

Estrutura de proteínas

Voet & Voet 4a edição, capítulo 8

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Sumário

- 1. Estrutura tridimensional**
- 2. Estabilidade**
- 3. Proteínas de membrana**
- 4. Determinação experimental**

Primary structure

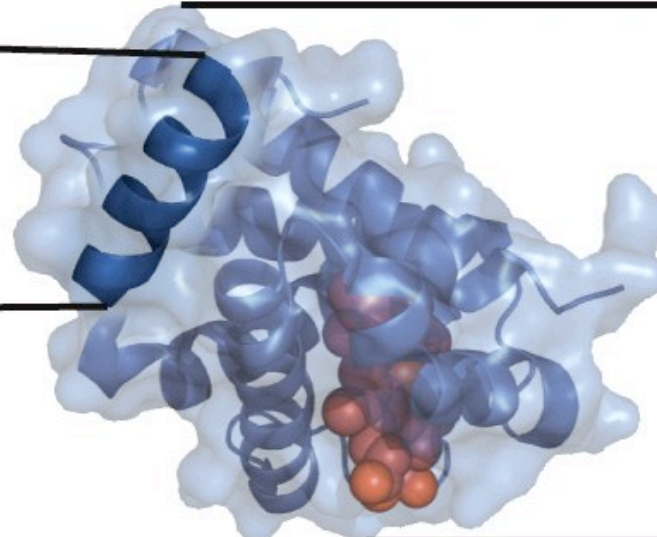
Pro
Ala
Asp
Lys
Thr
Asn
Val
Lys
Ala
Ala
Trp
Gly
Lys
Val

Amino acid residues

Secondary structure

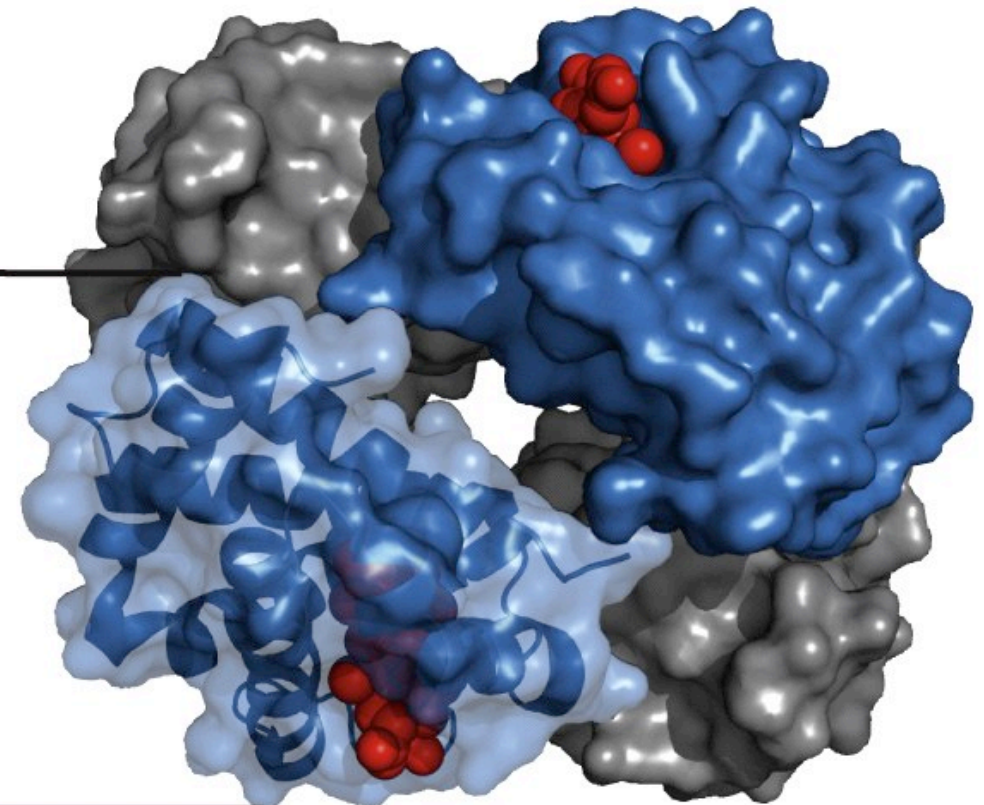


Tertiary structure

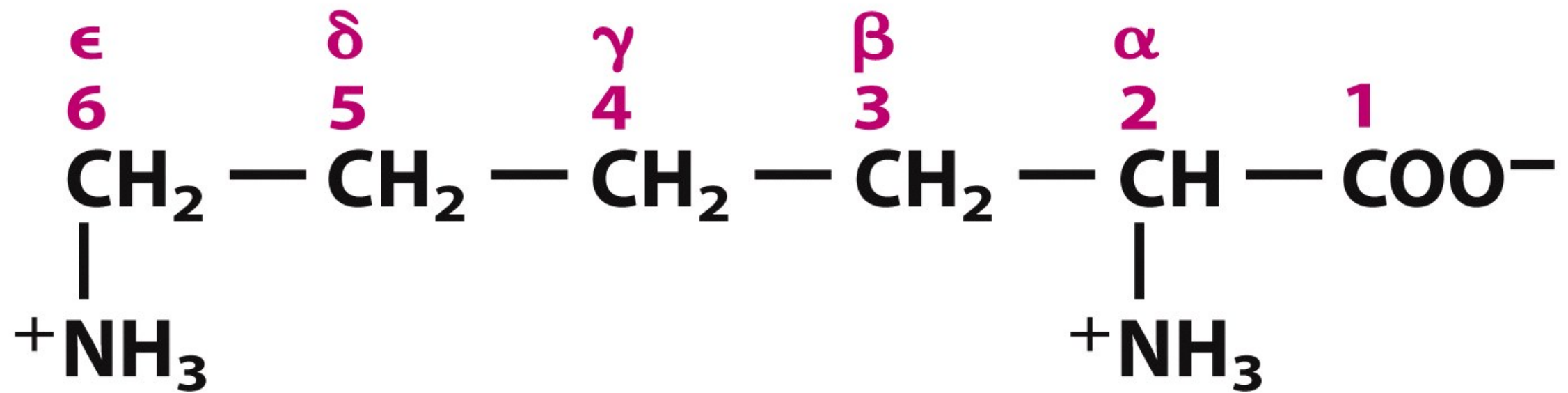


Polypeptide chain

Quaternary structure



Assembled subunits



Lysine

Unnumbered 3 p74

Lehninger Principles of Biochemistry, Fifth Edition

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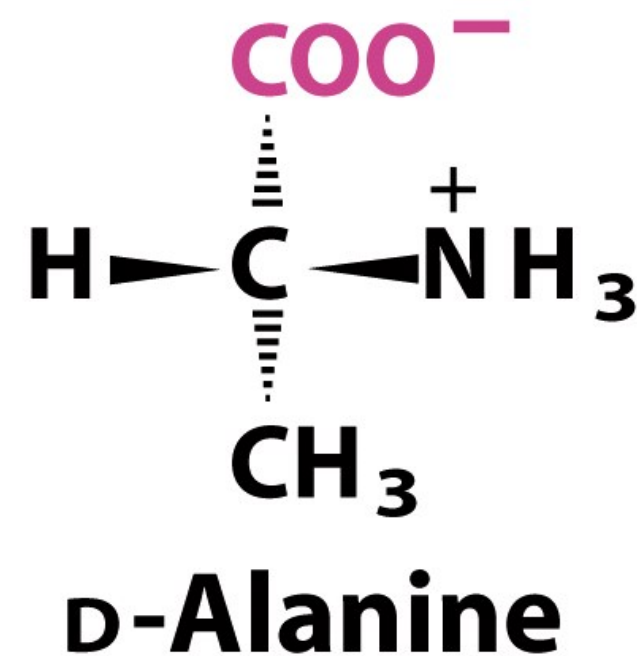
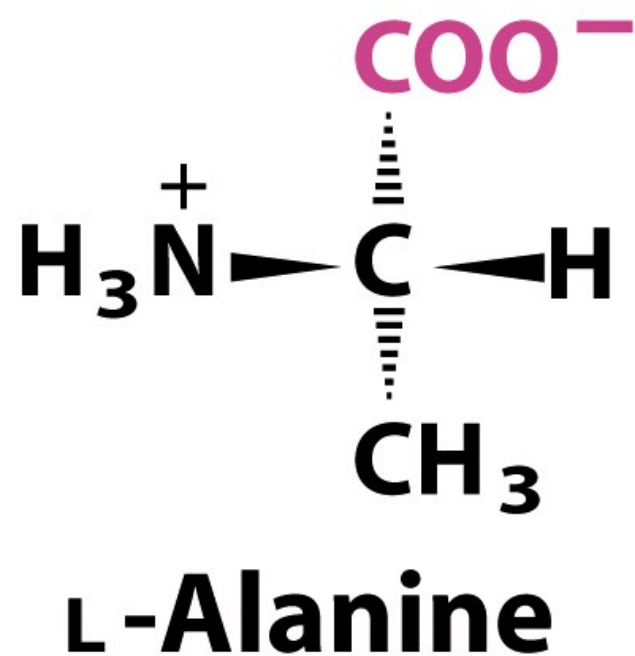
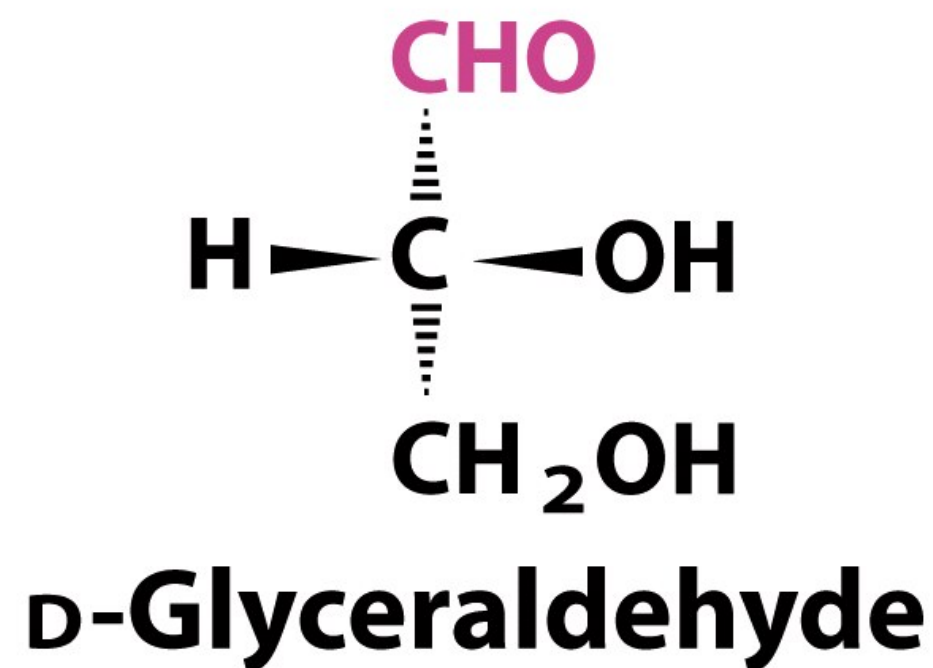
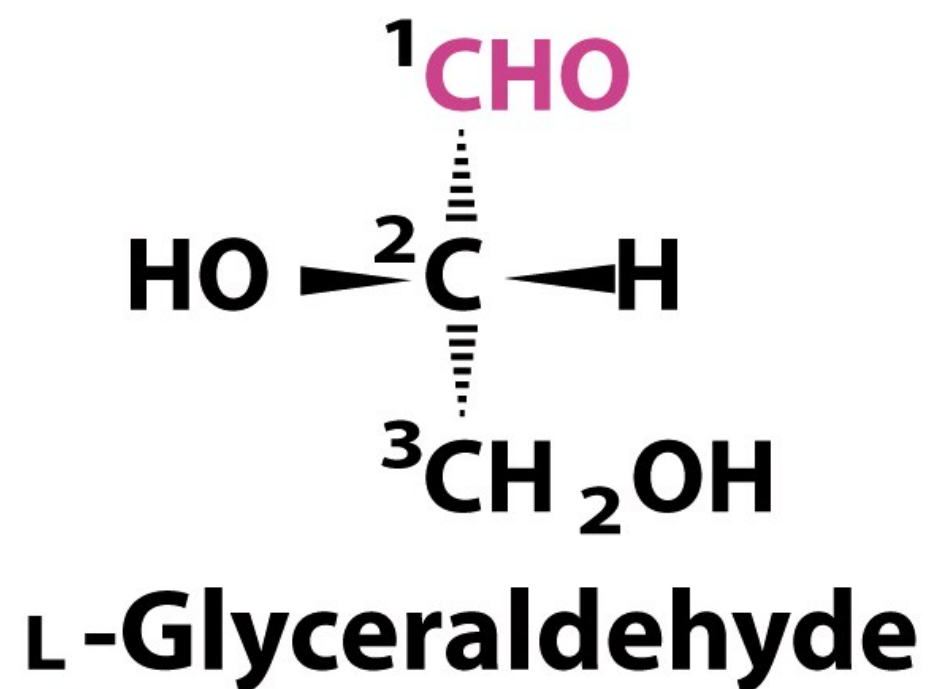


Figure 3-4
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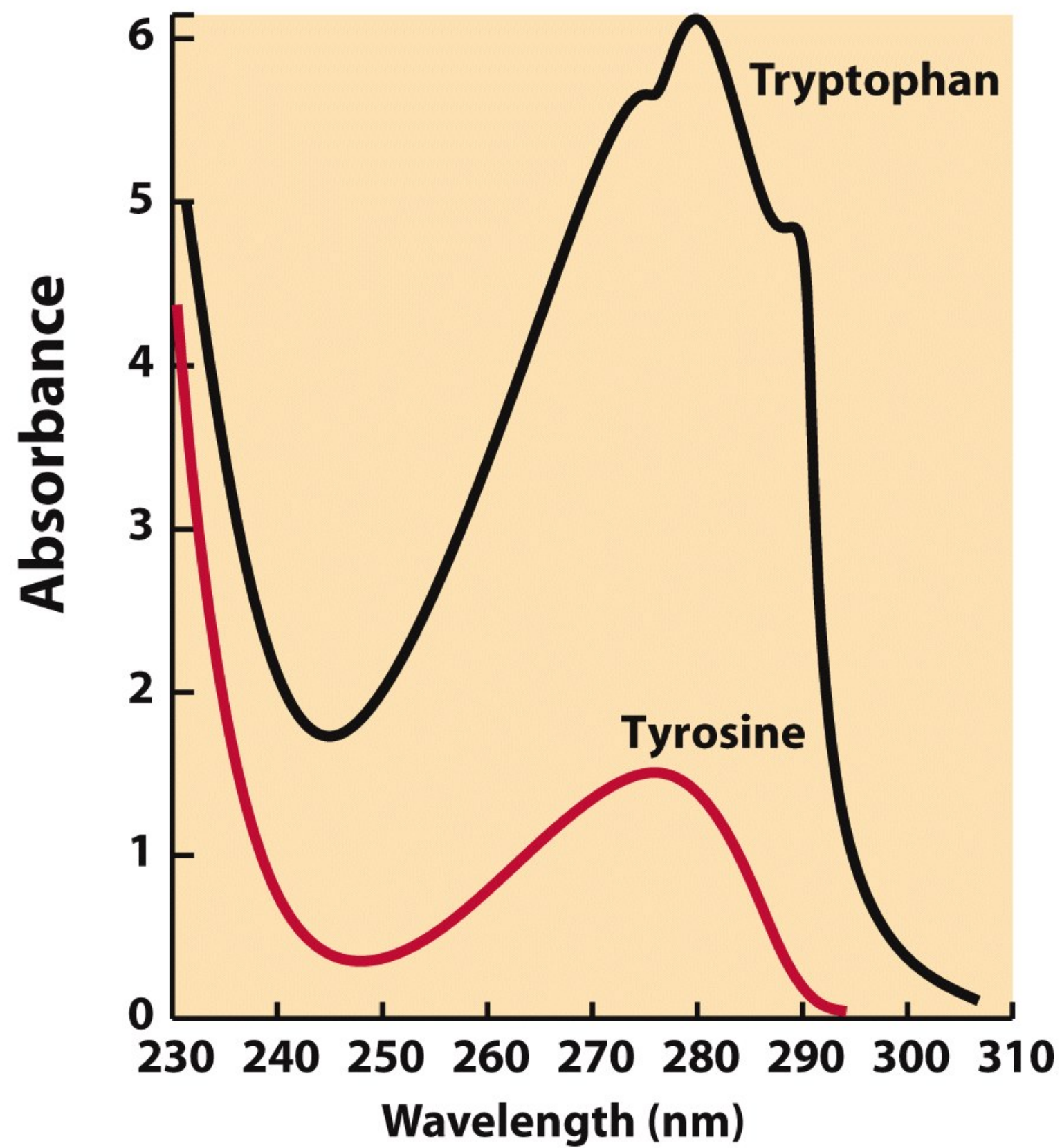


Figure 3-6
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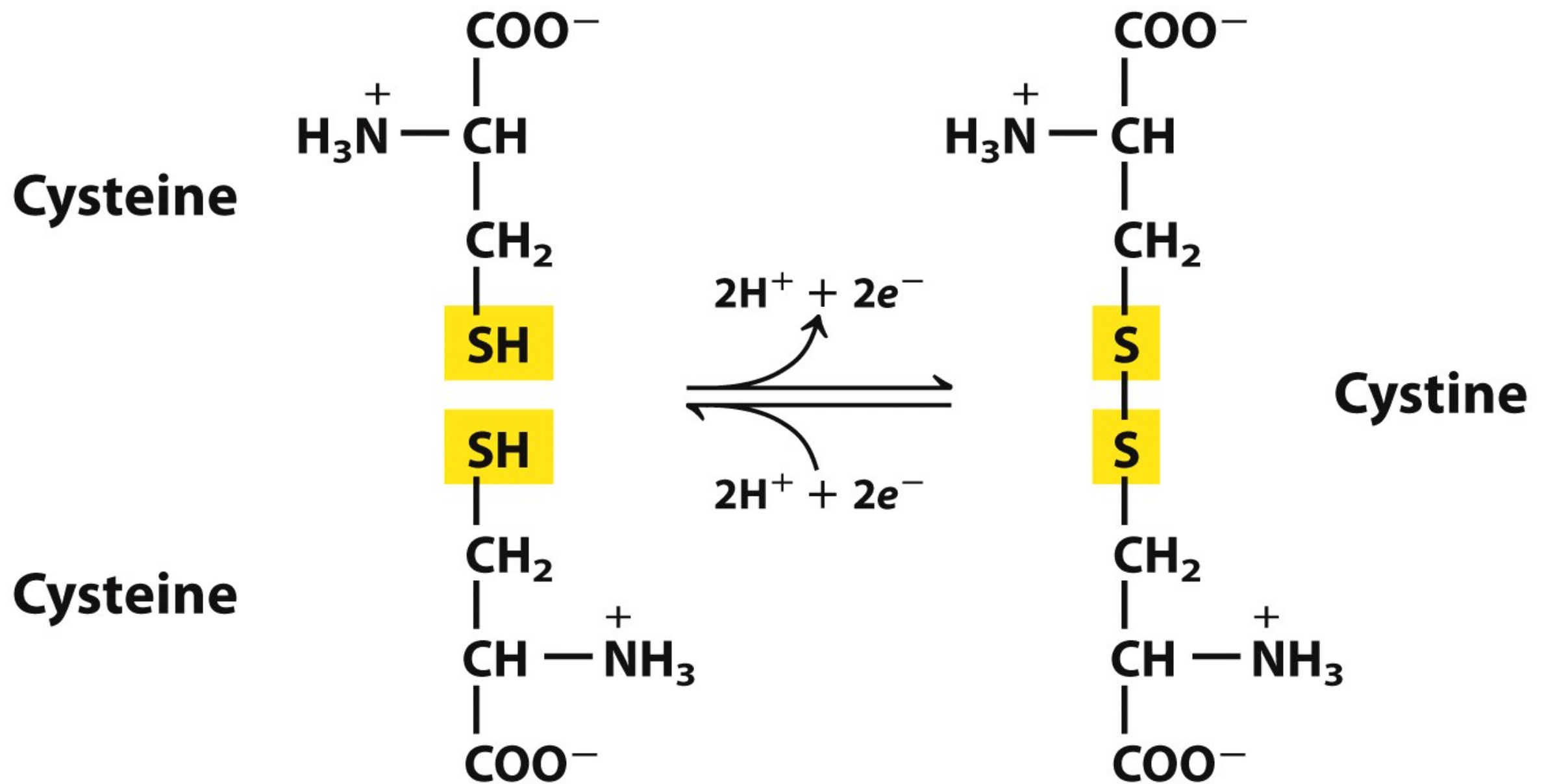


Figure 3-7

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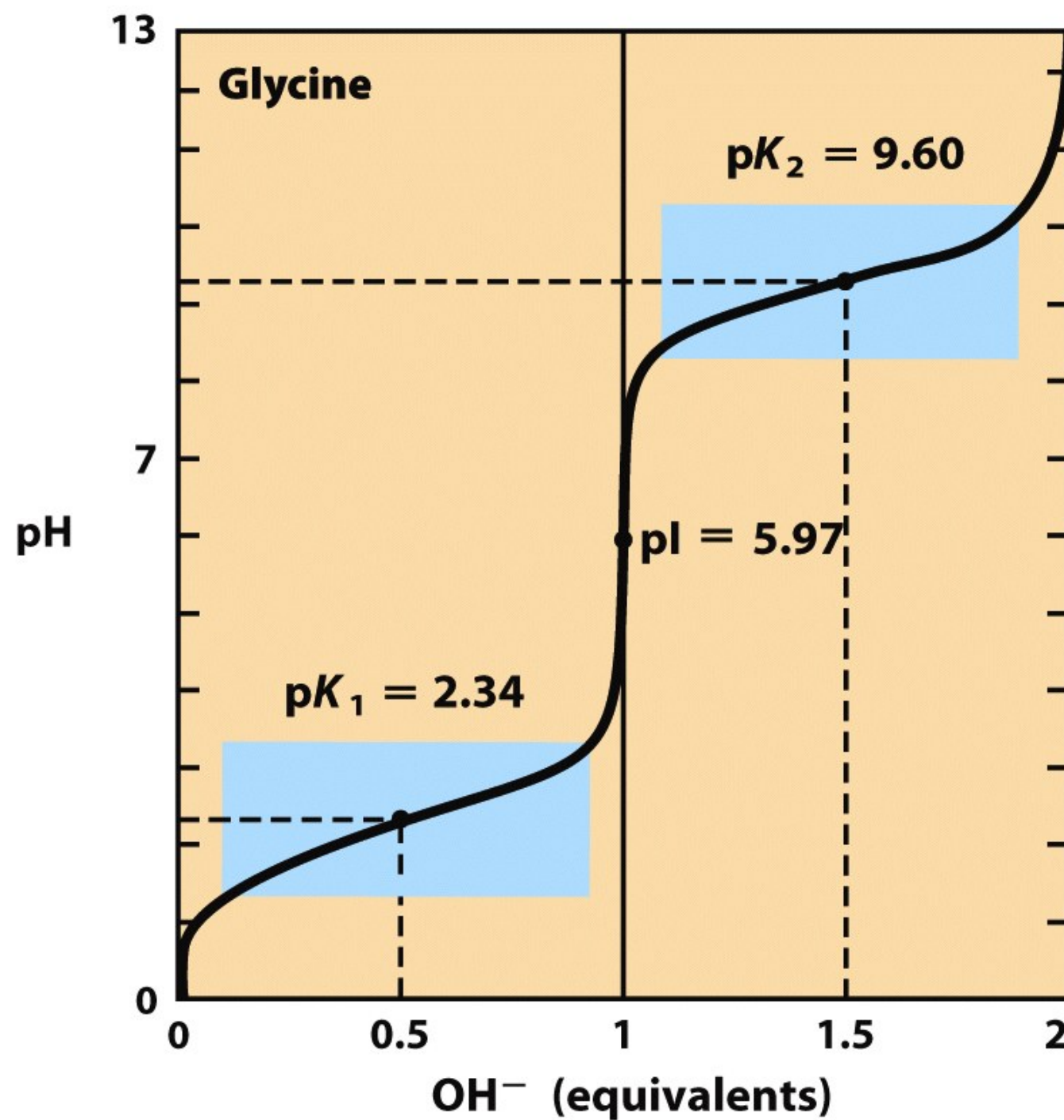
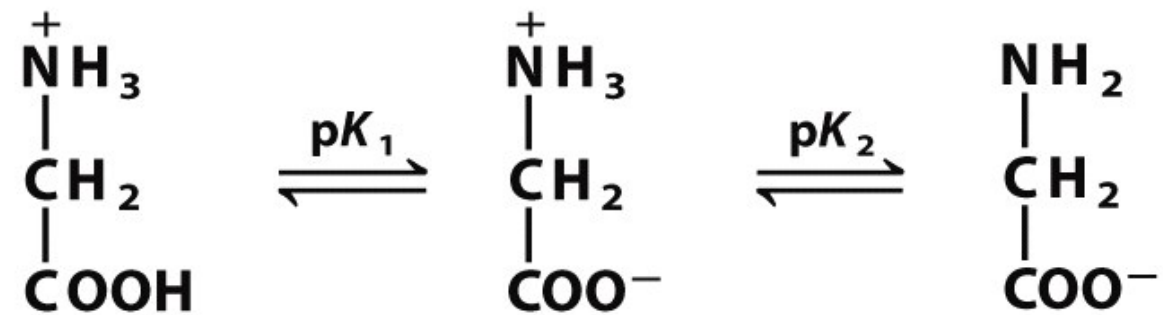


Figure 3-10
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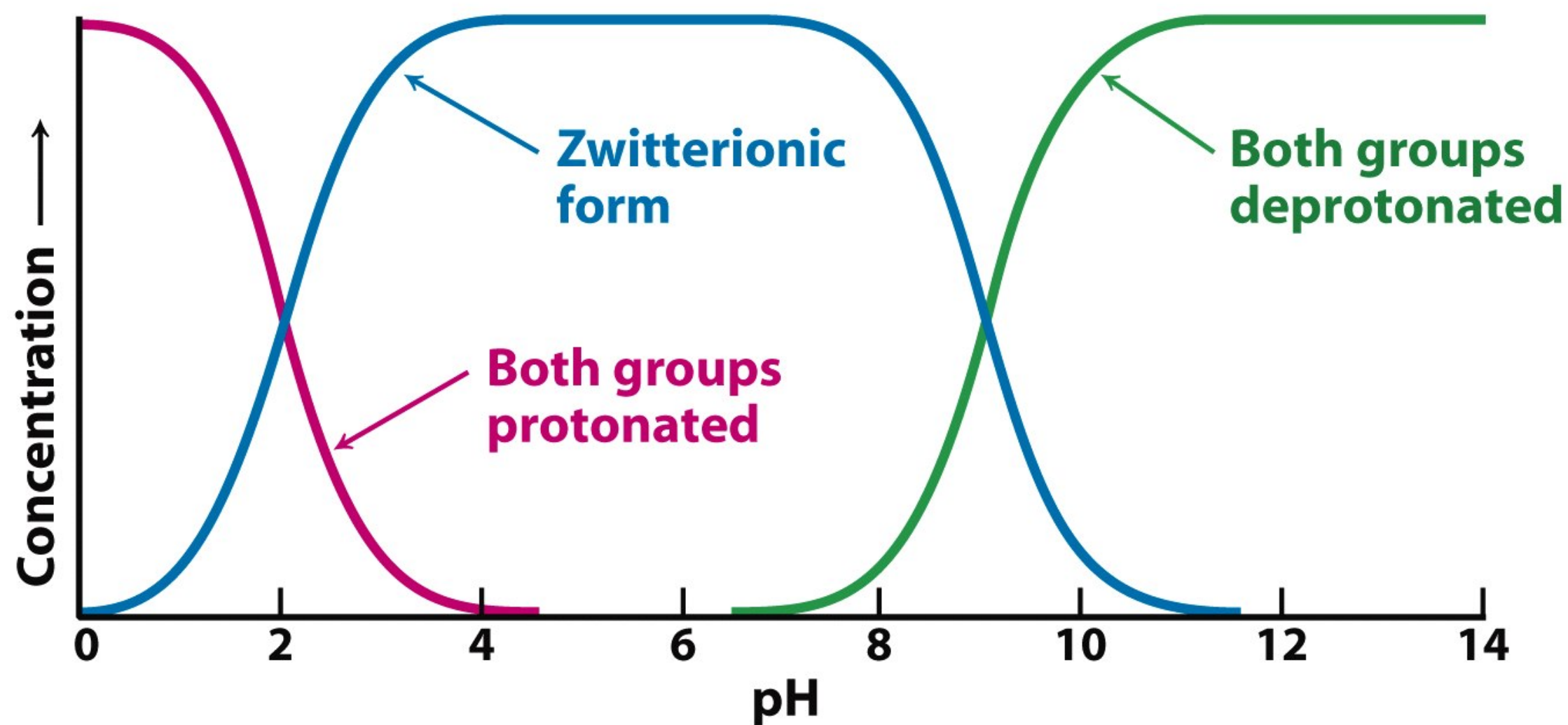
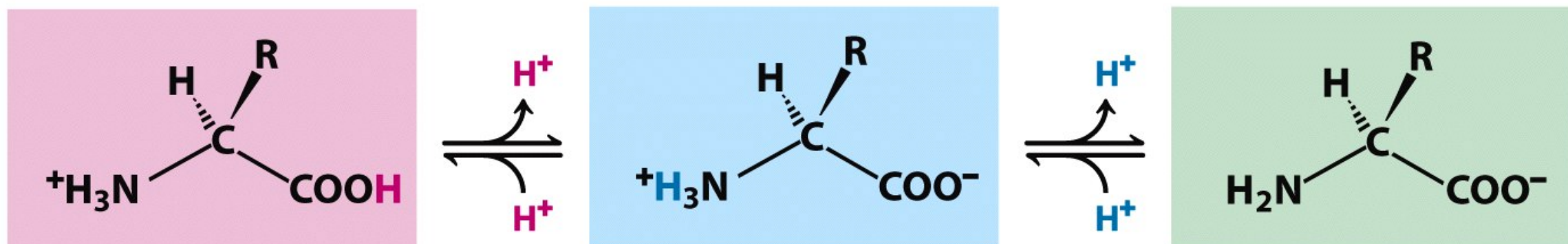


Figure 2-6
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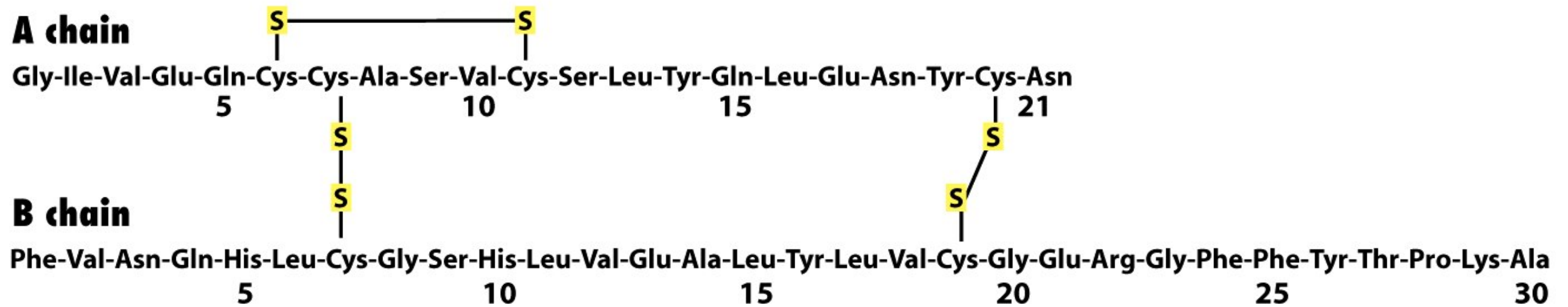
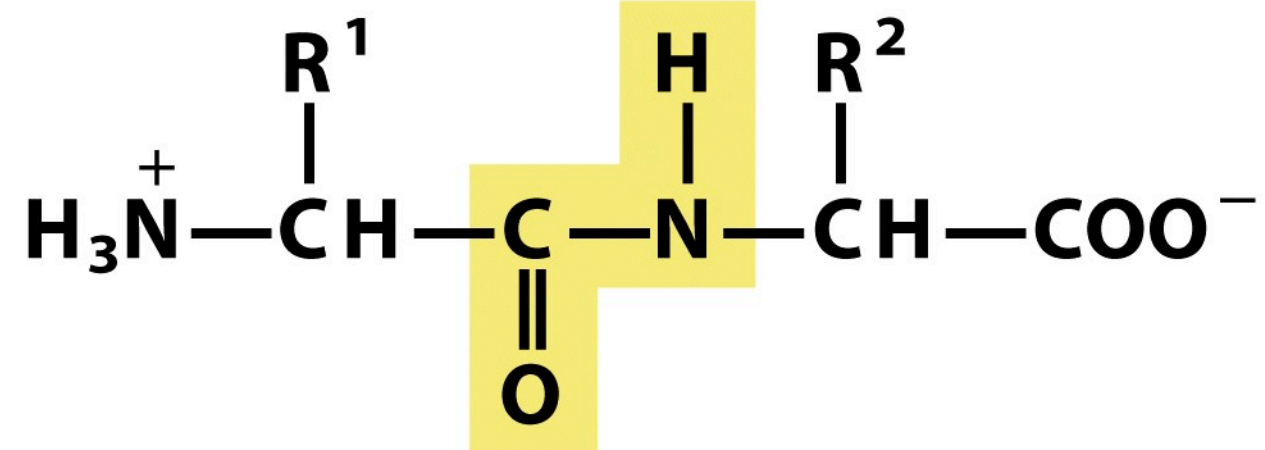
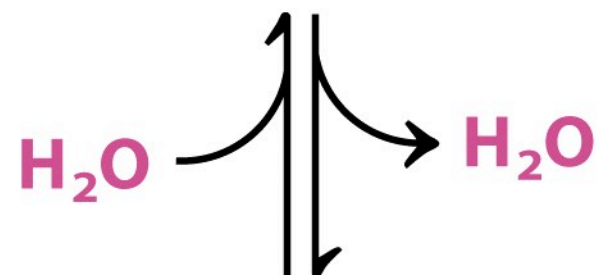
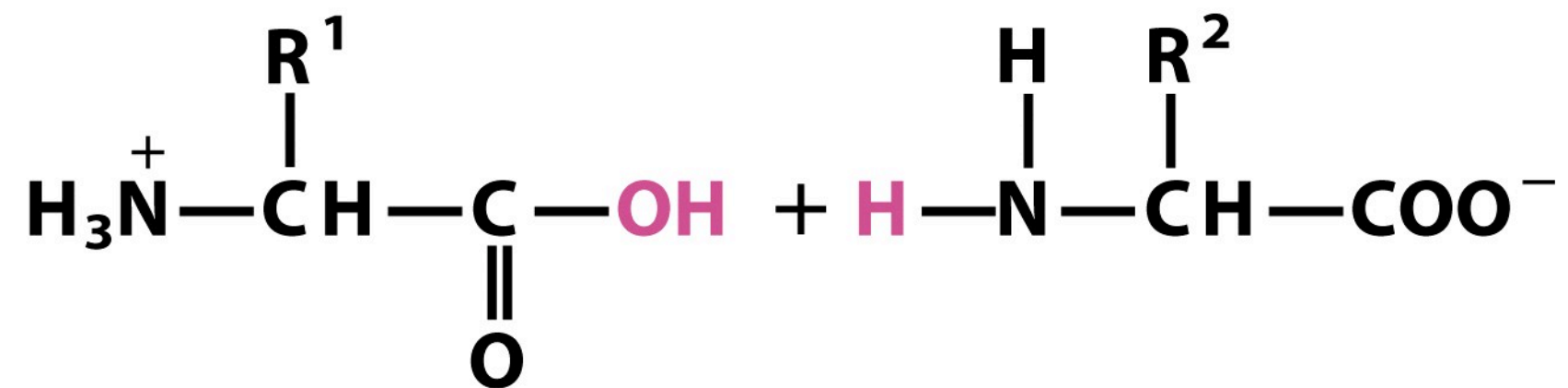
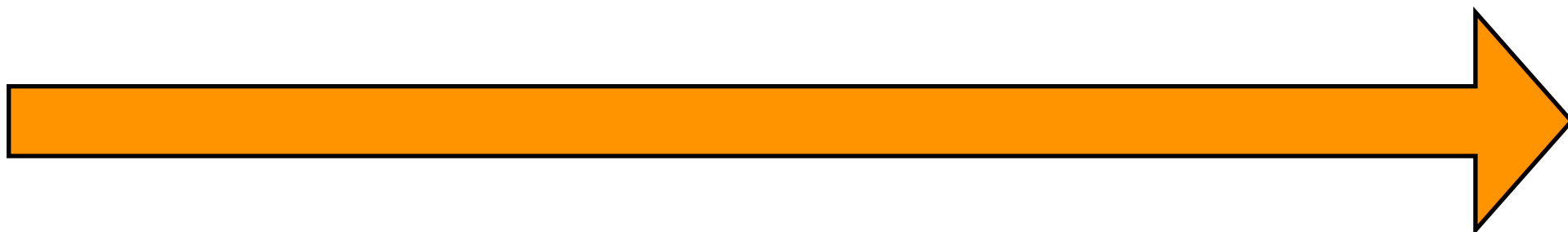
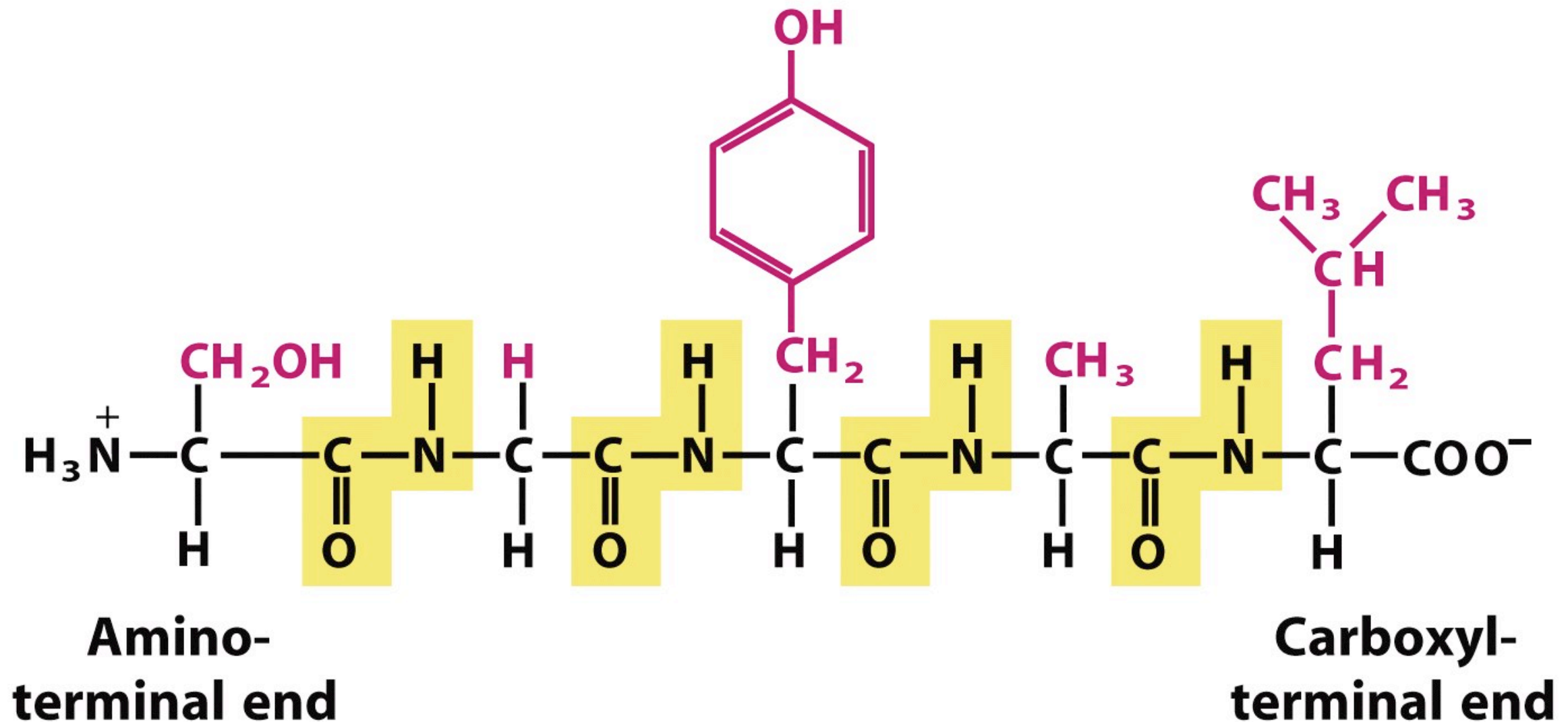


Figure 2-22
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Estrutura primária é a sequência de aminoácidos da proteína:



Polaridade: da extremidade NH_3^+ -terminal à extremidade COO^- -terminal

Pauling e Corey analisaram dados de difração de raios-X de peptídeos para propor modelos conformacionais para o esqueleto polipeptídico

VOL. 37, 1951

CHEMISTRY: PAULING AND COREY

251

THE PLEATED SHEET, A NEW LAYER CONFIGURATION OF POLYPEPTIDE CHAINS

BY LINUS PAULING AND ROBERT B. COREY

GATES AND CRELLIN LABORATORIES OF CHEMISTRY,* CALIFORNIA INSTITUTE OF TECHNOLOGY, PASADENA, CALIFORNIA

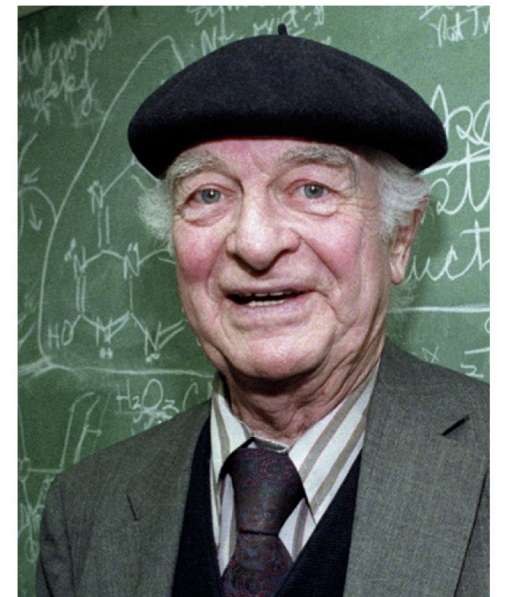
Communicated March 31, 1951

THE STRUCTURE OF PROTEINS: TWO HYDROGEN-BONDED HELICAL CONFIGURATIONS OF THE POLYPEPTIDE CHAIN

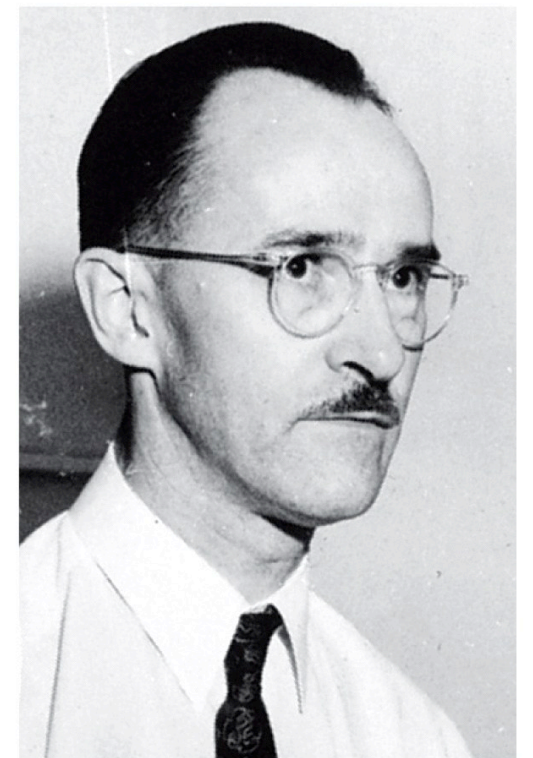
BY LINUS PAULING, ROBERT B. COREY, AND H. R. BRANSON*

GATES AND CRELLIN LABORATORIES OF CHEMISTRY,
CALIFORNIA INSTITUTE OF TECHNOLOGY, PASADENA, CALIFORNIA†

Communicated February 28, 1951

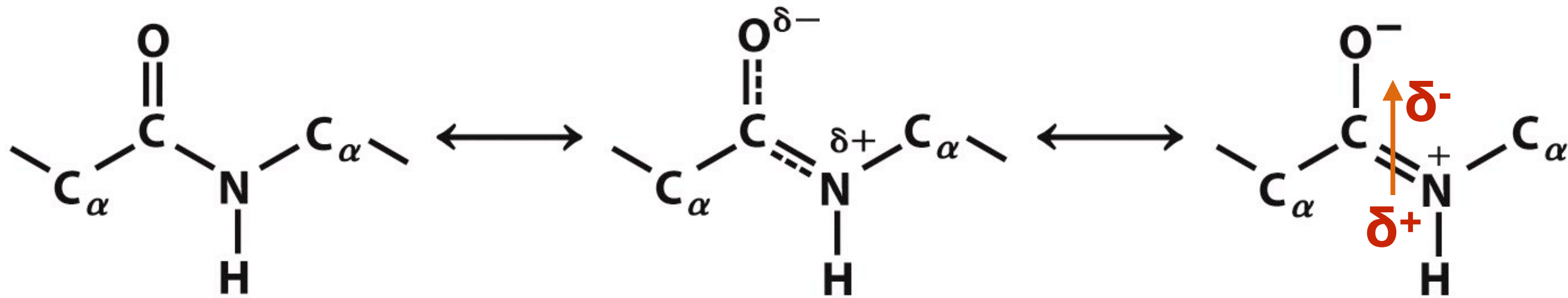


Linus Pauling, 1901–1994



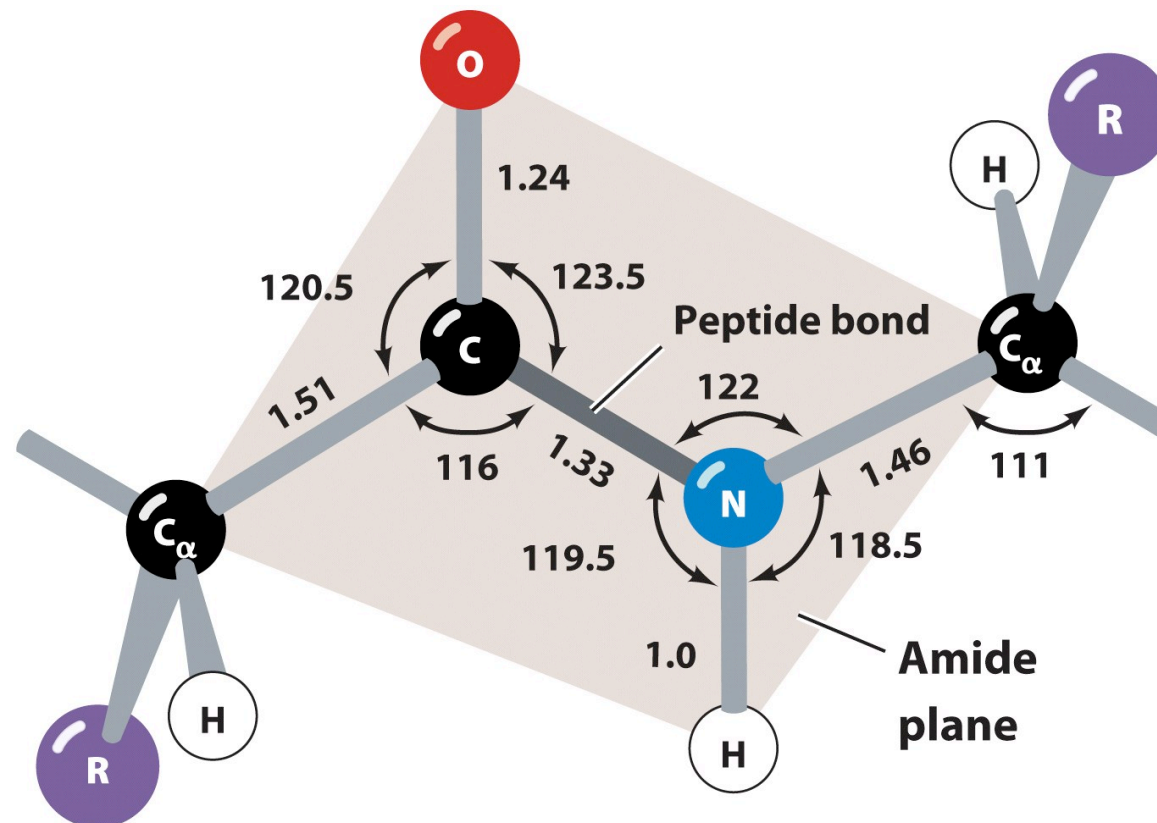
Robert Corey, 1897–1971

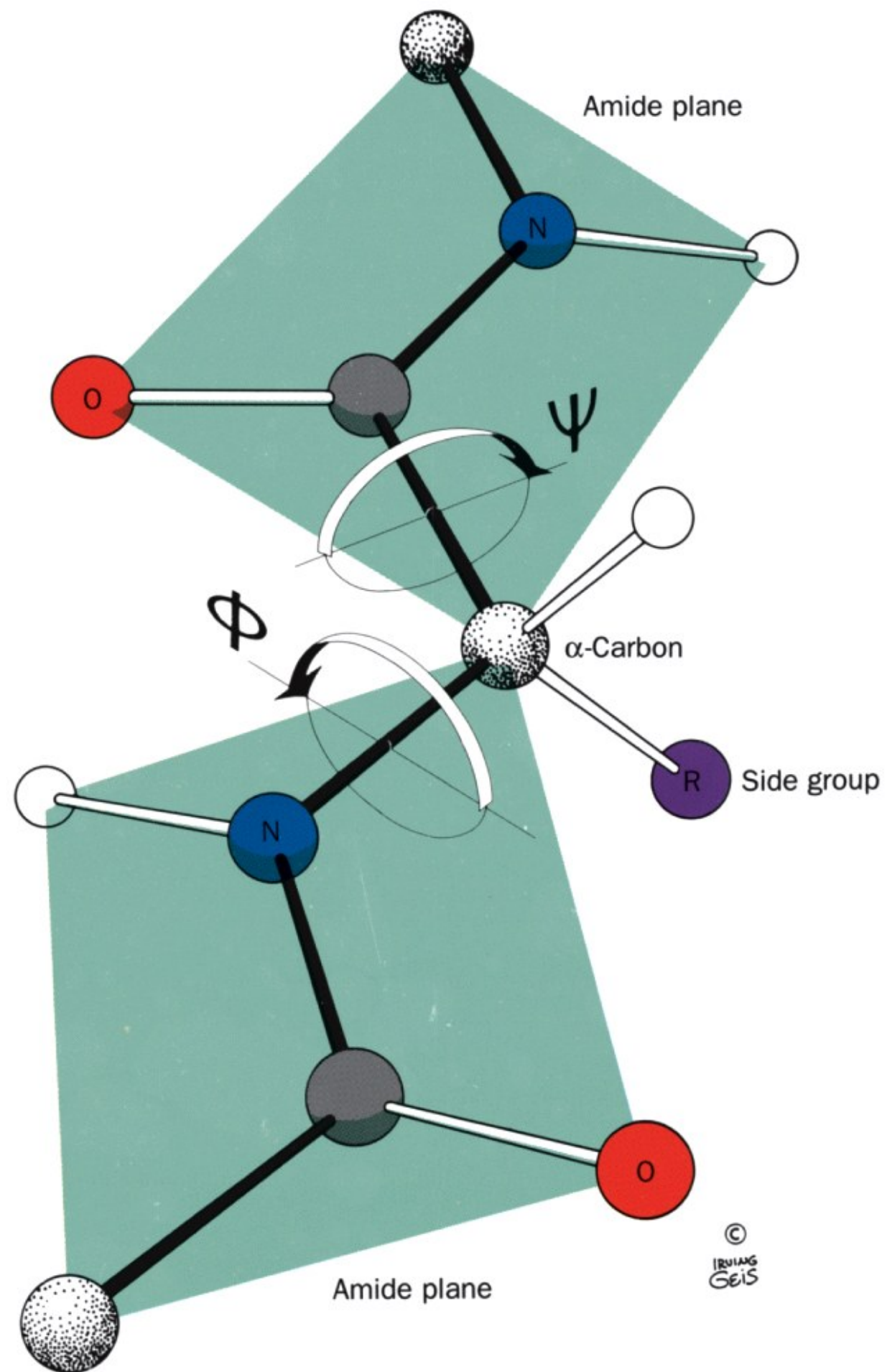
A ligação peptídica é rígida



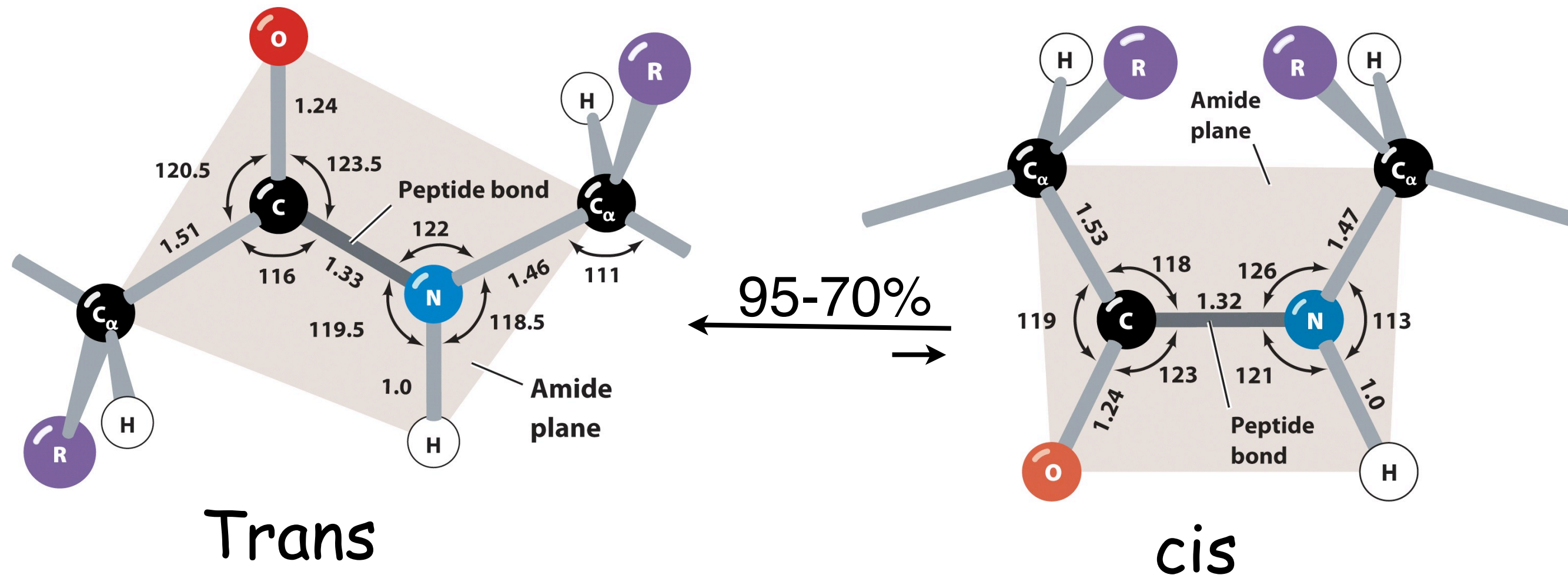
Obs.: Note o dipolo elétrico da ligação peptídica

...e planar



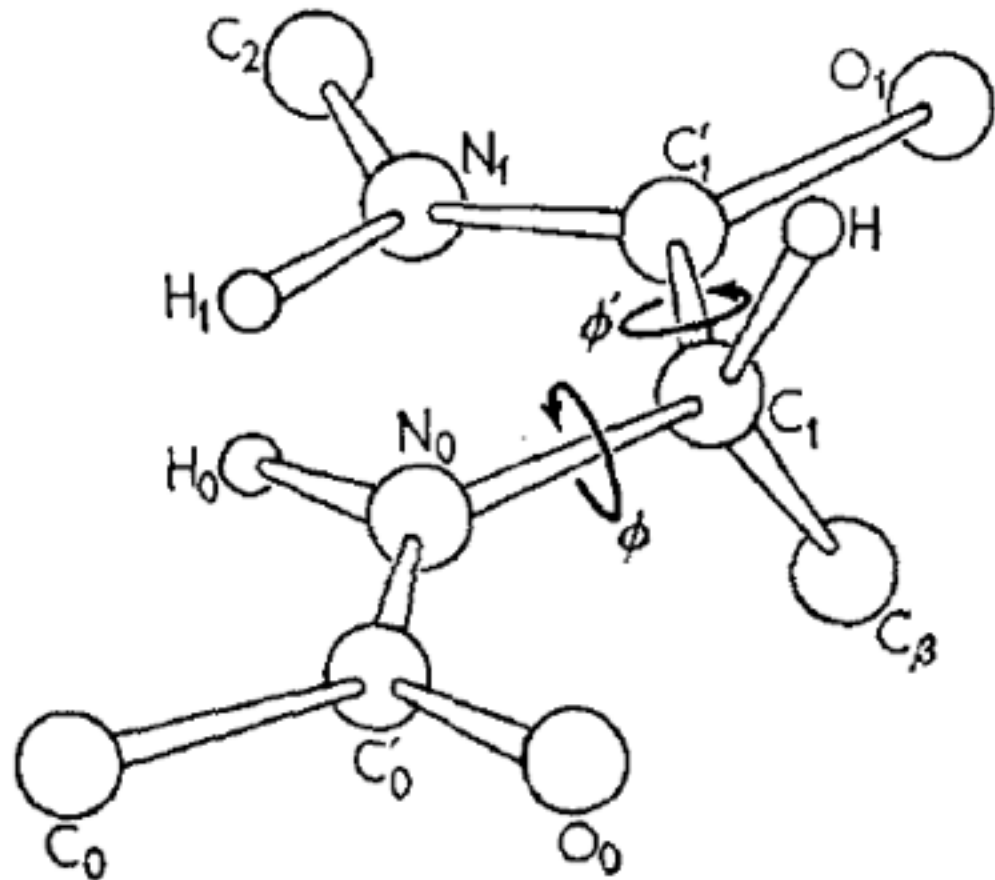


A ligação peptídica sempre assume conformação *trans* ($\omega=180^\circ$). Prolinas podem, eventualmente, assumir conformação *cis* ($\omega = 0^\circ$)

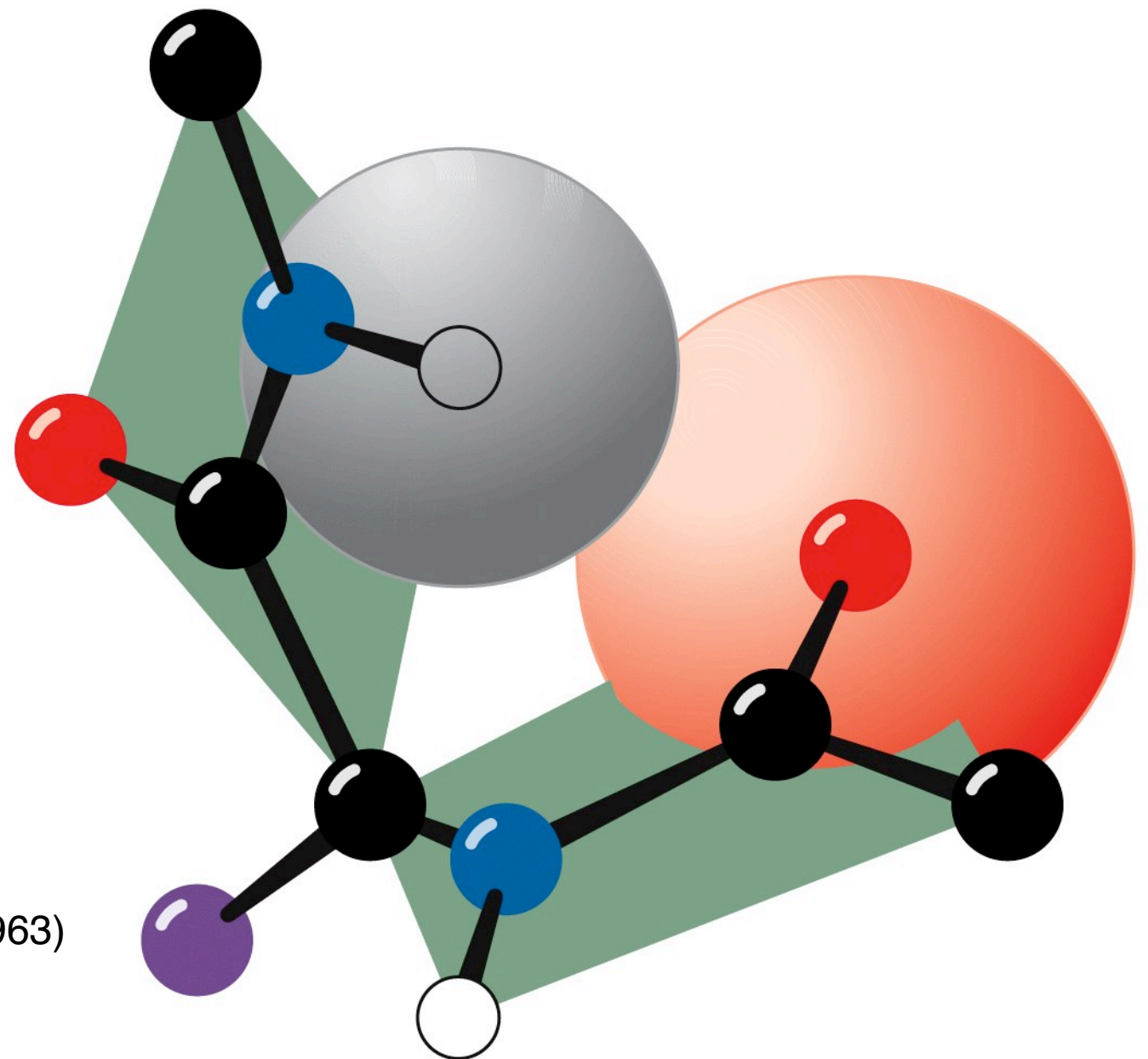


A lenta isomerização *cis-trans* das prolinas “retarda” o enovelamento de proteínas

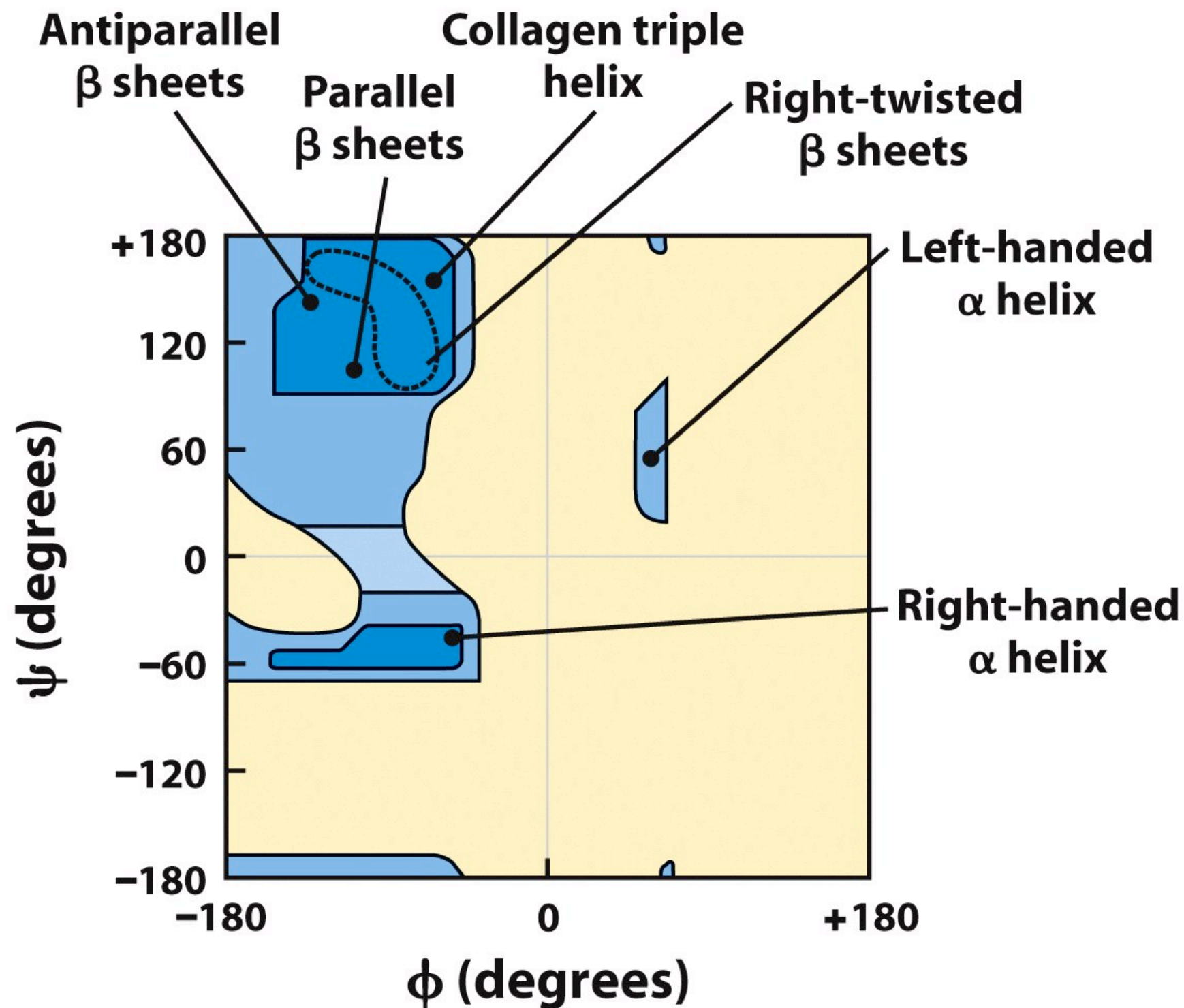
Gráfico de Ramachandran: nem todas as combinações de ϕ e ψ são permitidas



Stereochemistry of polypeptide chain configurations (1963)
J. Mol. Biol. 7, 95-99



Estruturas secundárias possuem valores ϕ e ψ muito bem definidos



Glicina permite conformações pouco usuais

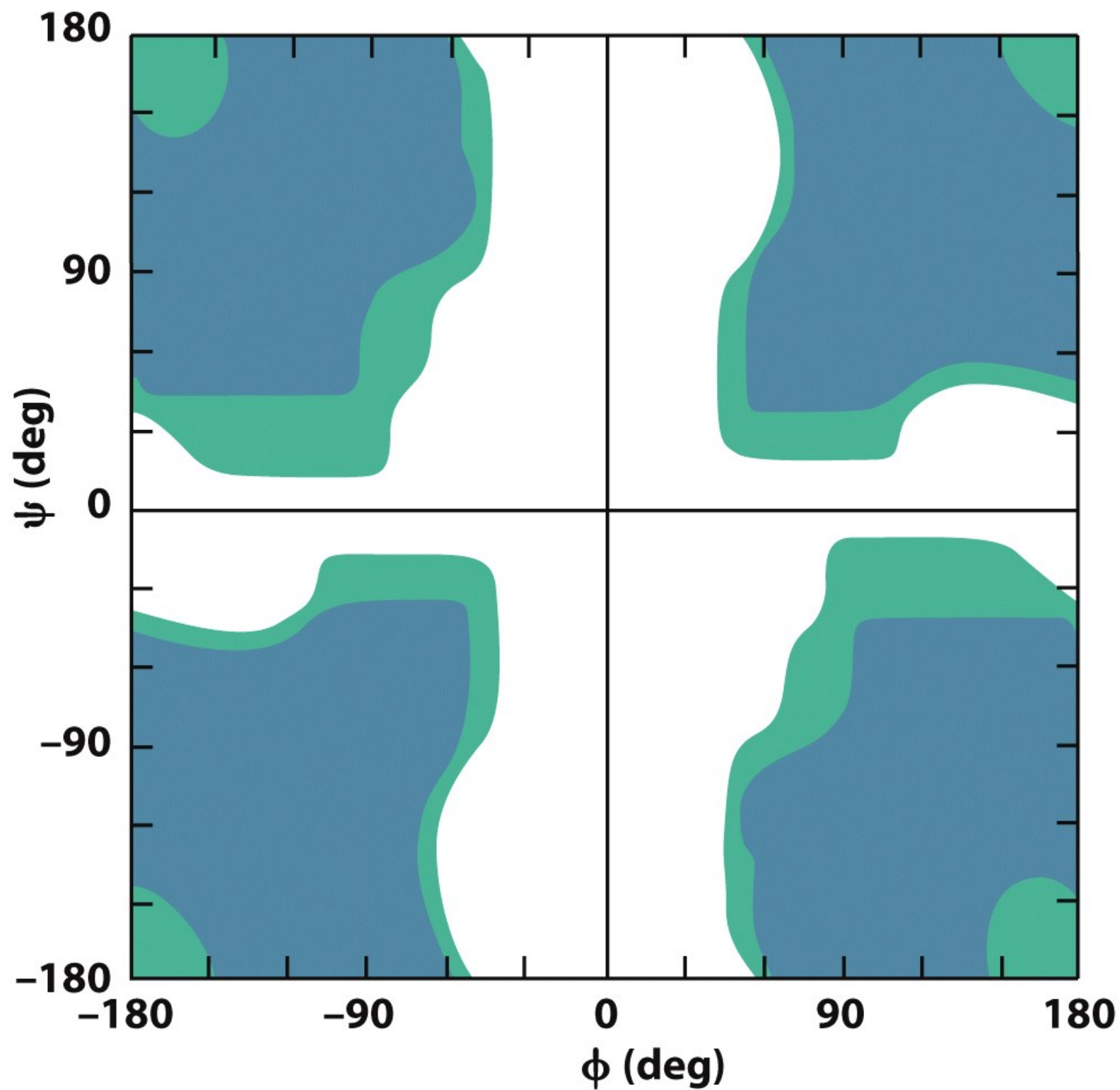
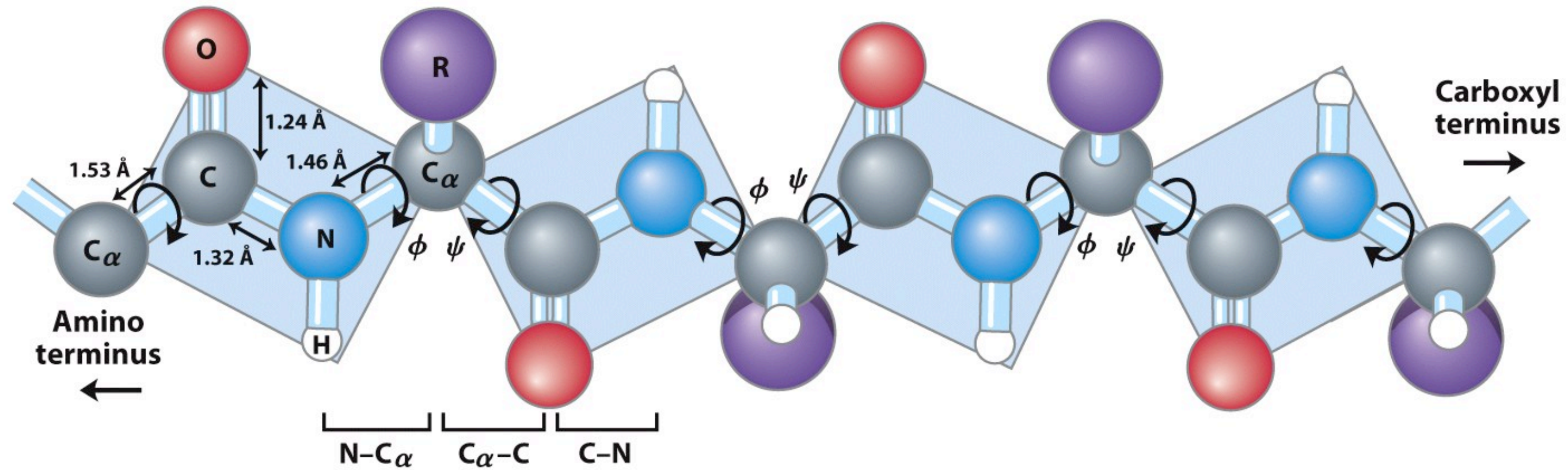
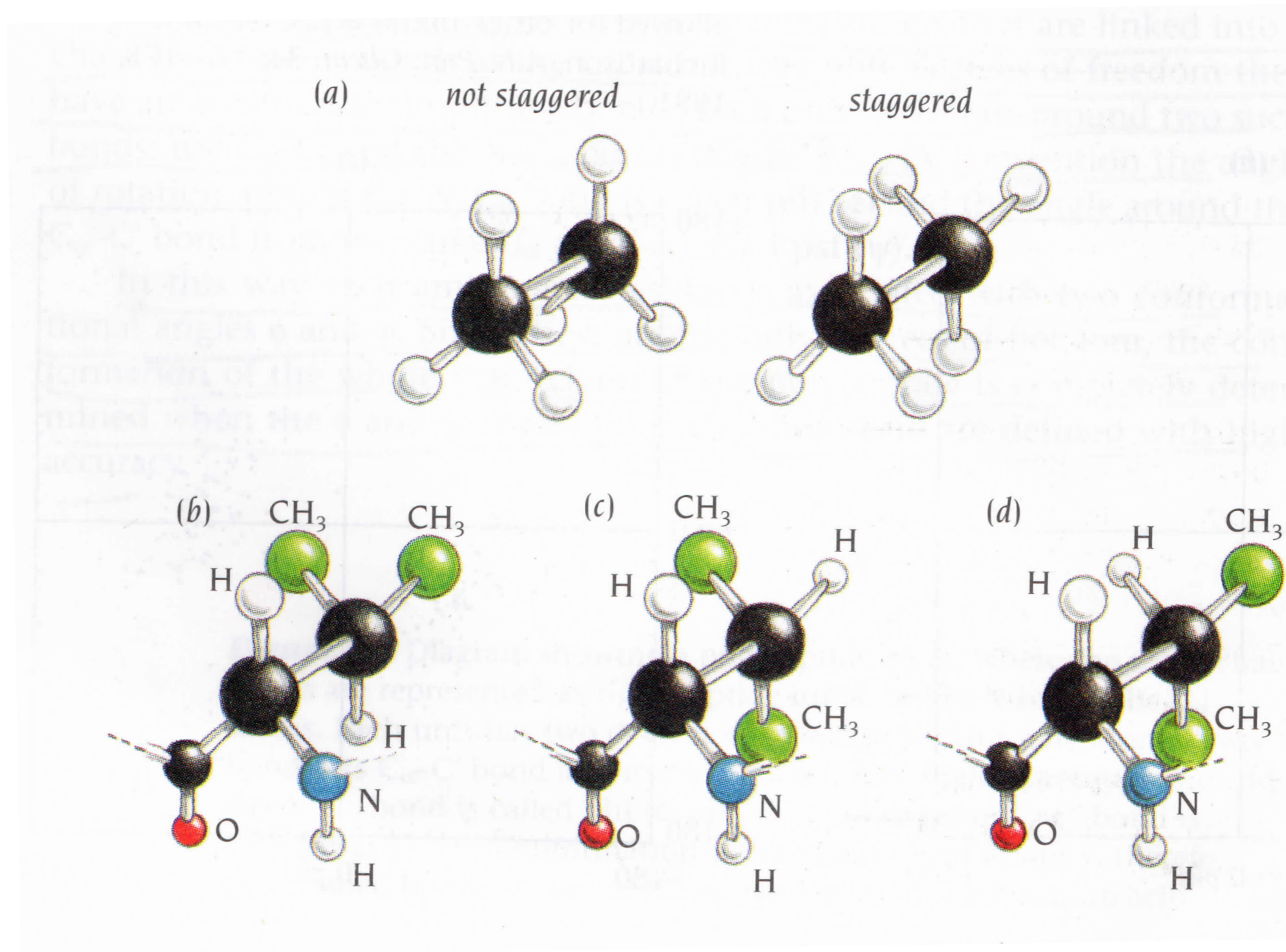


Figure 8-9
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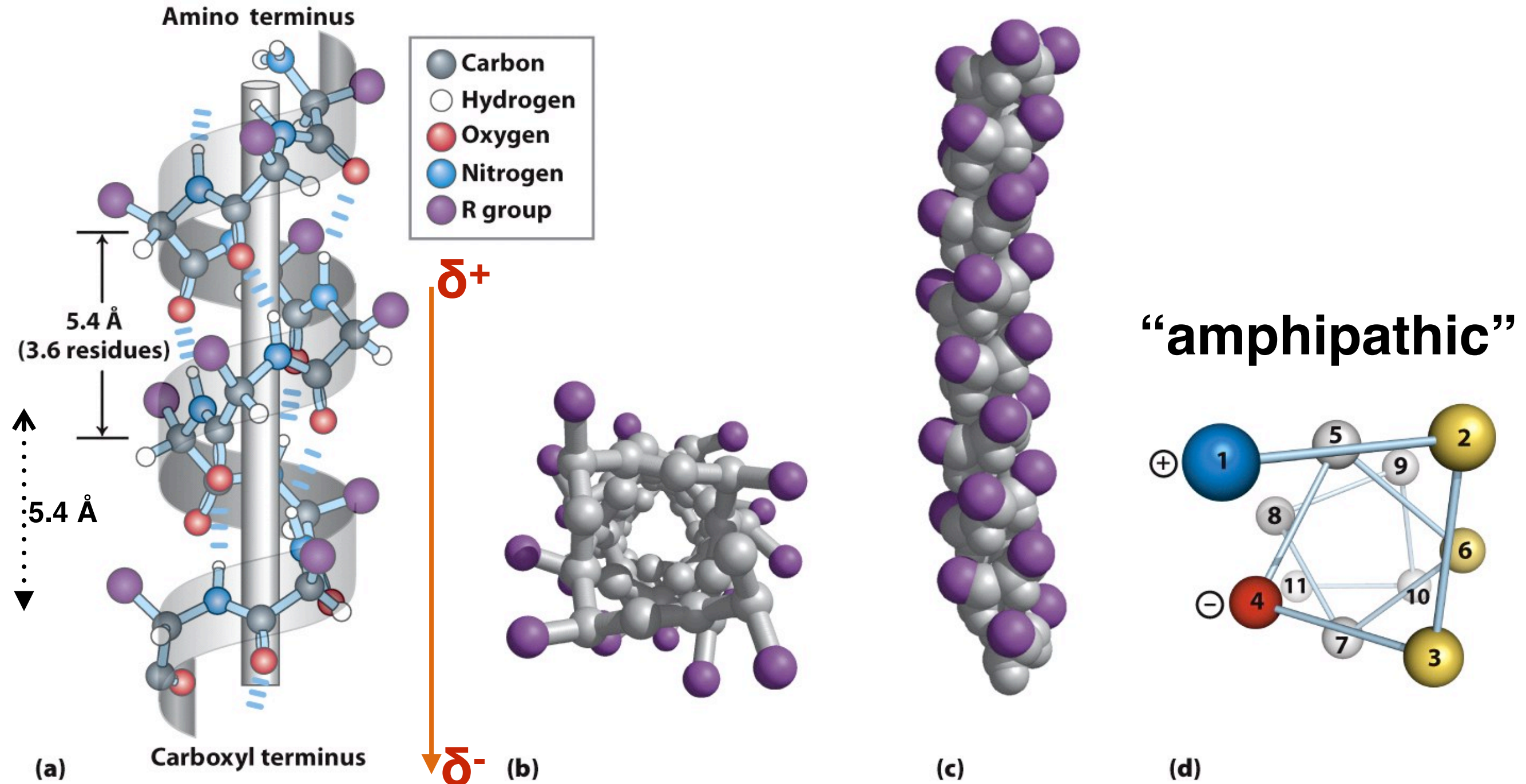
A conformação do esqueleto polipeptídico é descrita por três ângulos: ϕ , ψ , e ω



Nem todas as conformações das cadeias laterais são igualmente prováveis



α -hélice



Ligações de hidrogênio entre $\text{HN}_{(i+4)}$ e $\text{CO}_{(i)}$ estabilizam a hélice
Os primeiros 4 e os últimos 4 amino ácidos não formam ligações de hidrogênio (“helix fraying”)

Alguns aminoácidos estabilizam hélices, enquanto que outros destabilizam

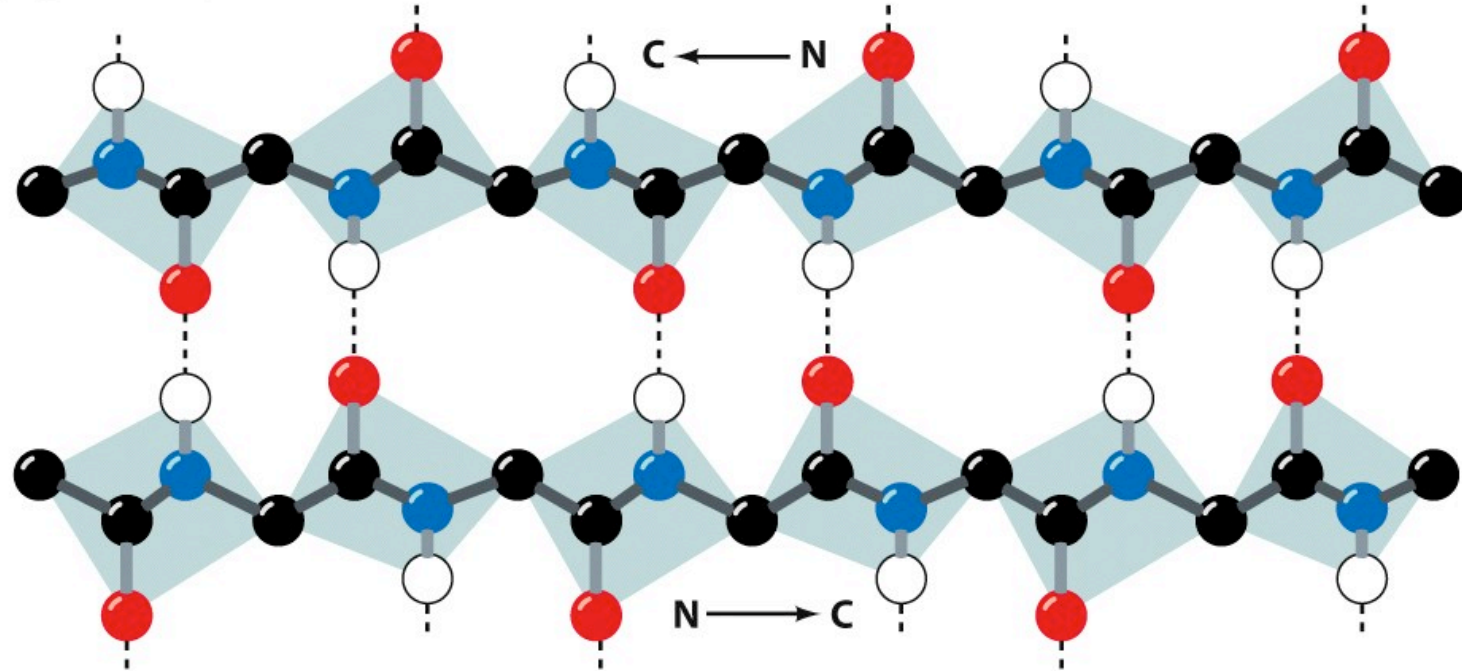
TABLE 4–1		Propensity of Amino Acids to Take Up an α -Helical Conformation	
Amino acid	$\Delta\Delta G^\circ$ (kJ/mol)*	Amino acid	$\Delta\Delta G^\circ$ (kJ/mol)*
Ala	0	Leu	0.79
Arg	0.3	Lys	0.63
Asn	3	Met	0.88
Asp	2.5	Phe	2.0
Cys	3	Pro	>4
Gln	1.3	Ser	2.2
Glu	1.4	Thr	2.4
Gly	4.6	Tyr	2.0
His	2.6	Trp	2.0
Ile	1.4	Val	2.1

Sources: Data (except proline) from Bryson, J.W., Betz, S.F., Lu, H.S., Suich, D.J., Zhou, H.X., O'Neil, K.T., & DeGrado, W.F. (1995) Protein design: a hierarchic approach. *Science* 270, 935. Proline data from Myers, J.K., Pace, C.N., & Scholtz, J.M. (1997) Helix propensities are identical in proteins and peptides. *Biochemistry* 36, 10,926.

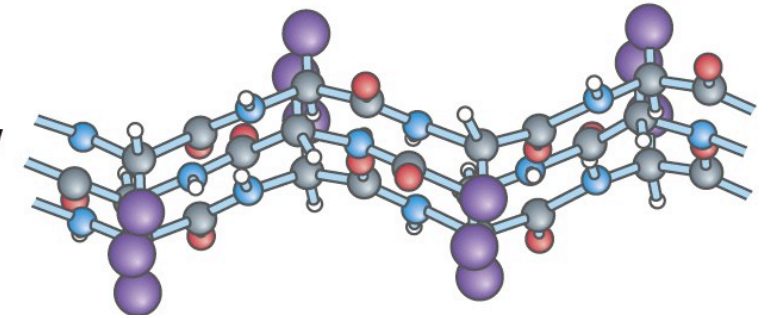
* $\Delta\Delta G^\circ$ is the difference in free-energy change, relative to that for alanine, required for the amino acid residue to take up the α -helical conformation. Larger numbers reflect greater difficulty taking up the α -helical structure. Data are a composite derived from multiple experiments and experimental systems.

Folha- β antiparalela e paralela

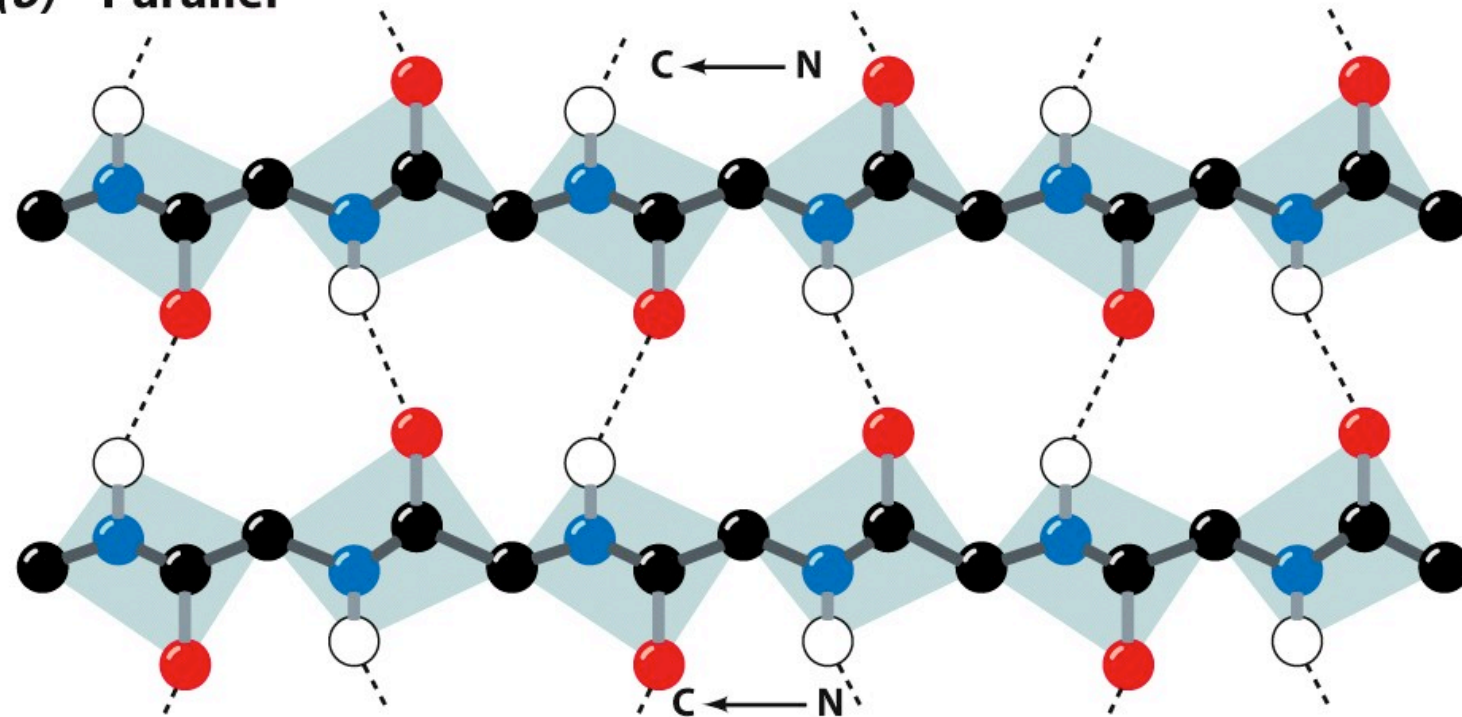
(a) Antiparallel



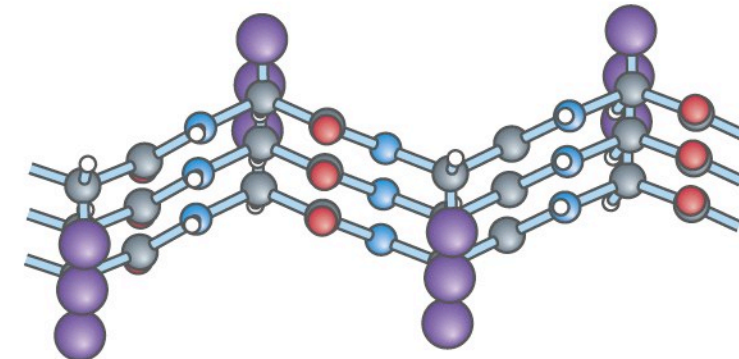
Side view



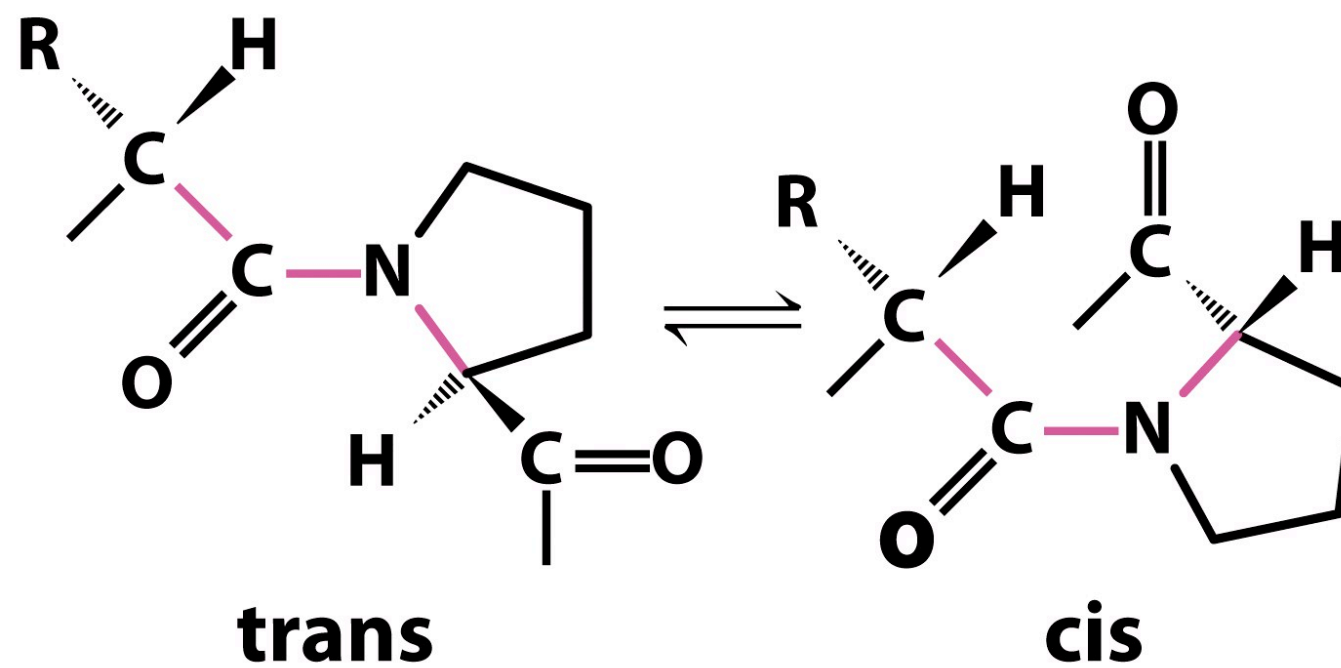
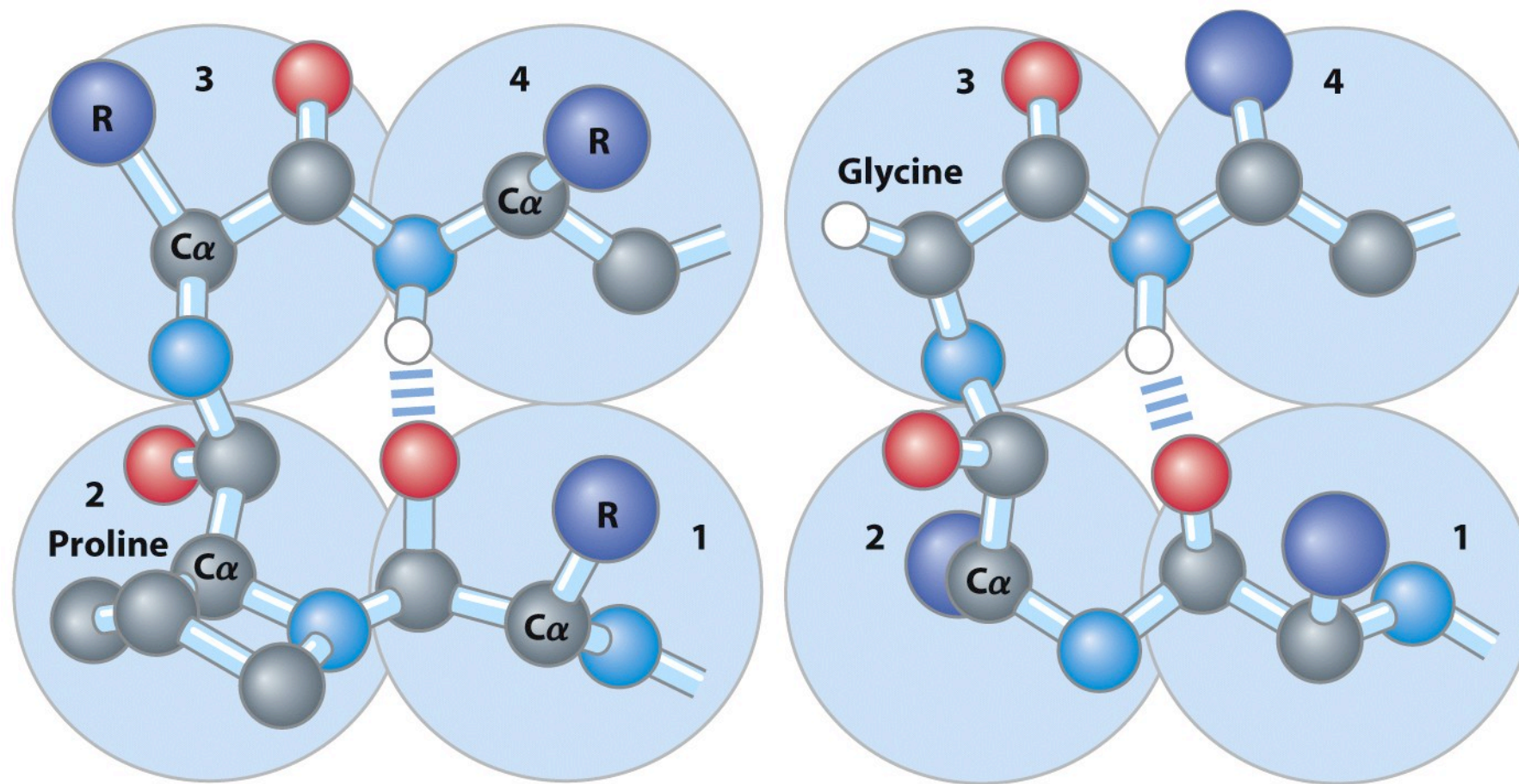
(b) Parallel



Side view



Dobras- β



Torsion angles for regular polypeptide conformations

Estrutura	ϕ	ψ
Hypothetically fully extended	+180°	+180°
Antiparallel β -sheet	-139°	+135°
Parallel β -sheet	-119°	+113°
Right-handed α -helix	-57°	-47°
Left-handed α -helix	+60°	+60°
3	-49°	-26°

Representação esquemática de estrutura secundária

Computer-Generated Schematic Diagrams of Protein Structures

Abstract. Computer-generated pictures are essential for studying and comparing the structures of proteins that have been solved by x-ray crystallography. Stereoscopic pairs produced by a computer program are particularly useful in providing an intelligible portrayal of the molecular topology.

ARTHUR M. LESK*

*Fairleigh Dickinson University,
Teaneck, New Jersey 07666*

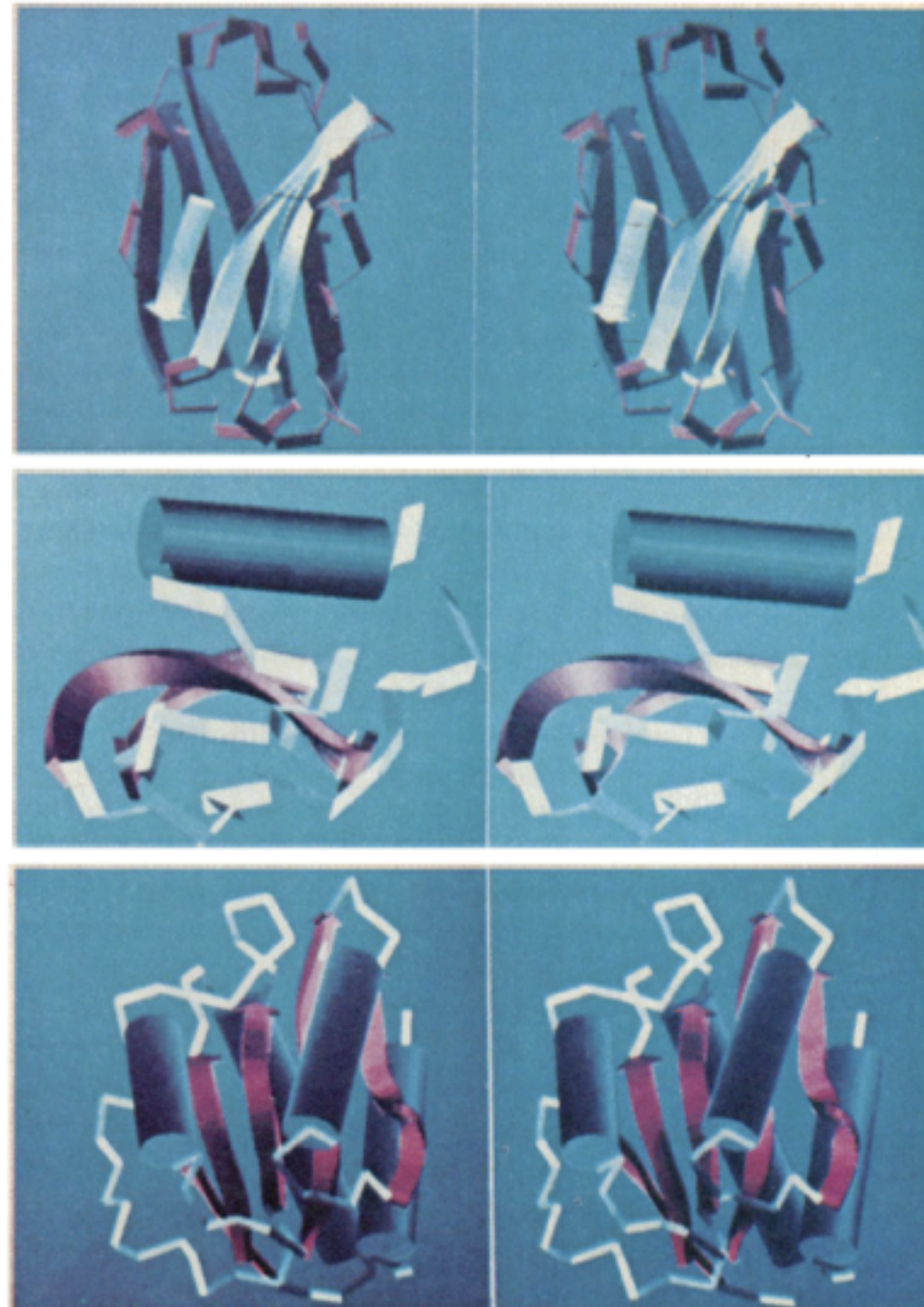
KARL D. HARDMANT†

*Thomas J. Watson Research Center,
IBM Corporation, P.O. Box 218,
Yorktown Heights, New York 10598*

SCIENCE, VOL. 216, 30 APRIL 1982



Arthur Lesk



Estrutura de proteínas - Prof. Bayardo Torres

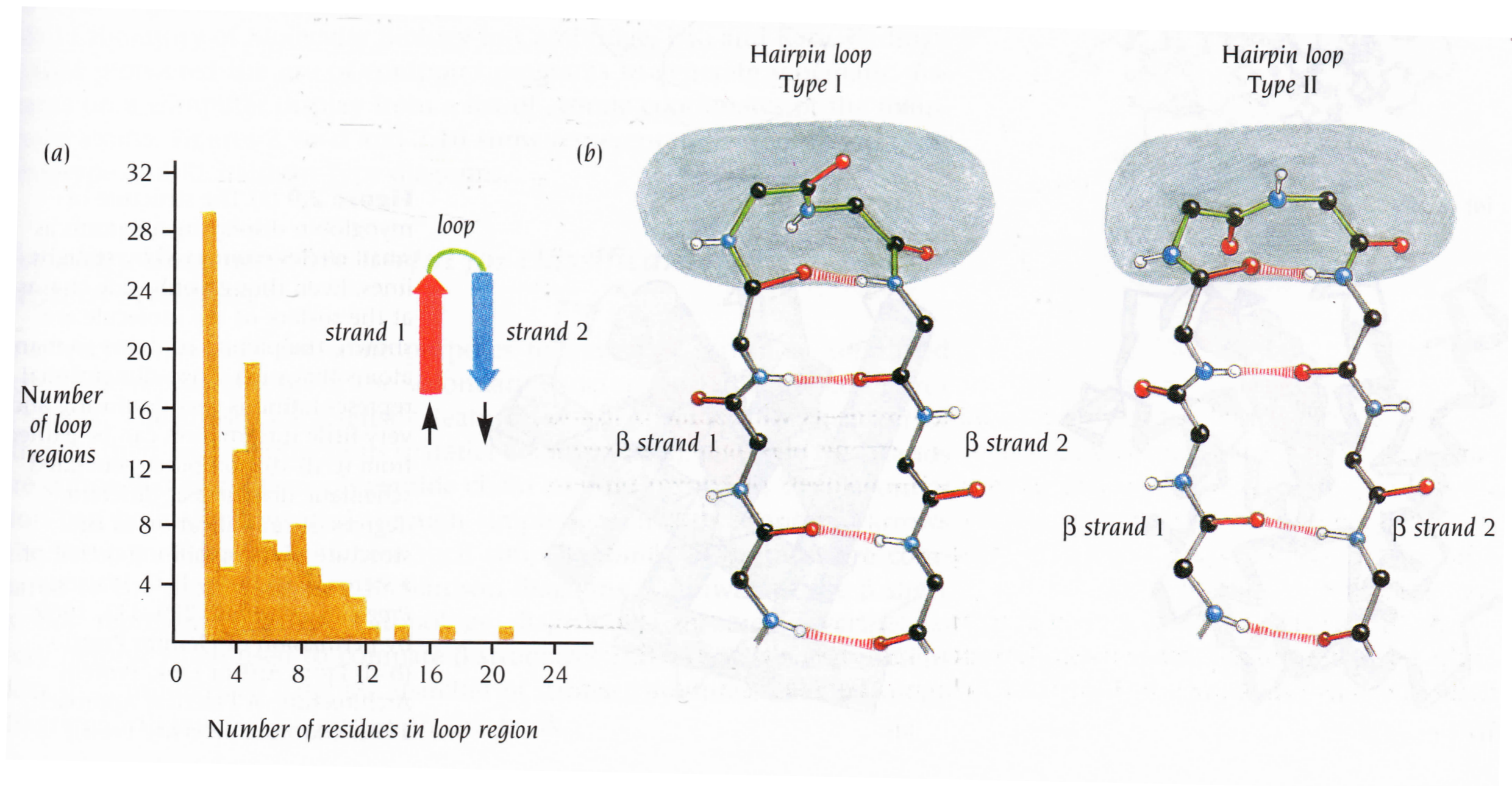
<http://www.iq.usp.br/bayardo/softwares/proteina/basic/cap1/main1.html>

Visualização de uma alfa-hélice

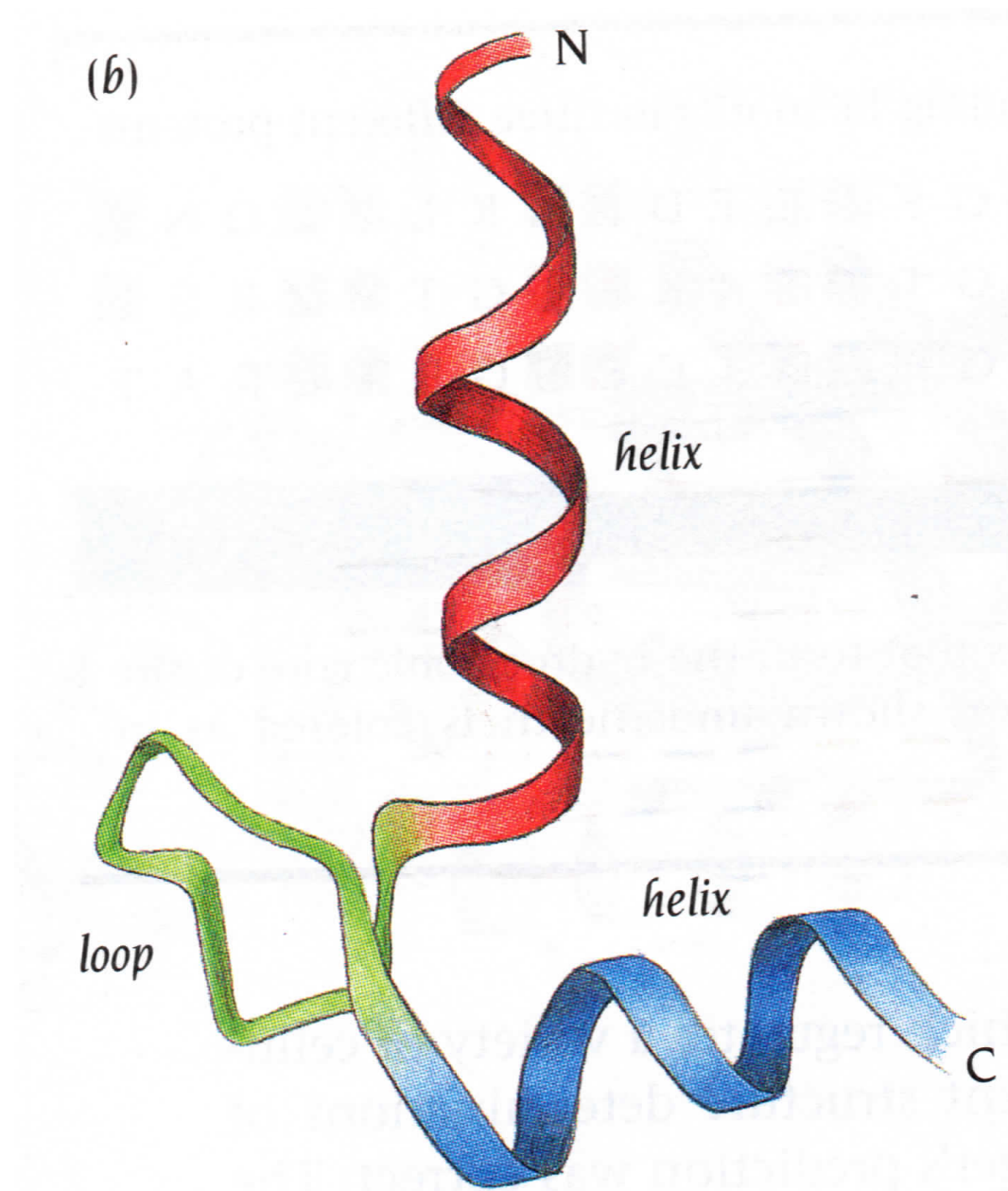
http://www.iq.usp.br/roberto/aulas_2014/qbq4010/pratica/pratica_validacao.html

Motivos

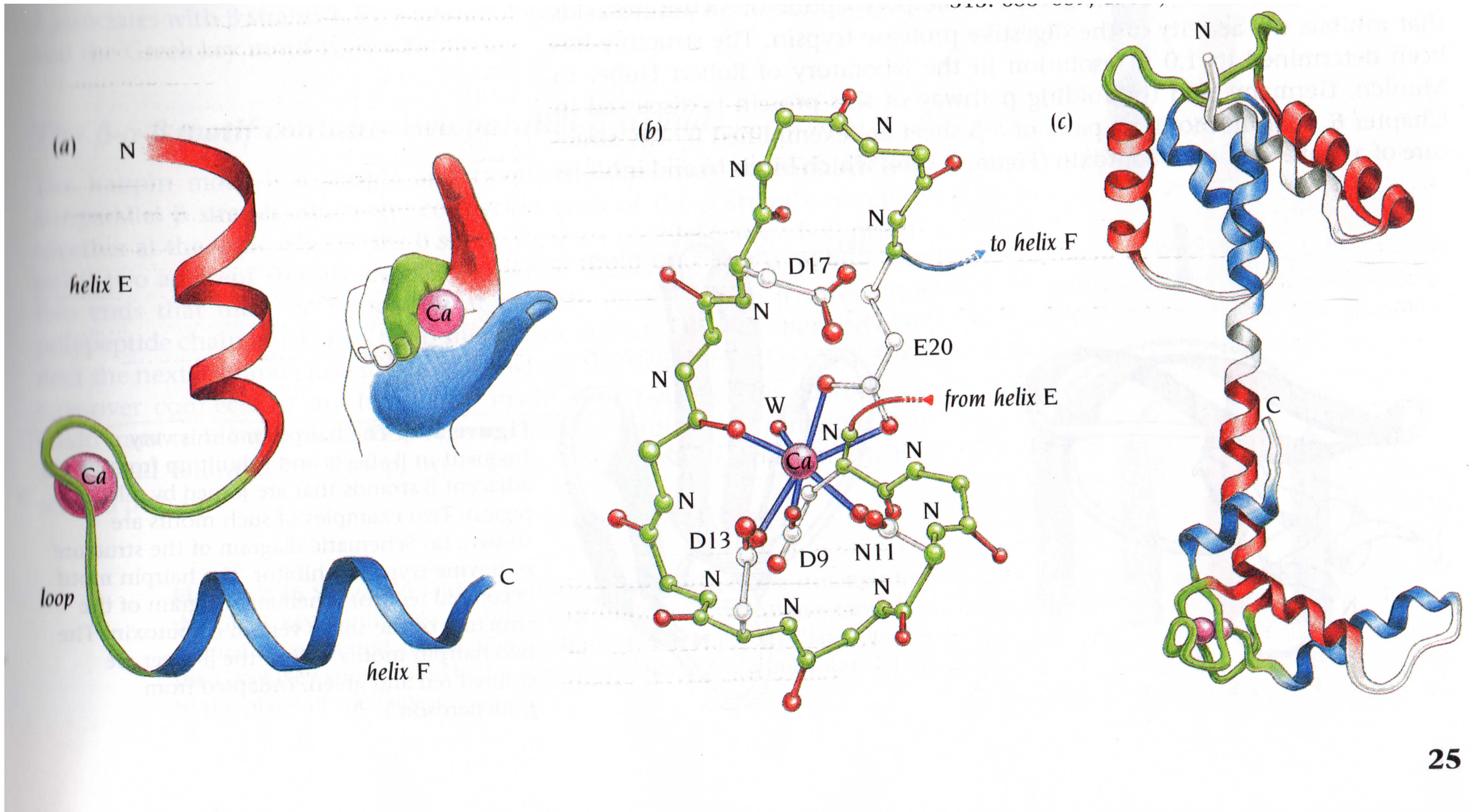
β -hairpin



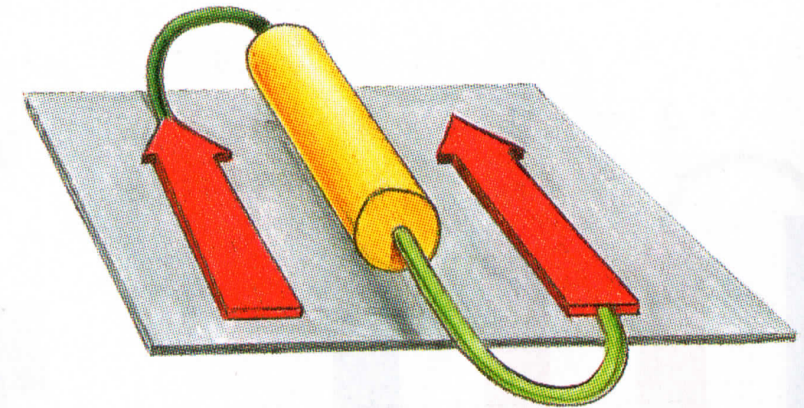
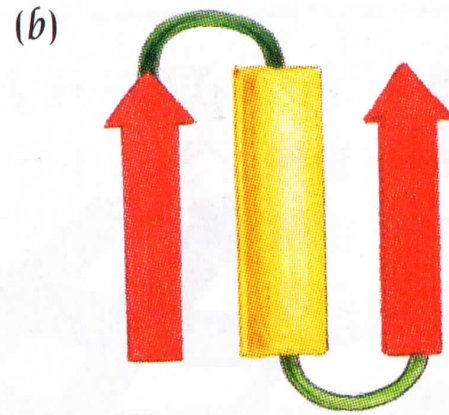
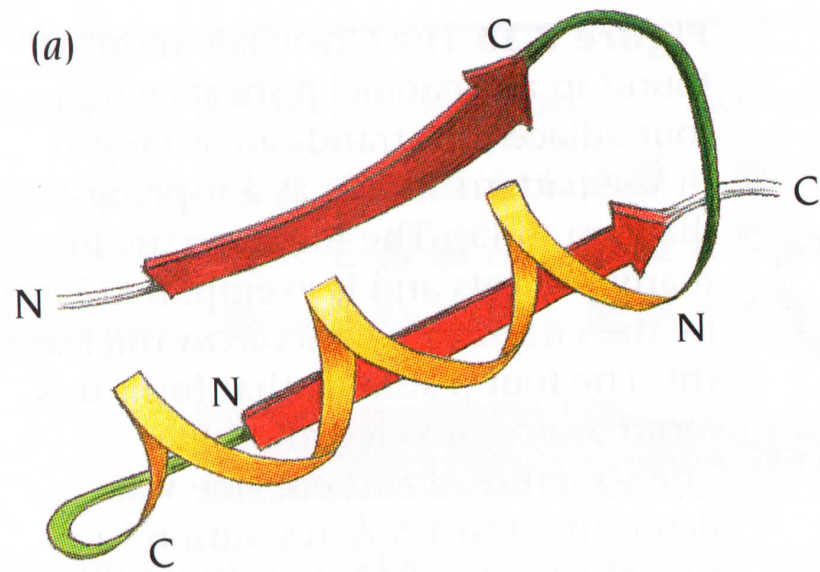
Helix-loop-helix Ca^{2+} -binding motif



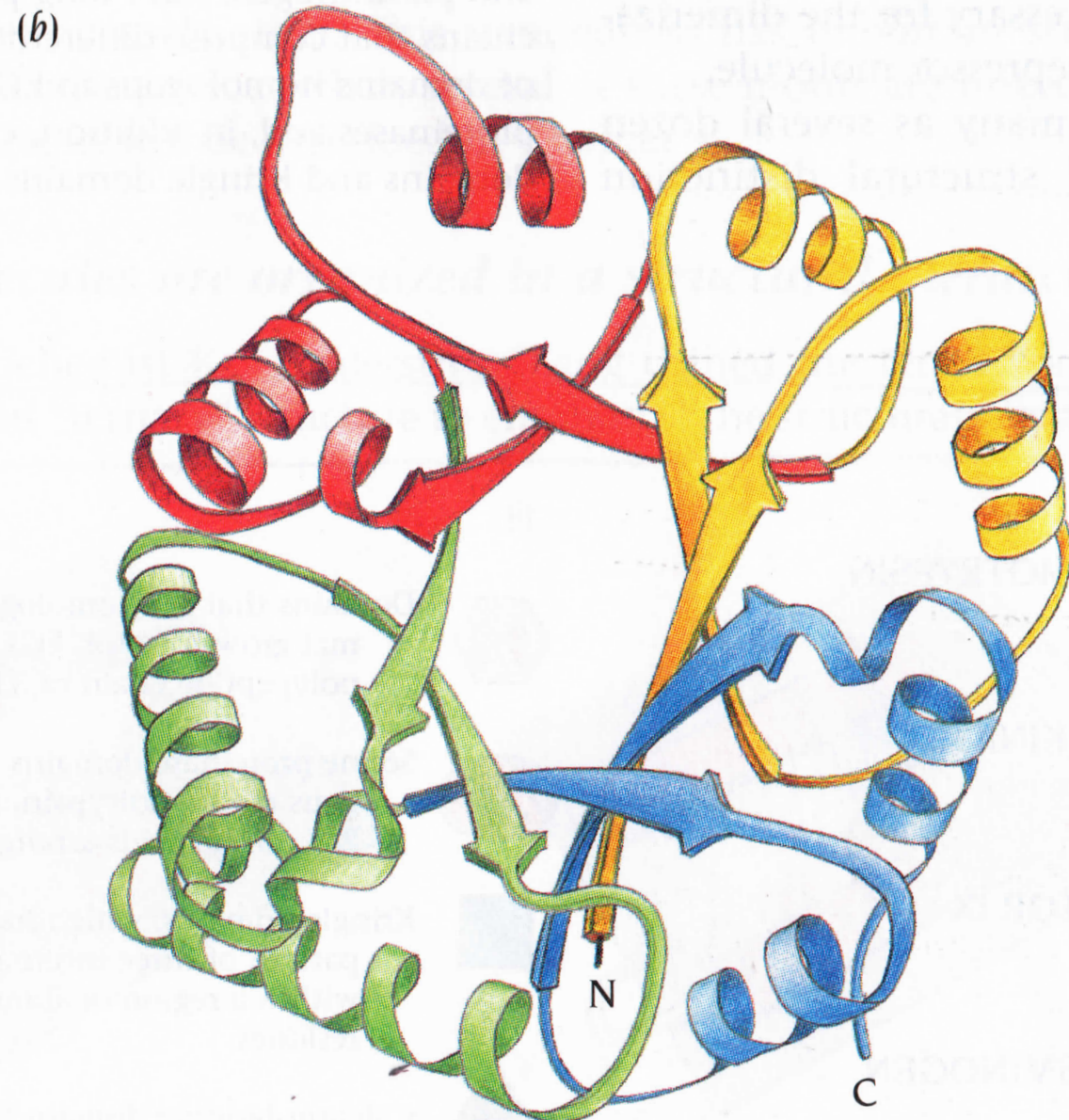
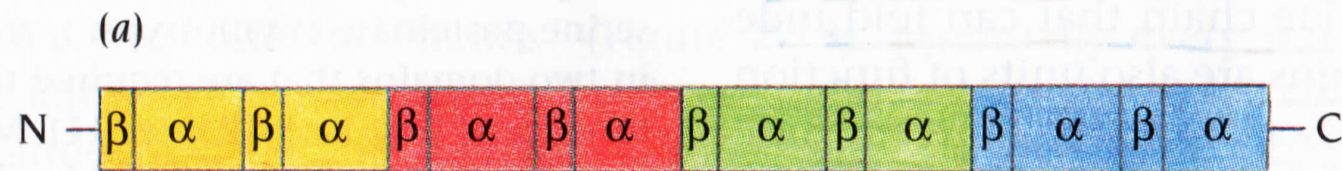
Helix-loop-helix Ca^{2+} -binding motif in calmodulin



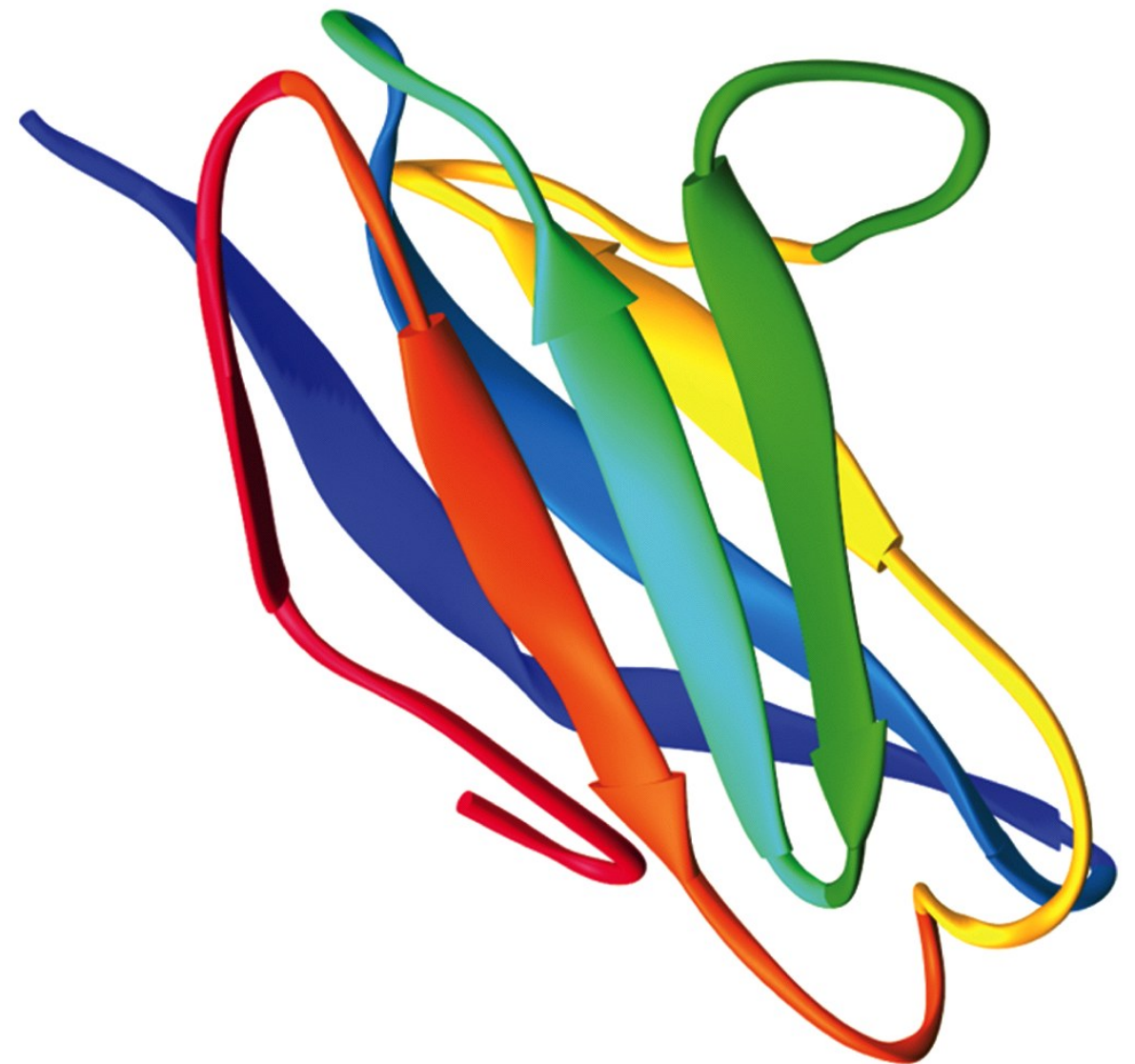
α - β - α motif



Domínios são formados a partir da combinação de motivos estruturais



Triose-fosfato-isomerase (TIM)



Imunoglobulina

Estrutura quaternária

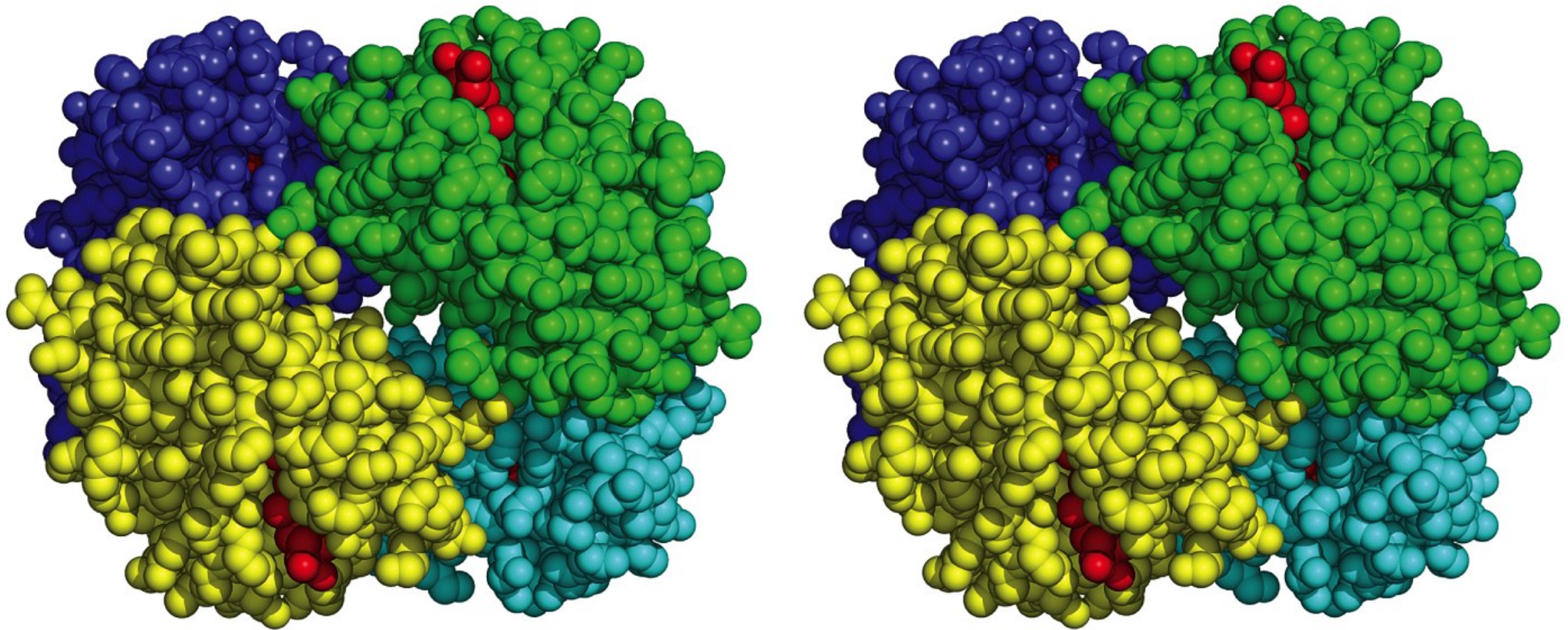
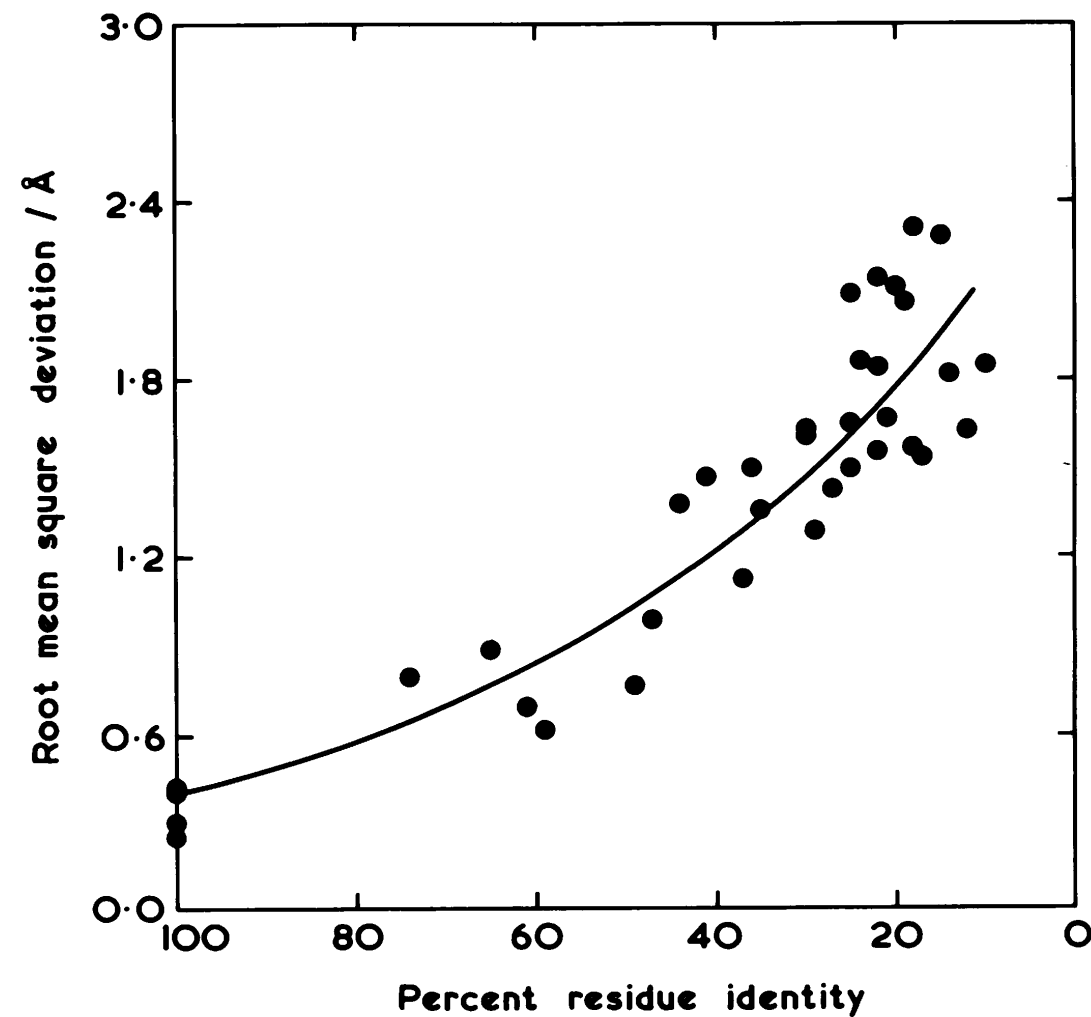


Figure 8-64
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Em geral, um grau de identidade maior que 30 % indica estrutura similar



Clothia and Lesk (1986) *EMBO J.* **5**: 823

Estabilidade

● A hipótese termodinâmica de Anfinsen

A sequência de aminoácidos determina a estrutura tridimensional

The studies on the renaturation of fully denatured ribonuclease required many supporting investigations (6–8) to establish, finally, the generality which we have occasionally called (9) the “thermodynamic hypothesis.” This hypothesis states that the three-dimensional structure of a native protein in its normal physiological milieu (solvent, pH, ionic strength, presence of other components such as metal ions or prosthetic groups, temperature, and other) is the one in which the Gibbs free energy of the whole system is lowest; that is, that the native conformation is determined by the totality of interatomic interactions and hence by the amino acid sequence, in a given environment. In terms of natural selection

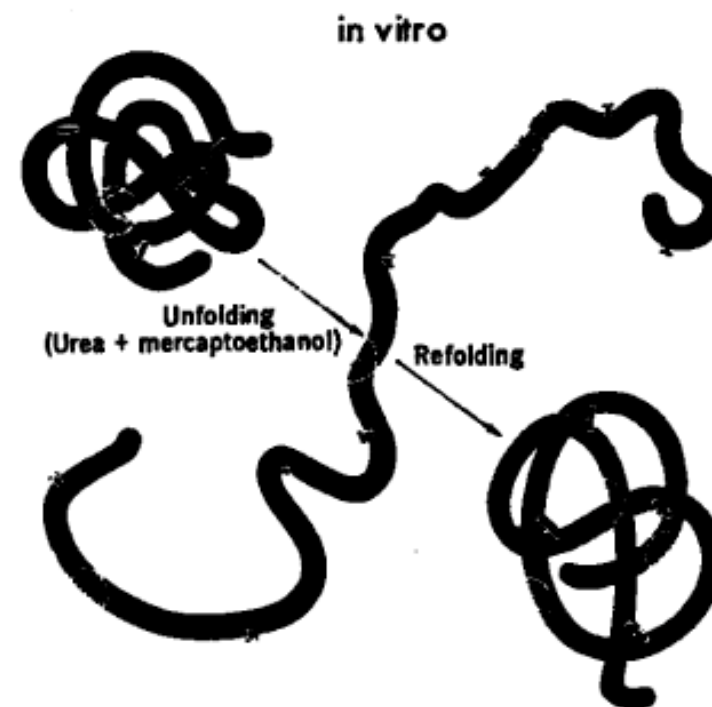


Fig. 2. Schematic representation of the reductive denaturation, in 8M urea solution containing 2-mercaptoethanol, of a disulfide-cross-linked protein. The conversion of the extended, denatured form to a randomly cross-linked, “scrambled” set of isomers is depicted at the lower right.

Interações que estabilizam a estrutura 3D

Não covalentes

Interações de van der Waals

Interações eletrostáticas

Ligações de hidrogênio

Covalente

Ligação dissulfeto

**Proteínas enovelam-se em consequência do efeito
hidrofóbico**

Ligações de disulfeto

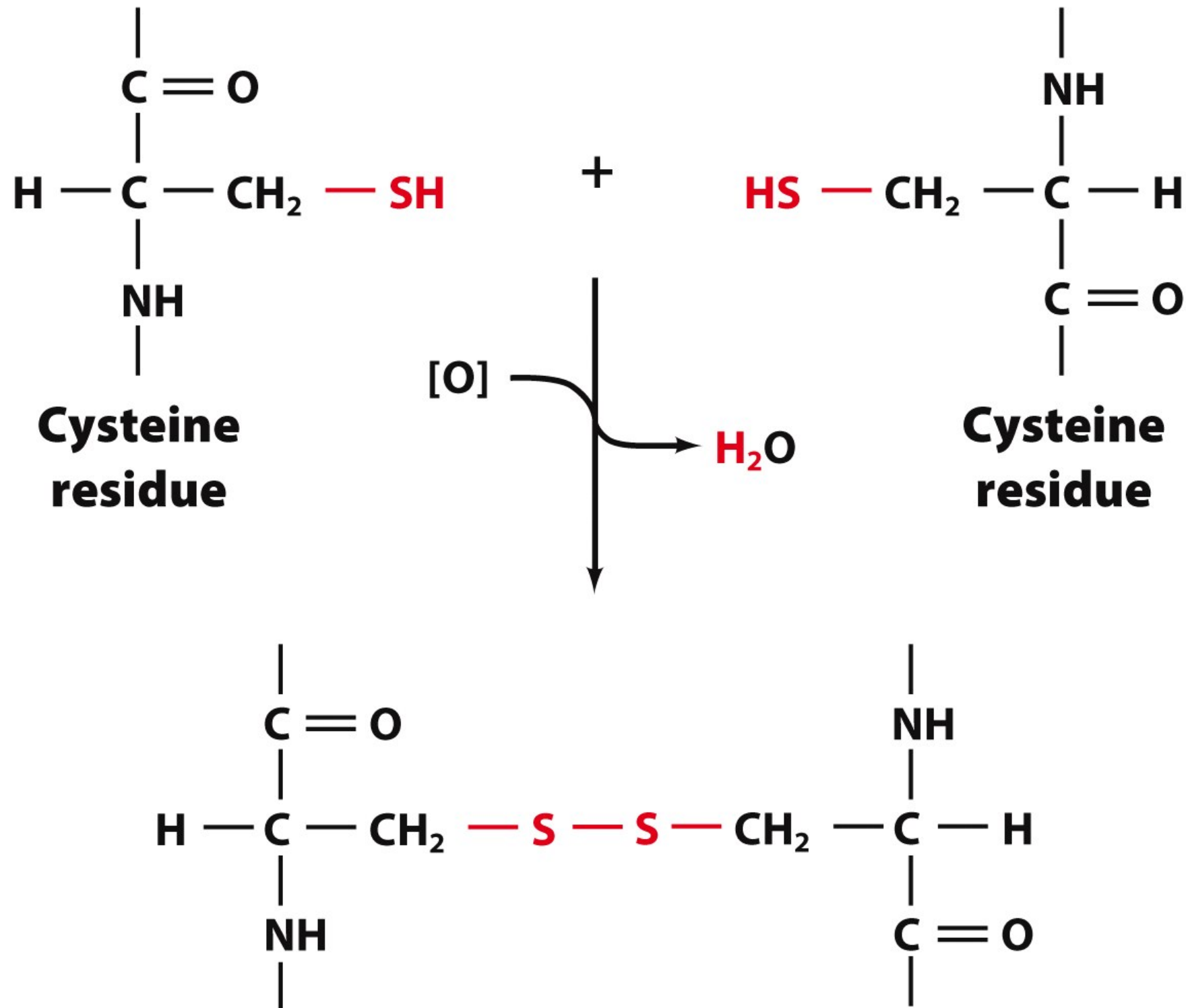


Figure 4-5
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Interações de van der Waals

$U_{\text{london}}(r) = -14.54 \times 10^{-21} \text{ J}$ para duas moléculas de HCl ($r=3 \text{ \AA}$) a 300K

$U_{\text{dipolo-dipolo}}(r) = -2.5 \times 10^{-21} \text{ J}$

$U_{\text{dipolo-dipolo induzido}}(r) = -0.77 \times 10^{-21} \text{ J}$

$k_B T = 4.1 \times 10^{-21} \text{ J}$ a 300K

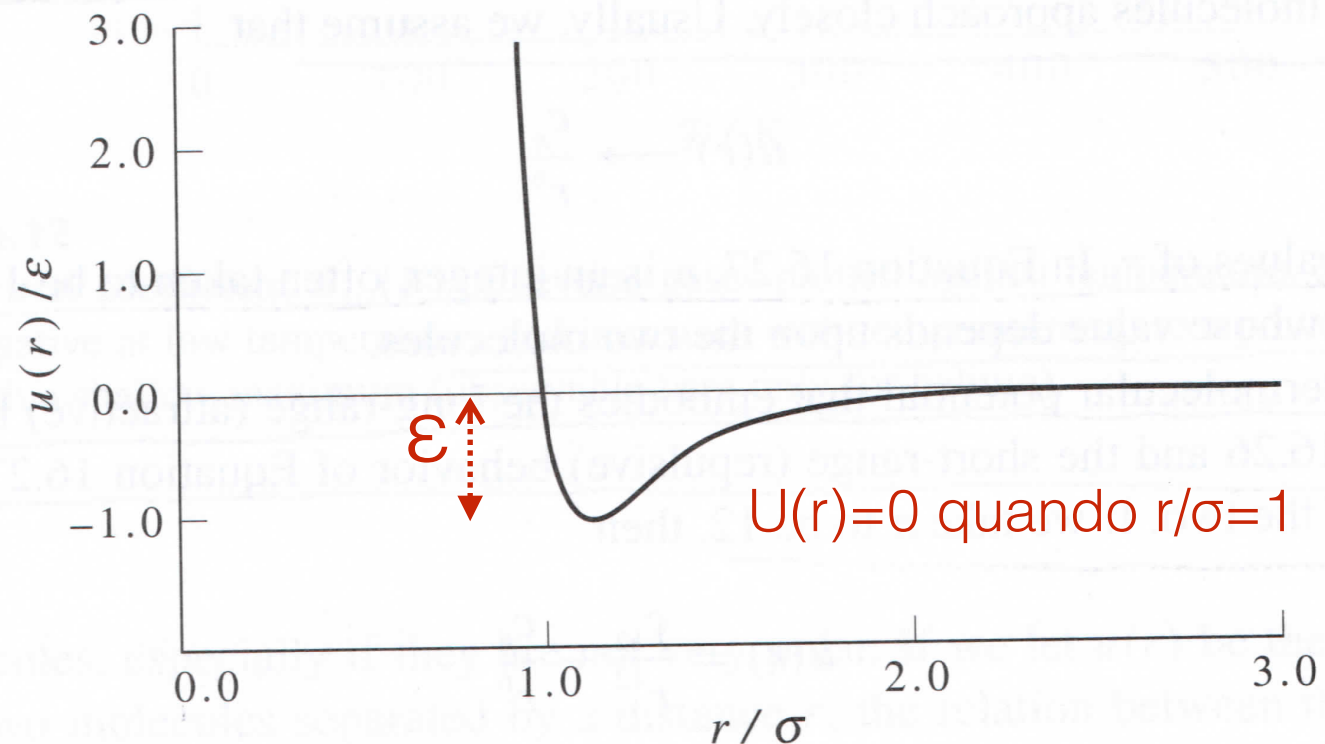
$C_6 = U_{\text{london}} + U_{\text{dipolo-dipolo}} + U_{\text{dipolo-dipolo induzido}}$ **(moléculas polares)**

$C_6 = U_{\text{london}}$ **(moléculas apolares)**

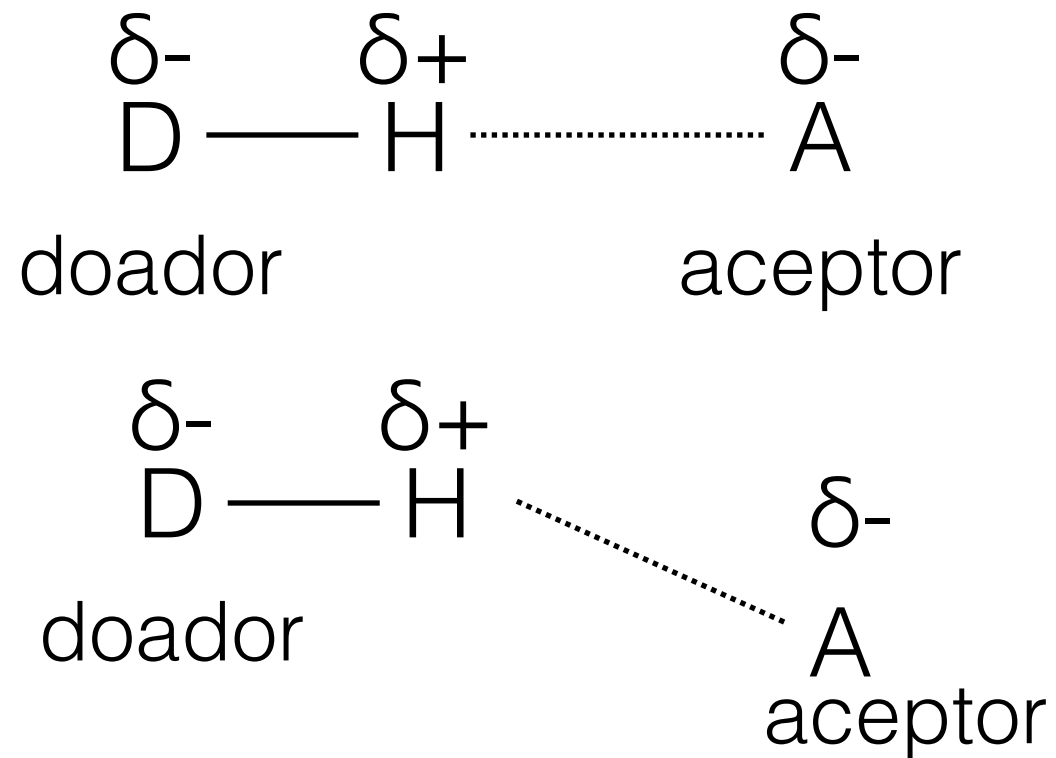
$$U(r) = \frac{C_{12}}{r^{12}} - \frac{C_6}{r^6}$$

$$U(r) = 4\epsilon \left[\left(\frac{\sigma}{r} \right)^{12} - \left(\frac{\sigma}{r} \right)^6 \right]$$

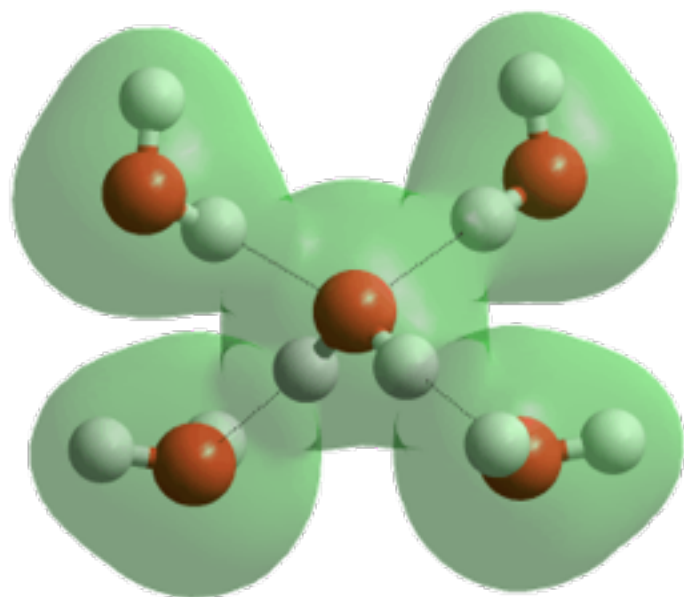
↑ ↑
repulsão atração



Ligações de hidrogênio



Caráter de ligação é 90% eletrostático e 10% covalente



Energia de ligações covalentes

Ligação	u (kcal/mol)
H—C	80.6
C—C	98.3

Ligação de hidrogênio:

Interação	Energia (kcal/mol)
Água/Água	5.5
Dipolo-dipolo	0.5
dipolo-dipolo induzido	0.012

Dill, K. et al. Molecular Driving Forces, Garland Science, 1ª. ed., 2003, Madison

Como as ligações de hidrogênio são muito mais fracas do que ligações covalentes o tempo de vida das ligações de hidrogênio é muito curto

Energias envolvidas na formação de interações não covalentes vs. covalentes

$$u(r) = (\text{constant})r^{-p}$$

Diferentes tipos de energia $u(r)$

Tipo	u (kcal/mol)	Dependência em r
iônica	66	$1/r$
íon/dipolo	4	$1/r^2$
dipolo/dipolo	0.5	$1/r^3$
dipolo/dipolo induzido	0.012	$1/r^6$

Energia de ligações covalentes

Ligação	u (kcal/mol)
H—C	80.6
C—C	98.3

Ponte de hidrogênio:

Água/Água	5.5 kcal/mol
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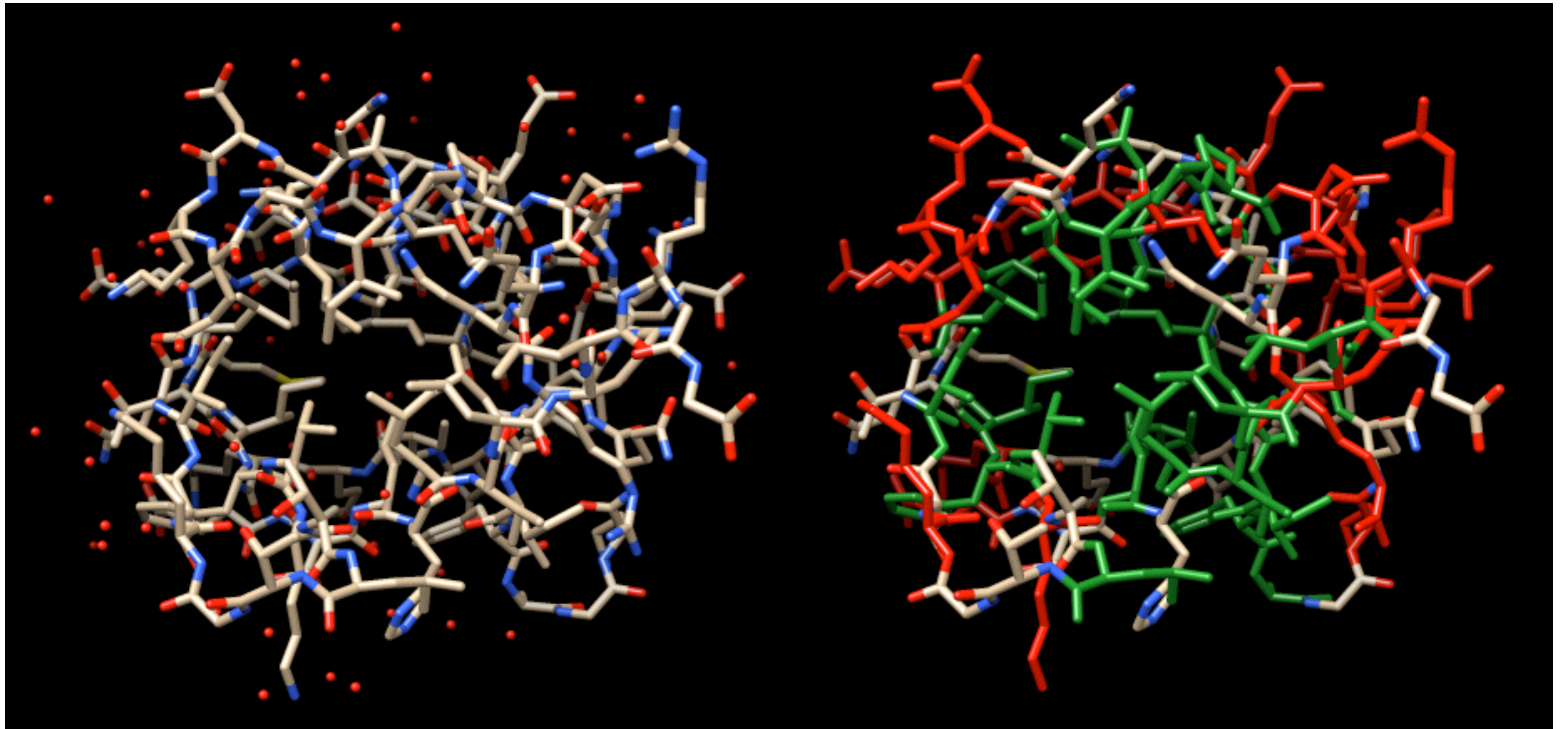
A estabilidade da proteína é da mesma ordem de grandeza que 4-5 ligações de hidrogênio

Termodinâmica de enovelamento de proteínas

Proteína	ΔG^0 (kcal/mol)	ΔH^0 (kcal/mol)	$T\Delta S^0$ (kcal/mol)
CI2	-6.62	-32.26	-25.64
EglinC	-8.82	-27.48	-18.66
RNAse T1	-8.96	-67.16	-58.22
Citocromo c	-8.87	-21.27	-12.40
Barnase	-11.69	-73.37	-61.71

Fonte: Lesk, A. *Introduction to protein science*. Oxford University Press, 2ª edição, p. 352 (2010)

Ubiquitina



- Apolares (A,L,I,V,P,F)
- Carregados (D,E,R,K)

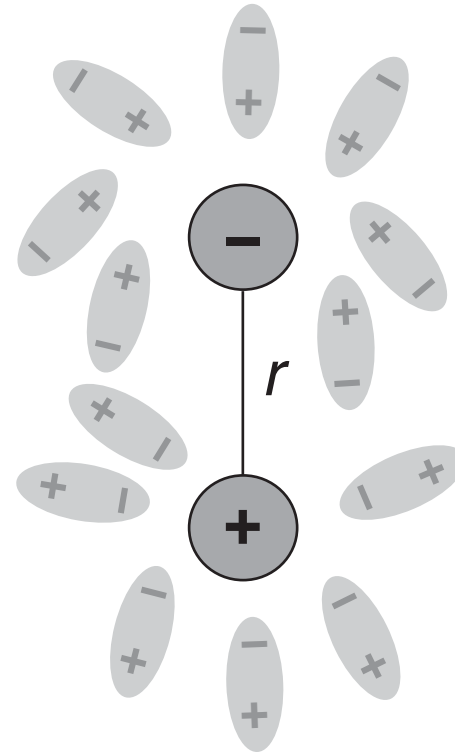
Interações eletrostáticas (longo alcance)

Energia electrostática no vácuo:

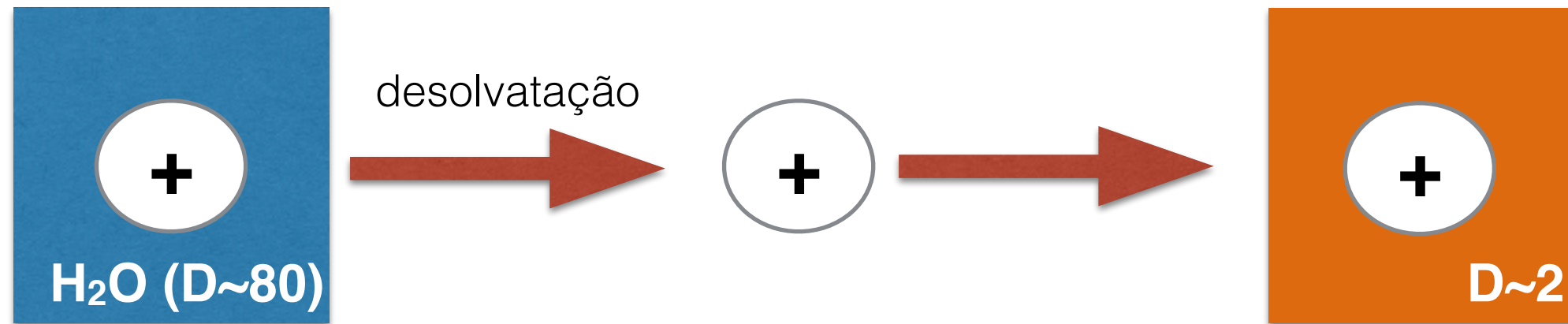
$$u(r) = C \frac{q_1 q_2}{r}$$

Energia electrostática em um
líquido de constante dielétrica D:

$$u(r) = C \frac{q_1 q_2}{Dr}$$



Transferência de uma esfera carregada da água para um meio apolar ($\mu=0$) consome muita energia



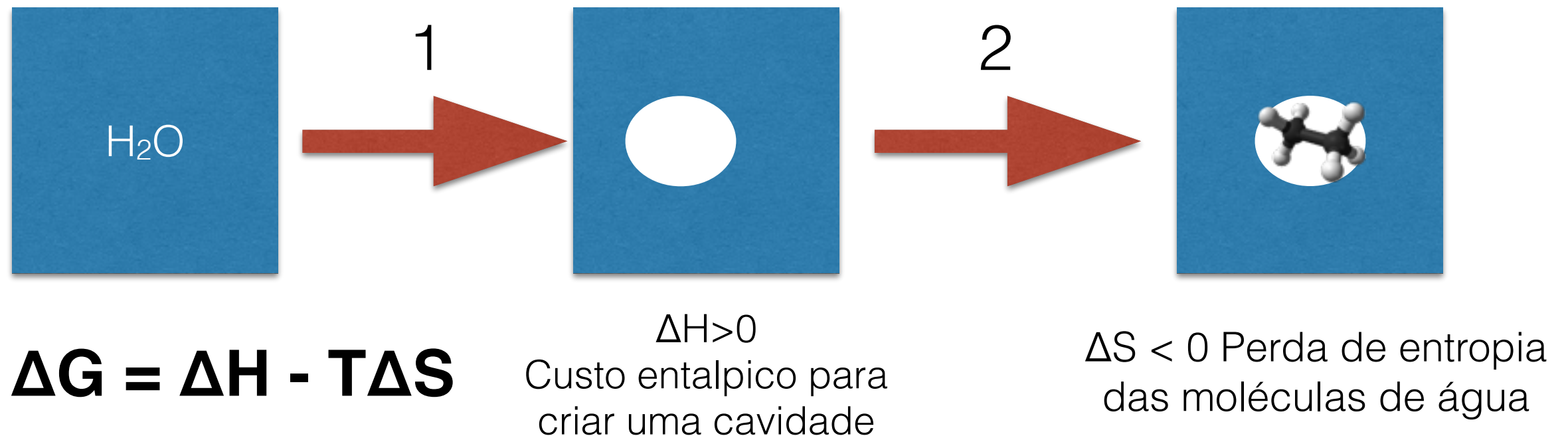
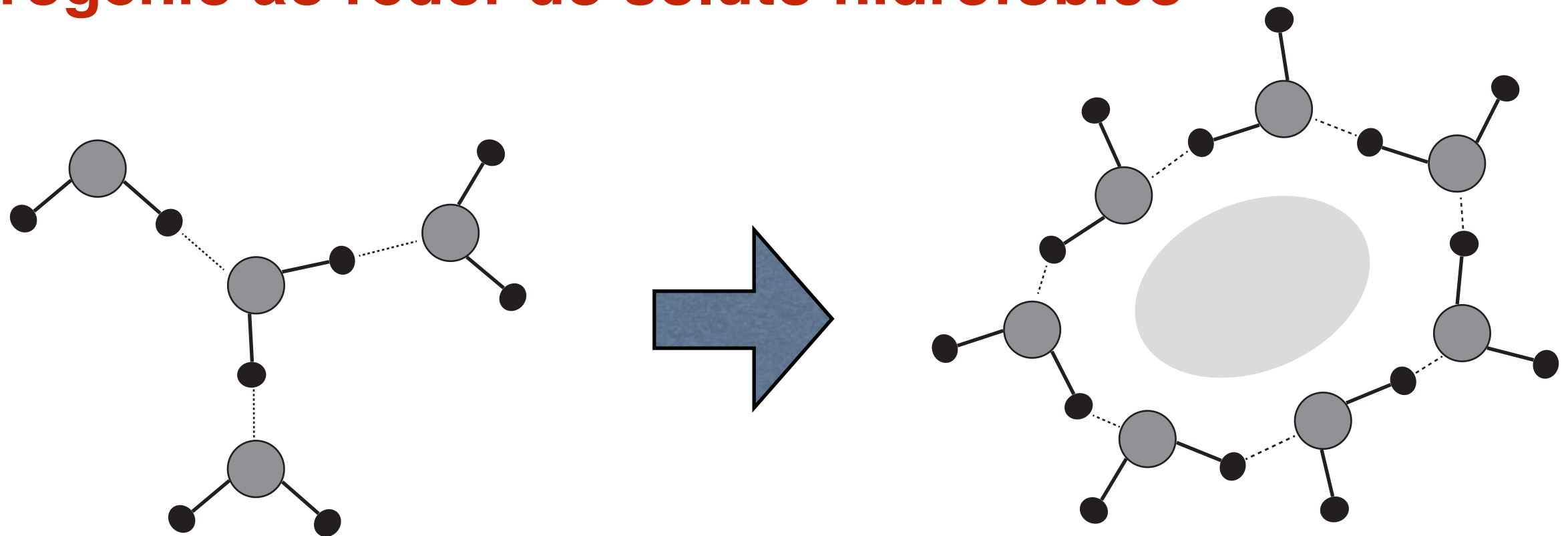
Energia eletrostática (considere carga “q” e raio “a”)

$$\Delta G_{elet} = \frac{Cq^2}{2a} \left(\frac{1}{D_{final}} - \frac{1}{D_{inicial}} \right) = \frac{Cq^2}{2a} \left(\frac{1}{2} - \frac{1}{80} \right) = +175 \text{ kJ/mol}$$

Energias típicas de interações não covalentes:

Tipo	u (kJ/mol)
iônica	276
íon/dipolo	16.7
dipolo/dipolo	2.09
dipolo/dipolo induzido	0.050

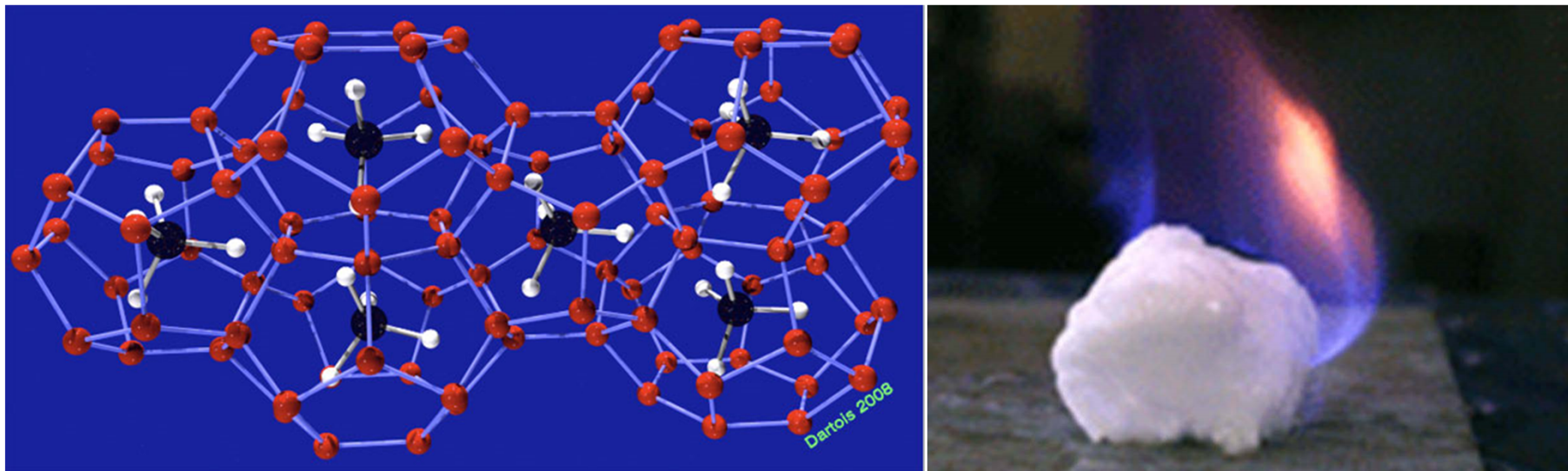
Moléculas de água mantêm a rede de ligações de hidrogênio ao redor do soluto hidrofóbico



Efeito hidrofóbico: enovelamento envolve a liberação de moléculas de água ($\Delta S_{\text{água}} > 0$)

Estrutura da água

Methane clathrate
Ultimate cluster formation



Efeito hidrofóbico

Propriedades termodinâmicas de transferência de hidrocarbonetos para a água, baseado nas solubilidades a 298K

	ΔG	ΔH	ΔS
benzeno	19.33	2.08	-57.8
pentano	28.62	-2	-102.7
hexano	32.54	0	-109.1
ciclohexano	28.13	-0.1	-94.7



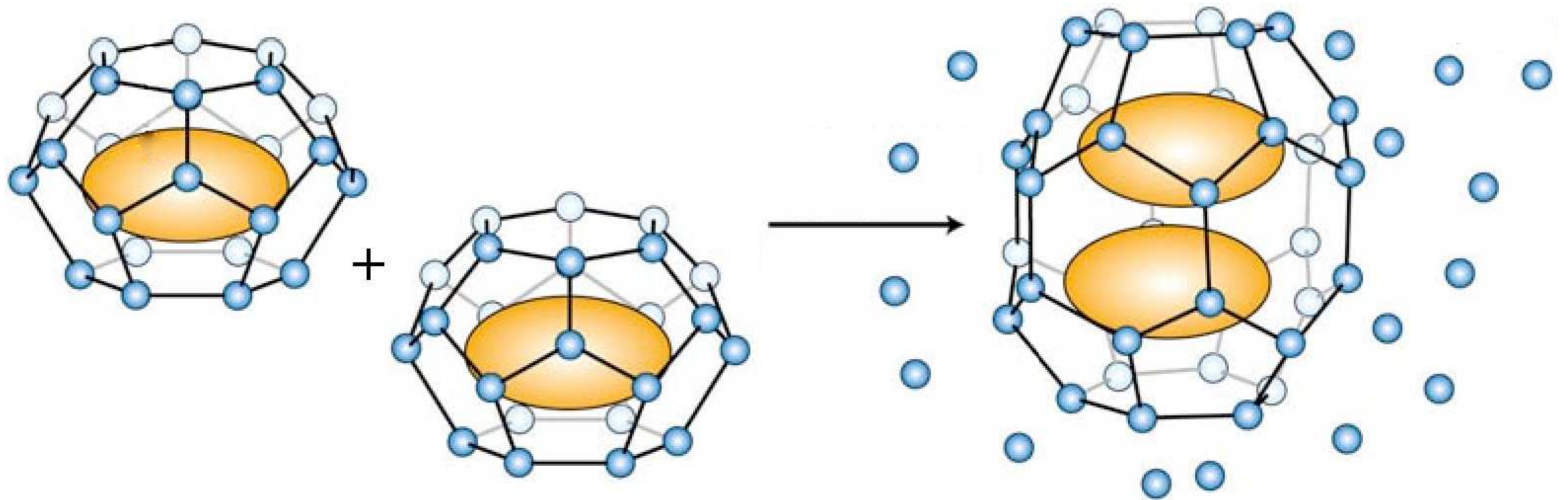
hidrofílico: dissolve em água
hidrofóbico: não dissolve em água

Dill, K. et al. Molecular Driving Forces, Garland Science, 1ª. ed., 2003, Madison (pág. 579)

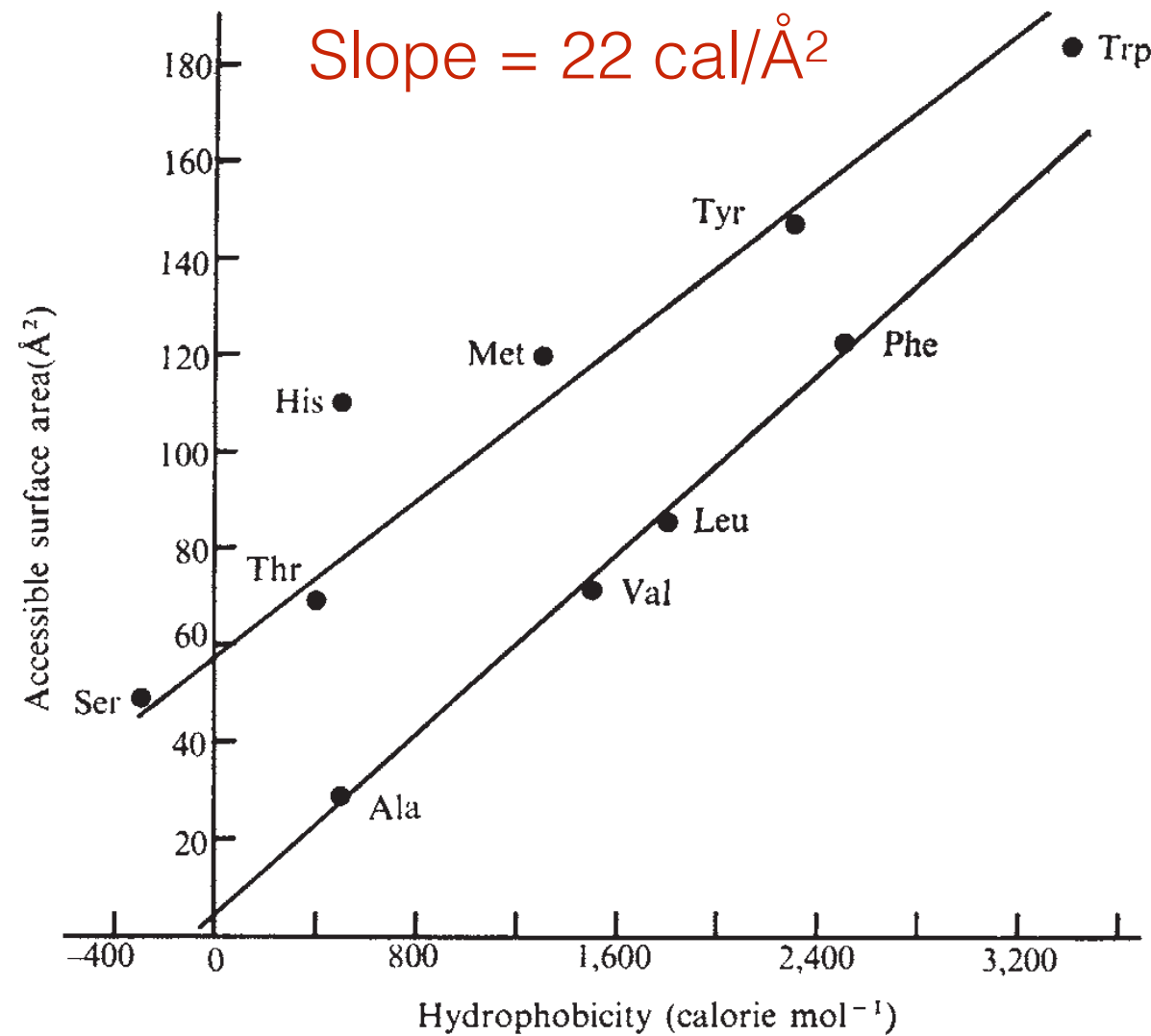
$$\Delta G = \Delta H - T\Delta S$$



Folding envolve o ganho de entropia do solvente



Cyrus Clothia quantificou o efeito hidrofóbico



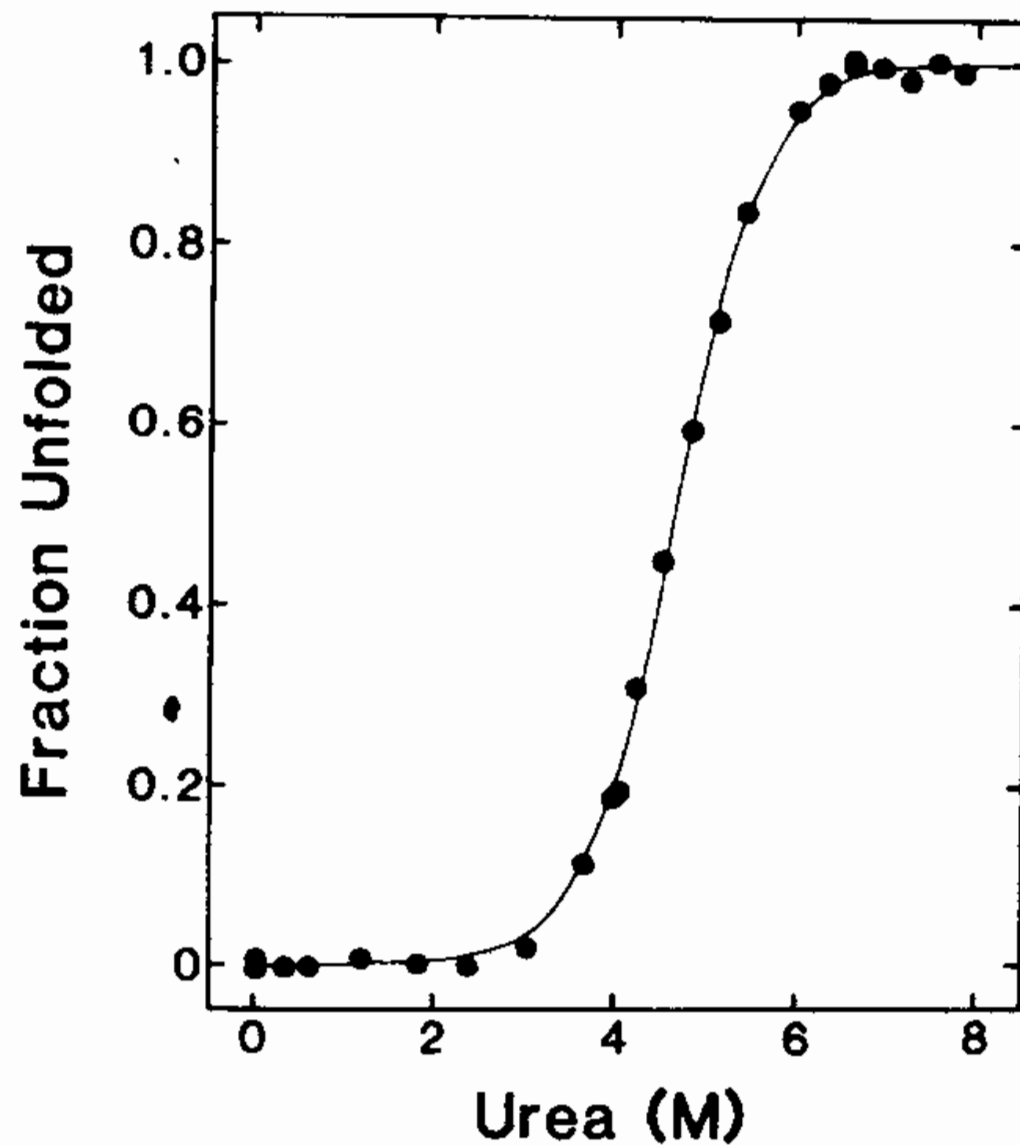
Cyrus Clothia (1974) *Nature* **248**:338

• Proteínas são marginalmente estáveis

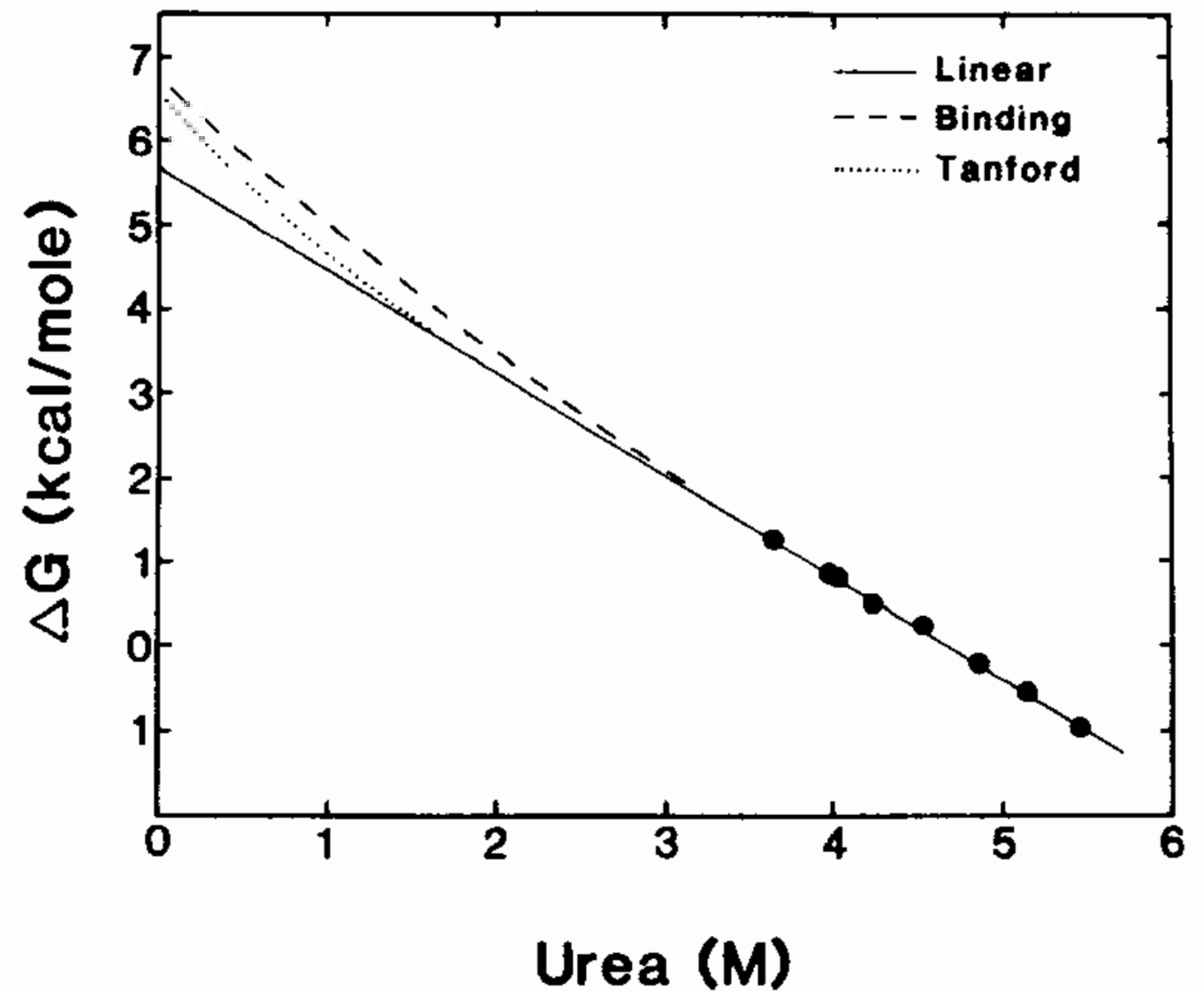
1. Estabilidade está intimamente conectada com enovelamento, proteínas precisam ser estáveis no estado enovelado (nativo)
2. Estado desnaturado (D) não é necessariamente o mesmo que desenovelado (U)
3. $\Delta G = \Delta H - T\Delta S$
4. A energia livre de enovelamento é a soma de termos entálpicos e entrópicos, resultando em um valor ΔG pequeno (tipicamente -5 a -15 kcal/mol), portanto **proteínas são marginalmente estáveis**
5. Durante o enovelamento, os ganhos entálpicos (pontes de hidrogênio, pontes salinas) são compensados por uma grande perda de entropia conformacional
6. Mas também existe um grande ganho de entropia do solvente (efeito hidrofóbico)
7. Para uma proteína de 100aa:
 - +167 kcal/mol = perda de entropia conformacional
 - 95 kcal/mol = efeito hidrofóbico
 - 83 kcal/mol = efeitos entálpicos (p. exemplo: pontes de hidrogênio)

- 11 kcal/mol

- Como medir a constante de equilíbrio de enovelamento/desenovelamento?



$$\Delta G = \Delta G(\text{H}_2\text{O}) - m [\text{denaturant}]$$



Proteínas de membrana

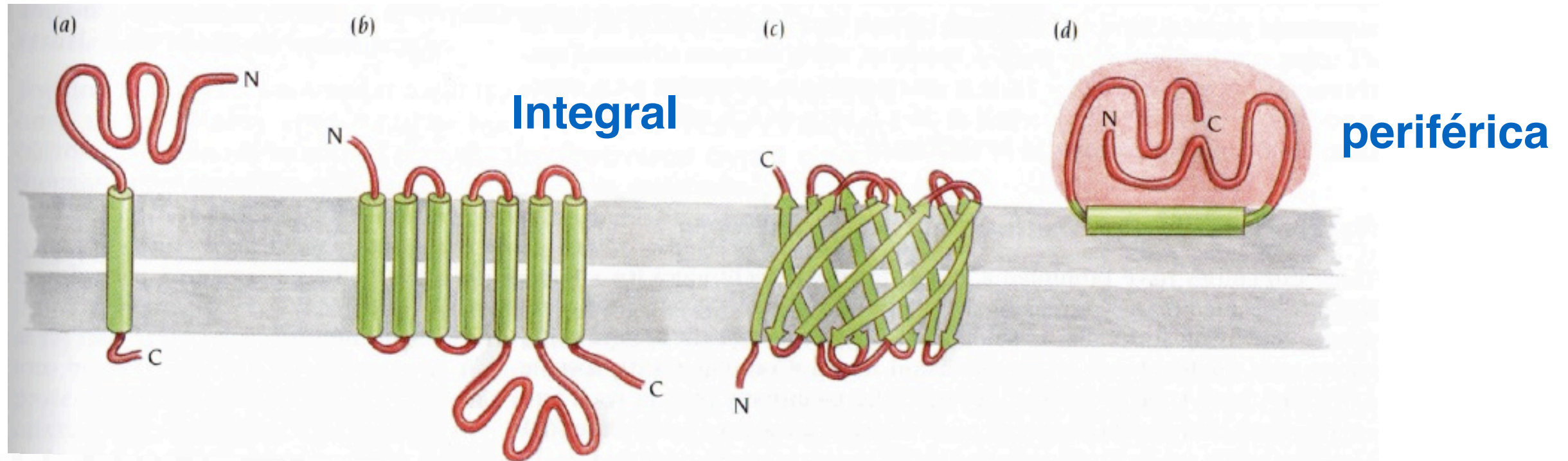
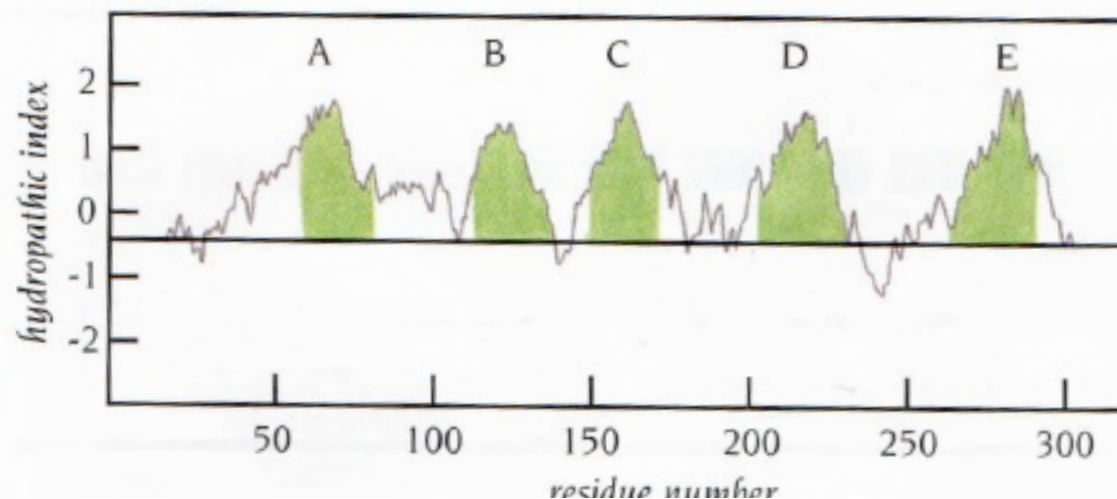


Table 12.1 Hydrophobicity scales

Amino acid	Phe	Met	Ile	Leu	Val	Cys	Trp	Ala	Thr	Gly	Ser	Pro	Tyr	His	Gln	Asn	Glu	Lys	Asp	Arg
A	2.8	1.9	4.5	3.8	4.2	2.5	-0.9	1.8	-0.7	-0.4	-0.8	-1.6	-1.3	-3.2	-3.5	-3.5	-3.5	-3.9	-3.5	-4.5
B	3.7	3.4	3.1	2.8	2.6	2.0	1.9	1.6	1.2	1.0	0.6	-0.2	-0.7	-3.0	-4.1	-4.8	-8.2	-8.8	-9.2	-12.3

Row A is from J. Kyte and R.F. Doolittle; row B, from D.A. Engelman, T.A. Steitz, and A. Goldman.



hidrofóbico

hidrofílico

Proteínas periféricas estão mais fracamente associadas à membrana

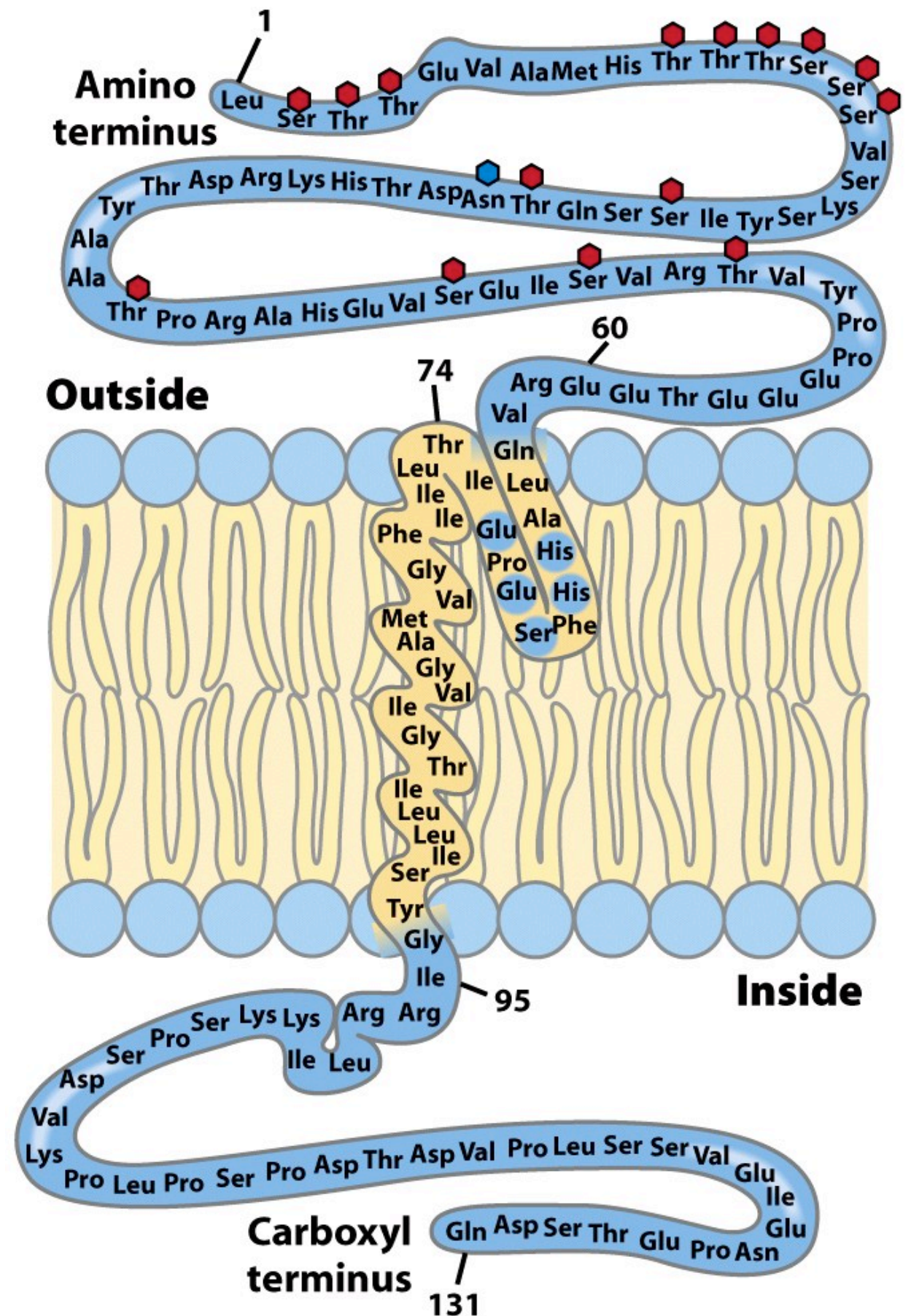
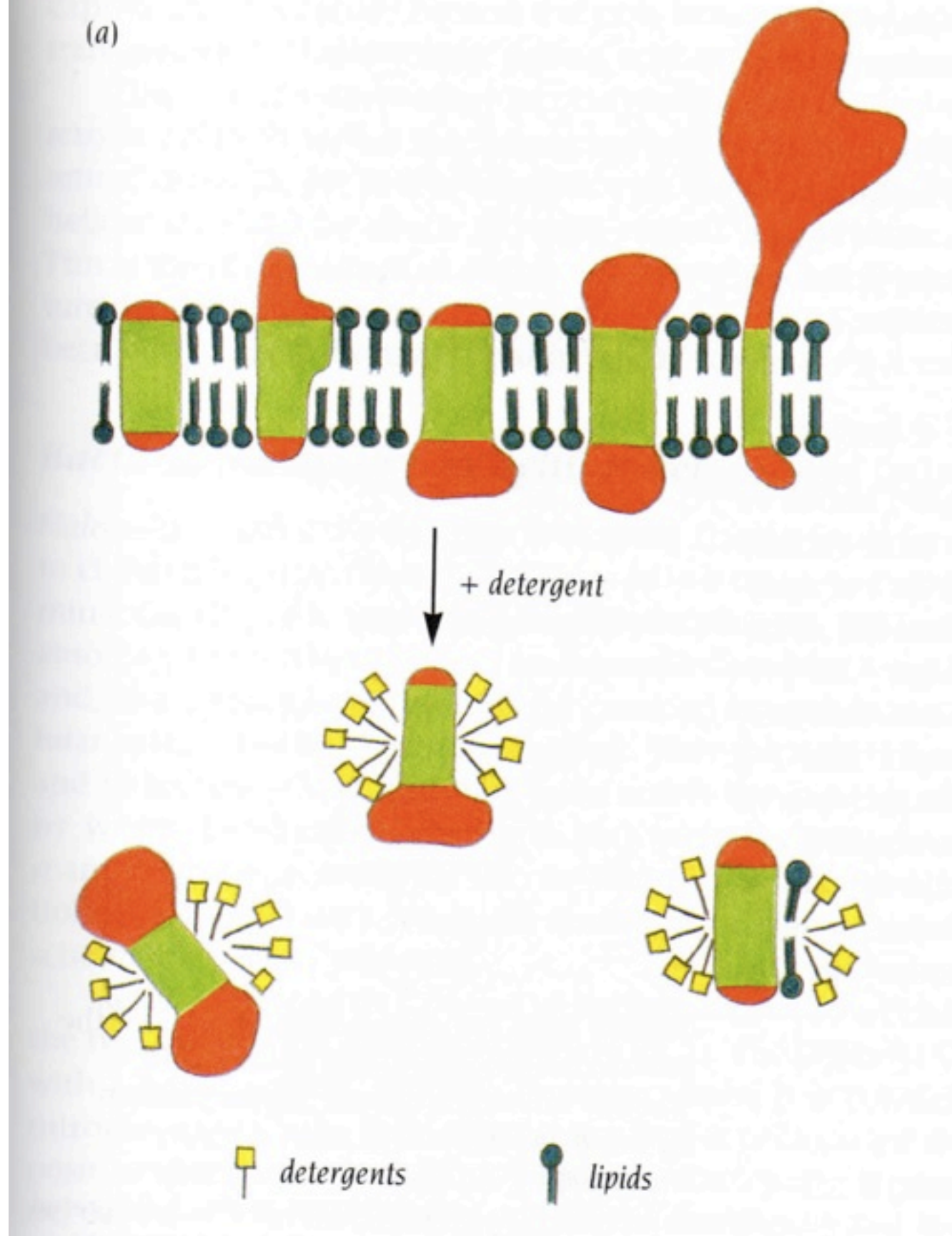
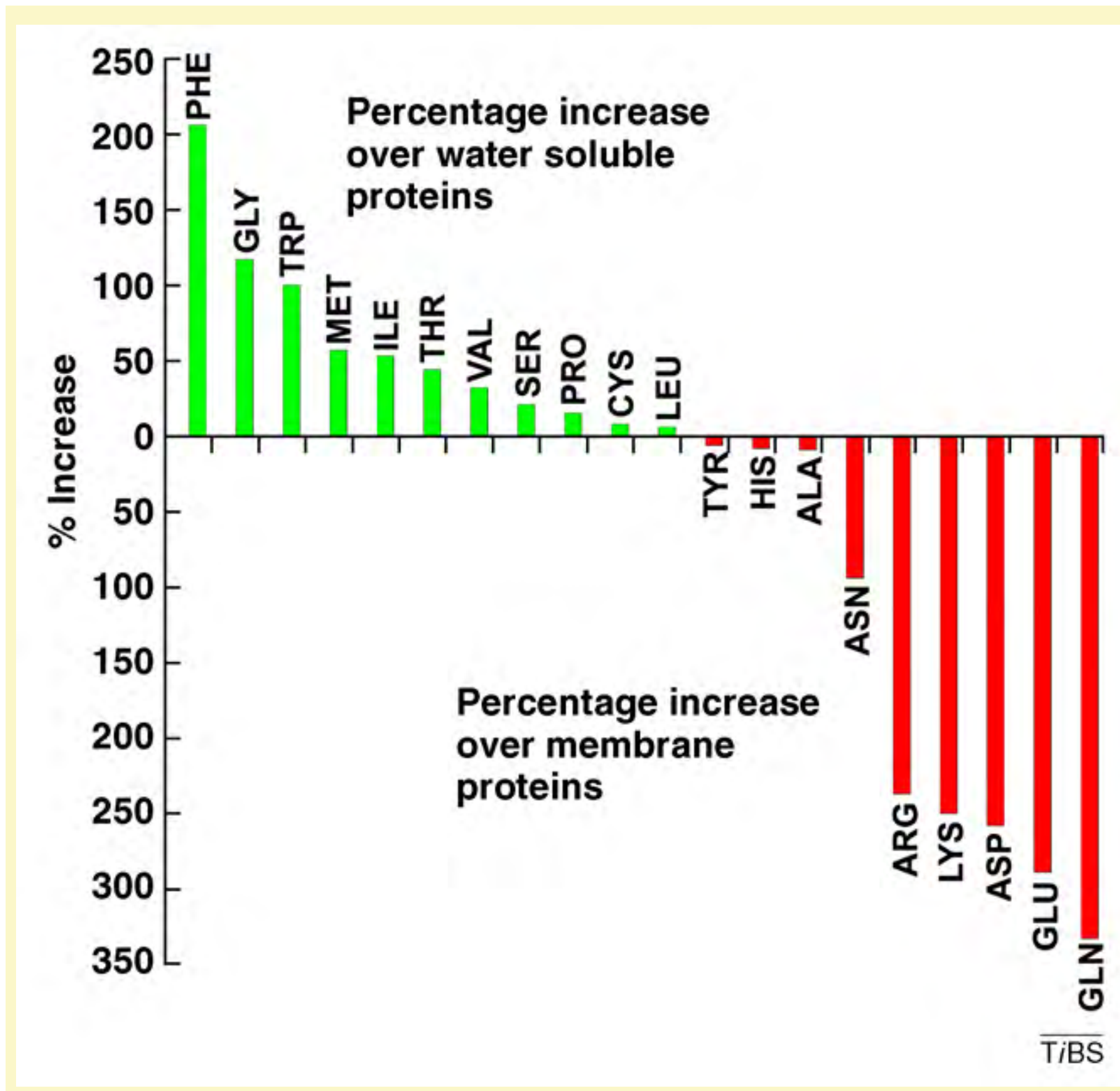
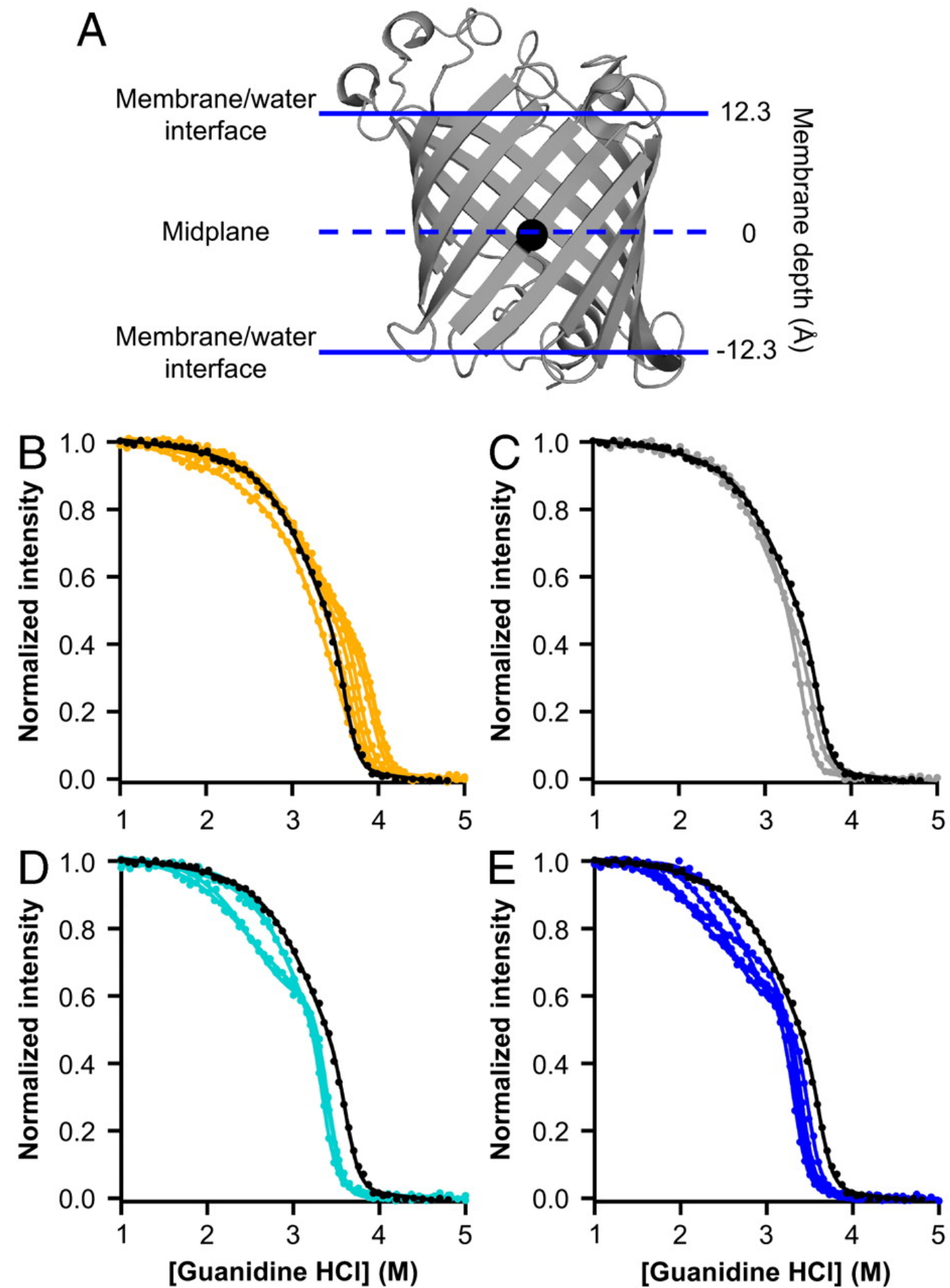
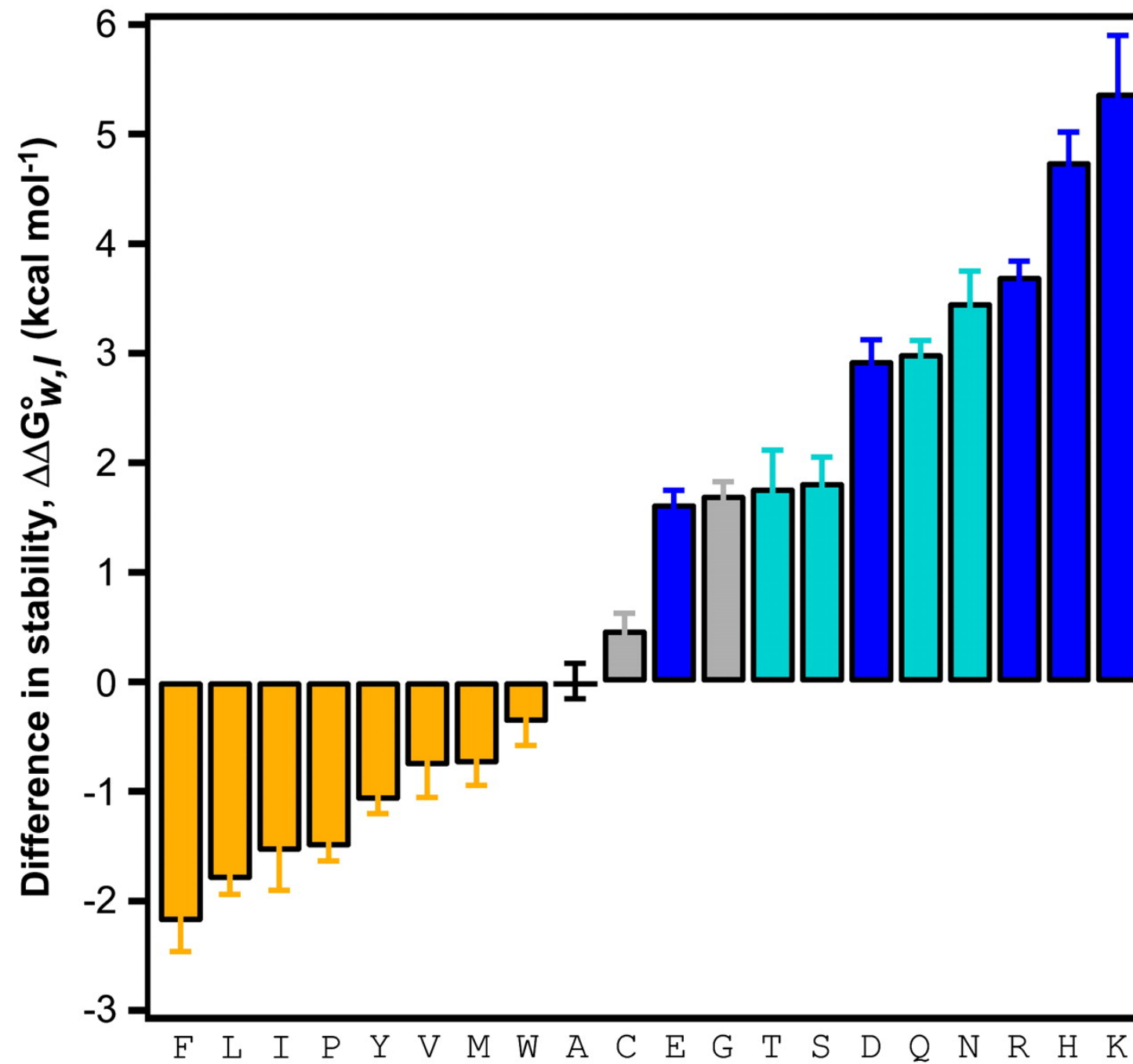


Figure 11-7
Lehninger Principles of Biochemistry, Fifth Edition

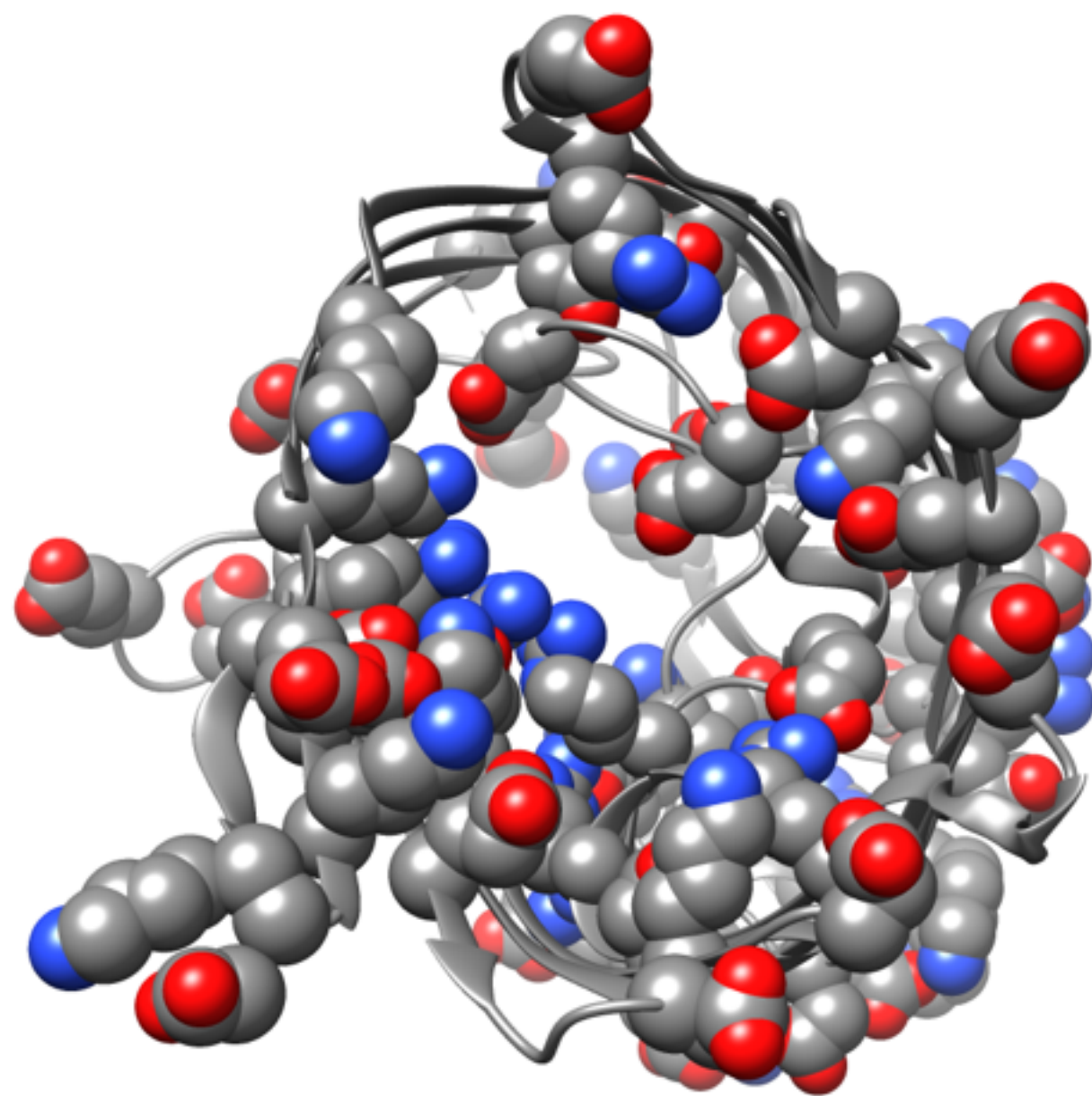
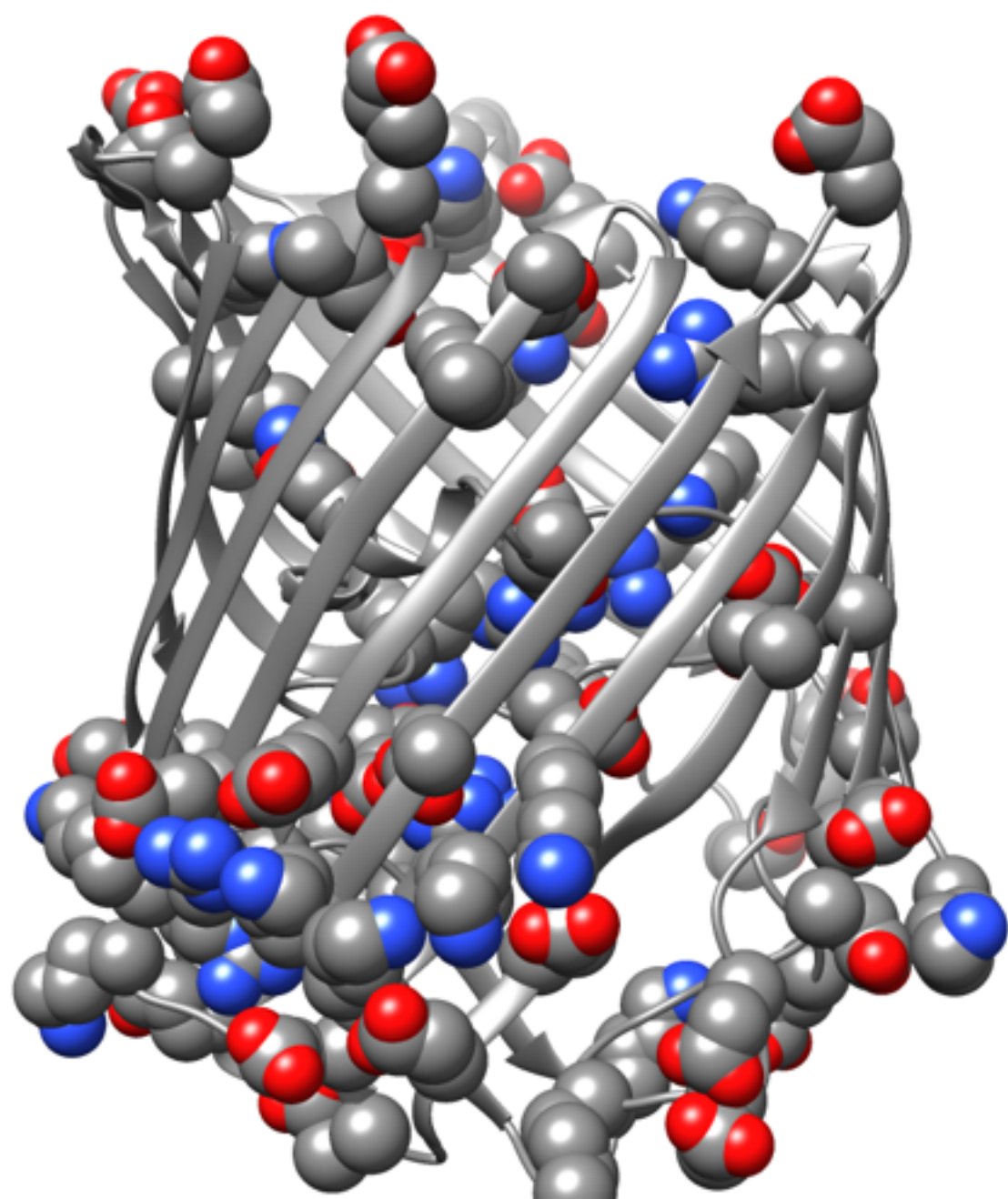




Whole-protein hydrophobicity scale determined from the OmpLA system.

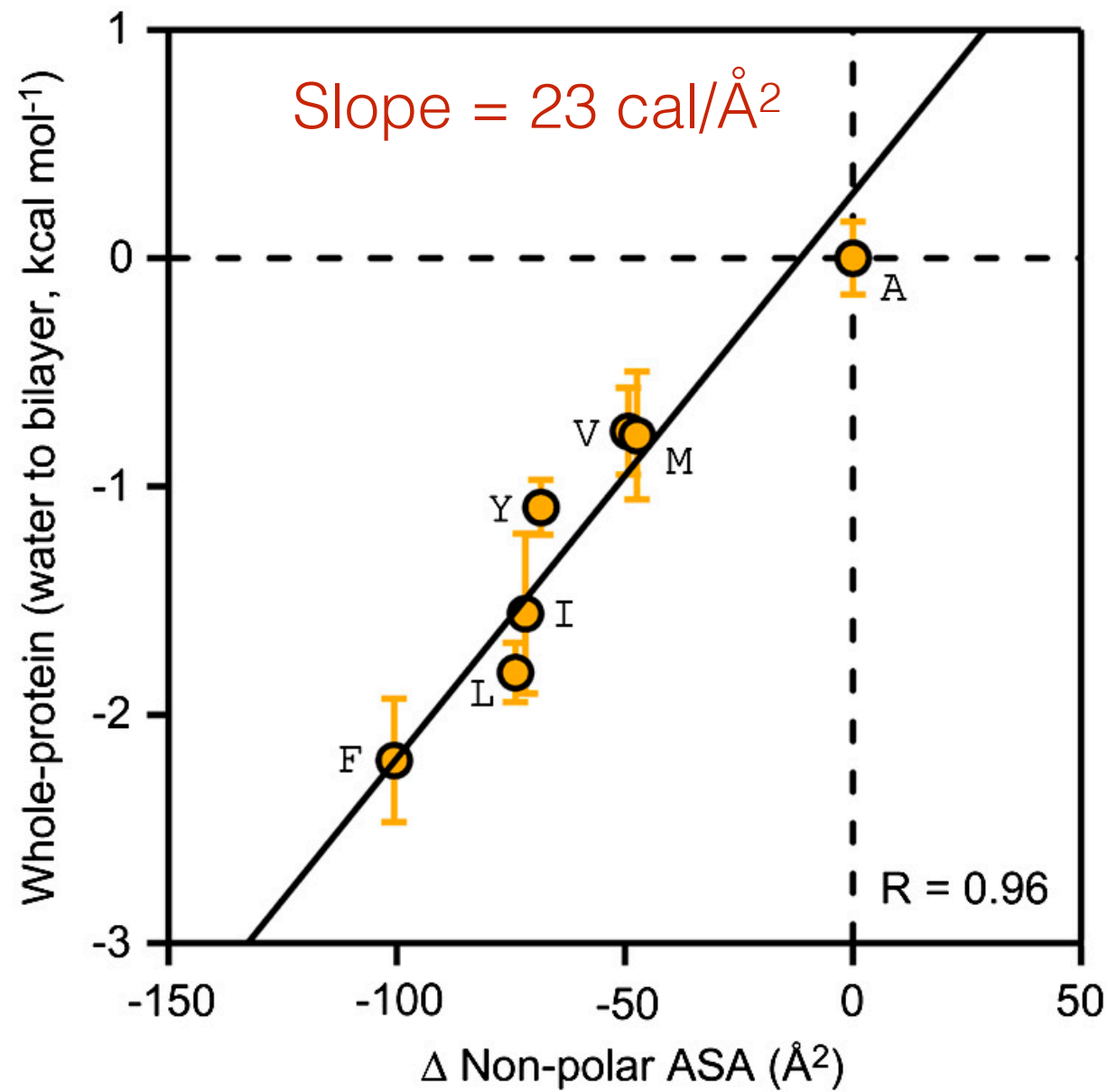


C. Preston Moon, and Karen G. Fleming PNAS
2011;108:10174-10177

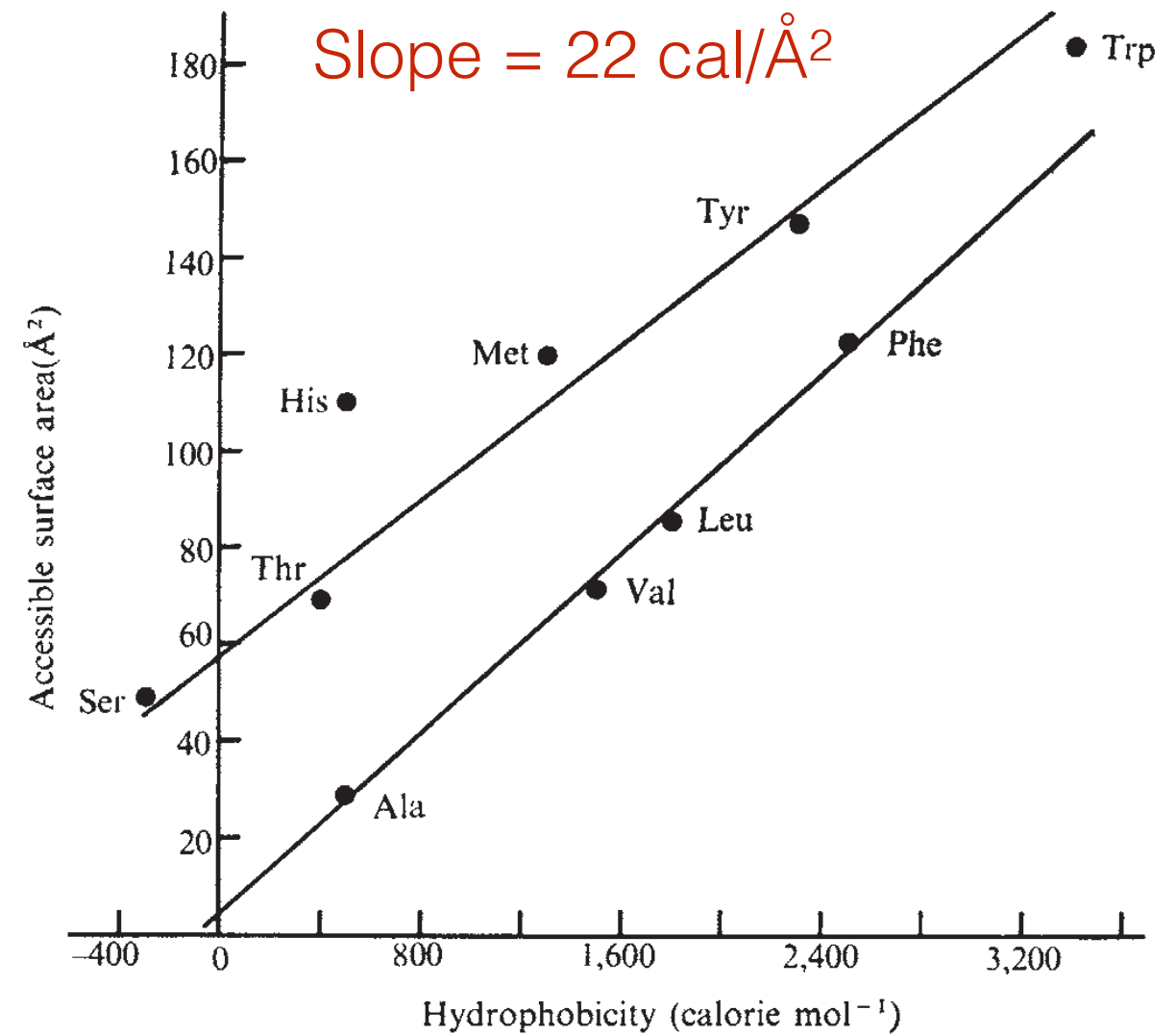


Porina

Energia de partição de cadeias laterais hidrofóbicas na bicamada lipídica



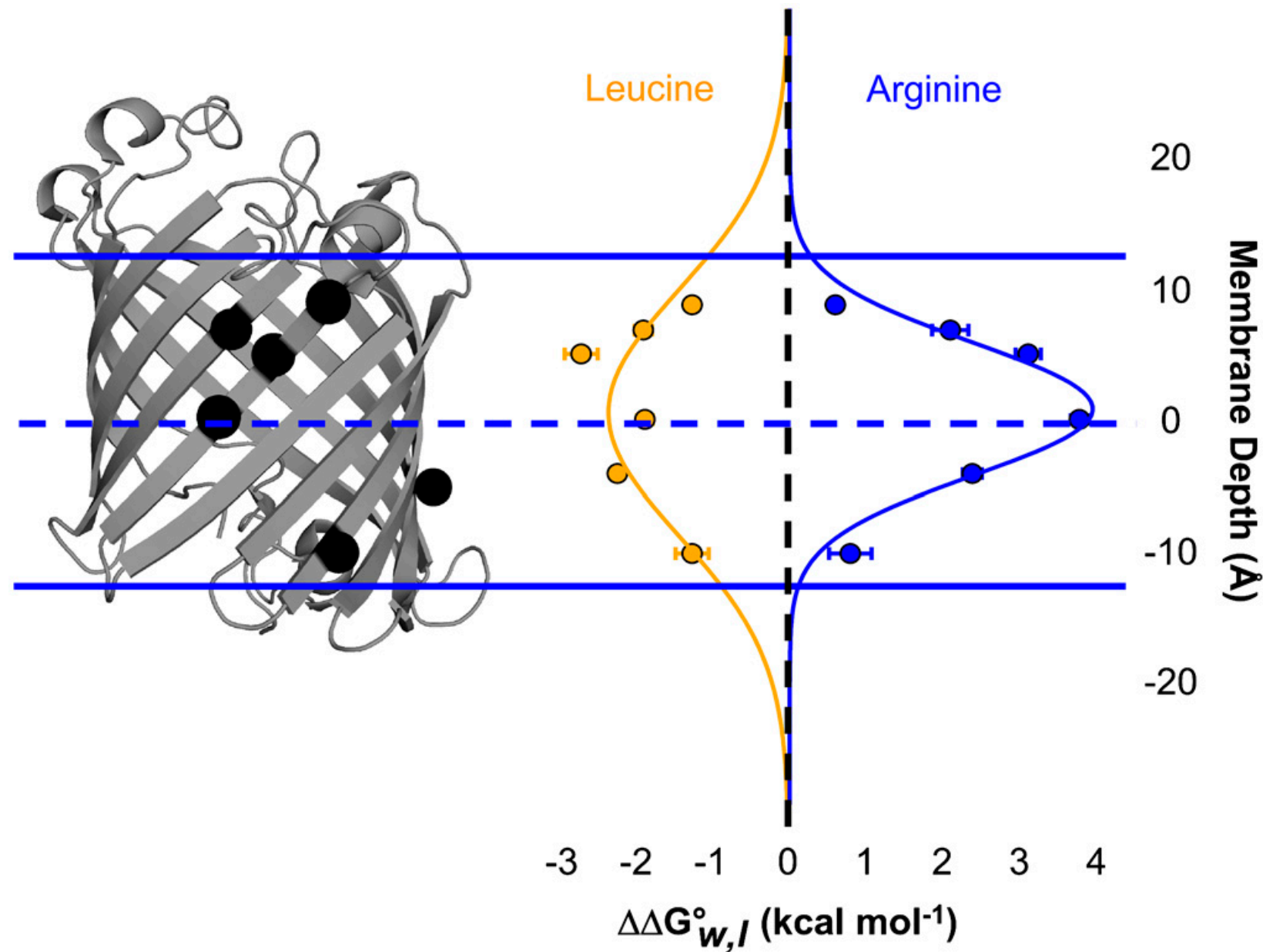
Moon e Fleming (2011) *PNAS* **108**: 10174



Cyrus Clothia (1974) *Nature* **248**:338

O efeito hidrofóbico também contribui para a estabilidade de proteínas de membrana

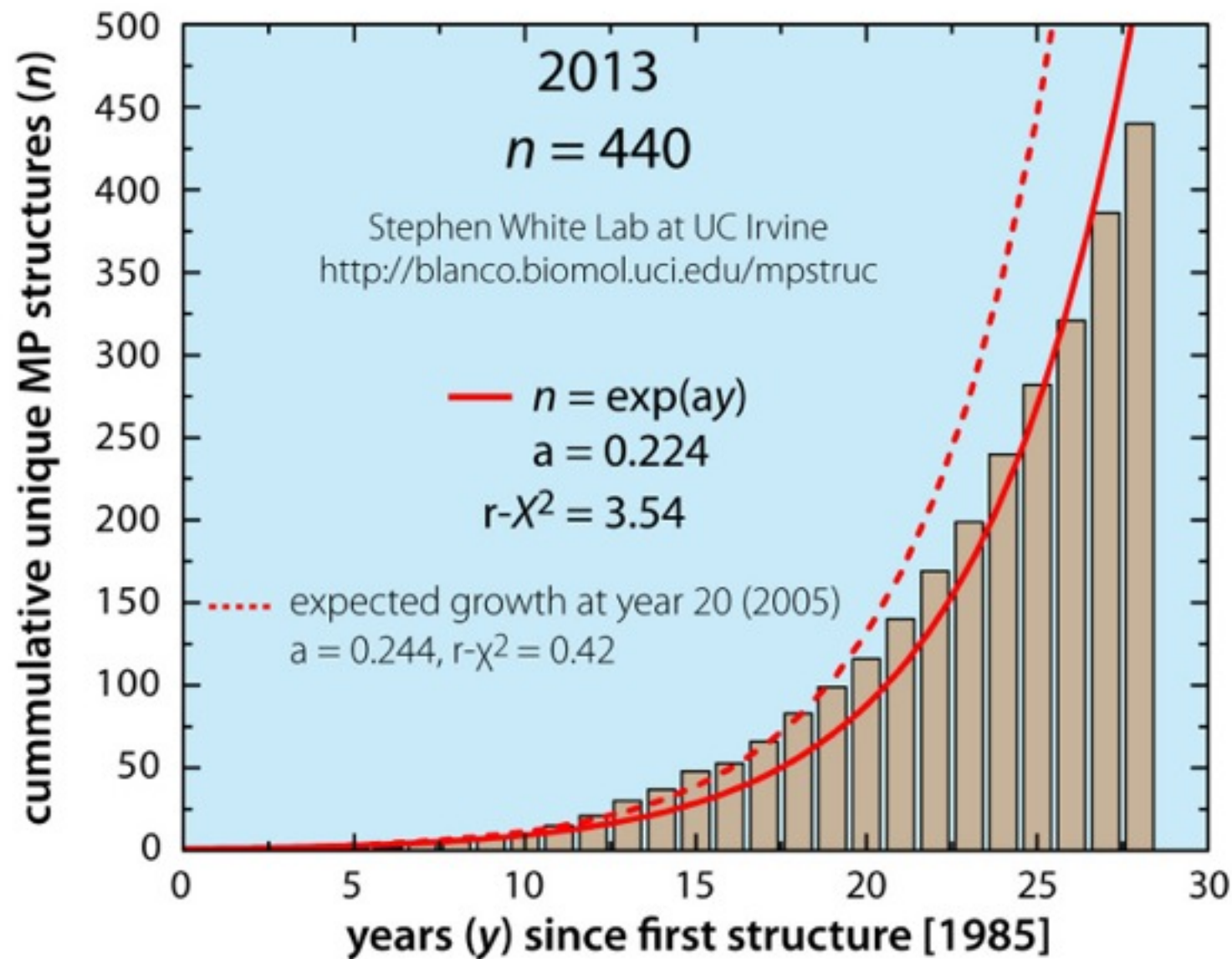
O custo energético de partição de cadeias laterais depende da profundidade na membrana



É difícil estudar proteínas de membrana

PDB Todas - 101245 estruturas

PDB TM - 2131 estruturas

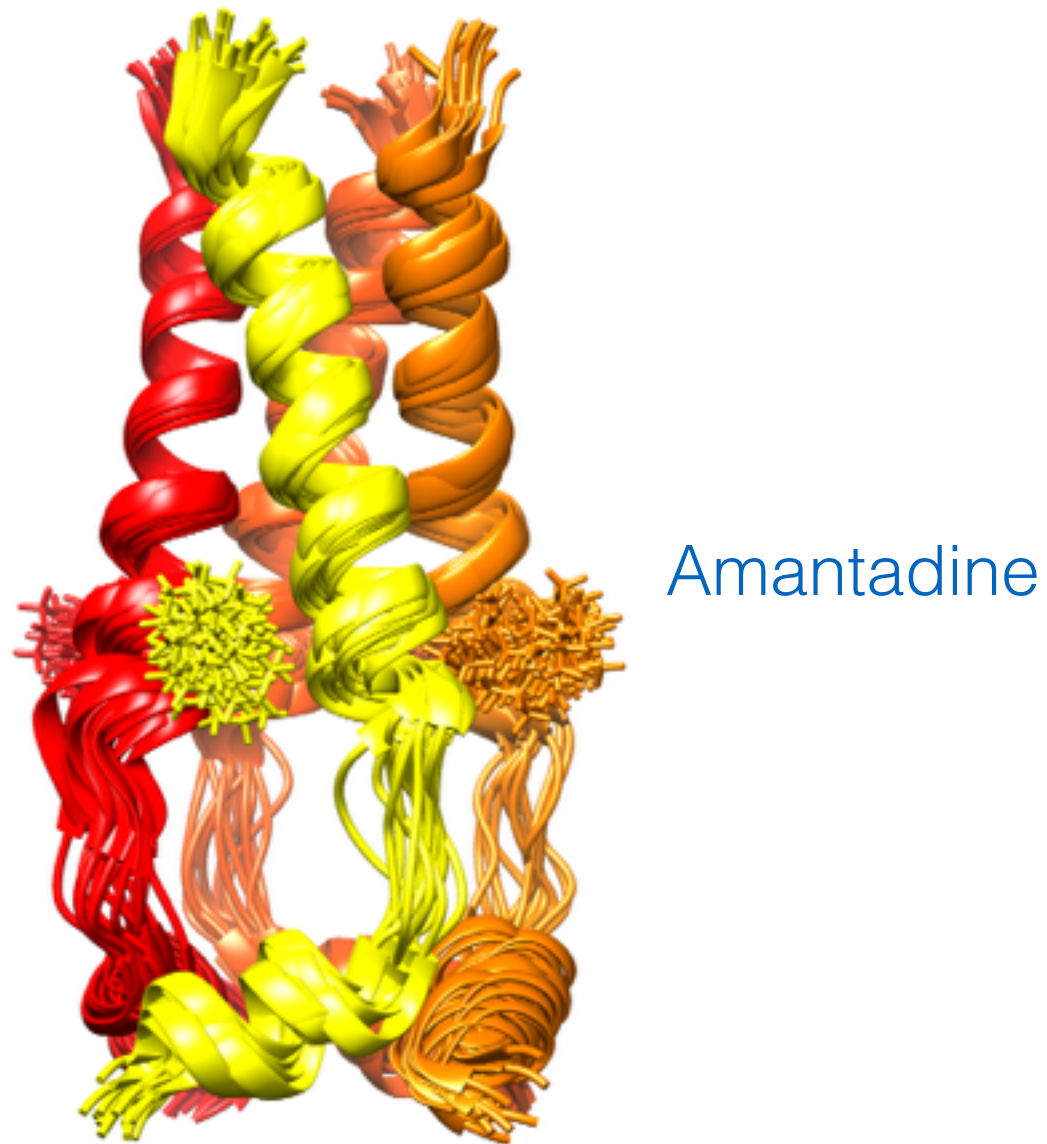


<http://blanco.biomol.uci.edu/mpstruc/>

<http://pdbtm.enzim.hu/?>

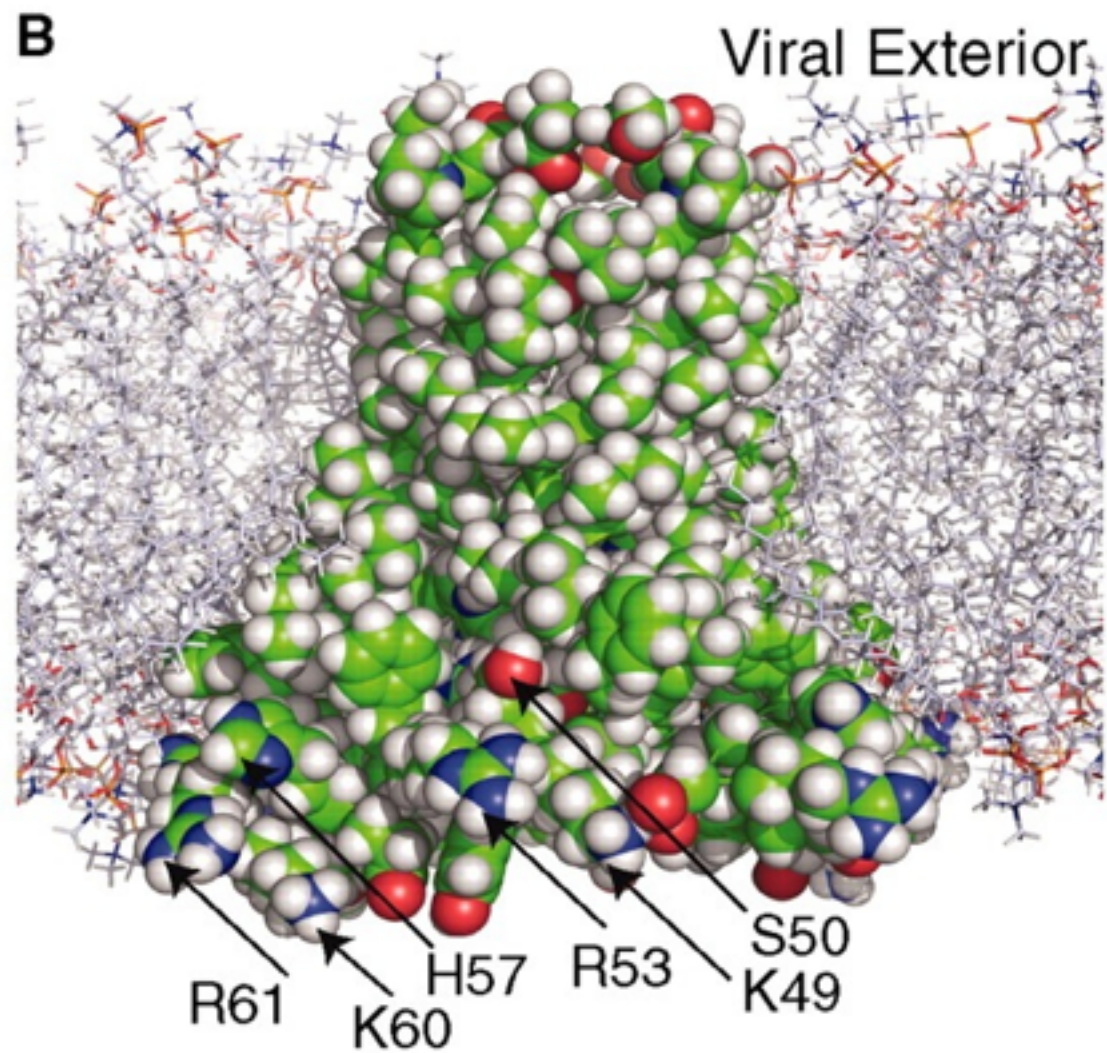
O ambiente importa

Estrutura do canal de H⁺ M2 do virus influenza A
RMN em solução com micelas de DHPC



Estrutura do canal de H⁺ M2 do virus influenza A

RMN em estado solido e bicamadas orientadas



Sharma et al. (2010) *Science* **330**: 509

● A hipótese termodinâmica de Anfinsen

A sequência de aminoácidos determina a estrutura tridimensional

The studies on the renaturation of fully denatured ribonuclease required many supporting investigations (6–8) to establish, finally, the generality which we have occasionally called (9) the “thermodynamic hypothesis.” This hypothesis states that the three-dimensional structure of a native protein in its normal physiological milieu (solvent, pH, ionic strength, presence of other components such as metal ions or prosthetic groups, temperature, and other) is the one in which the Gibbs free energy of the whole system is lowest; that is, that the native conformation is determined by the totality of interatomic interactions and hence by the amino acid sequence, in a given environment. In terms of natural selection

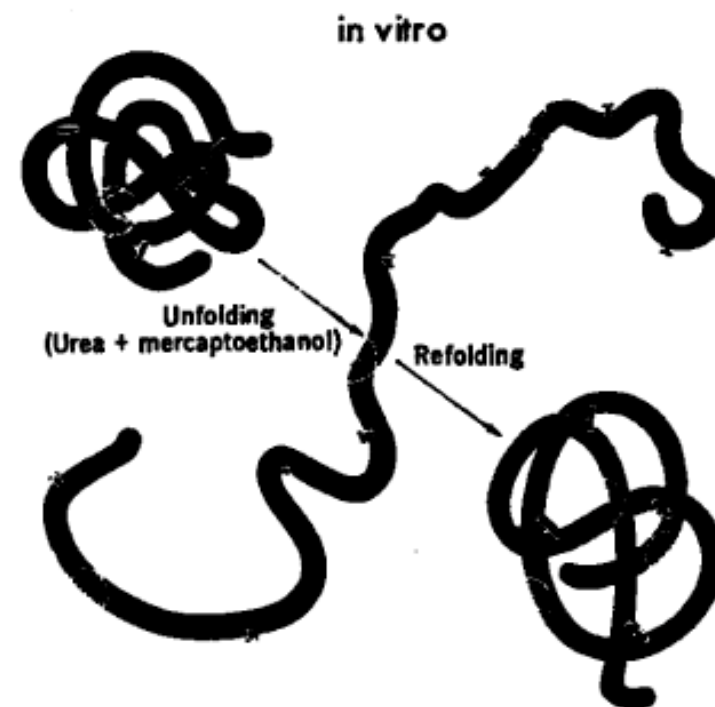


Fig. 2. Schematic representation of the reductive denaturation, in 8M urea solution containing 2-mercaptoethanol, of a disulfide-cross-linked protein. The conversion of the extended, denatured form to a randomly cross-linked, “scrambled” set of isomers is depicted at the lower right.

Determinação estrutural



Welcome

Deposit

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Visualize

Analyze

Download

RCSB PDB-101

Molecule of the Month

Structural View of
Biology

Educational Resources

Understanding PDB
Data

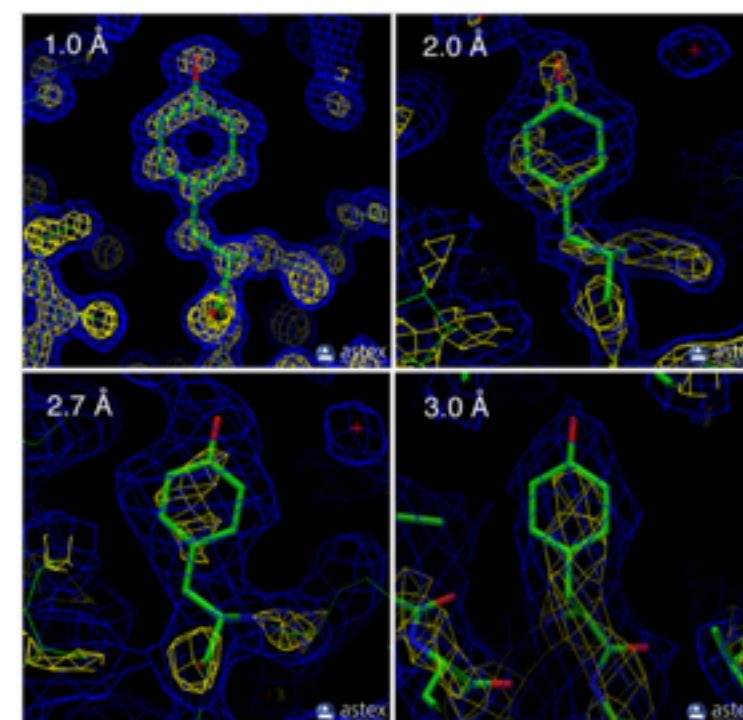
Author Profiles

Video Challenge

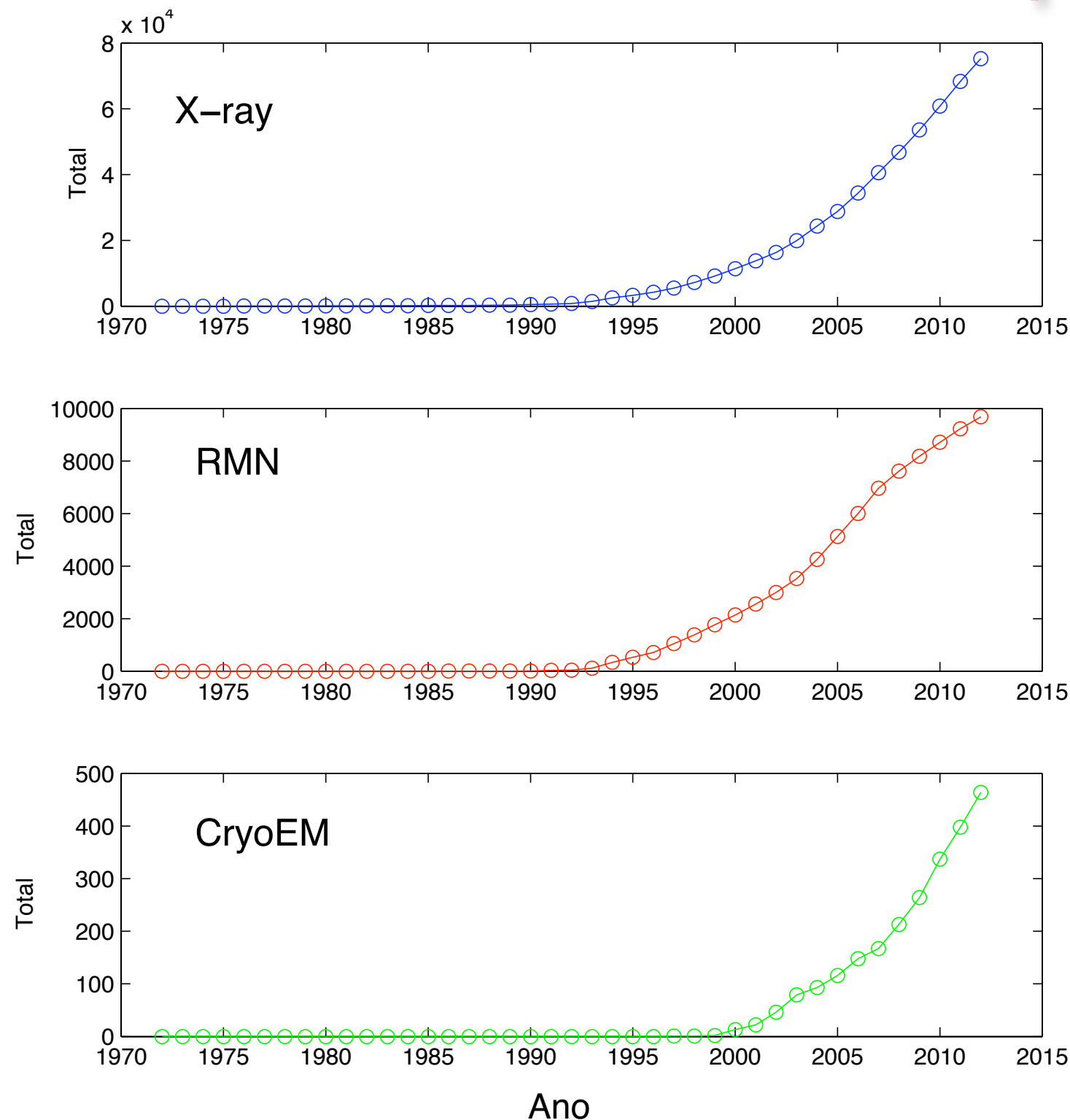
Protein Structure
Initiative Featured
System

Understanding PDB Data

Understanding PDB Data is a reference to help explore and interpret individual PDB entries. Broad topics include how to understand PDB data, how to visualize structures, how to read coordinate files, and potential challenges to exploring the archive.

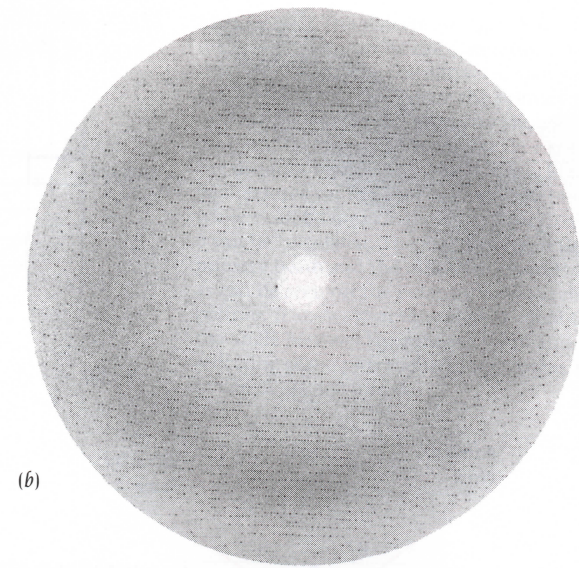
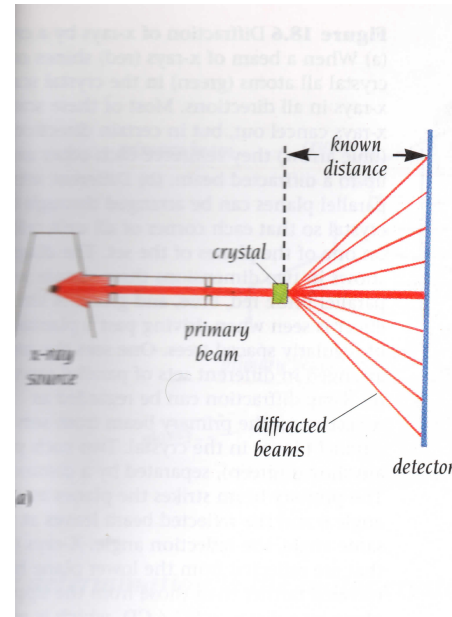
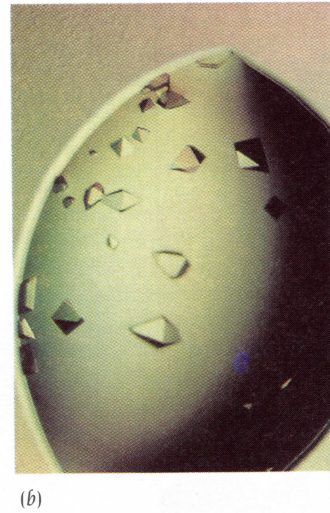
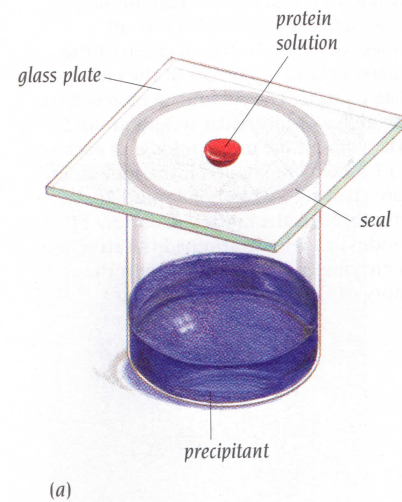
[Looking at Structures](#)[Dealing with Coordinates](#)[Resolution](#)Right image: Looking at Structures -
Resolution[Read More on PDB Data](#)

Estruturas de proteína depositadas no PDB e classificadas de acordo com o método experimental



Número de estruturas depositadas no PDB
ao longo dos anos

Xray crystallography

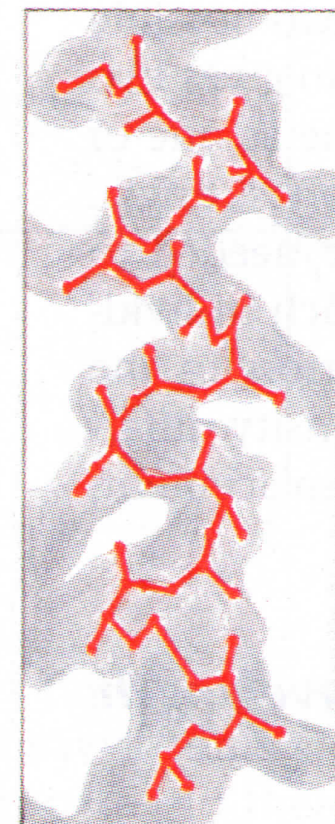


- Requires good quality crystals
- One needs to solve the phase problem
- Quality of the data may be examined by the resolution ($< 2.5 \text{ \AA}$) and the R-factor ($0.15 < R < 0.20$)

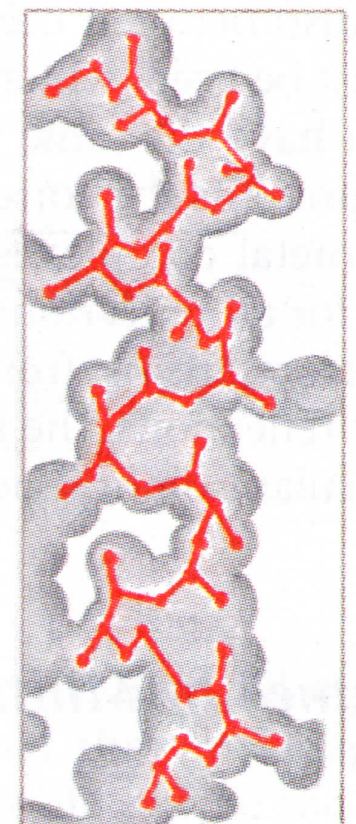
(a) $5.0 \text{ \AA}$

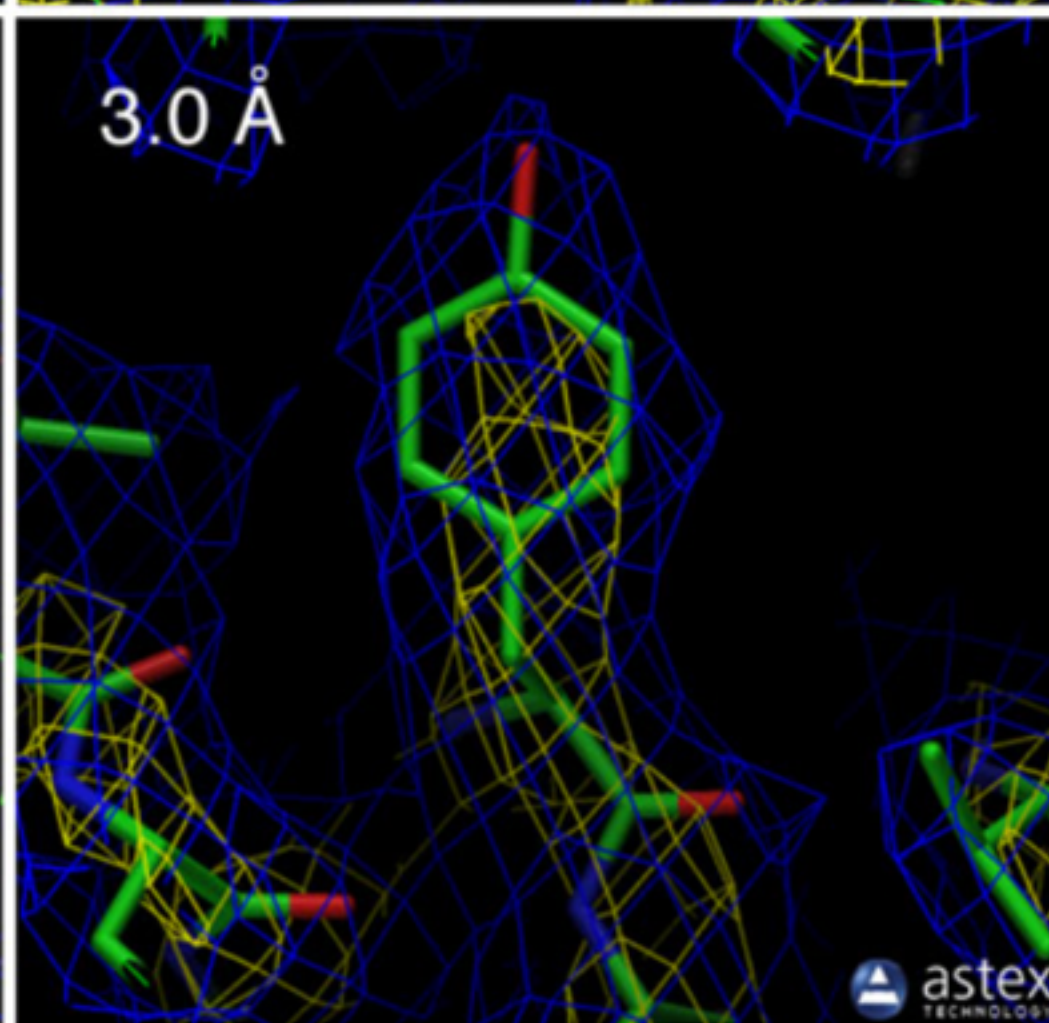
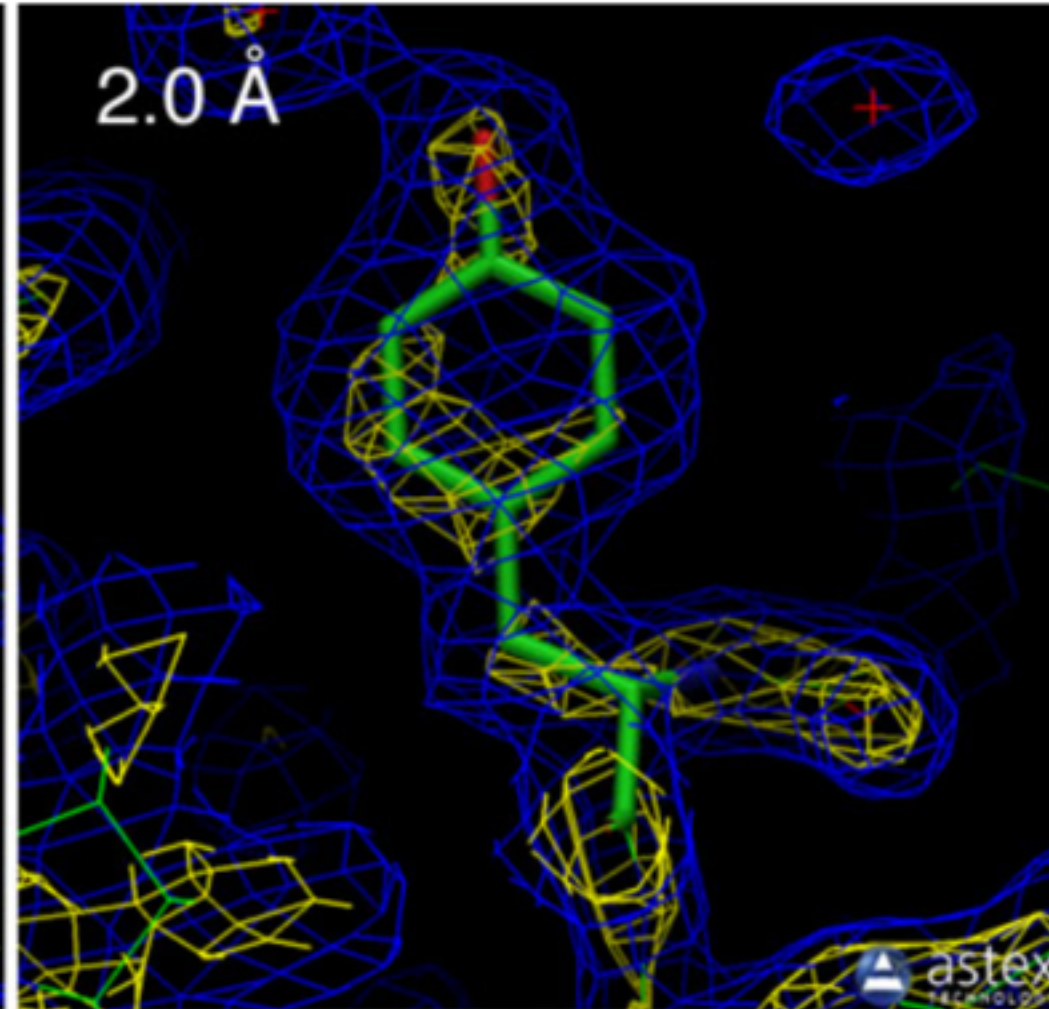
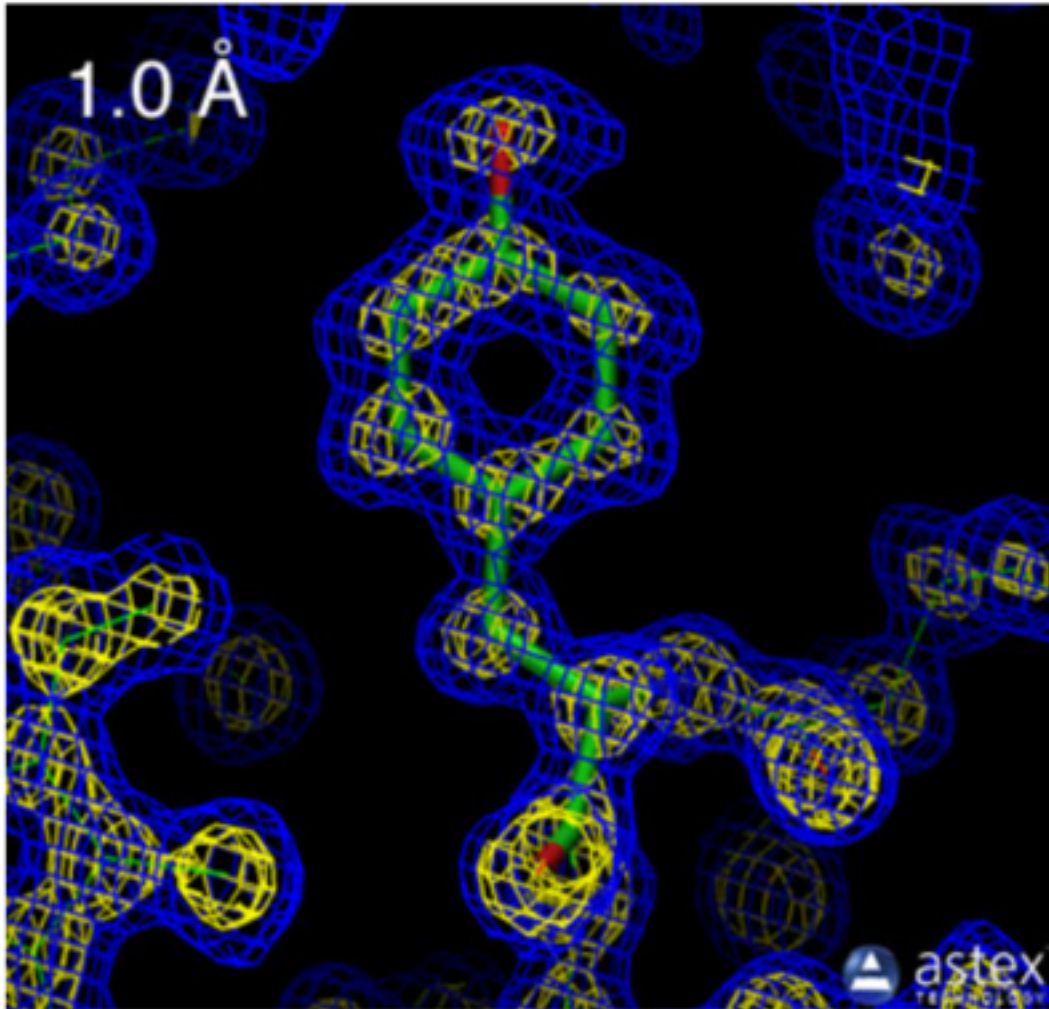


(b) $3.0 \text{ \AA}$



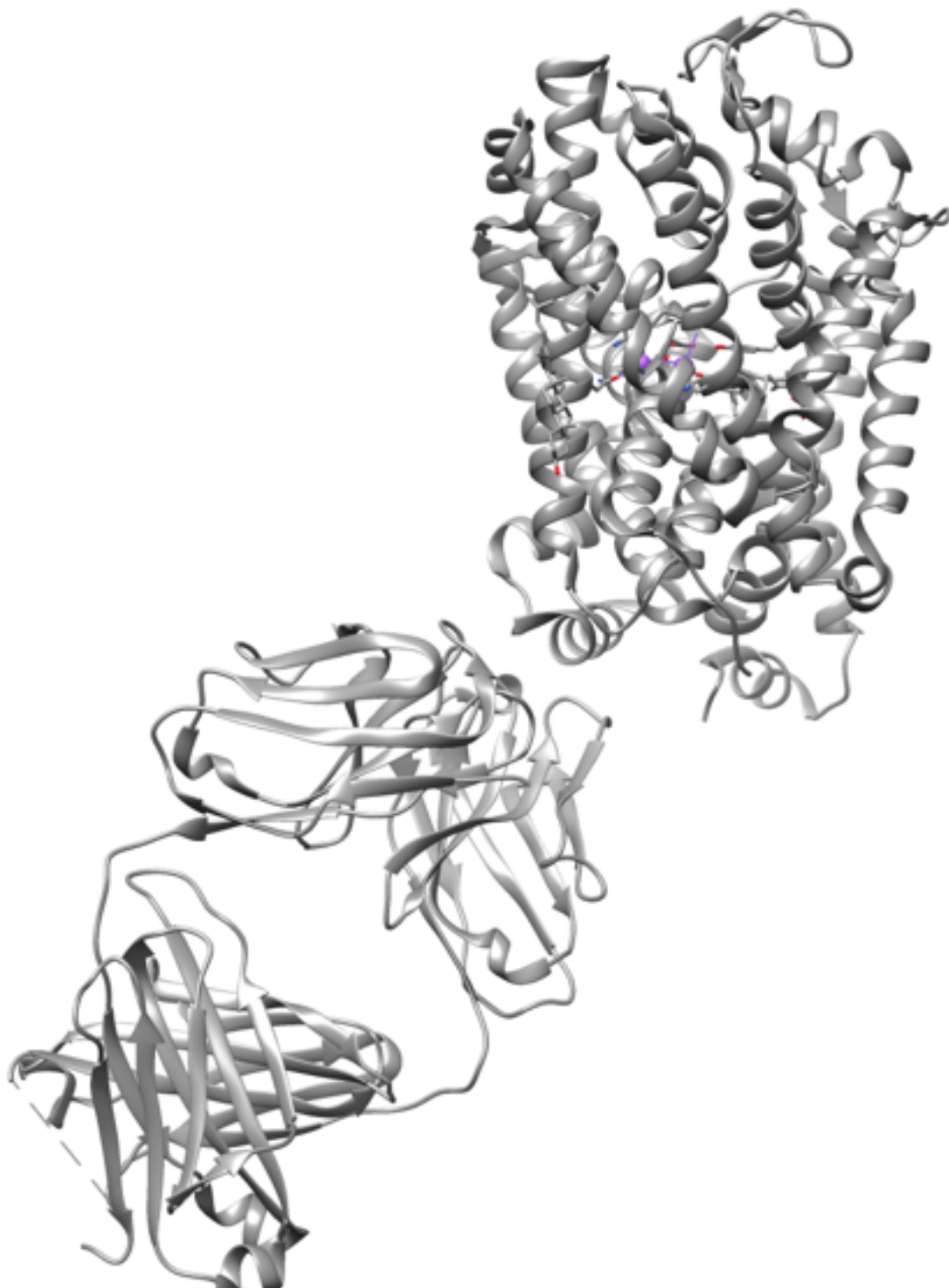
(c) $1.5 \text{ \AA}$



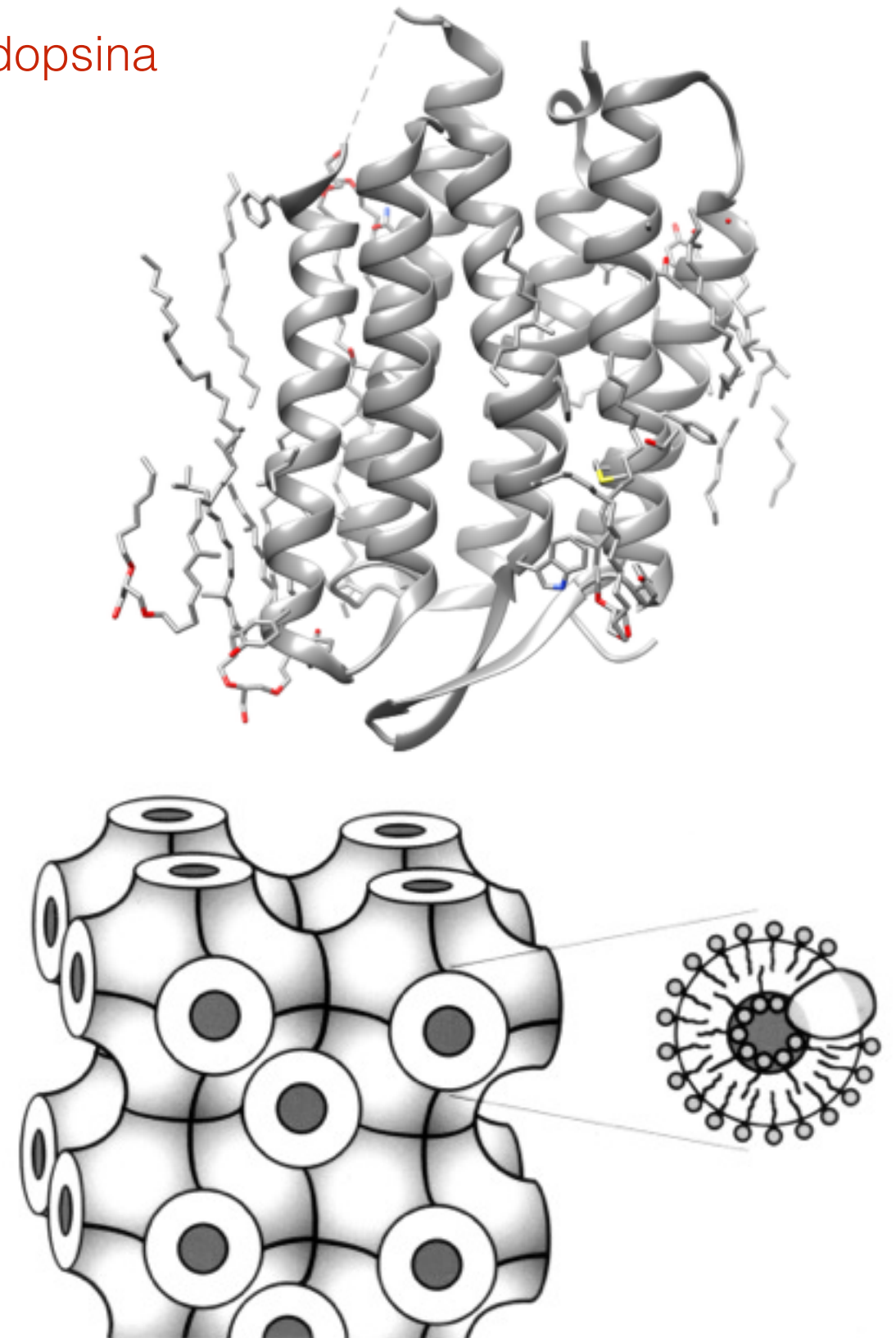


X-ray: fusão com anticorpos, lisozima, e introdução de mutações para tornar a estrutura mais rígida

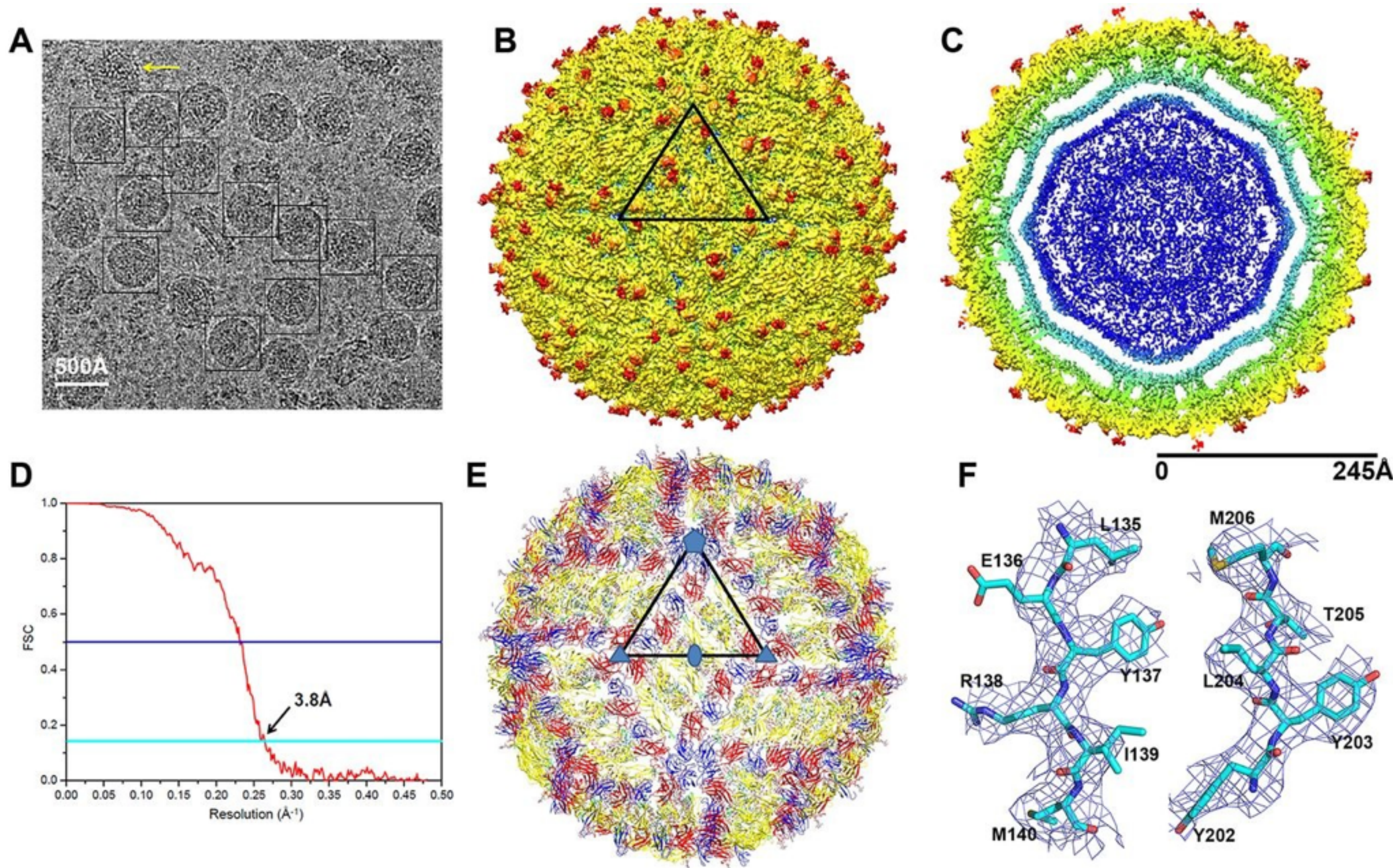
Transportador de dopamina



Bacteriorodopsina



Cryo-Electron Microscopy



NMR & Structural biology

Structure determination

- NMR enables the calculation of high-resolution protein 3D structures in solution



1. Unambiguous restraints

Intraresidual	535
Sequential	675
Medium range	239
Long range	767
Intermolecular	87

2. Talos dihedral angles

140

3. Consistent violations

Distances > 0.5 Å	0
Torsion angles > 5°	0

4. RMS deviation from the lowest energy structure

All core residues (30-38, 163-252)	
Backbone atoms (N,CA,CO)	0.58 ± 0.06
Heavy atoms	1.64 ± 0.07

5. Ramachandran plot (30-38, 163-252)

Most favored region (%)	86.31 ± 2.51
Additionally allowed region (%)	12.36 ± 2.59
Generously allowed region (%)	1.32 ± 0.37
Disallowed region (%)	0.00

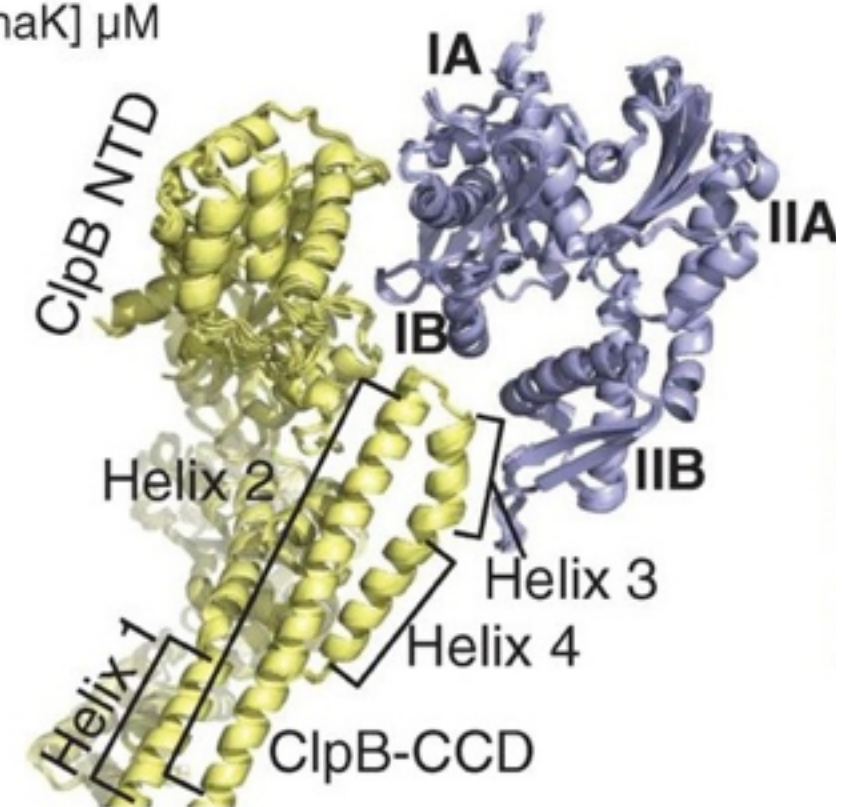
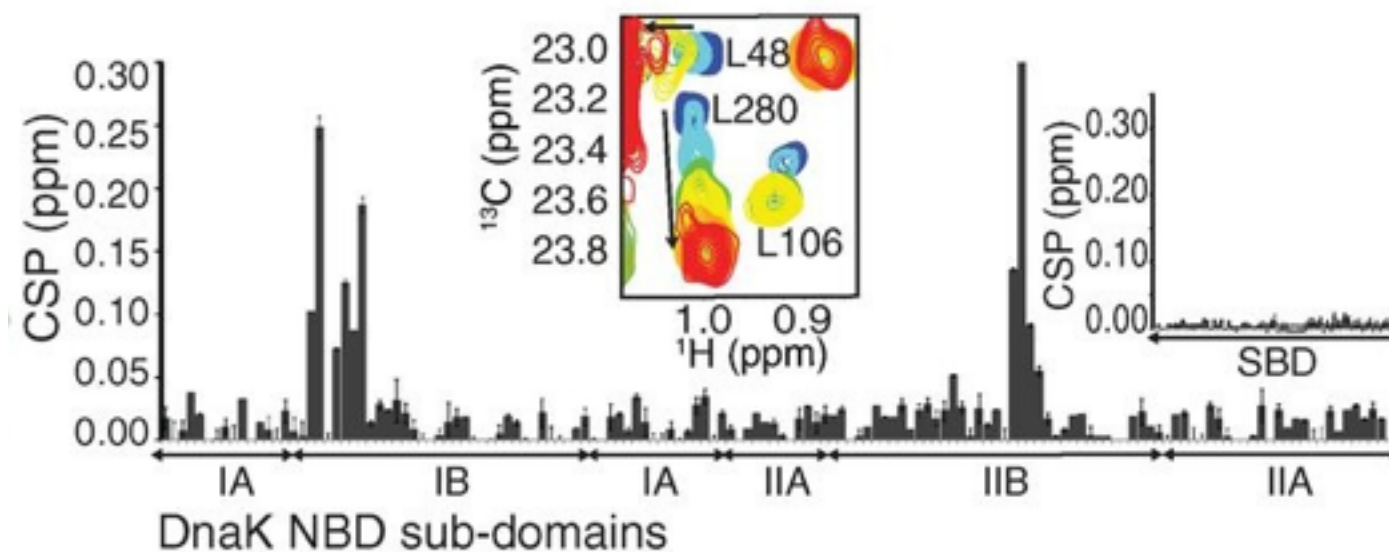
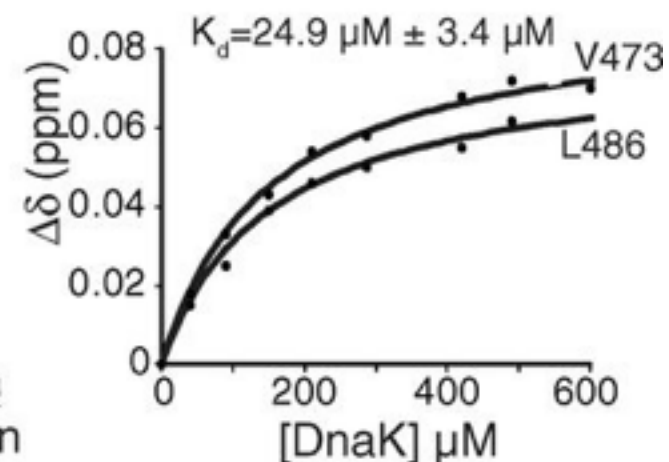
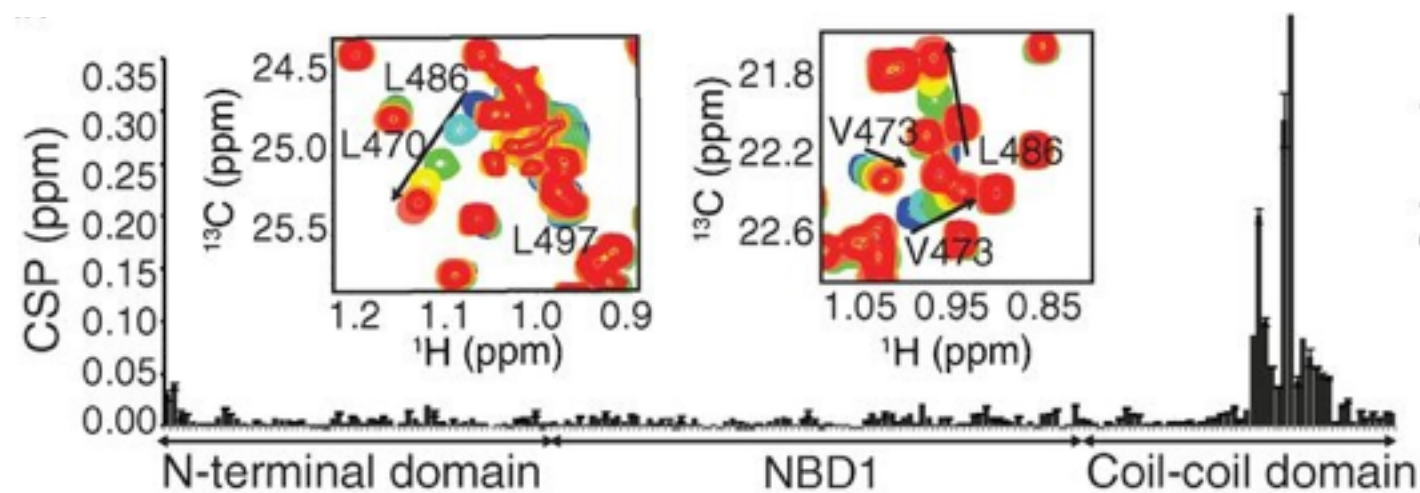
6. WHAT CHECK structure Z-scores

Ramachandran plot appearance	-2.42 ± 0.26
2nd generation packing quality	0.25 ± 0.28
χ ¹ - χ ² rotamer normality	-1.00 ± 0.35
Backbone conformation	-4.23 ± 0.29

NMR & Structural biology

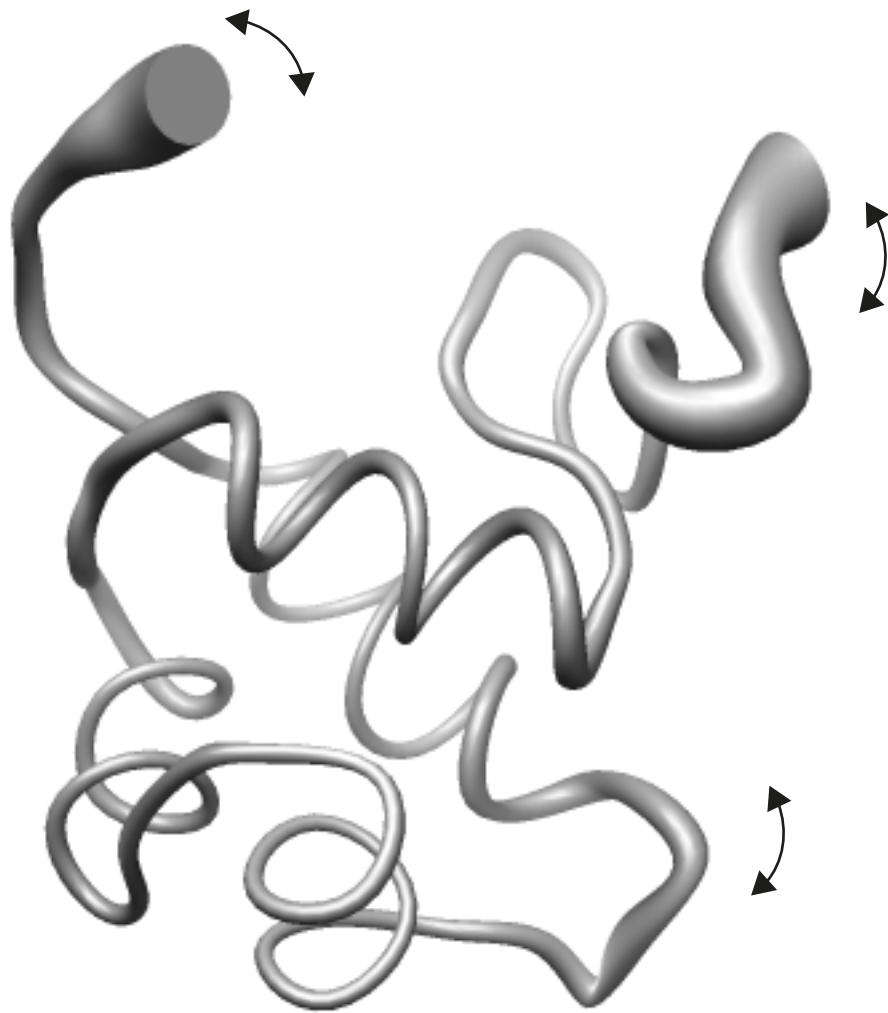
Biomolecular interactions

- Even weak and transient complexes can be studied

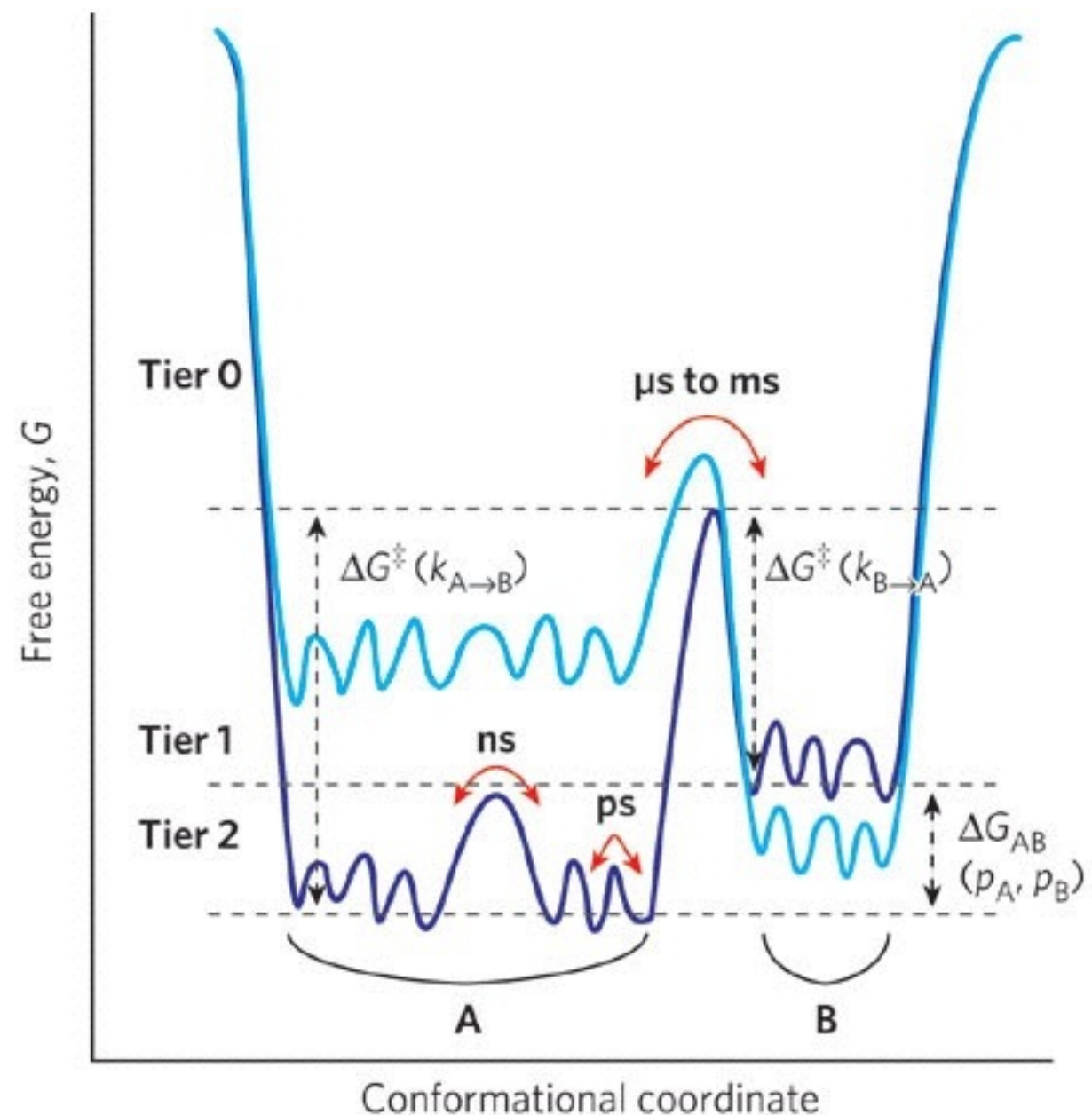


NMR provides essential information on protein dynamics

Proteins are not rigid, they consist of an ensemble of conformations that interconvert at different time scales

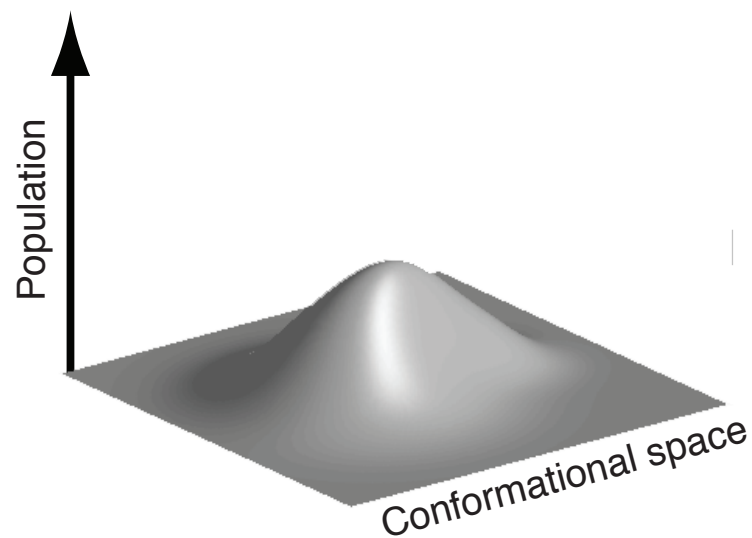
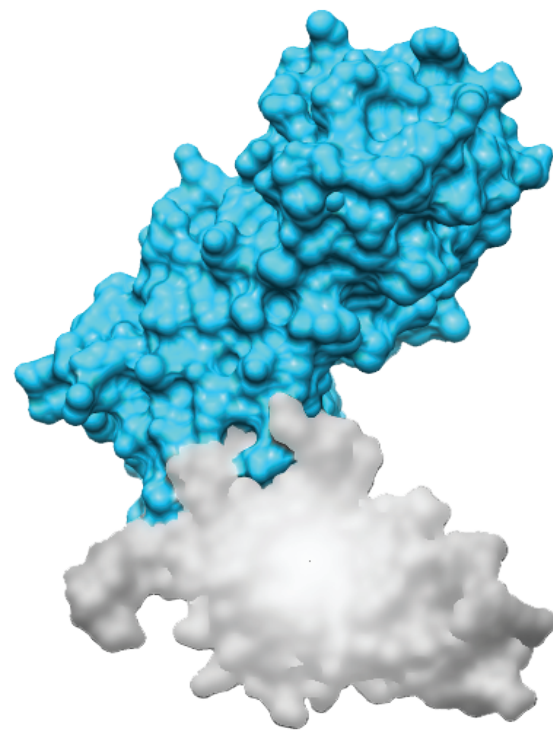


PDB 2JVL

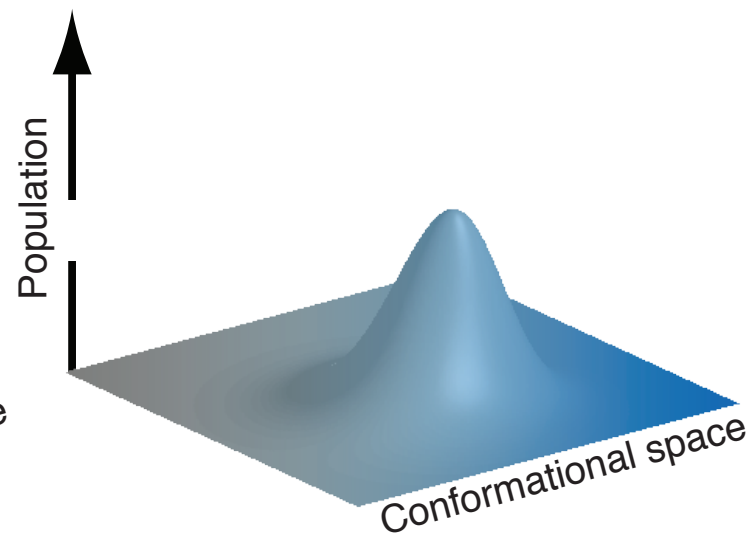
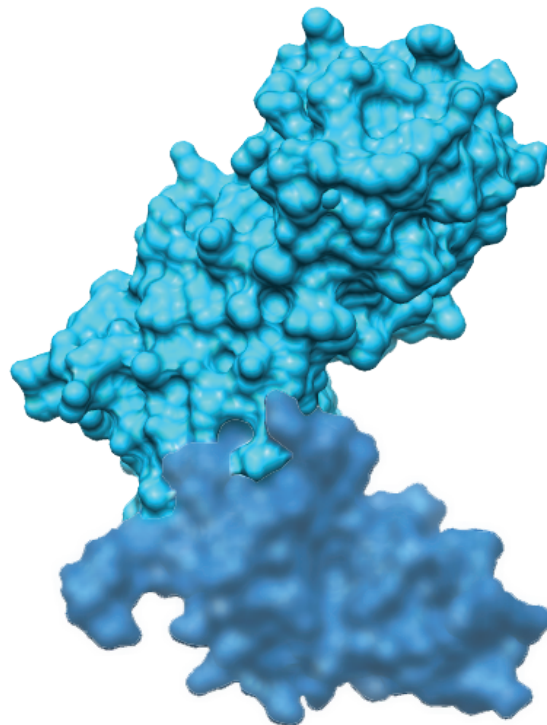


NMR & Structural biology

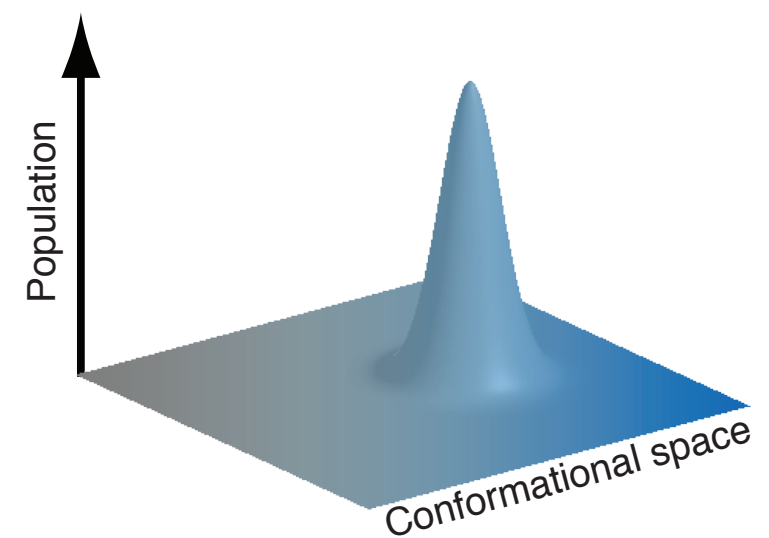
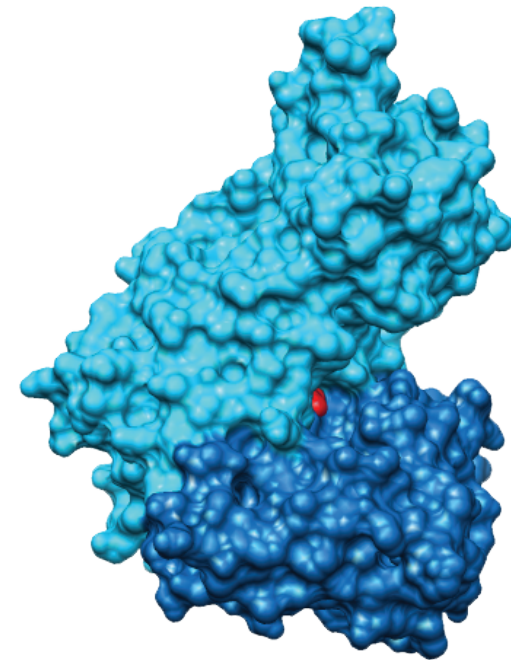
DYNAMICS



Unliganded



Activated



GK + Glucose $\pm$ AMP-PNP

Motivation

Multiple kinds of motions are relevant for different biological processes

- Fast backbone motion ($< \text{ns}$)
 - Side chain motion ($< \text{ns}$)
 - Aromatic ring flipping ($\text{ps} - \text{ms}$)
 - Inter-domain motion ($\mu\text{s} - \text{ms}$)
 - Overall tumbling (ns)
 - Folding/unfolding
 - Allosteric communication
 - Ligand recognition
 - Enzyme kinetics
- 

If we want to understand how proteins perform their tasks, it is important to understand the structure and the dynamics

Quanto maior for o campo magnético externo, mais fácil será para detectar o sinal de RMN da amostra

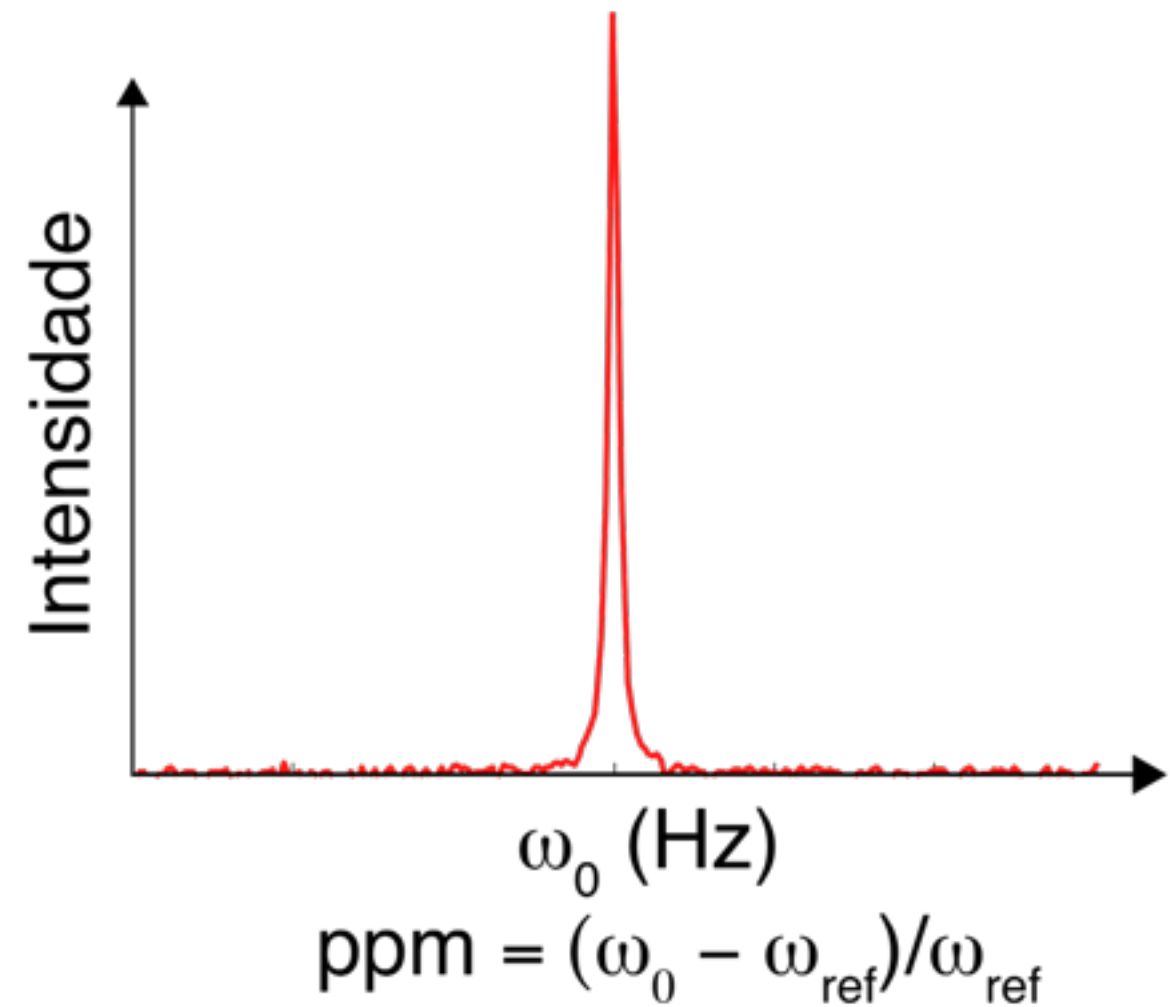
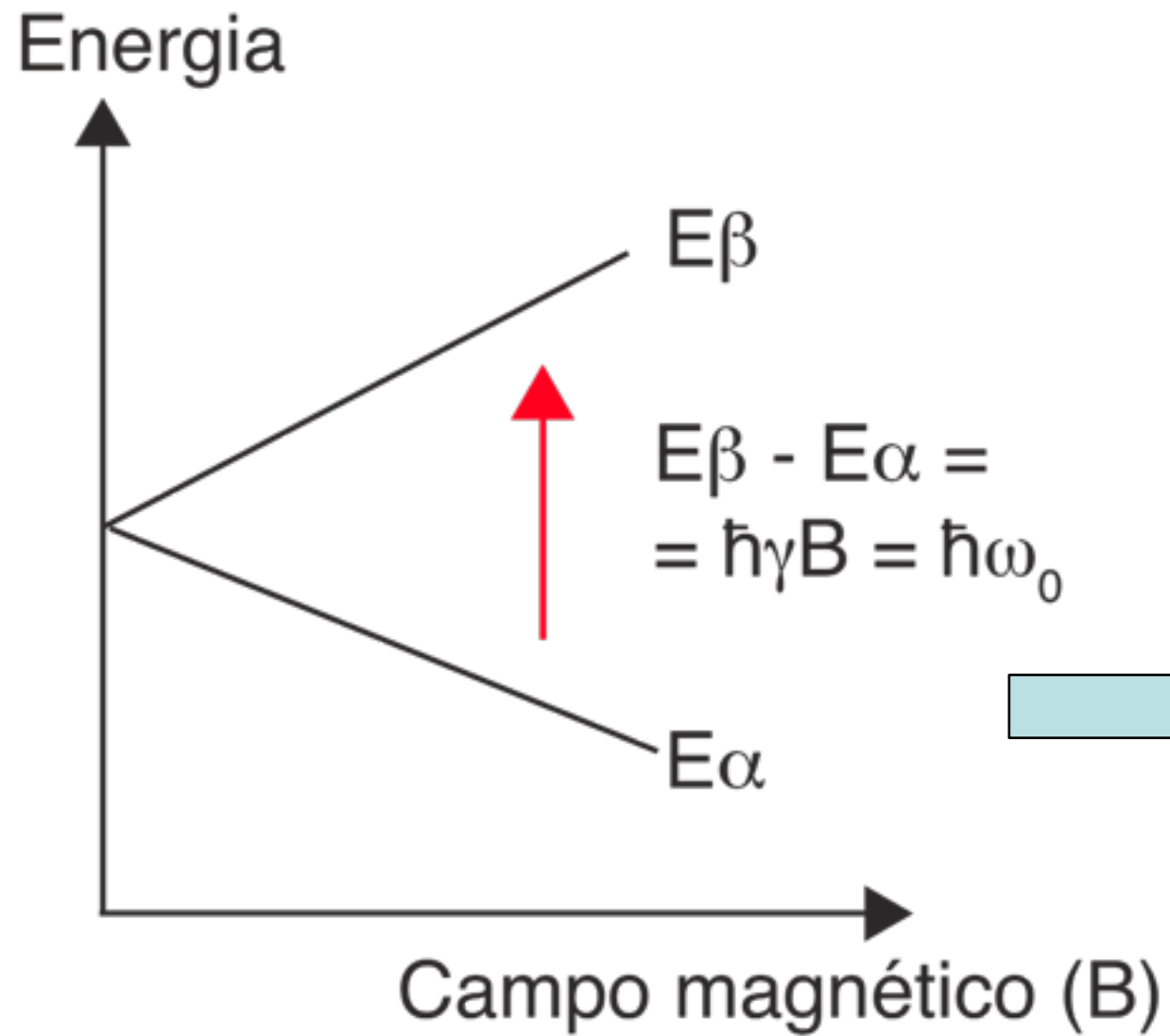


18.8 Tesla
 ω_0 (^1H) 800 MHz

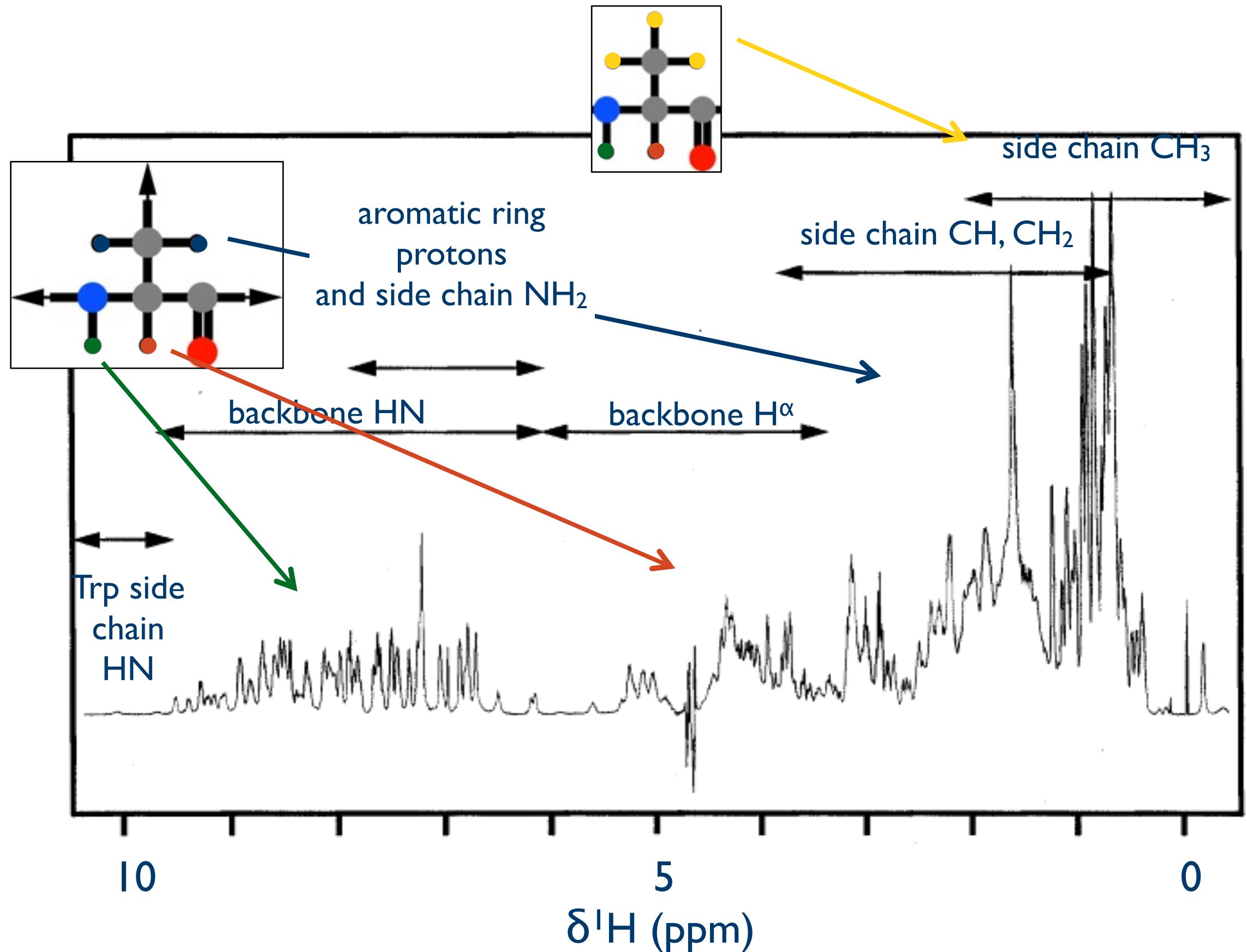


$\sim 500 \mu\text{l}$

RMN detecta transições entre os estados do spin nuclear com diferentes energias na presença de um campo magnético estático



Proton Chemical Shifts



O problema do assinalamento

Strategy 1: “Wüthrich's” approach (1980s)

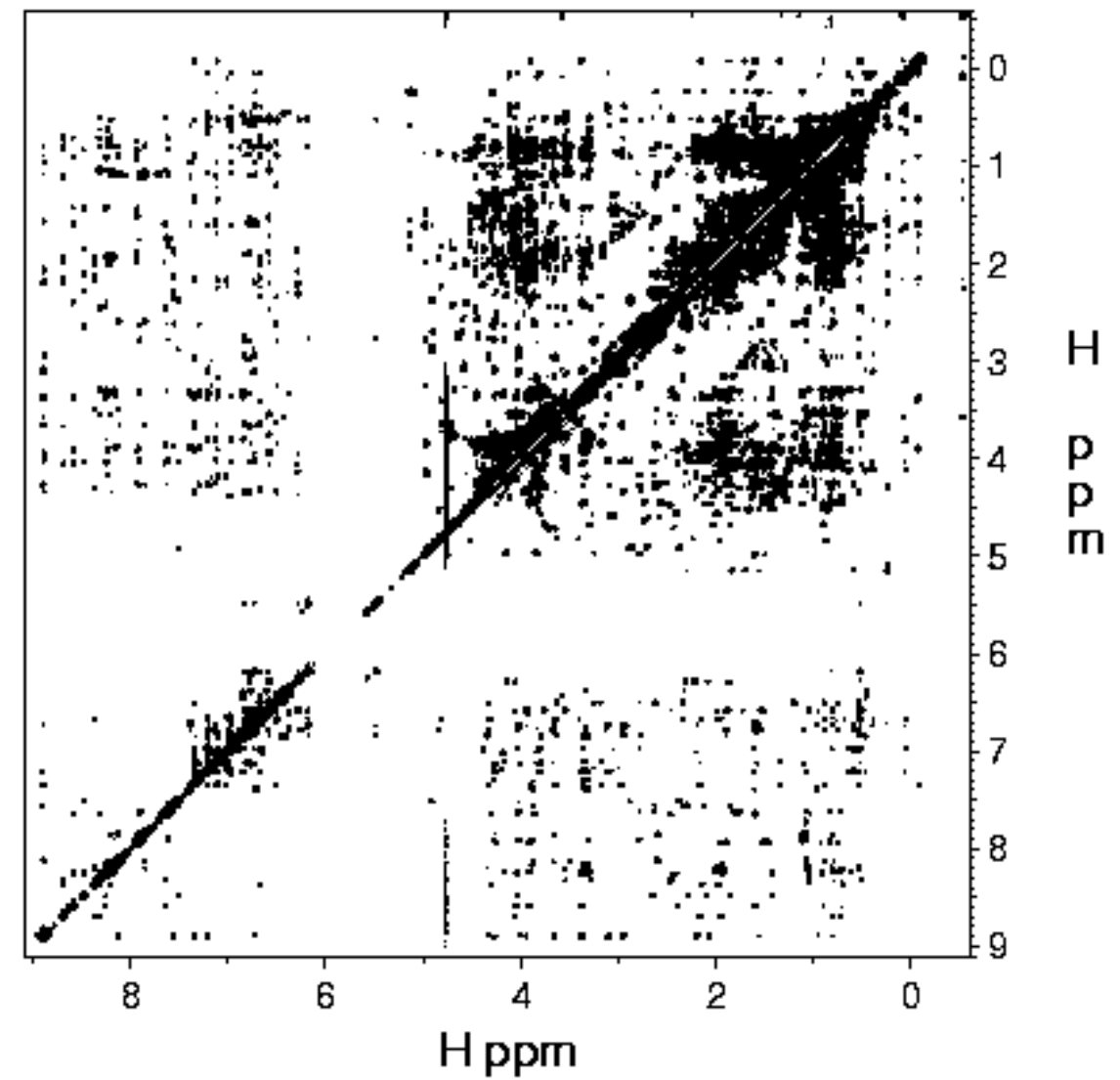
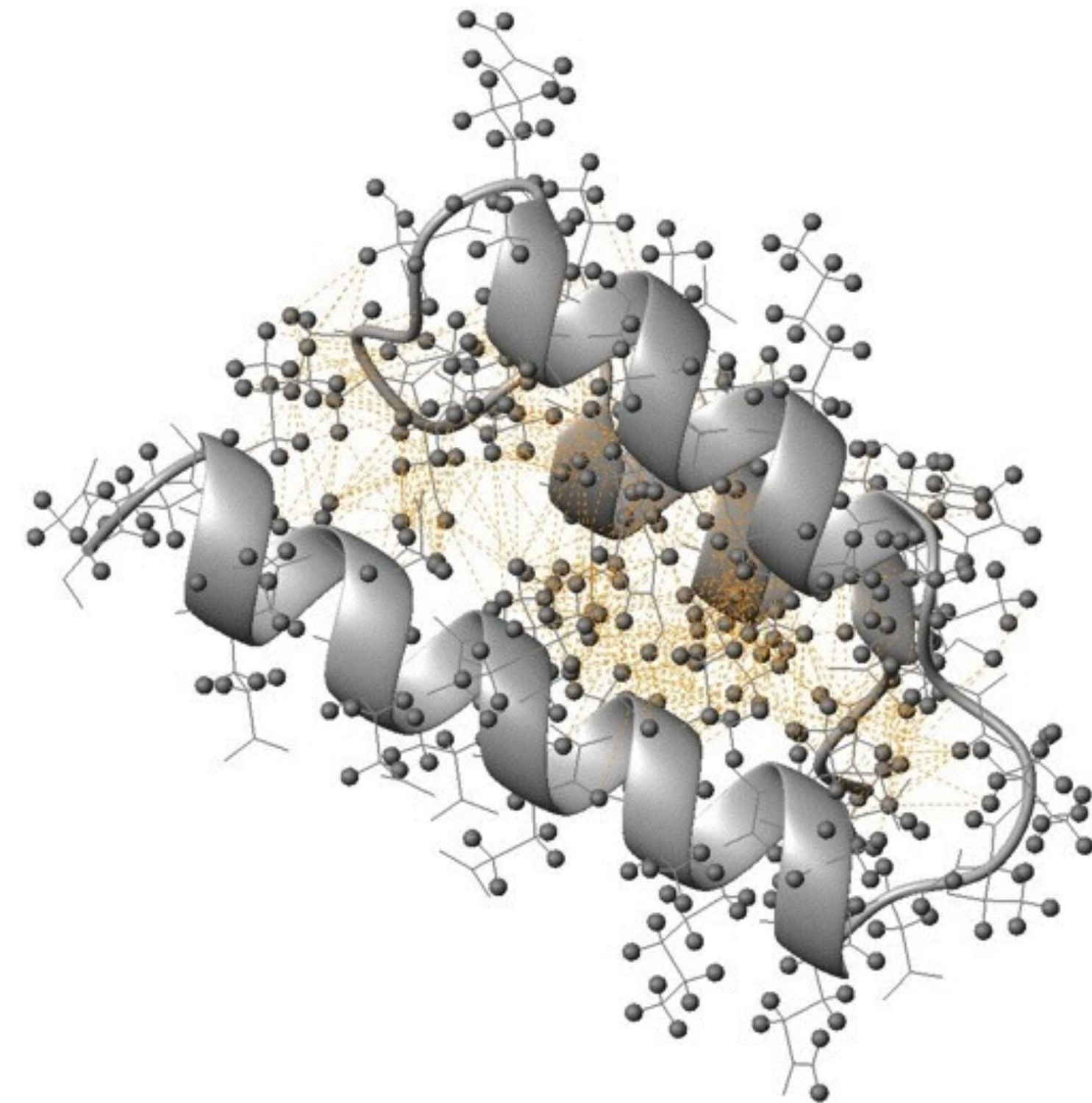
- Homonuclear 2D experiments ($^1\text{H} - ^1\text{H}$)
 - ✓ COSY / TOCSY - assign "spin systems" (residue-type)
 - ✓ NOESY - determine neighbors



Strategy 2: Multidimensional approach

- (Additional) Heteronuclear 2D & 3D experiments
 - ✓ Triple resonance - assign "spin systems" (residue-type)
 - ✓ Requires isotope labelling with ^{15}N and ^{13}C (< 20 kDa) or ^2H , ^{15}N and ^{13}C (20 - 50 kDa)
 - ✓ Use pairs of experiments to sequentially assign all resonances

RESTRAINTS: distances



A precisão melhora conforme maior o número de restrições

