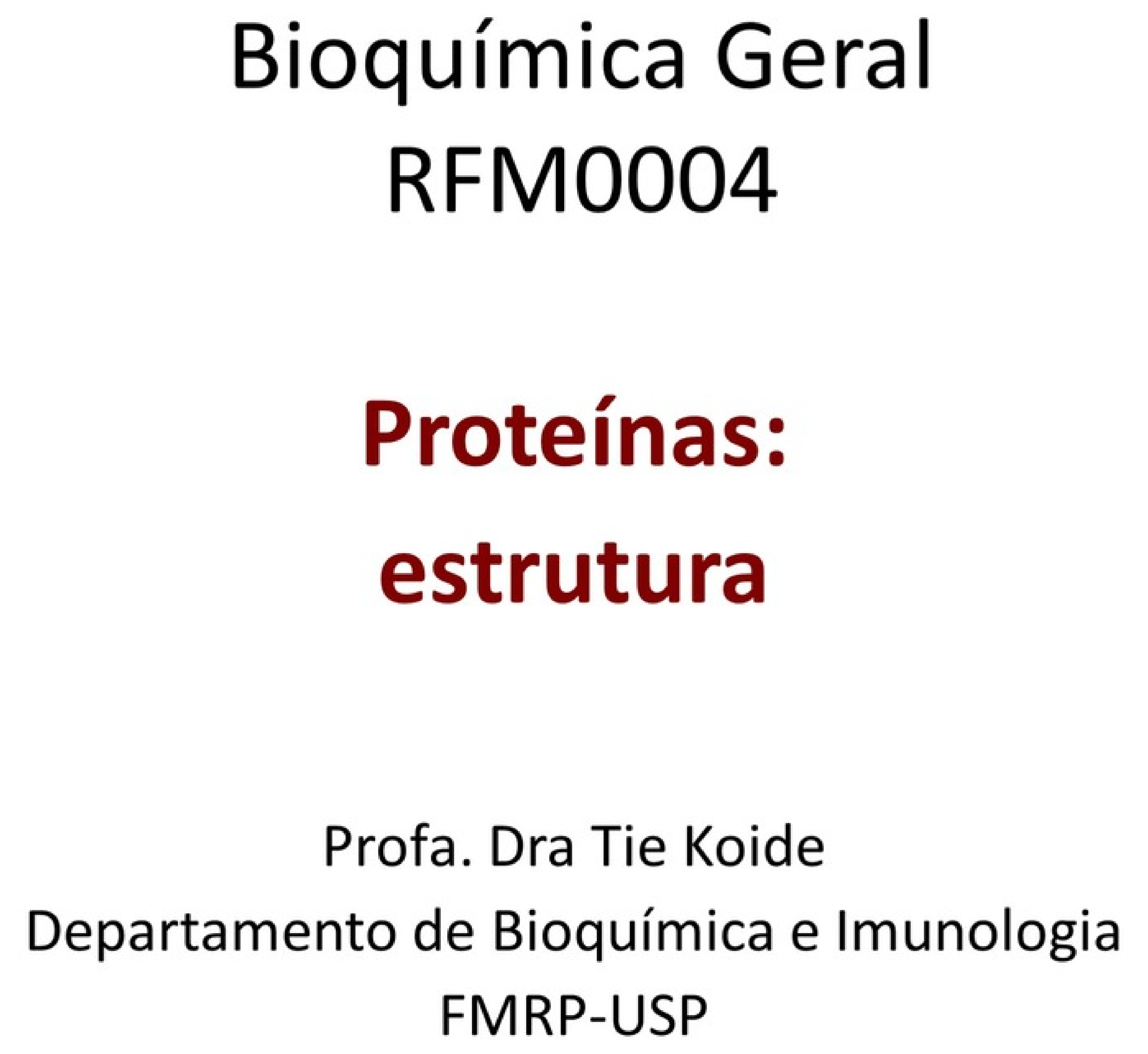
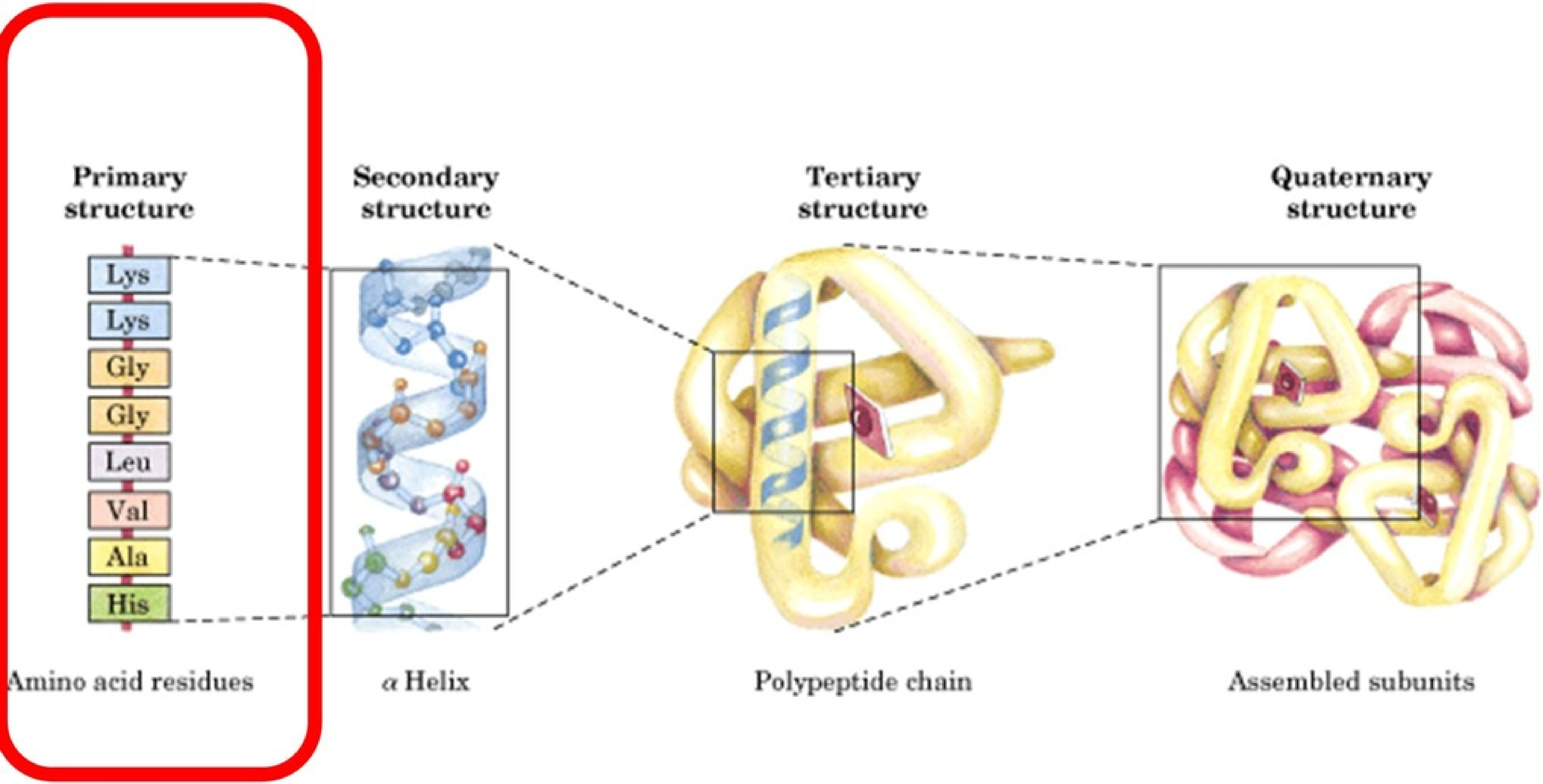


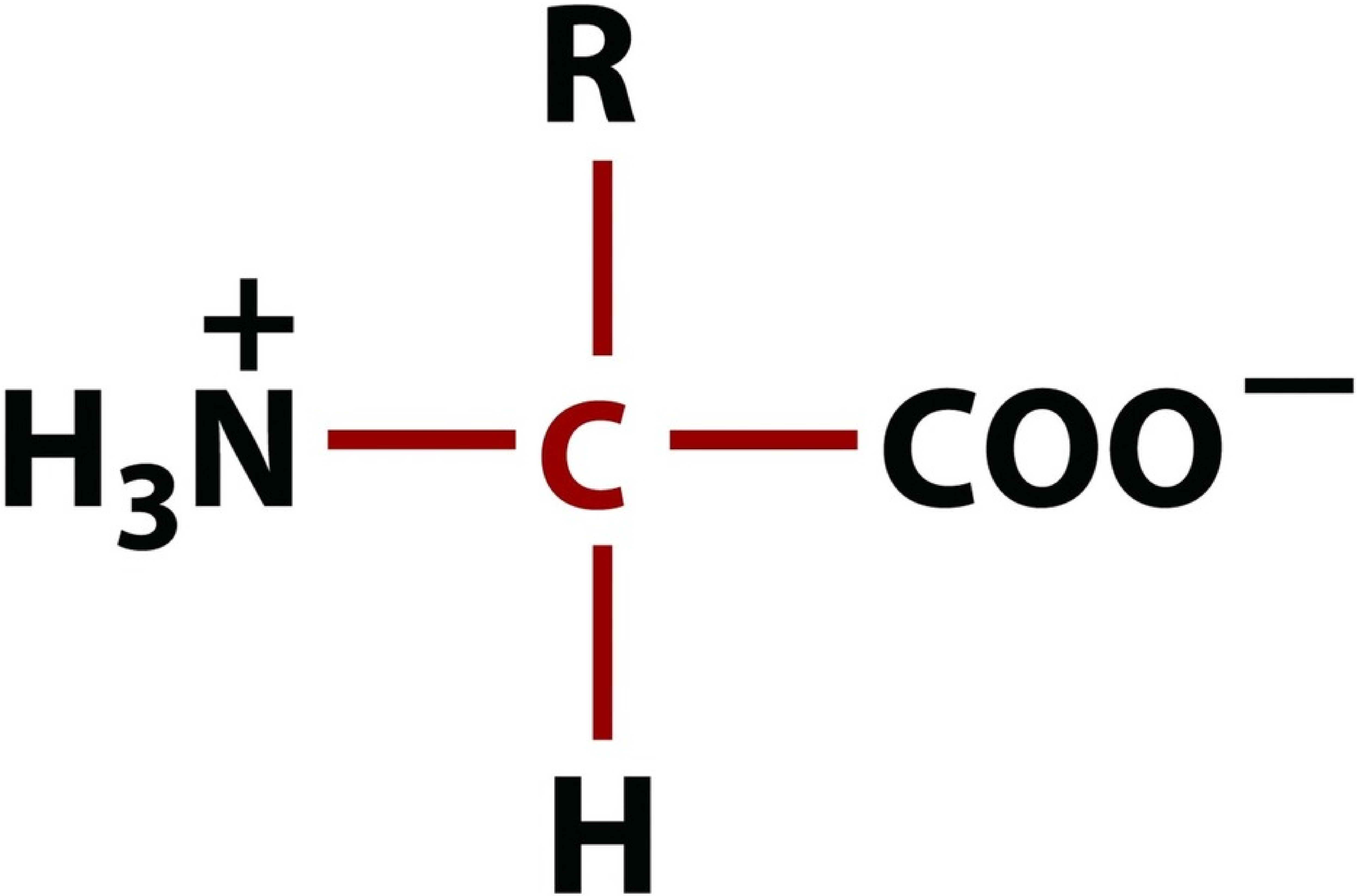
Bioquímica Geral
RFM0004

Proteínas: estrutura

Profa. Dra Tie Koide
Departamento de Bioquímica e Imunologia
FMRP-USP

Proteínas: polímeros de aminoácidos

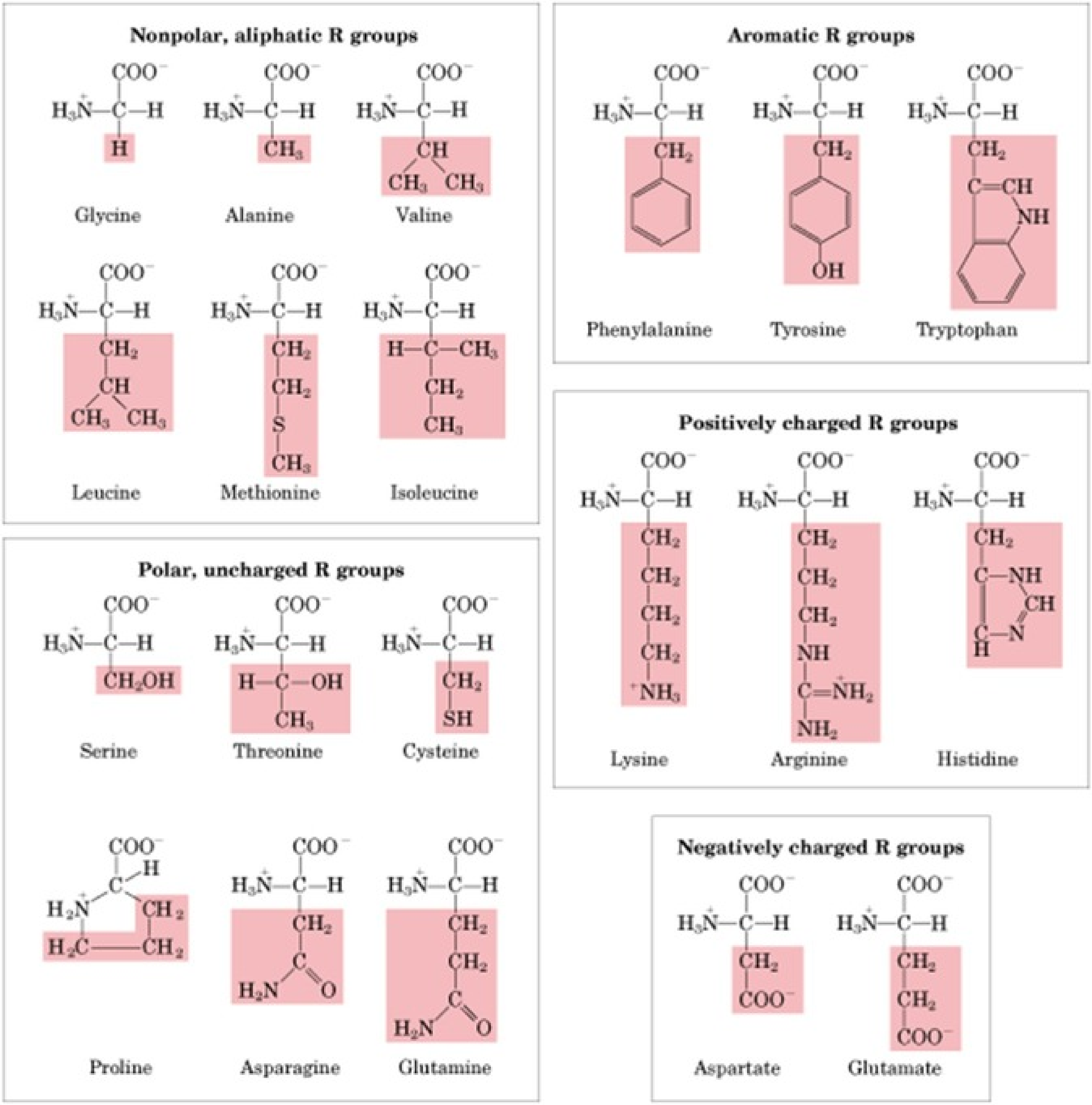


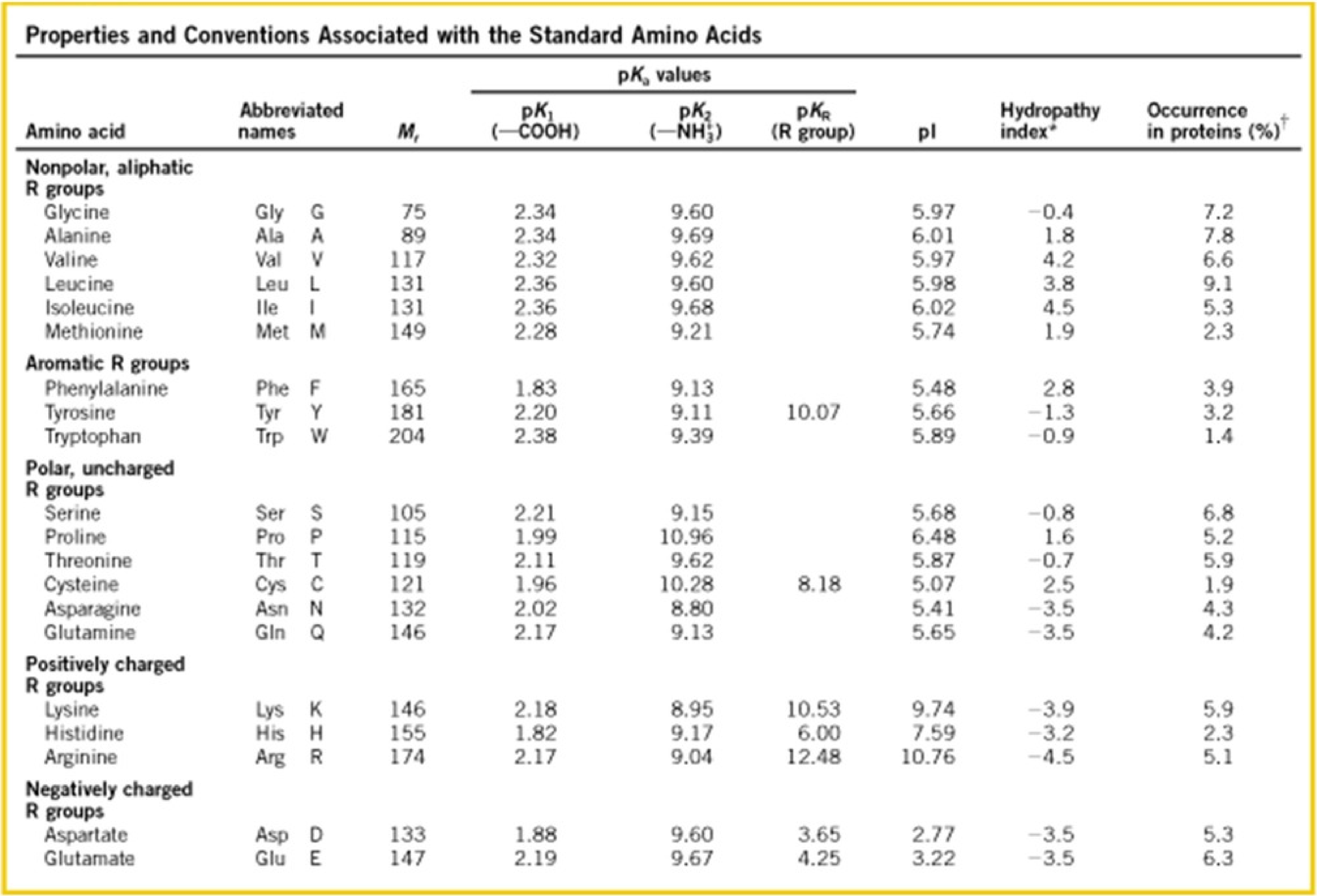


$$\begin{array}{ccccc} & & R & & \\ & & | & & \\ H_3\overset{+}{N} & - & C & - & COO^- \\ & & | & & \\ & & H & & \end{array}$$

Figure 4-2 Fundamentals of Biochemistry, 2/e

Os 20 aminoácidos

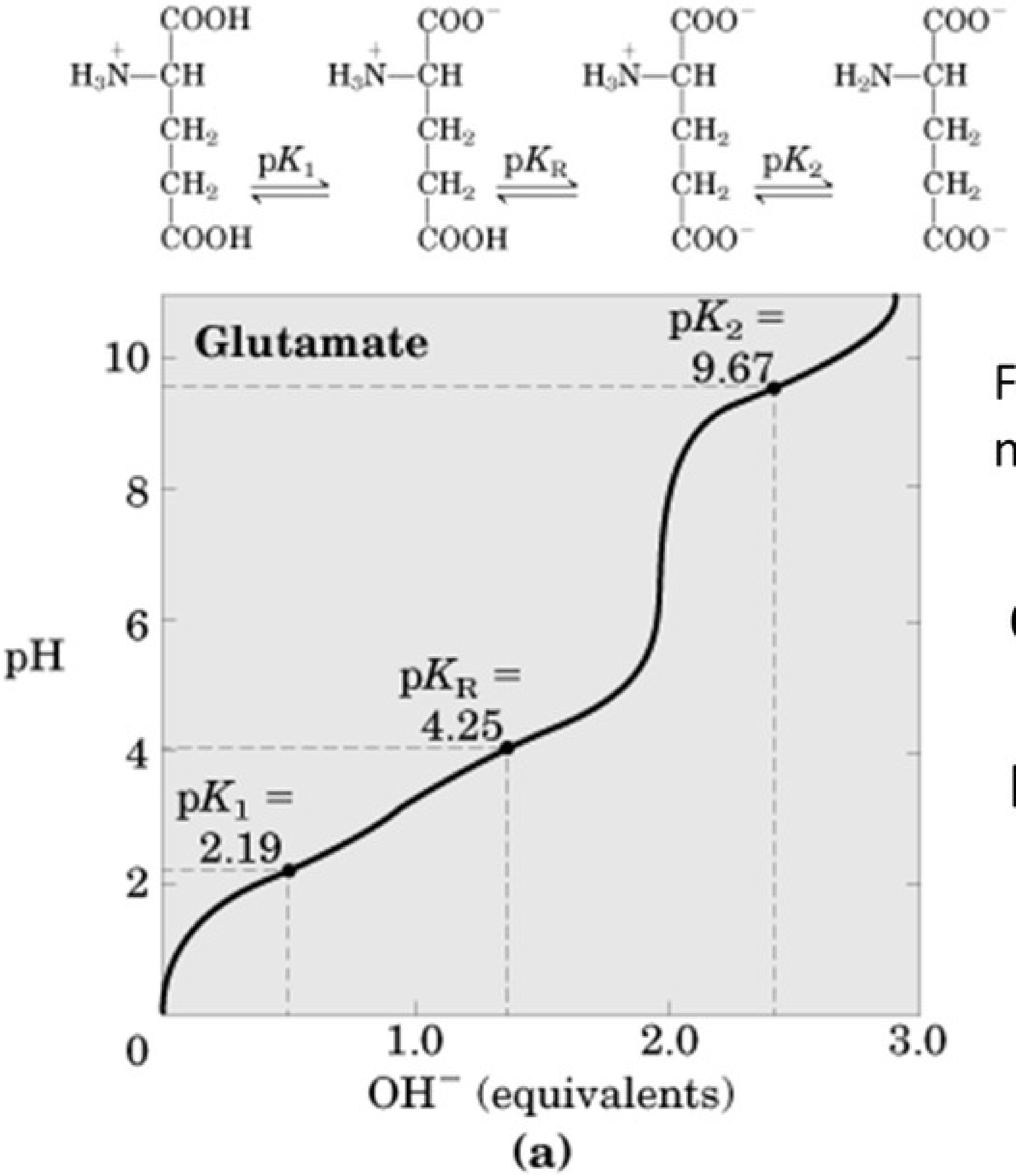




Properties and Conventions Associated with the Standard Amino Acids

				pK_a values					
Amino acid	Abbreviated names		M_r	pK_1 (—COOH)	pK_2 ($—NH_3^+$)	pK_R (R group)	pI	Hydropathy index*	Occurrence in proteins (%)†
Nonpolar, aliphatic R groups									
Glycine	Gly	G	75	2.34	9.60		5.97	−0.4	7.2
Alanine	Ala	A	89	2.34	9.69		6.01	1.8	7.8
Valine	Val	V	117	2.32	9.62		5.97	4.2	6.6
Leucine	Leu	L	131	2.36	9.60		5.98	3.8	9.1
Isoleucine	Ile	I	131	2.36	9.68		6.02	4.5	5.3
Methionine	Met	M	149	2.28	9.21		5.74	1.9	2.3
Aromatic R groups									
Phenylalanine	Phe	F	165	1.83	9.13		5.48	2.8	3.9
Tyrosine	Tyr	Y	181	2.20	9.11	10.07	5.66	−1.3	3.2
Tryptophan	Trp	W	204	2.38	9.39		5.89	−0.9	1.4
Polar, uncharged R groups									
Serine	Ser	S	105	2.21	9.15		5.68	−0.8	6.8
Proline	Pro	P	115	1.99	10.96		6.48	1.6	5.2
Threonine	Thr	T	119	2.11	9.62		5.87	−0.7	5.9
Cysteine	Cys	C	121	1.96	10.28	8.18	5.07	2.5	1.9
Asparagine	Asn	N	132	2.02	8.80		5.41	−3.5	4.3
Glutamine	Gln	Q	146	2.17	9.13		5.65	−3.5	4.2
Positively charged R groups									
Lysine	Lys	K	146	2.18	8.95	10.53	9.74	−3.9	5.9
Histidine	His	H	155	1.82	9.17	6.00	7.59	−3.2	2.3
Arginine	Arg	R	174	2.17	9.04	12.48	10.76	−4.5	5.1
Negatively charged R groups									
Aspartate	Asp	D	133	1.88	9.60	3.65	2.77	−3.5	5.3
Glutamate	Glu	E	147	2.19	9.67	4.25	3.22	−3.5	6.3

Titulação de aminoácidos

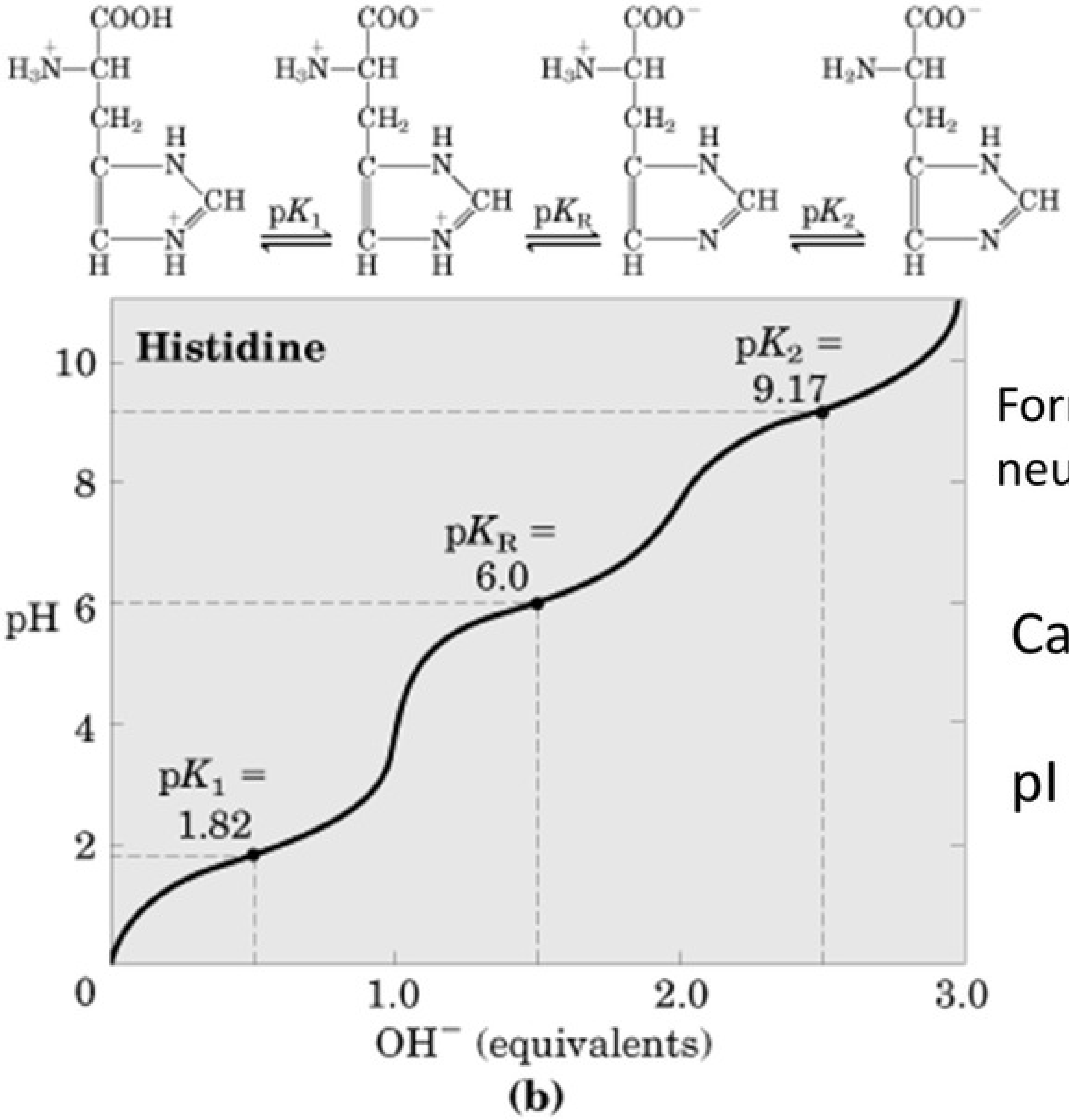


Forma eletricamente neutra?

Calcule o pI

pI = 3.22

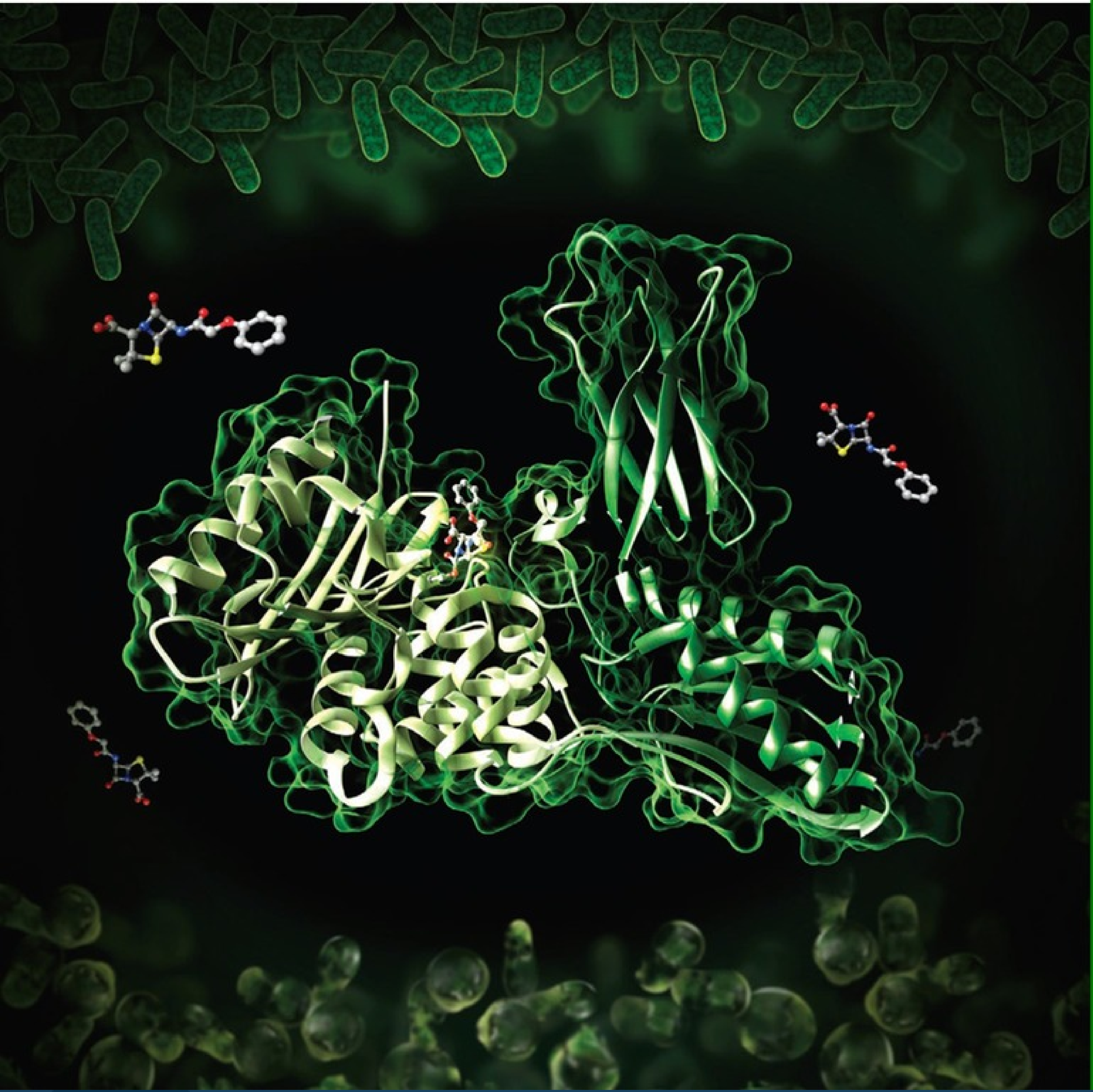
Titulação de aminoácidos



Forma eletricamente neutra?

Calcule o pI

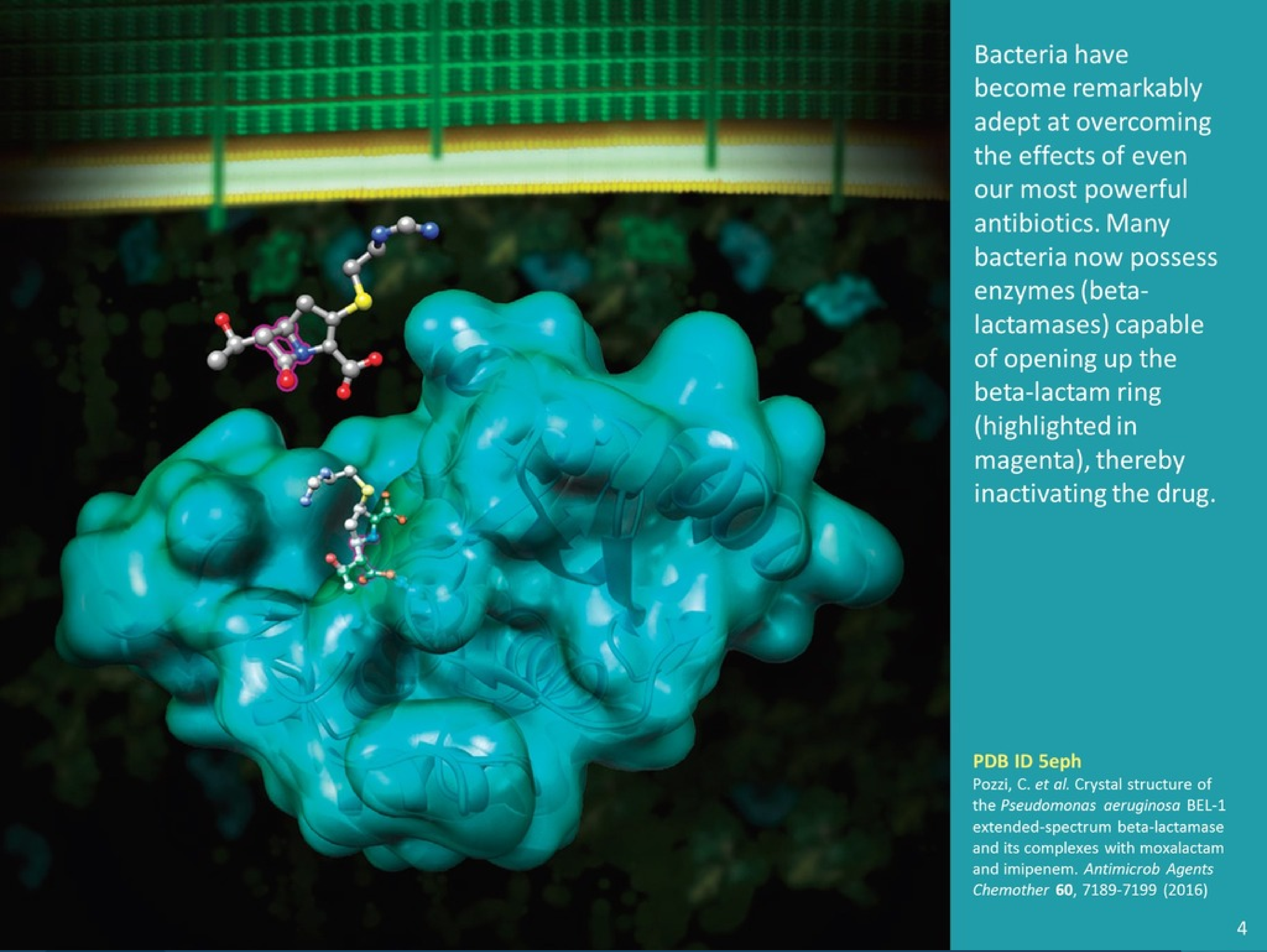
pI = 7.59



Bacteria build a tough network of peptidoglycan to support their cell walls. Penicillin V (a beta-lactam antibiotic shown in ball and stick representation) blocks one of the enzymes that builds this peptidoglycan sheath. Penicillin binds to the portion shown in light green.

PDB ID 2ex9

Kishida, H. *et al.* Crystal structure of penicillin binding protein 4 (dacB) from *Escherichia coli*, both in the native form and covalently linked to various antibiotics. *Biochemistry* **45**, 783-792 (2006)



Bacteria have become remarkably adept at overcoming the effects of even our most powerful antibiotics. Many bacteria now possess enzymes (beta-lactamases) capable of opening up the beta-lactam ring (highlighted in magenta), thereby inactivating the drug.

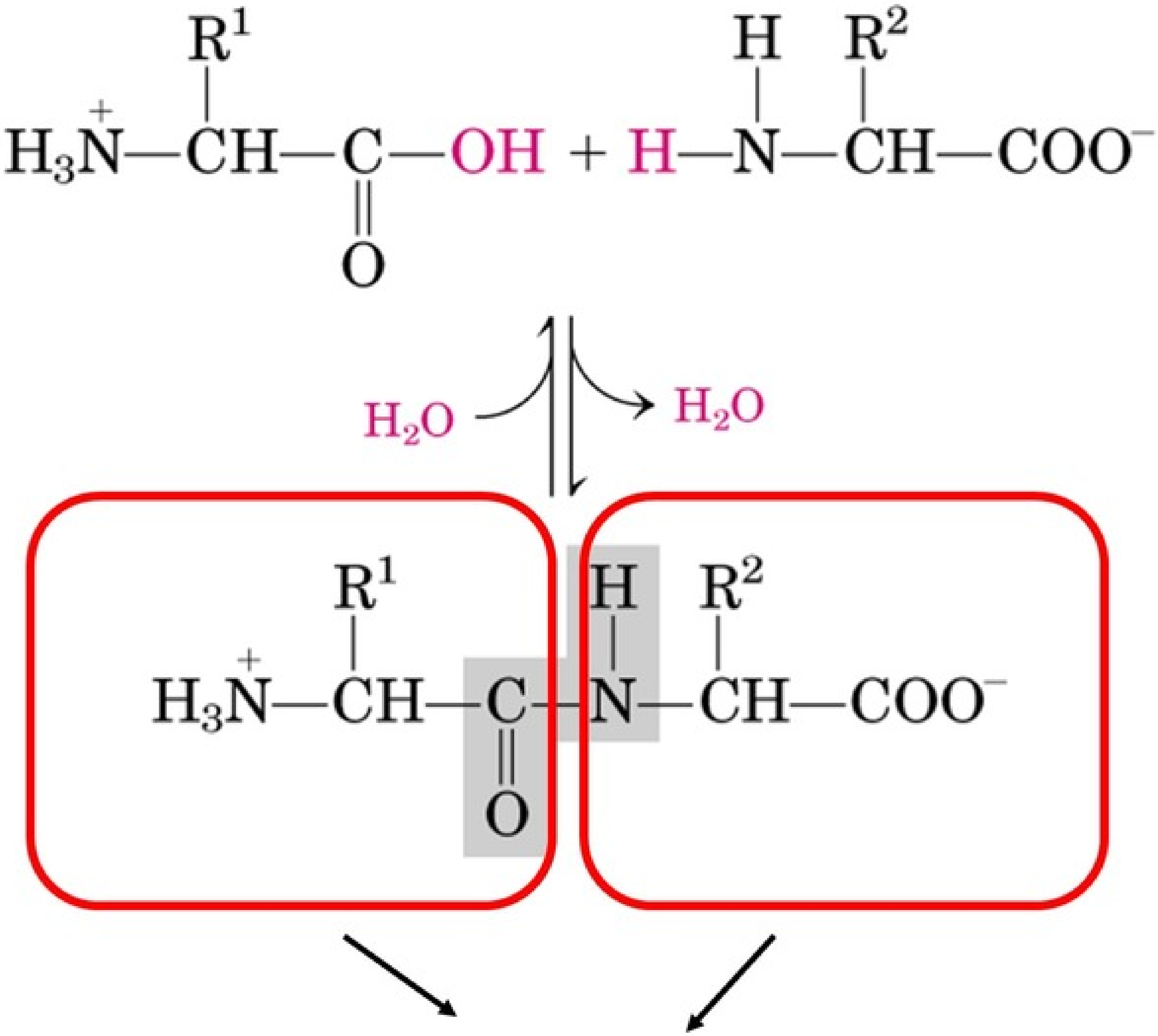
PDB ID 5eph

Pozzi, C. *et al.* Crystal structure of the *Pseudomonas aeruginosa* BEL-1 extended-spectrum beta-lactamase and its complexes with moxalactam and imipenem. *Antimicrob Agents Chemother* **60**, 7189-7199 (2016)

O que é uma proteína?

http://pdb101.rcsb.org/learn/videos/what-is-a-protein-video

Ligação peptídica



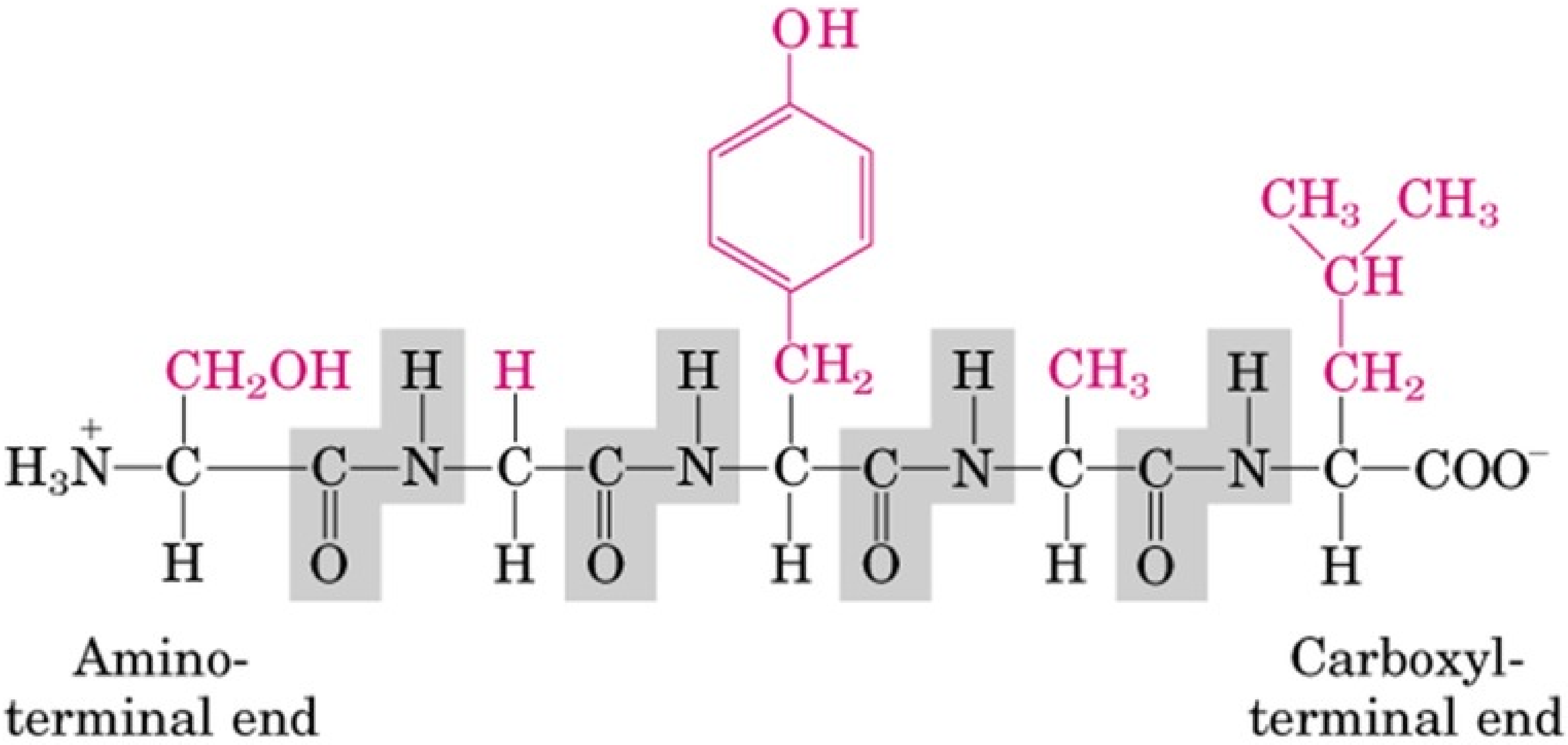
$$H_3\overset{+}{N}-\overset{R^1}{\overset{|}{CH}}-\underset{\underset{O}{\|}}{C}-OH + H-\overset{H}{\overset{|}{N}}-\overset{R^2}{\overset{|}{CH}}-COO^-$$

$$\underset{H_2O}{\overset{H_2O}{\rightleftharpoons}}$$

$$H_3\overset{+}{N}-\overset{R^1}{\overset{|}{CH}}-\underset{\underset{O}{\|}}{C}-\overset{H}{\overset{|}{N}}-\overset{R^2}{\overset{|}{CH}}-COO^-$$

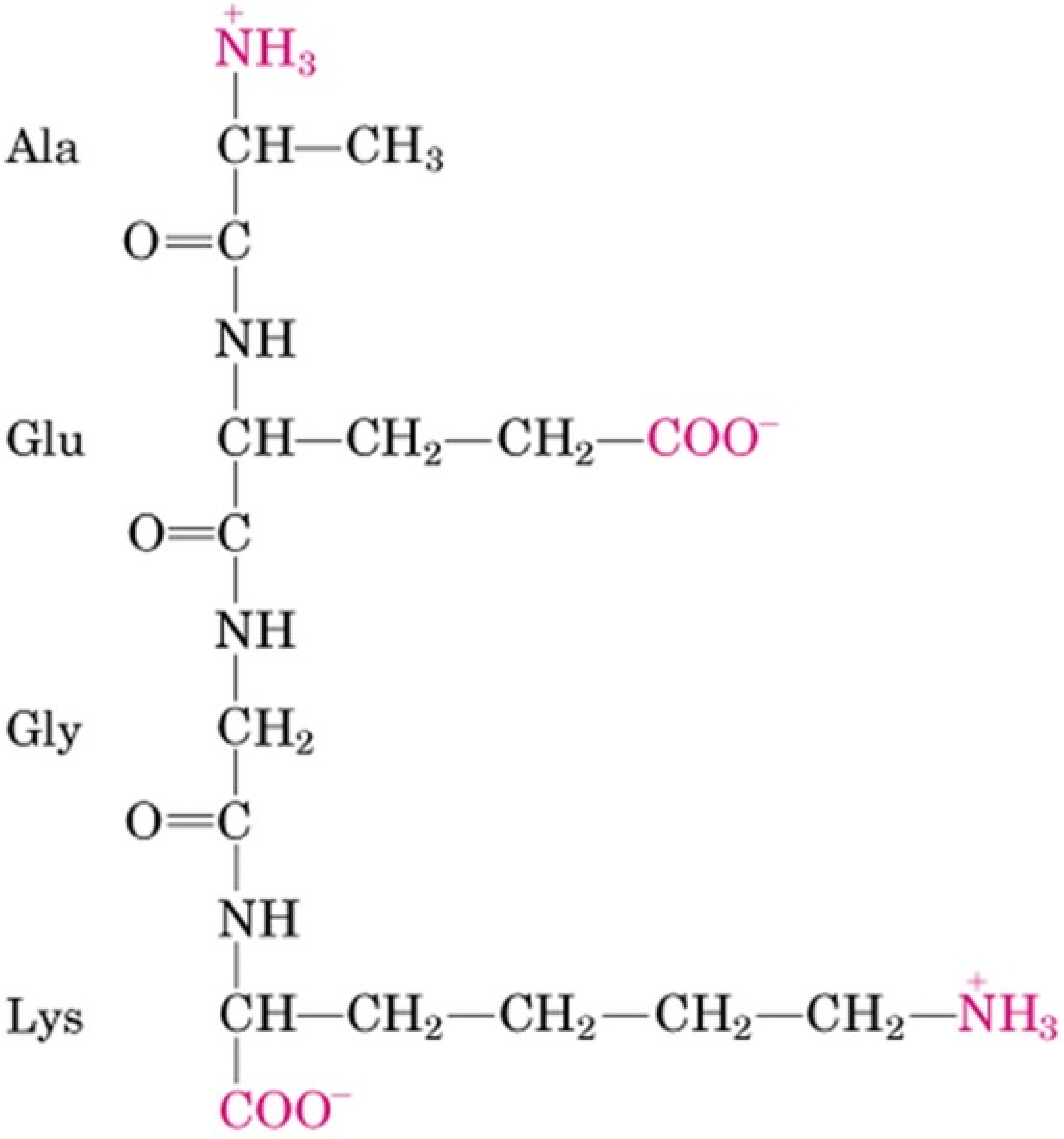
Resíduo de aminoácido

Um exemplo de peptídio



Oligopeptídeos
Polipeptídeos
proteínas

Peptídeo: ionização



Proteínas em todos os lugares

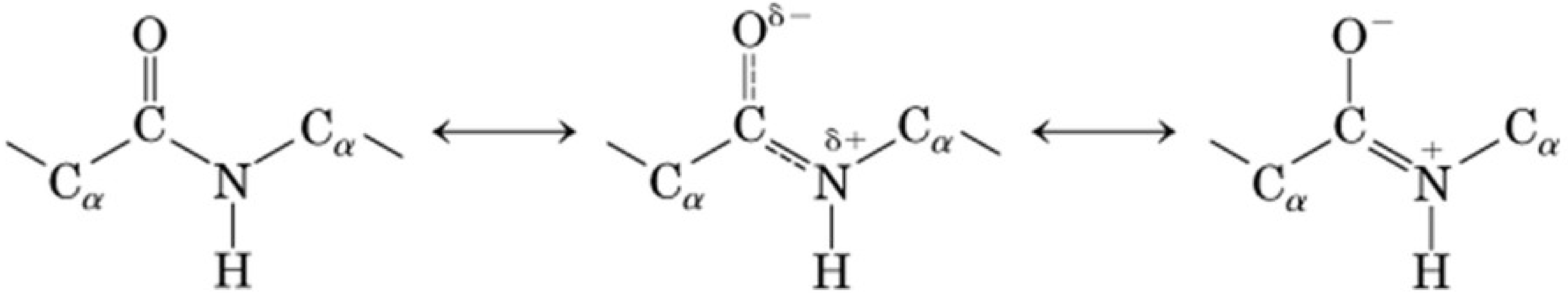
http://mm.rcsb.org/

Os 20 aminoácidos

Collaborate!

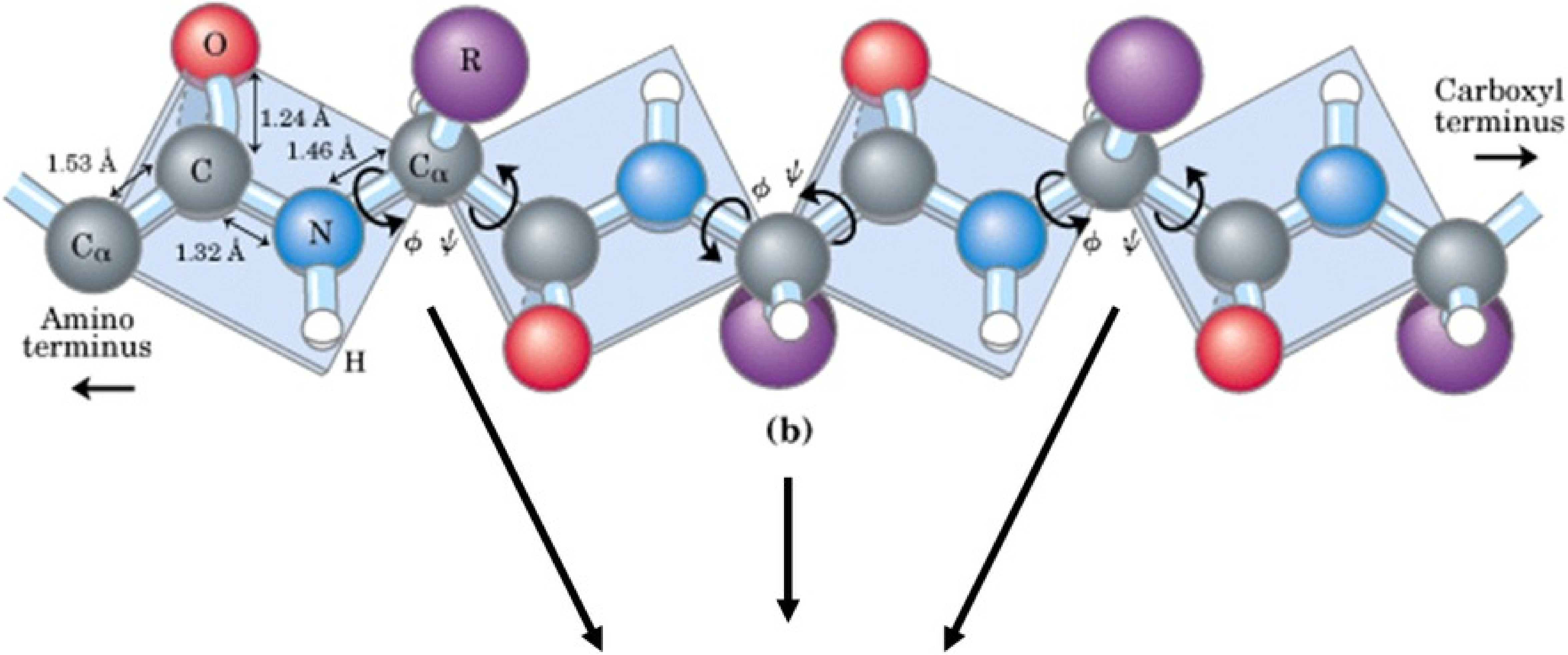
Ligação peptídica

Ligação peptídica:
caráter intermediário entre ligação simples e dupla ligação



(a)

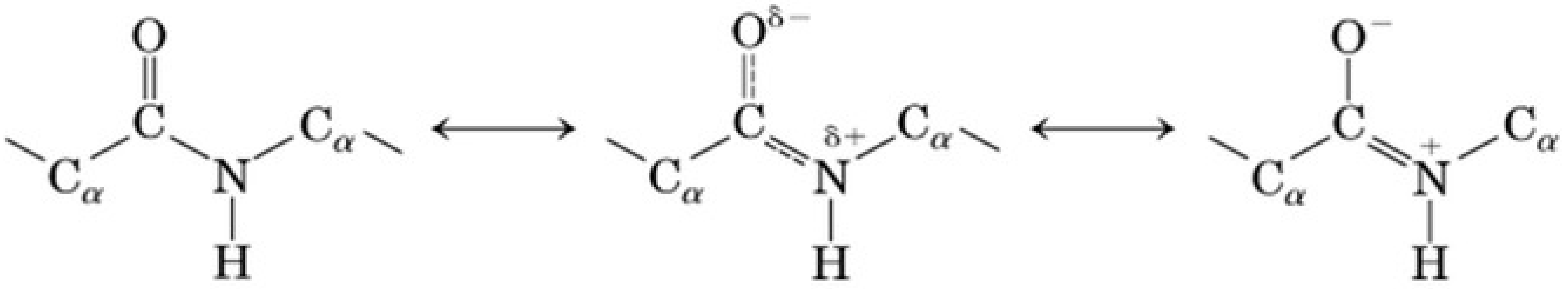
Ligação peptídica:
não há rotação em torno da ligação peptídica

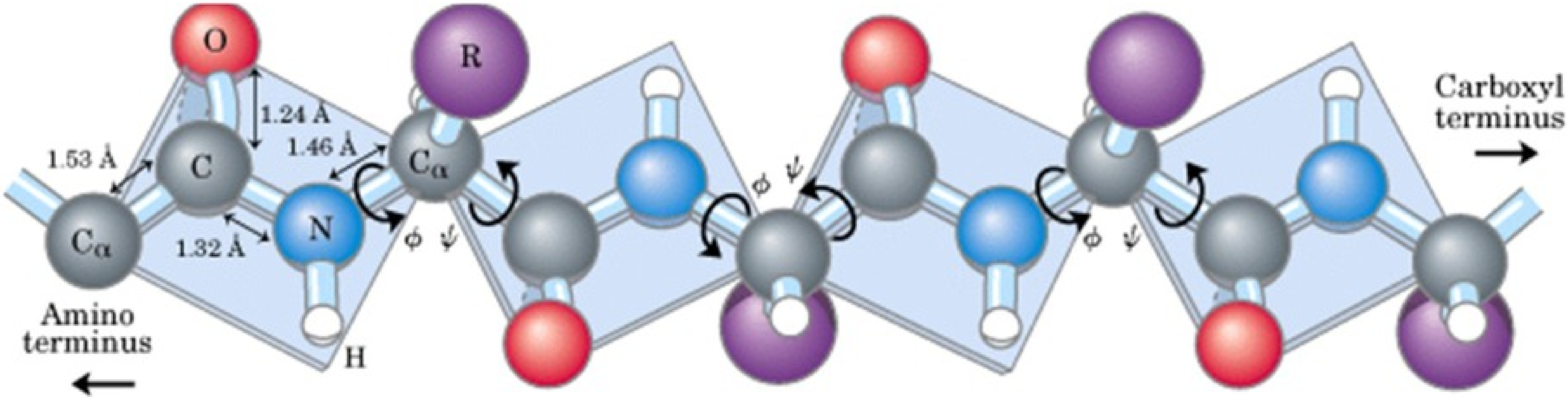


Pontos de dobramento entre as unidades peptídicas rígidas

CADEIA POLIPEPTÍDICA

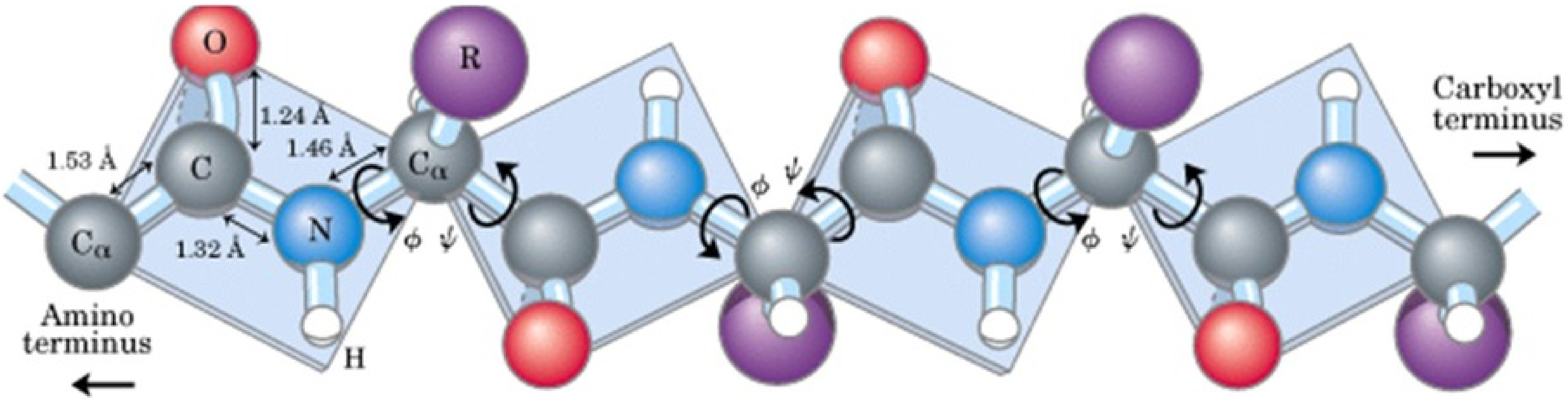
Ligação Peptídica



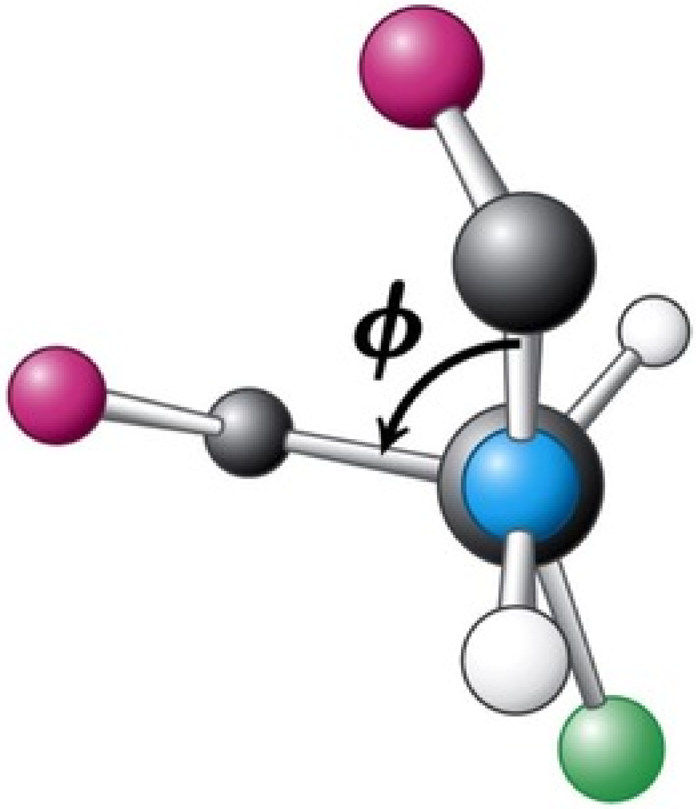


ϕ phi

ψ PSi



Ângulos de torção



$\phi = -80°$

Figure 2-27b
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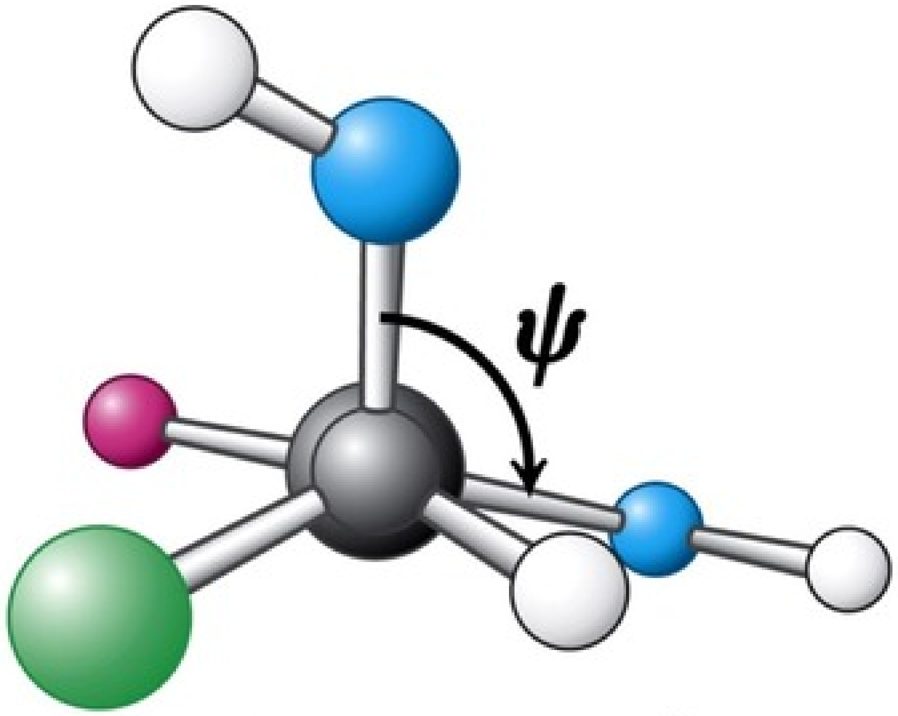

$\psi = +85°$

Figure 2-27c
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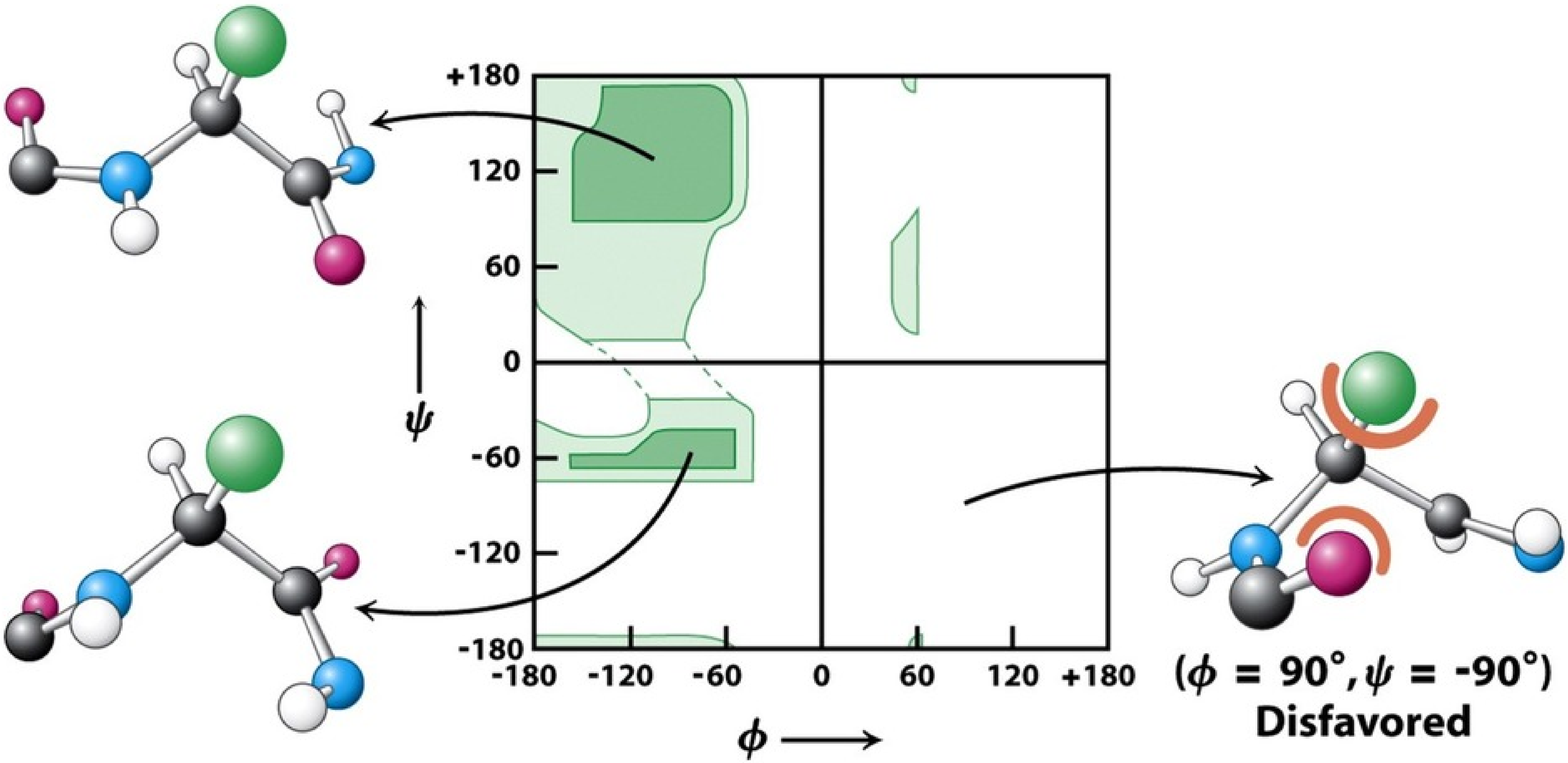

Figure 2-28
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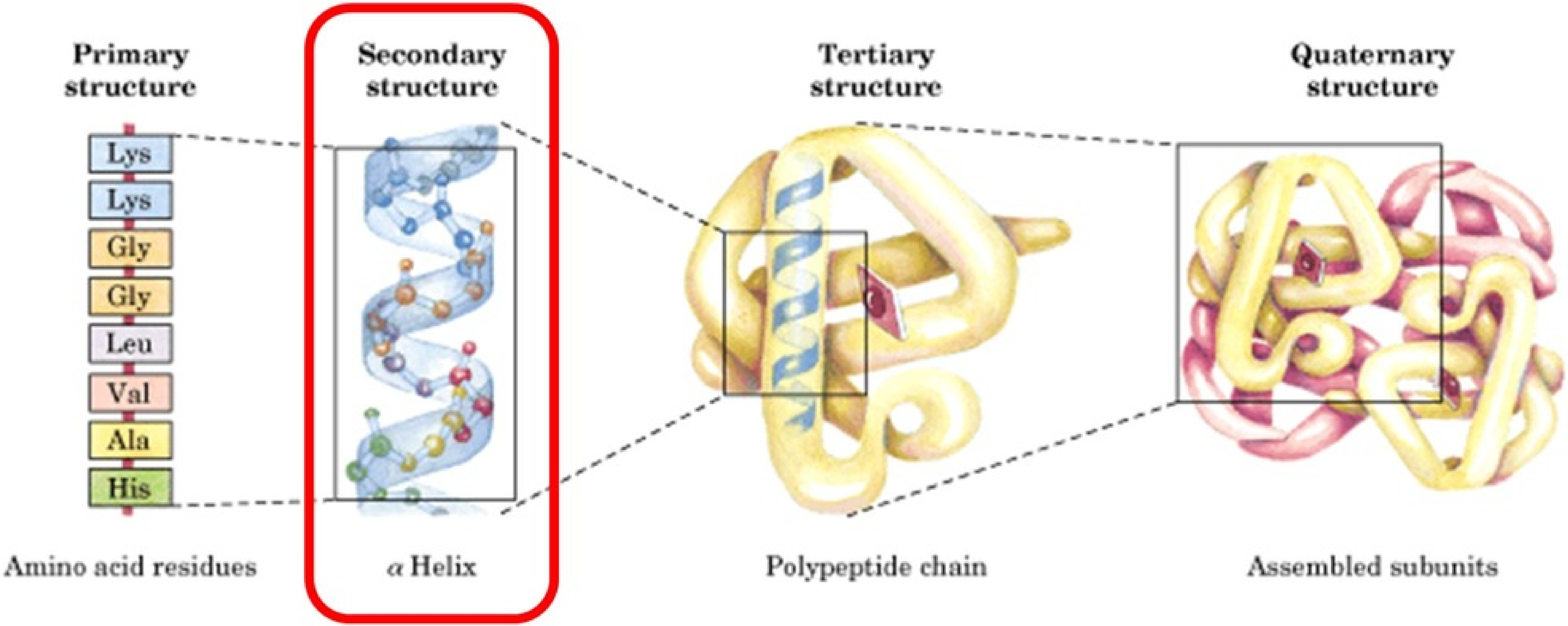
Diagrama de Ramachandran

Estrutura secundária



Estruturas regulares de segmentos da cadeia polipeptídica

α - hélice

Folhas β

Volta β

α - hélice

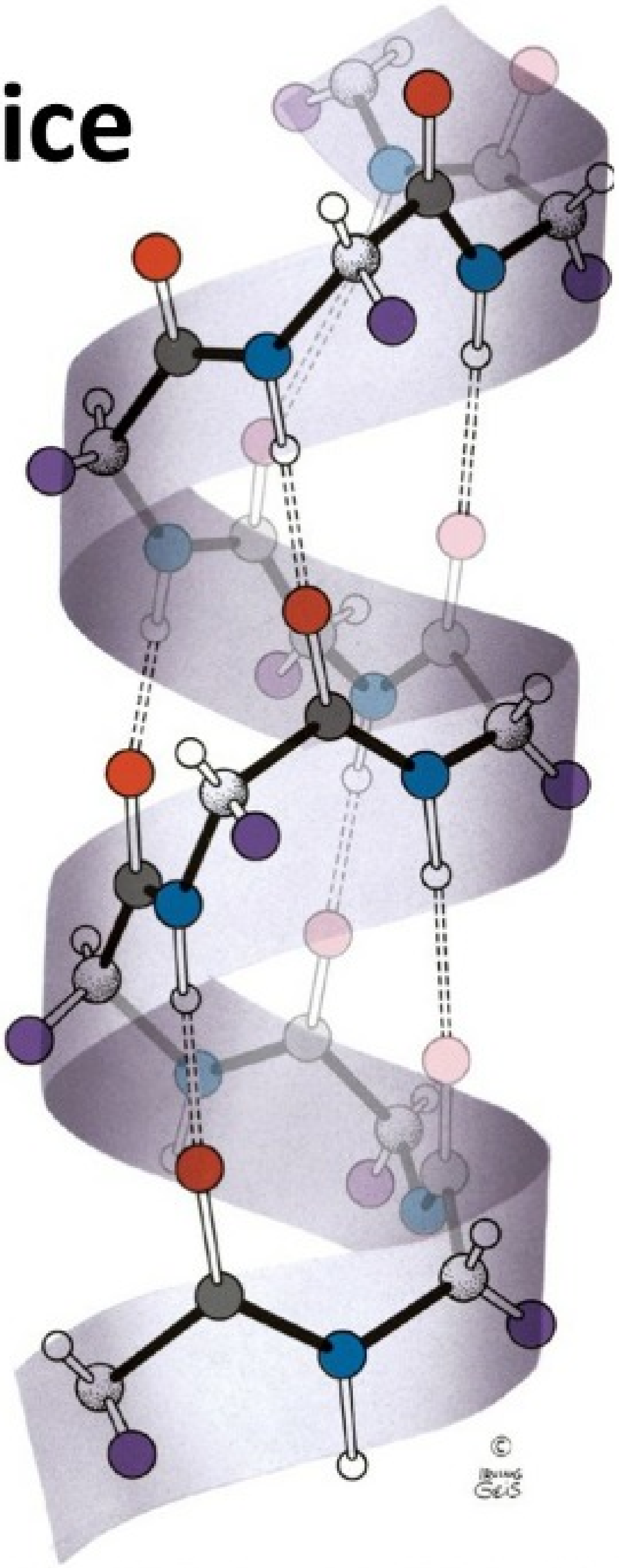
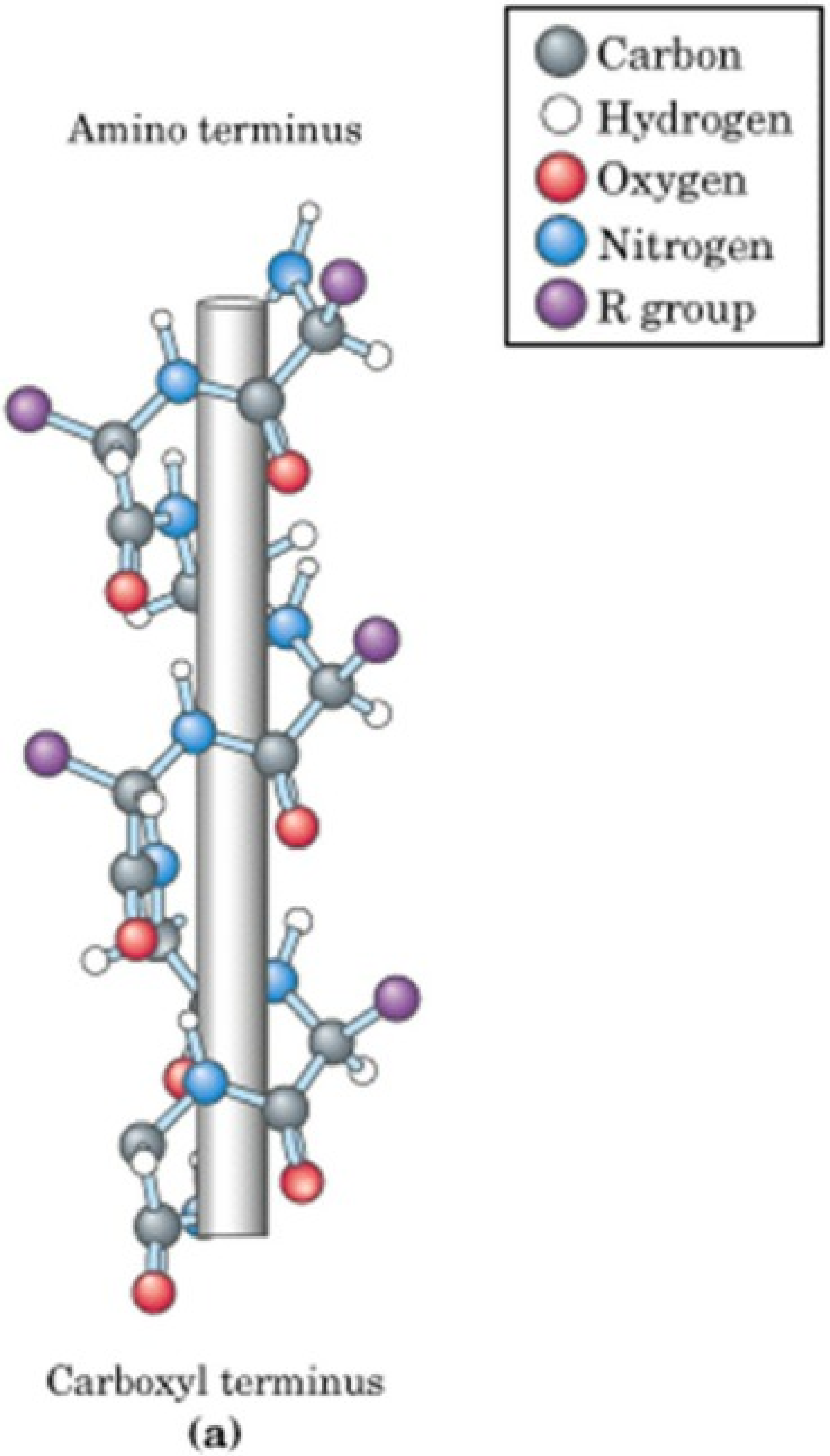


Figure 6-7 Fundamentals of Biochemistry, 2/e



Poll

Que tipo de interação intramolecular é responsável pela formação da alfa hélice?

○ ligação covalente

○ pontes de hidrogênio

○ ligação iônica

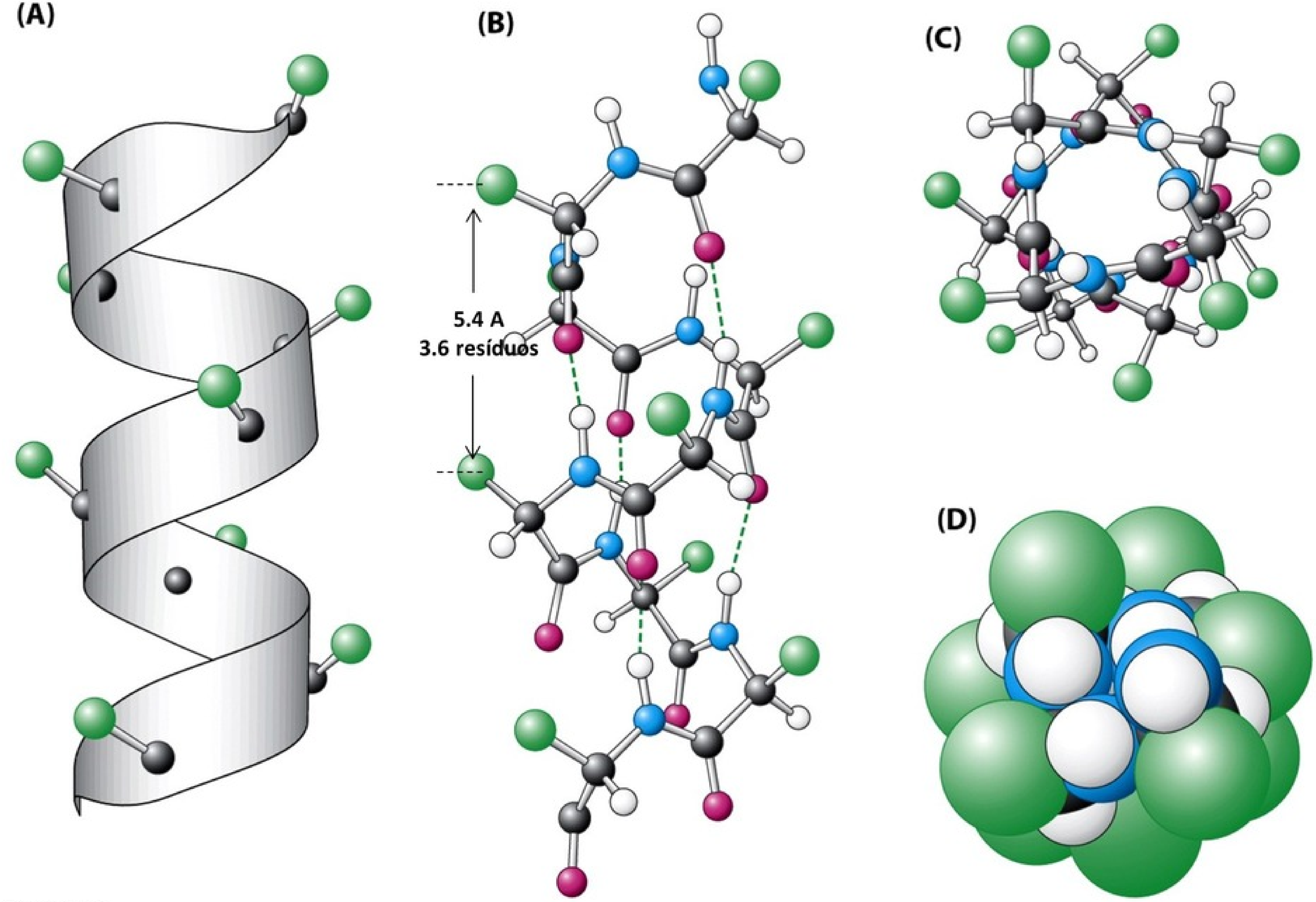


Figure 2-29
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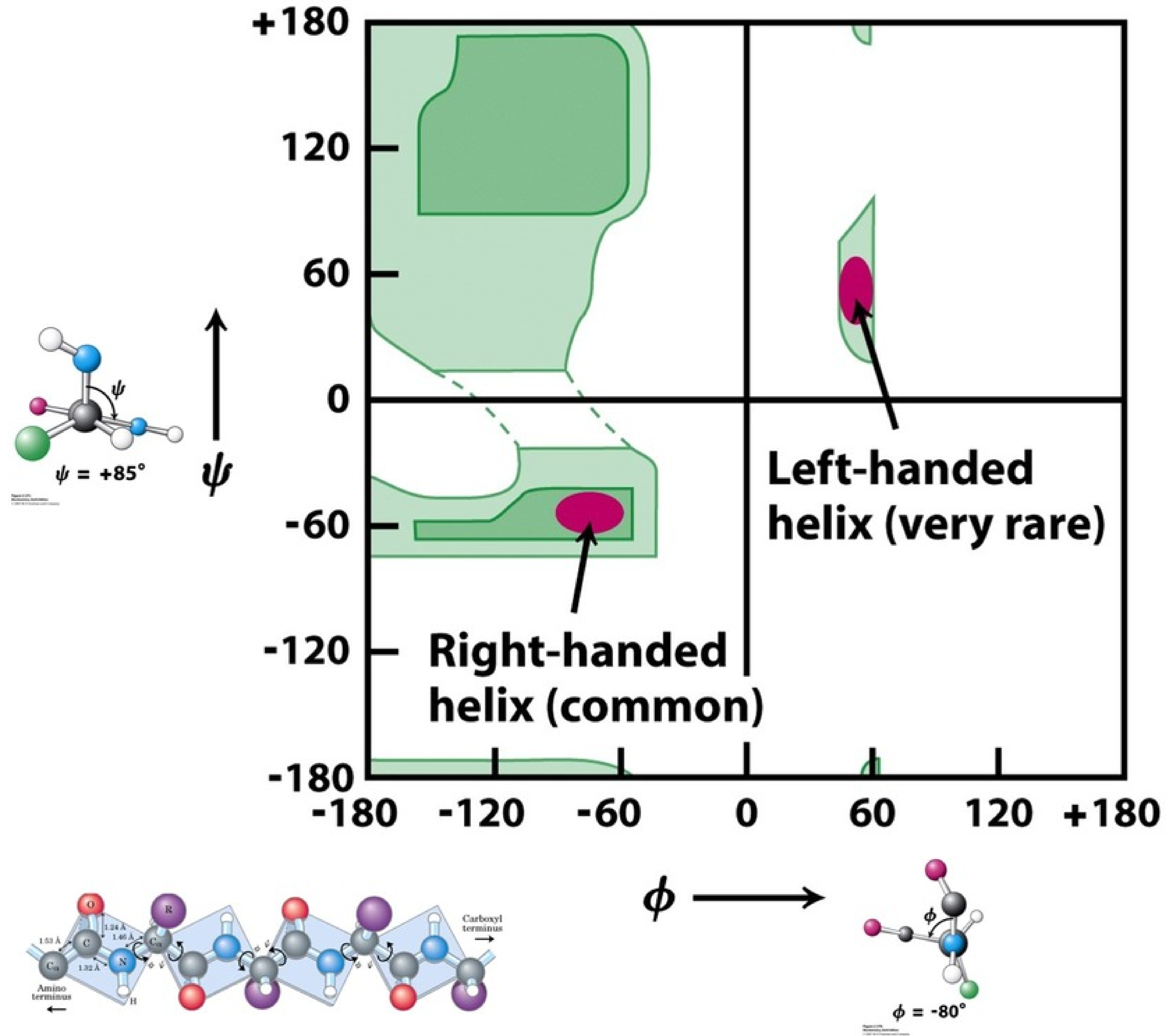
Poll

Um polipeptídeo formado por resíduos adjacentes de lisina (grupo R carregado positivamente em pH 7,0) poderia formar uma alfa-hélice?

○ **SIM**

○ NÃO


+180
120
60
0
-60
-120
-180
ψ
-180
-120
-60
0
60
120
+180
ϕ
Left-handed helix (very rare)
Right-handed helix (common)
ψ = +85°
ϕ = -80°
O
R
C
N
Cα
H
1.53 Å
1.24 Å
1.46 Å
1.32 Å
Carboxyl terminus
Amino terminus

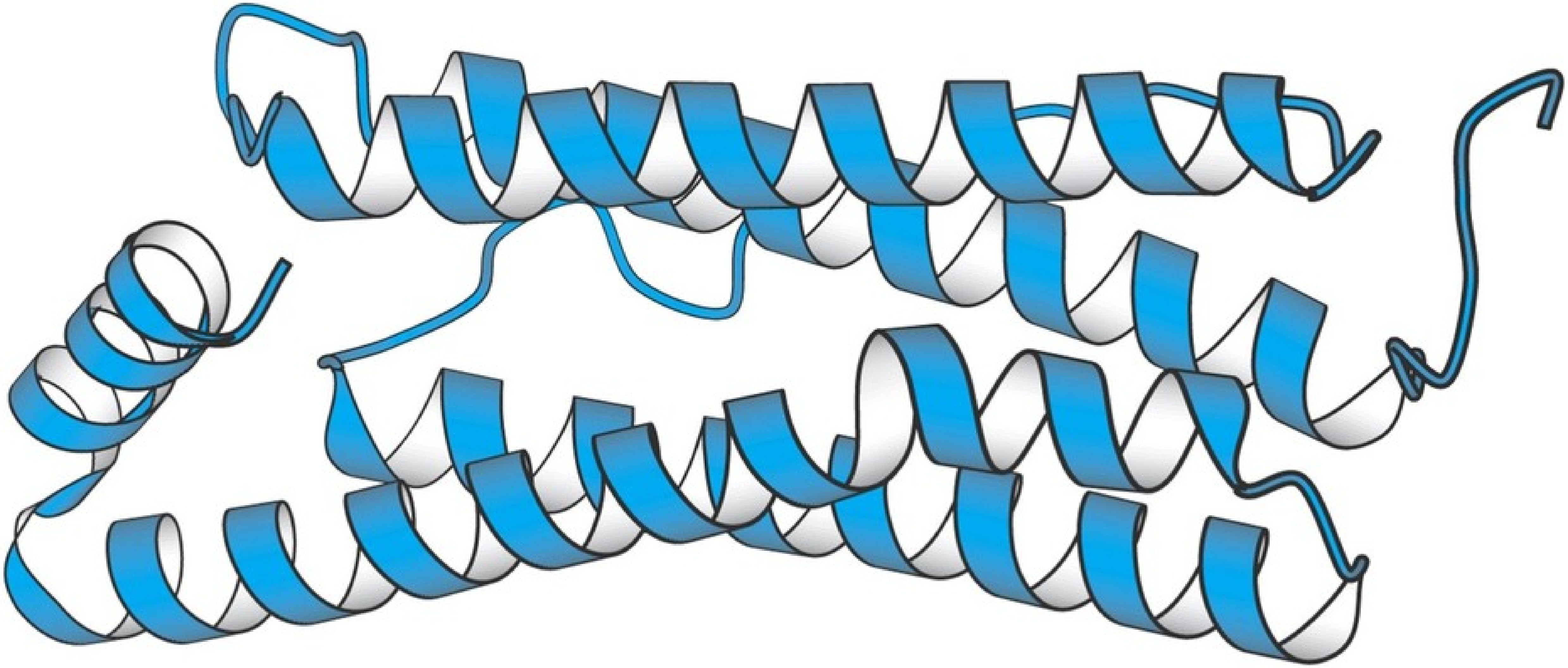


Figure 2-33
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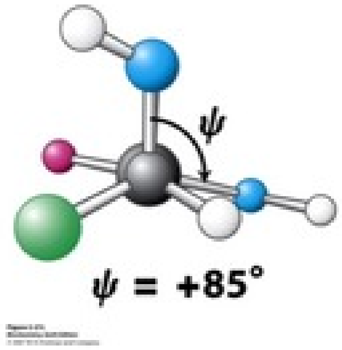
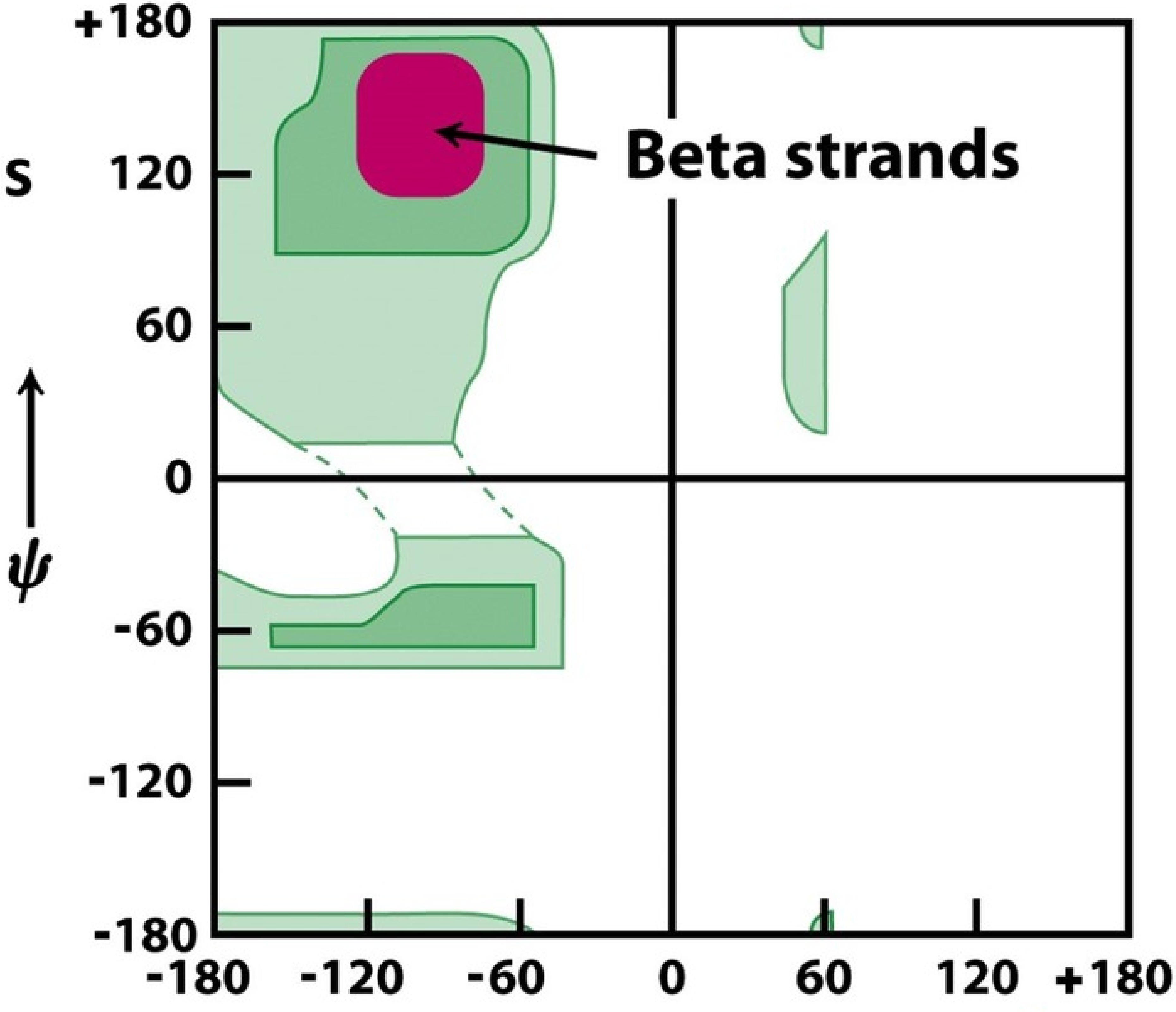
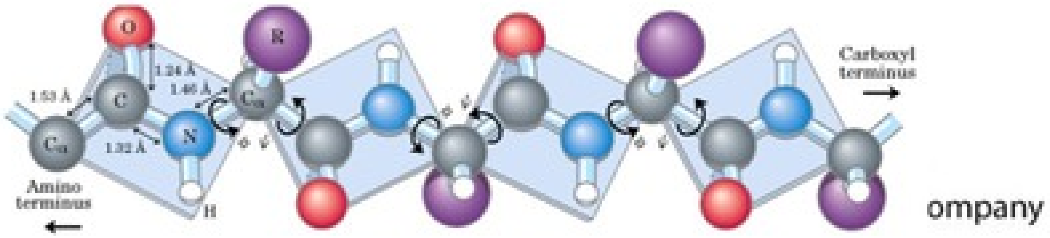
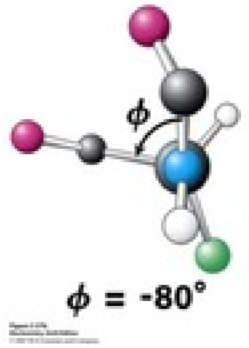
ferritina

Folhas β pregueadas









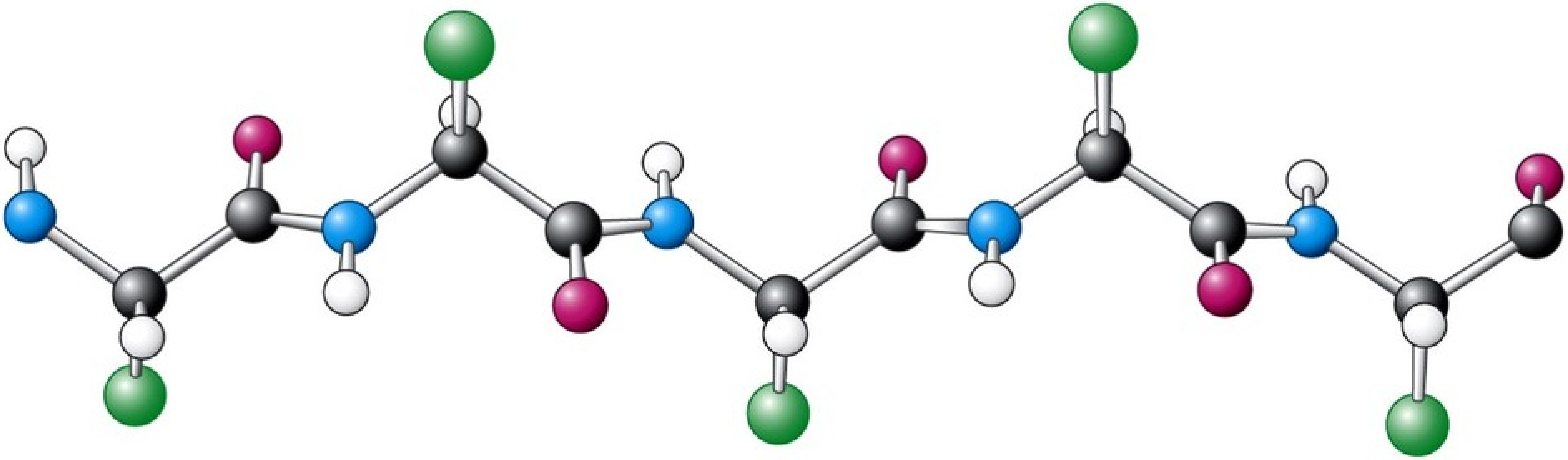


Figure 2-35
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Fita beta =
cadeia lateral alternadamente acima e abaixo do plano da fita

Folhas β pregueadas: antiparalelas

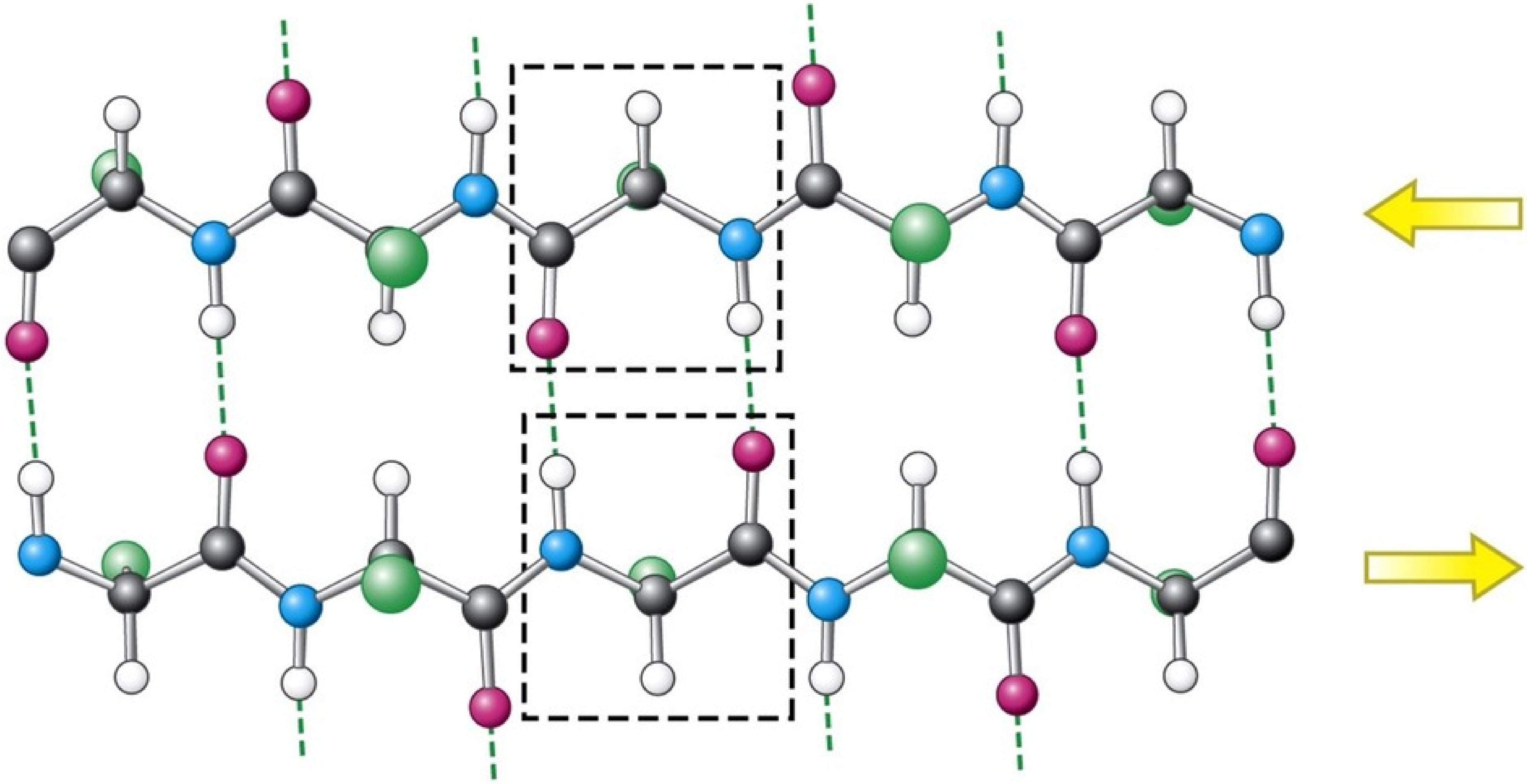

Figure 2-36
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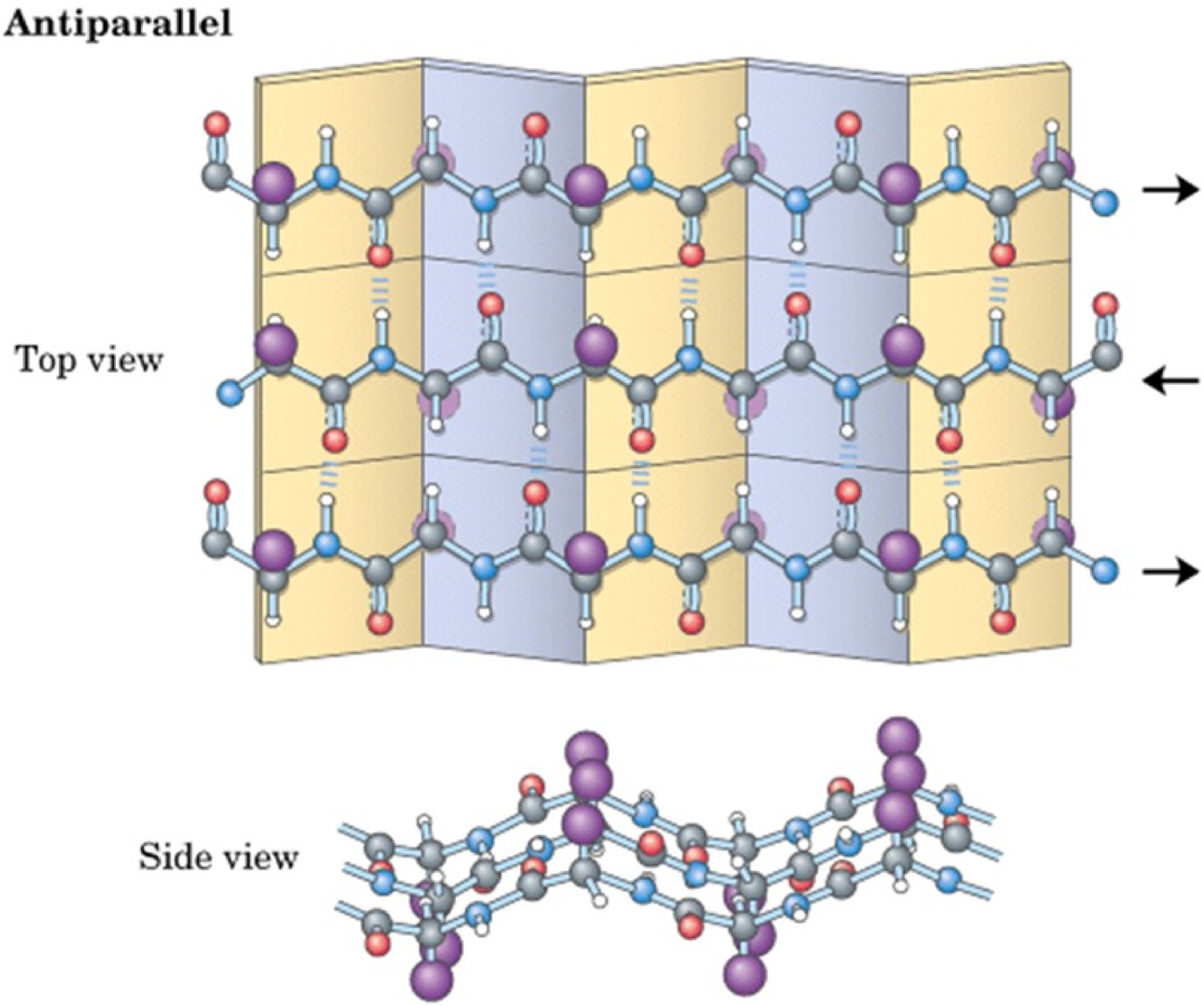

Antiparallel
Top view
Side view

Folhas β pregueadas: paralelas

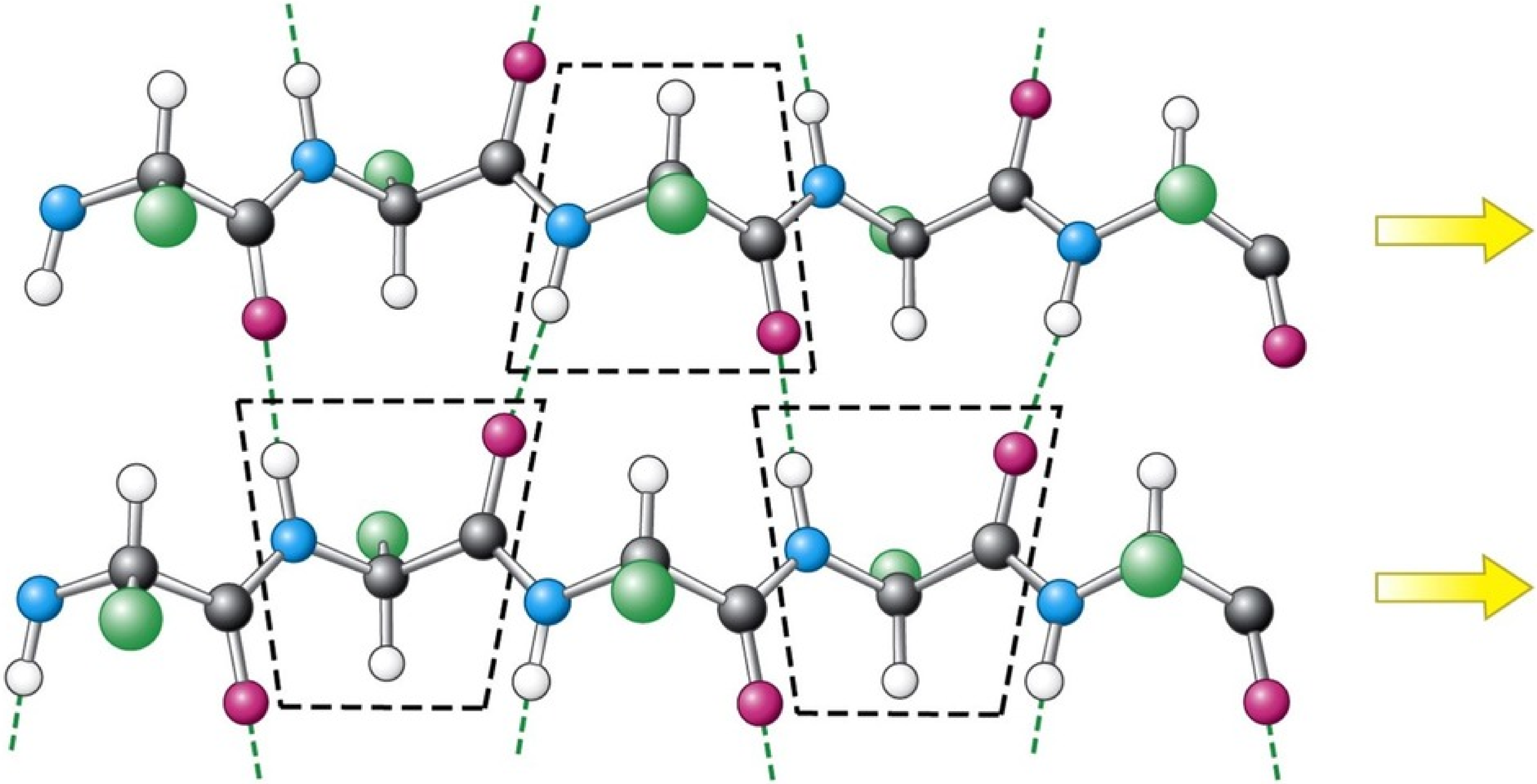
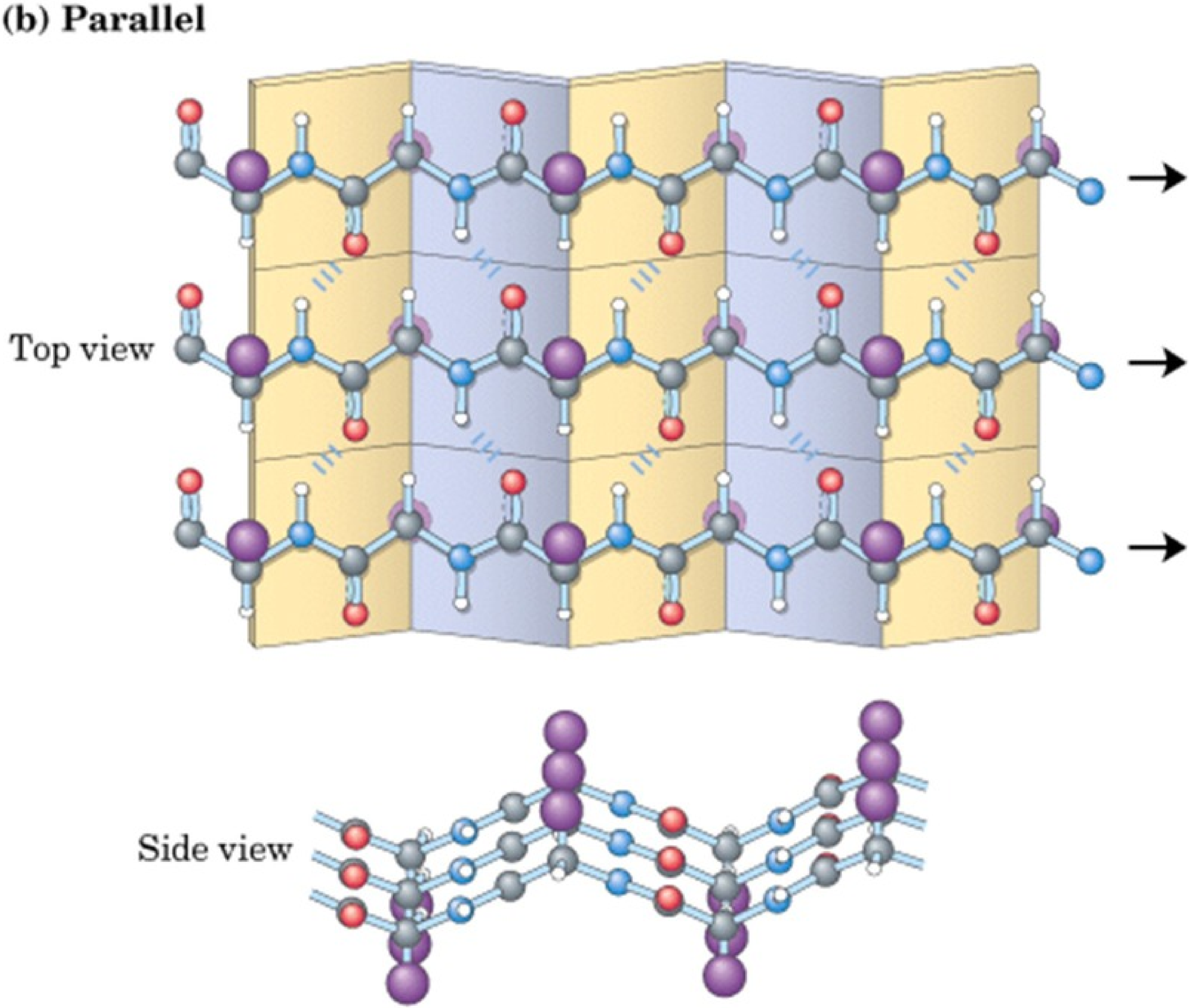


Figure 2-37
Biochemistry, Sixth Edition


(b) Parallel
Top view
Side view

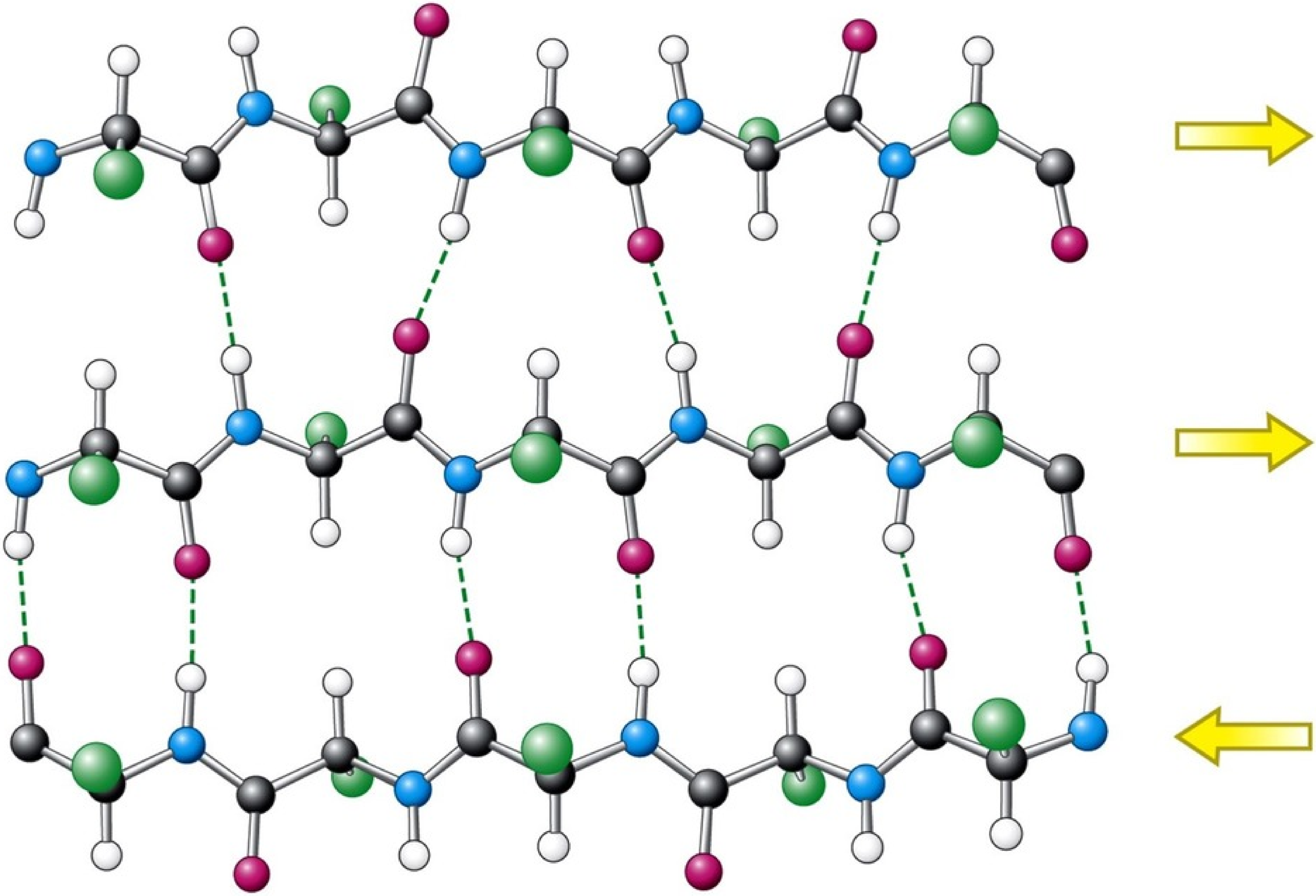


Figure 2-38
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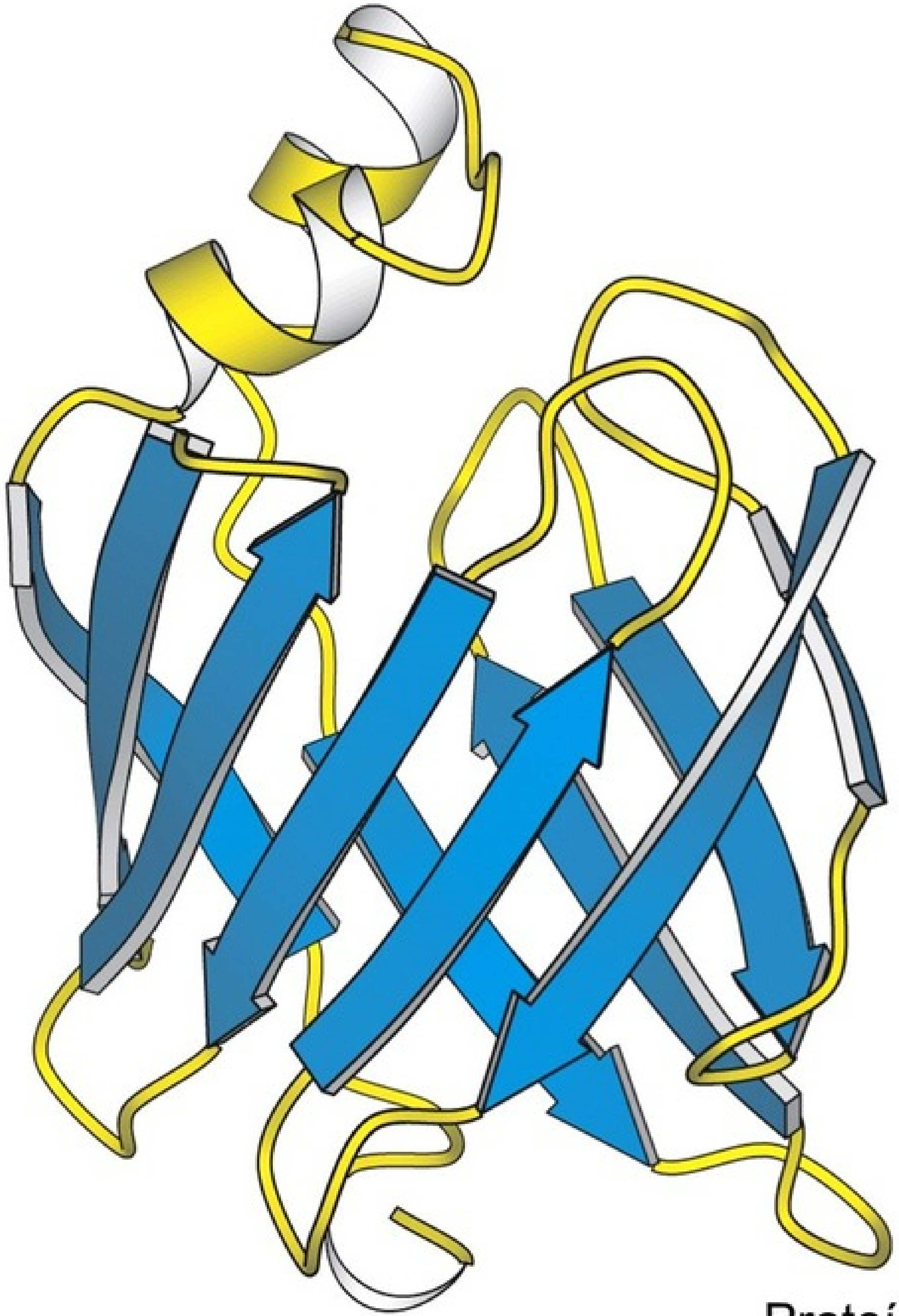

Figure 2-40
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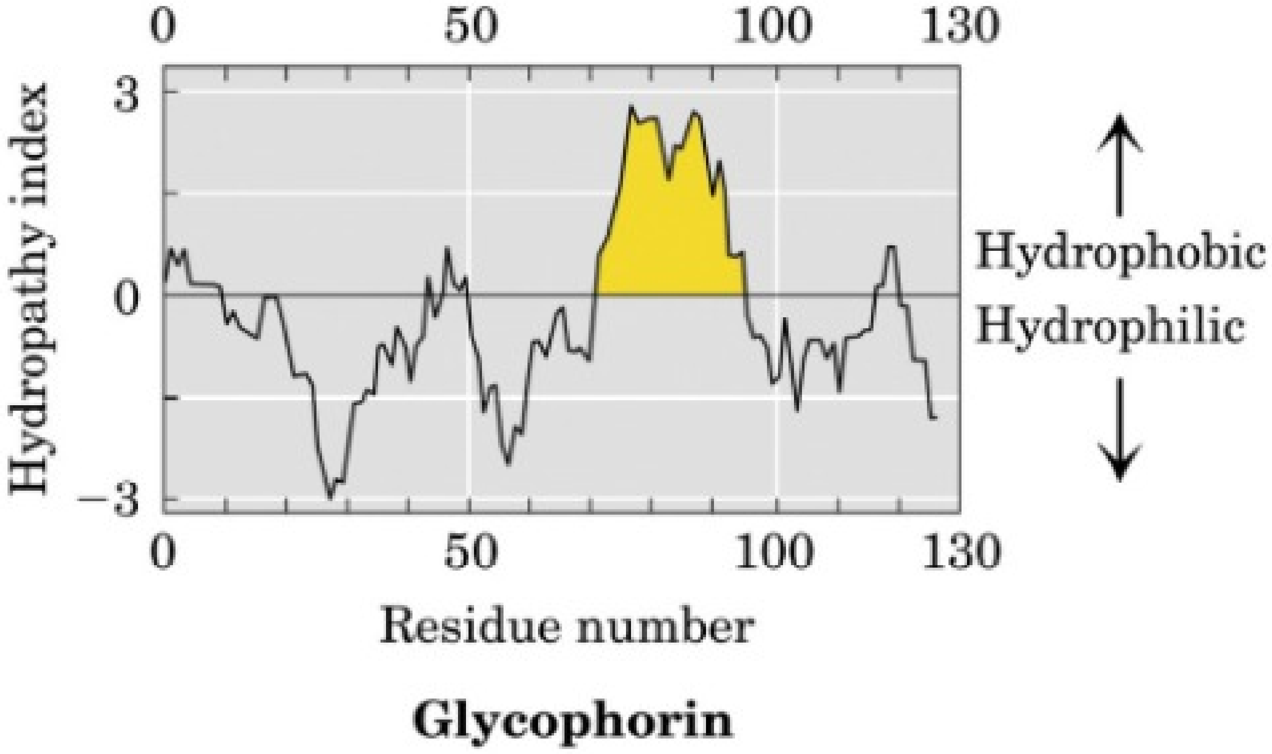
Proteína ligante a acido graxo

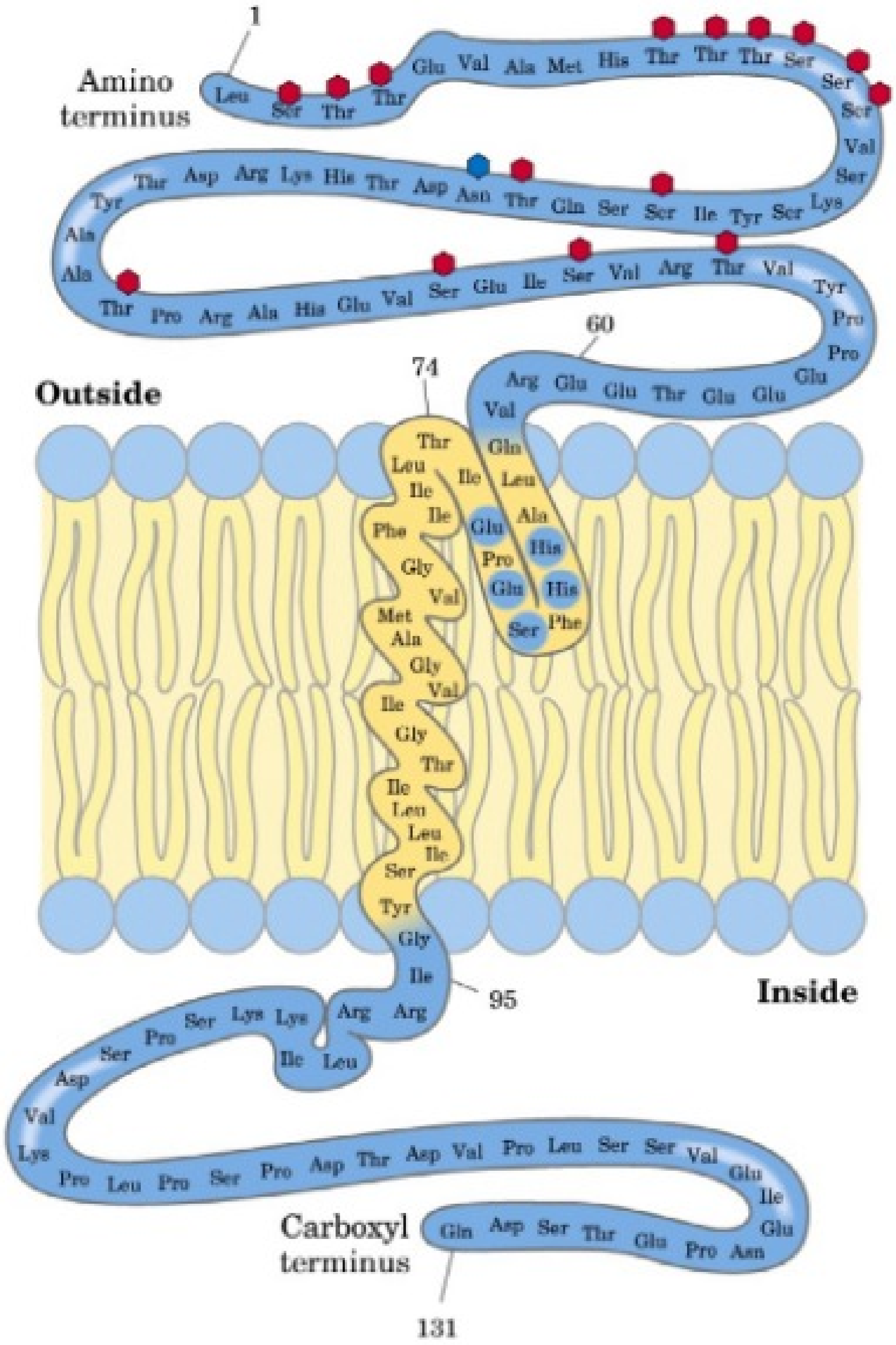
Poll

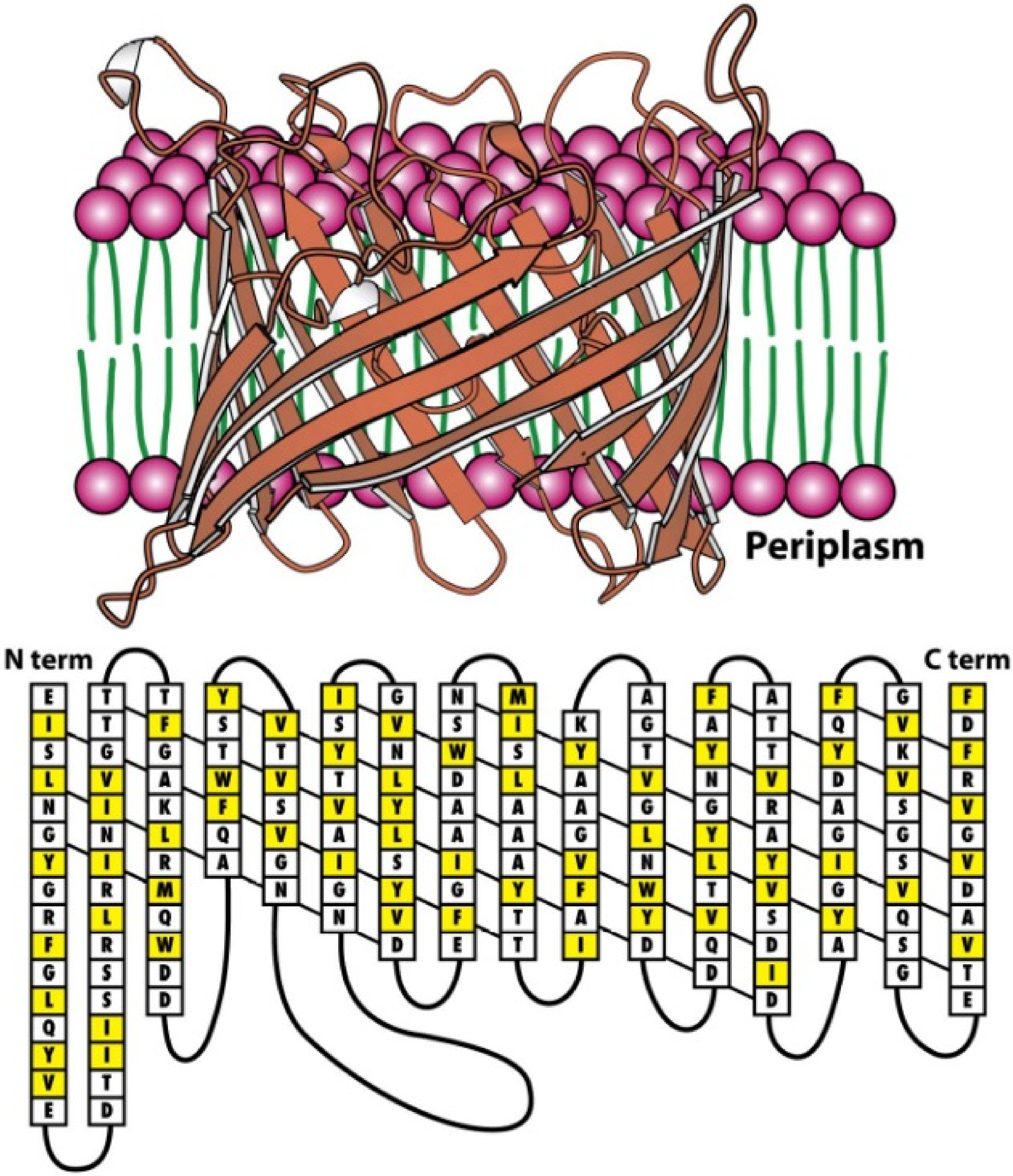
Numa proteína transmembrana, que tipos de resíduos de aminoácidos você esperaria encontrar na região da proteína que interage com a membrana?

- ○ hidrofílicos
- ○ hidrofóbicos
- ○ aromáticos
- ○ **sem carga**

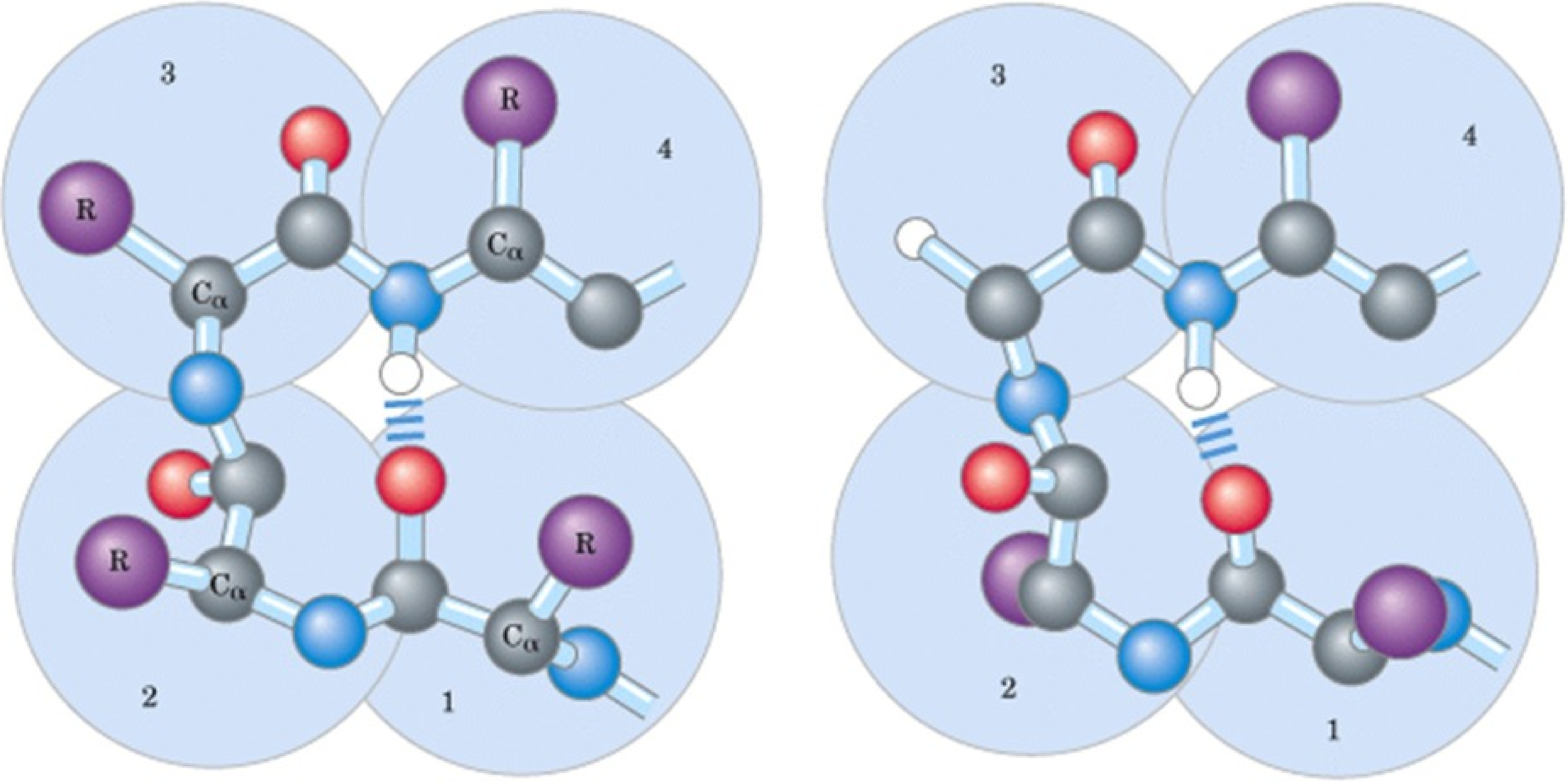
Glicoforina






Periplasm
N term
C term
EISLNGYGRFGLQYVE
TTGVINIRLRSSIITD
TFGAKLRMQWDD
YSTWFQA
VTVSVGN
ISYTVAIGN
GVNLYLSYVD
NSWDAAIGFE
MISLAAAYTT
KYAAGVFAI
AGTVGLNWYD
FAYNGYLTVQD
ATTVRAYVSDID
FQYDAGIGYA
GVKVSGSVQSG
FDFRVGVDAVTE

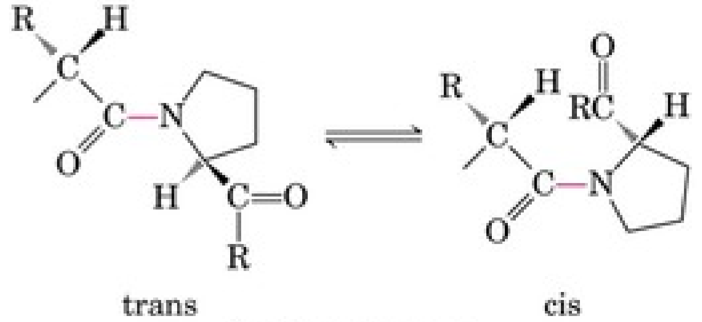
Voltas β



Type I

Type II

β Turns
(a)



Proline isomers
(b)

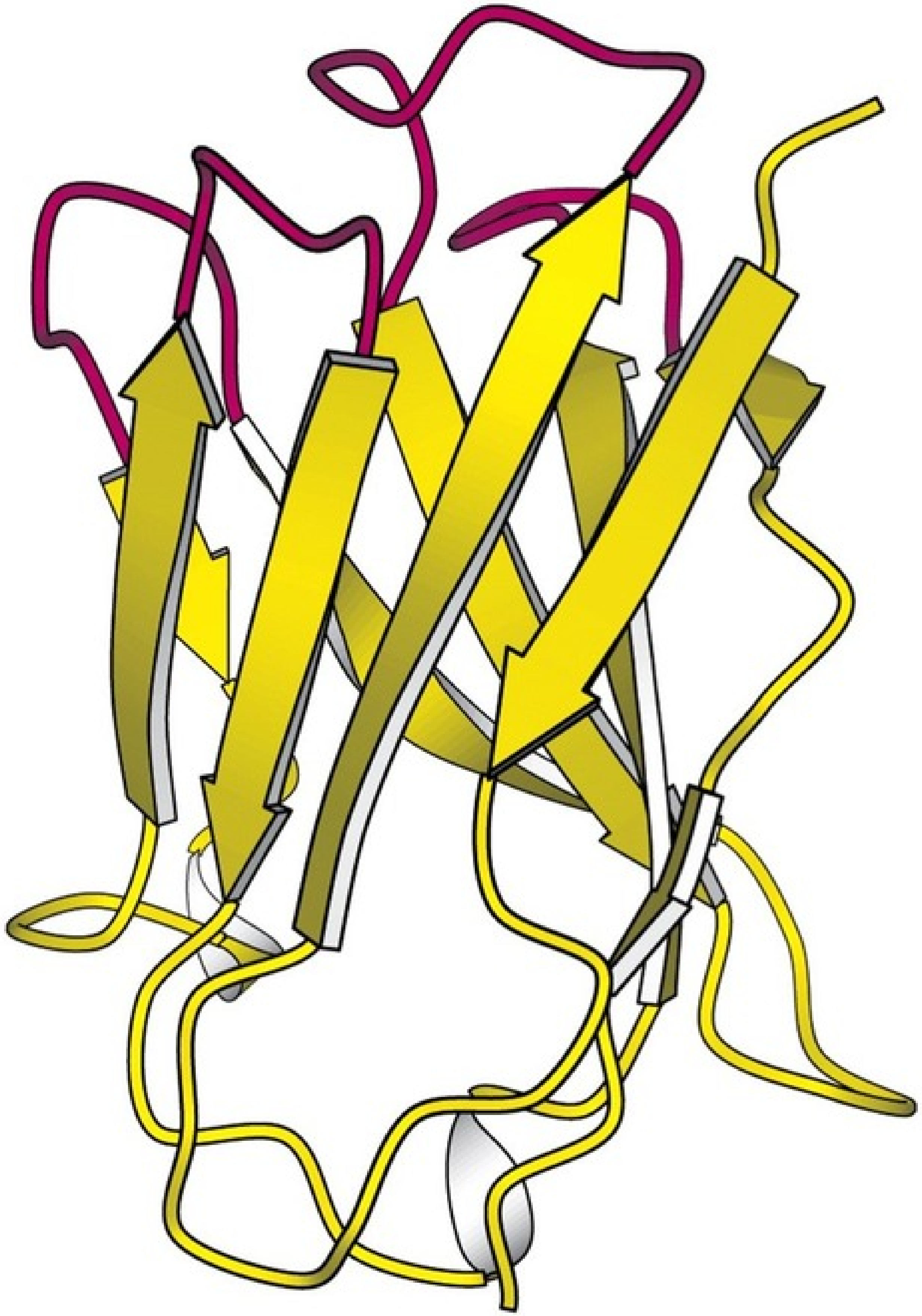


Figure 2-42
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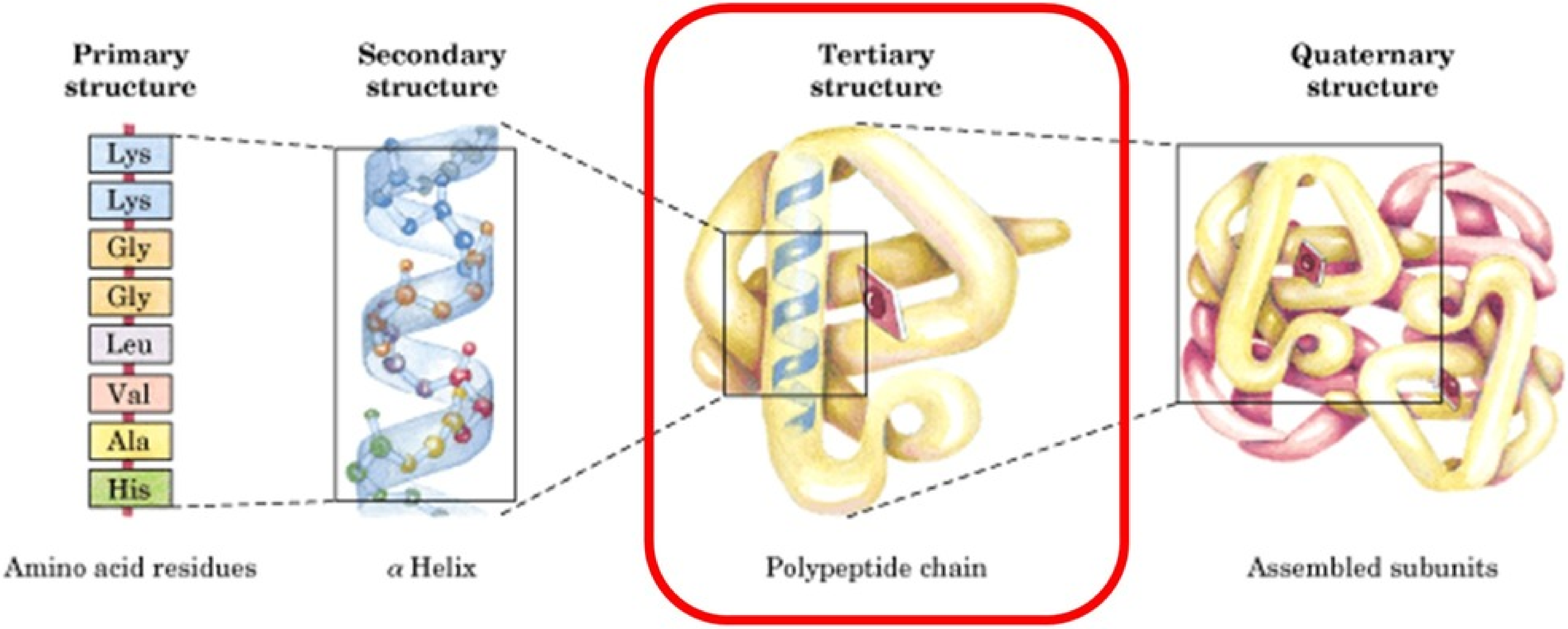
anticorpo

Albumina: 585 resíduos

Estrutura Terciária



Dobramento final da cadeia polipeptídica:

interações entre regiões com estrutura regular ou sem estrutura definida, em conjunto com a disposição das cadeias laterais – Forma 3D

Poll

Que tipos de forças mantem a estabilidade da estrutura de uma proteína?

○ Pontes de hidrogênio

○ forças de wan der Waals

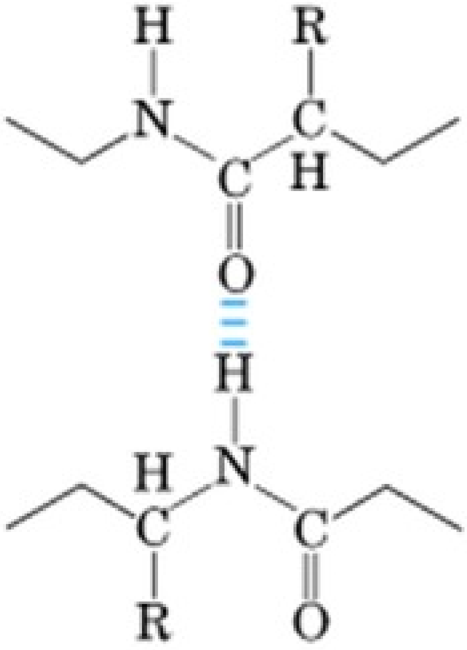
○ interações iônicas

○ interações hidrofóbicas

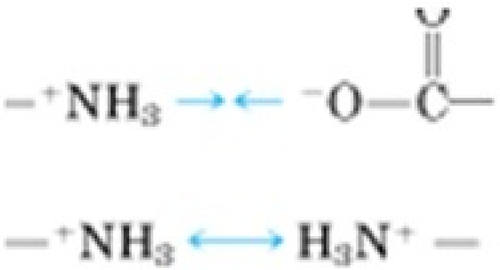
Forças que mantêm a estabilidade de uma proteína ???

Interações fracas

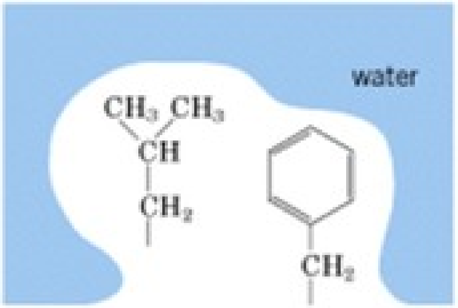
Pontes de Hidrogênio



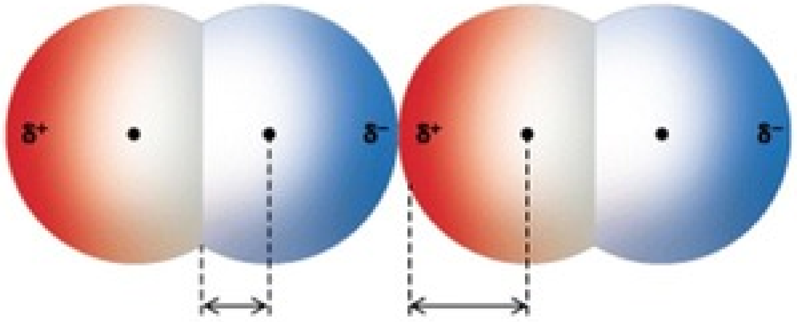
Interações iônicas



Interações hidrofóbicas



Forças de van der Waals



Pontes dissulfeto : ligação covalente

Pontes Dissulfeto

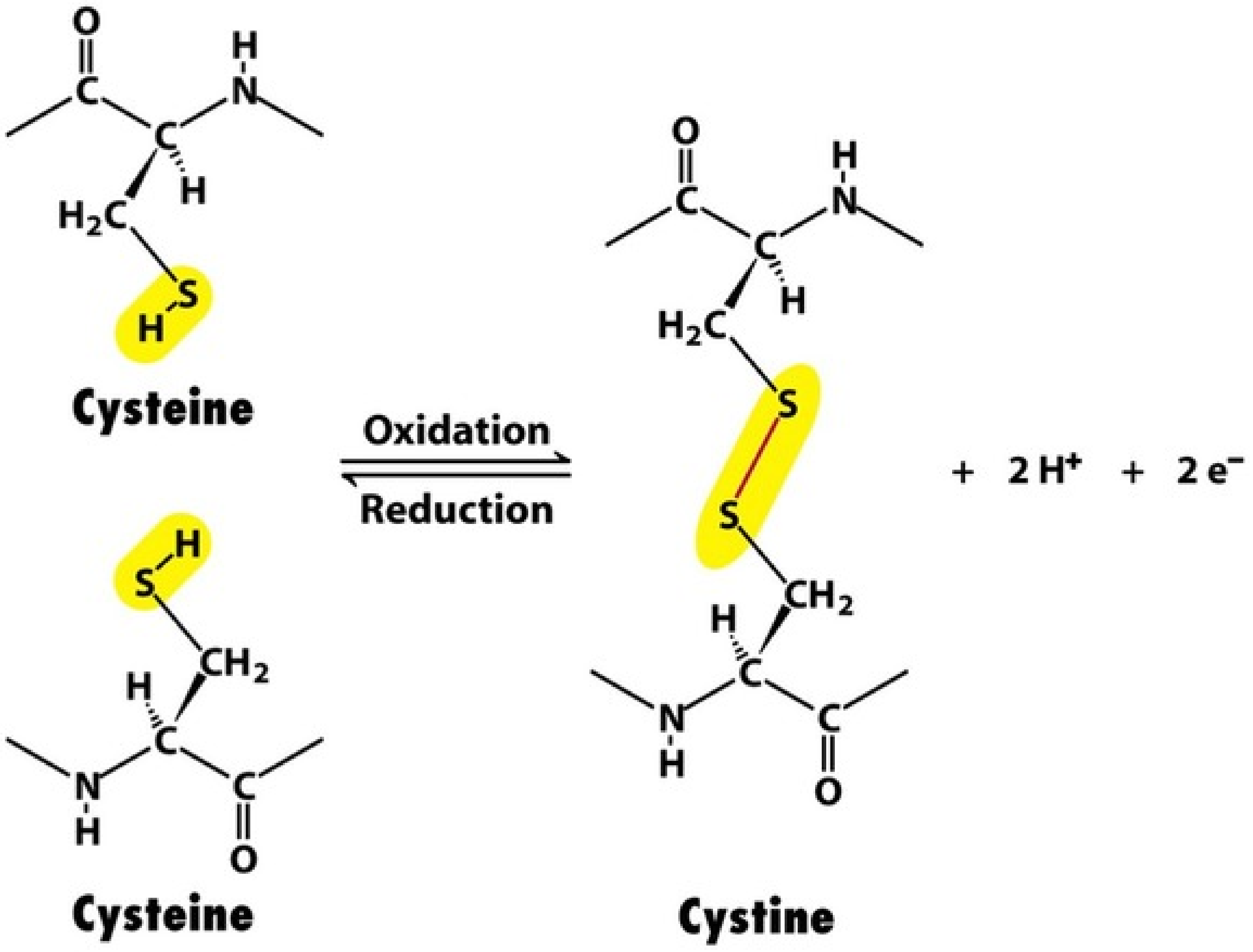


Figure 2-21
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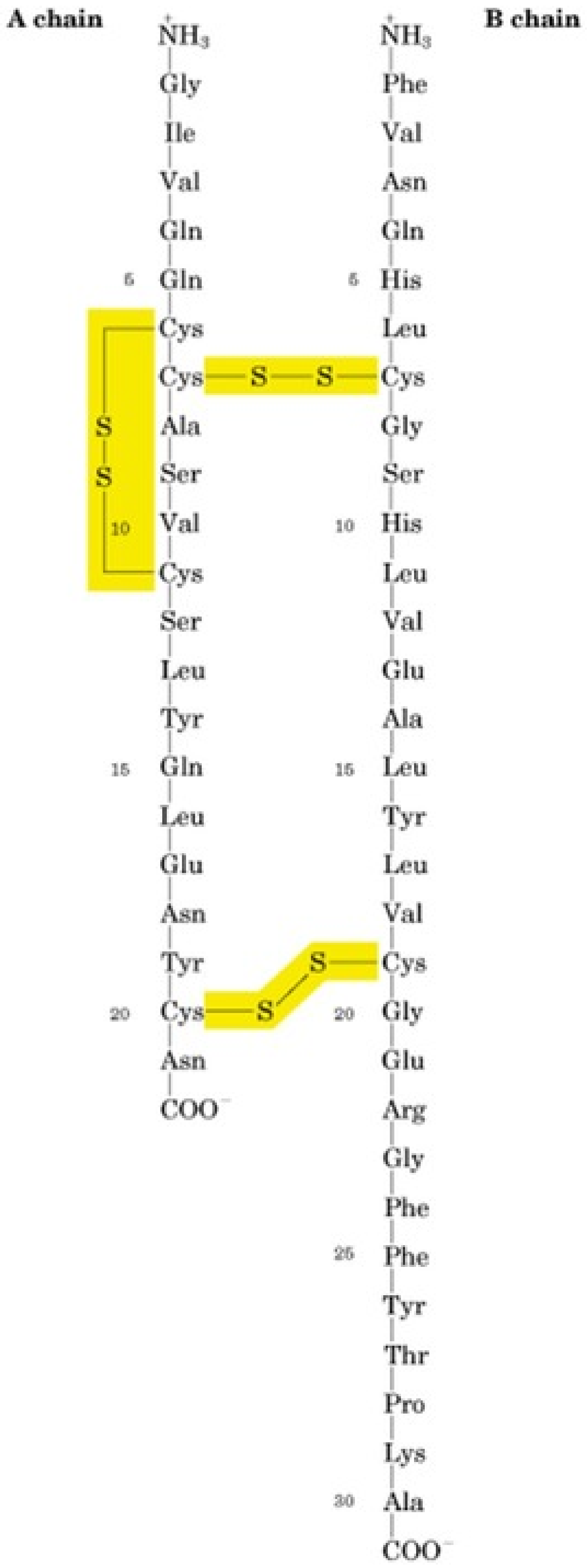
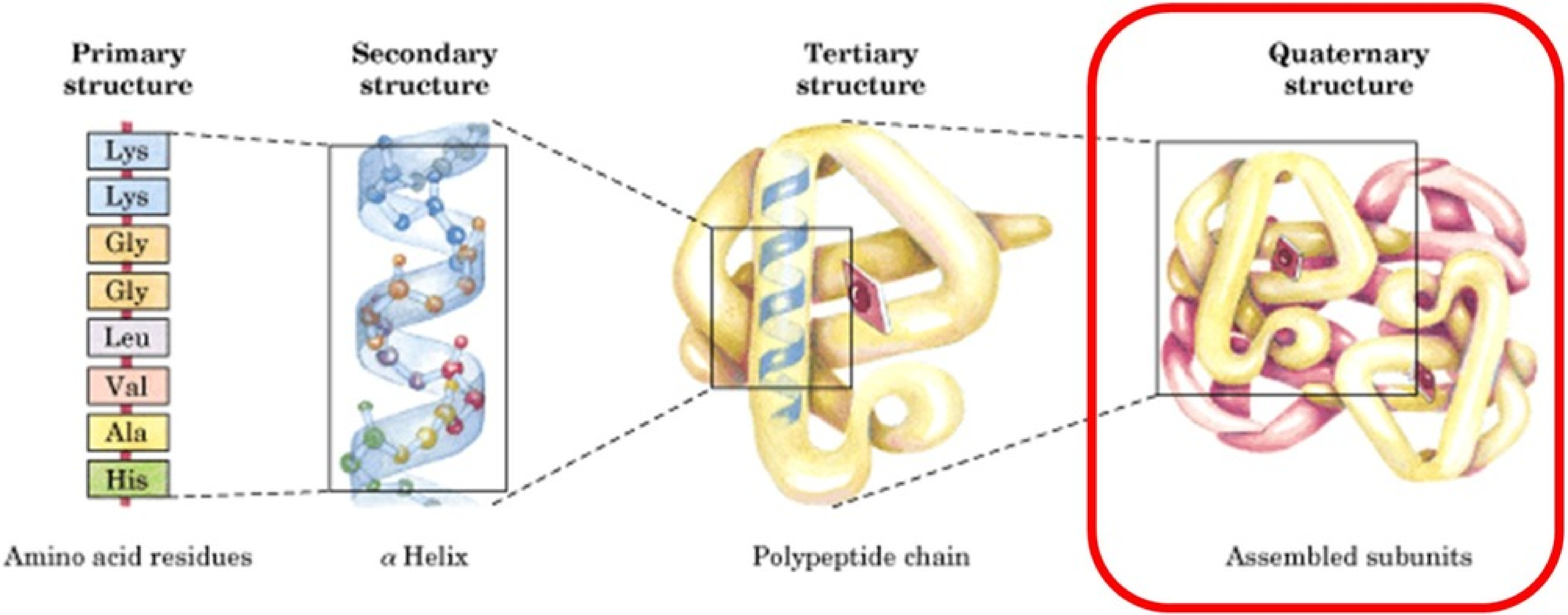
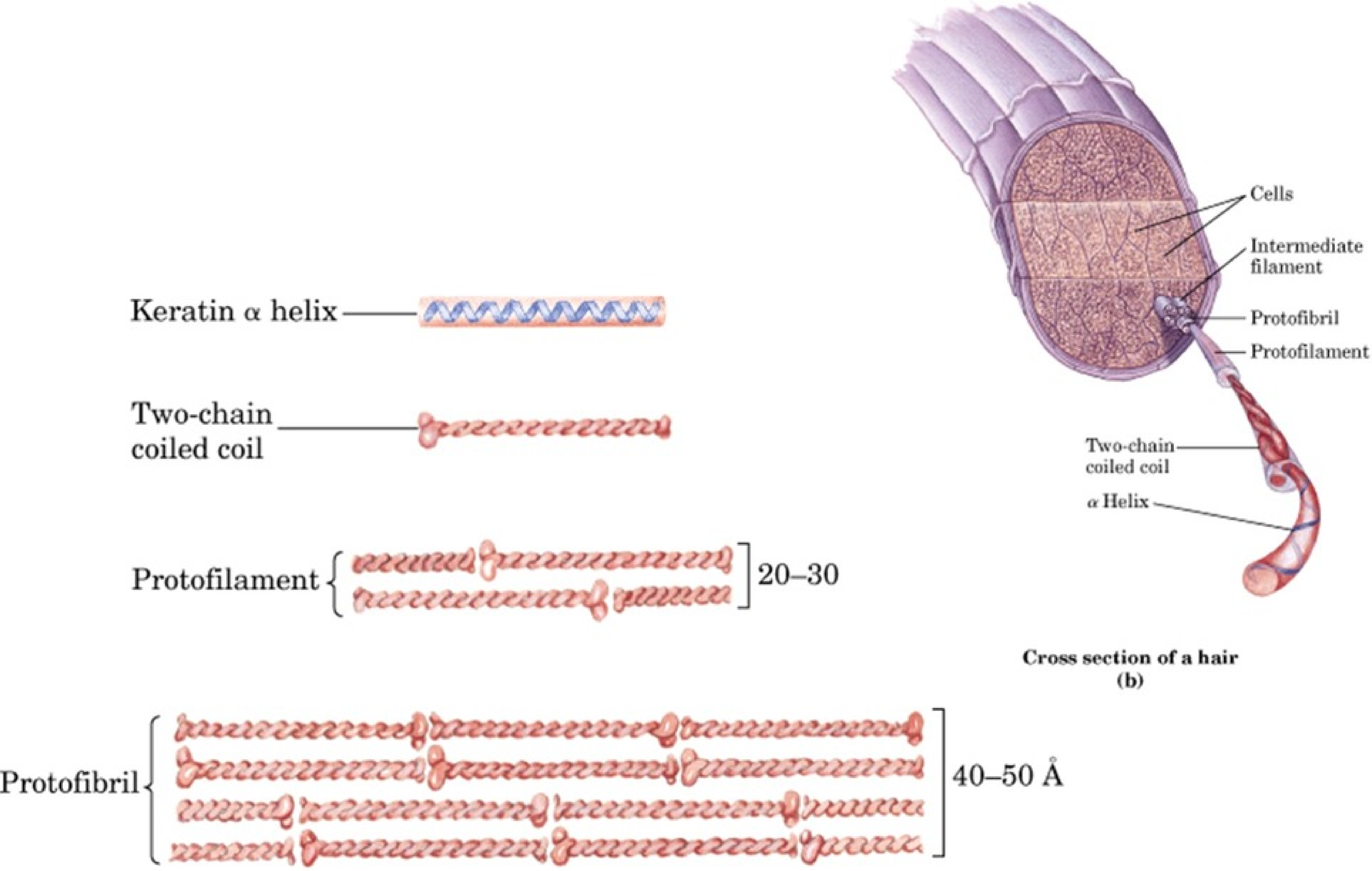


Estrutura Quaternária



Arranjo espacial entre subunidades

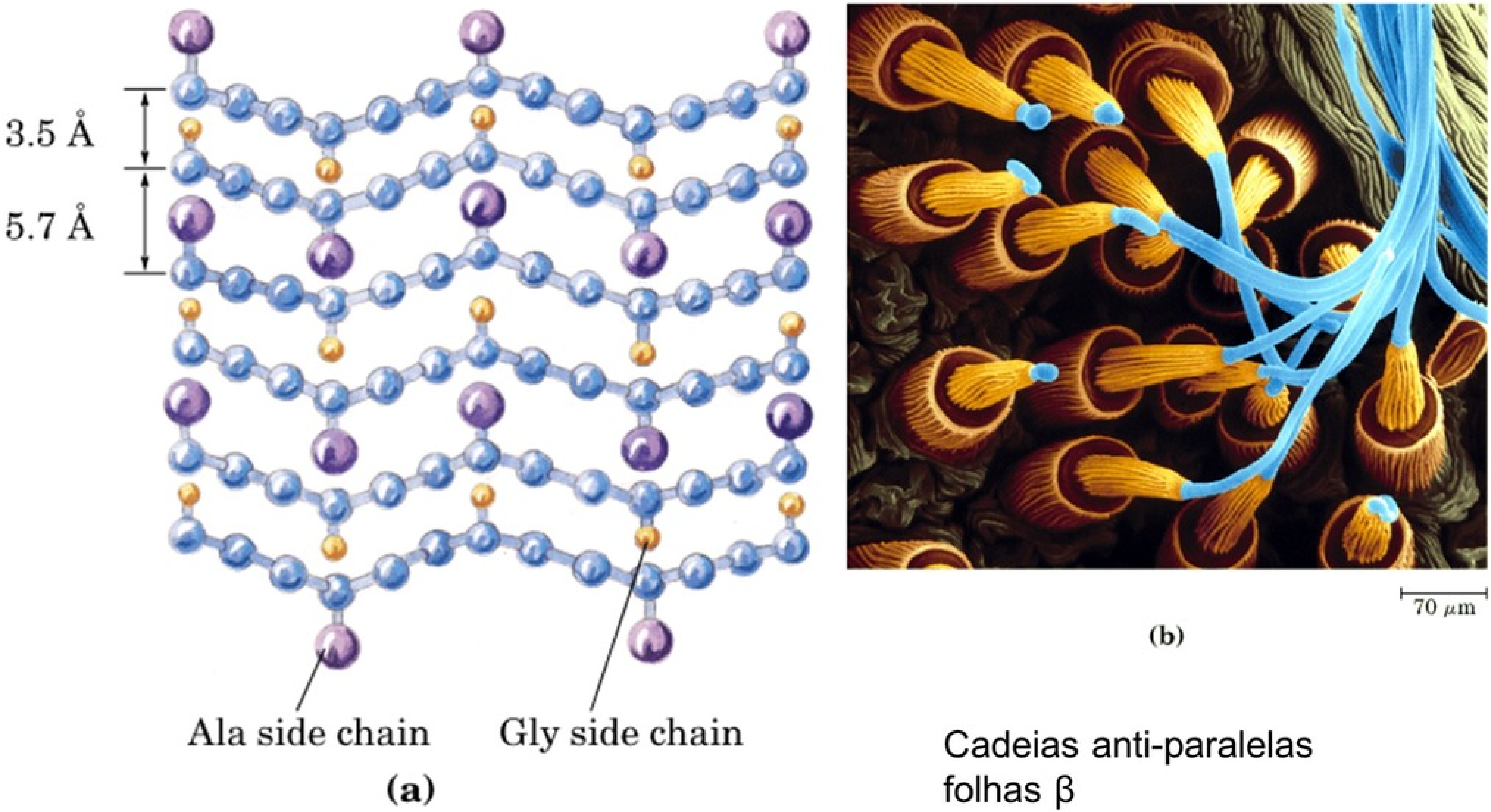
Proteínas fibrosas: α queratina



(a)

Cross section of a hair
(b)

Proteínas fibrosas: fibroína



Cadeias anti-paralelas
folhas β

Proteínas globulares: mioglobina

Grupo
prostético
(auxiliar)

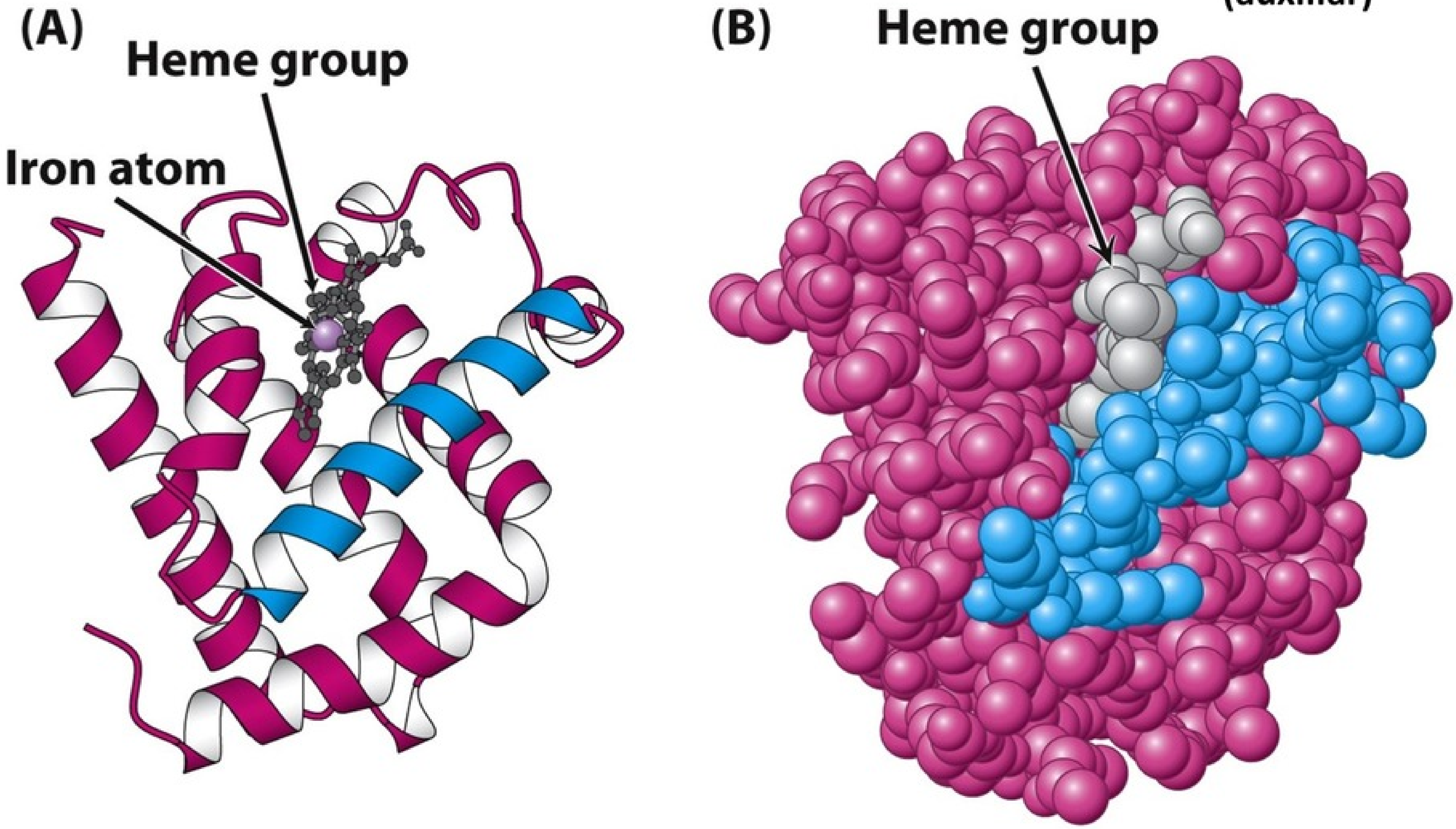


Figure 2-48
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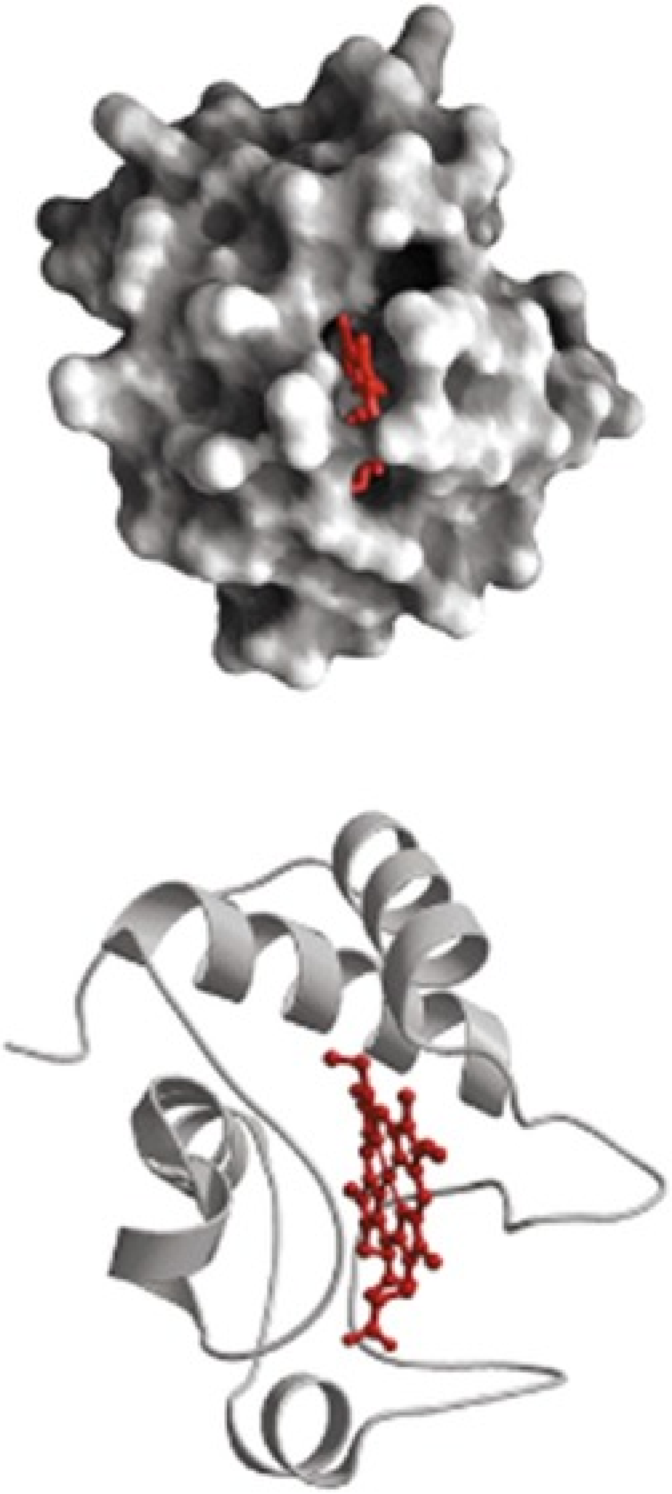
Poll

Numa proteína globular, que tipos de resíduos de aminoácidos você esperaria encontrar na parte interior e na superfície, respectivamente?

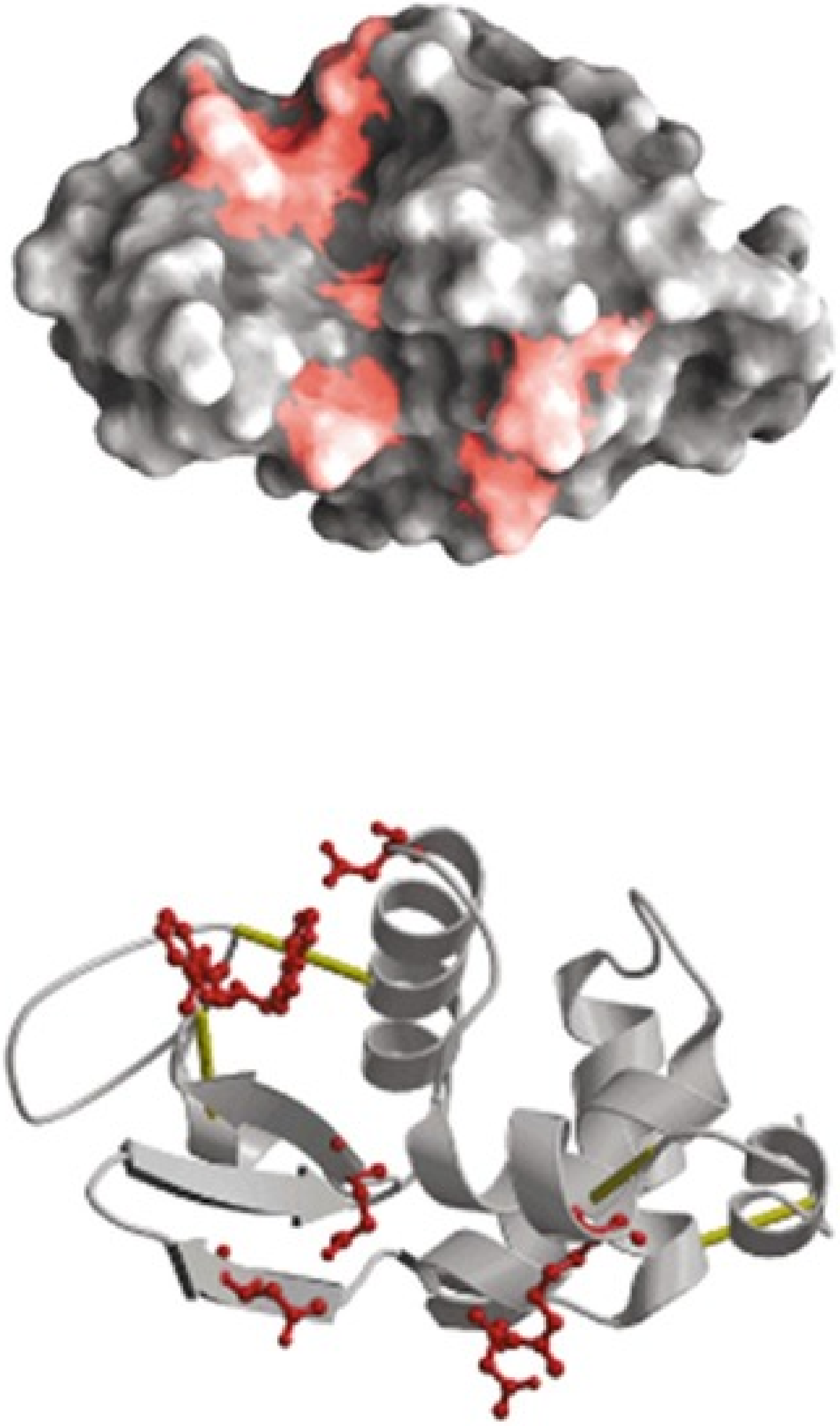
○ hidrofílicos, hidrofóbicos

○ hidrofóbicos, hidrofílicos

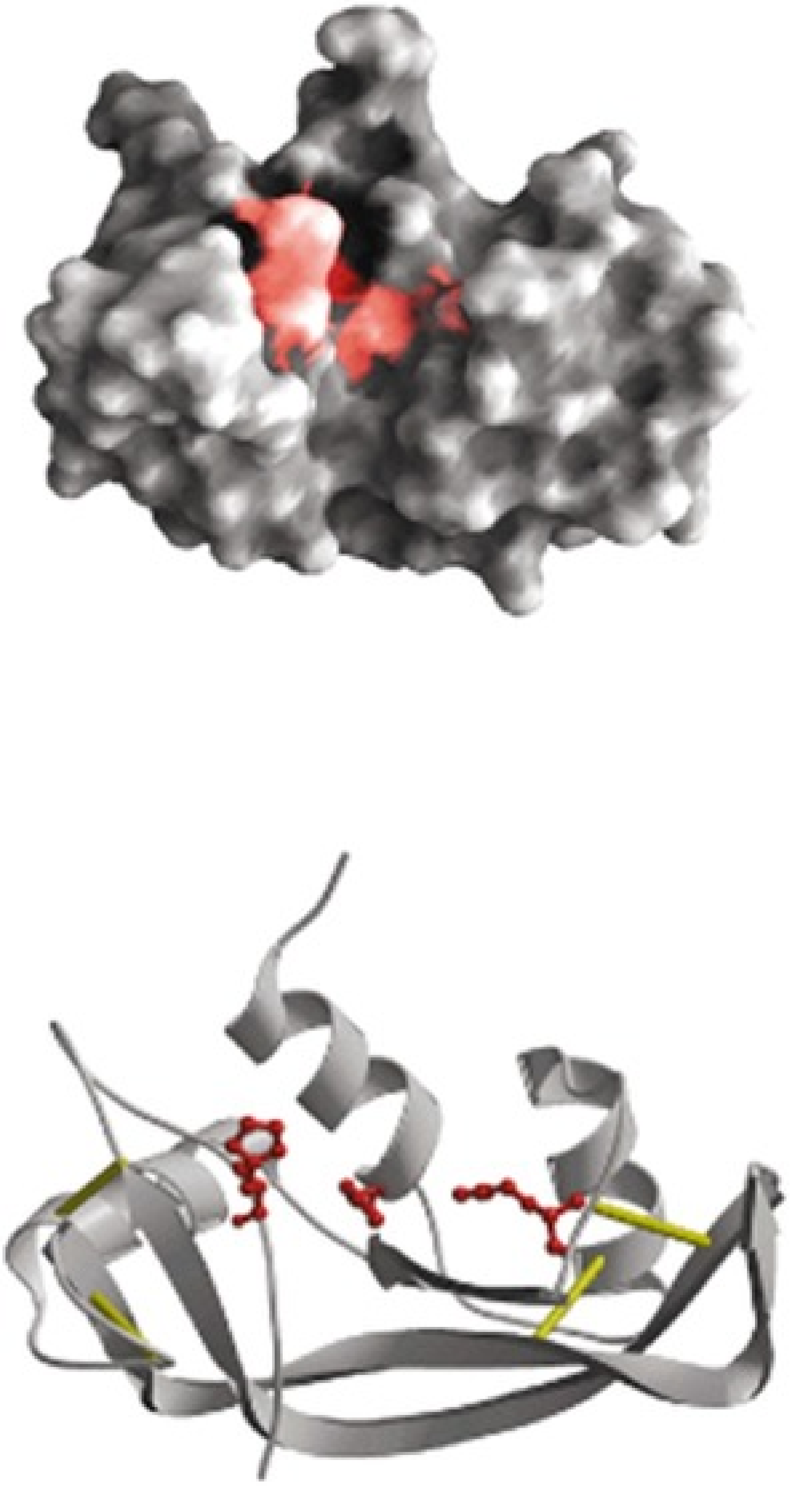
○ **sem carga, com carga**



Cytochrome *c*

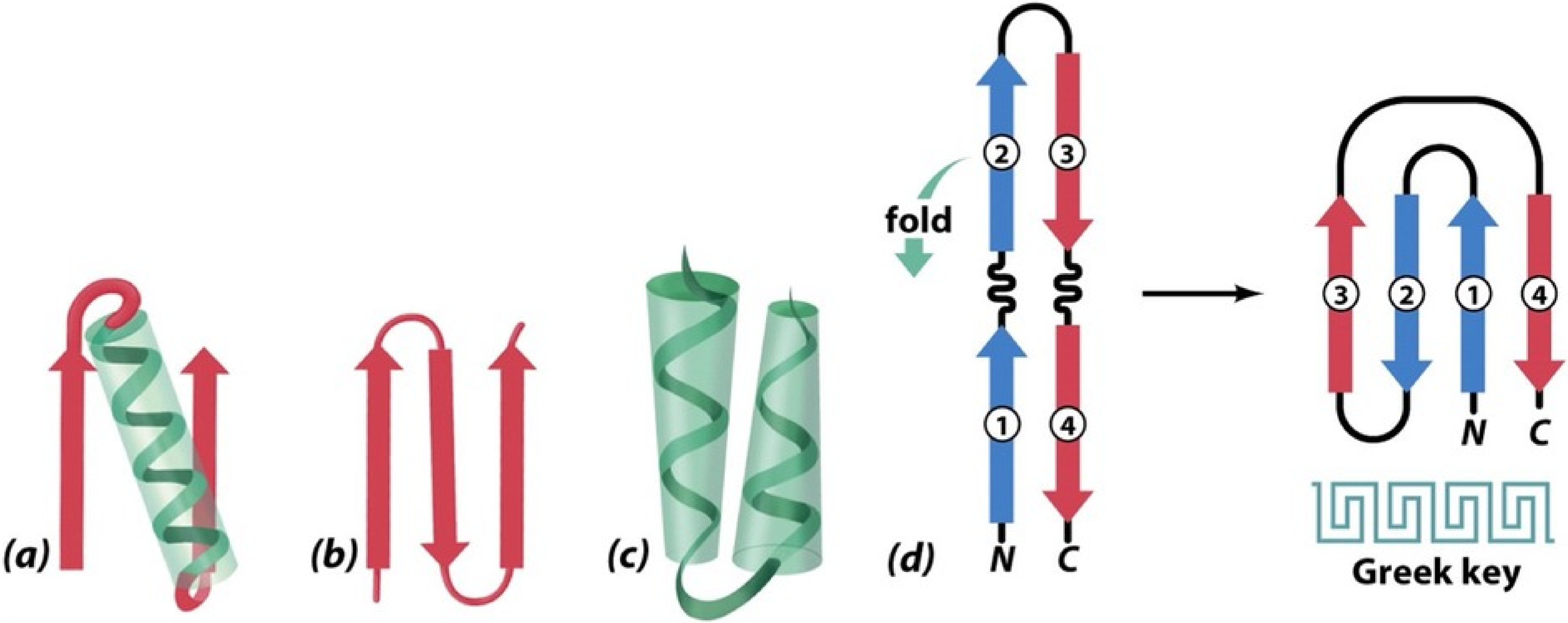


Lysozyme



Ribonuclease

Motivos
(ou estrutura supersecundária)



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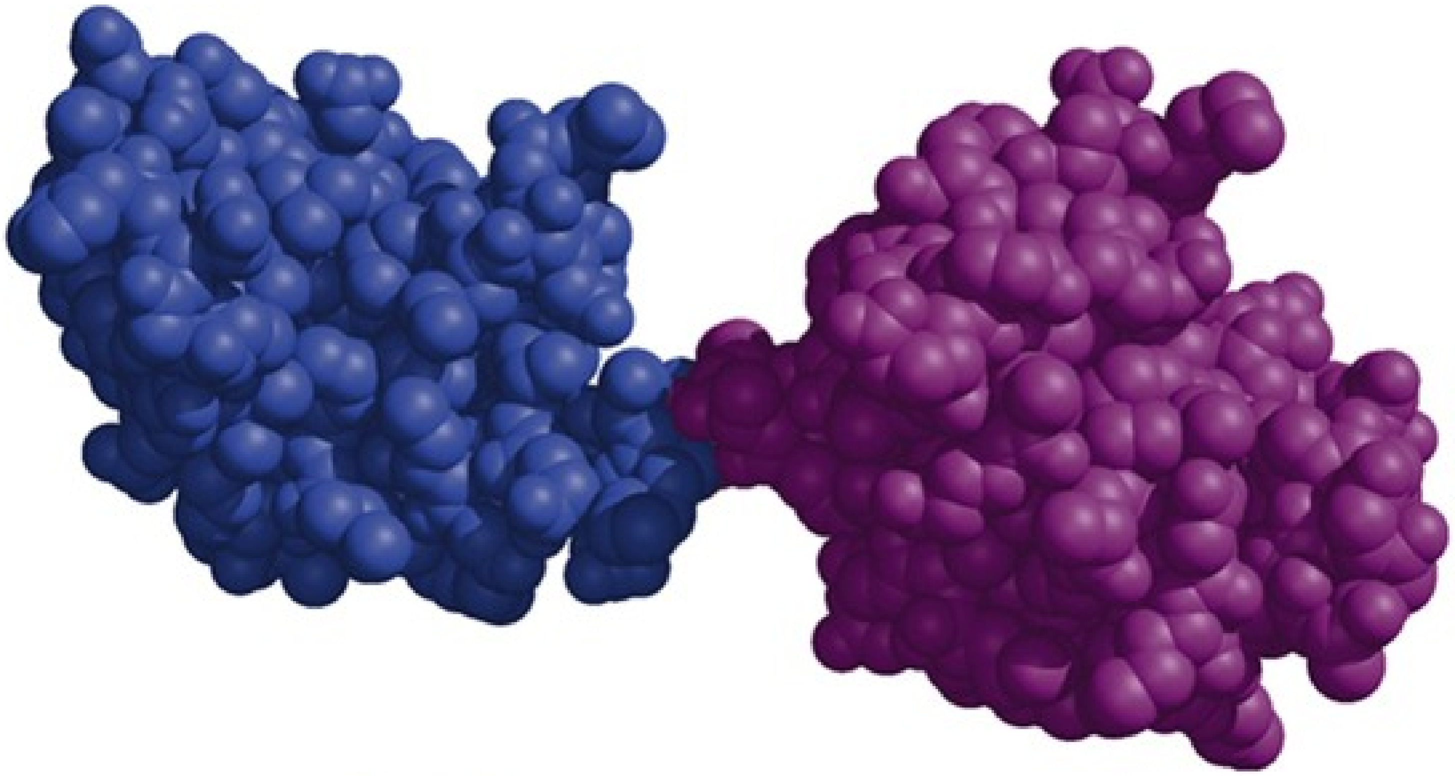
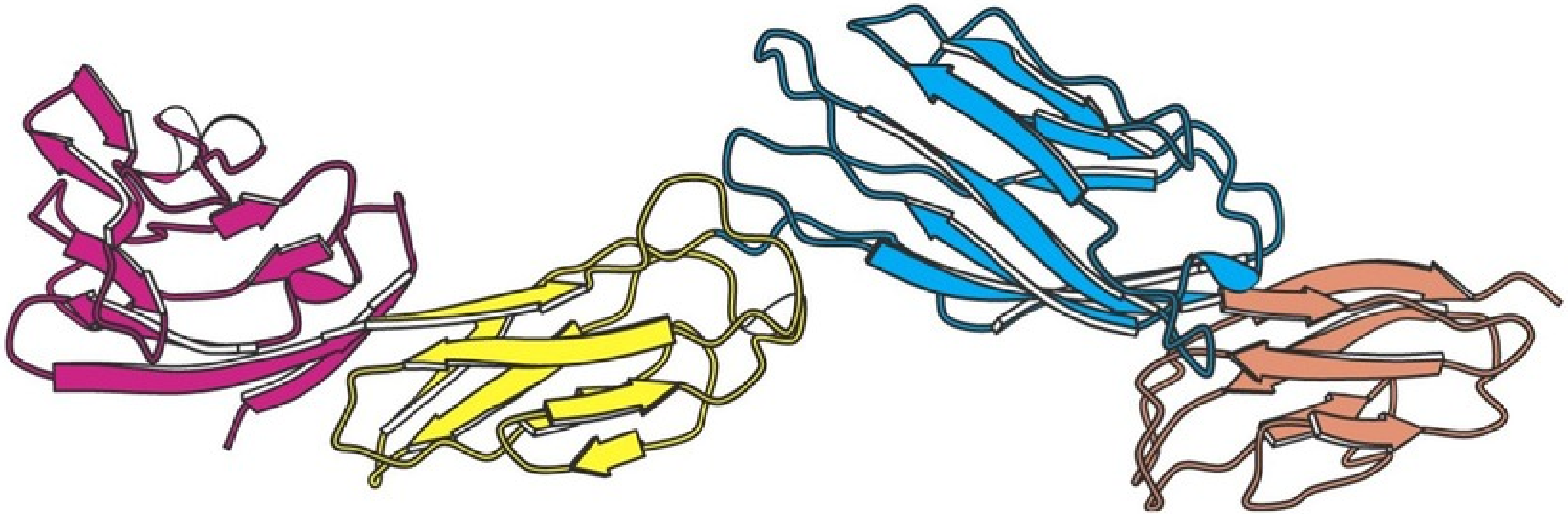
Grupos de elementos estruturais secundários

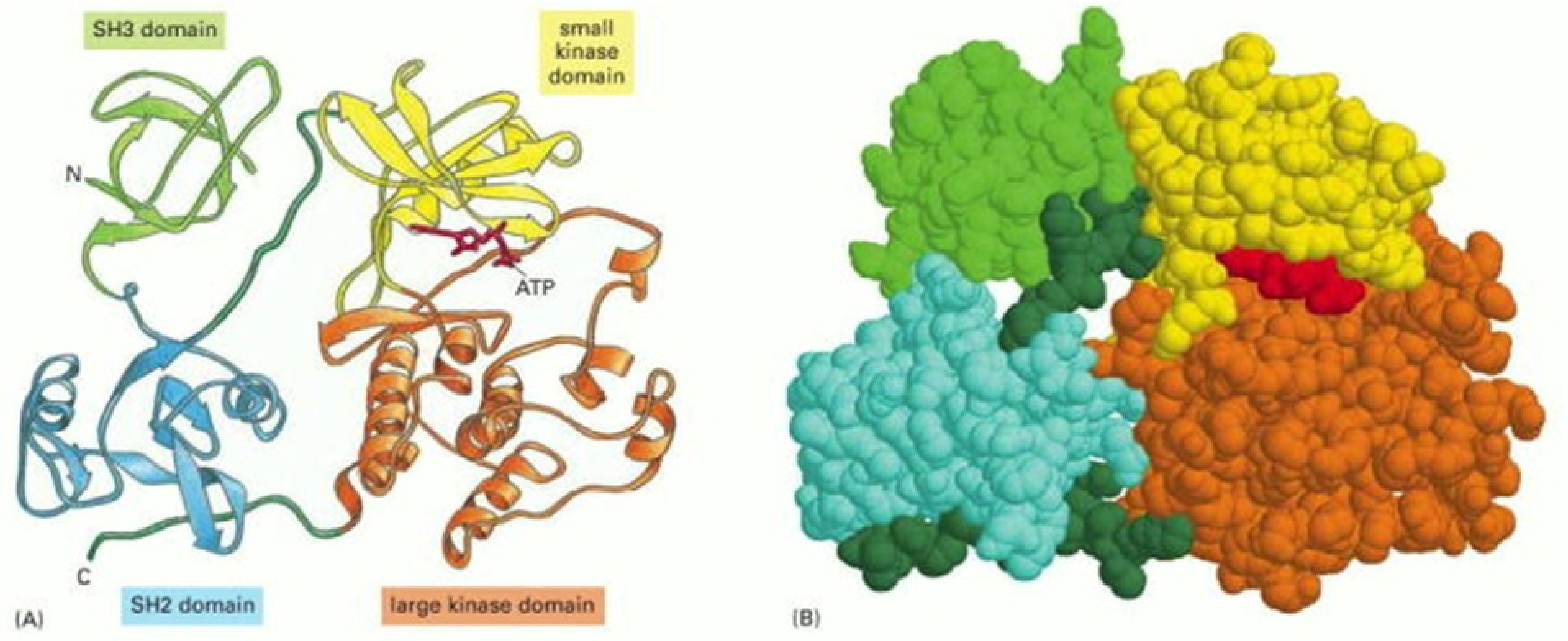
Domínios



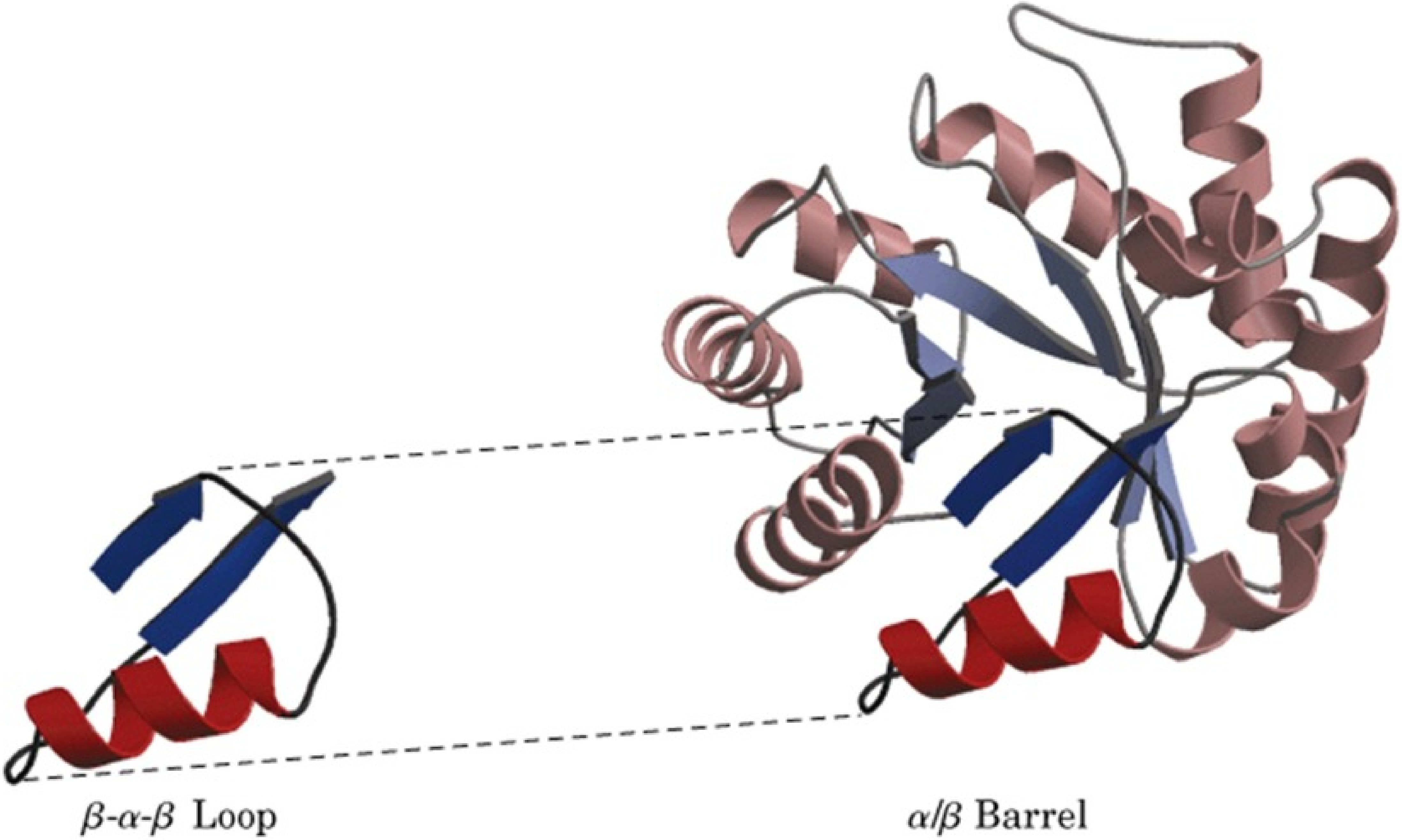


Proteínas globulares grandes, maiores q ~200 resíduos
Estruturalmente independentes
Funções específicas

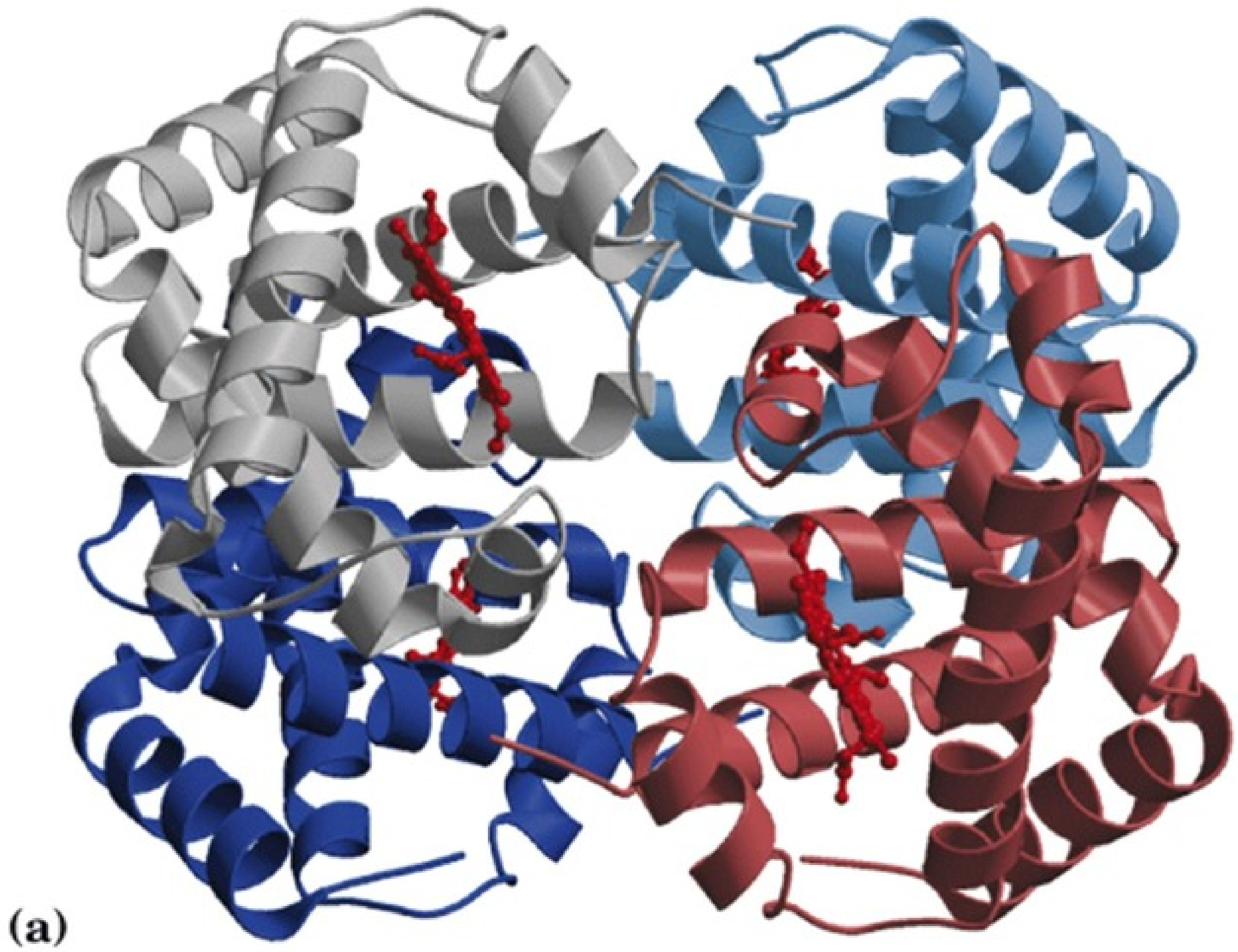
Proteína Formada por 4 Domínios

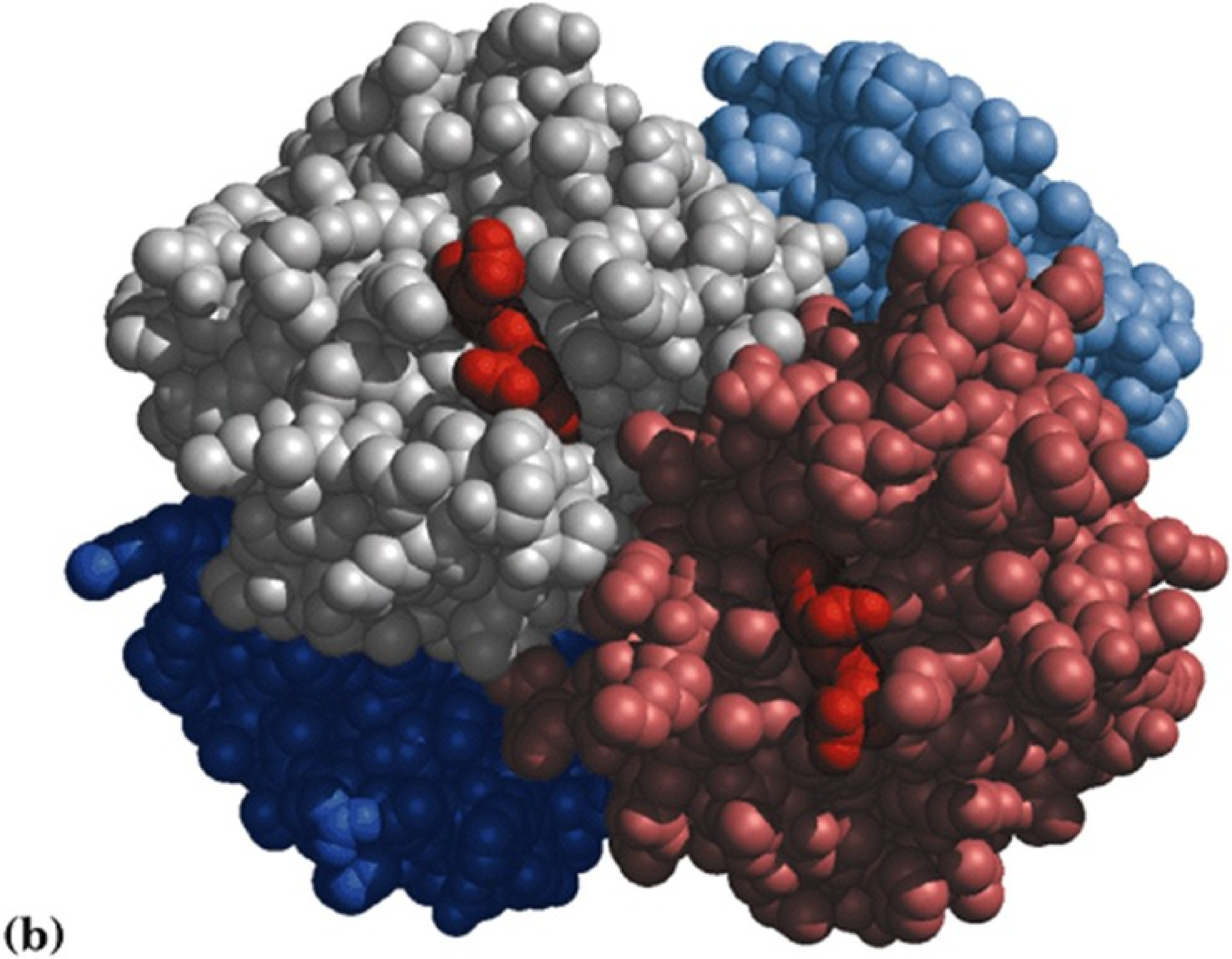


Base para classificação estrutural

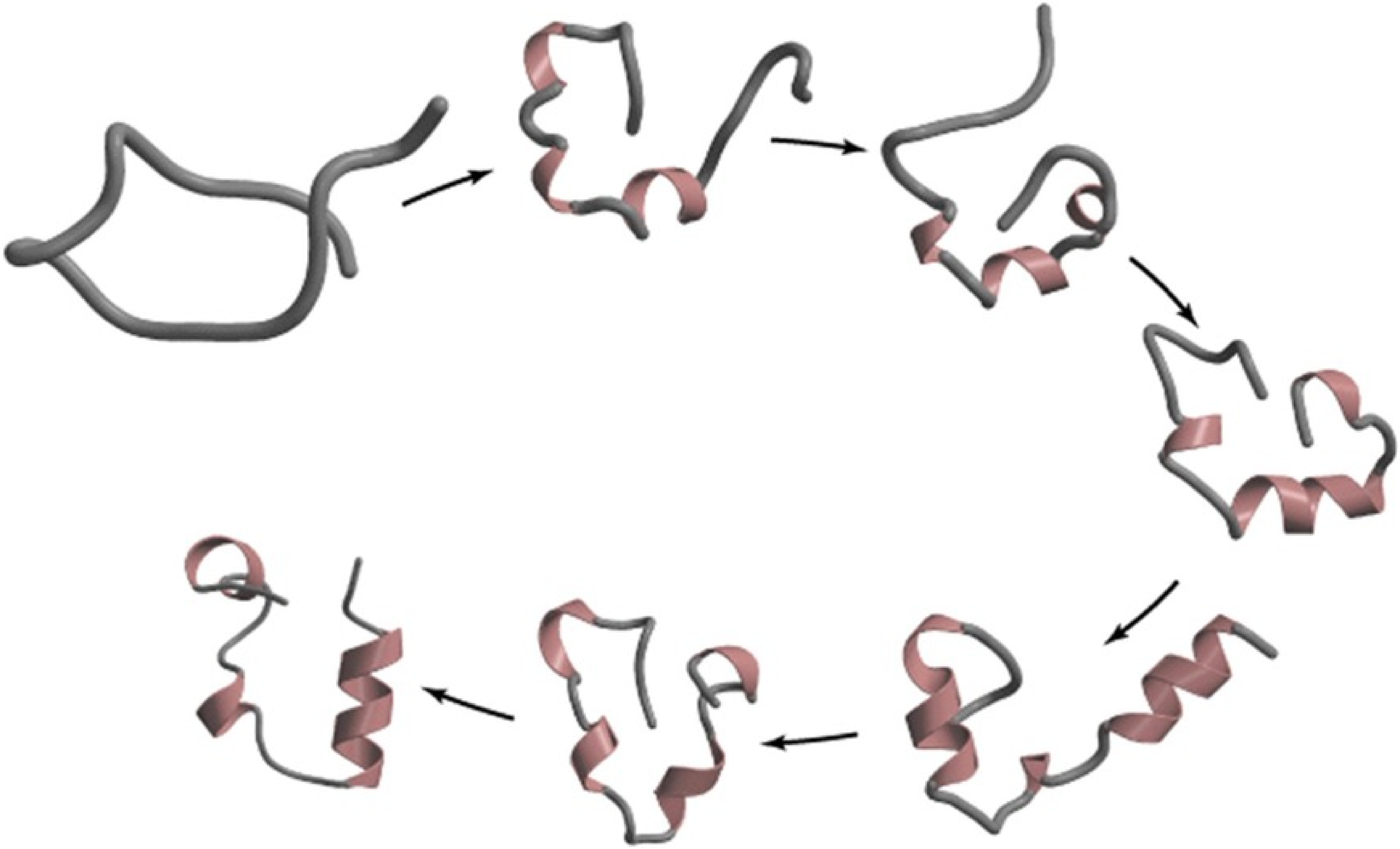


Hemoglobina

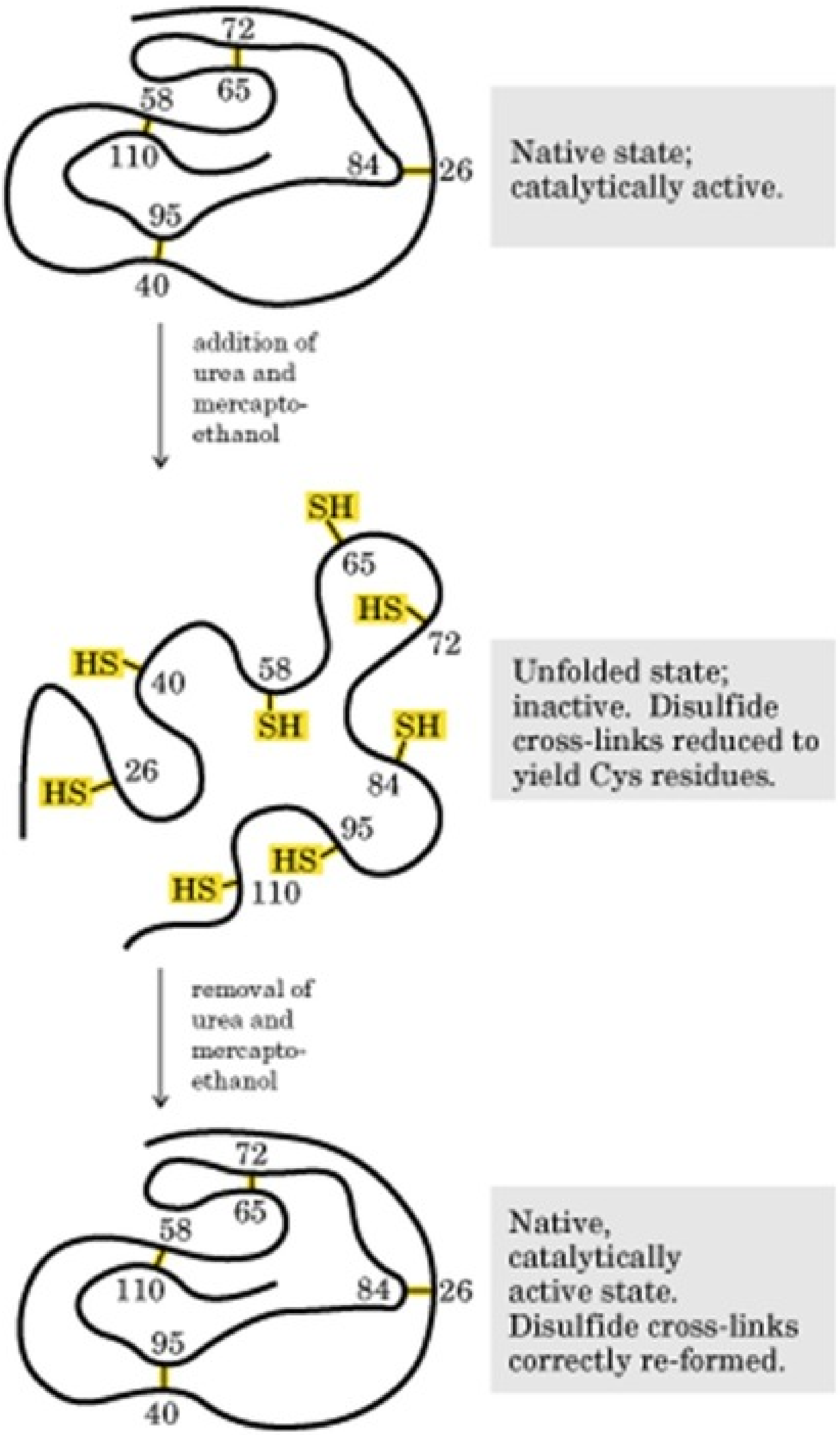



(b)

Enovelamento de proteínas



Sequencia de aa determina estrutura terciária

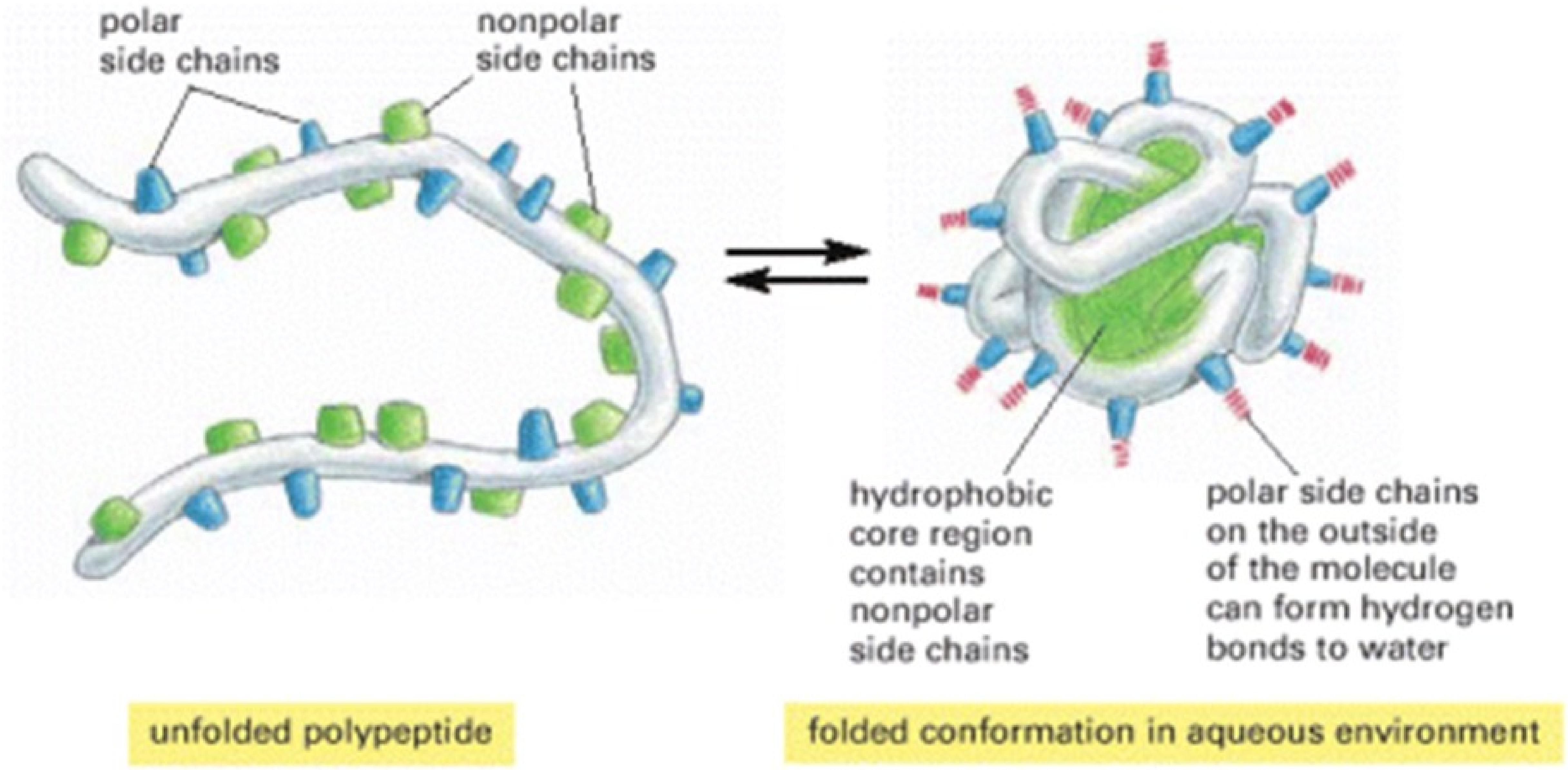


Native state; catalytically active.

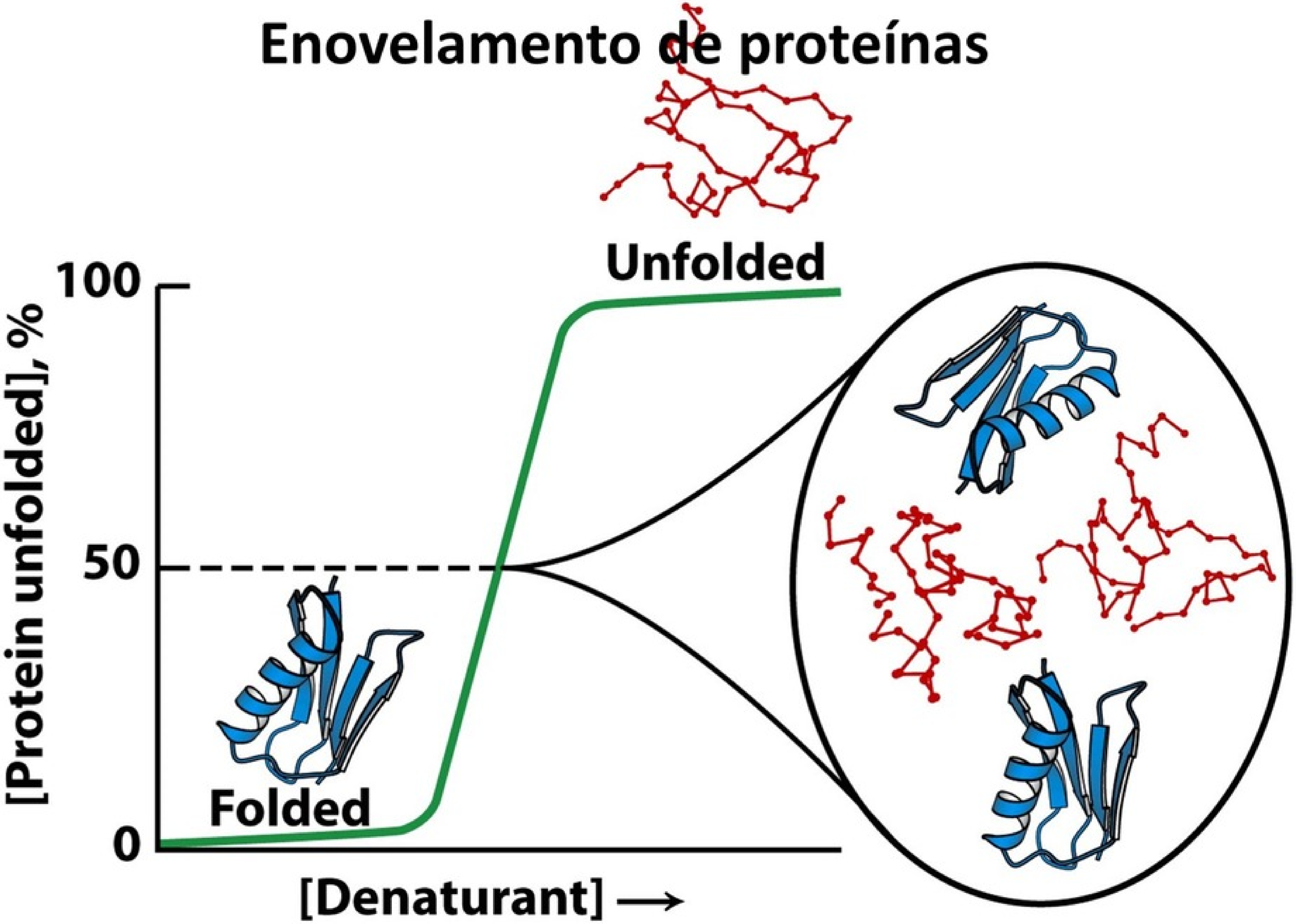
Unfolded state; inactive. Disulfide cross-links reduced to yield Cys residues.

Native, catalytically active state. Disulfide cross-links correctly re-formed.

Enovelamento de proteínas



Enovelamento de proteínas



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Enovelamento de proteínas

Proteína hipotética: 100 aa

Vamos supor que cada aa pode assumir 3 conformações
Número de estruturas possíveis = $3^{100} = 5 \times 10^{47}$

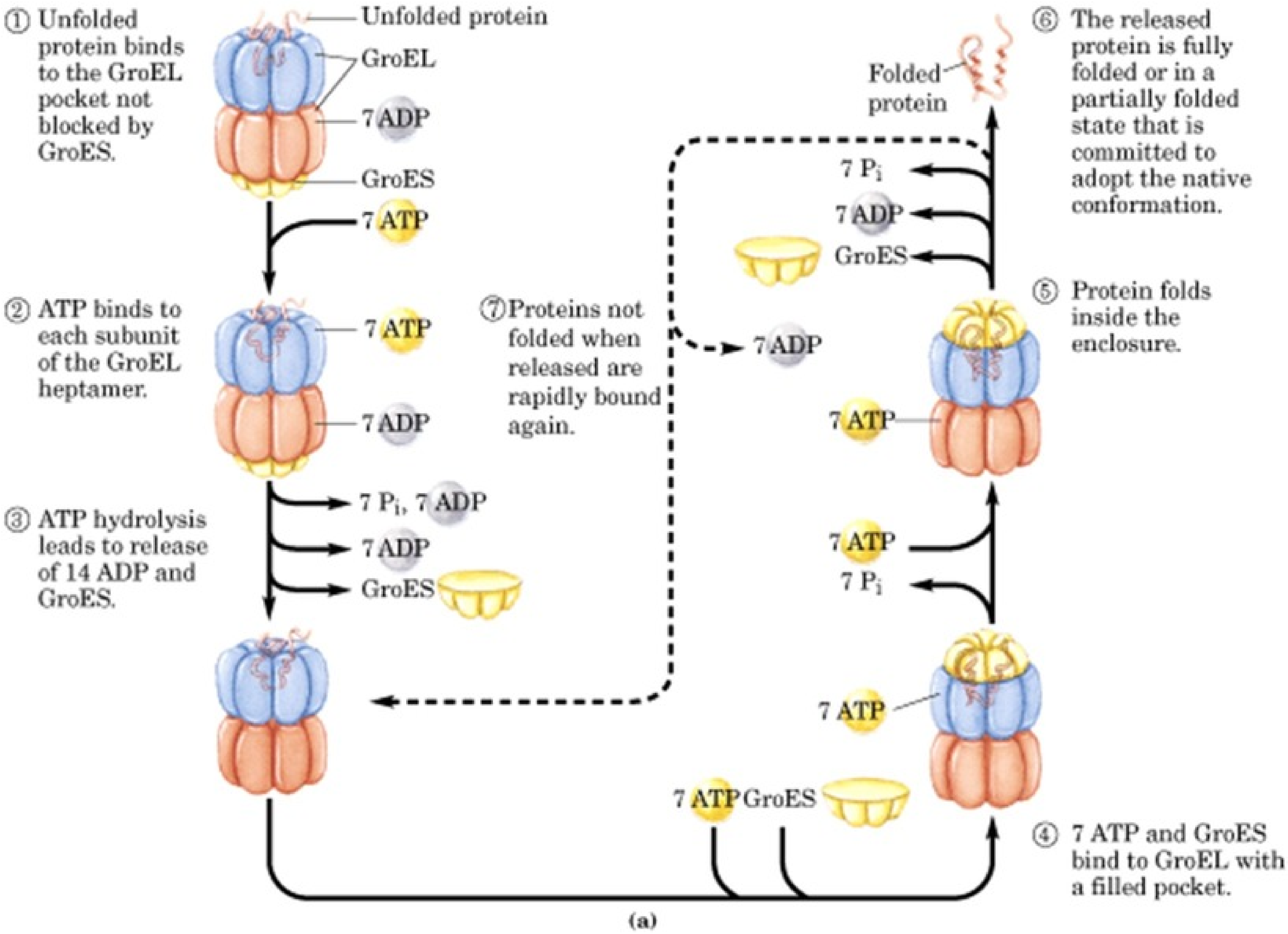
Tempo para transformar uma estrutura em outra = 10^{-13} s

Tempo total de busca = 5×10^{34} s = $1{,}67 \times 10^{27}$ anos!!!!

Processo cooperativo

Estabilização progressiva de intermediários

Chaperonas


① Unfolded protein binds to the GroEL pocket not blocked by GroES.
Unfolded protein
GroEL
7 ADP
GroES
7 ATP
② ATP binds to each subunit of the GroEL heptamer.
7 ATP
7 ADP
③ ATP hydrolysis leads to release of 14 ADP and GroES.
7 P_i, 7 ADP
7 ADP
GroES
⑦ Proteins not folded when released are rapidly bound again.
7 ADP
Folded protein
⑥ The released protein is fully folded or in a partially folded state that is committed to adopt the native conformation.
7 P_i
7 ADP
GroES
⑤ Protein folds inside the enclosure.
7 ATP
7 ATP
7 P_i
7 ATP
7 ATP
GroES
④ 7 ATP and GroES bind to GroEL with a filled pocket.
(a)

Predição de estruturas

CASP
Critical Assessment of Techniques for Protein Structure Prediction

community-wide, worldwide experiment (though it is commonly referred to as a competition) for protein structure prediction

Ab initio
Comparative protein modelling

http://predictioncenter.org/index.cgi

Determinação experimental de estrutura 3D

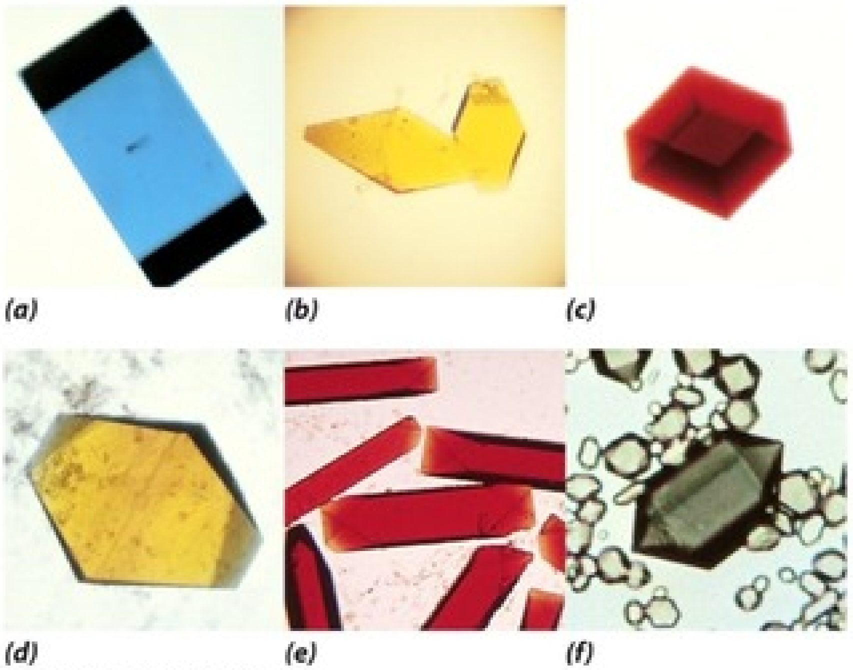
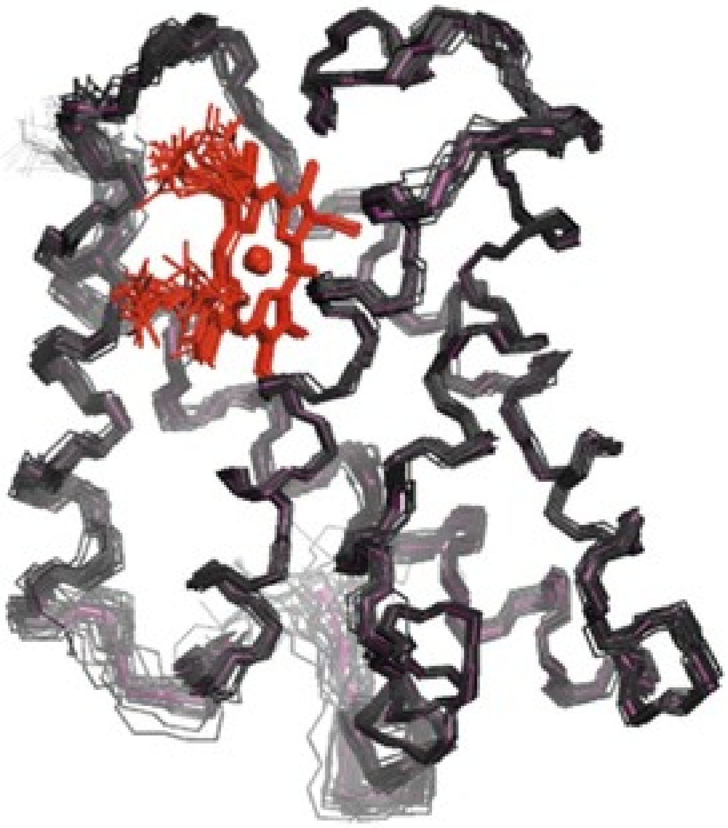
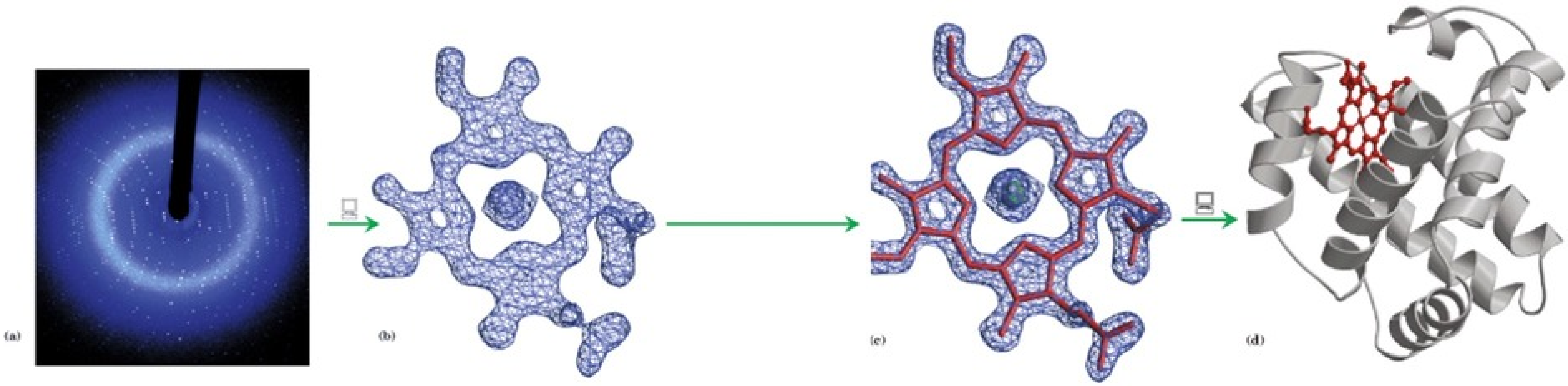
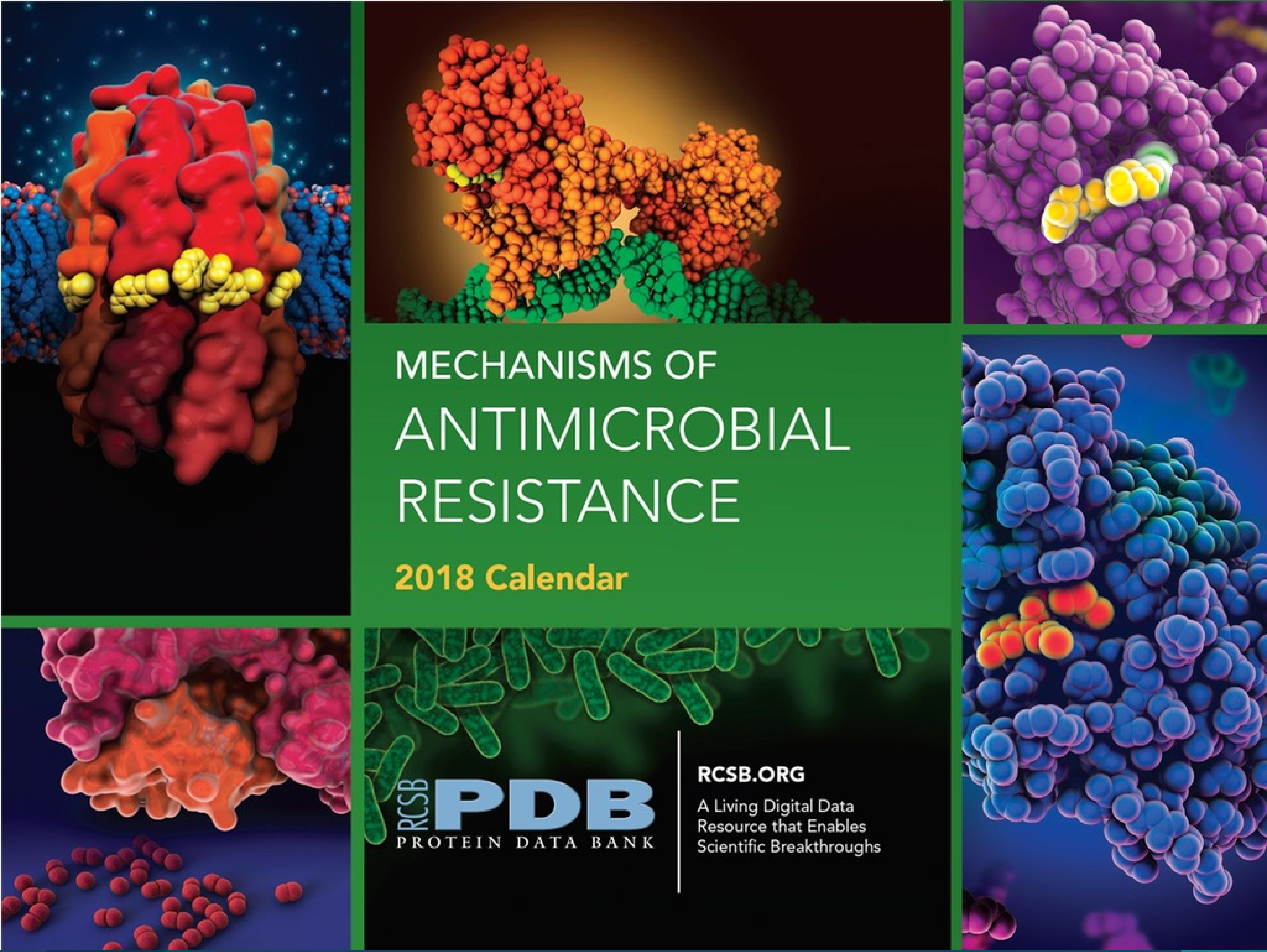


Figure 6-21 Fundamentals of Biochemistry, 2/e



(e)





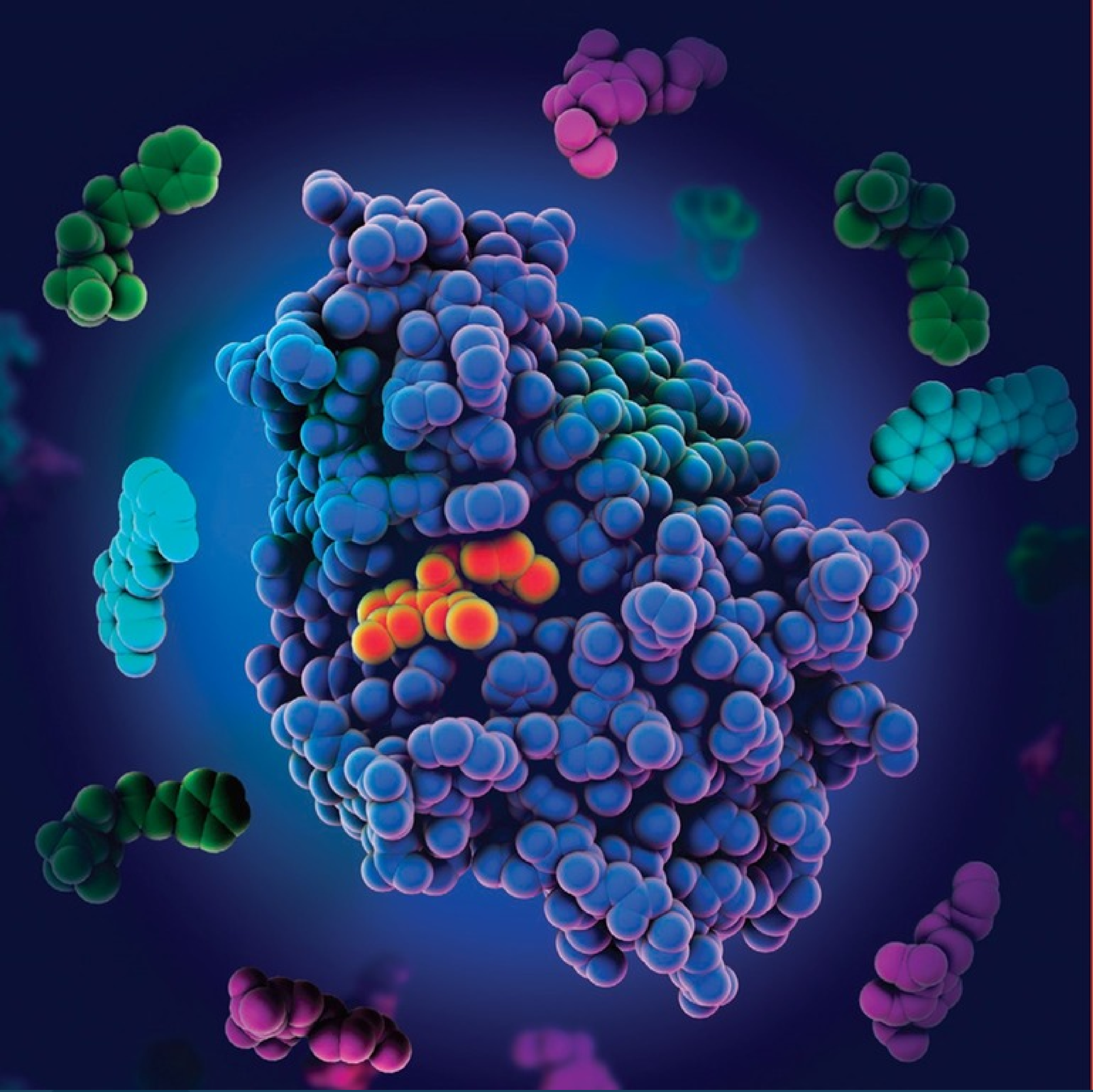
MECHANISMS OF
ANTIMICROBIAL
RESISTANCE

2018 Calendar

RCSB PDB
PROTEIN DATA BANK

RCSB.ORG

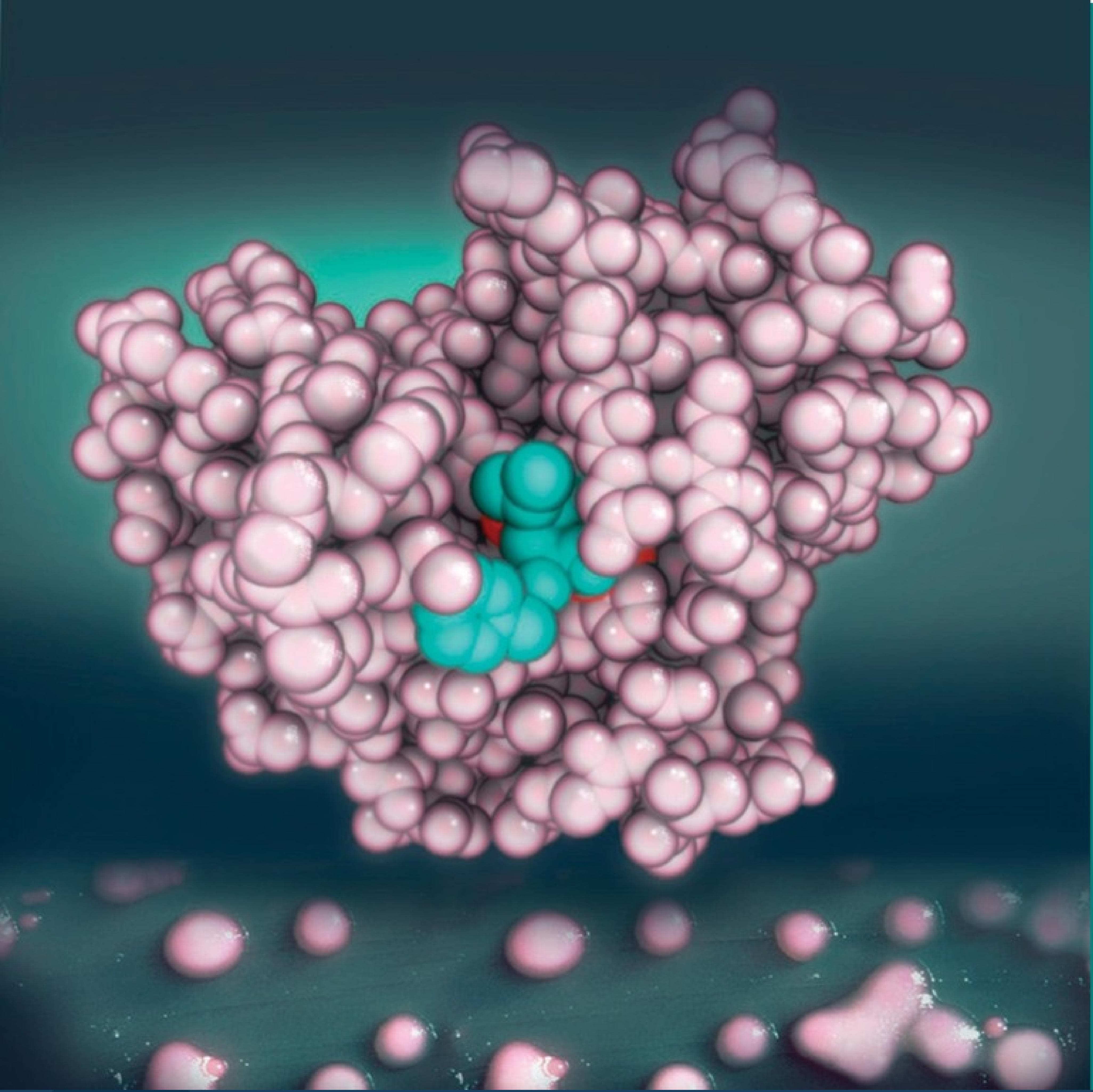
A Living Digital Data
Resource that Enables
Scientific Breakthroughs



KPC-2 is another "versatile beta-lactamase", with a large and shallow active site that breaks down nearly all known beta-lactam antibiotics. This structure captures the enzyme after it has destroyed a molecule of the broad-spectrum antibiotic cefotaxime.

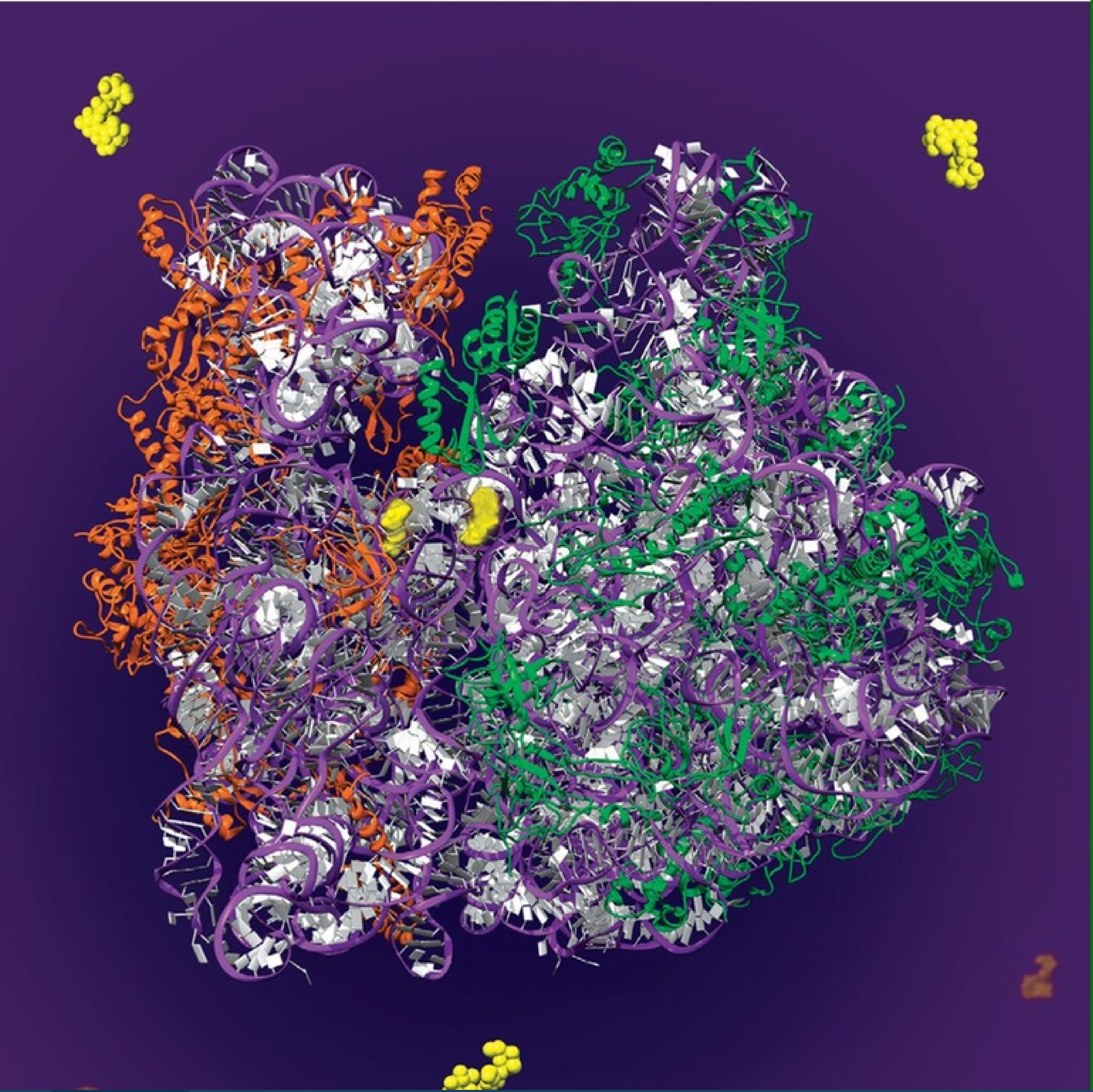
PDB ID 5uj3

Pemberton, O.A. *et al.* Molecular basis of substrate recognition and product release by the *Klebsiella pneumoniae* carbapenemase (KPC-2). *J Med Chem* **60**, 3525-3530 (2017)



Bacteria that possess NDM-1 metallo-lactamase enzymes pose a great danger because they can inactivate all approved penicillin-like antibiotics, including our most advanced carbapenems.
PDB structures, such as this one with benzylpenicillin, have revealed that NDM-1 uses zinc in its active site to inactivate a wide range of different antibiotics.

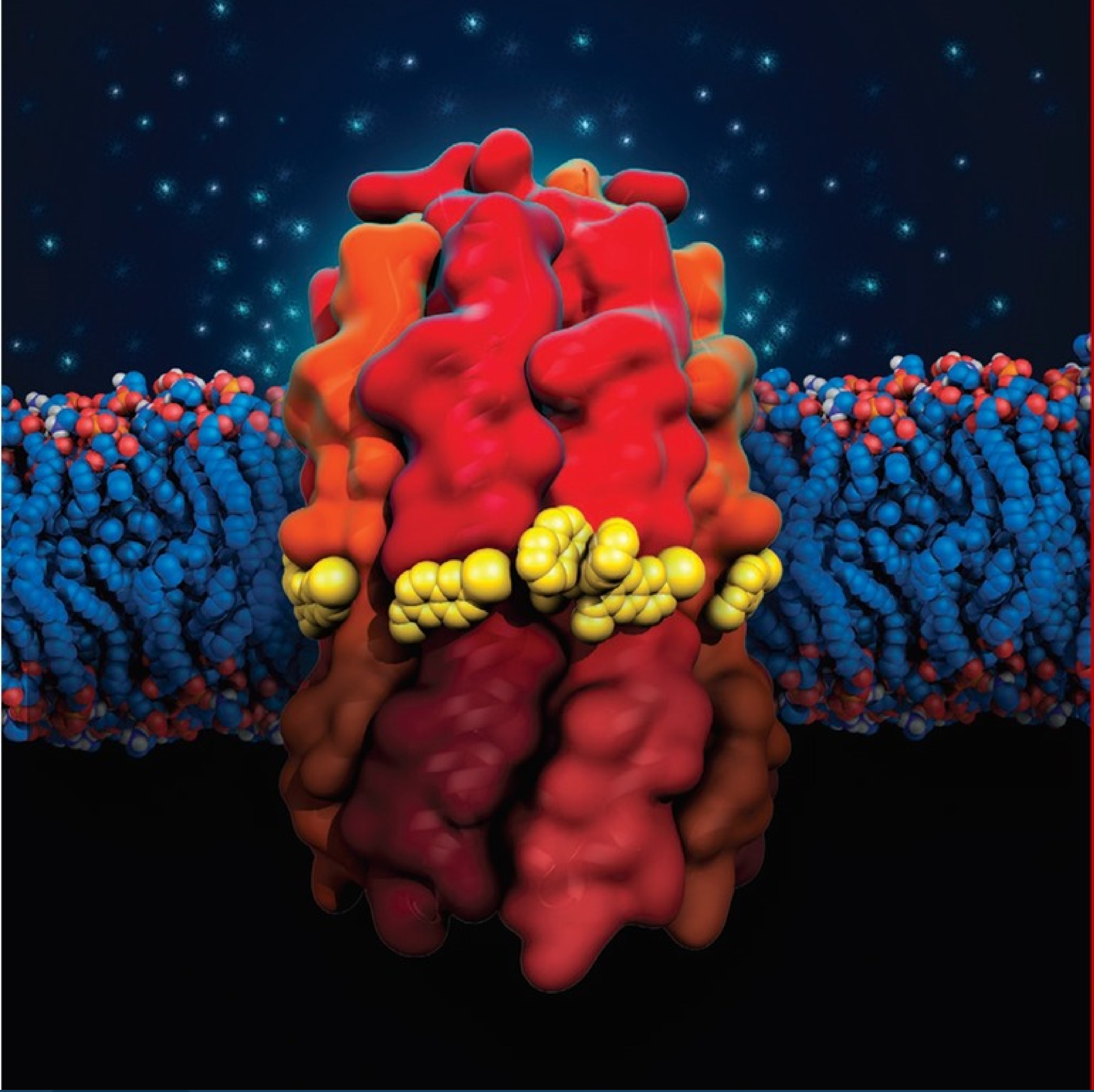
PDB ID 4eyf
King, D.T. *et al.* New Delhi metallo-beta-lactamase: structural insights into beta-lactam recognition and inhibition. *J Am Chem Soc* 134, 11362-11365 (2012)



Aminoglycosides are effective broad-spectrum antibiotics, because they target ribosomes in many types of bacteria. This structure shows how neomycin (yellow) binds to ribosomes and shifts their structure, blocking recycling of ribosomes after they finish building a protein.

PDB ID 4v52

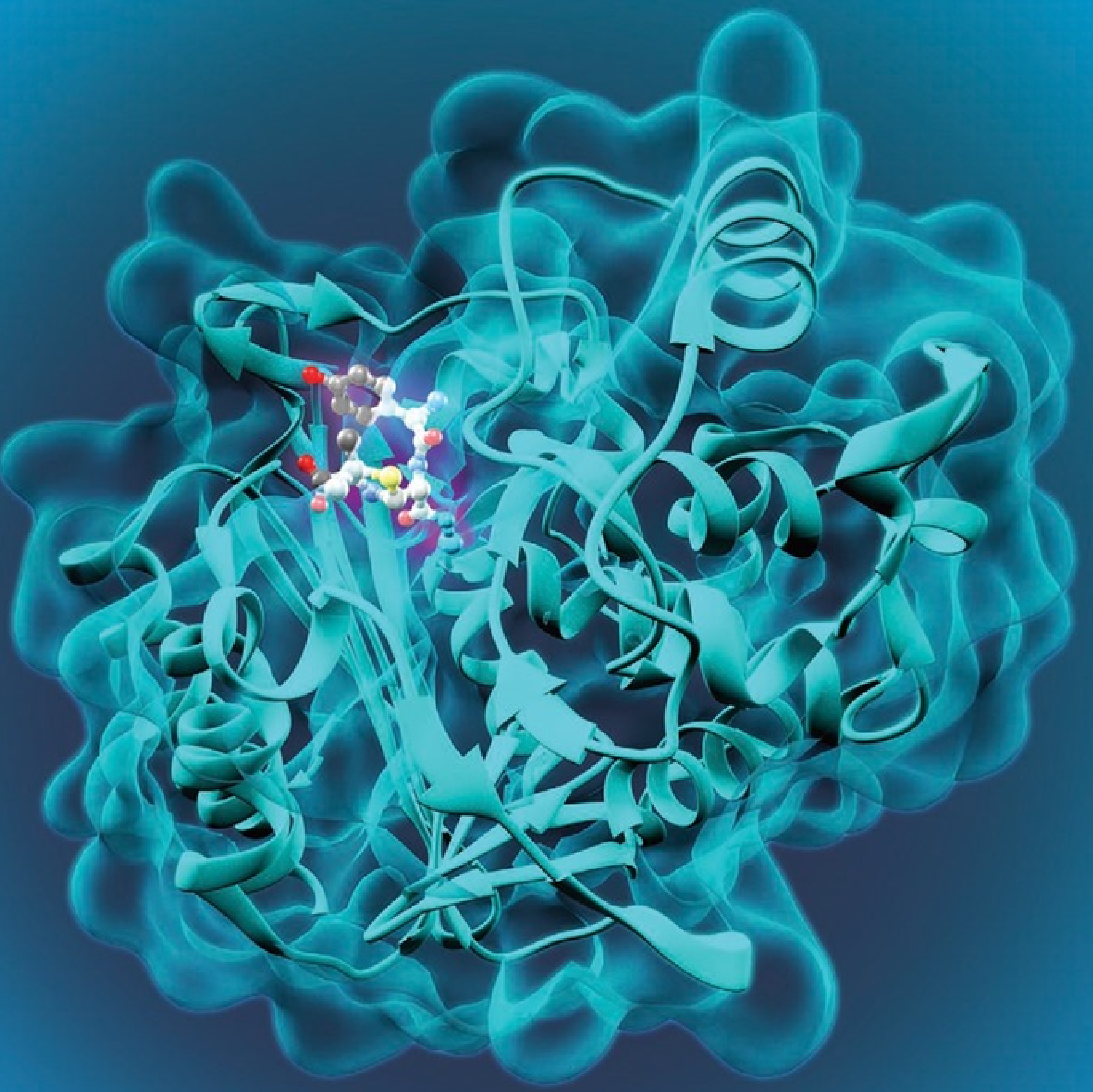
Borovinskaya, M.A. *et al.* Structural basis for aminoglycoside inhibition of bacterial ribosome recycling. *Nat Struct Mol Biol* **14**, 727-732 (2007)



After 40 years of searching, researchers have discovered bedaquiline (shown in yellow), a new drug now approved for the treatment of multidrug-resistant tuberculosis. It binds to the rotor of ATP synthase, blocking the central energy-production machinery of mycobacteria.

PDB ID 4v1f

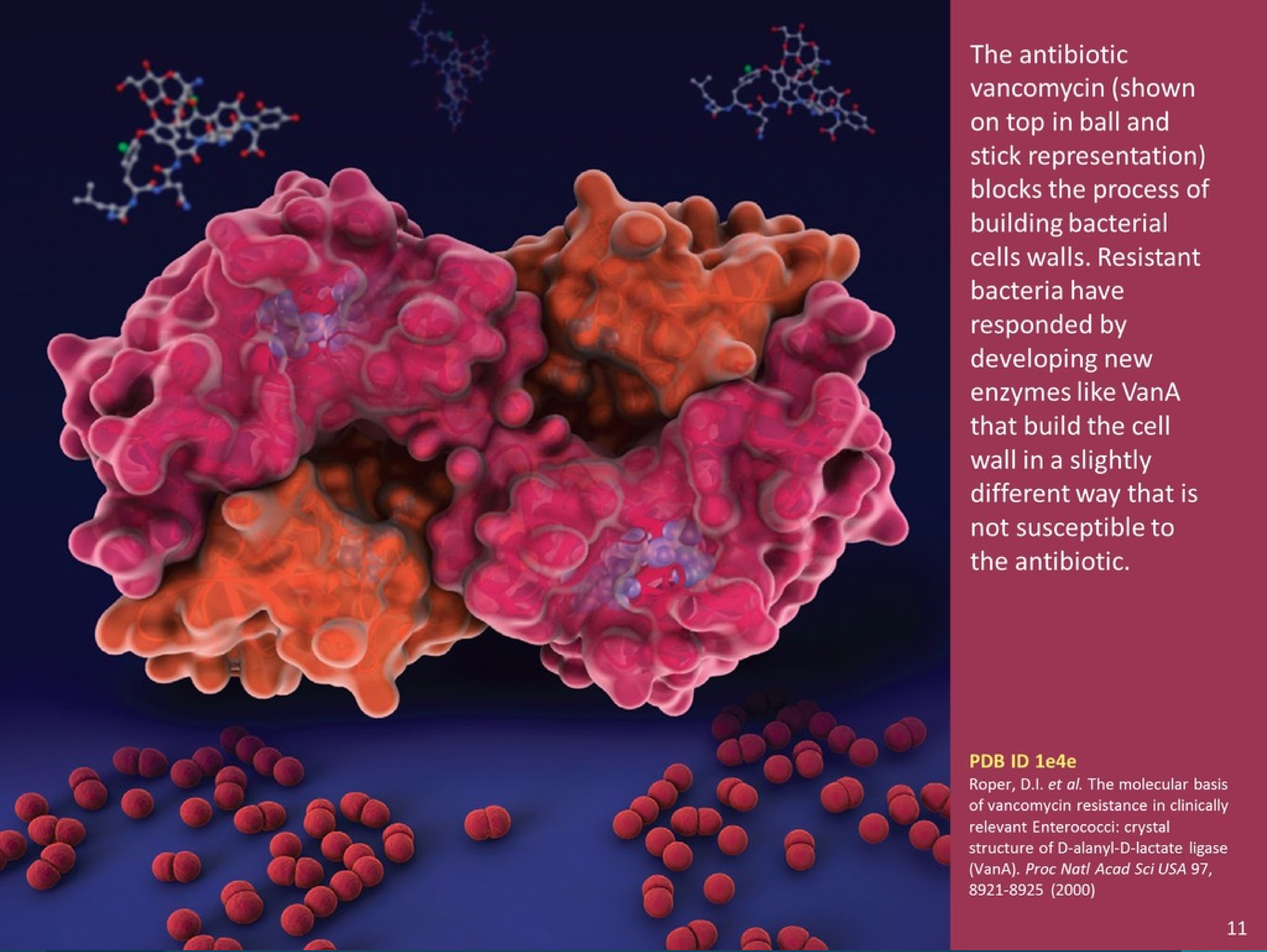
Preiss, L. *et al.* Structure of the mycobacterial ATP synthase Fo rotor ring in complex with the anti-TB drug bedaquiline. *Sci Adv* **1**, e1500106 (2015)



Beta-lactamases usually confer resistance by breaking the beta-lactam ring of penicillin-like antibiotics. To overcome resistance, researchers are working to design inhibitors of beta-lactamases that can be given together with established beta-lactam antibiotics.

PDB ID 1ll9

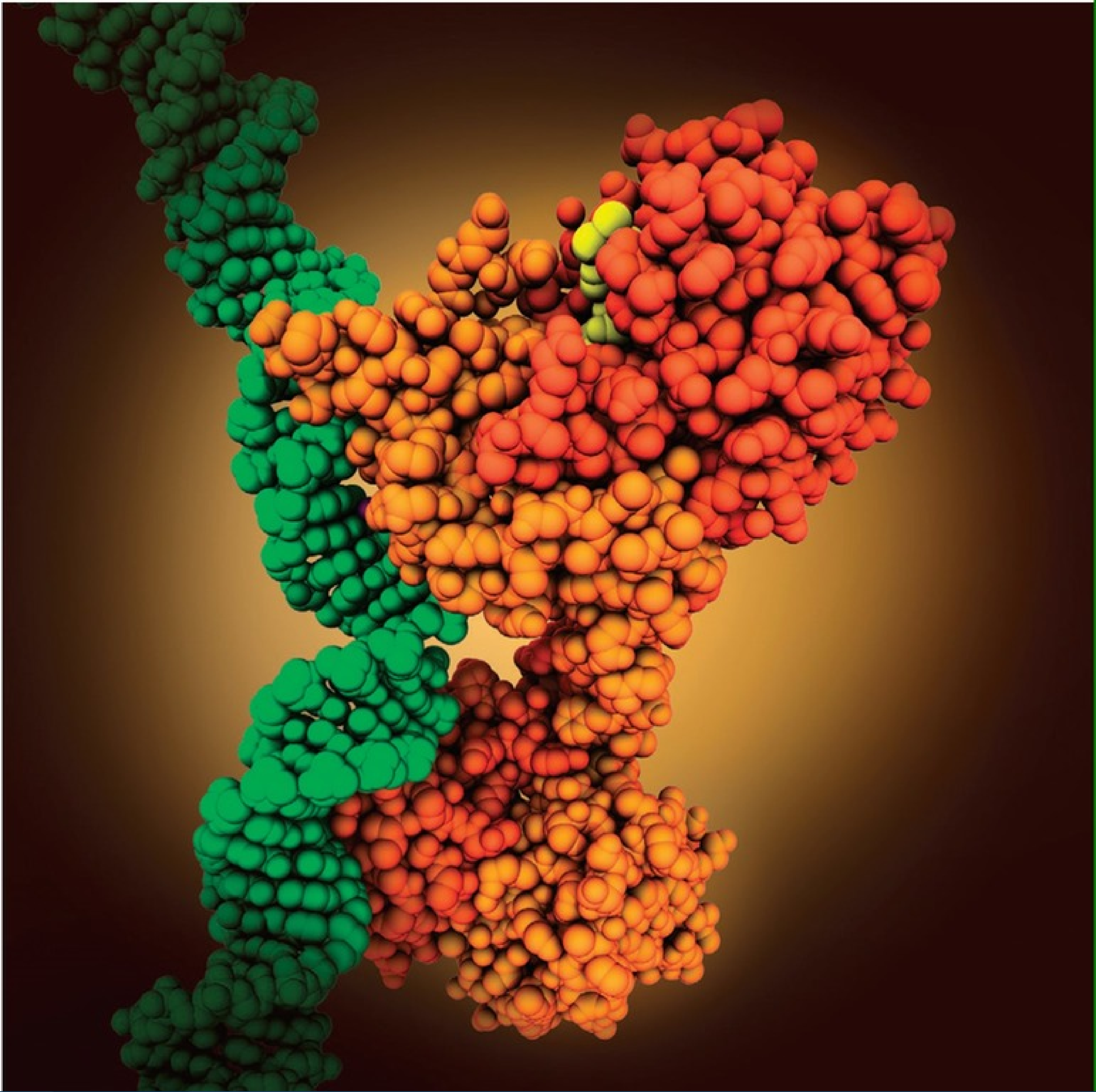
Trehan, I. *et al.* Using steric hindrance to design new inhibitors of class C beta-lactamases. *Chem Biol* 9, 971-980 (2002)



The antibiotic vancomycin (shown on top in ball and stick representation) blocks the process of building bacterial cells walls. Resistant bacteria have responded by developing new enzymes like VanA that build the cell wall in a slightly different way that is not susceptible to the antibiotic.

PDB ID 1e4e

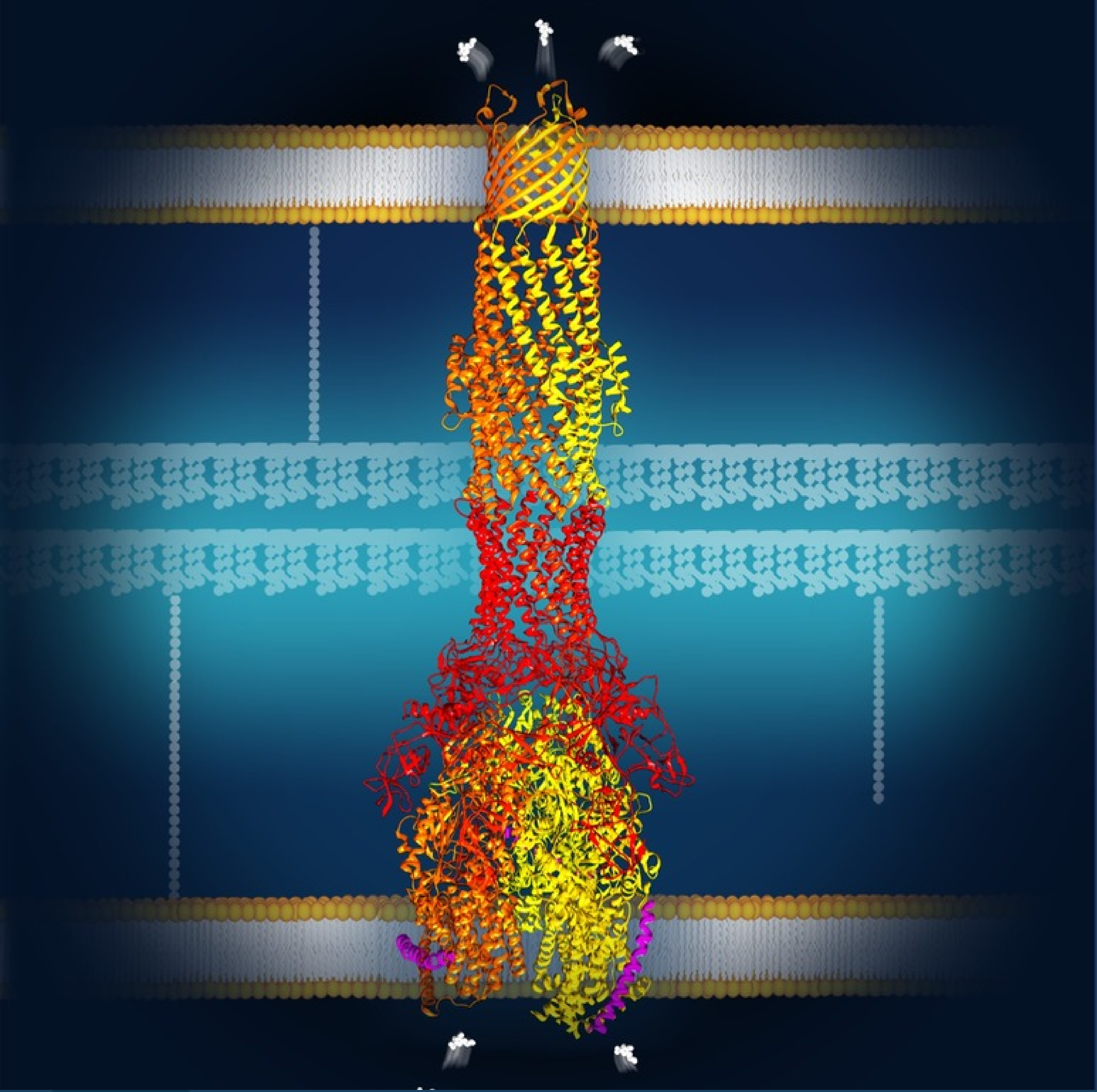
Roper, D.I. *et al.* The molecular basis of vancomycin resistance in clinically relevant Enterococci: crystal structure of D-alanyl-D-lactate ligase (VanA). *Proc Natl Acad Sci USA* 97, 8921-8925 (2000)



The gene regulator BmrR senses when antibiotics (such as puromycin, in yellow) get into a bacterial cell, directing the synthesis of multidrug efflux pumps to eject them out of the cell.

PDB ID 3q3d

Bachas, S. *et al.* Structural contributions to multidrug recognition in the multidrug resistance (MDR) gene regulator, BmrR. *Proc Natl Acad Sci USA* **108**, 11046-11051 (2011)



Bacteria build huge protein pumps that span their cell walls and expel many types of antibiotics. This cryoEM structure reveals how the pump works to expel antibiotics from bacterial cells.

PDB ID 5o66

Wang, Z. *et al.* An allosteric transport mechanism for the AcrAB-TolC multidrug efflux pump. *Elife* 6, eLife.24905 (2017)