❑Proteins: why are so important?

❑From the Greek: “Being of Primary Importance”

No better world could have been chosen

Enzymatic catalysis

Transport and Storage

Movement

Immunitary Defense

Transmission of nervous signals

Hormon activity

Forming tissues

Albuminoids: these materials were identified (1800) in natural processes as the coagulation of egg white by heat, the curdling of milk with acid or the spontaneous clotting of blood

Gerardus Mulder (1802-1880) proposed a molecular form: $C_{40}H_{62}N_{10}O_{12}$

$+ S$

Justus von Liebig(1803-1873):

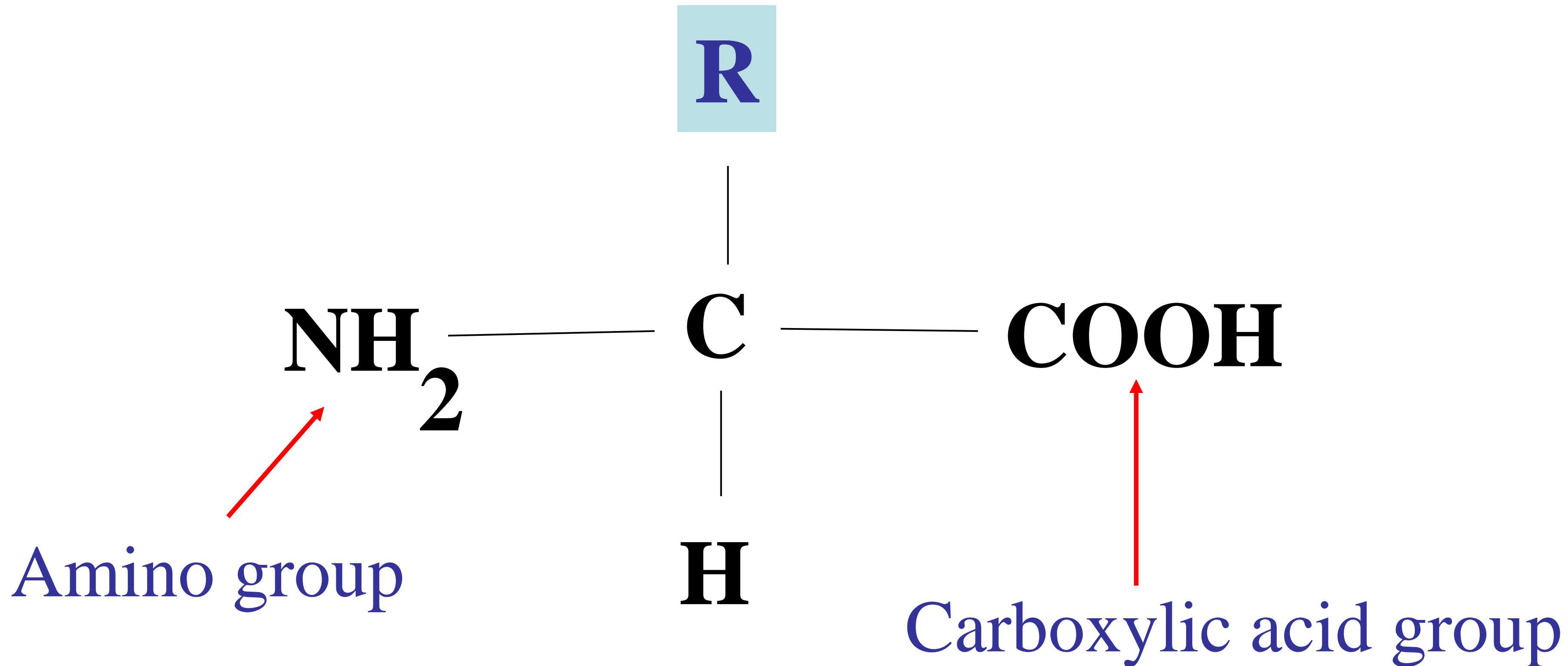
We cannot isolate the particular substance described by Mr. Moulder. And so, it is a source of despair, after so much has been prattled and written about "protein", to have to say there is no such thing.

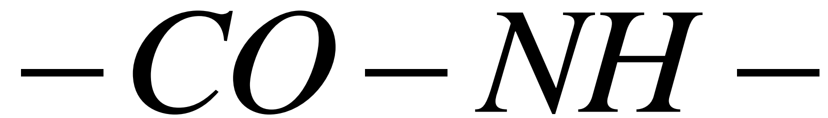
Mulder again: $C_{36}H_{54}N_{8}O_{12}$

Von Liebig (working with casein) purified to small molecules:

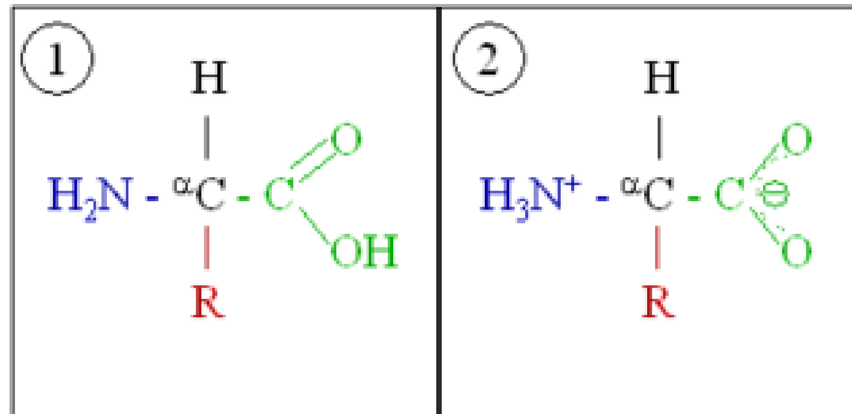
first amino-acids: *leucine* and *tyrosine*

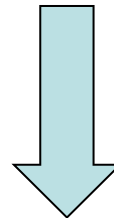
20 KIND OF AMINO-ACIDS

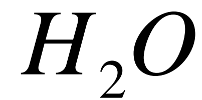




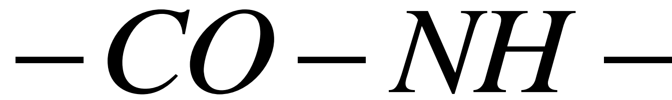
Covalent Bond







Covalent Bond



Amino group

Carboxyl group

H_2O

Peptide linkage

N terminus

C terminus

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Protein Chain

Carbon alpha, Carbon’, Nitrogen, …. Hydrogen omitted

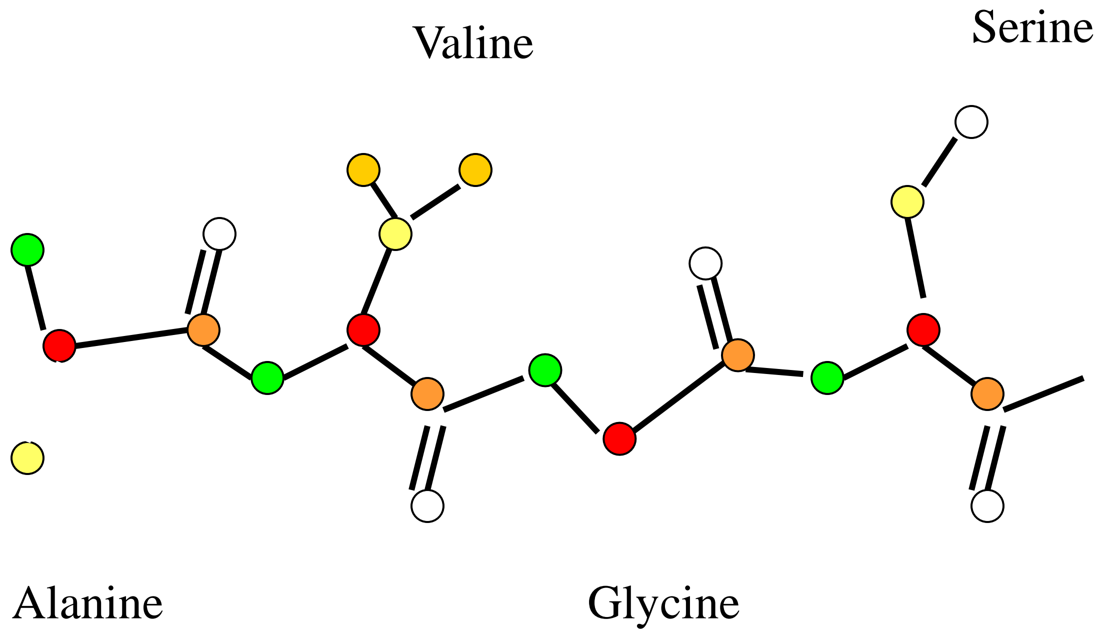


Table 1

Amino acid names and abbreviations		
Amino acid	Three letter code	Single letter code
Alanine	Ala	A
Arginine	Arg	R
Aspartic acid	Asp	D
Asparagine	Asn	N
Cysteine	Cys	C
Glutamic acid	Glu	E
Glutamine	Gln	Q
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V

Languages

Each monomer is identified by a symbol

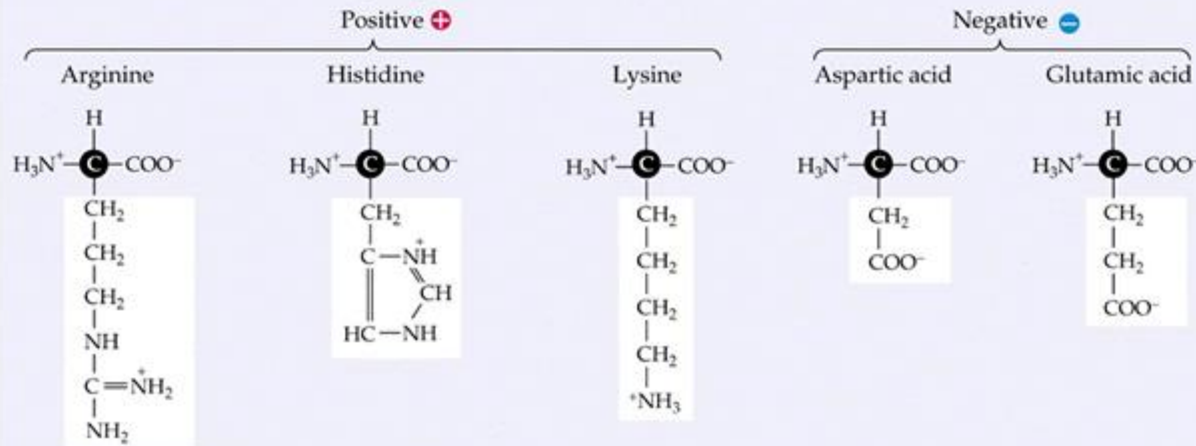
2nd base in codon

1st base in codon

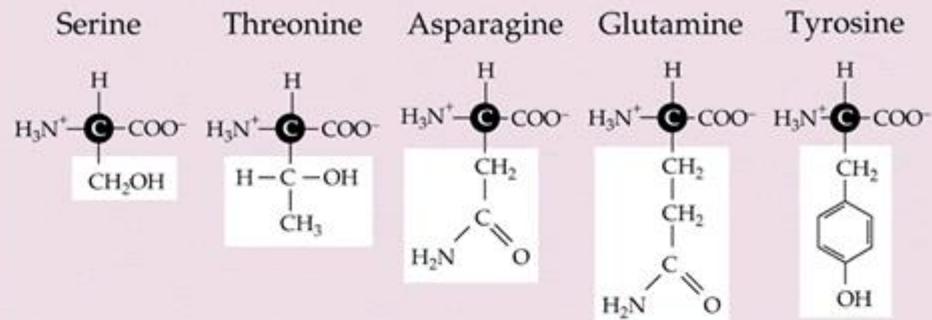
3rd base in codon

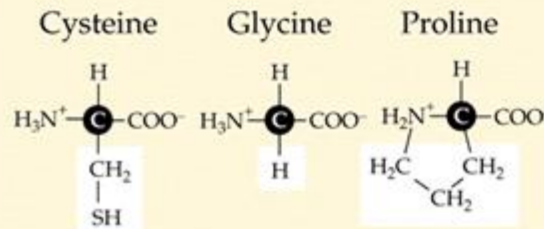
	U	C	A	G	
U	Phe Phe Leu Leu	Ser Ser Ser Ser	Tyr Tyr STOP STOP	Cys Cys STOP Trp	U C A G
C	Leu Leu Leu Leu	Pro Pro Pro Pro	His His Gln Gln	Arg Arg Arg Arg	U C A G
A	Ile Ile Ile Met	Thr Thr Thr Thr	Asn Asn Lys Lys	Ser Ser Arg Arg	U C A G
G	Val Val Val Val	Ala Ala Ala Ala	Asp Asp Glu Glu	Gly Gly Gly Gly	U C A G

Amino-acidi Carichi



Amino-acidi Polari (idrofilici)





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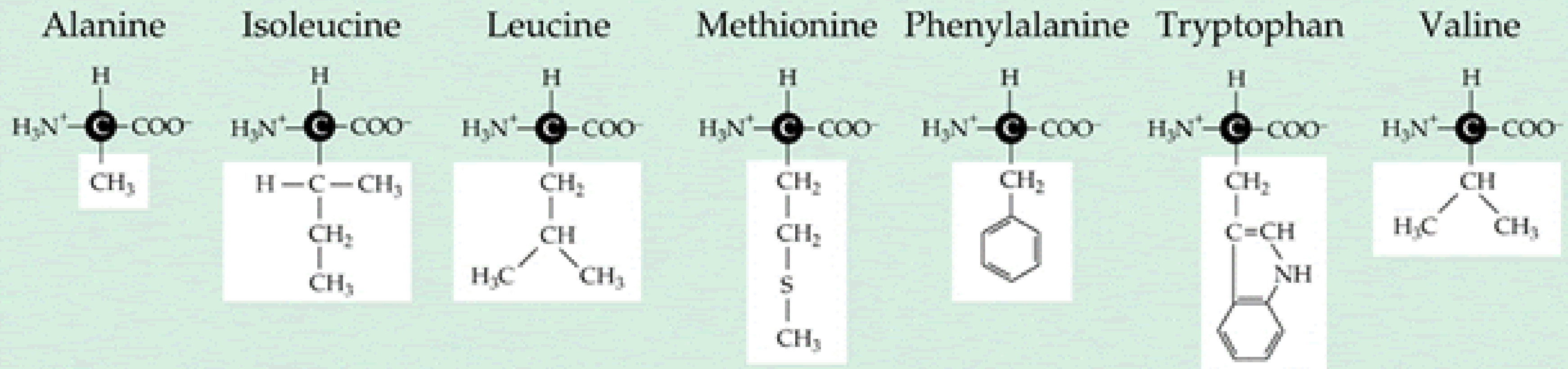
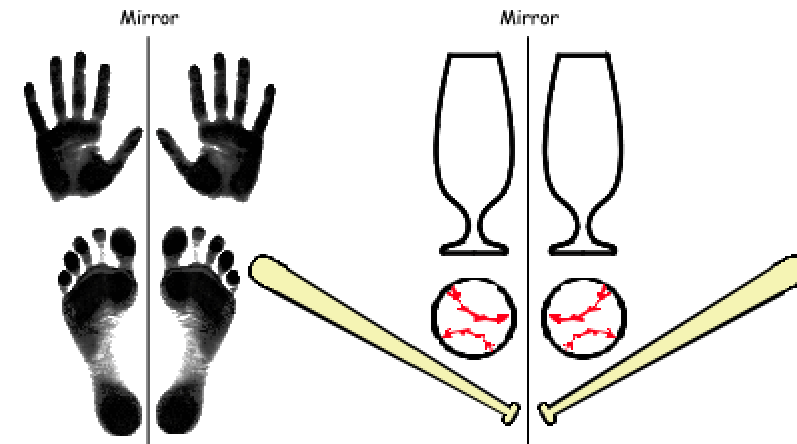
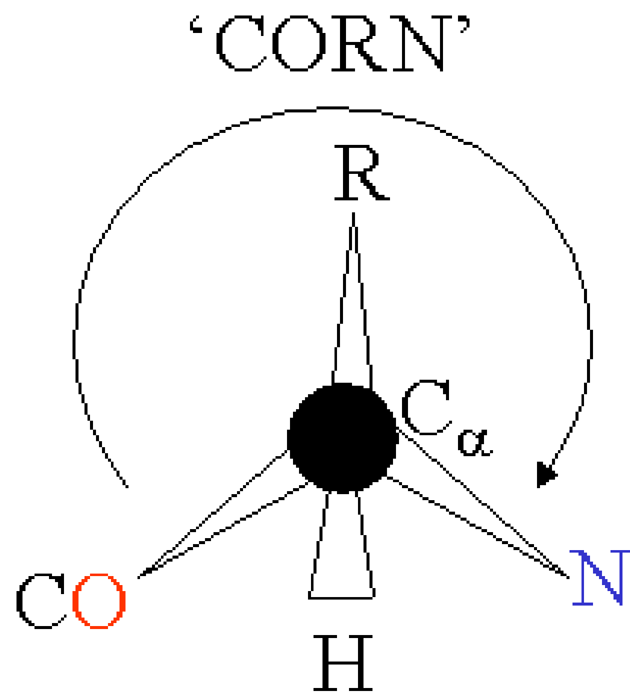
Amino-acidi non polari (idrofobici)

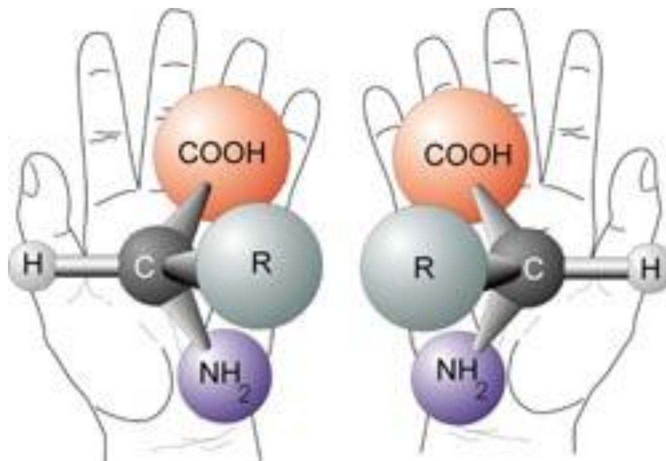



CHIRALITY
An object that cannot be superimposed
on its mirror image is called chiral
Mirror
Mirror
Chiral objects
Nonsuperimposable
mirror images
Nonchiral objects
Superimposable
mirror images



Guardando C_α da H

A **chiral molecule** is a type of molecule that lacks an internal plane of symmetry and has a non-superimposable mirror image. The feature that is most often the cause of chirality in molecules is the presence of an asymmetric carbon atom.[



Although most amino acids can exist in both left and right handed forms, Life on Earth is made of left handed amino acids, almost exlusively. No one knows why this is the case. However, Cronin and Pizzarello have shown that some of the amino acids that fall to earth from space are more left than right. Thus, the fact that we are made of L amino acids may be because of amino acids from space.

La prima proteina (myoglobina) fu cristalizzata nel 1961.

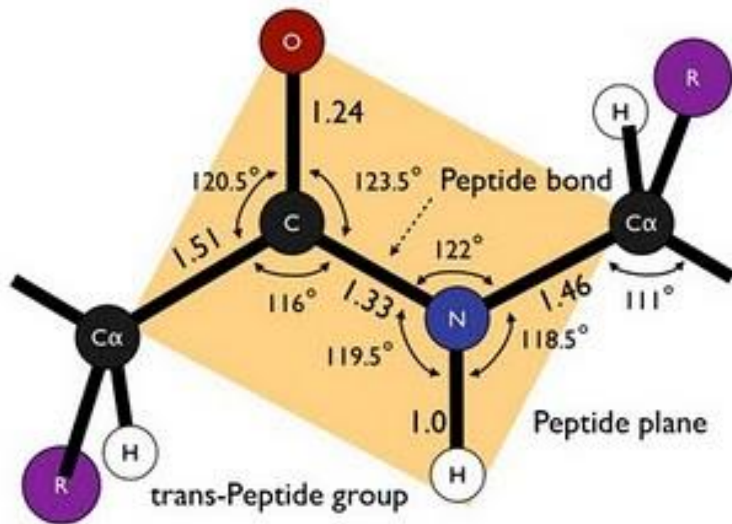
La selezione di una proteina di N aminoacidi non puo’ essere avvenuta per trial and error.

Eta’ dell’universo 5*10^(17) secondi

Tempo necessario per esplorare 20^N enorme 20^300=10^400

Una proteina con nuova funzione puo’ risultare dalla fusione di mRNA di sequenze piu’ corte, ognuna delle quali selezionata per una funzione piu’ semplice.

$$C - N = 1.47\,A$$
$$C == N = 1.25\ \mathrm{A}$$
nel legame peptidico:
$$\mathrm{C - N} = 1.32\,A$$



Trans favorito rispettoa Cis

4 kcal mol^{-1}

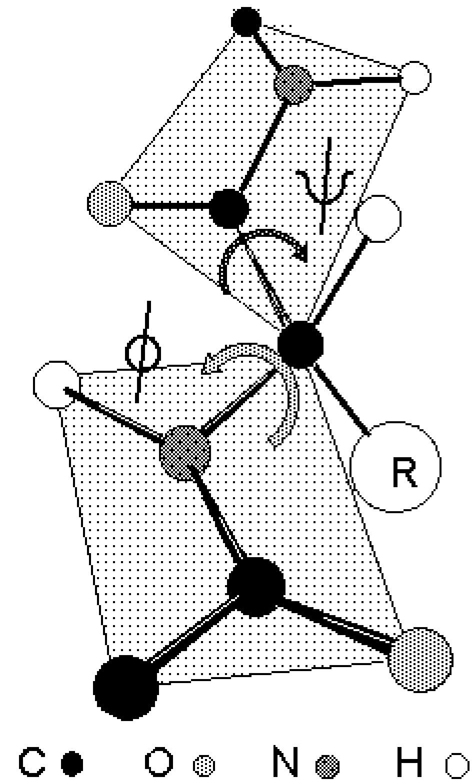
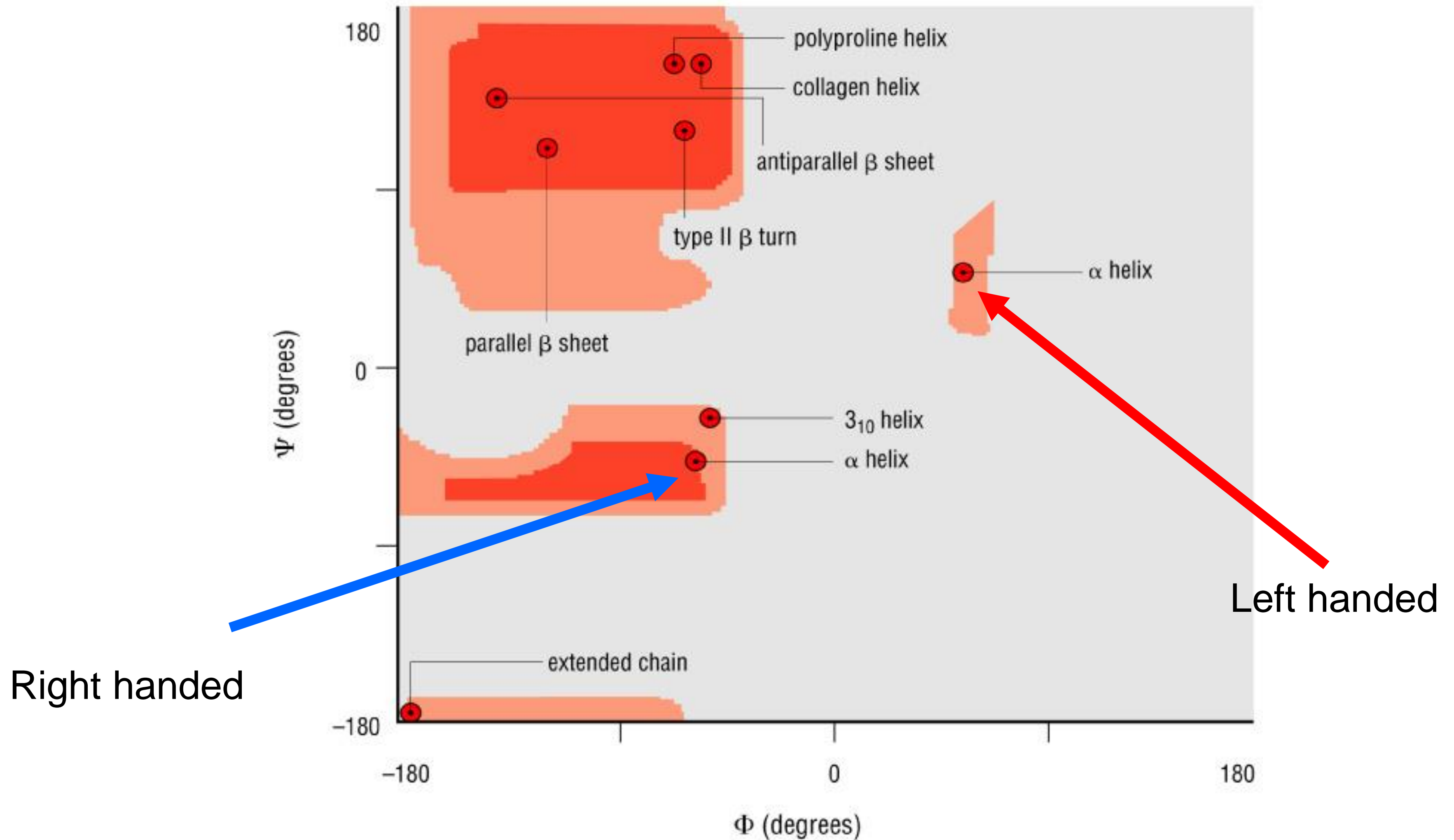


Grafico di Ramachandran

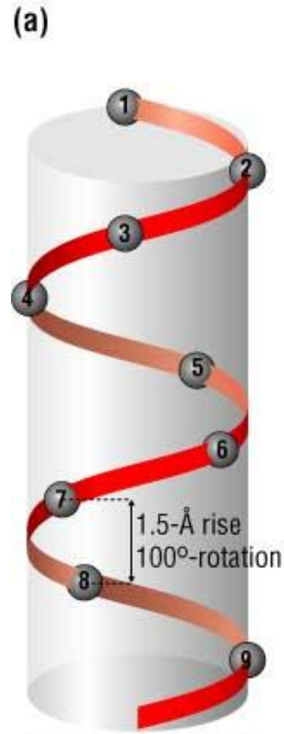


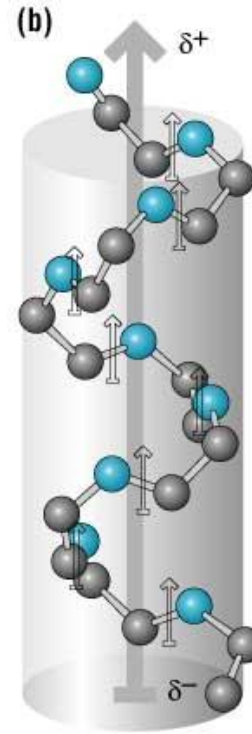
α helices

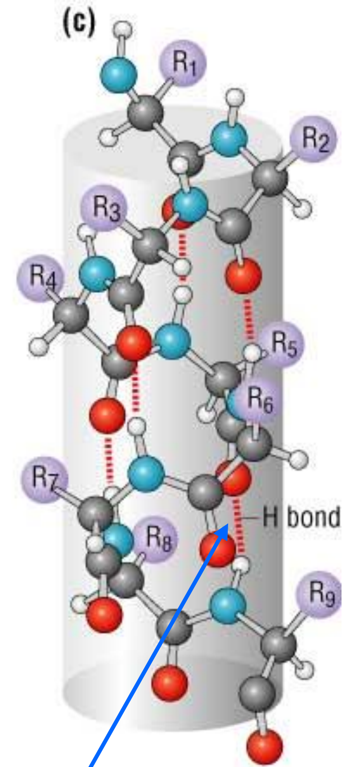
p=1.5 A

P/p=3.6

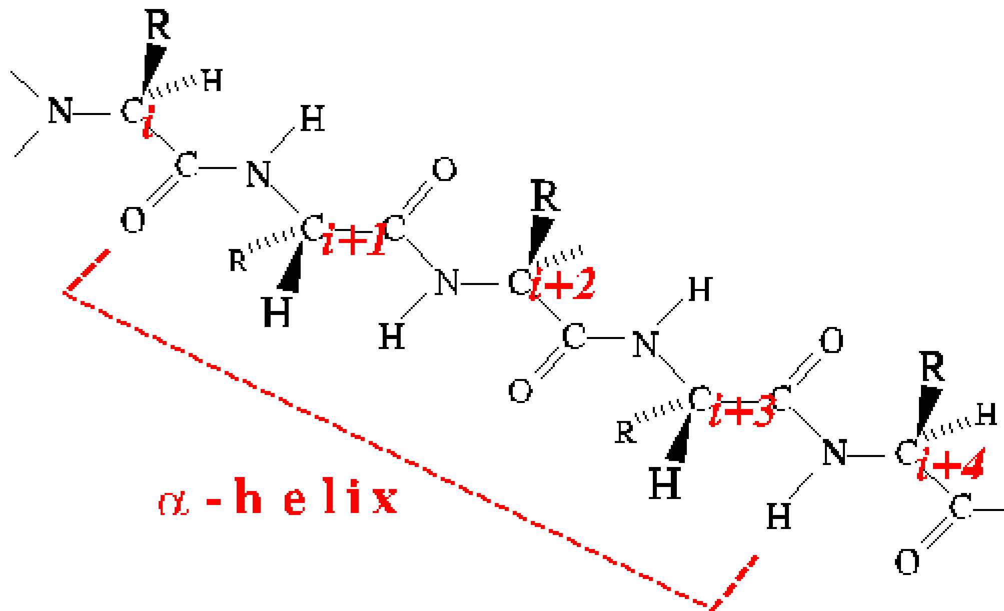
Hb {i,i+4}







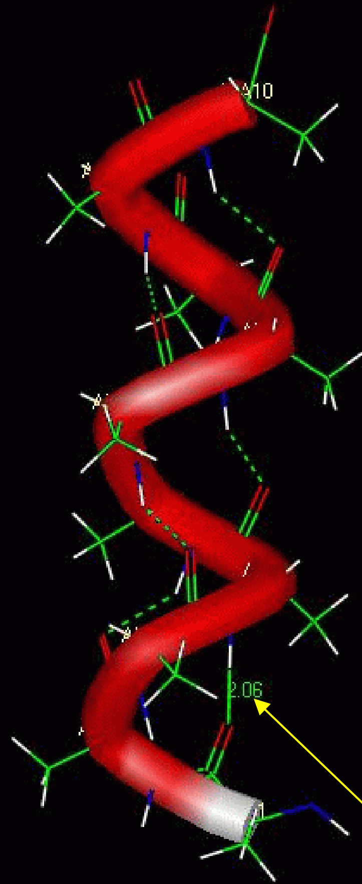
Parallel to the axis



Right-handed

Elica 3-10

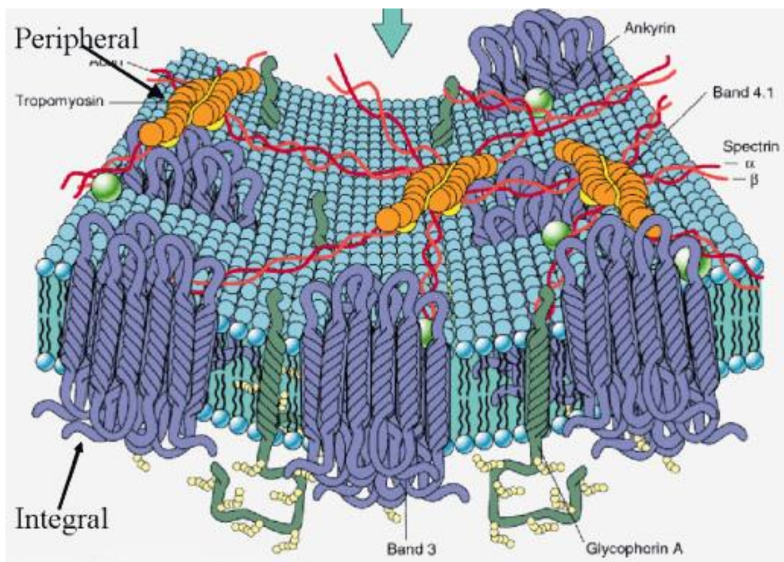
3–10 helix

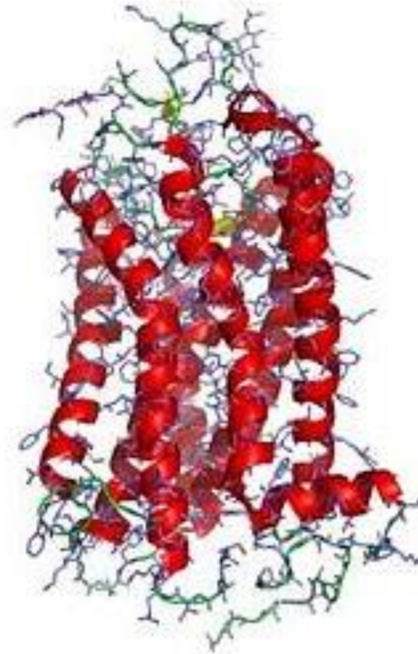


Hb {i.i+3}

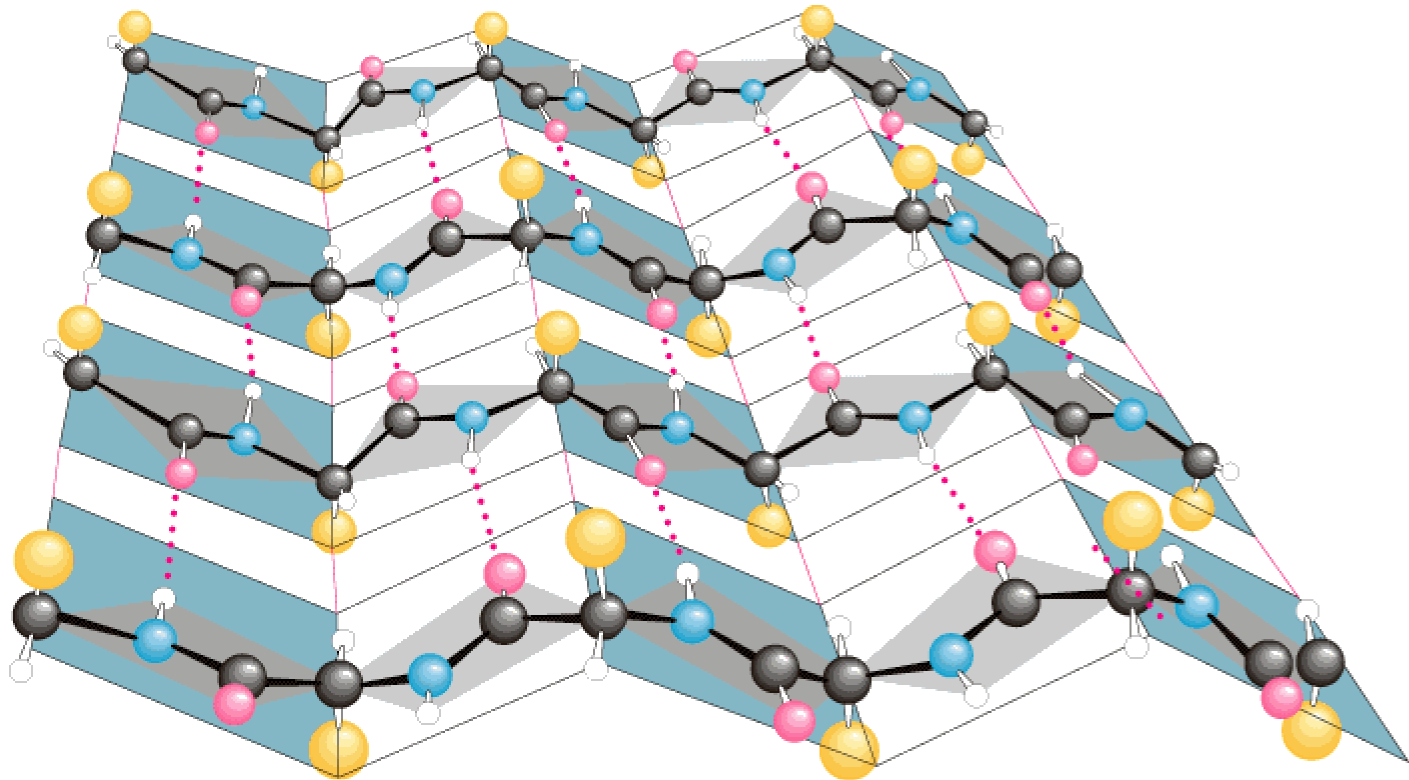
Not parallel

Proteine di membrana



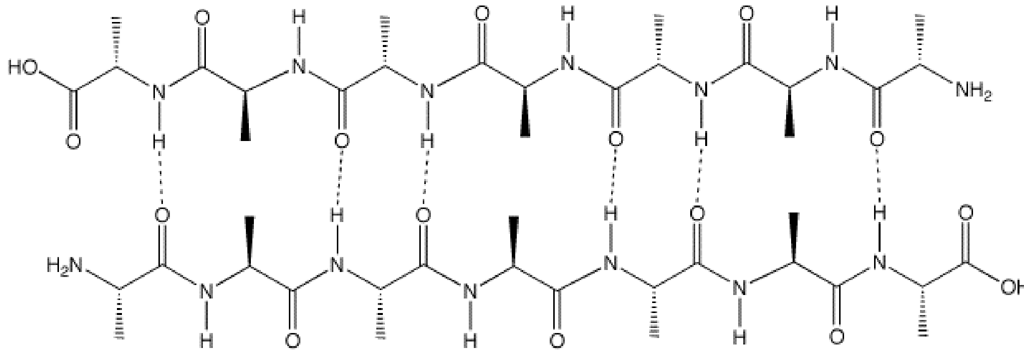


Foglietti β

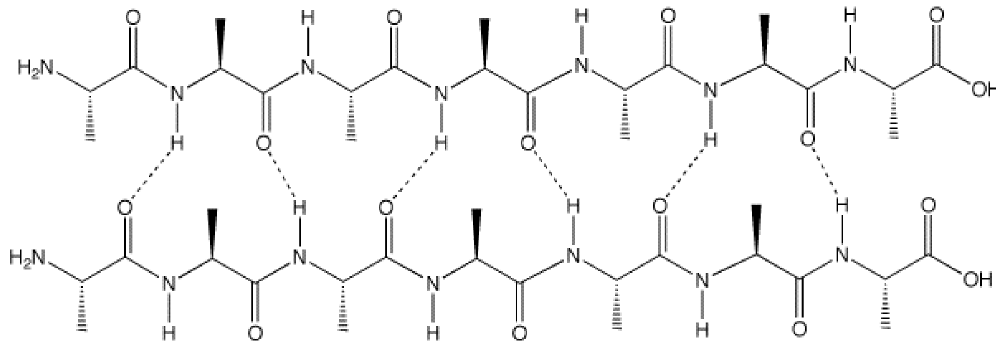


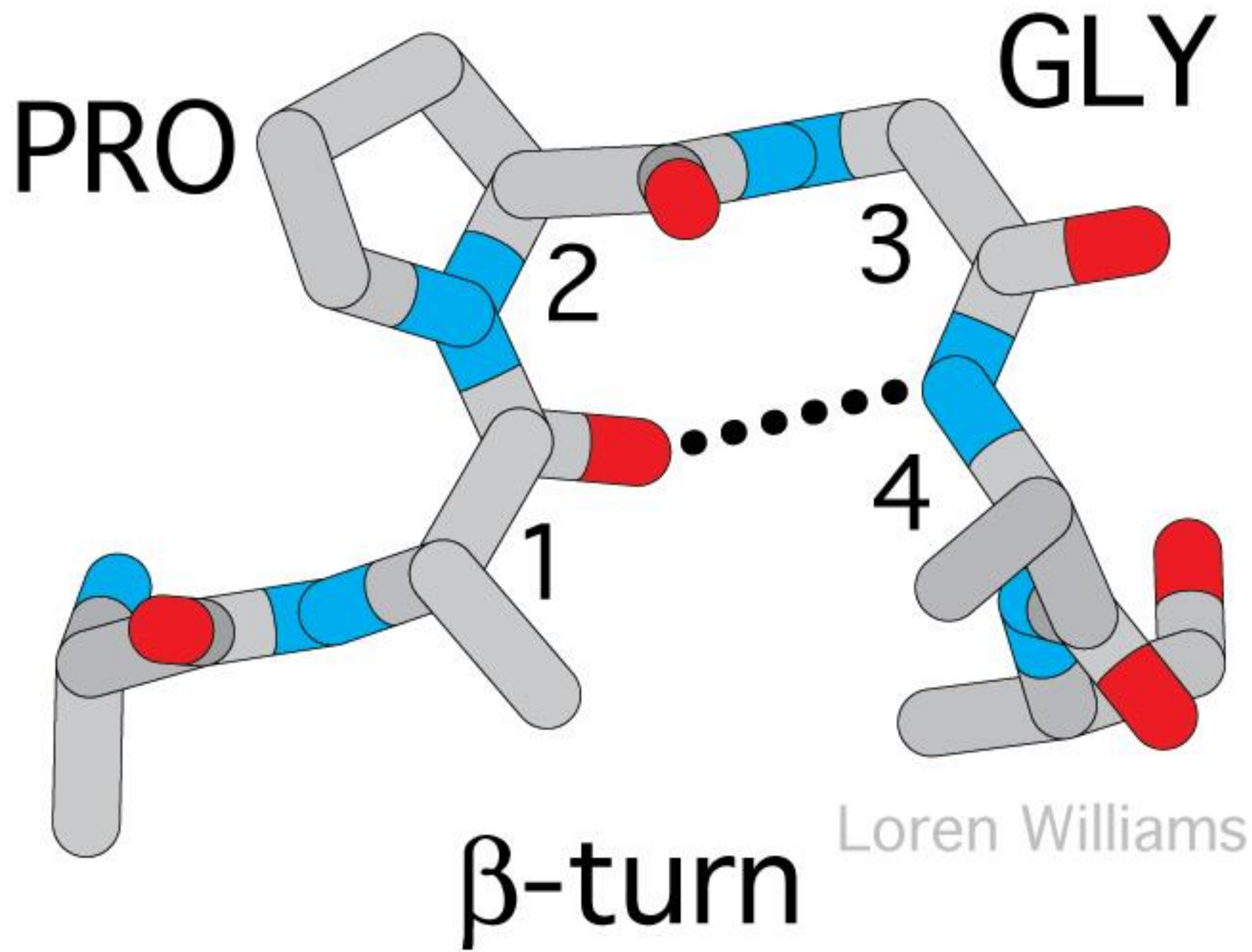
Foglietti β

Anti-parallel



Parallel




PRO
GLY
2
3
1
4
Loren Williams
β-turn

Propensita' degli ammino acidi nelle strutture secondarie

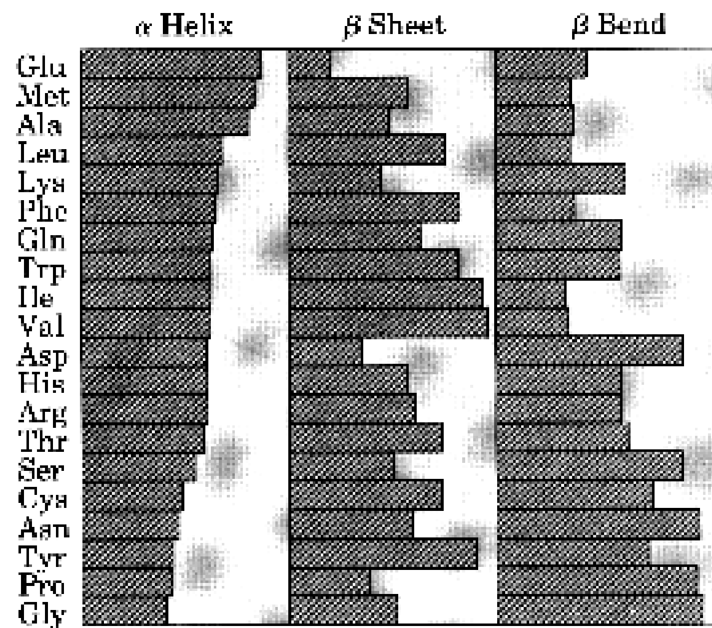
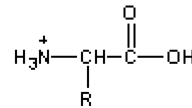


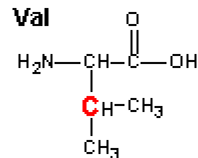
Figure 7–12 Relative probabilities that a given amino acid will occur in the three common types of secondary structure.

AMINO ACID PROPENSITIES FOR SECONDARY STRUCTURE

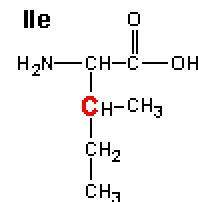


Branched at C_β

Val



Ile

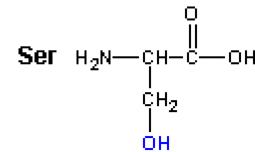


Branch at C_β
destablizes α helix

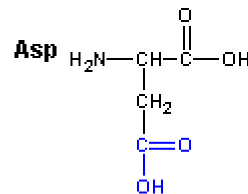
OK in β Sheet
side chain projects out of plan of main chain

No branch at C_β and R group can from H bonds

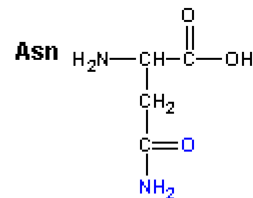
Ser



Asp



Asn



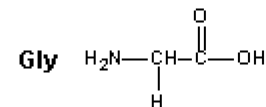
destablizes α helix
H Bond donor/acceptors compete with main chain

No branch at C_β and R group can't form H bonds

OK in α helix
side chain projects out of

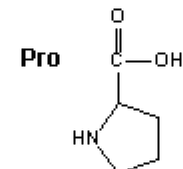
Exceptions:

Gly



- too conformationally flexible

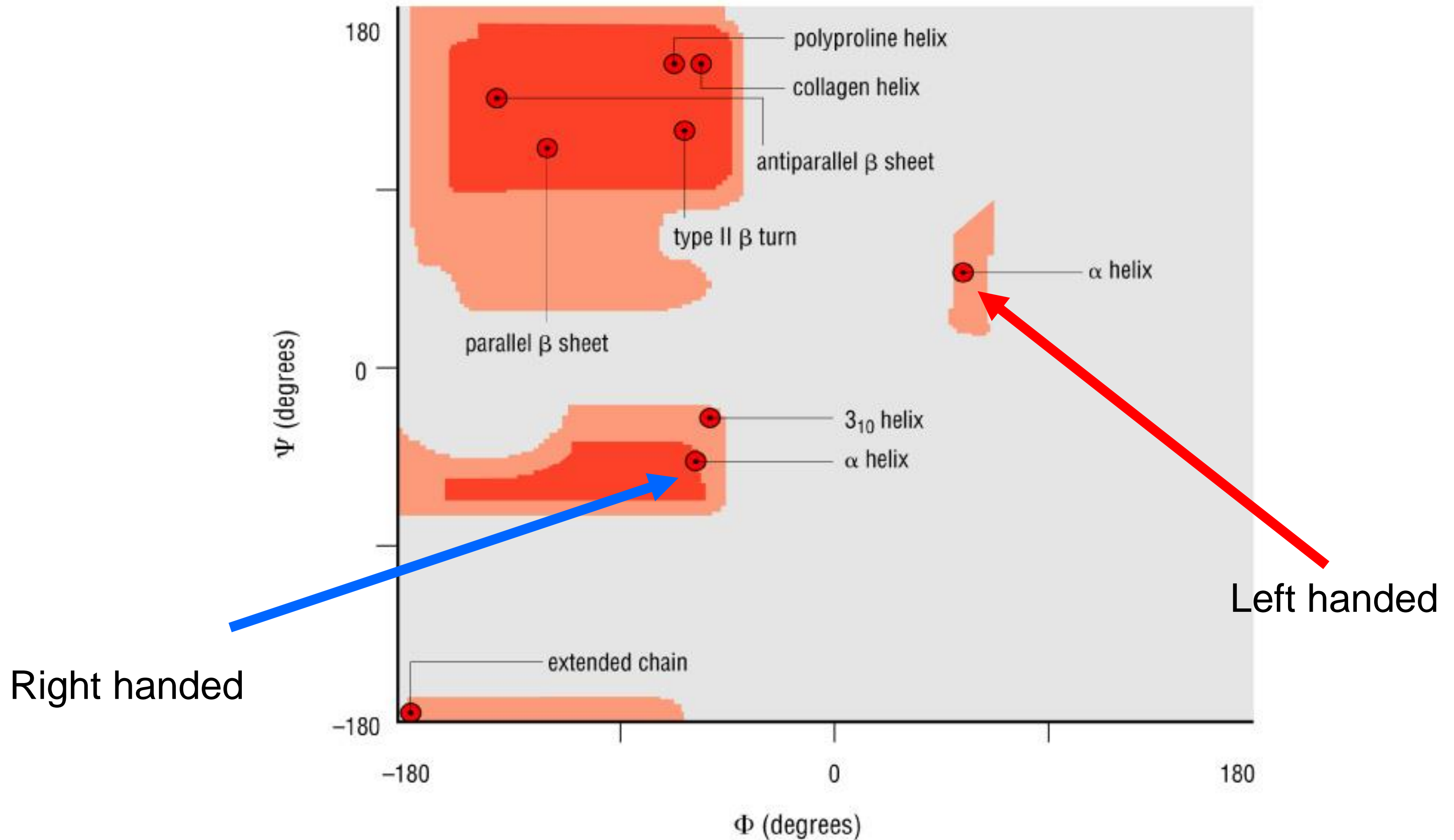
Pro



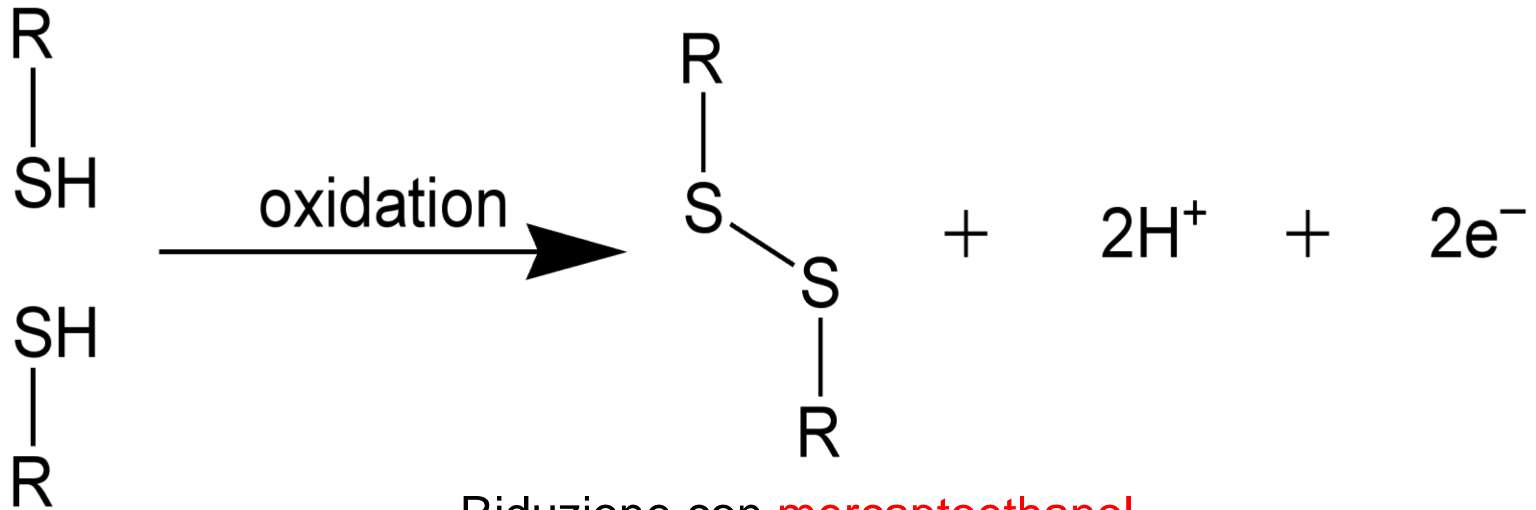
- too rigid

Ala and Leu

Grafico di Ramachandran



Legami disolfuro



Riduzione con mercaptoethanol,

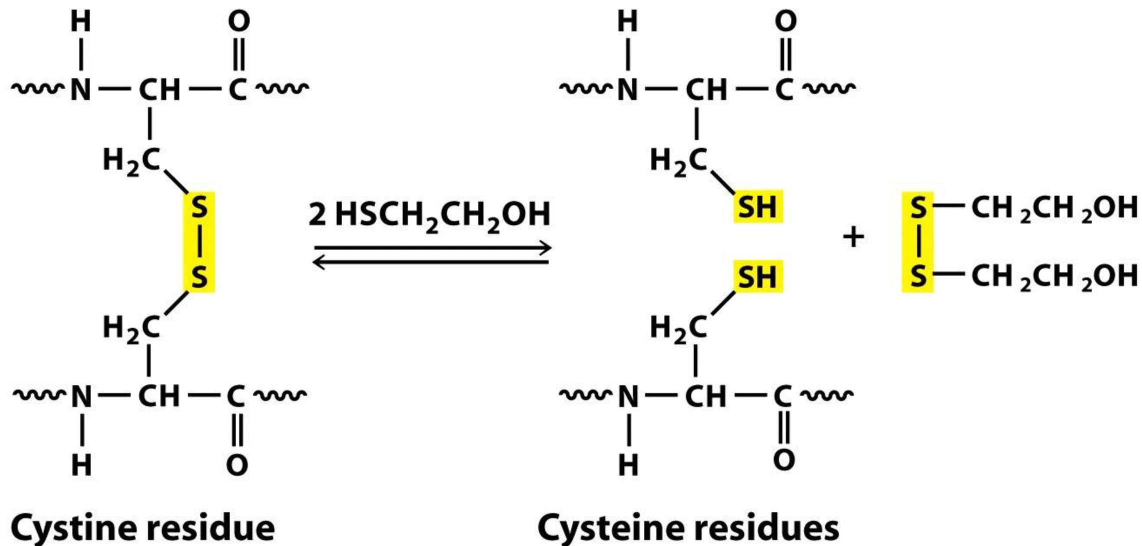


Figure 3-19a Principles of Biochemistry, 4/e
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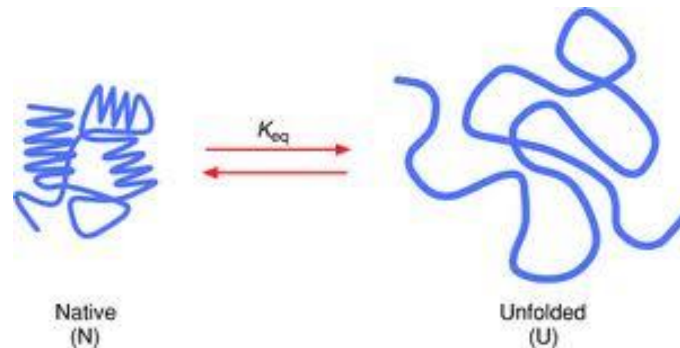
Unfolding by denaturants



As substances such as urea,guanidinium amd many alchols are added to a solution, a protein is converted to a state that is qualitatively similar to the unfolded state induced by heating..

A protein unfolded by denaturants as almost no residual structure

Denaturants unfold proteins by interacting favourably with the protein interior

For example urea can hydrogen bond with backbone amides and carbonyls of the peptide chain

Buried residues of a protein have an unfovarable interaction with water.

As the denaturant concentration increases, the unfavorable interaction with water is offset by an attractive interaction with the denaturant.

Esperimento di Anfinsen (1960’s)

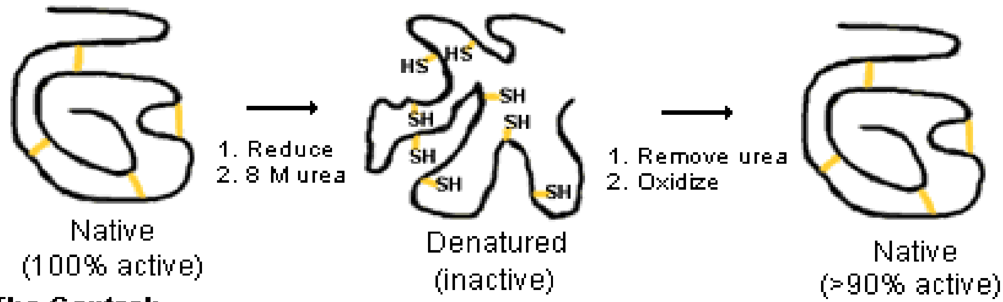
His summary of the experiments was presented as a Nobel Prize Lecture and published in:
Anfinsen, C.B. (1973) "Principles that govern the folding of protein chains." *Science* **181** 223-230.

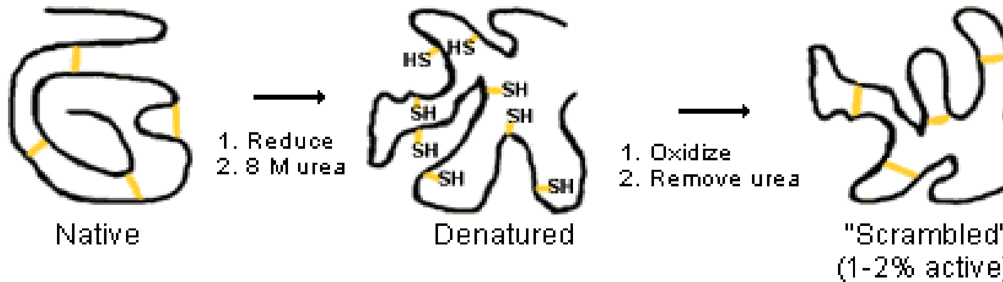
KUNGLIGA
VETENSKAPSAKADEMIEN
HAR VID SIN SAMMANKOMST DEN 20 OKTOBER 1972
I ENLIGHET MED FÖRESKRIFTERNA I DET AV
ALFRED NOBEL
DEN 27 NOVEMBER 1895 UPPRÄTTADE TESTAMENTET

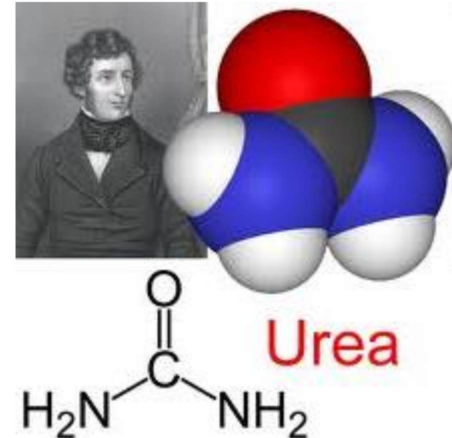
BESLUTAT ATT UTDELA DET PRIS SOM DETTA ÅR
BORTGIVES ÅT DEN SOM GJORT DEN VIKTIGASTE
KEMISKA UPPTÄCKT ELLER FÖRBÄTTRING
MED ENA HÄLFTEN TILL
CHRISTIAN B ANFINSEN
FÖR HANS ARBETEN ÖVER RIBONUKLEAS, SÄRSKILT
RÖRANDE SAMBANDET MELLAN AMINOSYRASEKVENSEN
OCH DEN BIOLOGISKT AKTIVA KONFORMATIONEN,
OCH MED ANDRA HÄLFTEN, LIKA DELAD, GEMENSAMT TILL
STANFORD MOORE OCH WILLIAM H STEIN
FÖR DERAS BIDRAG TILL FÖRSTÅELSEN AV SAMBANDET
MELLAN KEMISK STRUKTUR OCH KATALYTISK AKTIVITET
HOS RIBONUKLEASMOLEKYLENS AKTIVA CENTRUM
STOCKHOLM DEN 10 DECEMBER 1972

Esperimento di Anfinsen (1960's)

1



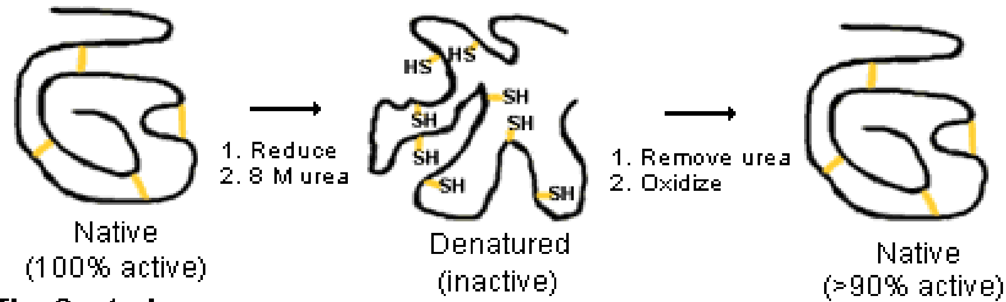


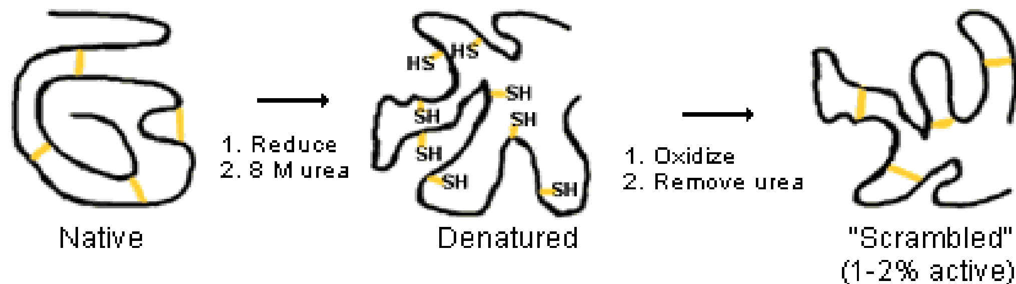


Ribonuclease A (RNaseA) is an extracellular enzyme of 124 residues with four disulfide bonds. In the first phase of the experiment, the S-S bonds were reduced to eight -SH groups (using mercaptoethanol, HS-CH2-CH2-OH); the protein was then denatured with 8 M urea. Under these conditions, the enzyme is inactive and becomes a flexible random polymer. In the second phase, the urea was slowly removed (dialysis); then the the -SH groups were oxidized back to S-S bonds. If the protein was able to regain its native structure spontaneously after removal of the urea, we expect that it would also regain its activity. In fact, the activity was >90% of the untreated enzyme. Moreover, sequence analysis showed that nearly all of the correct S-S bonds had been formed.

And if RNaseA was not completed unfolded???

Esperimento di Anfinsen (1960's)

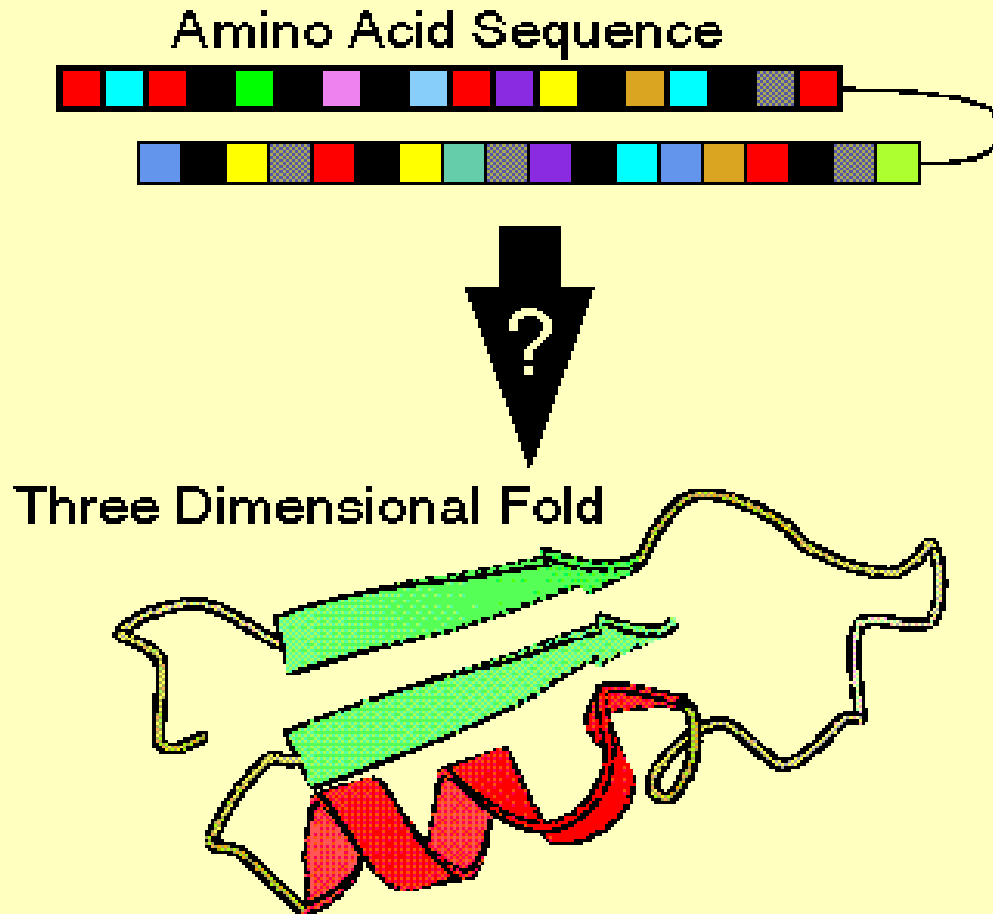




2

A reasonable objection can be raised to the above result by suggesting that perhaps RNaseA was not completely unfolded in 8 M urea. To address this class of objections, RNAseA was first reduced and denatured as above. But in the second phase, the enzyme was first oxidized to form S-S bonds, and **then** the urea was removed, *i.e.* the order of steps in the second phase of the experiment was reversed. The resulting activity was only about 1-2% of the untreated enzyme. Sequence analysis showed a random assortment of S-S bonds ("Scrambled" in the diagram). [Question: Can we account for the 1-2% recovery of activity in the "Scrambled" sample?] .

The Protein Folding Problem



The native state is uniquely determined by the sequence

- **The native state is thermodynamically stable and reachable from different starting conditions.**
- **Only few sequences are proteins**
- **Only few conformations are native states**
- **The folding time is very rapid (0.01-100 sec)**

Contributions to the total free energy of the protein

1. Conformation entropy corresponding to the loss of degrees of freedom due to the bonding of amino acids and the restricted motion of side chains
2. Energy of intramolecular hydrogen bonds and of hydrogen bonds between the protein and external water molecules
3. Energy of van der Waals bonds
4. Coulomb energy of electrostatic bonds and coupling energy between dipoles formed by helices
5. Valence bond energy in disulphide bridges
6. Hydrophobic effect

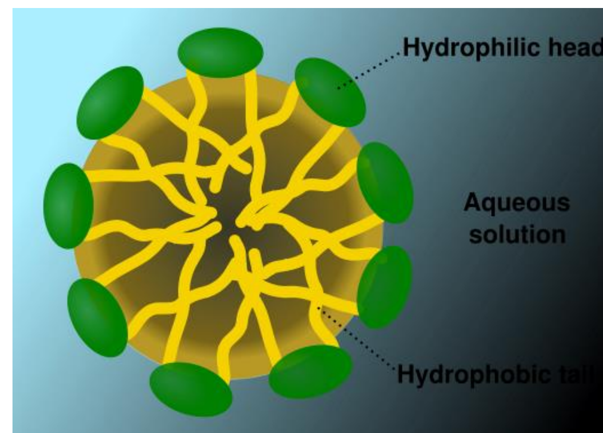
STABILITY: $10 kcal \cdot mol^{-1} = 20 k_B T \quad 100\,\mathrm{aa}$

Effetto idrofobico

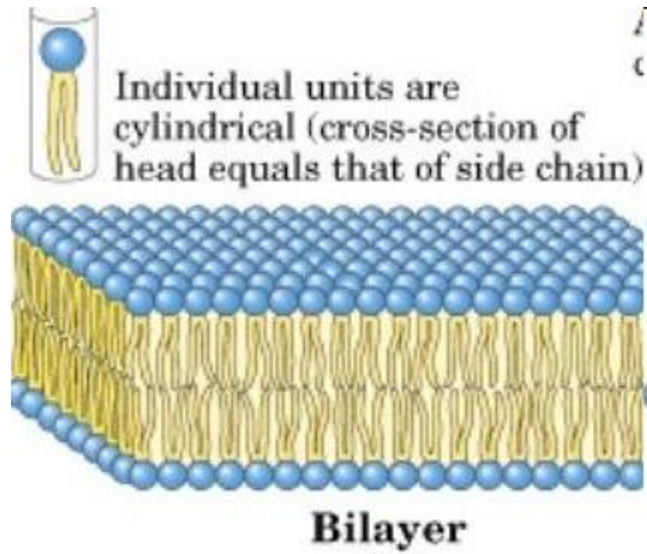
Le interazioni fra acqua e superfici non polari non sono favorevoli: proprio come l'olio disperso in acqua tende a raccogliersi in una unica goccia, anche I gruppi non polari nelle proteine tendono ad aggregarsi, per ridurre la superficie apolare a contatto con l'acqua.

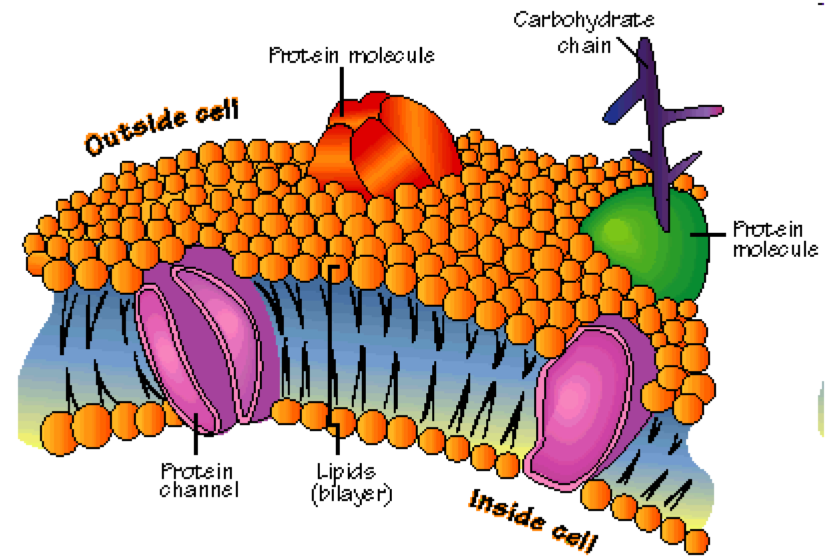
Questa preferenza di specie non polari per ambienti non acquosi viene detto effetto idrofobico: esso e' uno dei principali fattori di stabilita' delle proteine.

L'effetto idrofobico fa si che sostanze non polari minimizzino il loro contatto con l'acqua, e molecole anfipatiche formino micelle in soluzioni acquose



Lipid bilayer

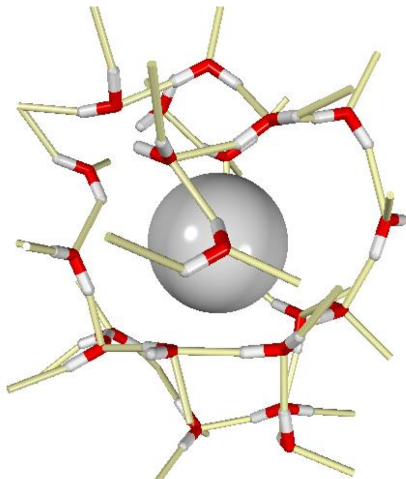




Effetto idrofobico

Le molecole d’acqua allo stato liquido formano dinamicamente un alto numero di legami idrogeno.

L’introduzione di una molecola non polare nell’acqua, che temporaneamente rompe alcuni legami idrogeno fra le molecole d’acqua, poiche’ un gruppo non polare non puo’ ne’ accettare ne’ donare legami idrogeno con le molecole d’acqua



Le molecole d’acqua spostate si orientano per formare il maggior numero di nuovi legami idrogeno, creando una struttura ordinata, una specie di gabbia, detto clatrato, intorno alla molecola non polare

Effetto idrofobico

Poiche’ il numero di modi con cui le molecole d’acqua formano legami idrogeno sulla superficie di un gruppo non polare e’ inferiore a quello che farebbero in sua assenza si ha una diminuzione di entropia del sistema.

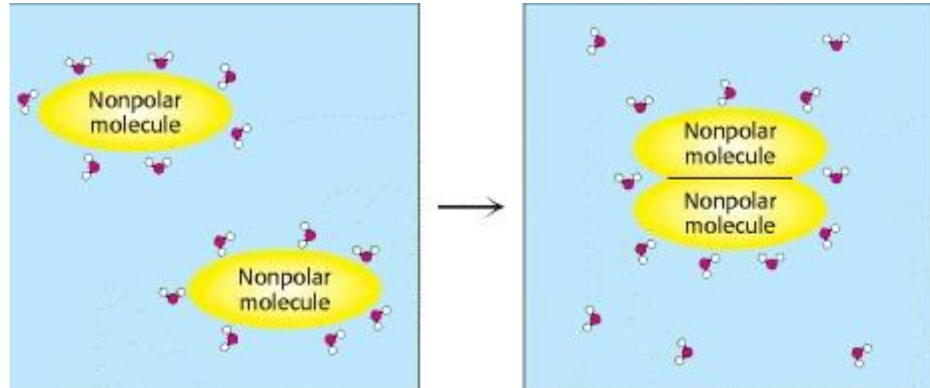
Anche se da un punto di vista entalpico il sistema clatrato e’ piu’ stabile

$$\Delta H < 0$$

per una debole liberazione di energia dovuto alla formazione di legami idrogeno ed interazioni di van der Waals, globalmente

$$\Delta G = \Delta H - T\Delta S > 0$$

Effetto idrofobico

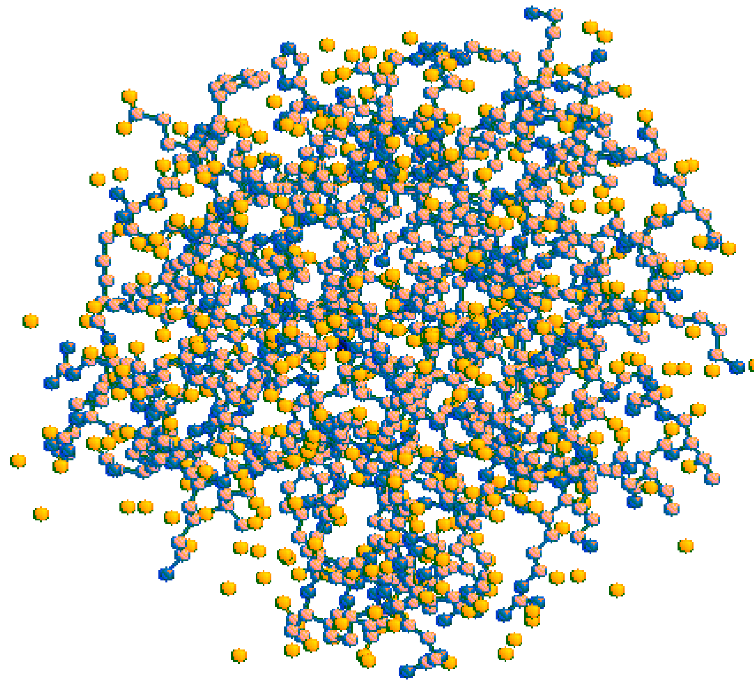


Perche' il processo sia spontaneo occorre l'aggregazione dei gruppi non polari in modo da minimizzare l'area superficiale della cavita' occupata dal gruppo apolare e quindi la perdita di entropia del sistema

Effetto idrofobico e' favorito dall'aumento della temperatura

Integrin Cell Adhesion Protein

184 Aminoacids 1491 Atoms



Integrin Cell Adhesion Protein

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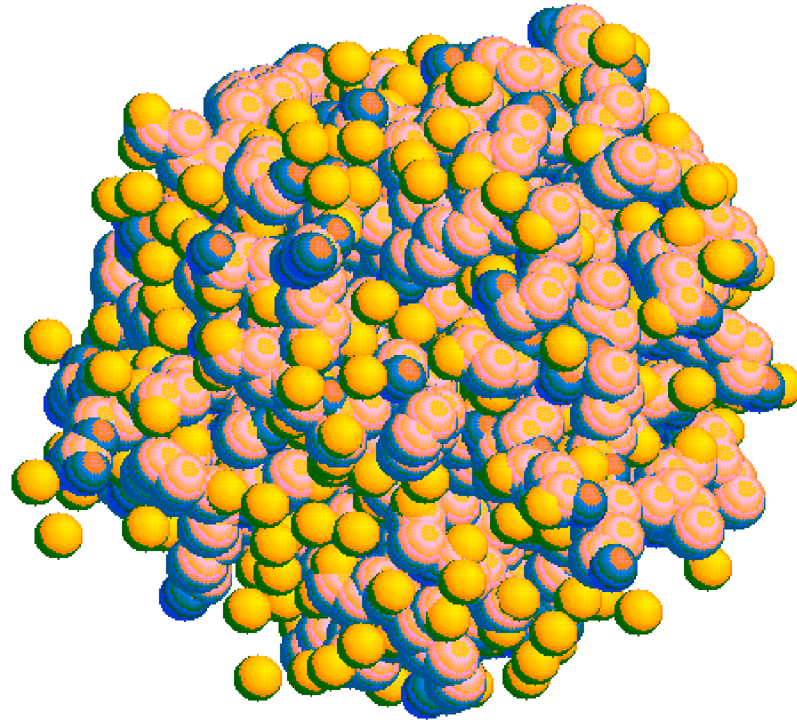

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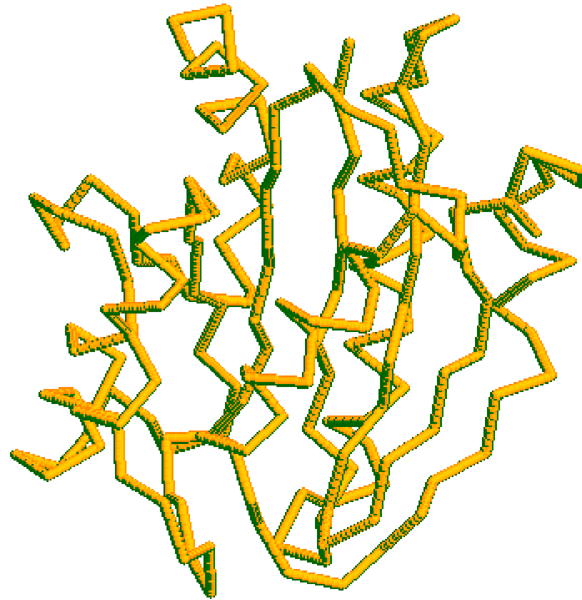
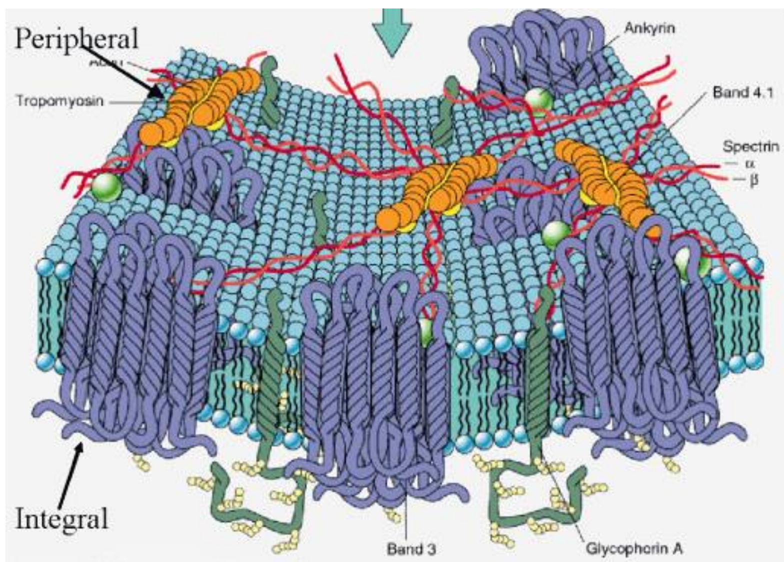
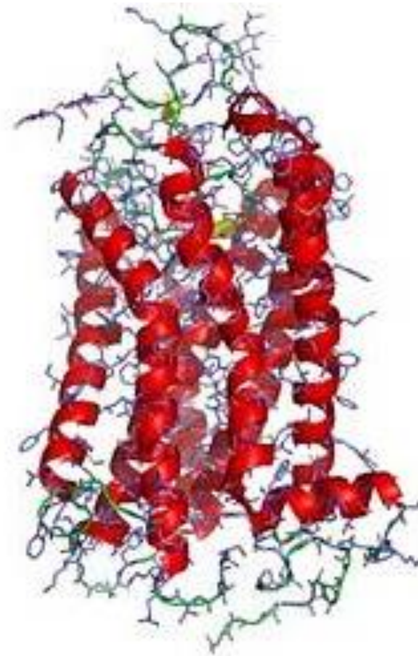


Proteine di membrana





Only 1000 folds!!!!