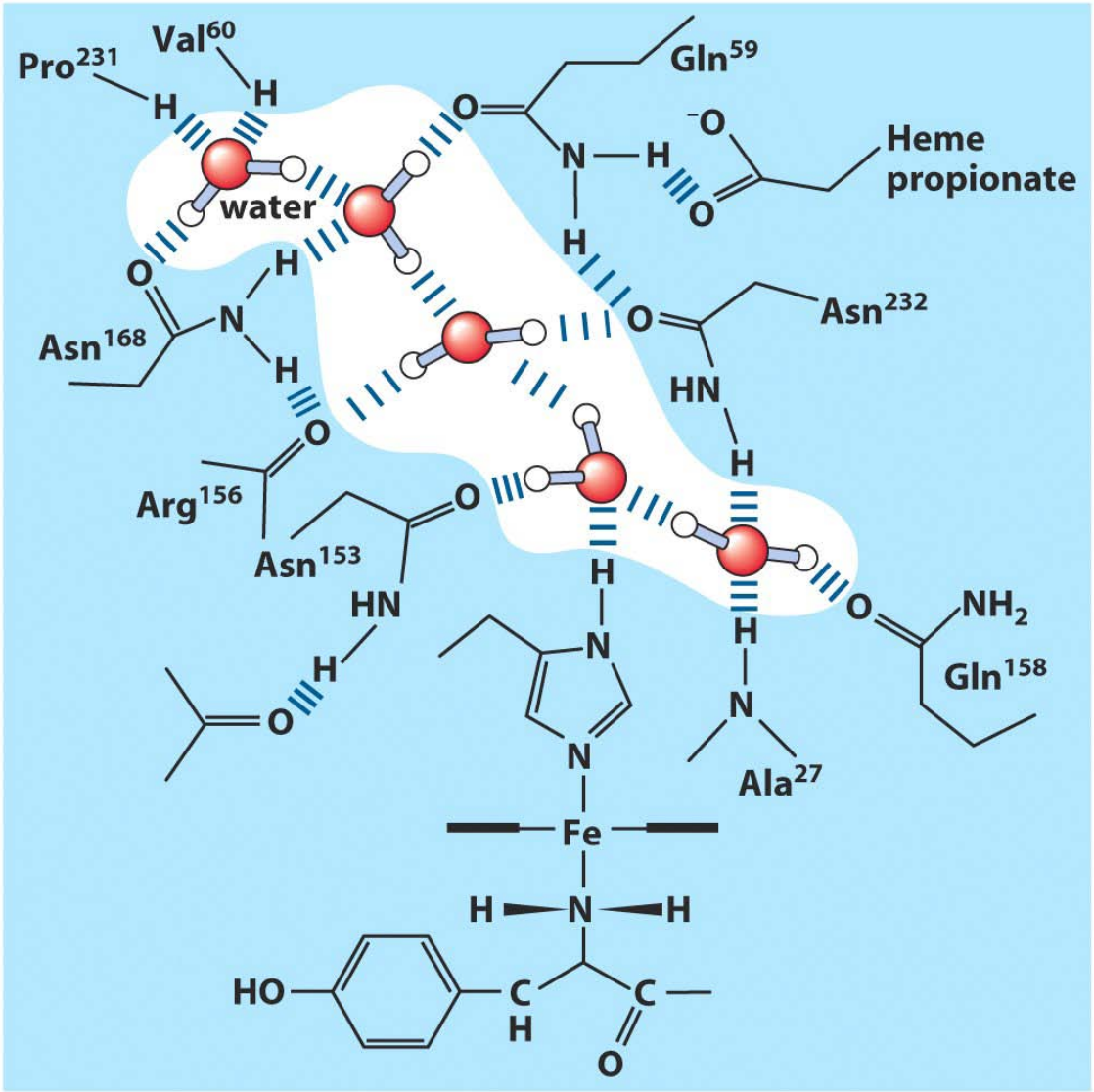


A



B

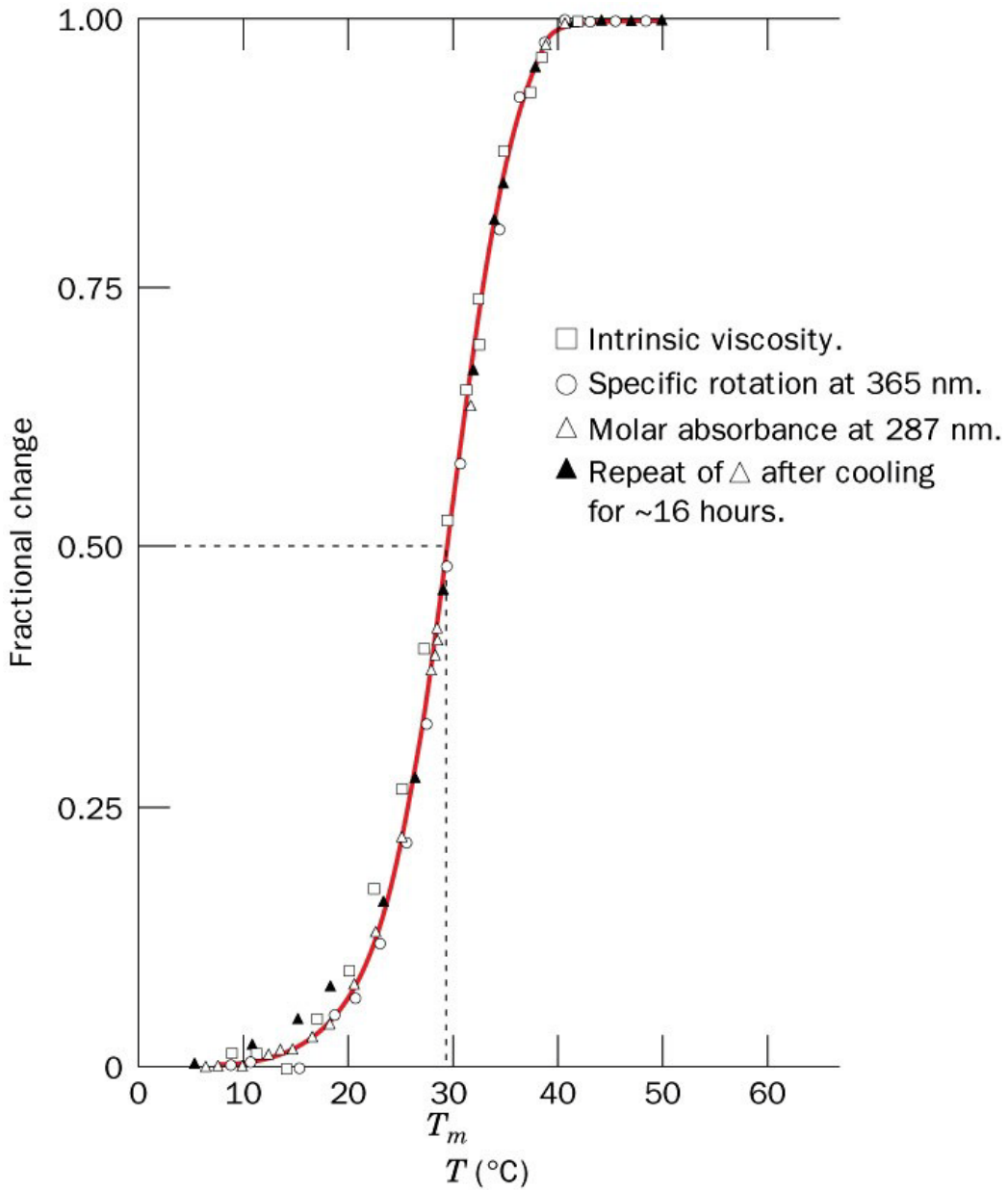
Voda je integralni del proteinske strukture in pomembno prispeva k njeni stabilnosti.



Proteine lahko denaturiramo – ključ do poznavanja zvijanja

Denaturanti, pH, T in visok P porušijo strukturo. Proces je lahko reverzibilen.

POKAŽI DIAGRAM (NE)STABILNOSTI!

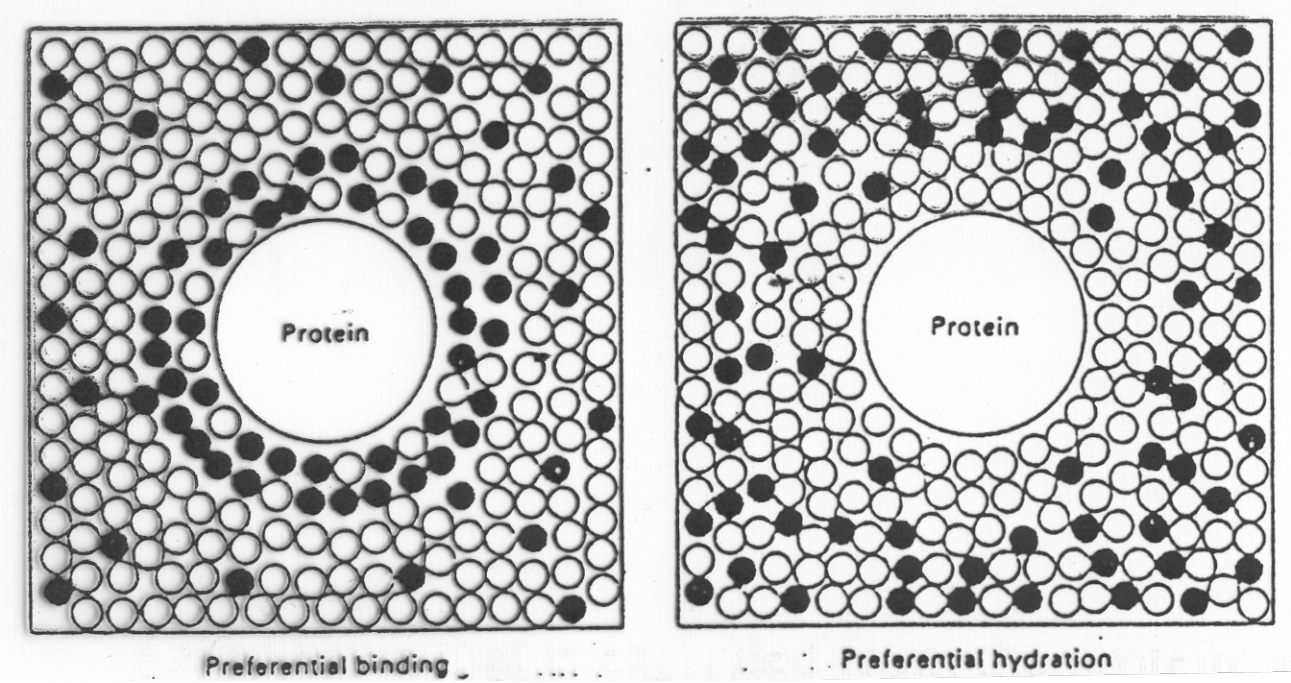


Zakaj denaturacija:

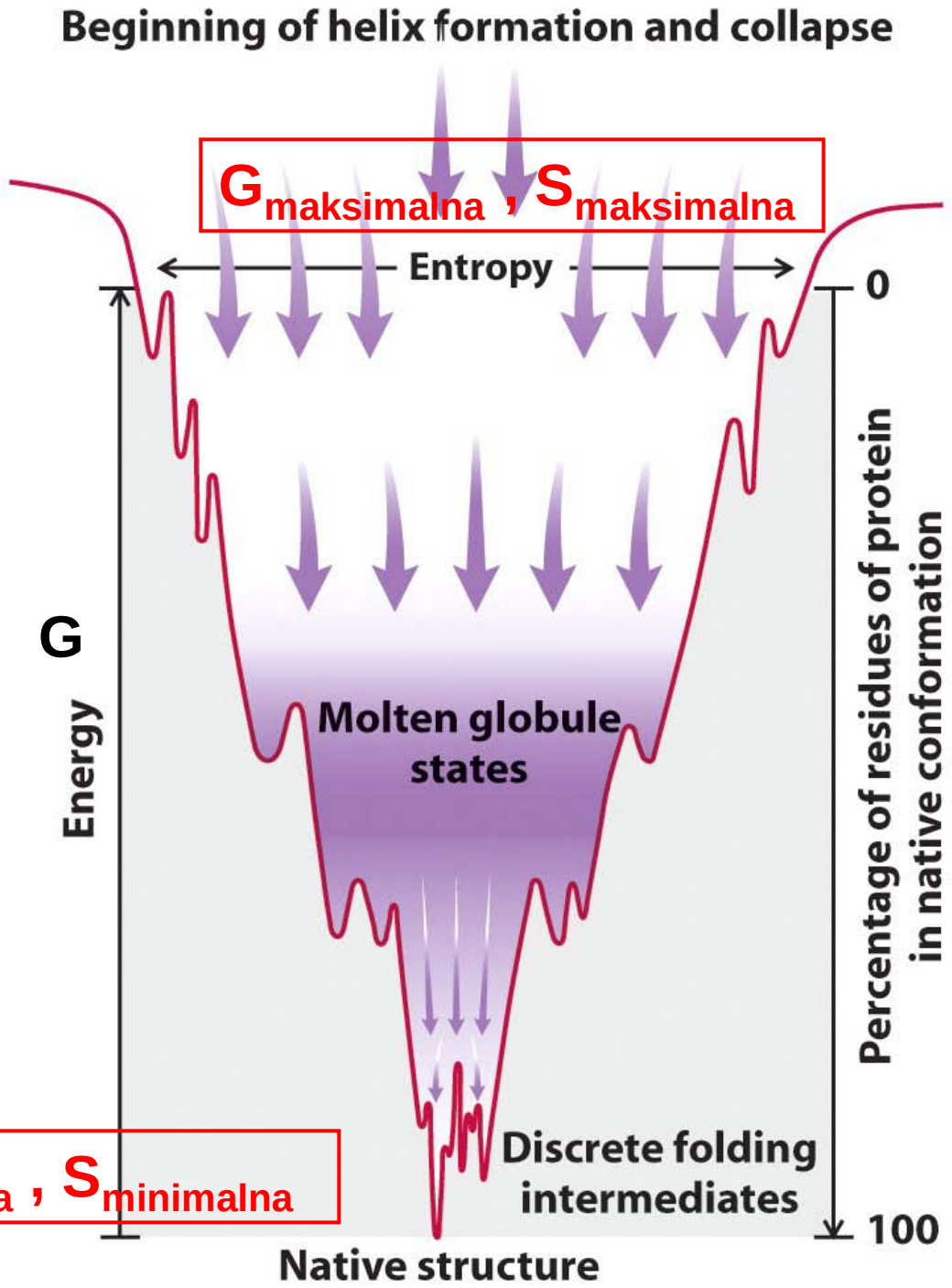
• toplota (visoka temperatura): neposreden vnos energije

• ekstremni pH: ionizacija le v razvitem stanju (premik ravnotežja); elektrostatski odboji na površini proteina; porušenje ionskih interakcij.

• denaturanti (urea, gvanidinijev klorid): preferenčna vezava (majhen vpliv na H-vezi!)



Prerez skozi 3D energijski profil zvijanja proteina. Nativna struktura ima najmanjšo energijo (prosto entalpijo G), povsem razvit protein pa ima največjo energijo (G) in tudi največjo entropijo (S), ker je ta struktura najbolj neurejena.



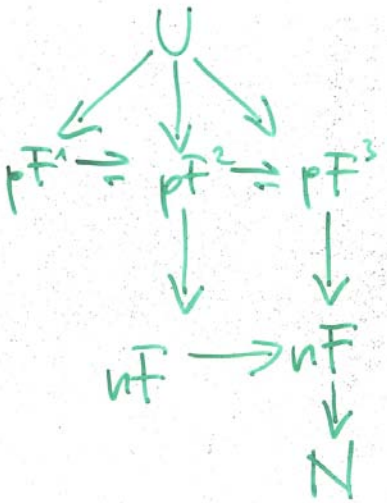
prispevek vezi

Prispevek (ne)urejenosti

$$G=H-T.S$$

$G_{minimalna}$, $S_{minimalna}$

Poti zvijanja lizozima



denatured states

< ms

< ms

< ms

neither domain correct

α–domain correct

α– and β–domains correct

~ 50 ms

~ 300 ms

interconverting collapsed states

~ 10 ms

~ 10 ms

~ 300 ms

protected states

persistent α–domain

near–native state

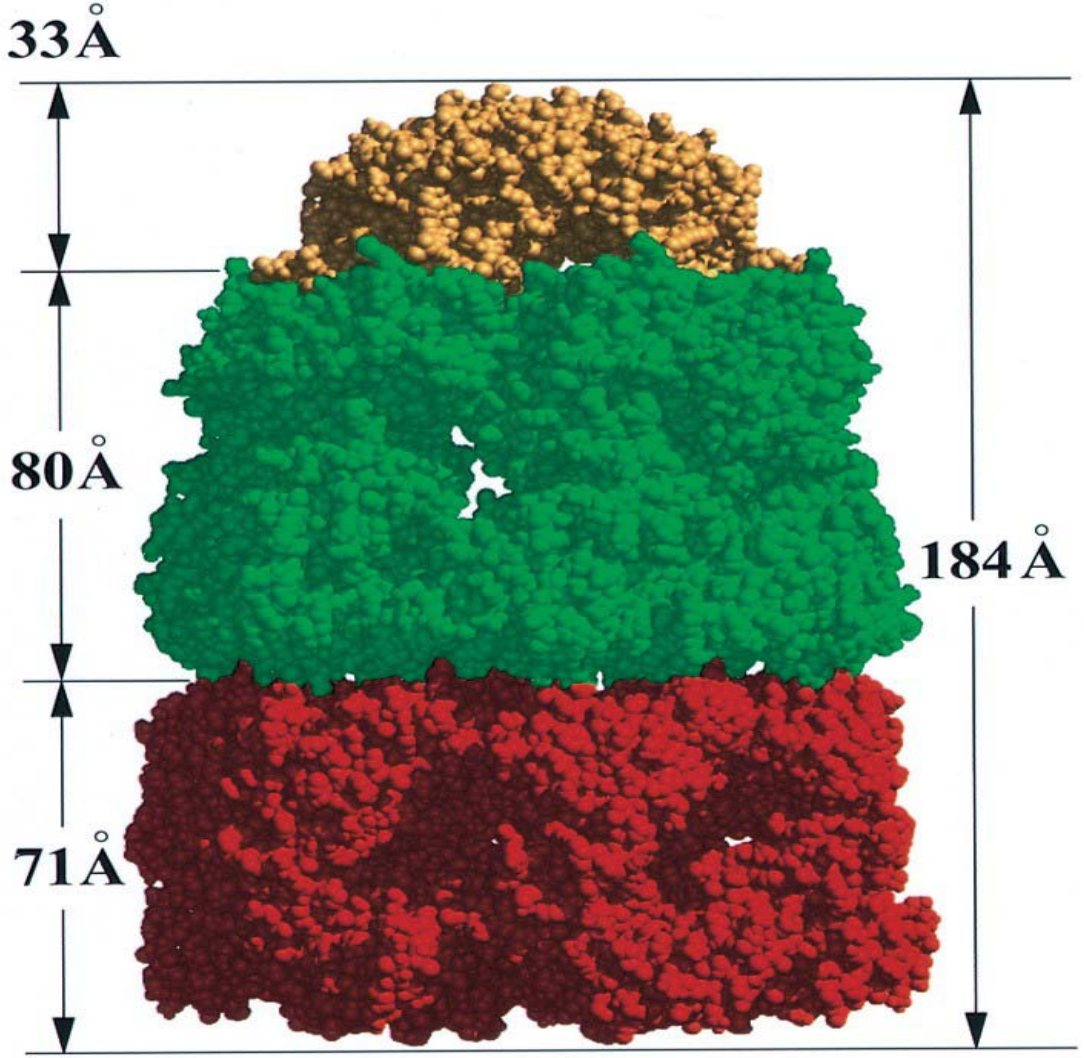
~ 100 ms

native state

native state

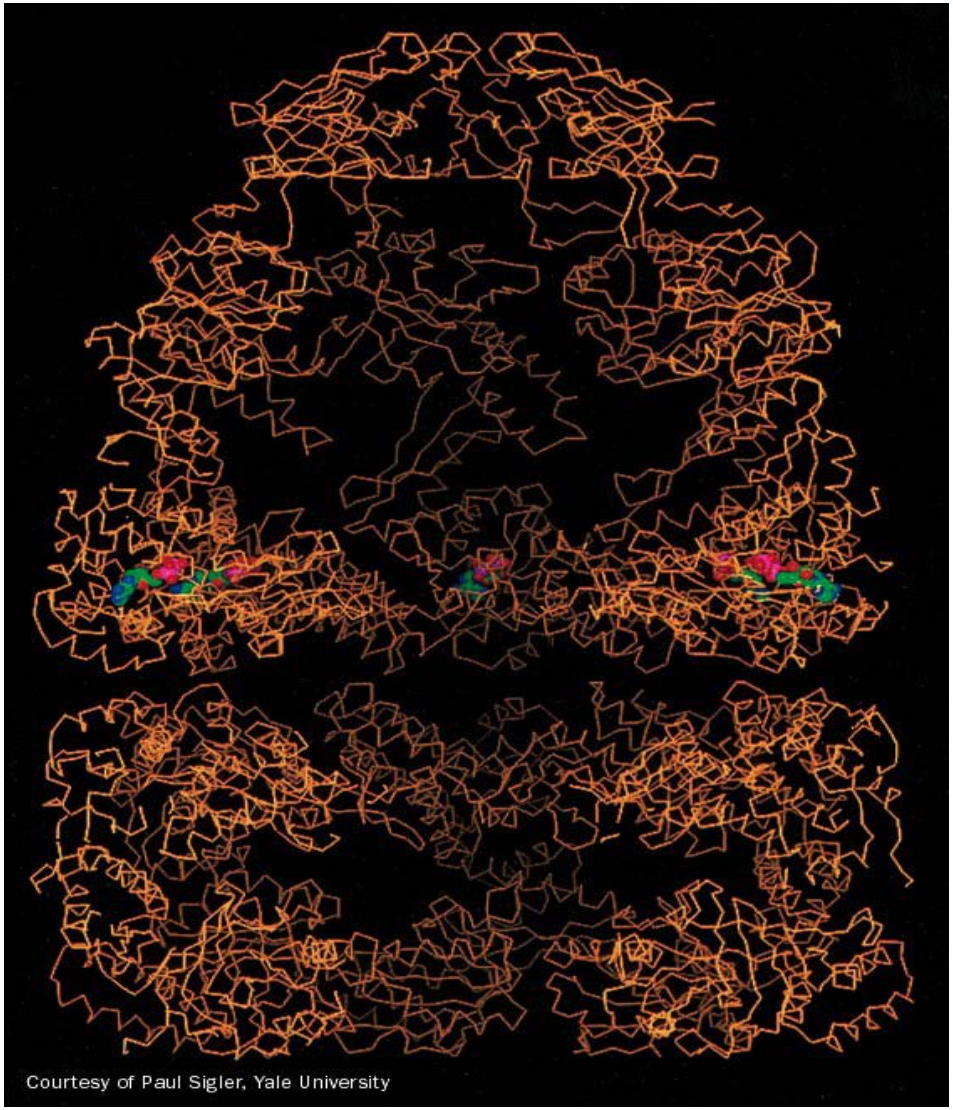
lysozyme deduced from the eight
remain intact throughout the folding

Šaperoni pomagajo proteinom pri zvijanju in skušajo ponovno zviti razvite proteine.

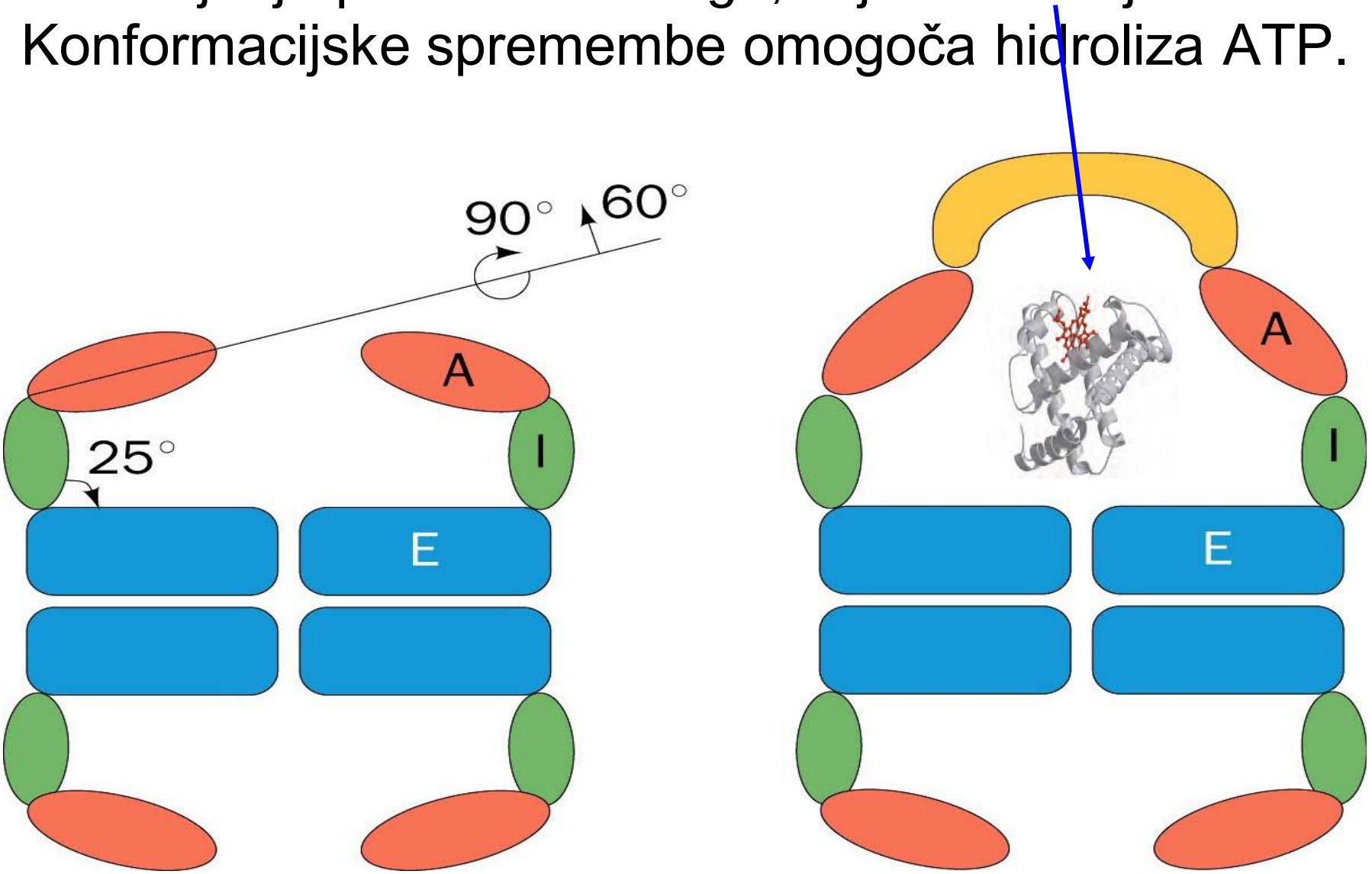


Courtesy of Paul Sigler, Yale University

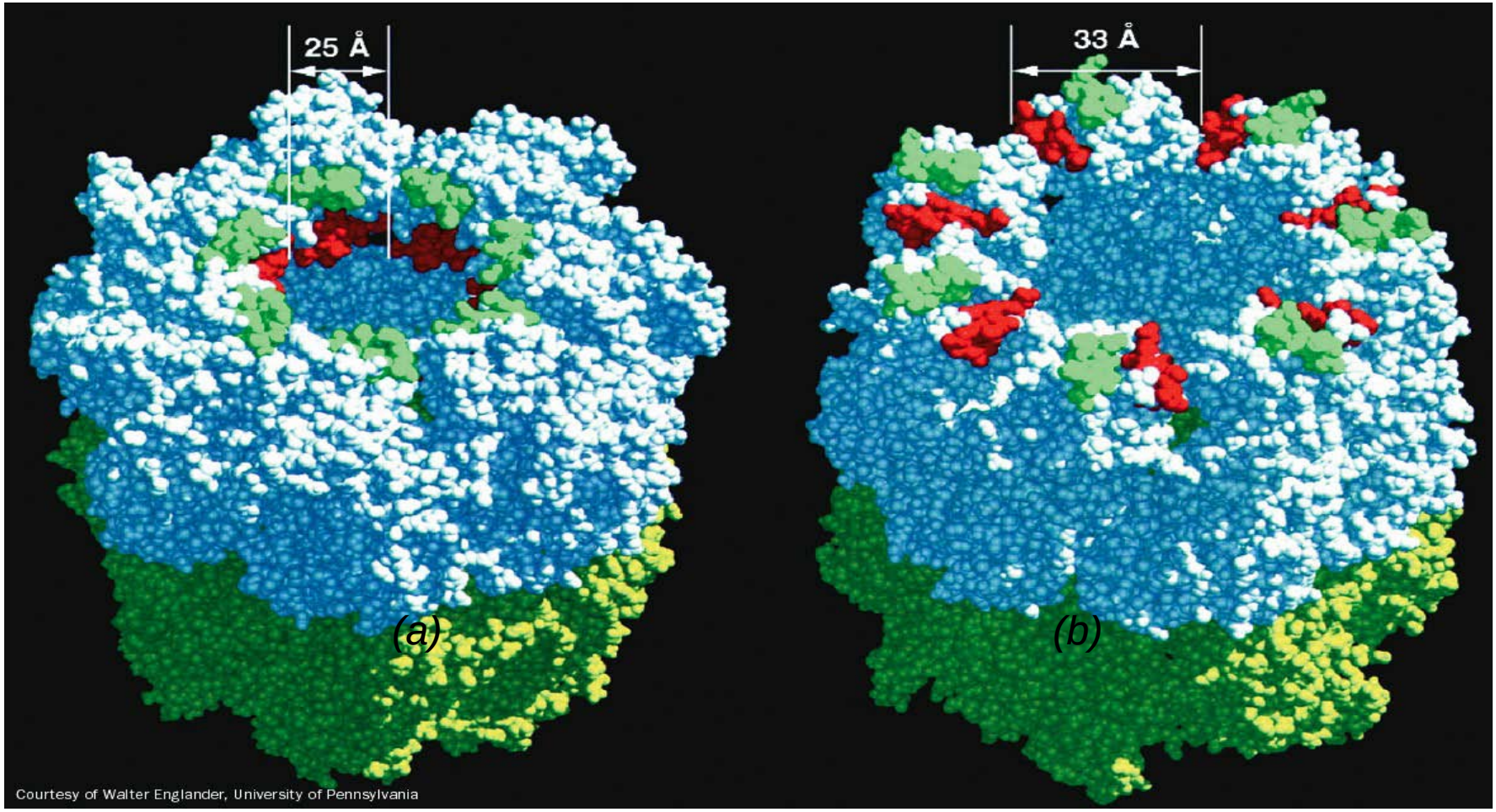
Notranjost šaperona je specifično okolje, ugodno za zvitje proteinske verige.



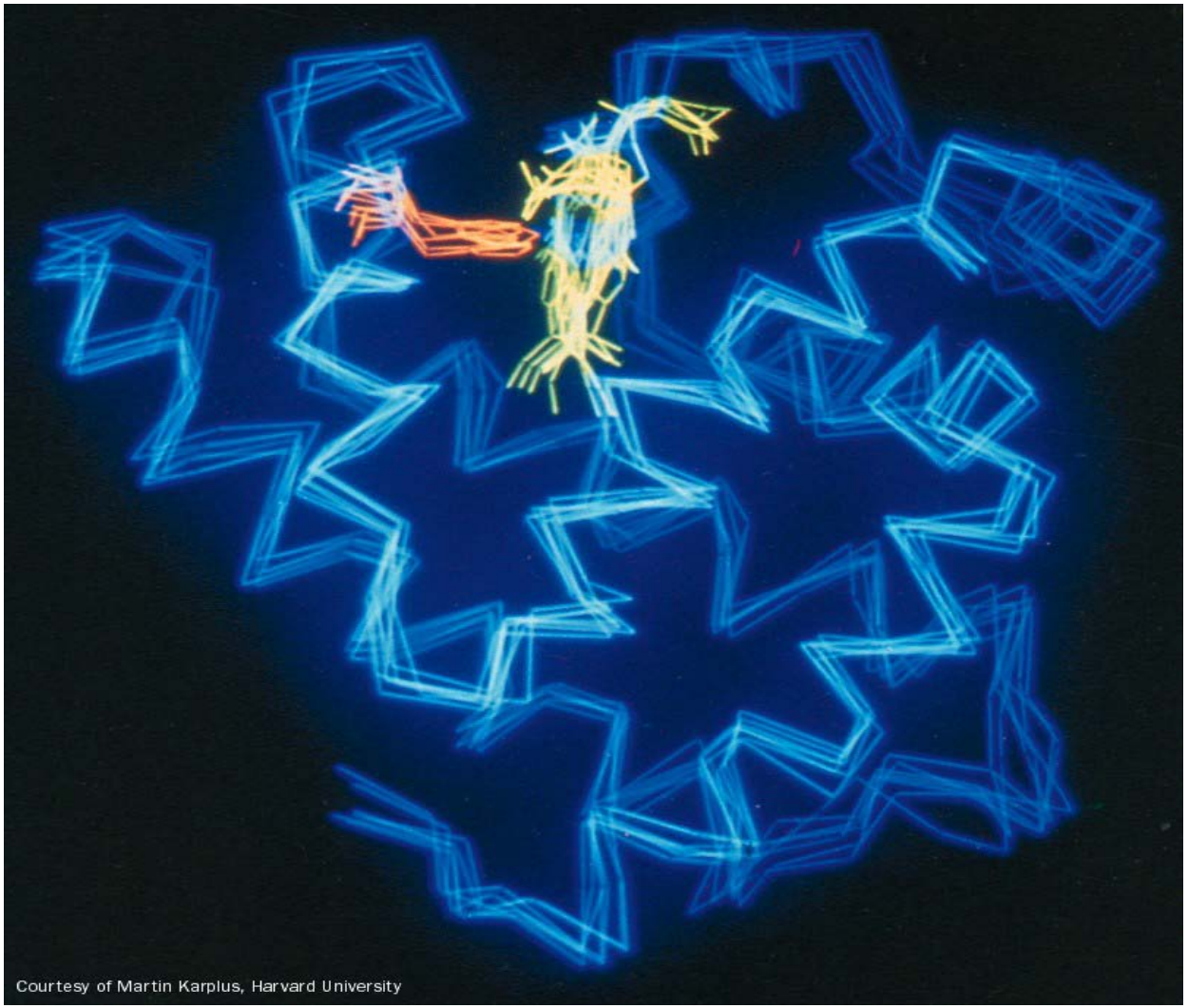
Premikanje domen v plašču šaperona pomaga pri zvijanju proteinske verige, ki je v notranjosti. Konformacijske spremembe omogoča hidroliza ATP.



Premikanje heliksov (rdeče), ki vežejo protein v notranjosti šaperona.



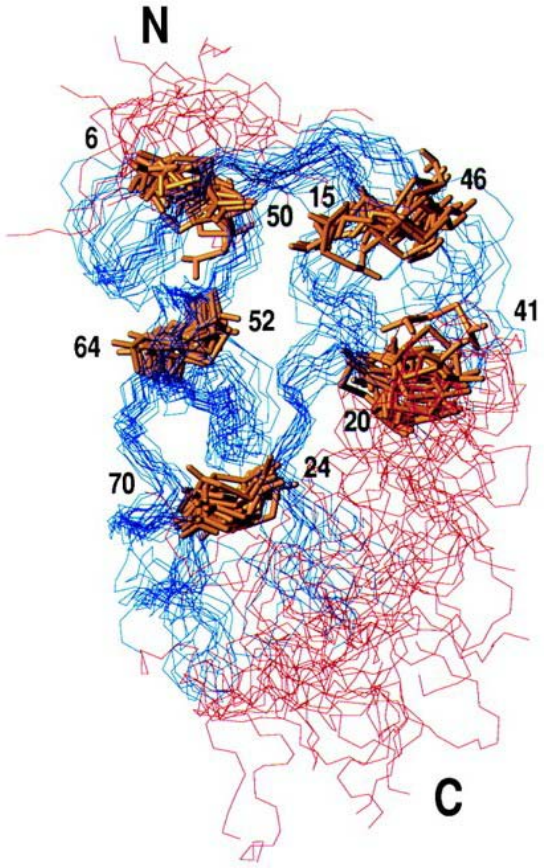
Fleksibilnost molekule Mb – hrbtenica polipeptida (modro), hem (rumeno) in proksimalni His (rdeče).



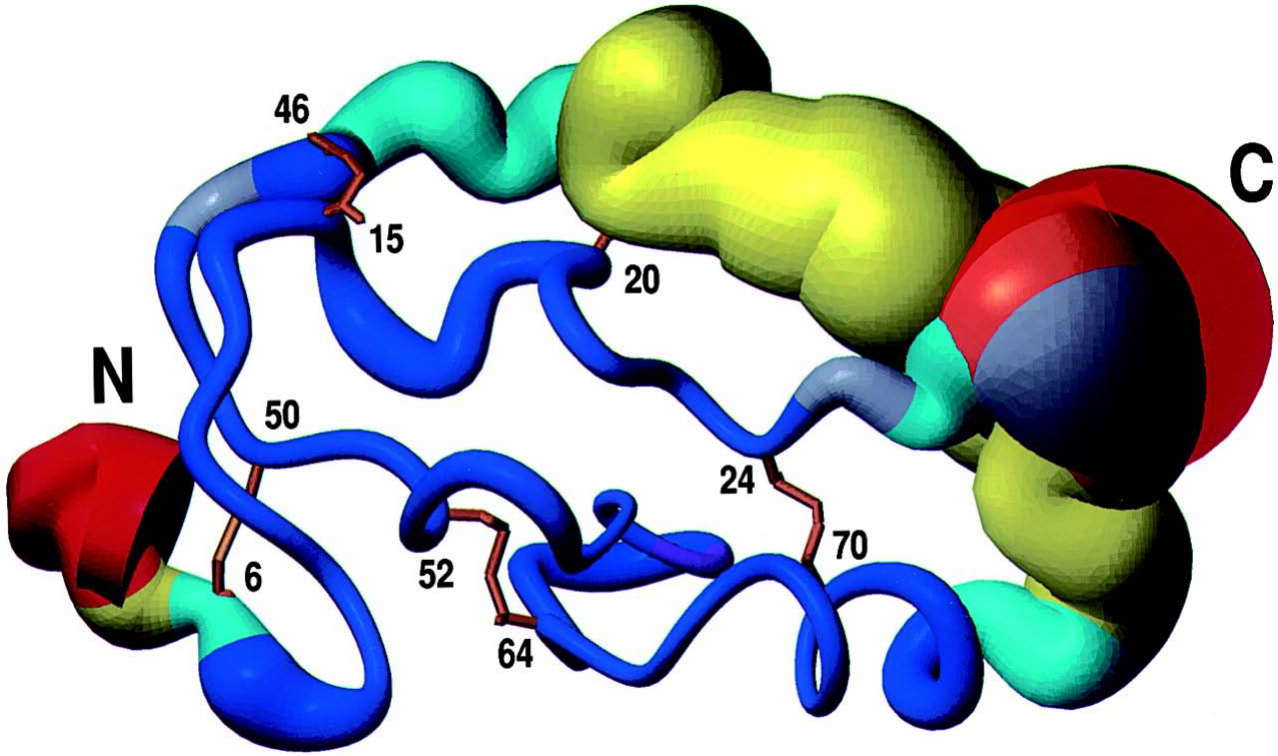
Nekateri proteini so ‘namerno’ nestabilni (vsaj lokalno); to omogoča:

• konformacijske spremembe

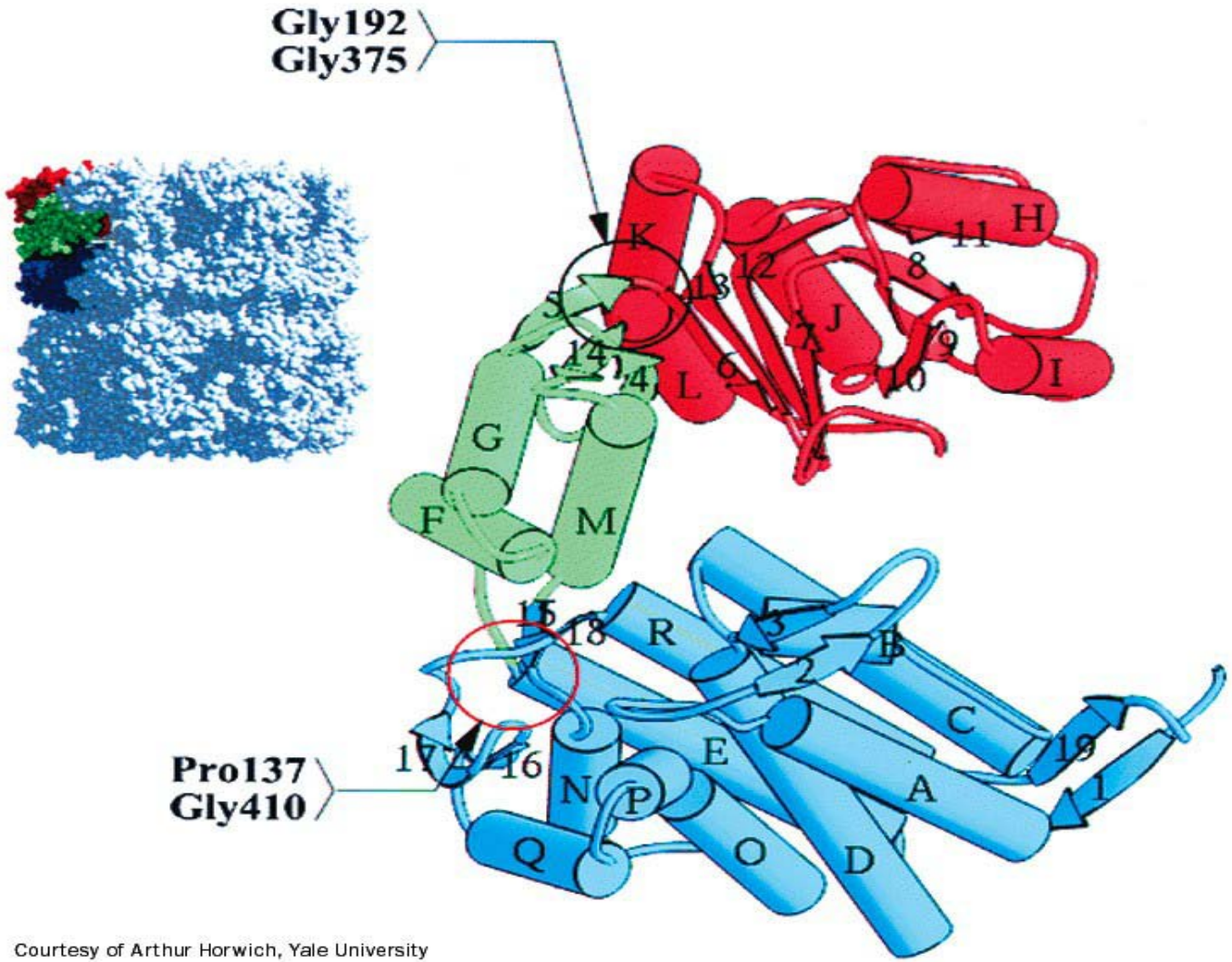
• prilagoditve pri vezavi na druge molekule (interakcije protein - protein in protein -nukleinska kislina)



transkripcijski faktor (P. Wright, Scripps)

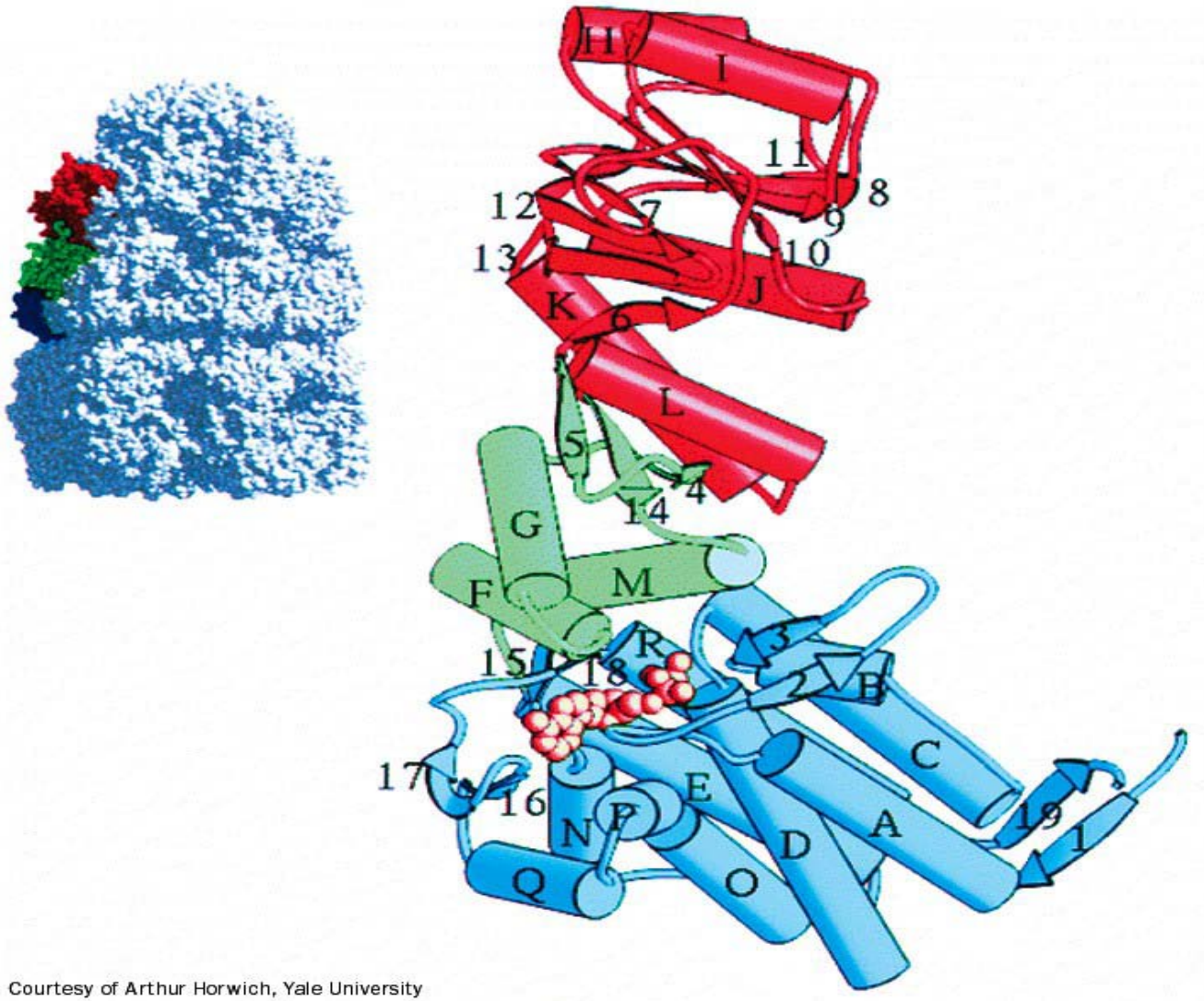


Premikanje domen v šaperonu GroEL (1).



Courtesy of Arthur Horwich, Yale University

Premikanje domen v šaperonu GroEL (2).



Courtesy of Arthur Horwich, Yale University

Metabolično ‘obračanje’ proteinov:

Proteini ‘živijo’ v celici različno dolgo.

N.pr.: razpolovni čas encimov v jetrih je od 0.2 do 150 ur.

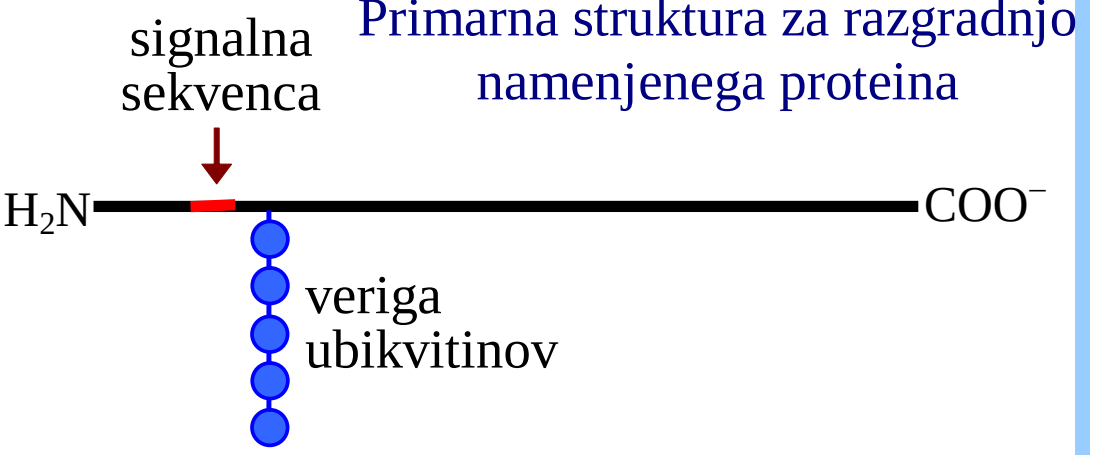
Pravilo N-konca: Razpolovni čas proteinov je povezan s strukturo N-terminalne AK:

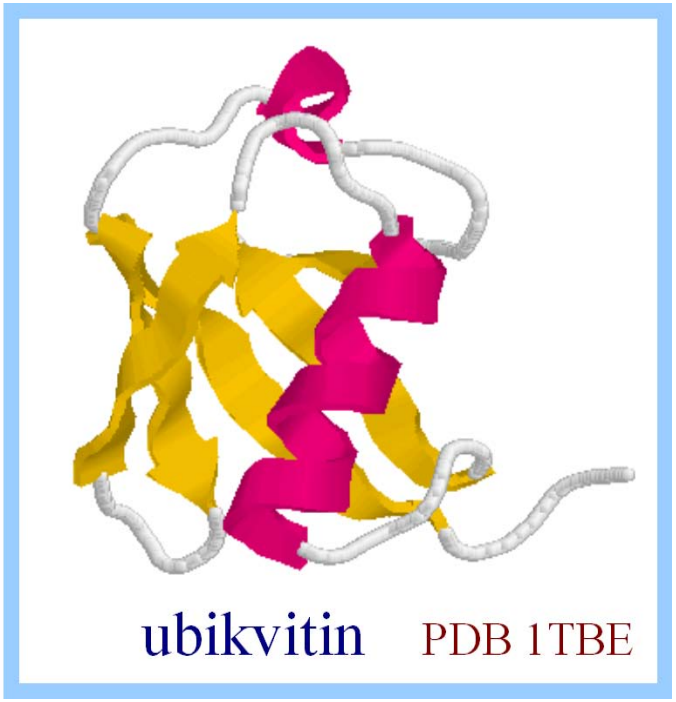
- Za proteine z N-terminalnimi Met, Ser, Ala, Thr, Val ali Gly je razpolovni čas večji od 20 ur.
- Za proteine z N-terminalnimi Phe, Leu, Asp, Lys ali Arg je razpolovni čas 3 min ali manj.

Pravilo PEST: proteini, ki imajo veliko Pro (P), Glu (E), Ser (S) ali Thr (T) se hitreje razgradijo kot drugi proteini.

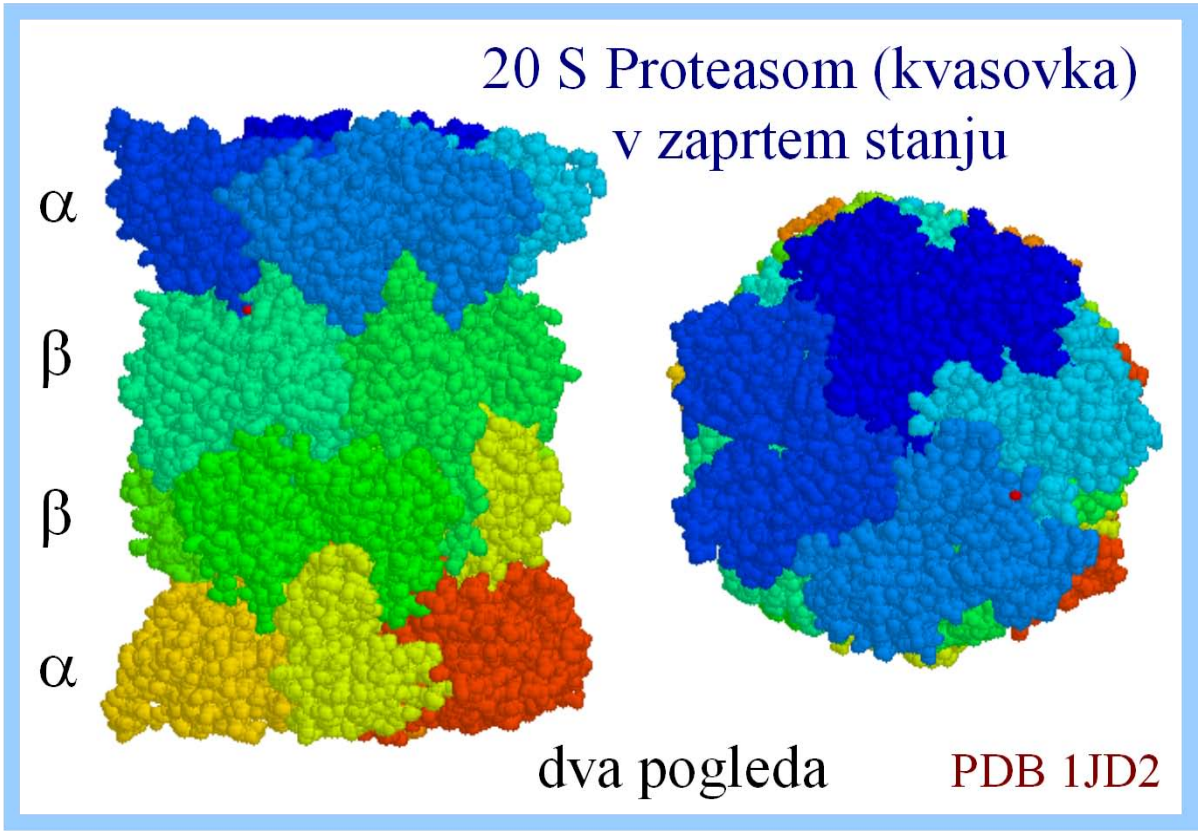
Selektivno razgradnjo proteinov uravnavajo znotrajcelični in zunajcelični signali.

Eden od njih je vezava **ubikvitina** (majhen protein) na za razgranjo namenjen (predvsem delno razvit) protein. Razgradnja ubikvitiniranih proteinov poteka v **proteasomih**.



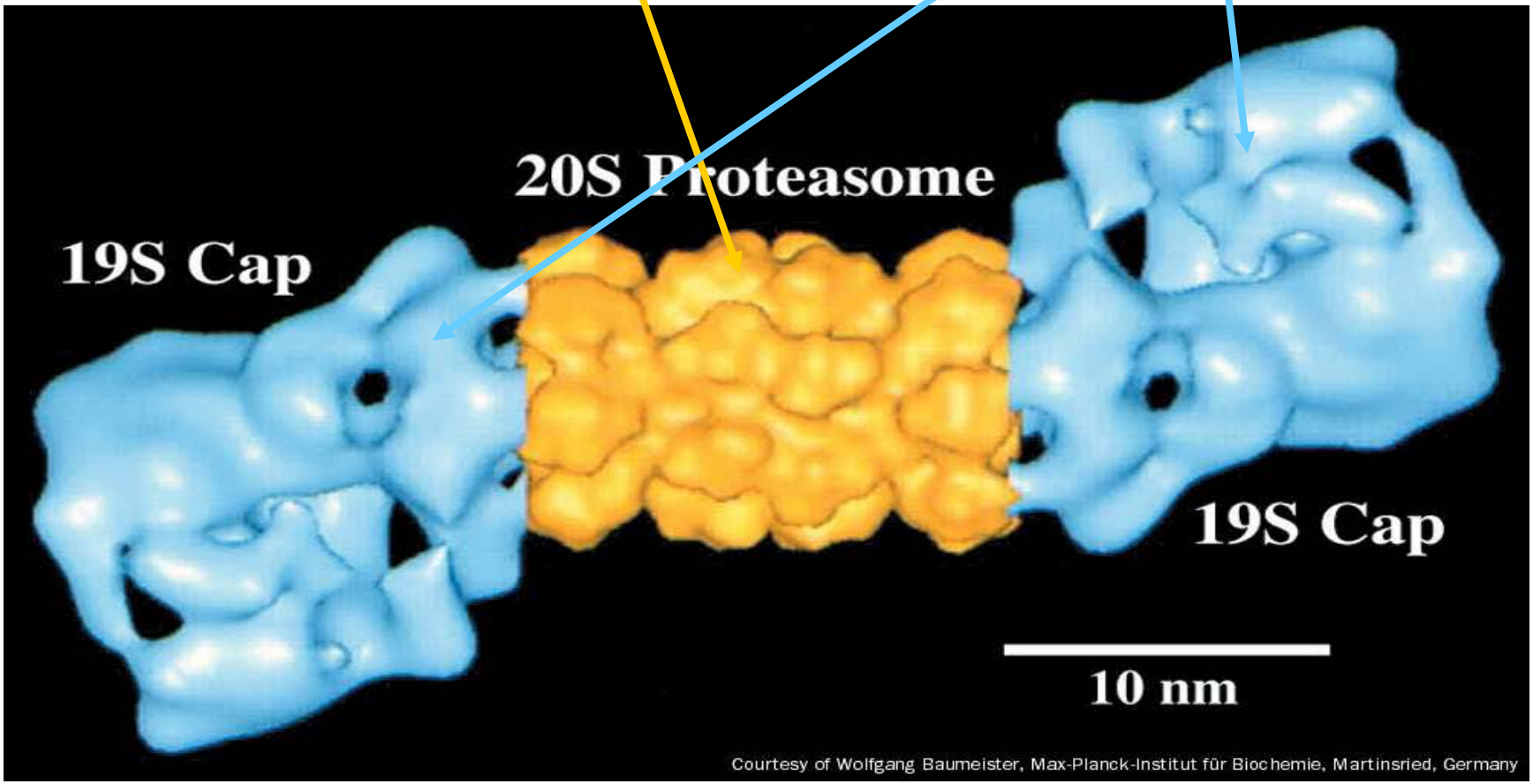


Veriga **4 ali več** ubikvitinov usmeri protein v razgradnjo.



Proteasome je velik kompleks. Jedro ima 4 obroče (2 α in 2 β), vsak je iz 7 proteinov, znotraj pa je votlina kjer se razgrajujejo ubikvitirani proteini. Po 3 podenote v β obročih so proteolitični encimi.

Elektronskomikroskopska slika 26S proteasoma *Drosophile melanogaster*. Poleg jedra (20S) sta prikazani dve ‘kapi’ (19S), ki usmerjata ubikvitinirane proteine v jedro, kjer se razgradijo.



Kontrola kvalitete proteinske sinteze

• Proteasomi prepoznajo in razgrajujejo poli-ubikvitinirane proteine, ki so celici lastni.

• Nekateri celici lastni proteini se le mono-ubikvitinirajo; ti verjetno doživijo razgradnjo v lizosomih.

• Lizosomi so organeli, ki vsebujejo proteolitične encime (katepsine); namenjeni so razgradnji predvsem izvenceličnih proteinov.

• Oba sistema sta potrebna za odstranjevanje ‘izrabljenih’ in napačno zvitih ali razvitih proteinov.

• Okrog 50 % vseh sintetiziranih proteinov se takoj razgradi!

Kako encimi delujejo?

nekatalizirana reakcija

S ↔ P

substrat produkt

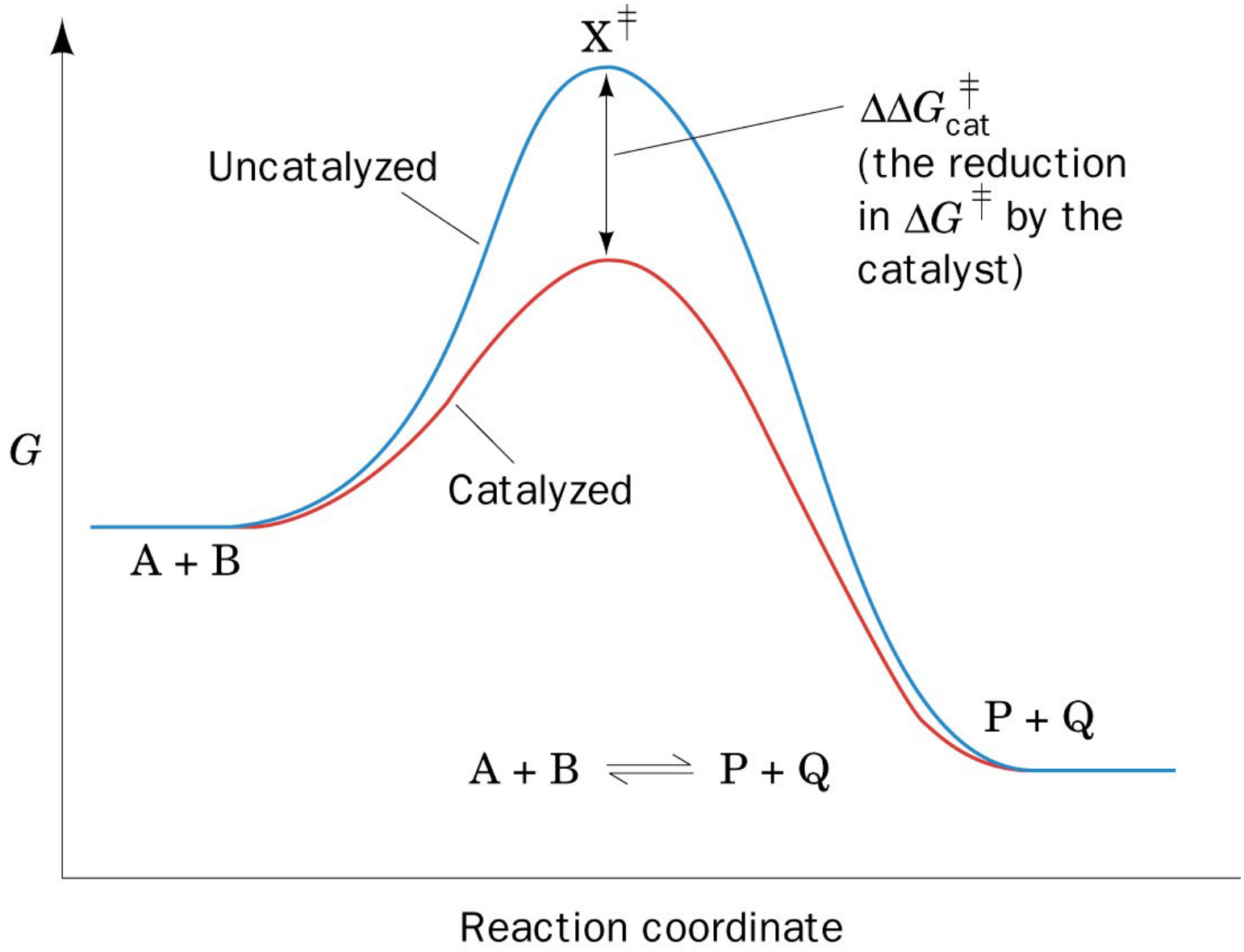
encimsko katalizirana reakcija

E + S ↔ ES ↔ EP ↔ E + P

E – encim
S – substrat, **P –** produkt
ES – kompleks encim-substrat
EP – kompleks encim-produkt

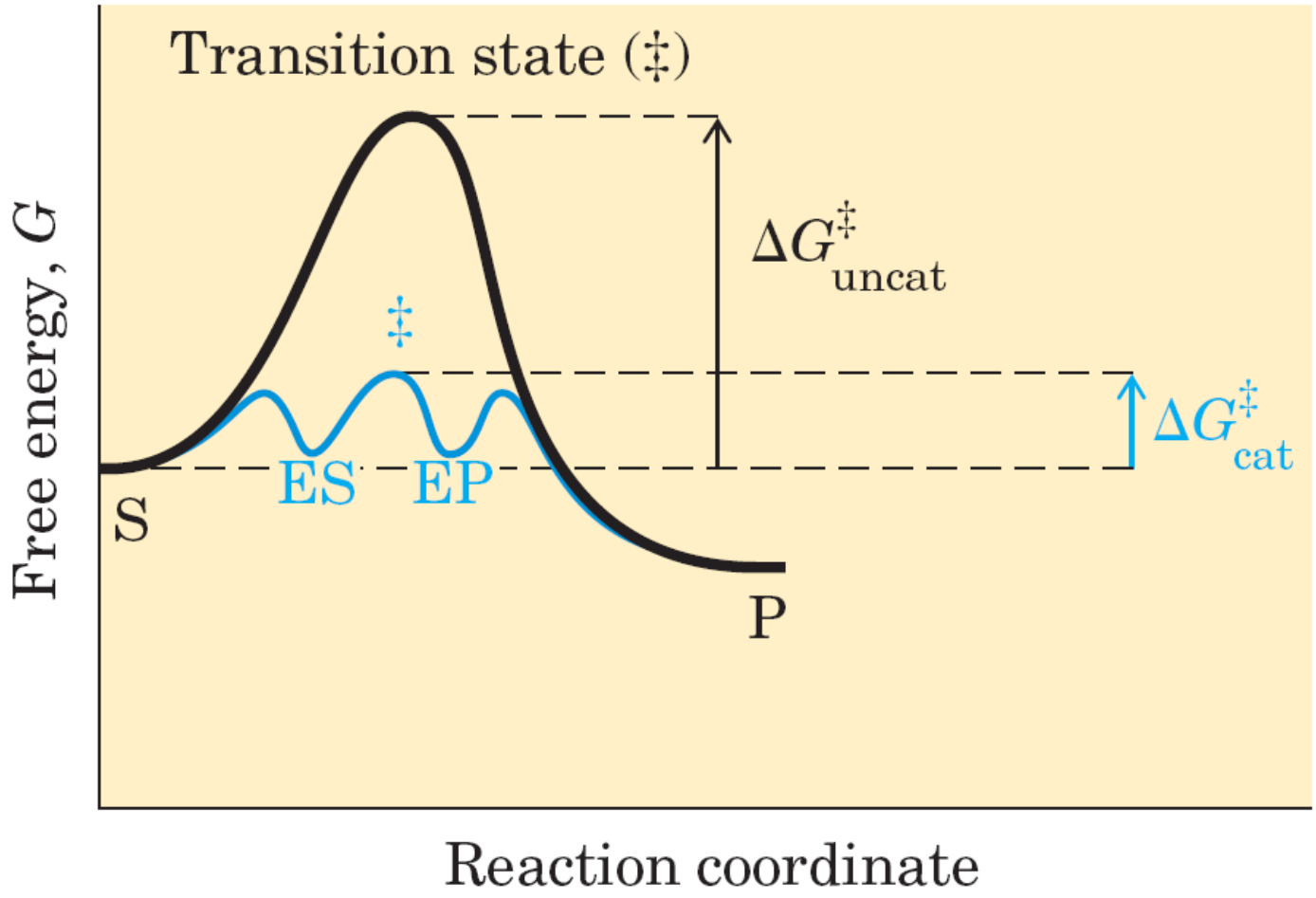
} POZOR, nista aktivirana kompleksa!

Encimi so bio-katalizatorji, ki zmanjšajo aktivacijsko energijo reakcije



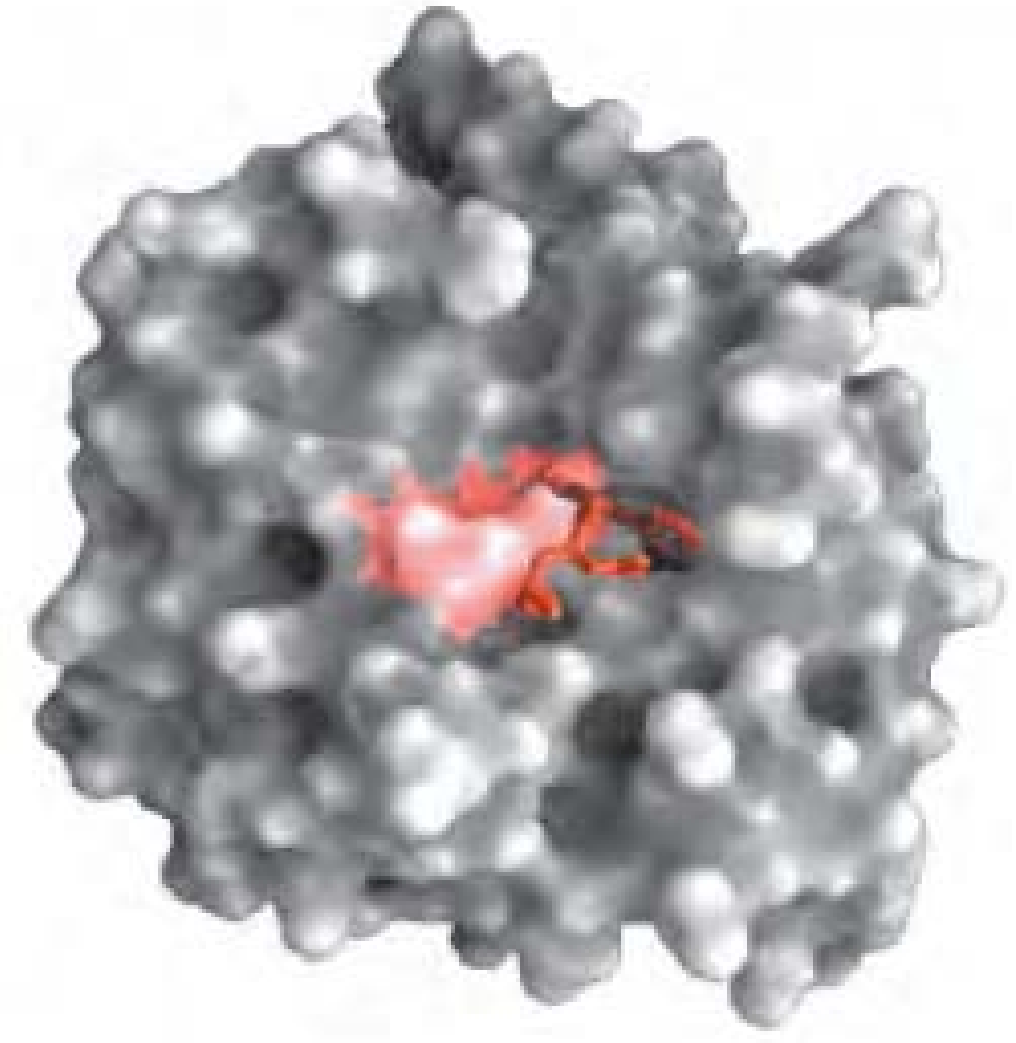
$S \leftrightarrow P$

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



$\Delta G^{o} = \Delta G^{o} = - RT \ln K$ $\Delta G^{\#} < \Delta G^{\#}$ $v > v$

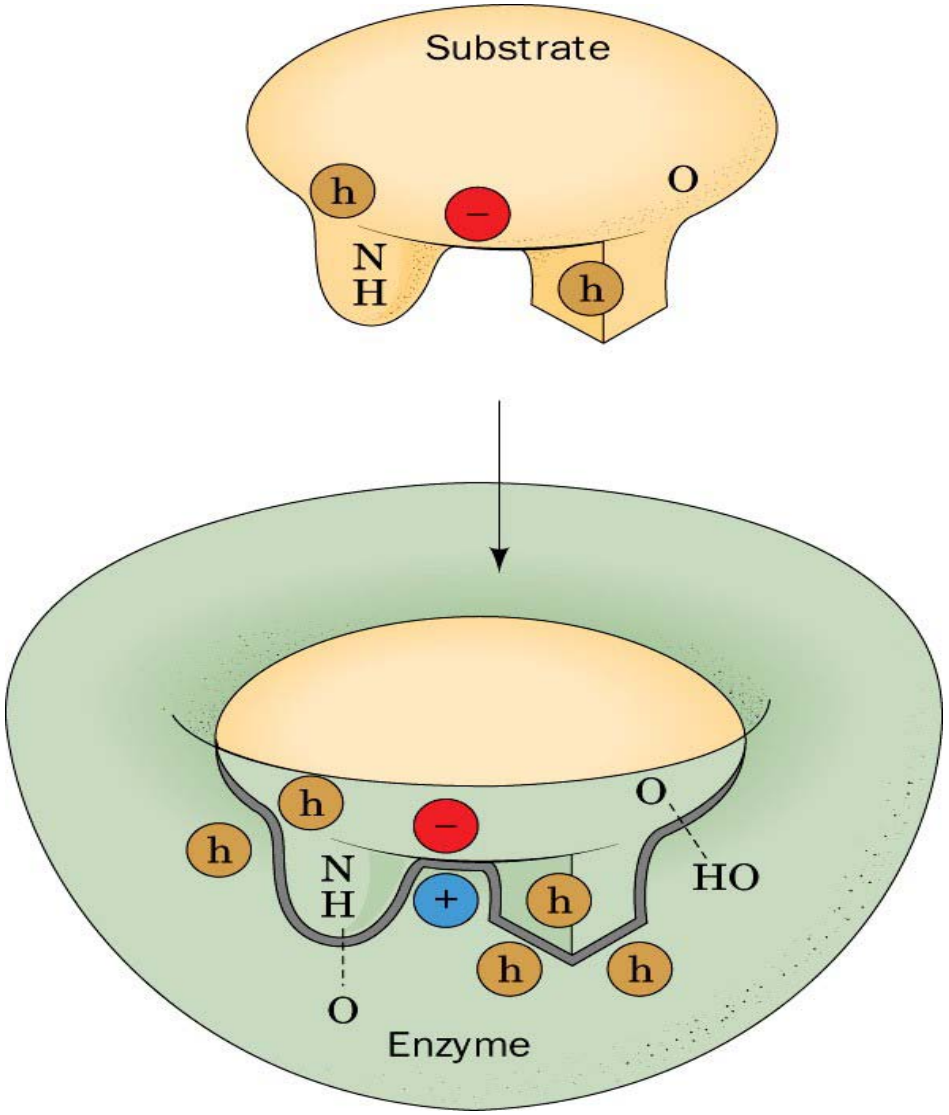
Substrat se veže na aktivno mesto, ki je navadno le manjši del encimske molekule



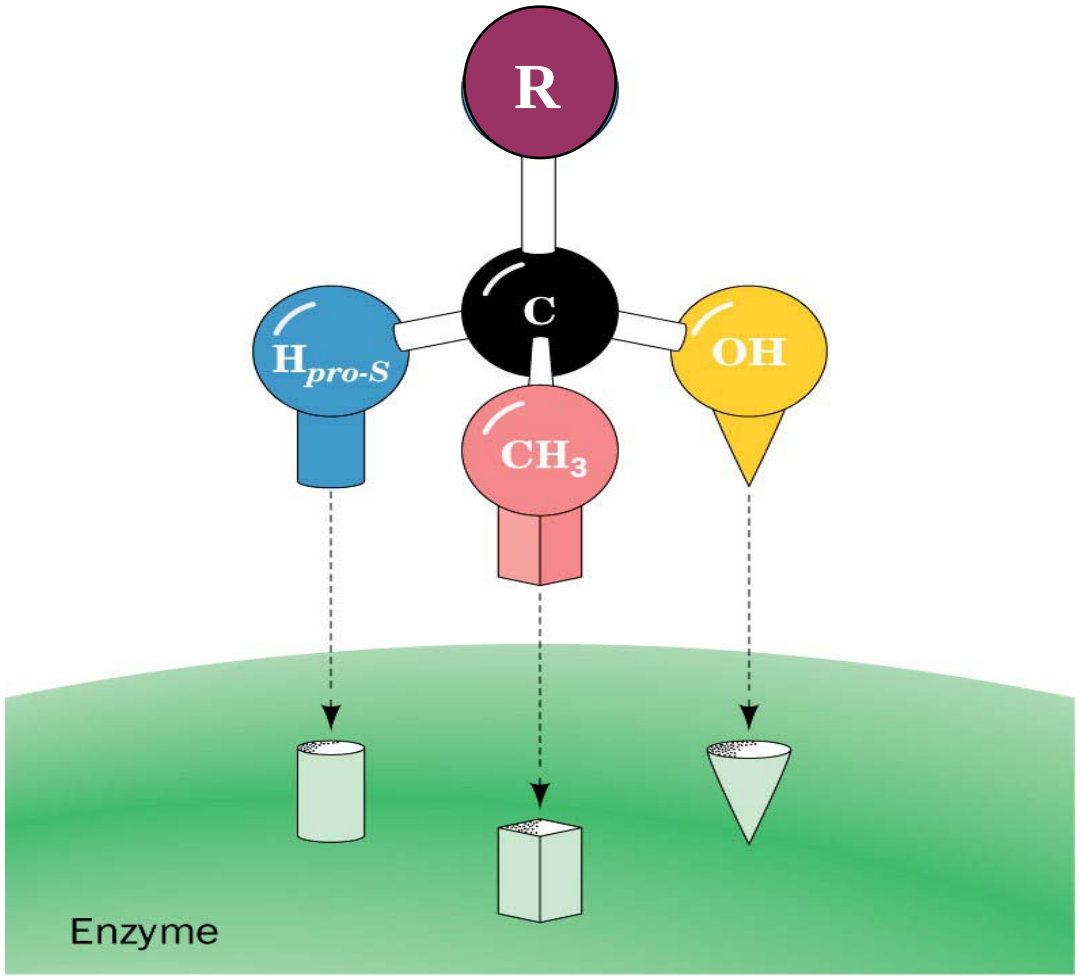
Encim predstavlja specifično okolje za molekulo substrata.

Koncept aktivnega mesta

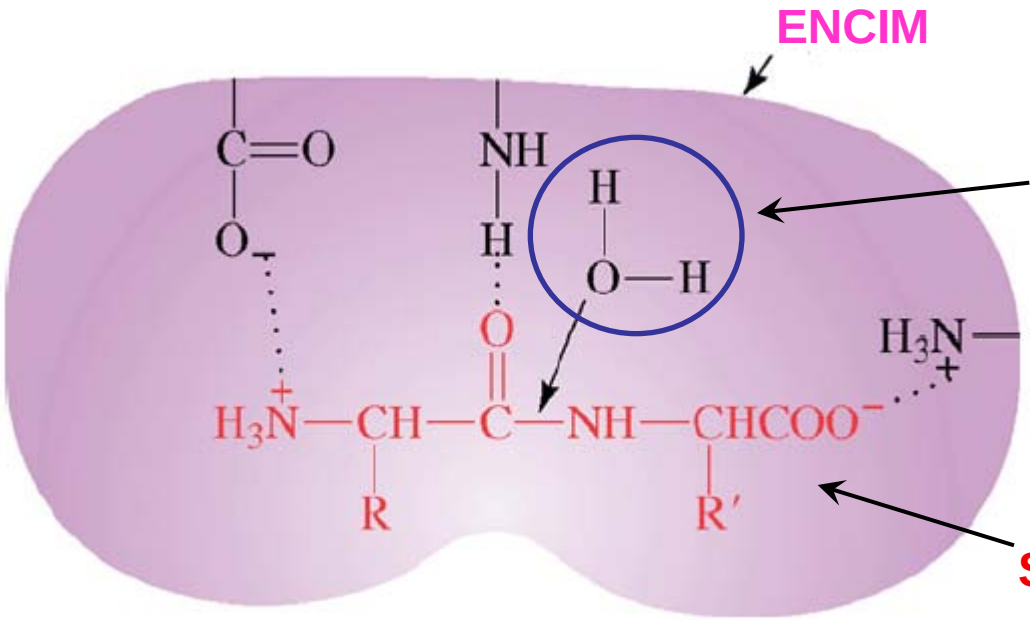
Nastanek kompleksa encim–substrat omogoča geometrijska in fizikalno-kemijska komplementarnost encima in substrata. Skupine na substratu in v aktivnem mestu encima si ustrezajo tako, da se lahko medsebojno povežejo.



Za razlikovanje enantiomerov mora imeti encim vsaj tri specifična mesta za vezavo substrata.

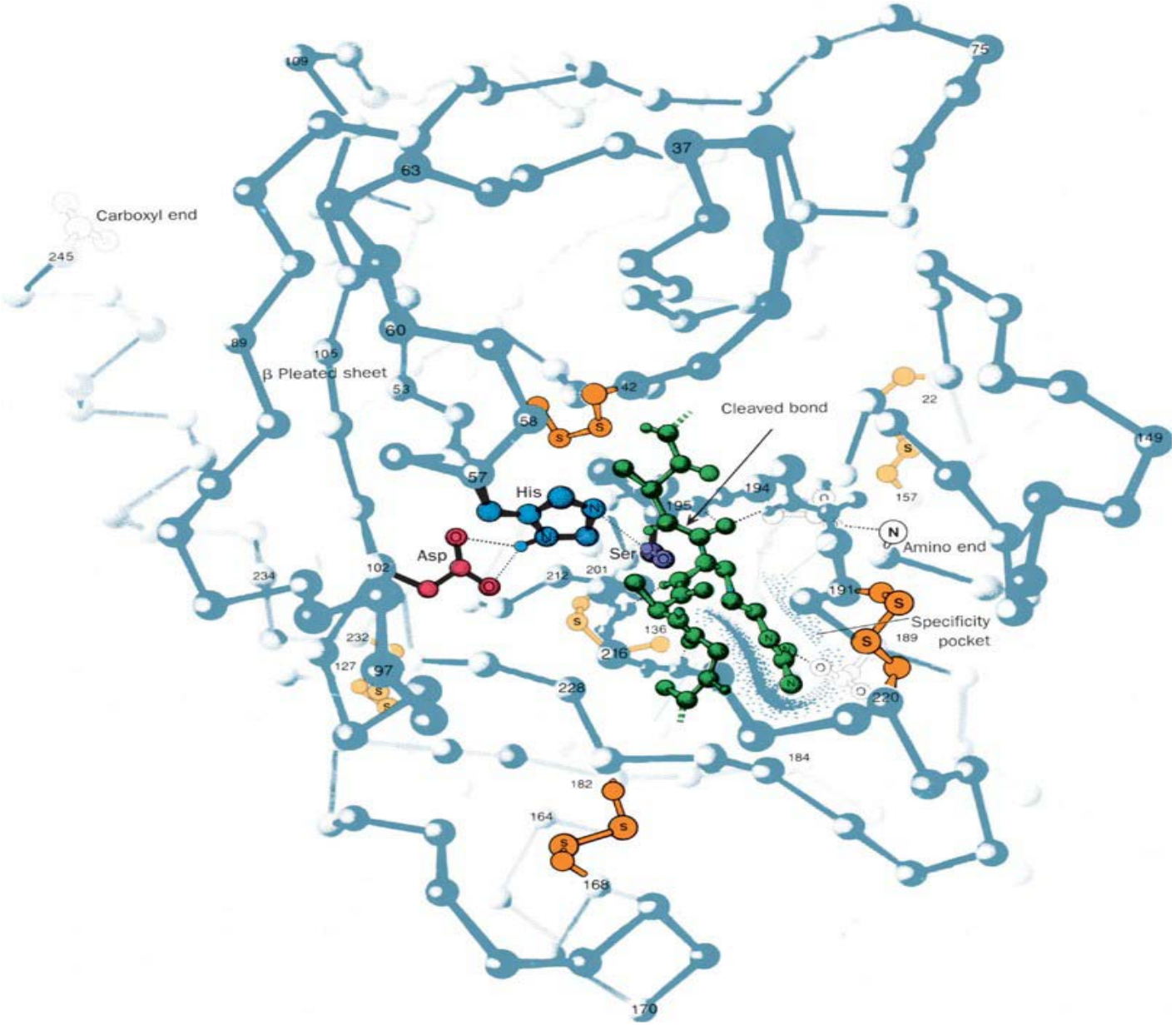


Peptidaza veže substrat (dipeptid) s tremi šibkimi vezmi, vsili substratu novo konformacijo, kar destabilizira peptidno vez (prehodno stanje!)



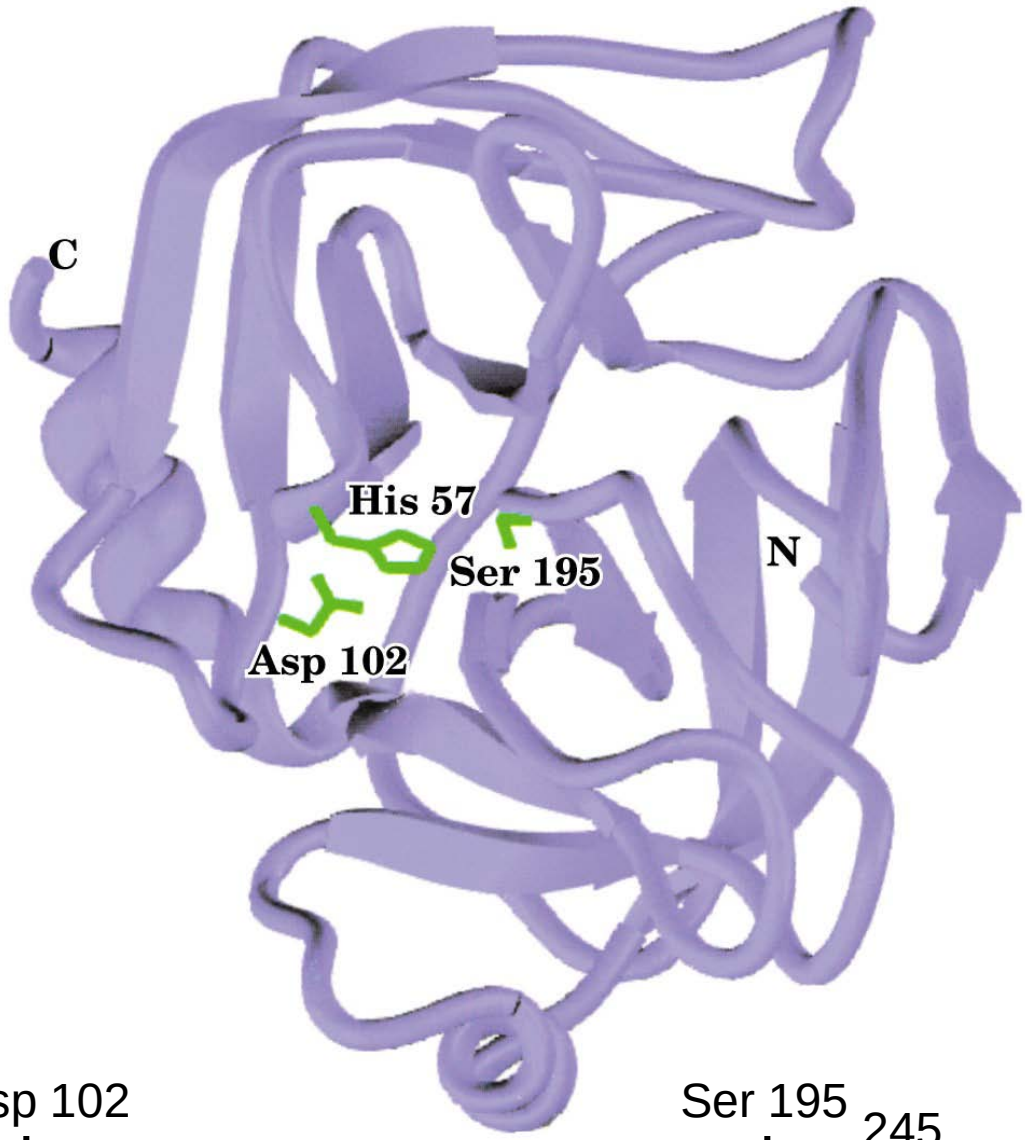
S_1 + S_2 ⟶ P_1 + P_2

Struktura tripsina s substratom



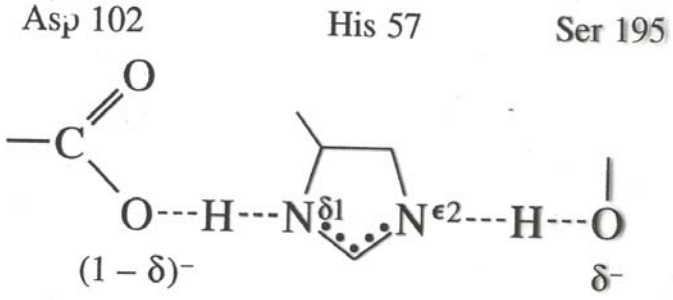
Struktura tripsina

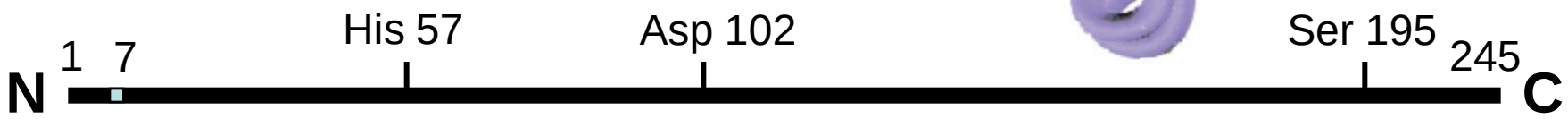
V aktivnem mestu pridejo zaradi III. strukture skupaj AK, ki so navadno v I.strukturi med seboj precej oddaljene.



Katalitična triada

Namen: ‘aktivacija’ Ser (OH)

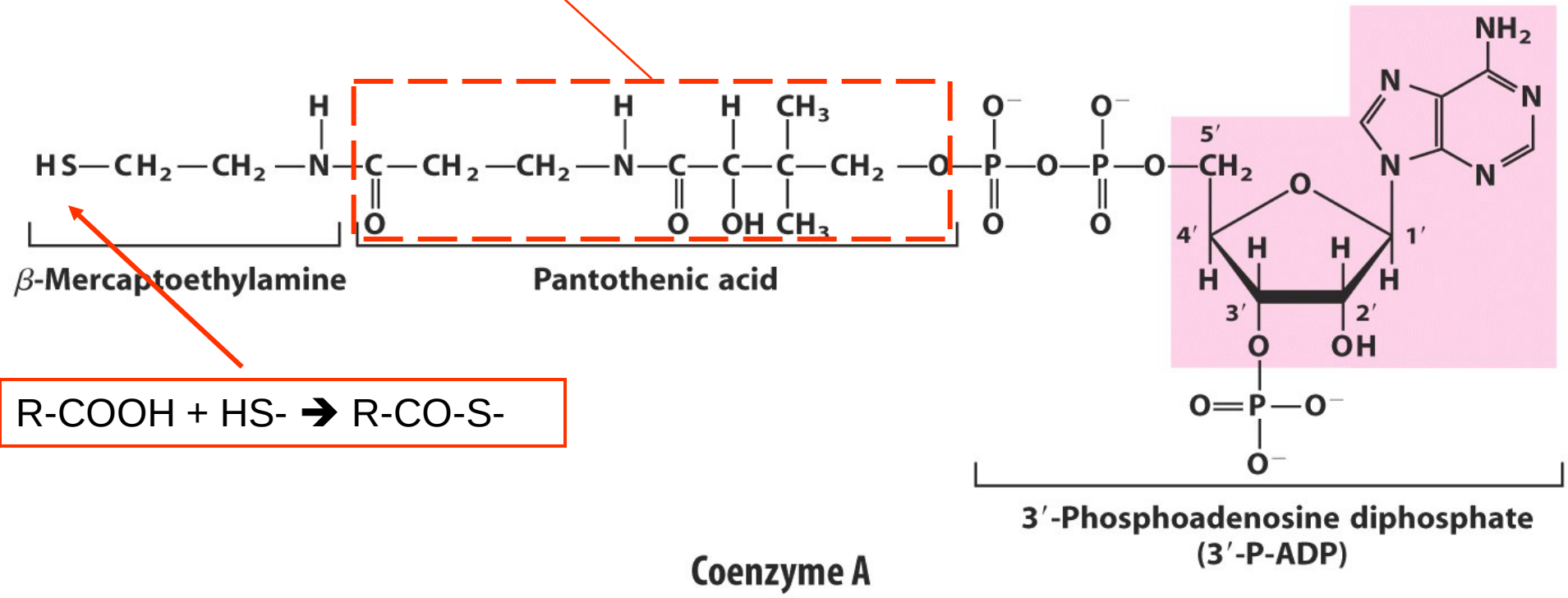




KOENCIMI IN PROSTETIČNE SKUPINE (nastanejo iz vitaminov), ZAGOTOVIJO DODATNE FUNKCIONALNE SKUPINE

PREKURZOR (VITAMIN)	SKUPINA PRENOSA	KOENCIM
tiamin (vitamin B1)	aldehidna skupina	tiamin pirofosfat
riboflavin (vitamin B2)	elektroni in protoni	FMN, FAD
nikotinska kislina (niacin)	hidridni ion (H^-)	NAD
pantotenska kislina in druge molekule	acilne skupine	koencim A
piridoksin (vitamin B6)	amino skupine	piridoksal fosfat
vitamin B12	H-atomi in alkilne skupine	(ciano)kobalamin
biotin	CO_2 (-COOH)	biocitin
folna kislina	skupine z enim C-atomom	tetrahidrofolna kislina
lipojska kislina	elektroni in acilne skupine	lipojska kislina

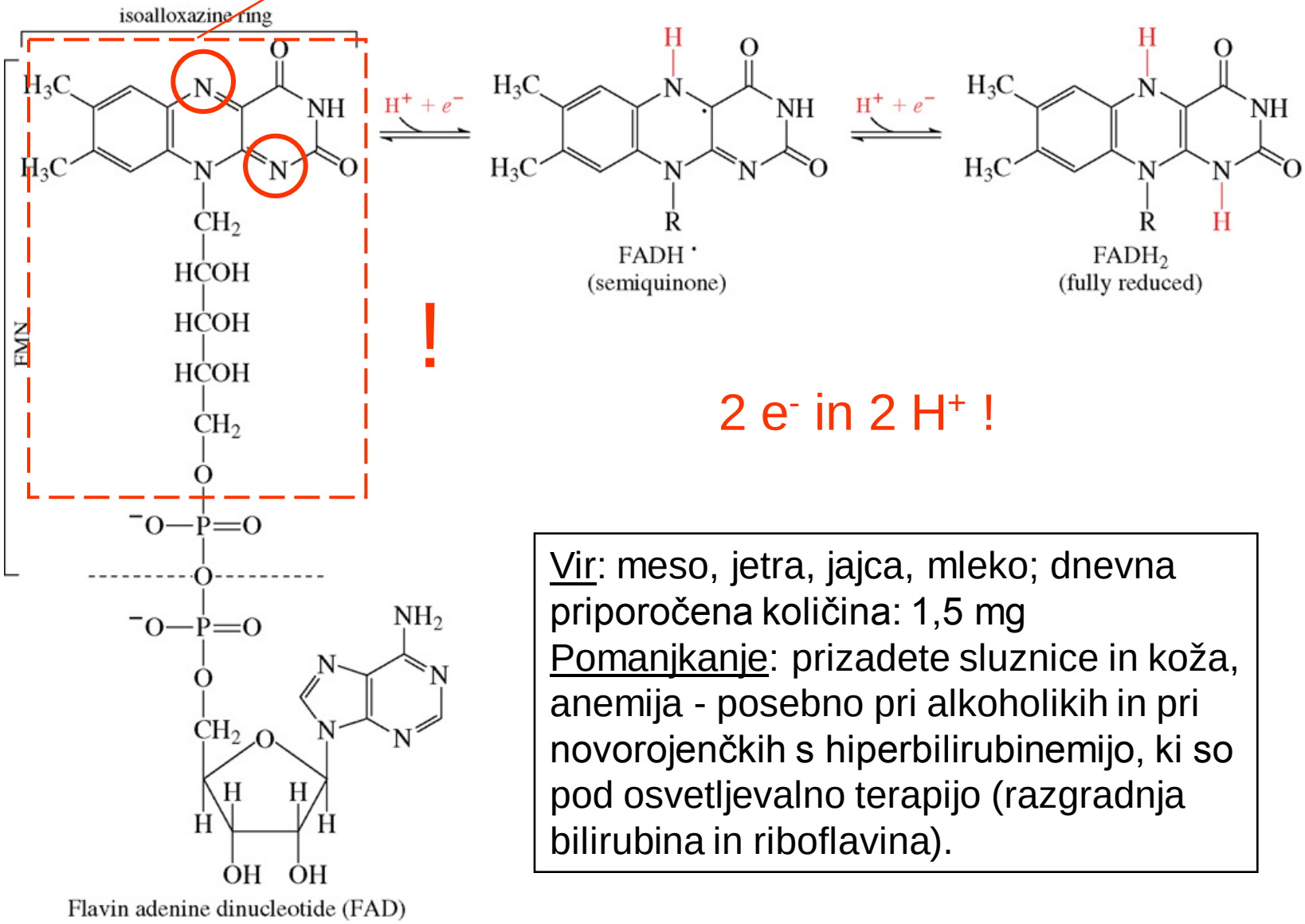
PREKURZOR (VITAMIN)	SKUPINA PRENOSA	KOENCIM
pantotenska kislina in druge molekule	acilne skupine	koencim A



R-COOH + HS- ➔ R-CO-S-

Vir: vsaka hrana; dnevna priporočena količina: 4 - 7 mg.
Pomanjkanja niso nikoli opazili, s težavo se ga da umetno izzvati v strogo nadzorovanih eksperimentalnih razmerah.

PREKURZOR (VITAMIN)	SKUPINA PRENOSA	KOENCIM
riboflavin (vitamin B2)	elektroni in protoni	FMN, FAD

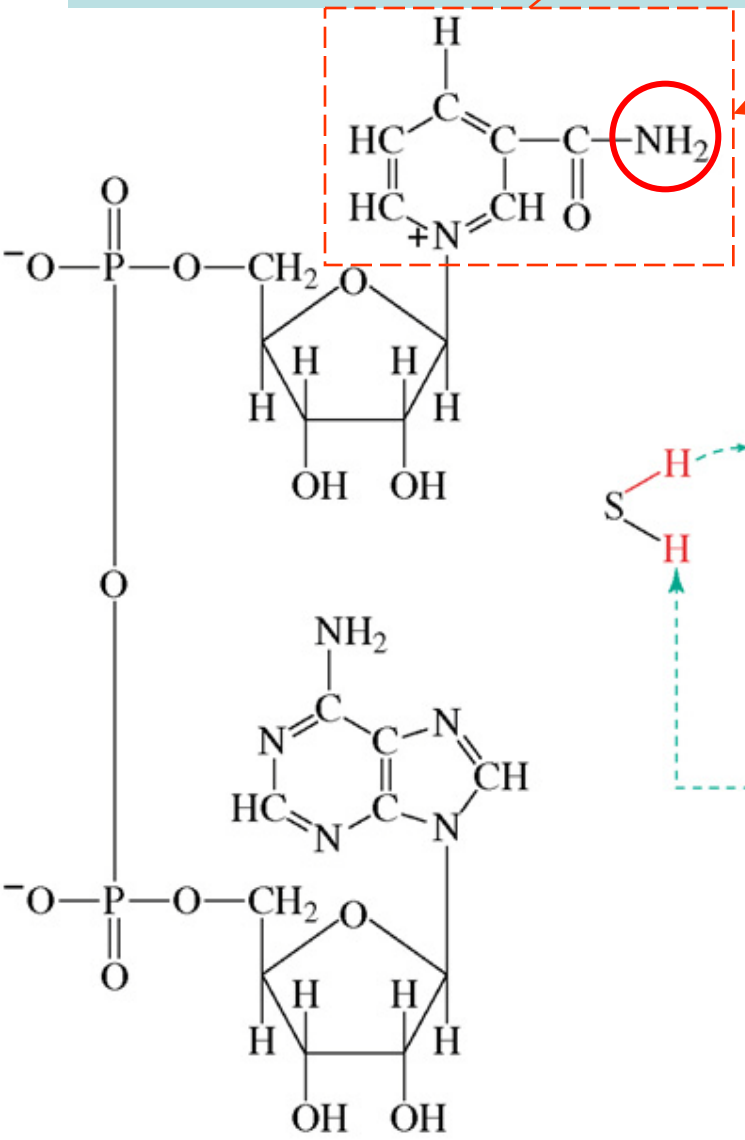


!

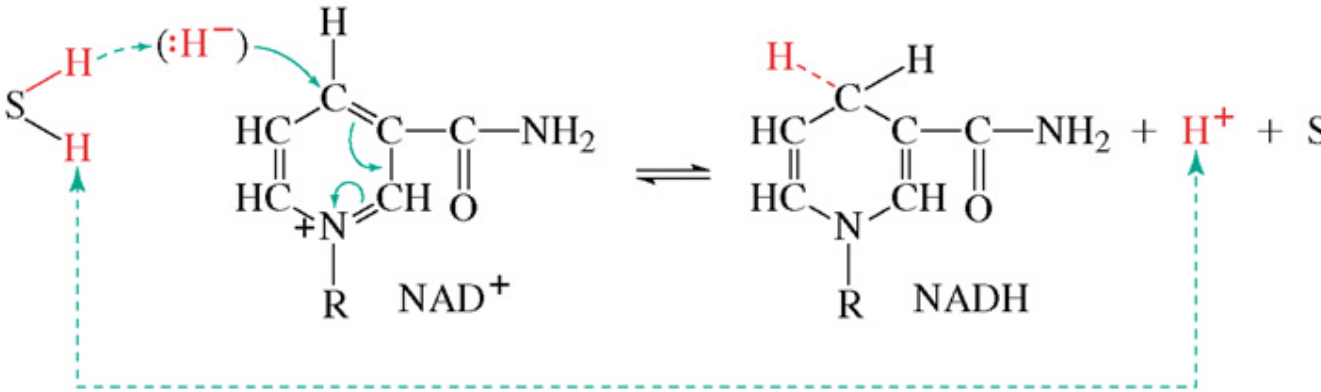
2 e^- in 2 H^+ !

Vir: meso, jetra, jajca, mleko; dnevna priporočena količina: 1,5 mg
Pomanjkanje: prizadete sluznice in koža, anemija - posebno pri alkoholikih in pri novorojenčkih s hiperbilirubinemijo, ki so pod osvetljevalno terapijo (razgradnja bilirubina in riboflavina).

PREKURZOR (VITAMIN)	SKUPINA PRENOSA	KOENCIM
nikotinska kislina (niacin)	hidridni ion (H^-)	NAD



2 e^- in 1 H^+ = H^- = hidridni ion!



Vir: meso, jetra, kvas, semena; dnevna priporočena količina: 15 - 20 mg (tudi neuč. sinteza iz Trp).
Pomanjkanje (koruza!): **pelagra** - prizadeta je koža, v resnejši obliki pa dermatitis, diareja in demenca (lahko tudi ireverzibilna). V razvitem svetu redka.