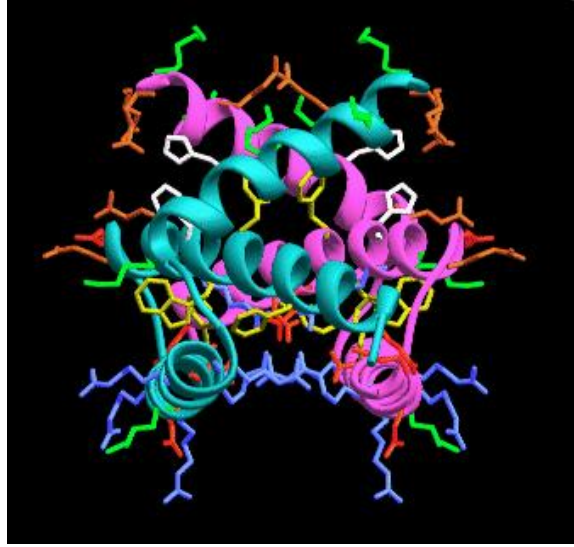
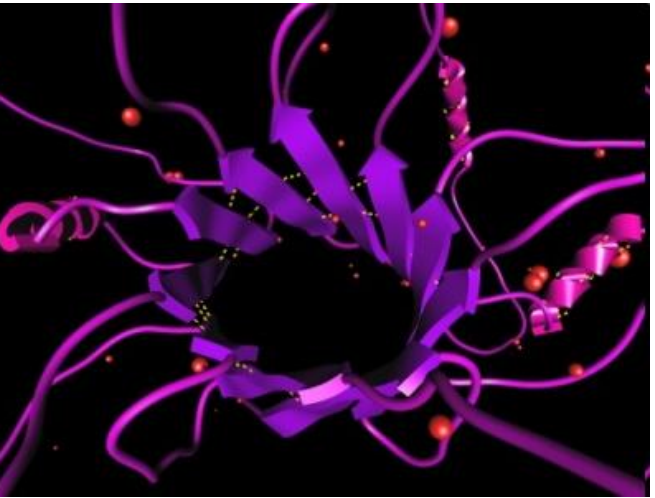
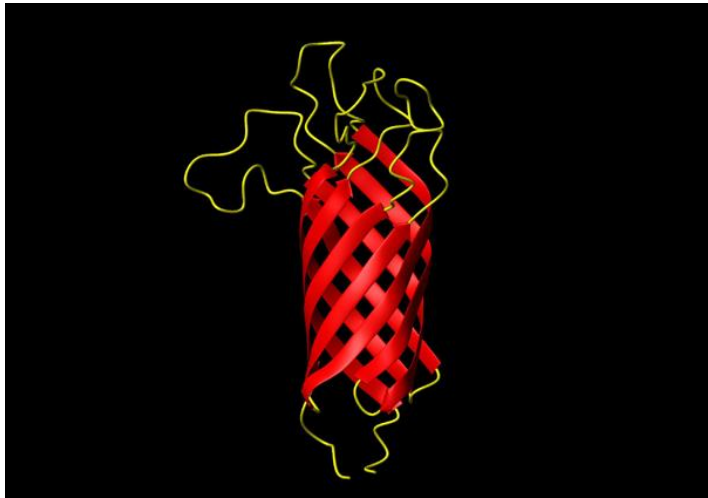


Tridimensional structure of proteins





Como as proteínas podem ser tão diversas?

Como elas realizam suas funções?

Estrutura primária:

- **proteínas com diferentes funções tem diferentes sequências**

- **a estrutura primária é que determina como a sequência será enovelada**

- **Cada proteína tem uma única estrutura tridimensional e consequentemente uma única função.**

RATO

TORRA

TORA

TATO

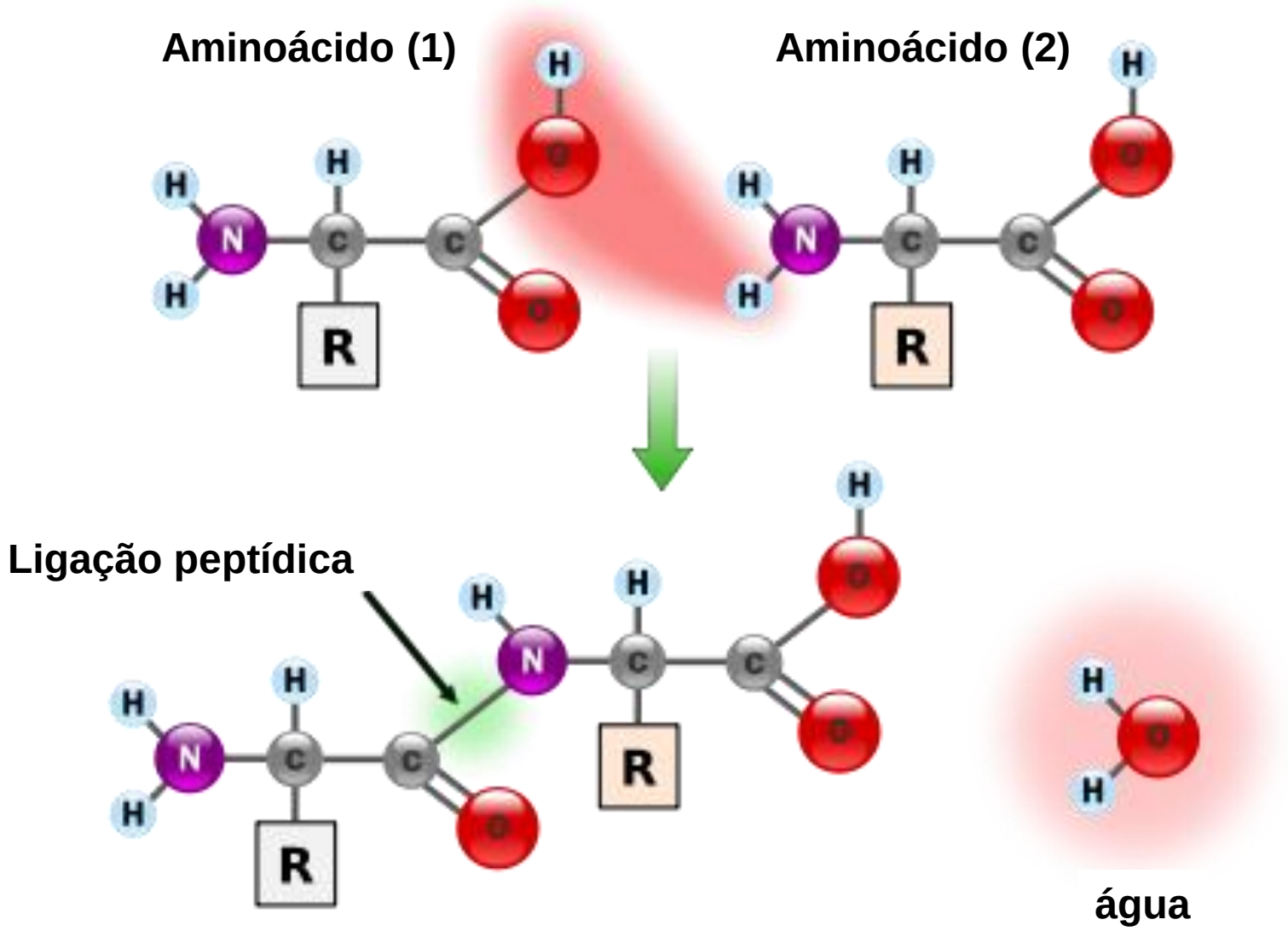
ROTA

ROTOR

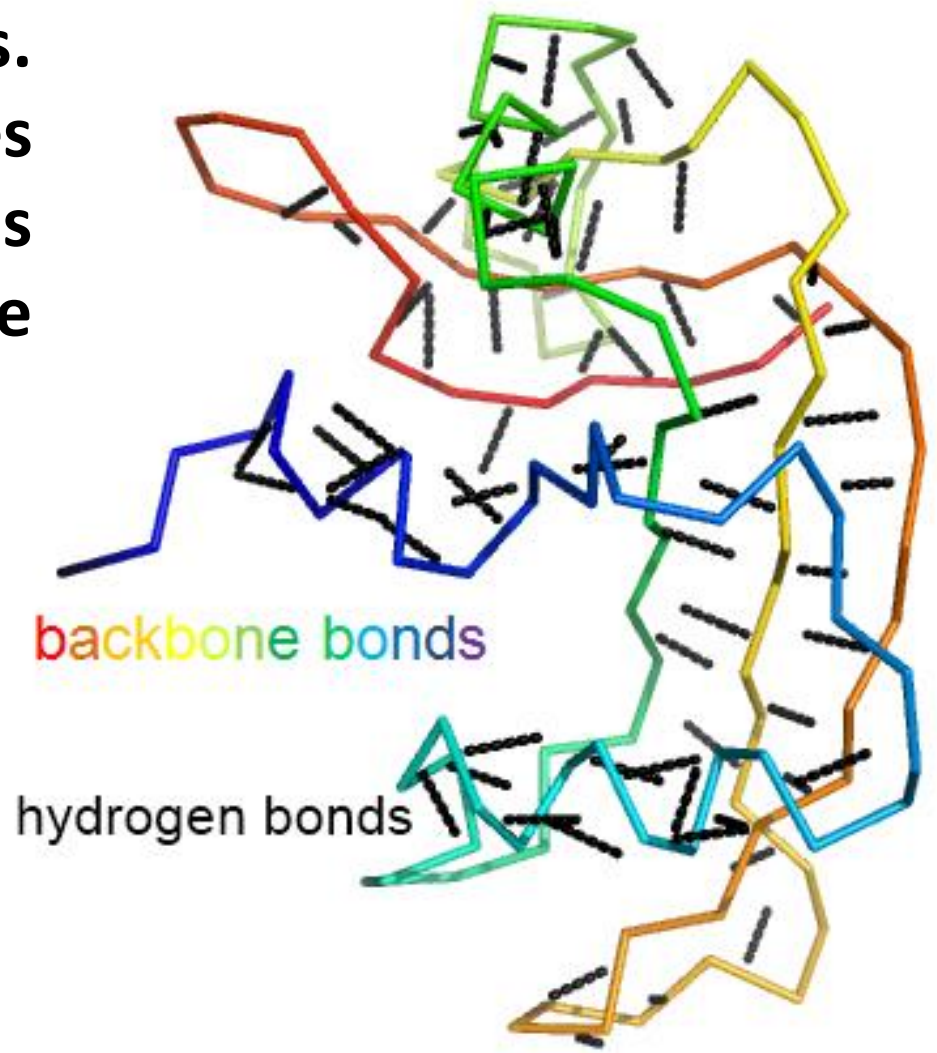
ORAR

ARARA

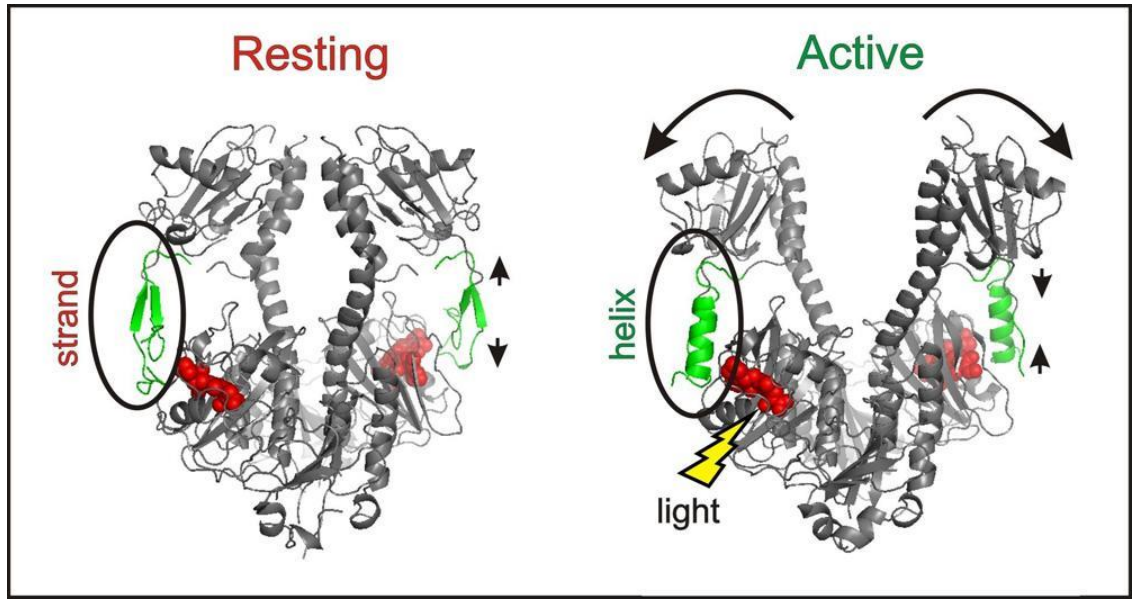
A ligação peptídica

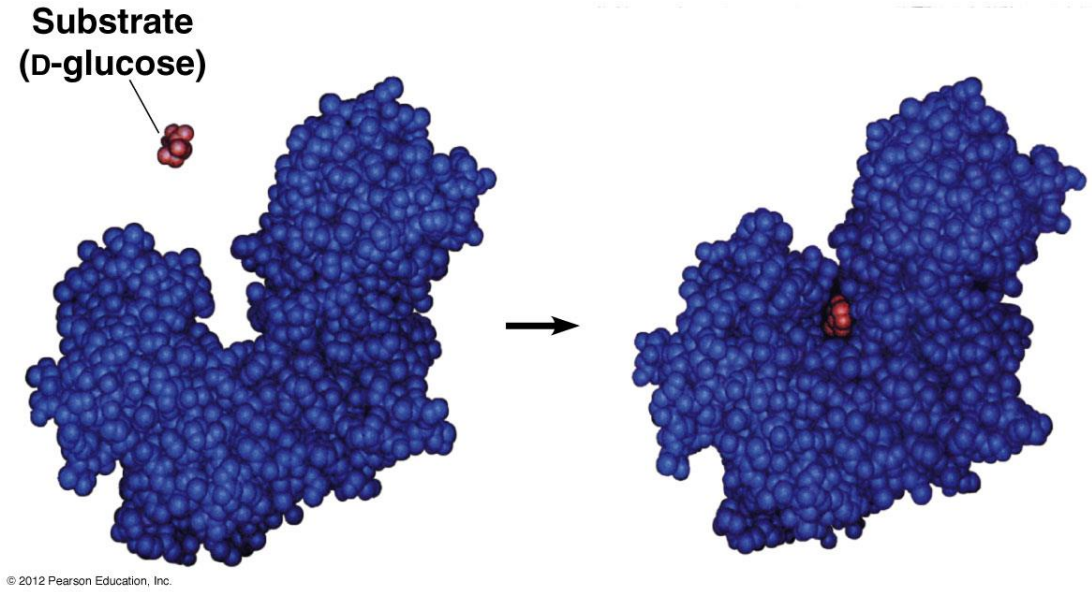


O esqueleto covalente de uma proteína contem milhares de ligações individuais. Essas ligações tem ligações tem rotações livres e consequentemente as proteínas podem adotar um largo número de conformações.



Conformação = arranjamento espacial dos átomos de uma proteína

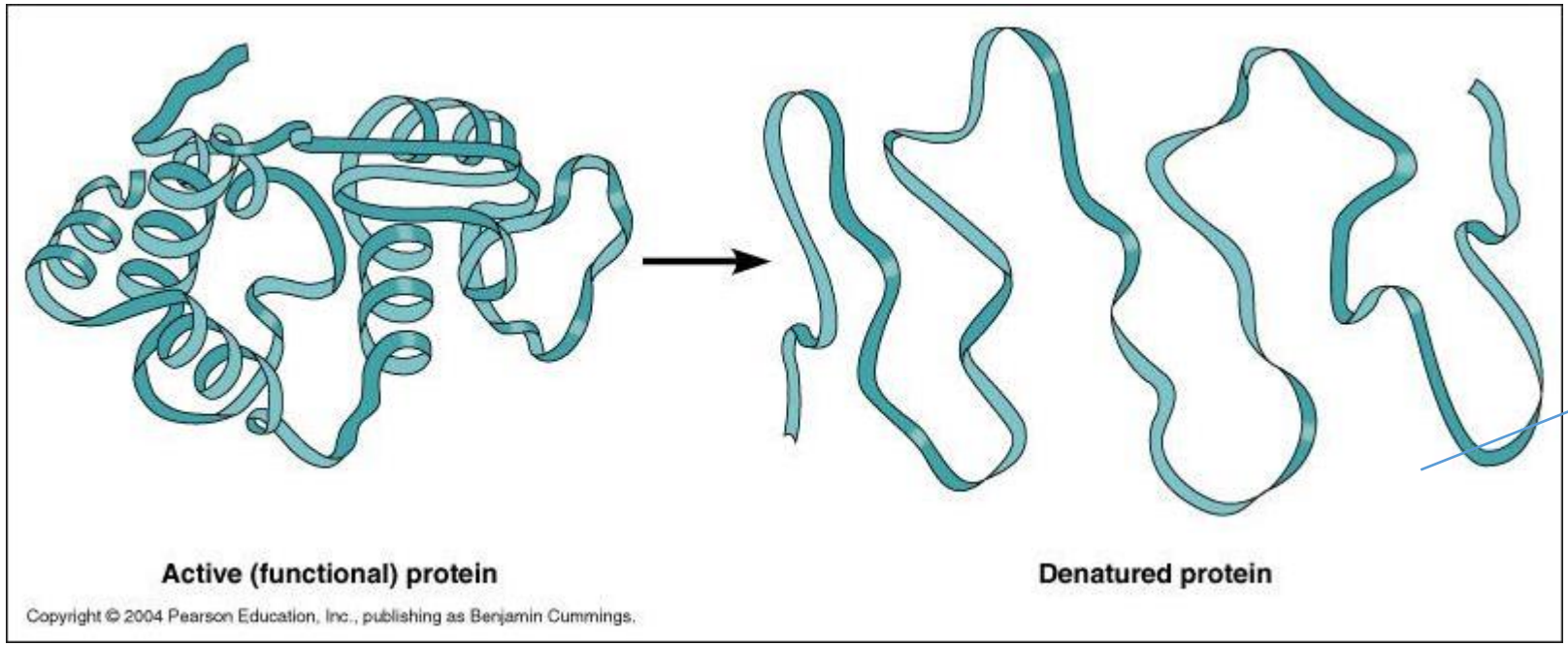




As conformações possíveis de uma proteína incluem qualquer estado estrutural que pode ser atingido sem quebrar as ligações covalentes

Por que muitas conformações são necessárias para uma proteína?

As conformações possíveis de uma proteína refletem os estados mais termodinamicamente estáveis, isto é, o que apresenta mais baixa energia livre de Gibbs (*G*)

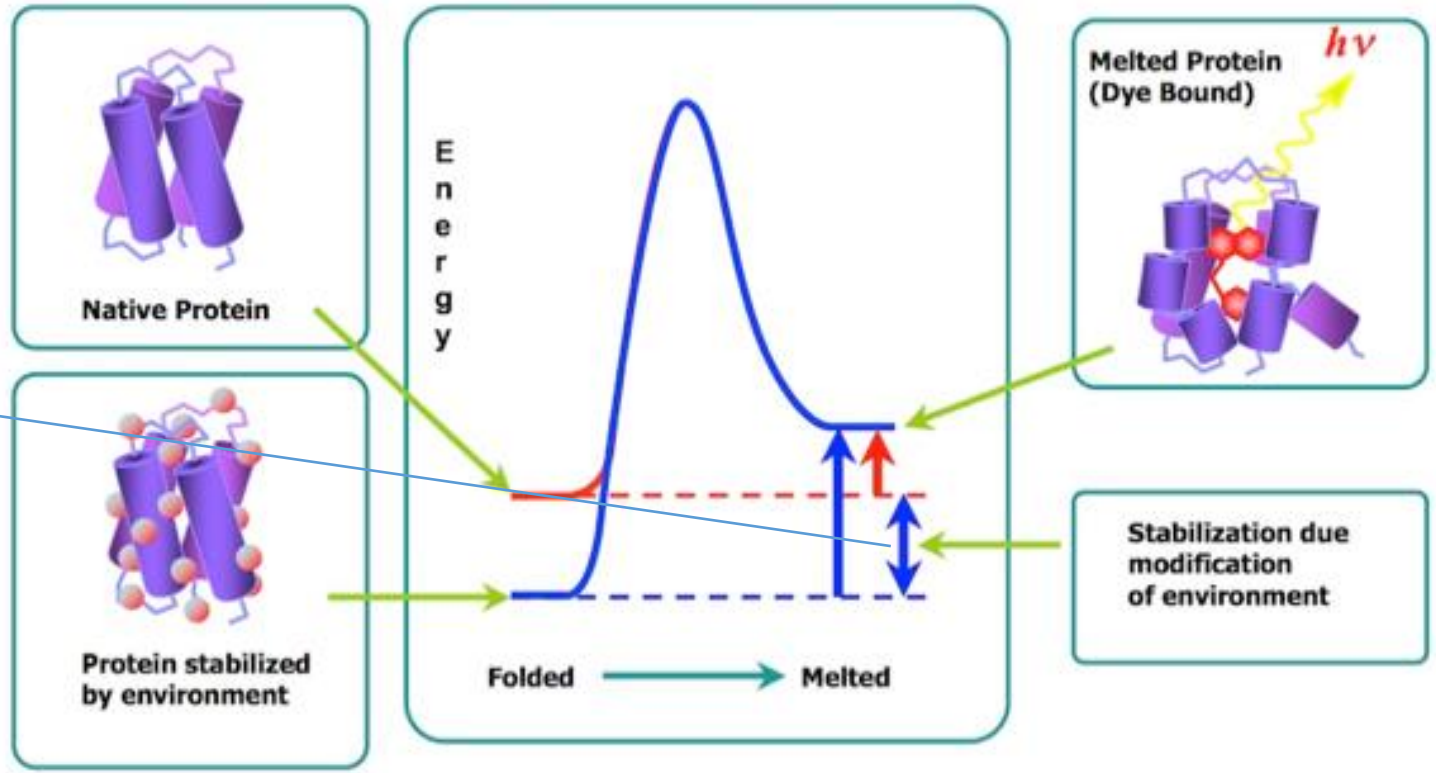


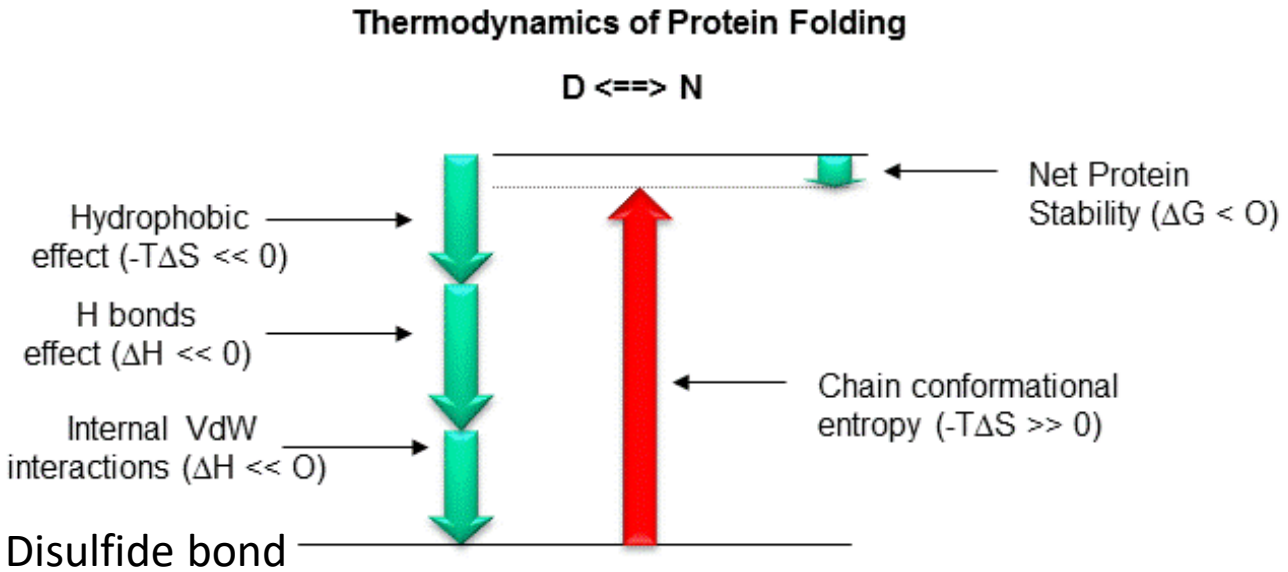
High level of entropy

A estabilidade é a tendência da proteína manter sua conformação nativa, isto qualquer estado funcional da proteína.

Proteínas são apenas marginalmente estáveis

20 to 65 kJ/mol

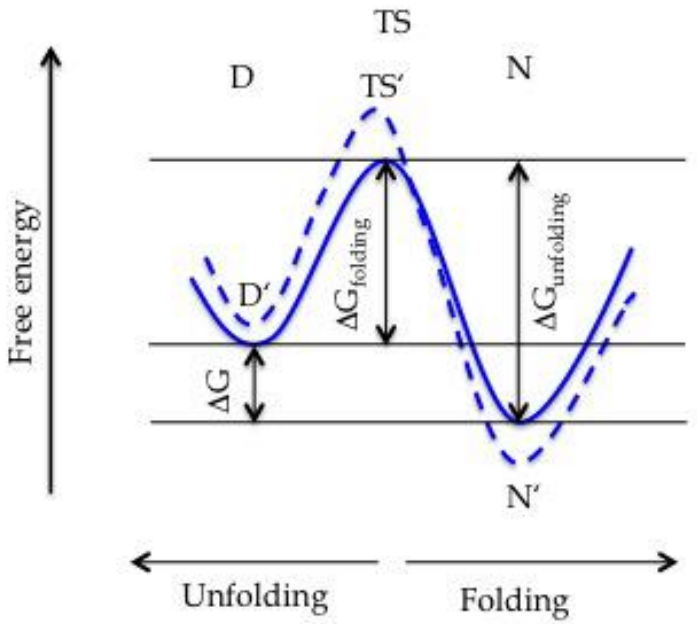




As conformações de mais baxia energia ou as são as conformações mais estáveis. Essas são aquelas apresentam o número máximo de interações

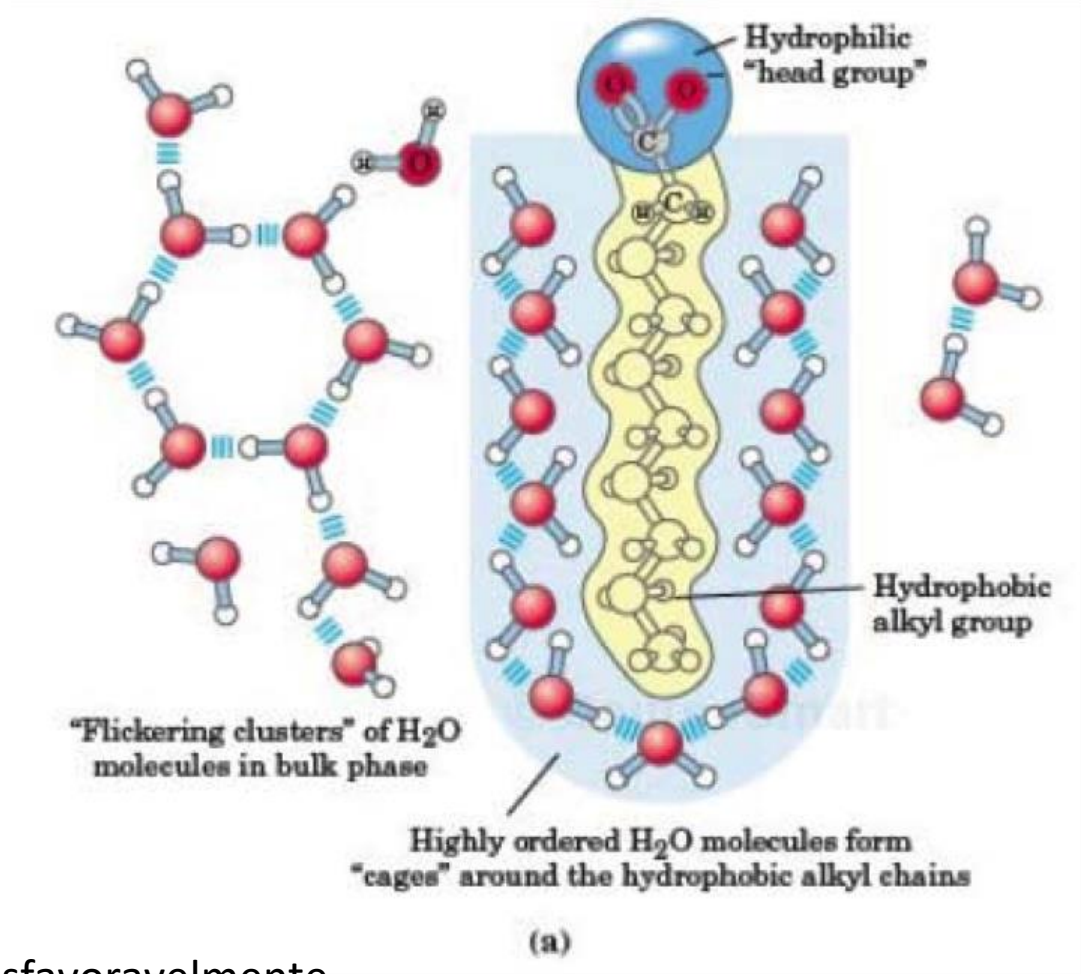
Para cada ligação de hidrogênio formada durante o enovelamento de uma proteína, uma ligação de hidrogênio entre o mesmo grupo e água é quebrada

A diferença entre as energias livres do estado enovelado e não enovelado é próximo de zero.



Isso garante que as proteínas na sua forma nativa possam existir.

As interações hidrofóbicas são as interações predominantes que contribuem para as conformações nativas



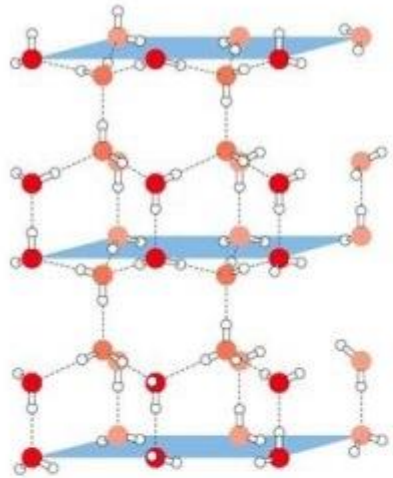
As moléculas de água ao redor de uma molécula hidrofóbica, formam uma camada de solvatação

Essa camada apresenta moléculas de água mais ordenadas ao redor da cadeia polipeptídica

Apresenta efeito sobre a entropia das moléculas de água

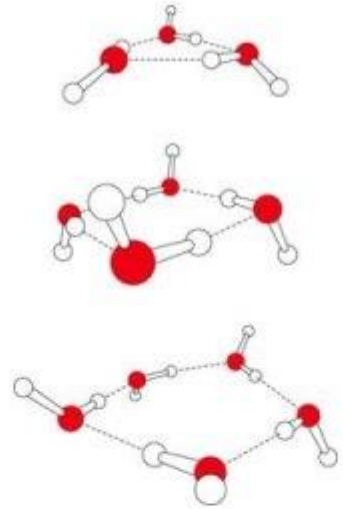
Grupos não polares agrupam-se diminuindo a camada de solvatação

Desfavoravelmente aumenta a entropia para a associação dos grupos hidrofóbicos



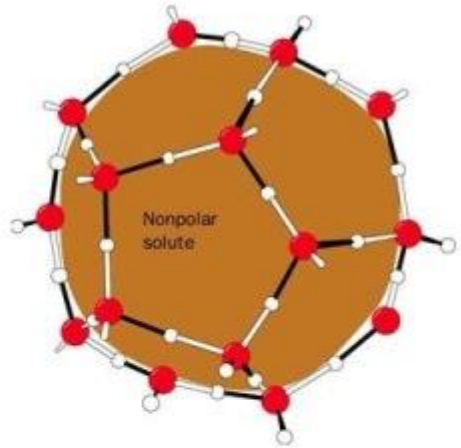
tetrahedral structure of ice

after L. Pauling, *The Nature of the Chemical Bond* (Cornell University Press 1960)



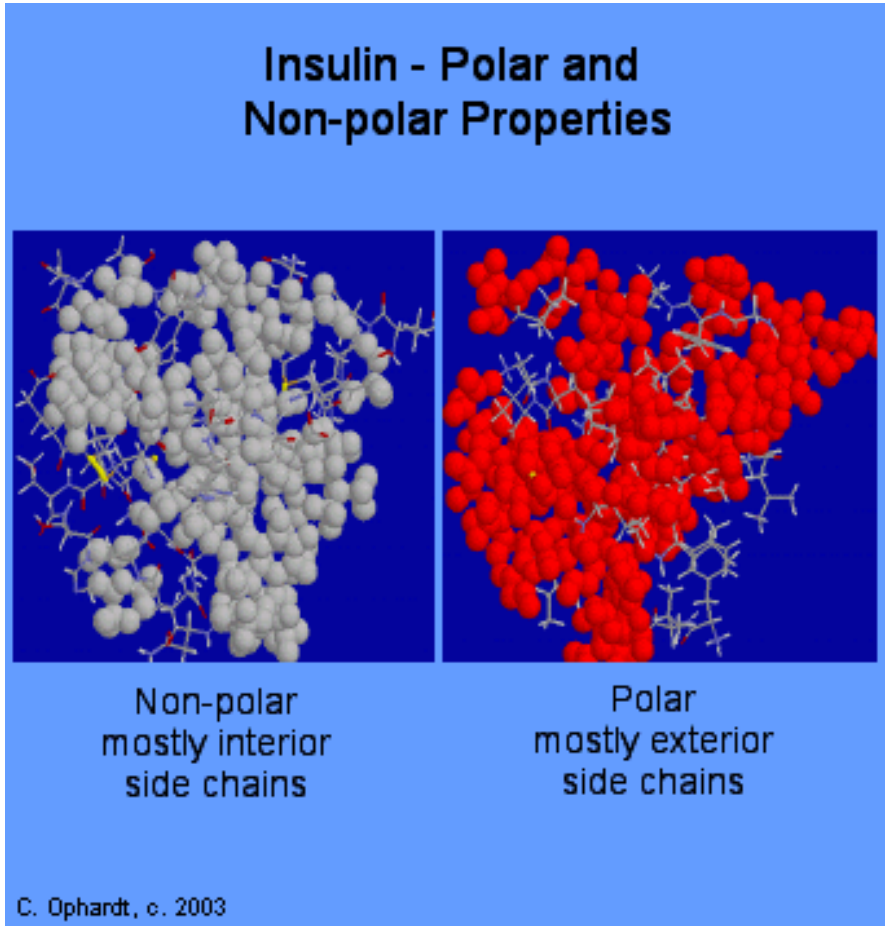
hydrogen bond networks in liquid water

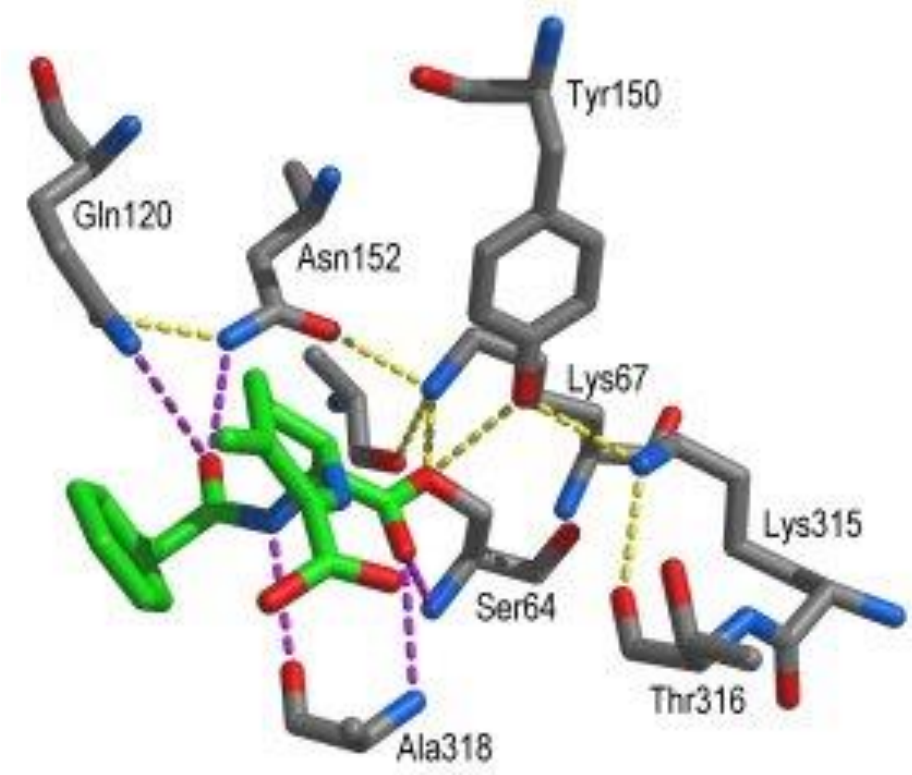
Liu, K., Cruzan, J.D. and Saykelly, R.J. *Science* **271**, 930 (1996)



ordering of water around a nonpolar solute

O interior de proteínas apresentam um núcleo com resíduos de aminoácidos hidrofóbicos

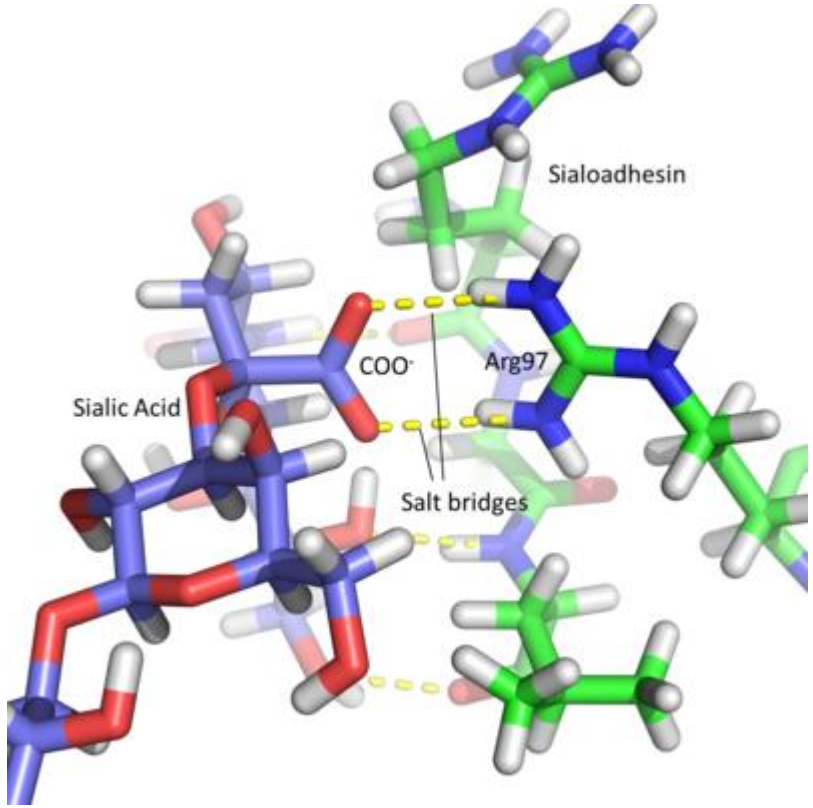




Os resíduos polares no interior da proteína devem ter parceiros para formação de interações de hidrogênio ou interações iônicas

Em contraste, ligações de hidrogênio entre grupos de uma proteína são importantes para a formação de estruturas secundárias, e consequentemente para o processo de enovelamento

Interações iônicas também são maximizadas no interior de proteínas



Os padrões estruturias, portanto seguem duas regras:

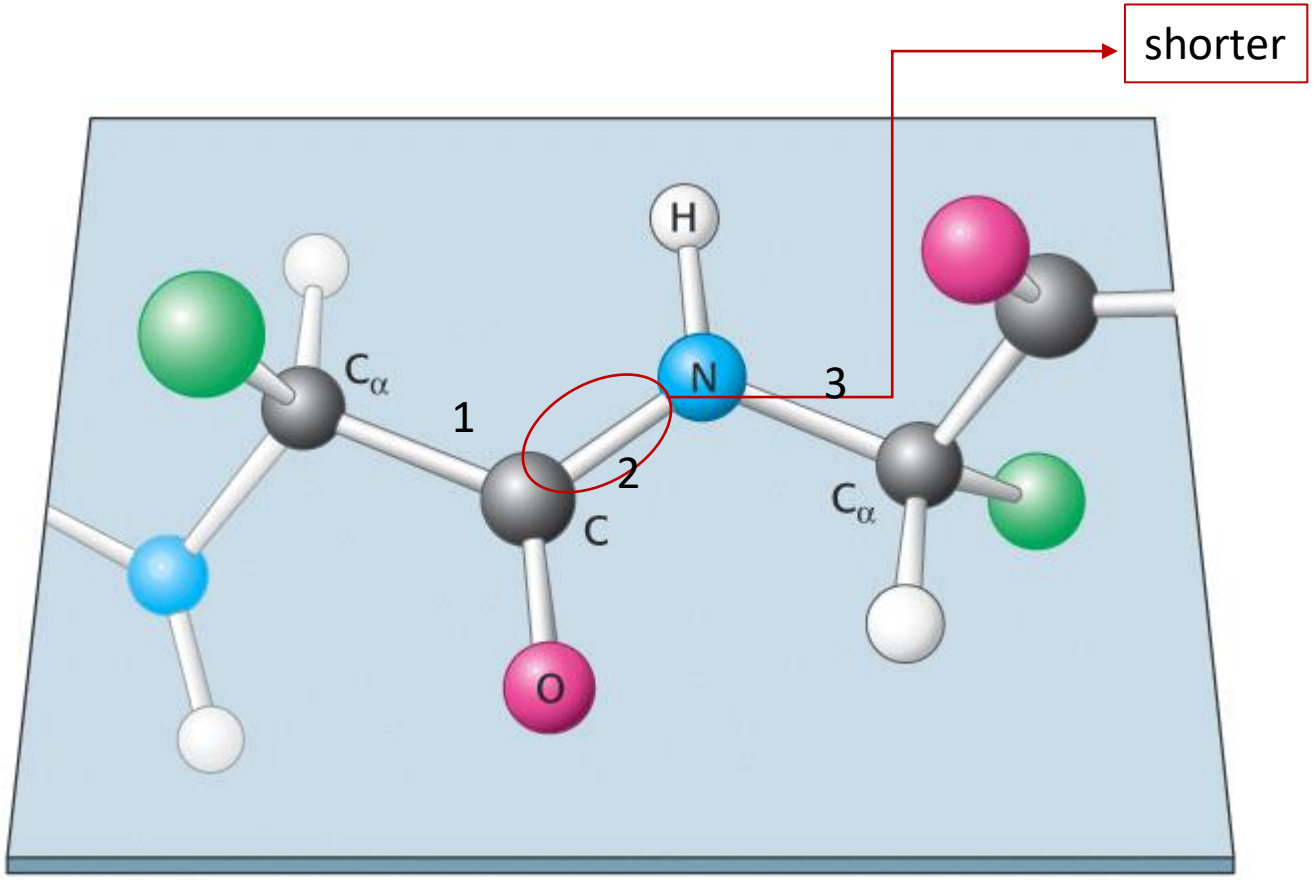
- **Resíduos hidrofóbicos são enterrados**

- **Ligações de hidrogênio e iônicas são maximizadas**

A ligação peptídica

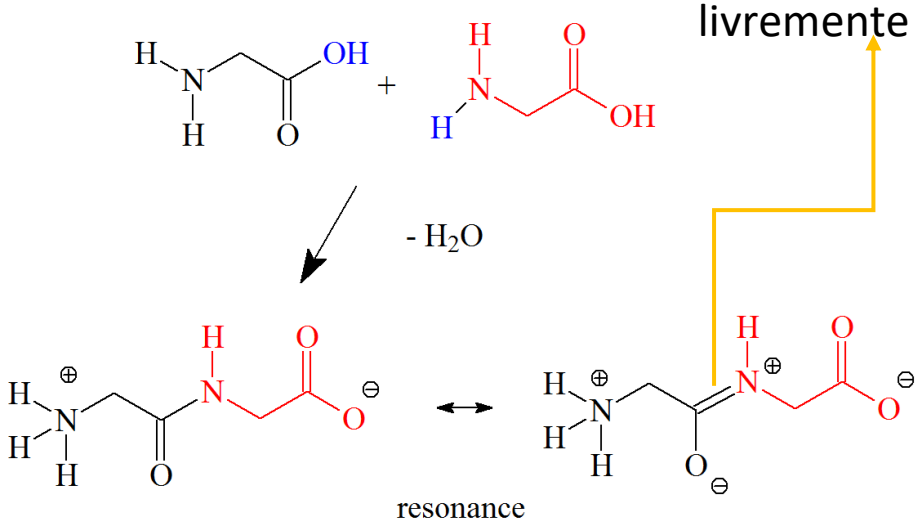
Linus Pauling e Robert Corey nos anos 1930s

Cristal de aminoácidos e peptídeos



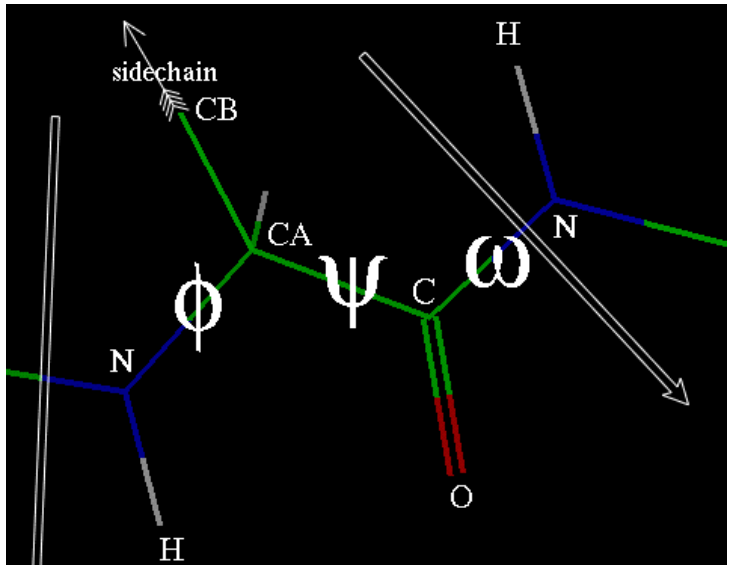
Os átomos da ligação peptídica são coplanares, o que significa que eles estão no mesmo plano de geometria

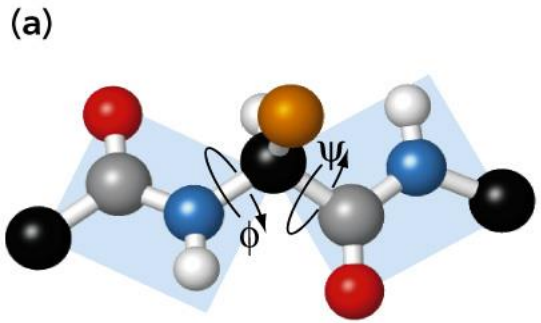
Esta não pode rotacionar livremente

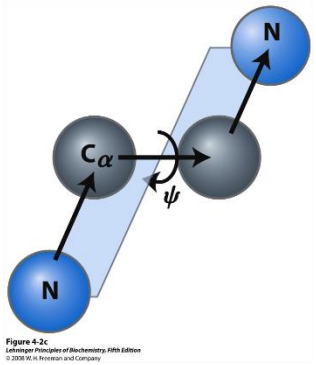


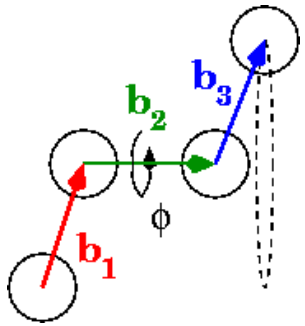
Ângulos torcionais e restrições espaciais

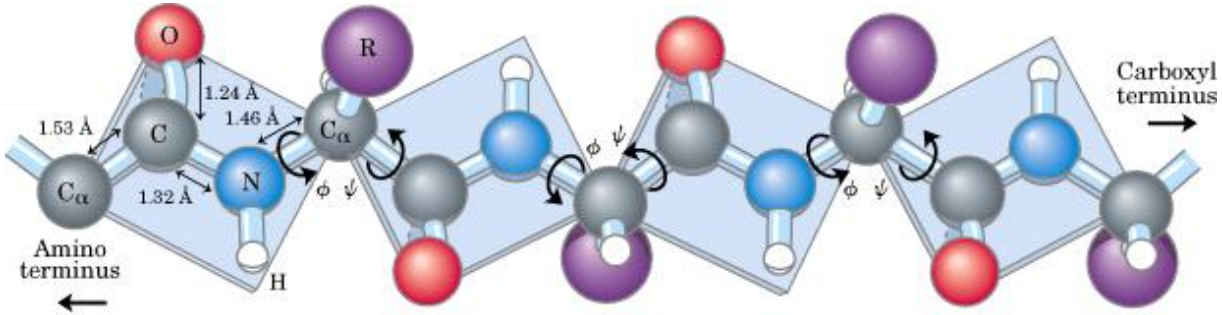
Conformações peptídicas são definida por três ângulos diédricos, que são ângulos formados poer nterceção de planos

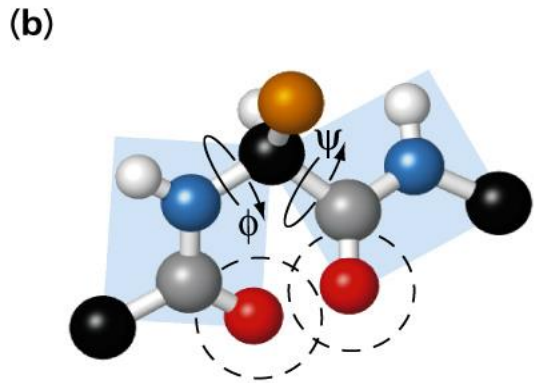






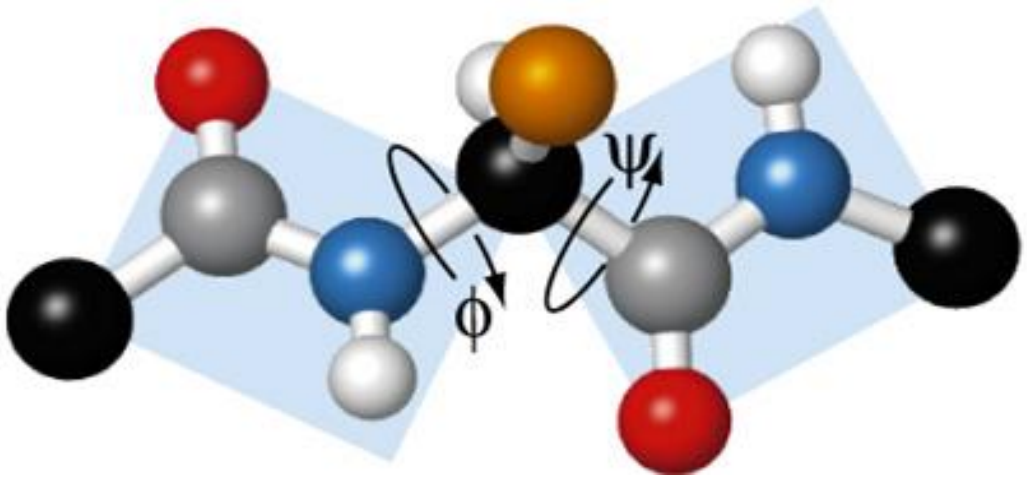




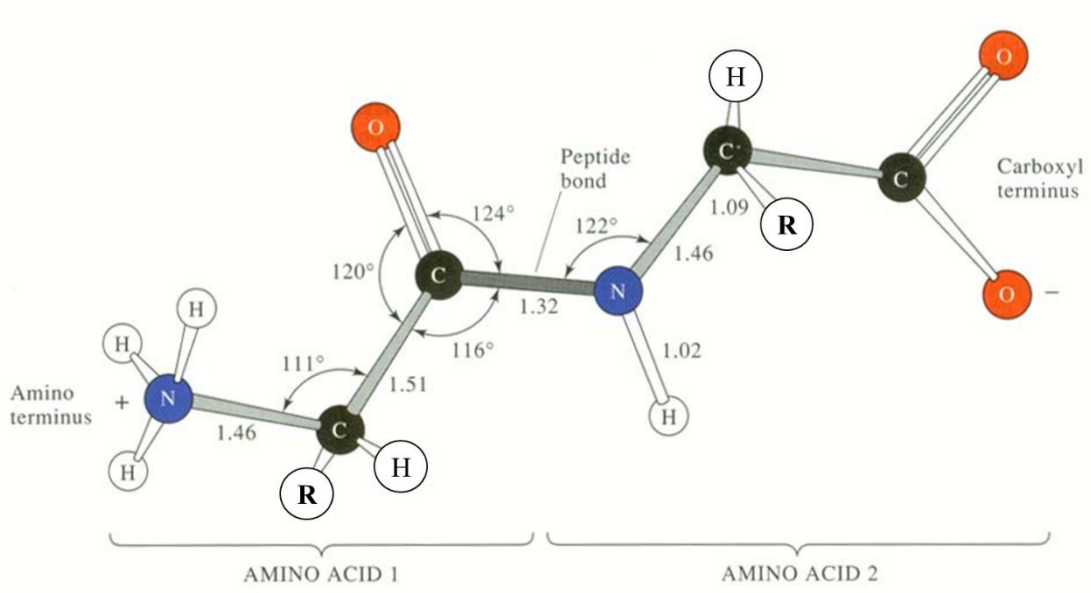


α-carbon
Carbonyl carbon
Hydrogen
Nitrogen
Oxygen
Side chain

Os ângulos entre dois planos descreve a conformação da proteína



Os valores de phi and psi podem ser qualquer um entre -180º to 180º ?



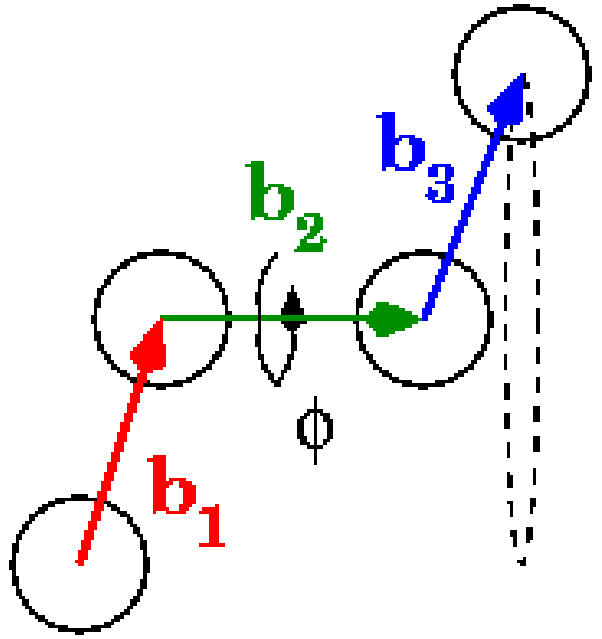
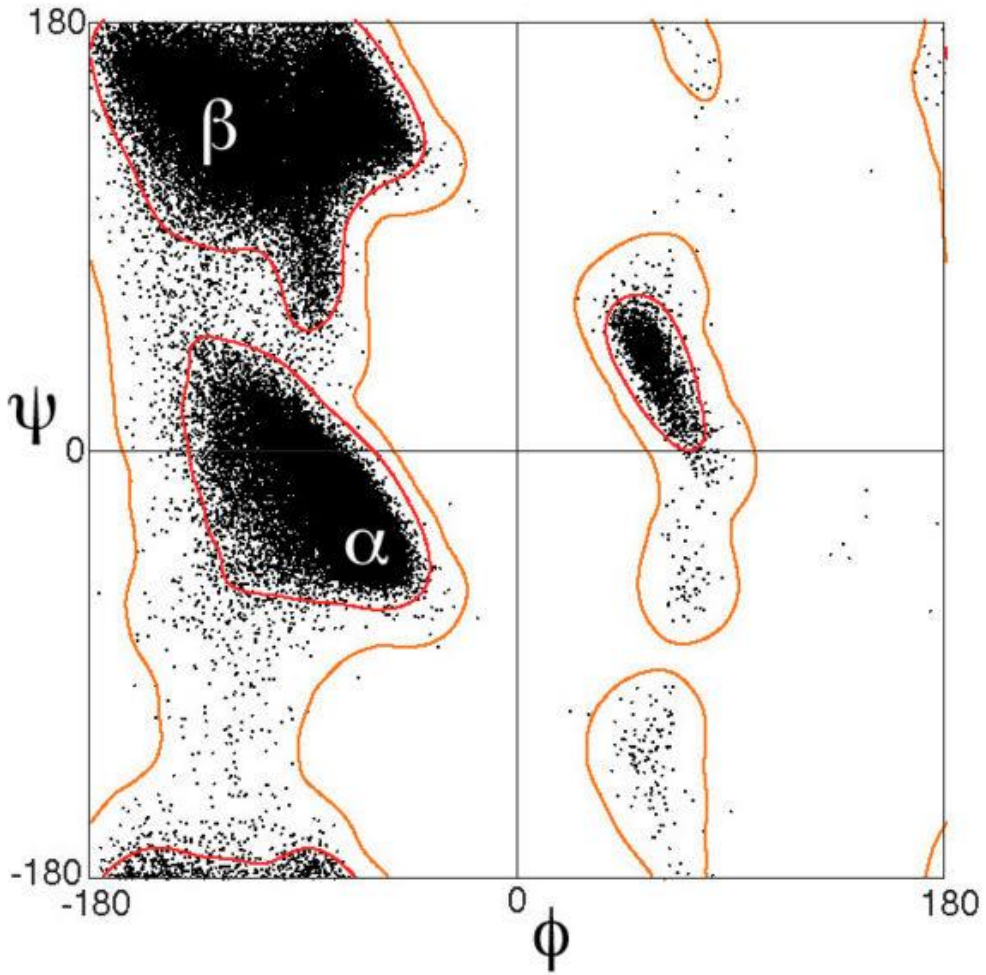
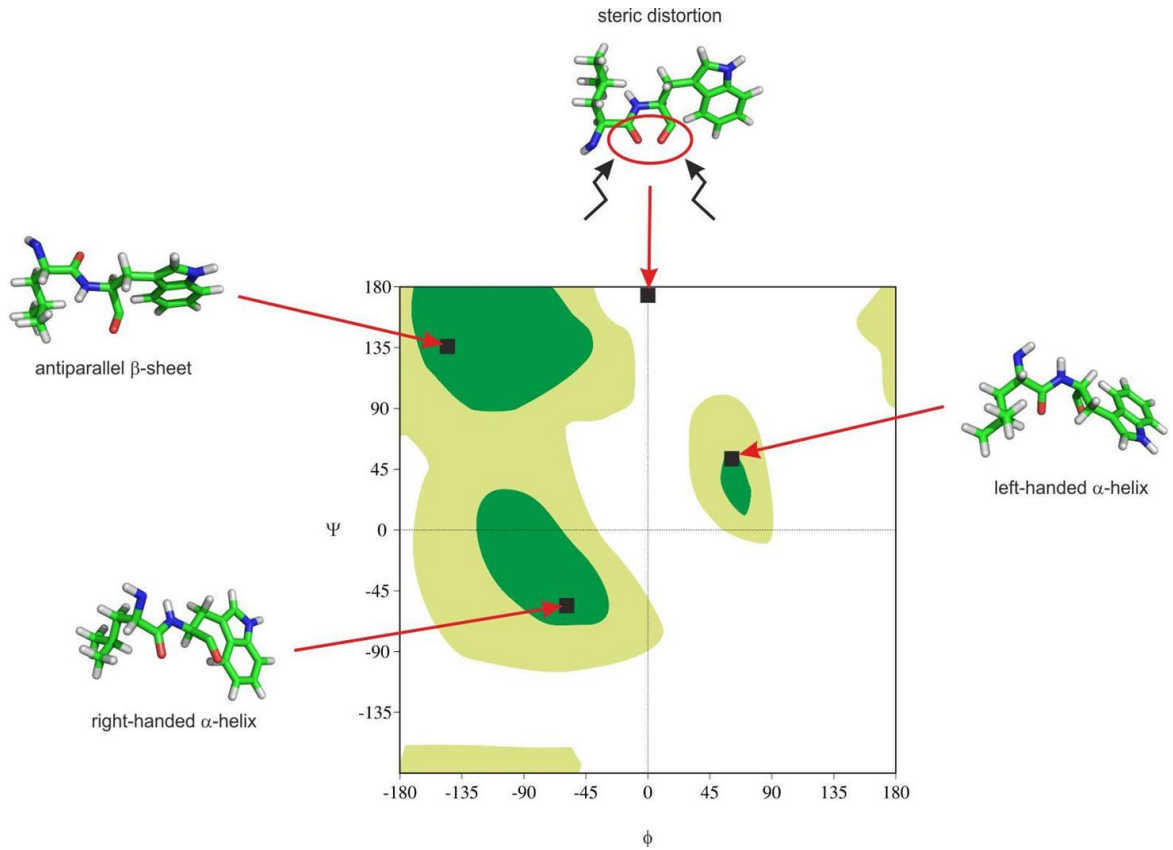


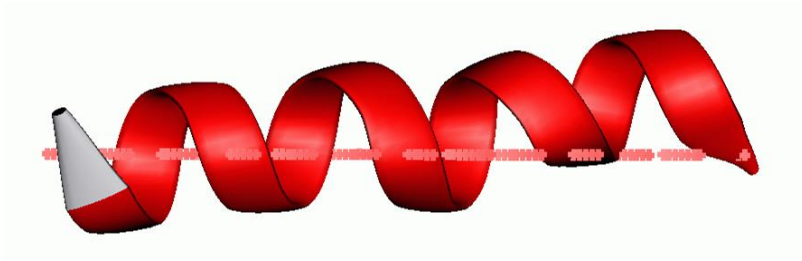
Diagrama de Ramachandran

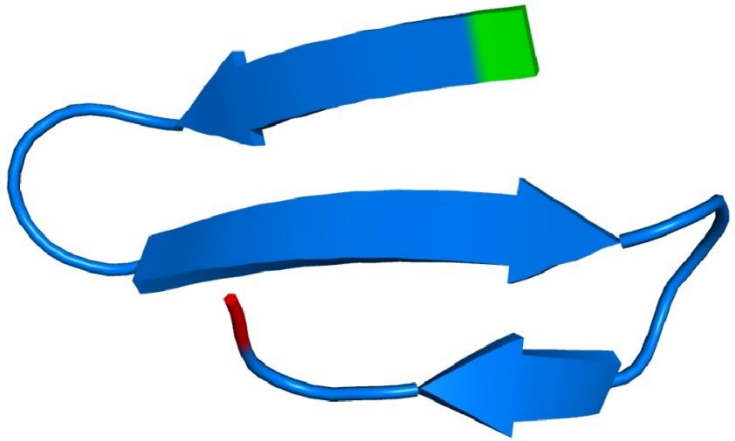


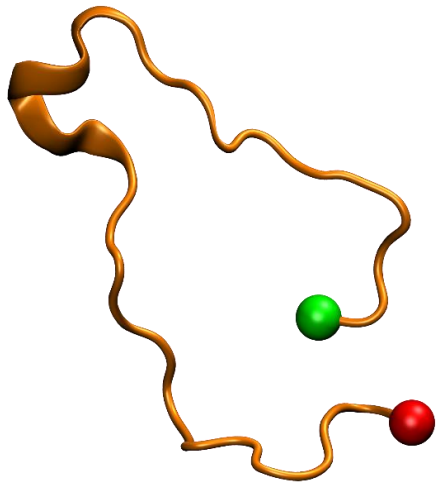


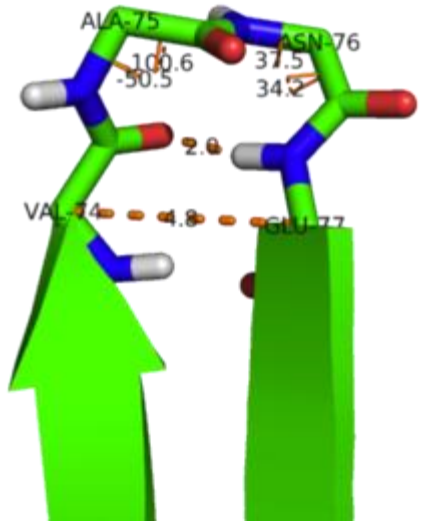
Protein Secondary Structure

É um seguimento da cadeia polipeptídica que descreve o arranjamento espacial dos átomos da cadeia principal. Geralmente uma estrutura secundária regular ocorre entre os ângulos diedros (phi e psi) que são restritos no mesmo seguimento



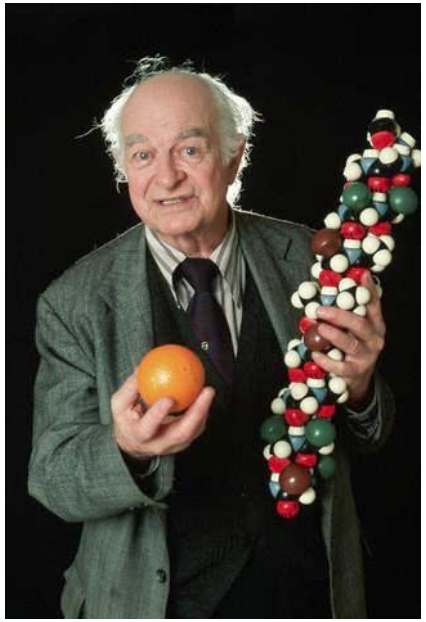






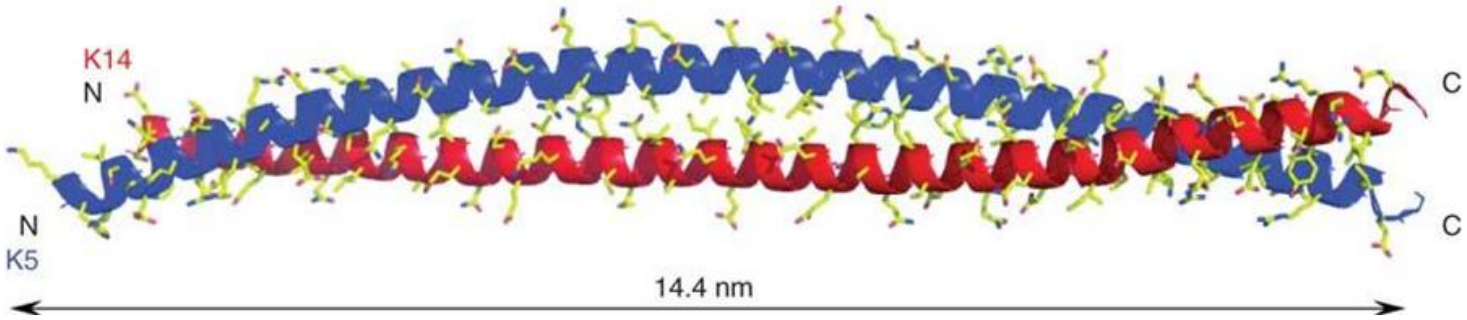
Helix α

William Astbury e Linus Pauling



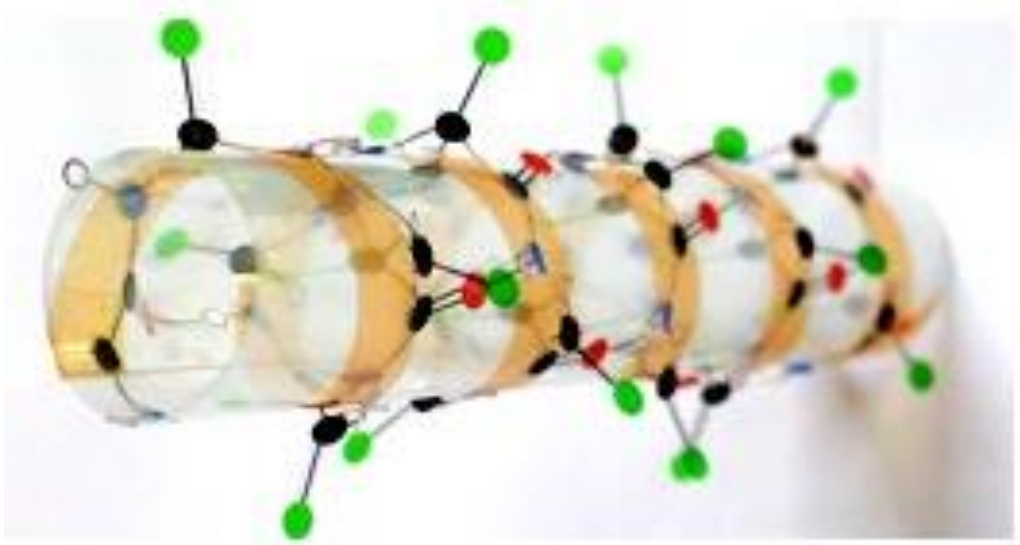
X-ray Crystallography of α-keratin

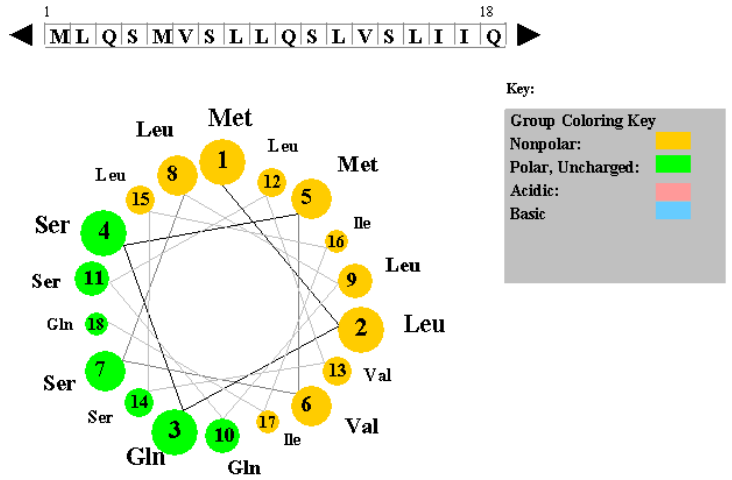
A-keratin has a regular structure that repets every 5.15 to 5.2Å



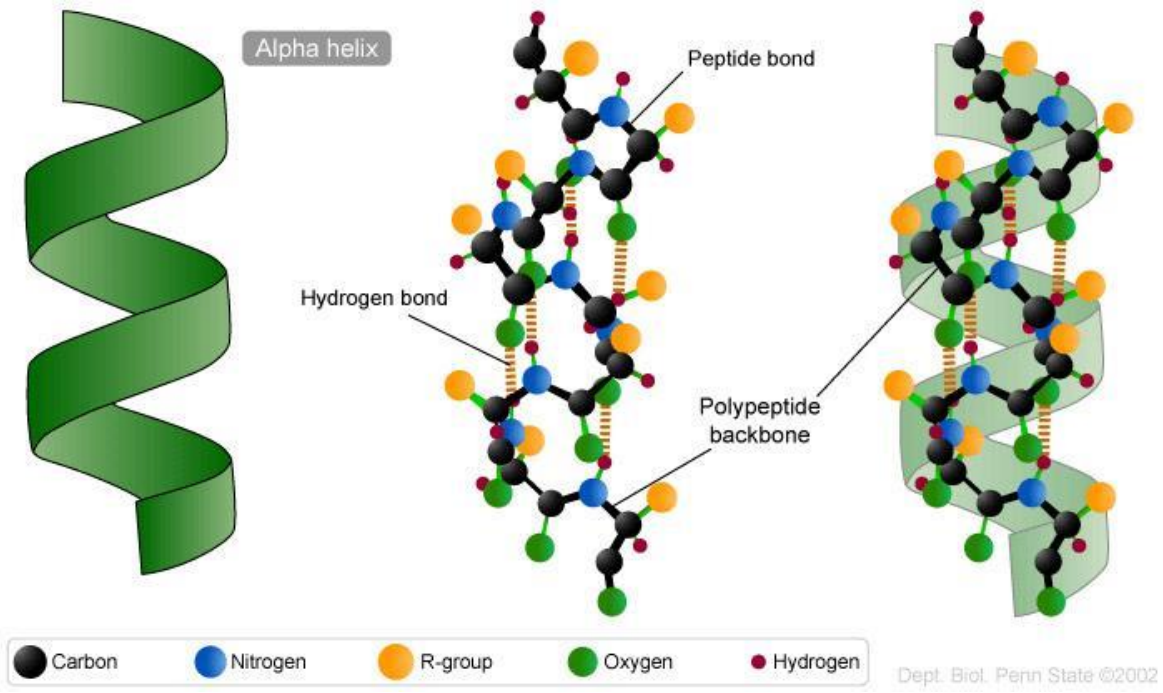
Helix α

About 25% of all residues in protein are found in alpha helix



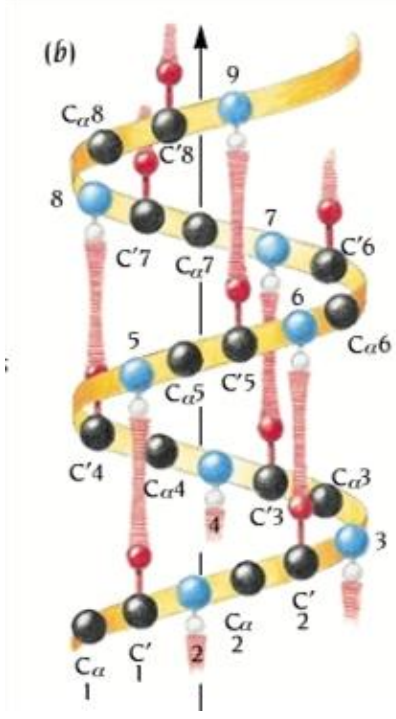


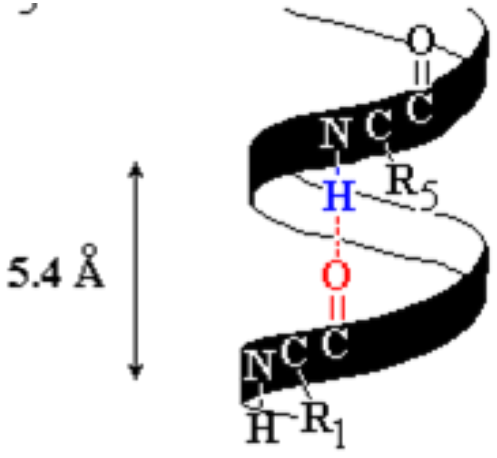
Polypeptide is wound around a imaginary axis drawn longitudinally through the middle of the helix and the R groups protrude outward

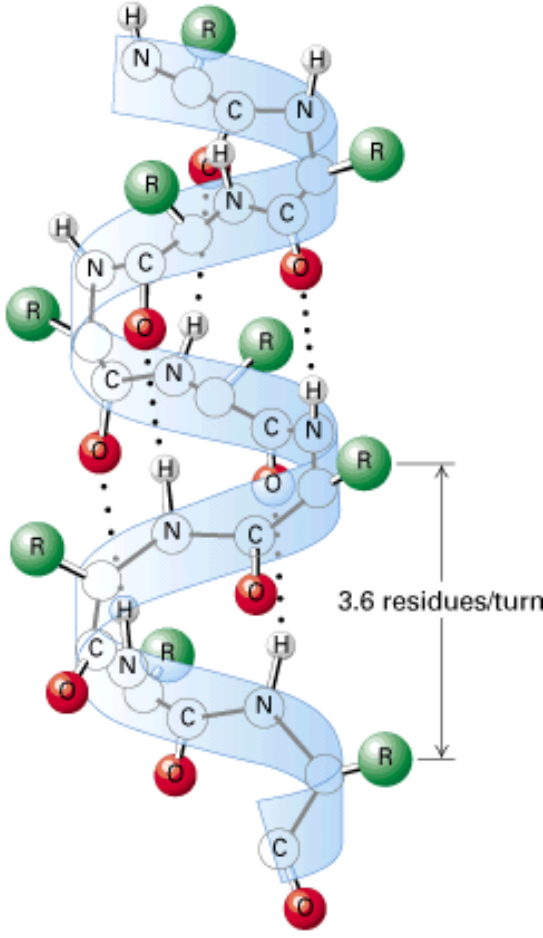


Alpha helix makes optimal use of internal hydrogen bond

Every peptide bond participates with 3 or 4 hydrogen bonds







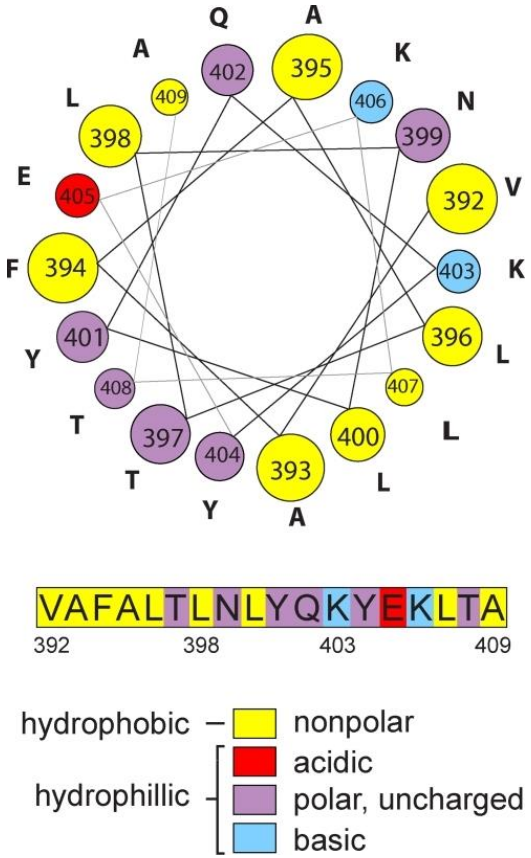
Not all amino acids can participate in alpha helix

Amino Acid	α-Helical Preference
Glutamic acid	1.59
Alanine	1.41
Leucine	1.34
Methionine	1.30
Glutamine	1.27
Lysine	1.23
Arginine	1.21
Phenylalanine	1.16
Isoleucine	1.09
Histidine	1.05
Tryptophan	1.02
Aspartic acid	0.99
Valine	0.90
Threonine	0.76
Asparagine	0.76
Tyrosine	0.74
Cysteine	0.66
Serine	0.57
Glycine	0.43
Proline	0.34

The position of an amino acid residue relative to its neighbors is also importante. Ex blocks of several glutamics will not form alpha helix.

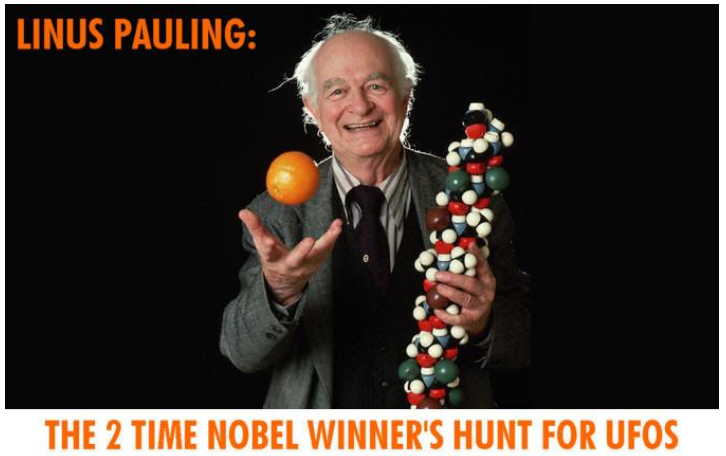
The shape of amino acids also can destabilize a alpha helix, as Asn, Ser, Thr and Cys.

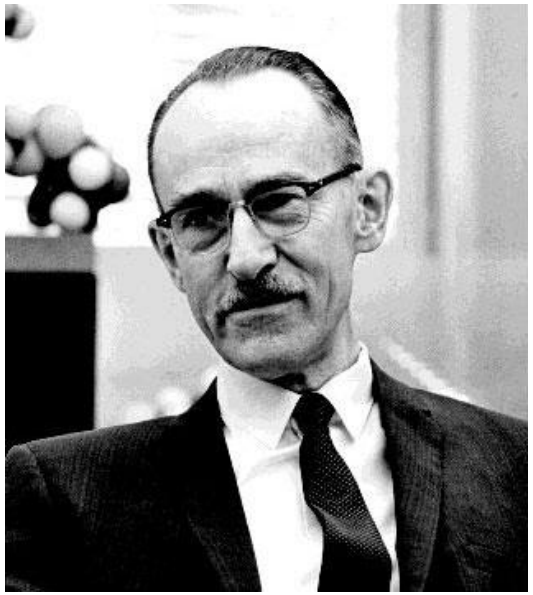
Positively charged residues are found 3 residues away of negative charged



β conformation

Predicted in 1951





Robert Corey

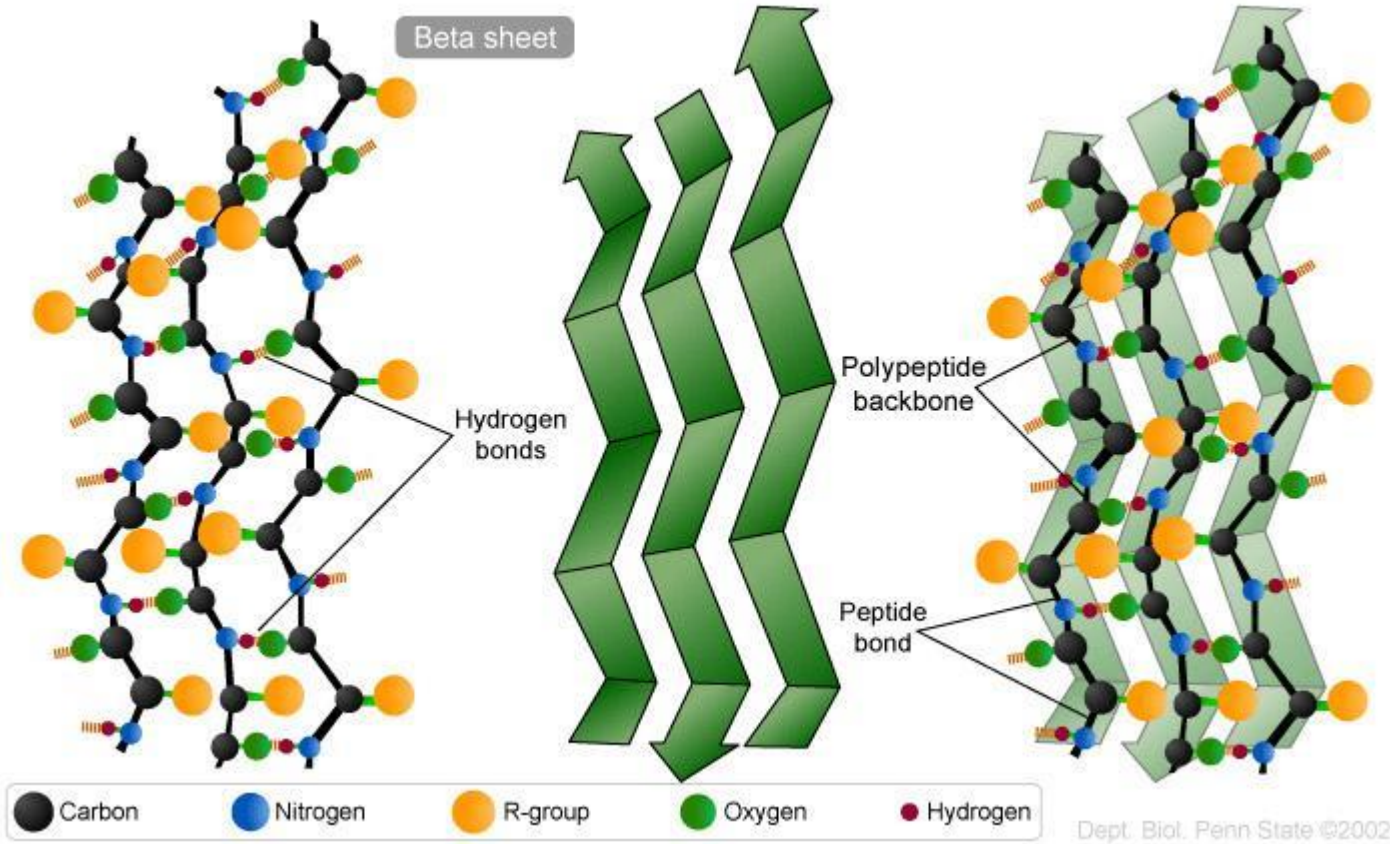
β conformation

The backbone of the polypeptide chain is extended into a zigzag

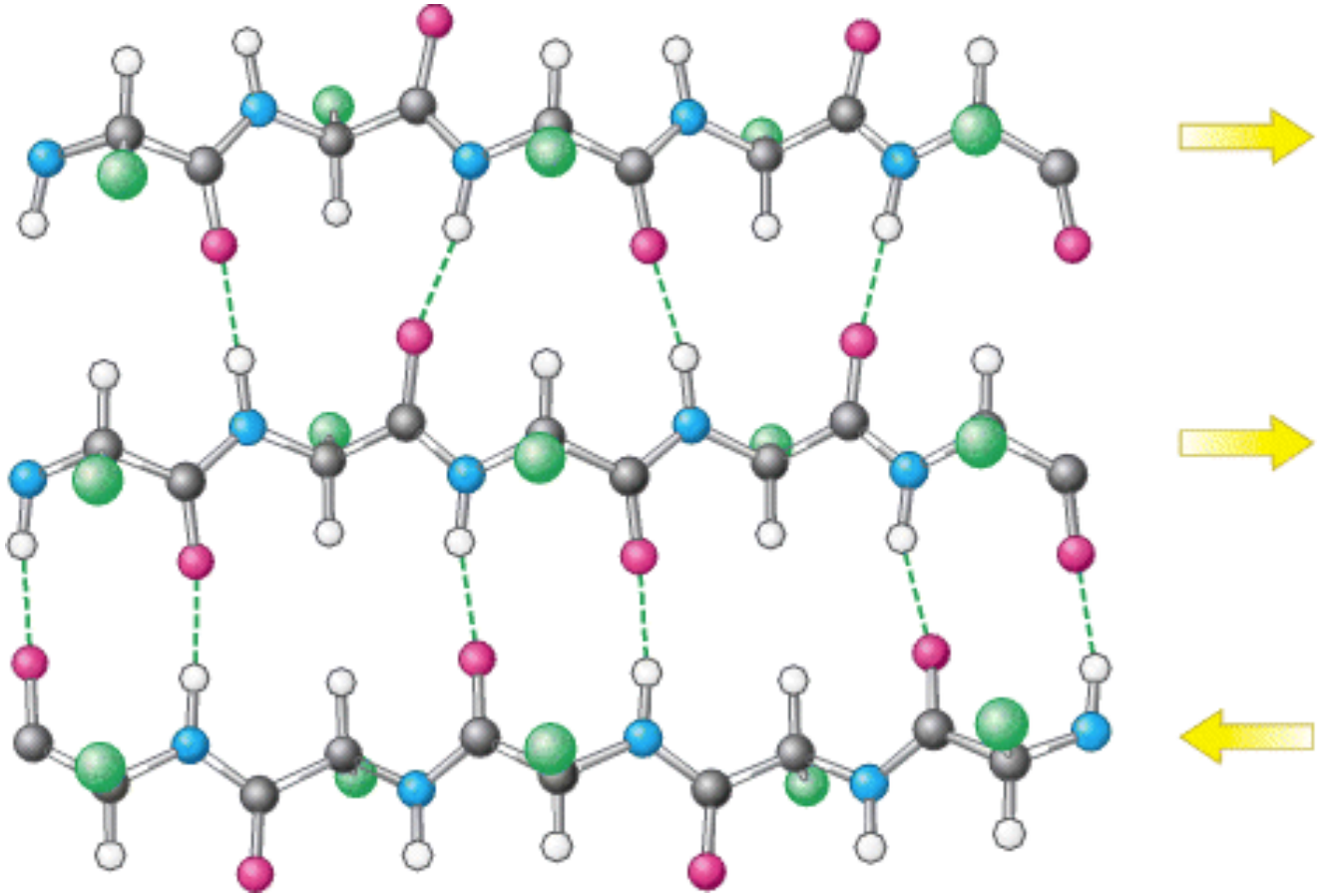
The zigzag polypeptide chains can be arranged side by side to form a structure resemble a series of pleats, the β-sheet

The hydrogen bonds are formed by adjacent segments of polypeptide chain.

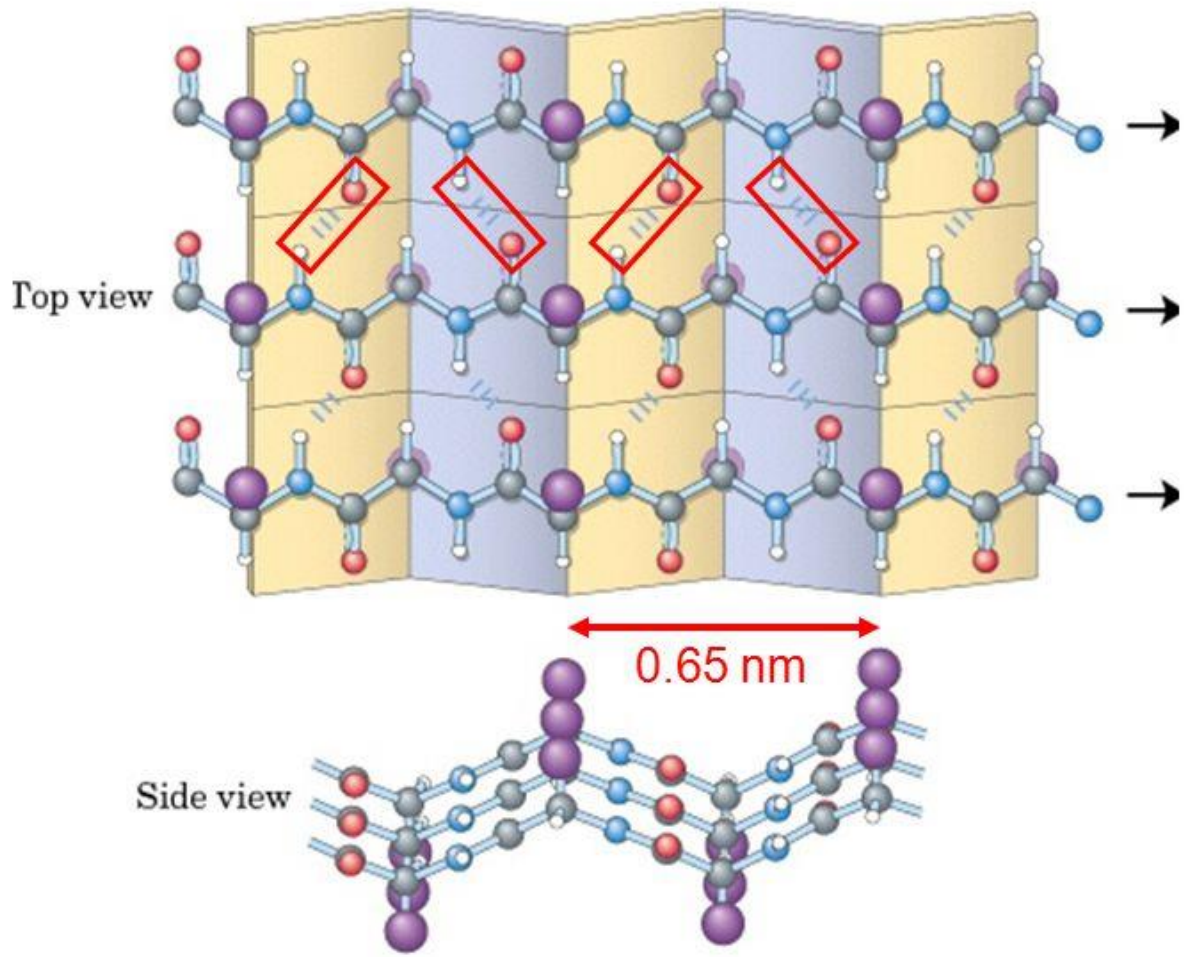
The R groups from adjacent residues protrude in opposite directions



β Conformatio: parallel and antiparallel

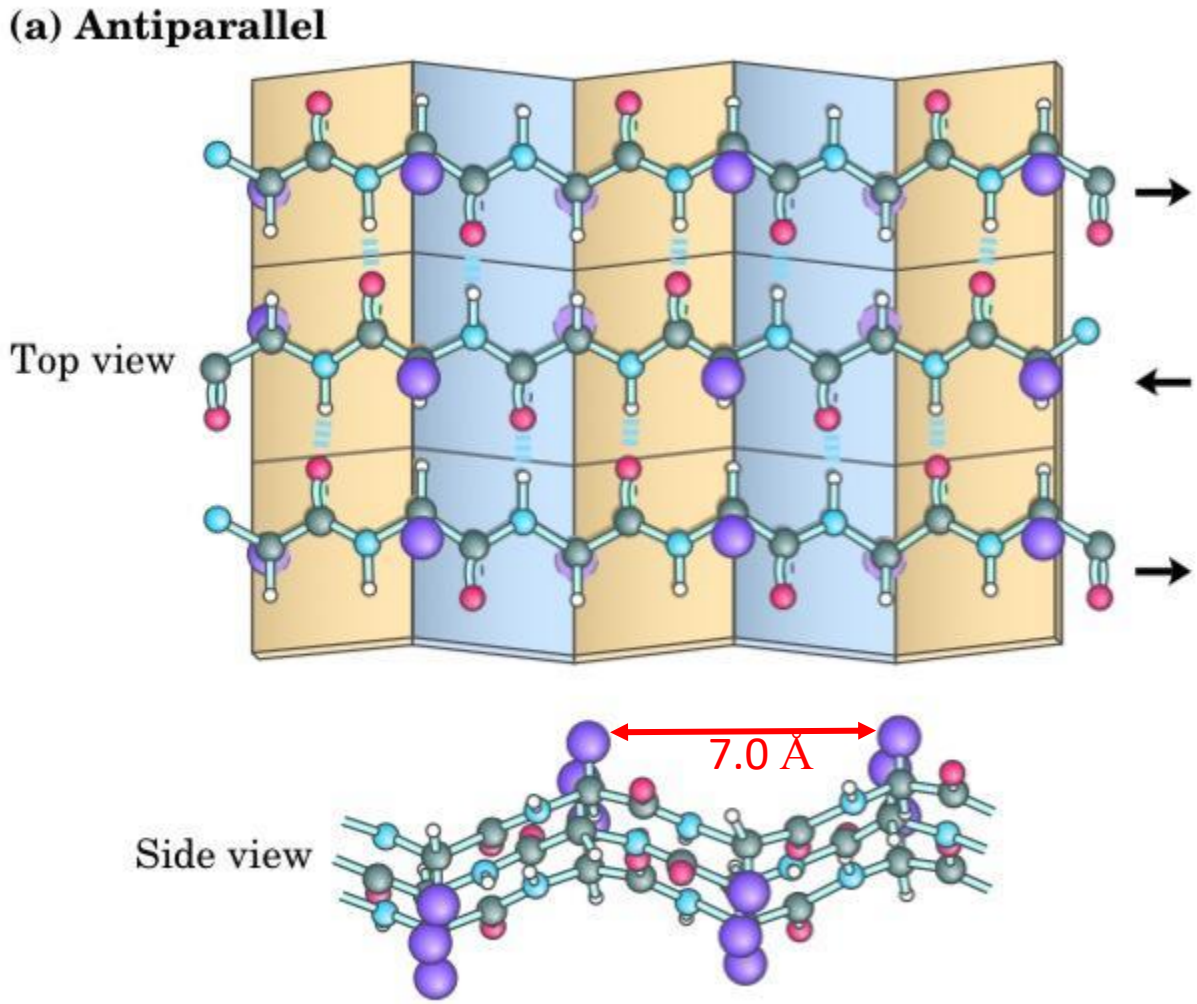


The β-sheet

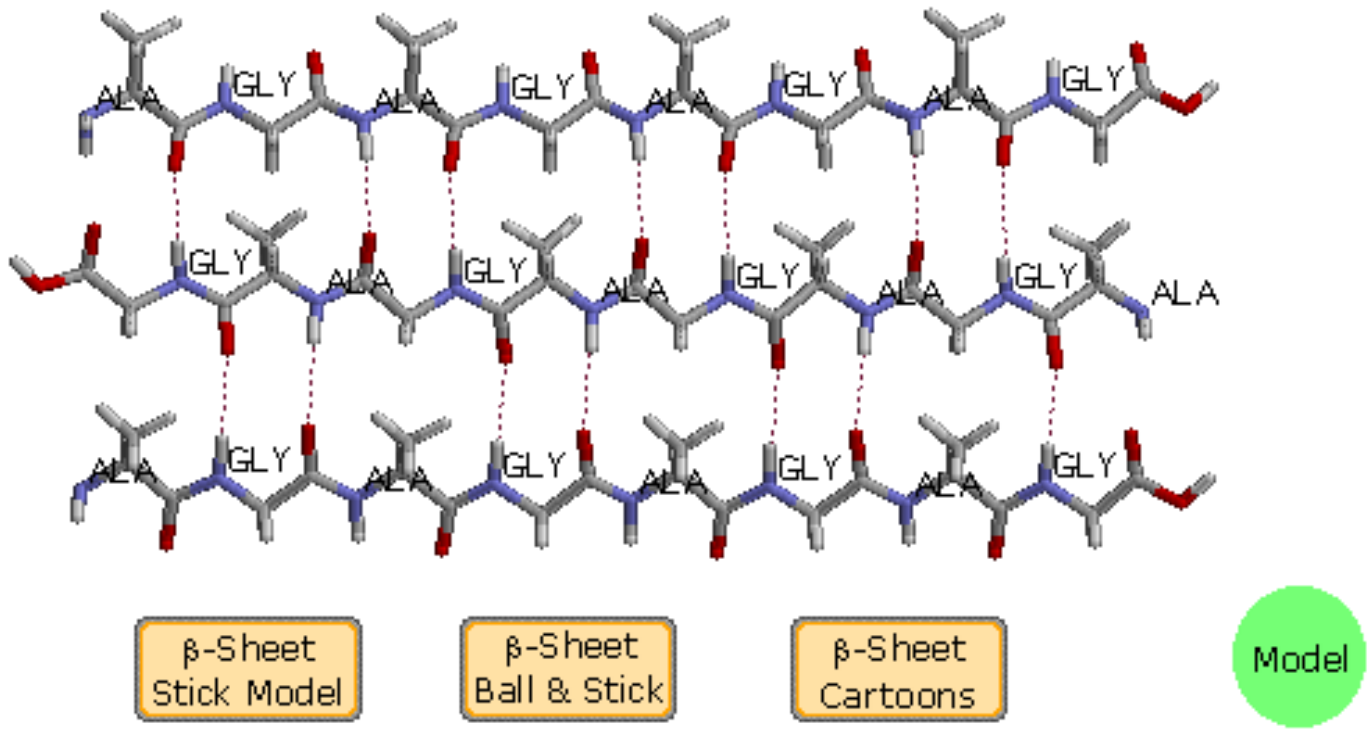


Parallel

Note:
- H-bonds not at optimal angle.
- R-group orientations, distances
- Pleated character
- Avg. Strand length ~ 6 aa.


(a) Antiparallel
Top view
Side view
7.0 Å

When two beta sheets are layered close together the groups R should be small



Structure of fibroin from spiders

Table 2.3 Relative frequencies of amino acid residues in secondary structures

Amino acid	α helix	β sheet	Reverse turn
Glu	1.59	0.52	1.01
Ala	1.41	0.72	0.82
Leu	1.34	1.22	0.57
Met	1.30	1.14	0.52
Gln	1.27	0.98	0.84
Lys	1.23	0.69	1.07
Arg	1.21	0.84	0.90
His	1.05	0.80	0.81
Val	0.90	1.87	0.41
Ile	1.09	1.67	0.47
Tyr	0.74	1.45	0.76
Cys	0.66	1.40	0.54
Trp	1.02	1.35	0.65
Phe	1.16	1.33	0.59
Thr	0.76	1.17	0.96
Gly	0.43	0.58	1.77
Asn	0.76	0.48	1.34
Pro	0.34	0.31	1.32
Ser	0.57	0.96	1.22
Asp	0.99	0.39	1.24

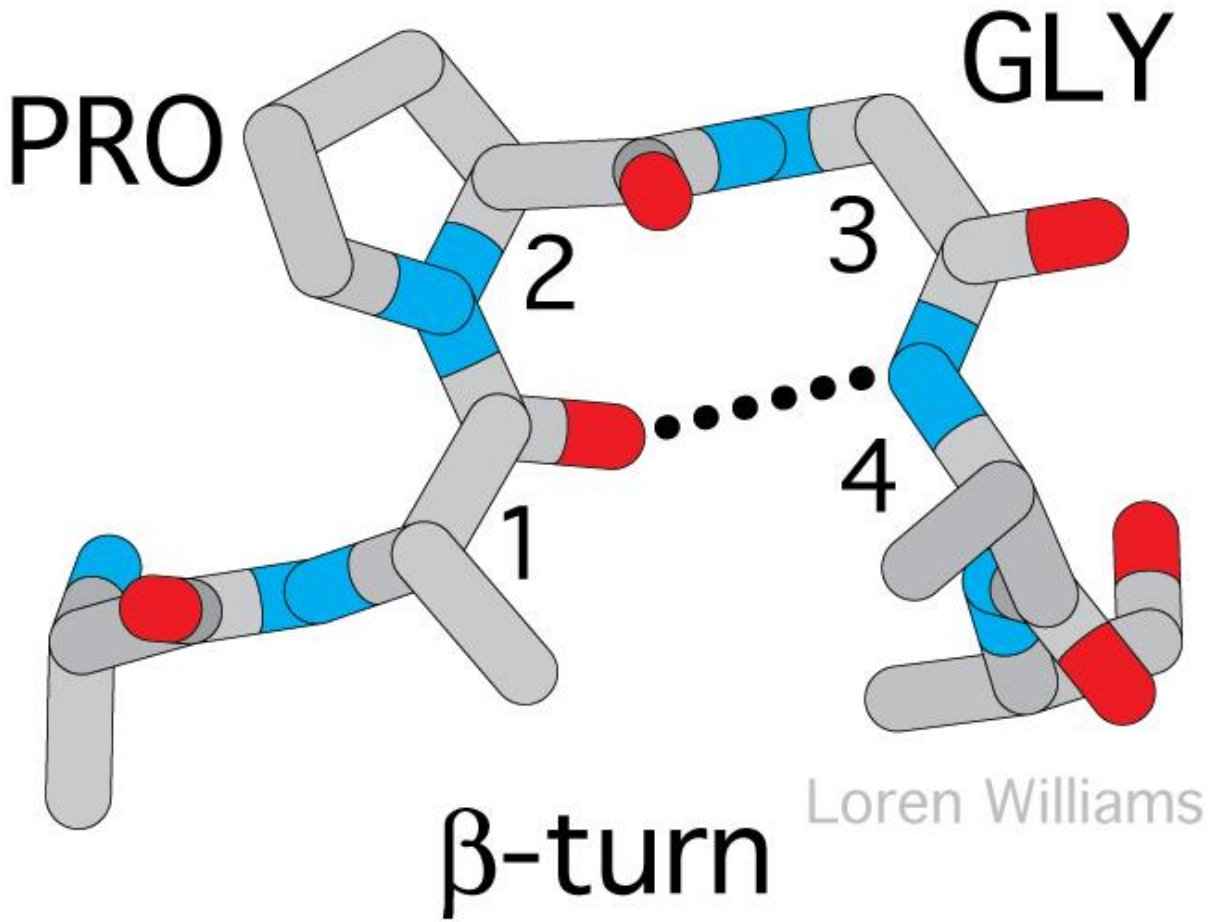
Note: The amino acids are grouped according to their preference for α helices (top group), β sheets (middle group), or turns (bottom group).
Source: T. E. Creighton, *Proteins: Structures and Molecular Properties*, 2d ed. (W. H. Freeman and Company, 1992), p. 256.

Table 2.3
***Biochemistry*, Seventh Edition**

beta- turn

One-third of amino acid residues are in turns and loops, where the polypeptide chain change the direction.

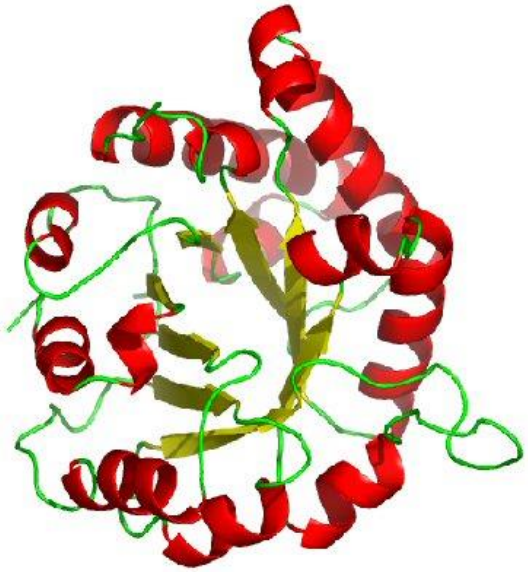
- Beta turns connect two adjacent segments of beta sheet
- Involves 4 amino acids residues
- There are the formation of oly one hydrogen interaction between 1 and 1+3
- High frequence of Pro and Gly
- Often find in the surface of protein and the two central residues are hydrogen bonding with water

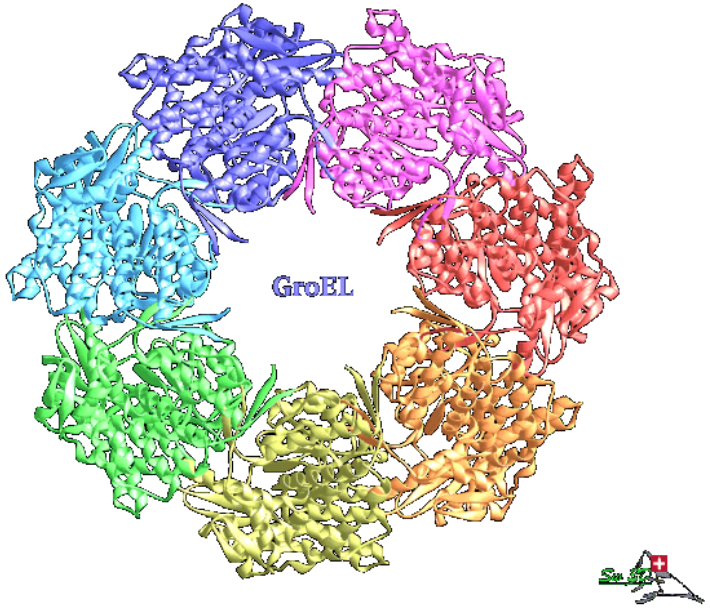


Protein tertiary and quaternary structure

Tertiary structure – overall three-dimensional arrangement of all atoms in a protein

Quaternary structure – arrangement of protein in subunits in three-dimentional complexes

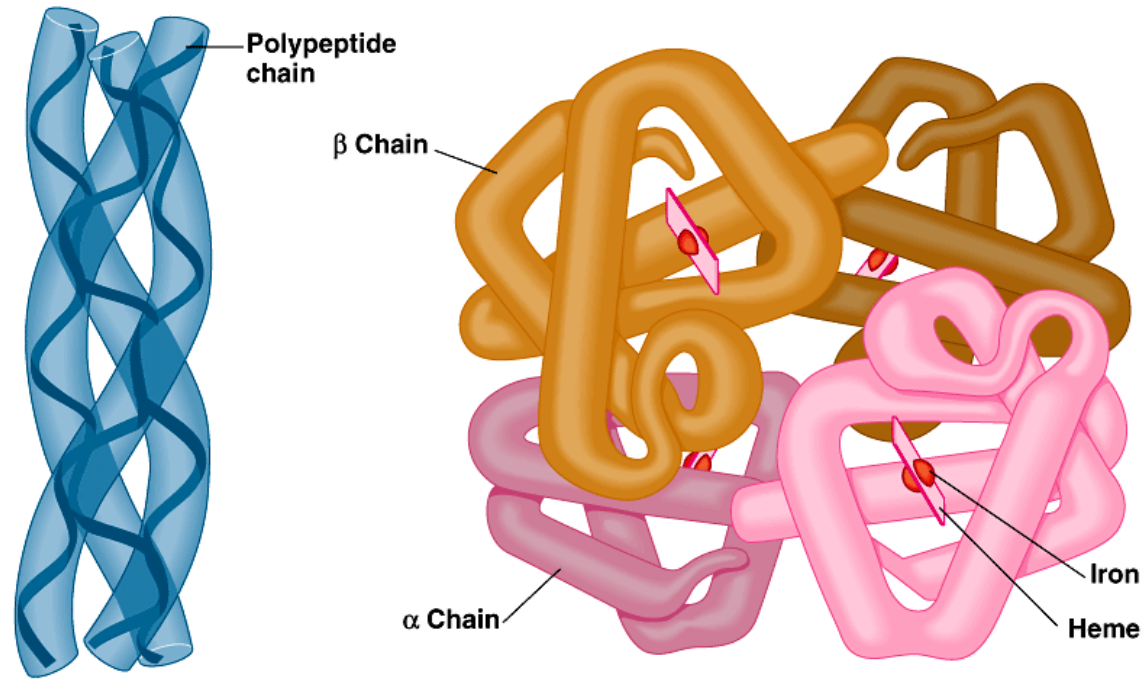




Protein classification

Fibrous protein – long strands or sheets

Globular protein – polypeptide chain folded into a spherical or globular shape



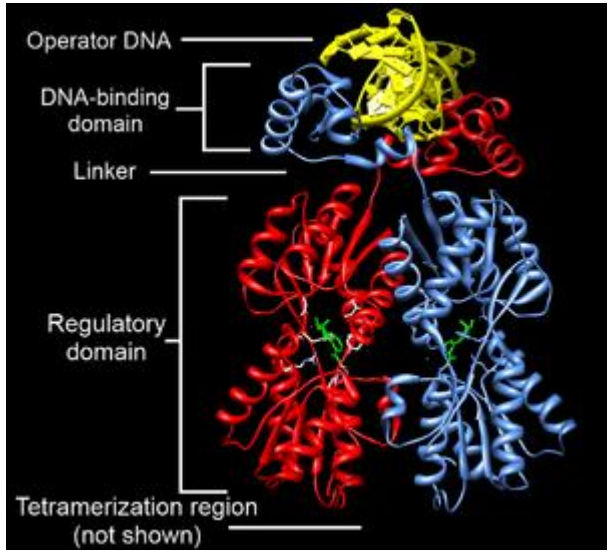
Protein quaternary structure

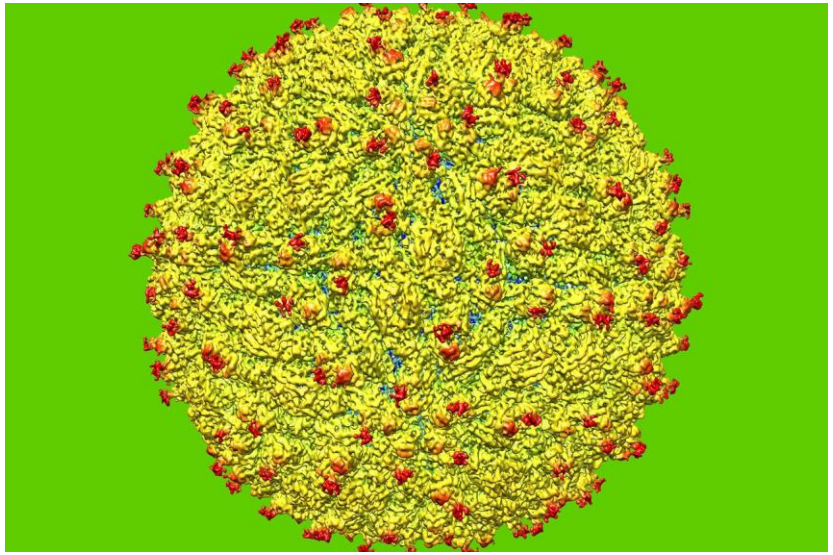
What is role to have multiple polypeptide chains?

- Regulatory roles

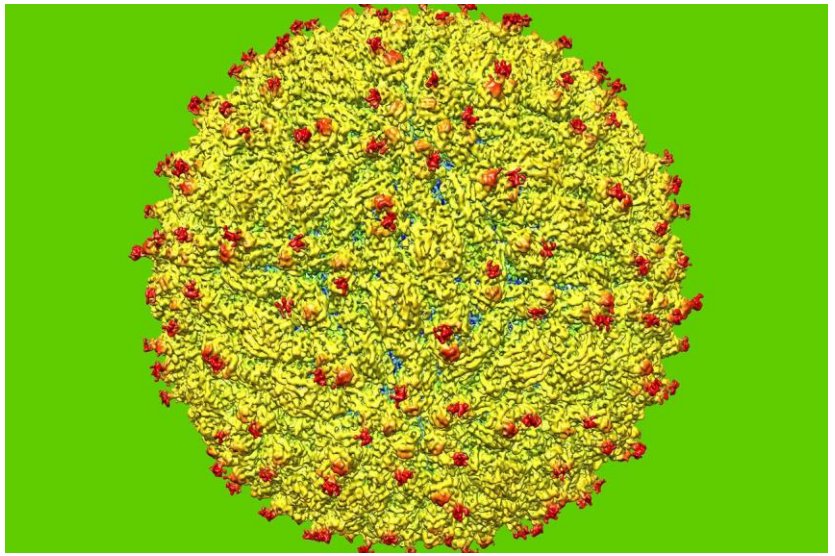
- Separate regulation

- Structural roles

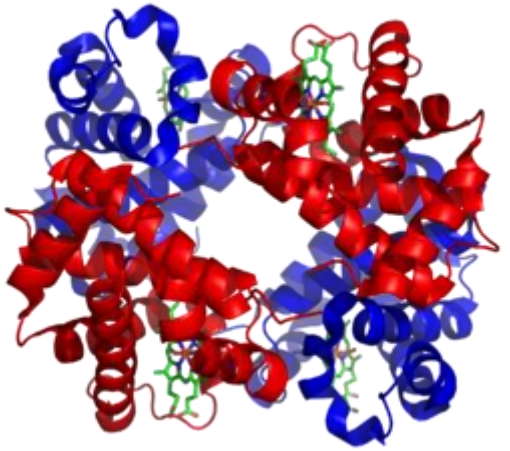




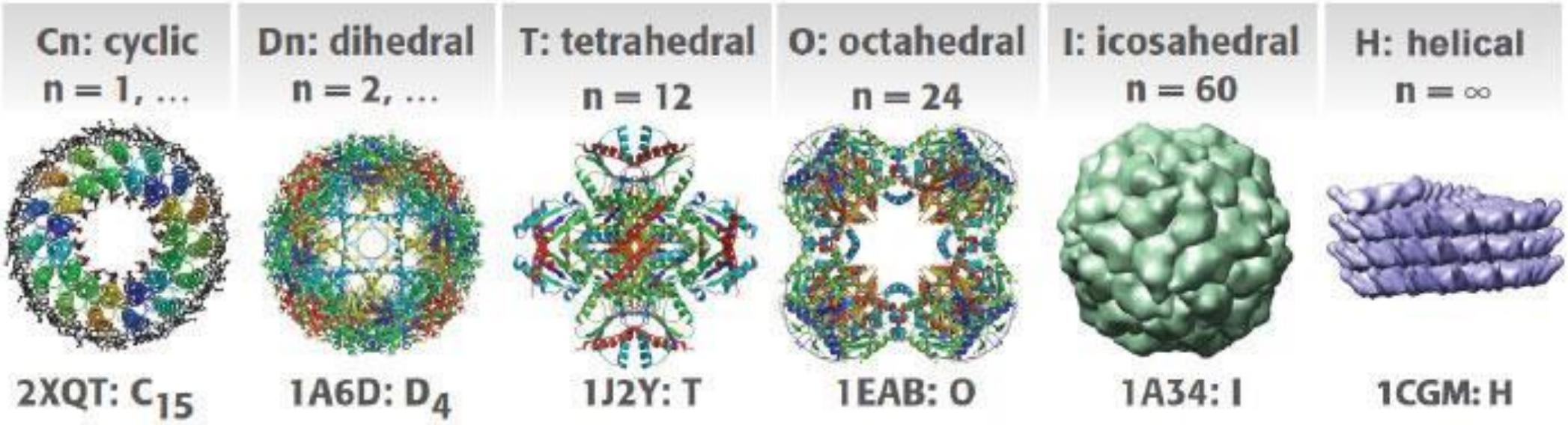
Multimer – protein with multi subunits



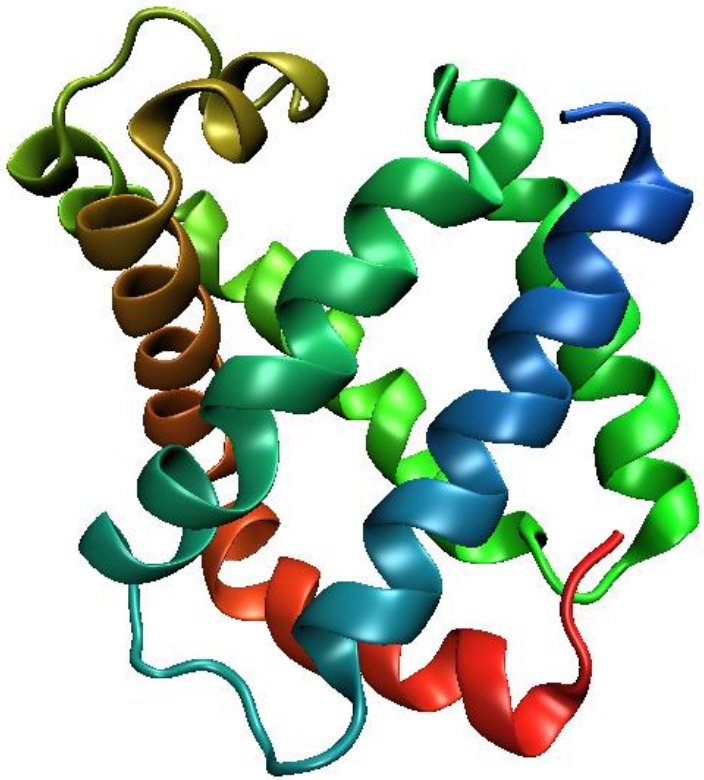
Oligomer – multimer with few subunits

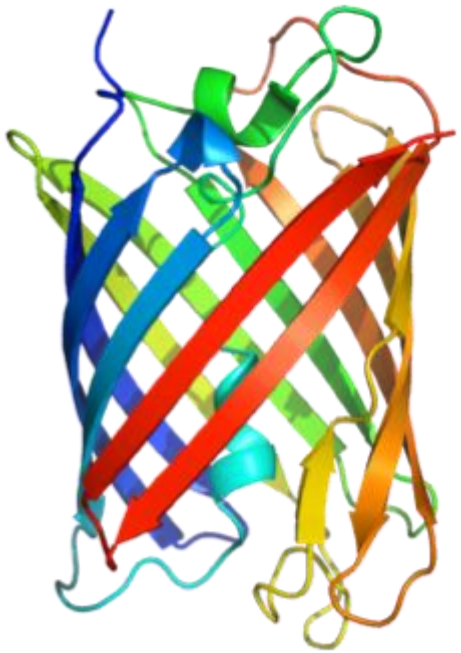


Most of proteins have symmetric arrangements



The structure of a protein can defined as the alpha helices and beta sheet segments stack on one another and how the segments that connect them are arranged





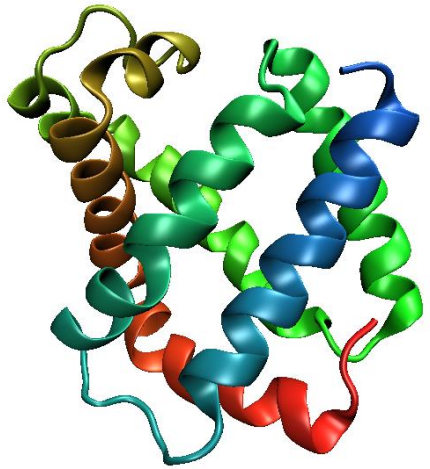
Protein classification based in the structure (SCOP – Structural classification of proteins)

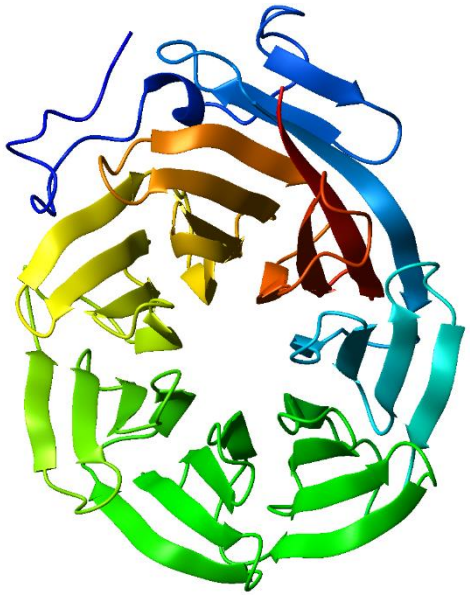
- All alpha proteins

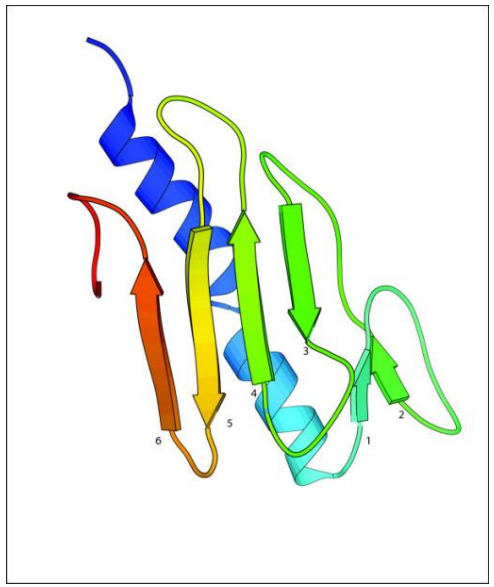
- All beta proteins

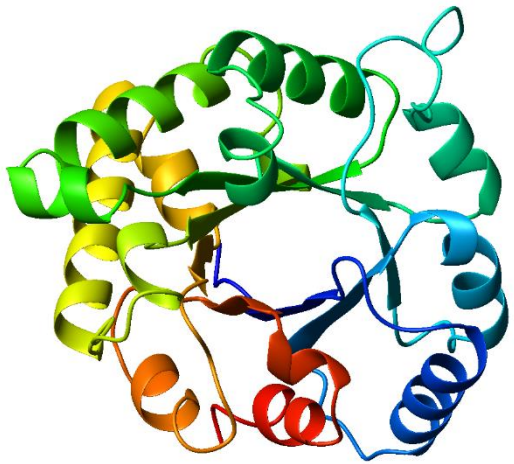
- alpha+ beta proteins

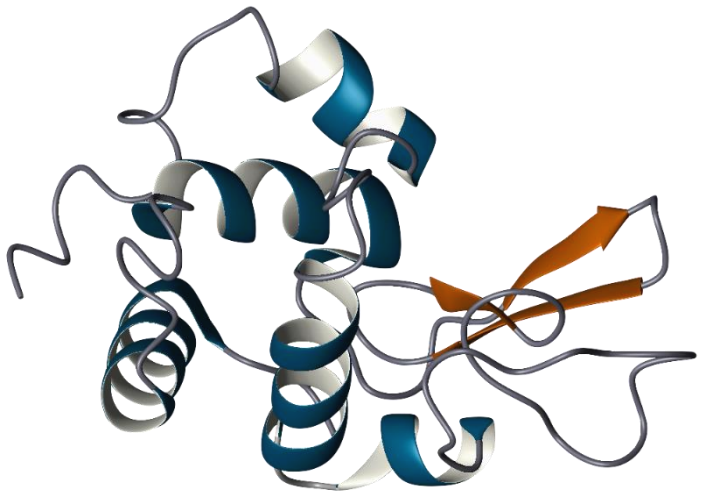
- Alpha/beta proteins









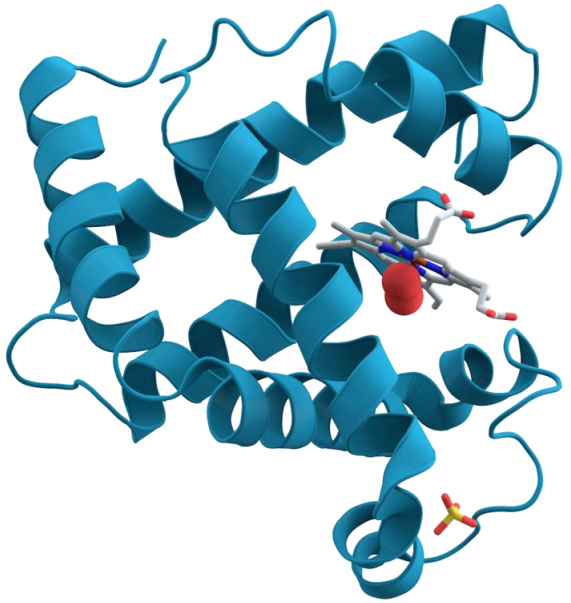


α- helical proteins

About 70 different folds

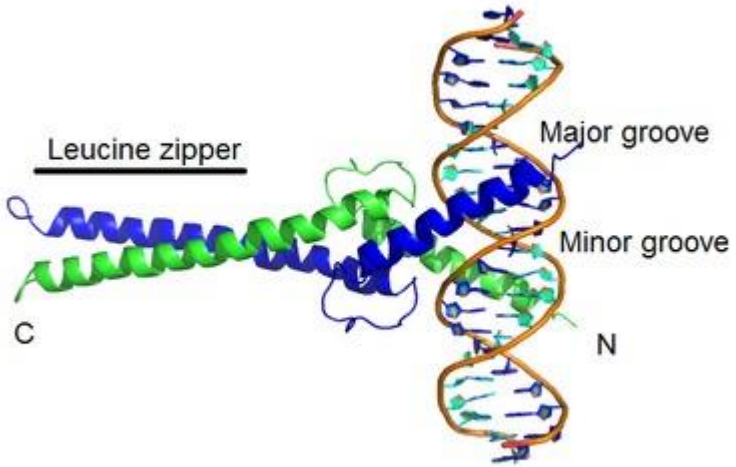
First determined structure was myoglobin

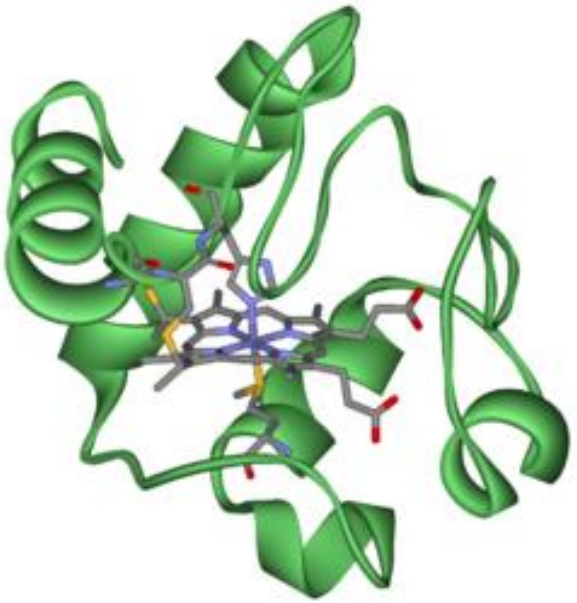
Cytochrome C is another Family of haem proteins



Myoglobin and cytochrome C have different folding topology

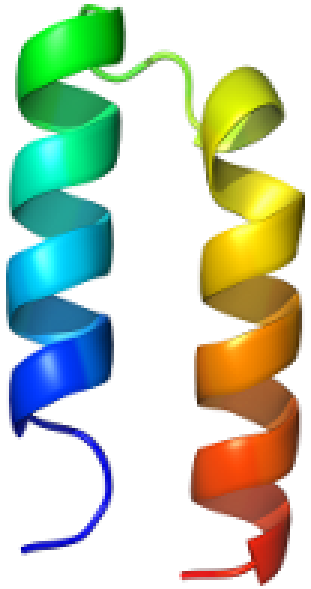
Leucine zipper is another member of α-helical protein



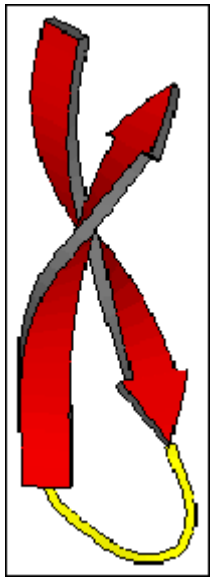


To understand the complete three-dimensional structure we have to analyse its folding patterns

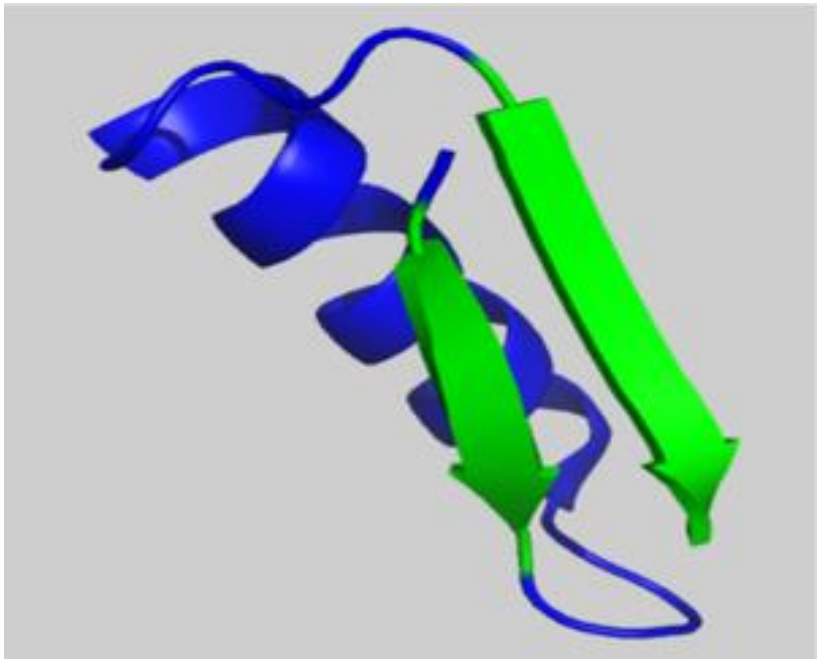
Motif ou supersecondary structure or fold – recognizable folding pattern involving two or more elements of secondary structure and the connections between them: α-helix hairpin, β-hairpin, β-α-β, β-barrel, coiled coil



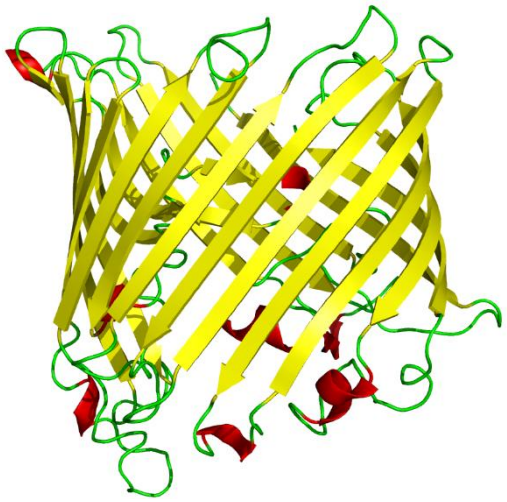
α-helix hairpin



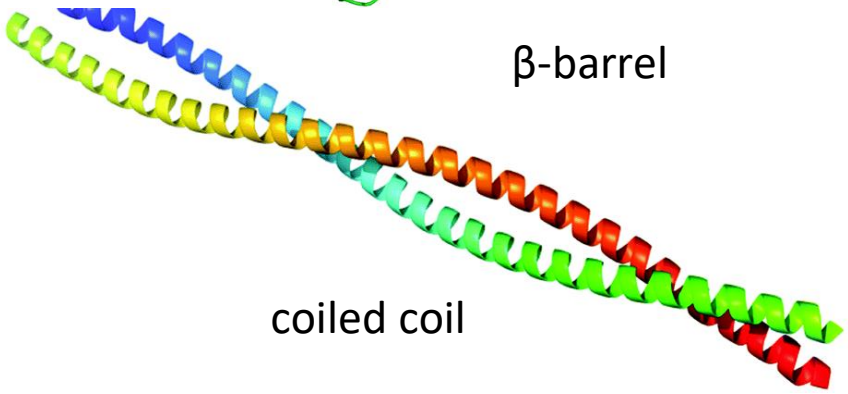
β-hairpin



β-α-β

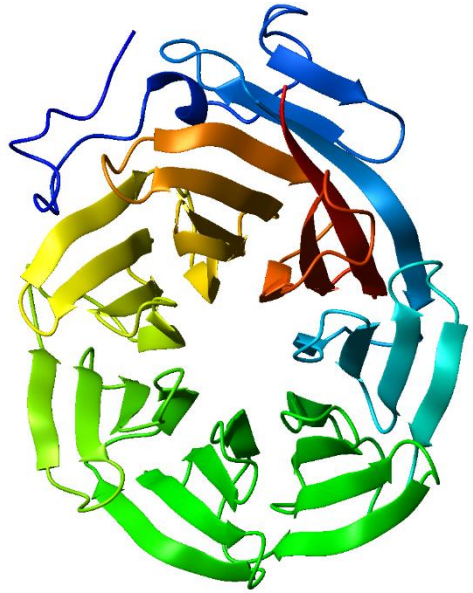


β-barrel

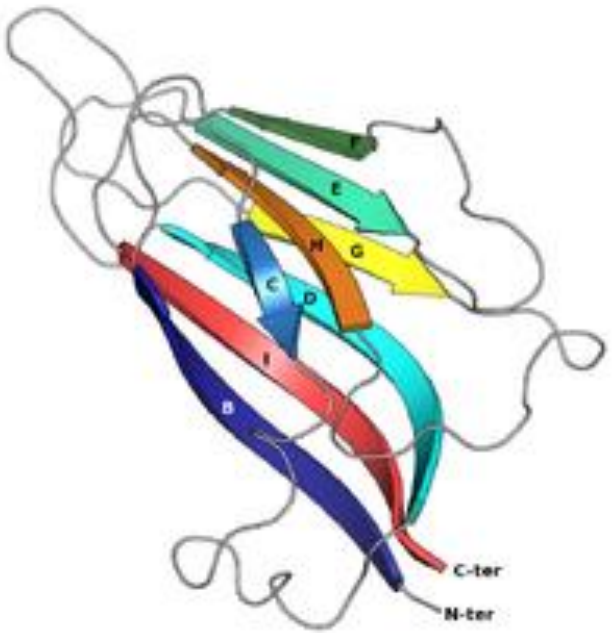


coiled coil

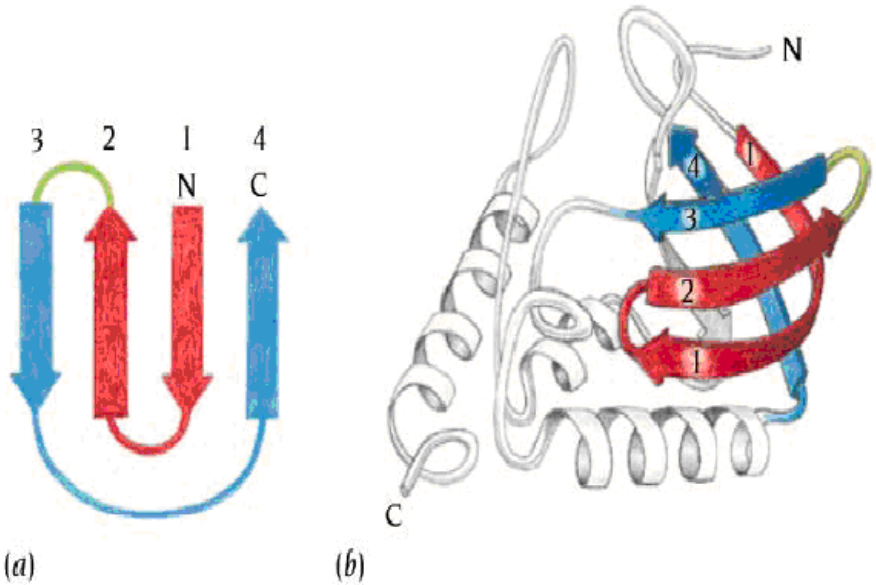
β-propellers



Jellyroll



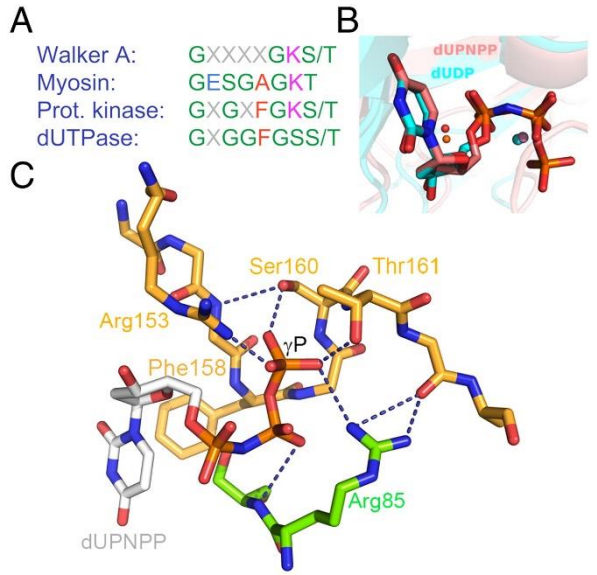
Greek Key

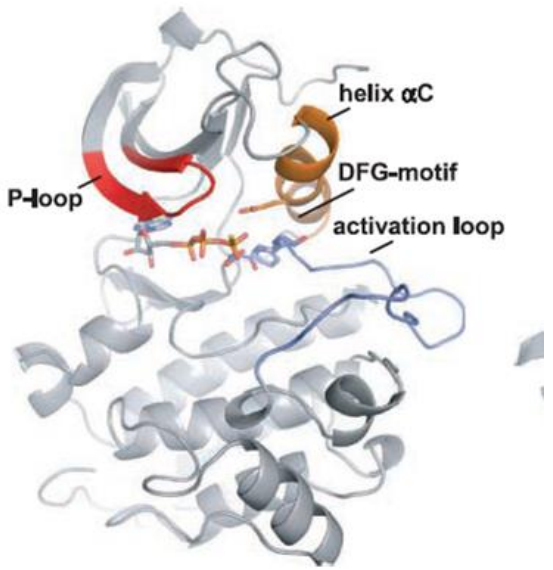


Sequential motifs in proteins: amino acid sequence pattern that is widespread and has a biological significance

ATP Binding site motifs:

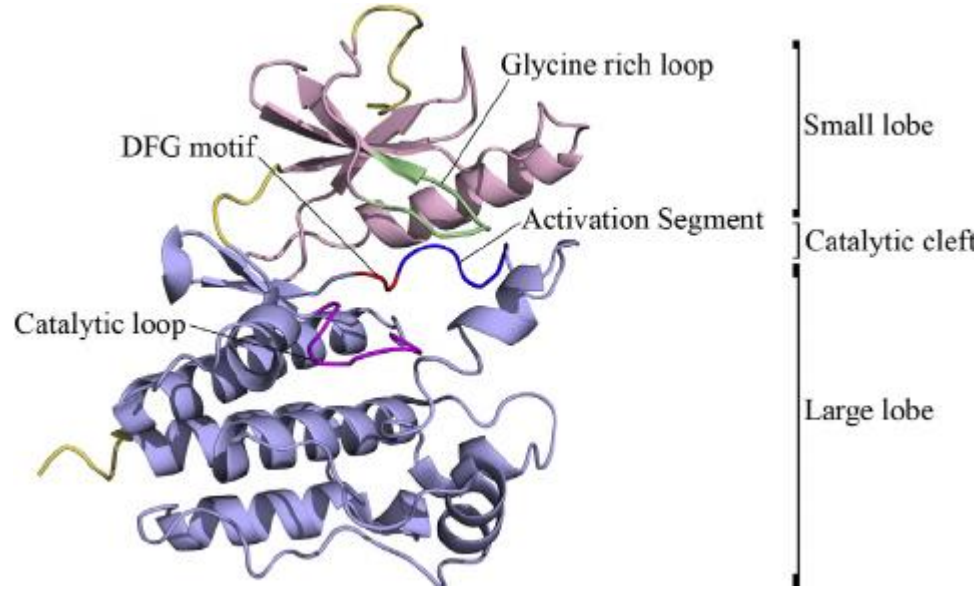
P-loop (phosphate binding loop) or Walter A motif – have the consensus sequence Gly-X_4-Gly-Lys-(Thr/Ser), where X is amino acid. Glycine residues have a key role in the binding of phosphoryl groups. Ser or Thr generally coordinates a divalent cation for phosphotransference

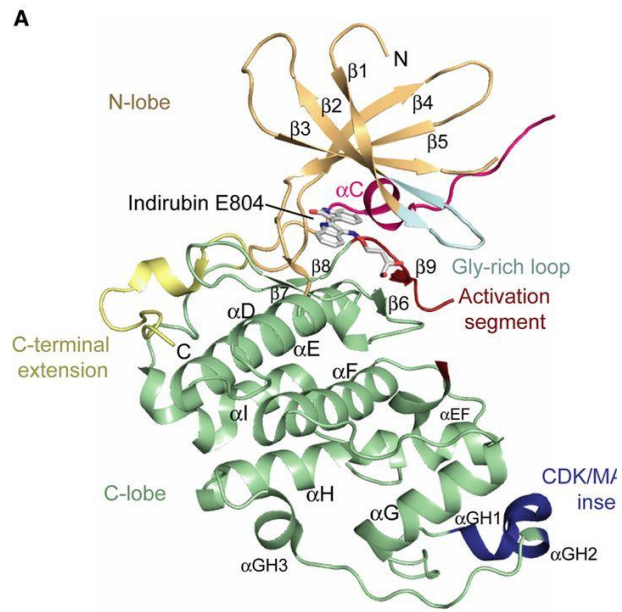




Glycine-rich loop

The glycine rich loop consensus sequence is Y-Gly-X-Gly-X-(Phe/Tyr)-Gly-X-Val, where Y is hydrophobic residue, and X is a less well defined residue. Generally, it is located in a β-turn between two antiparallel β- strands, which cover the nucleotide





Domains – Firstly defined by Jane Richardson as a compact units within the folding pattern of a single chain or a part of the polypeptide chain that is independently stable or could undergo movements as a single entity.

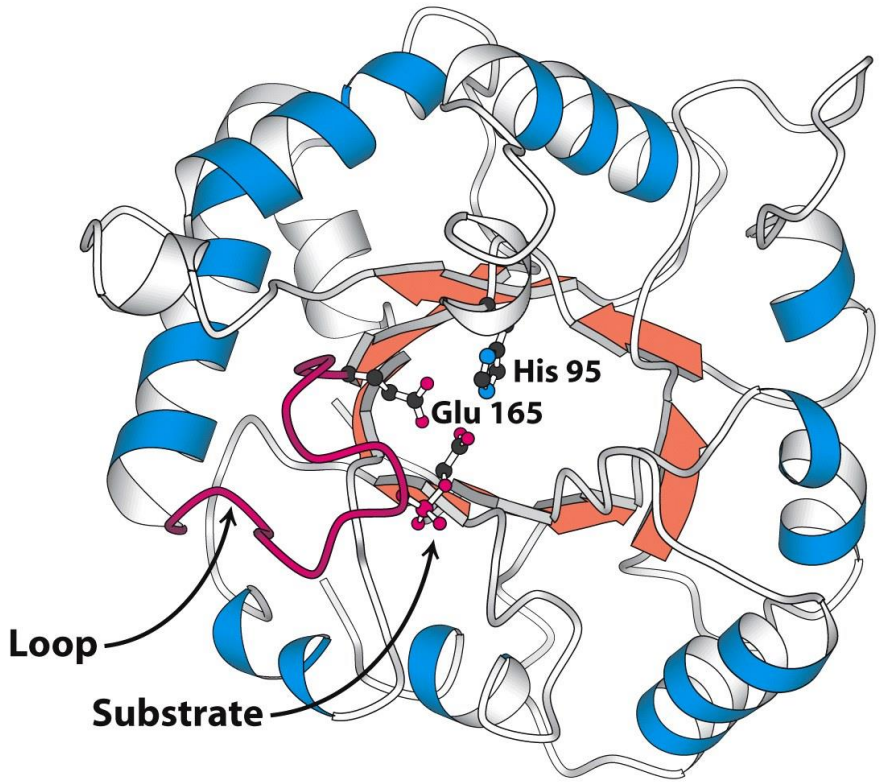
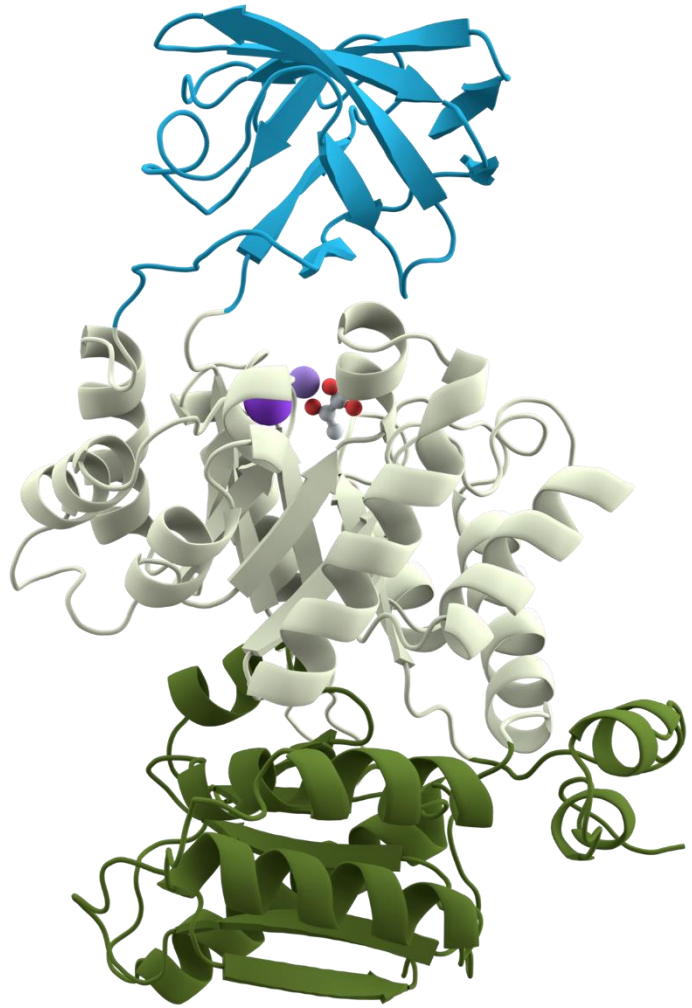


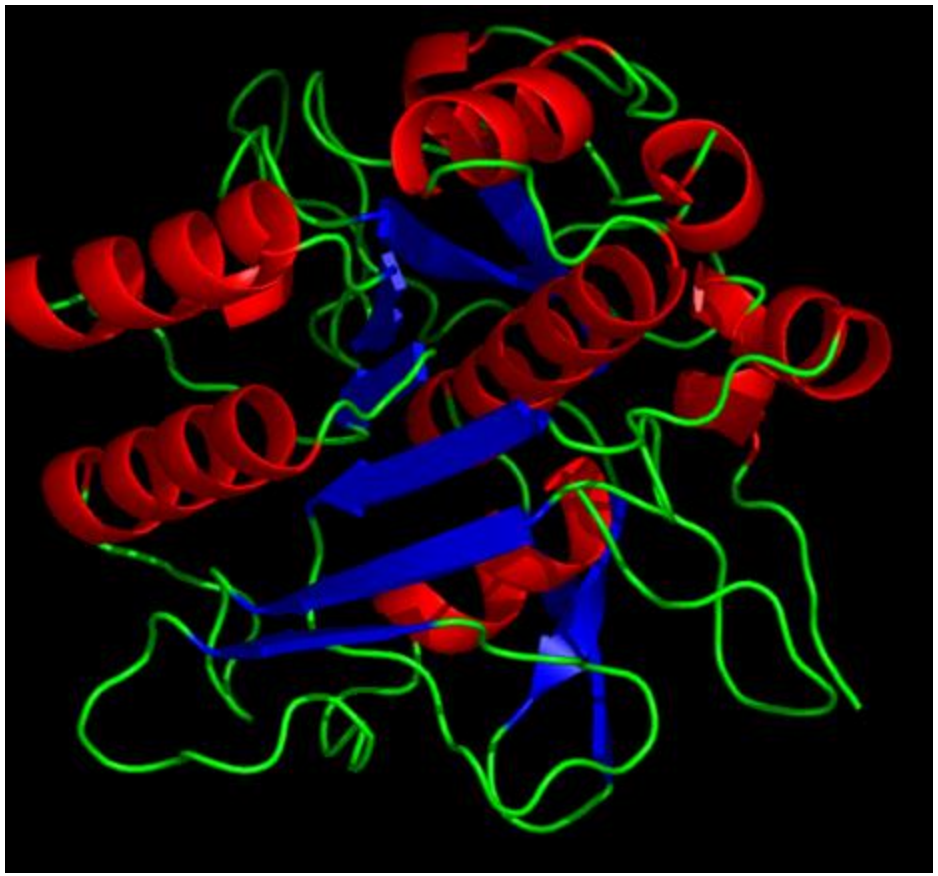
Figure 16.4
Biochemistry, Seventh Edition

TIM Barrel

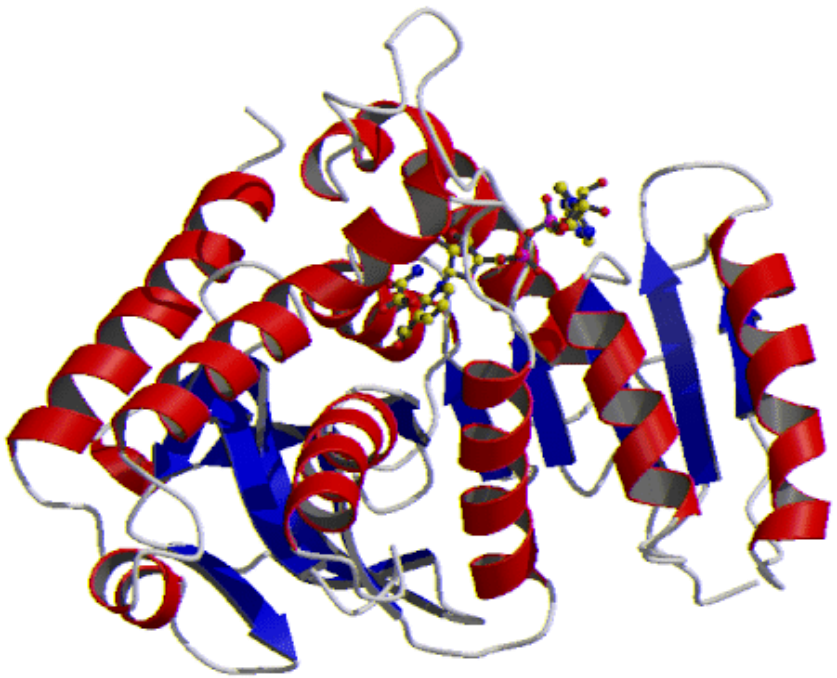


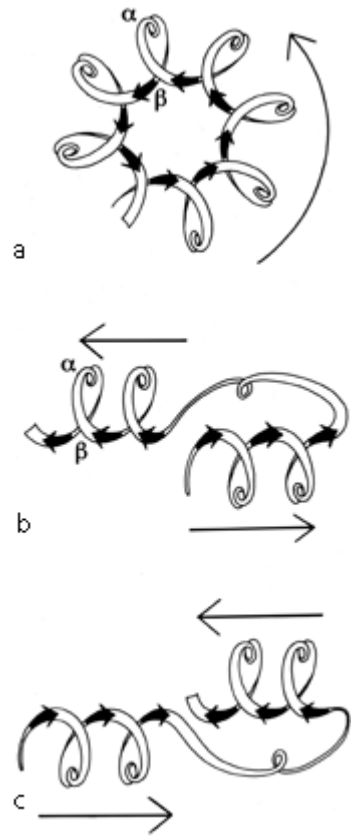
Common folds:

α/β doubly wound – mostly parallel sheet with helices on both sides



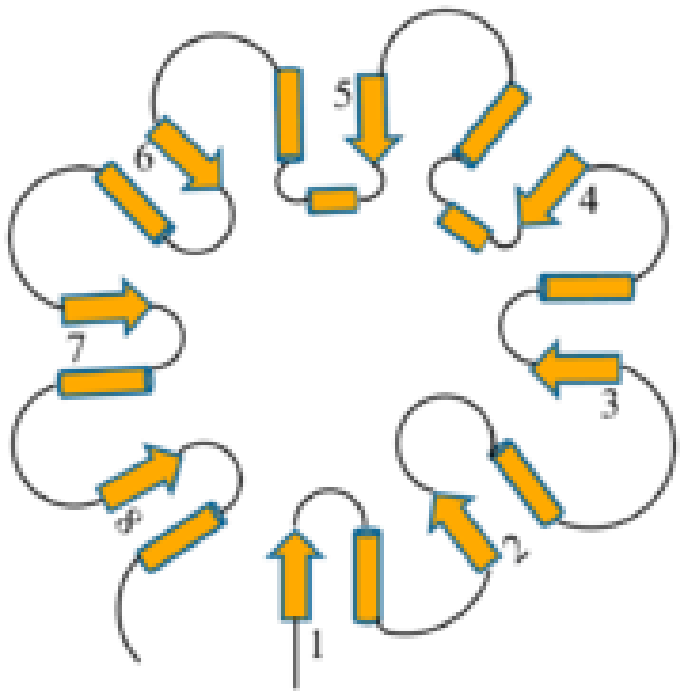
subtilisin

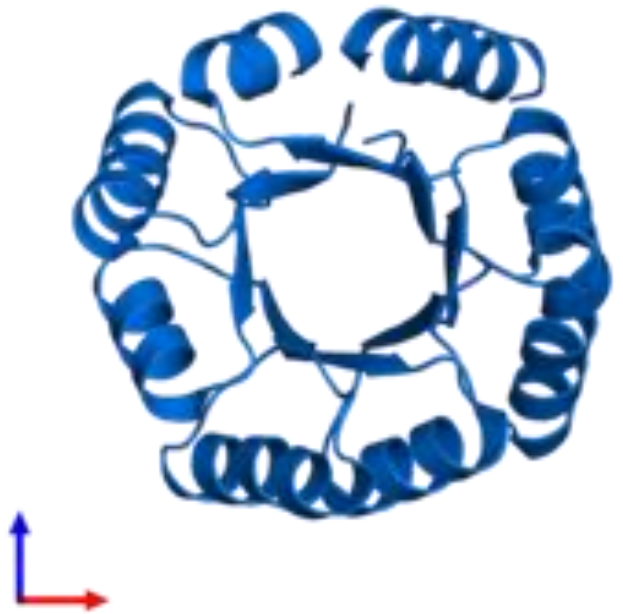




TIM barrel

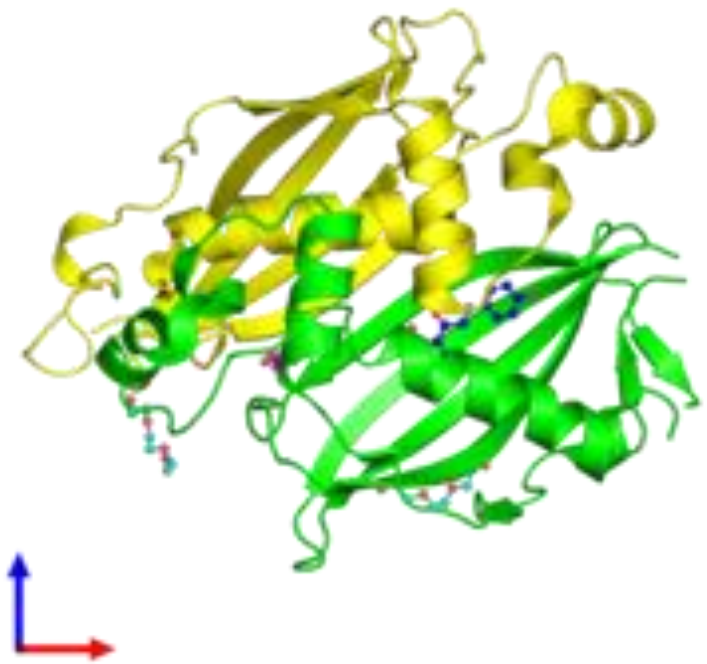
Cylinder of eight β-strands interconnected by helices





Split α/β sandwich

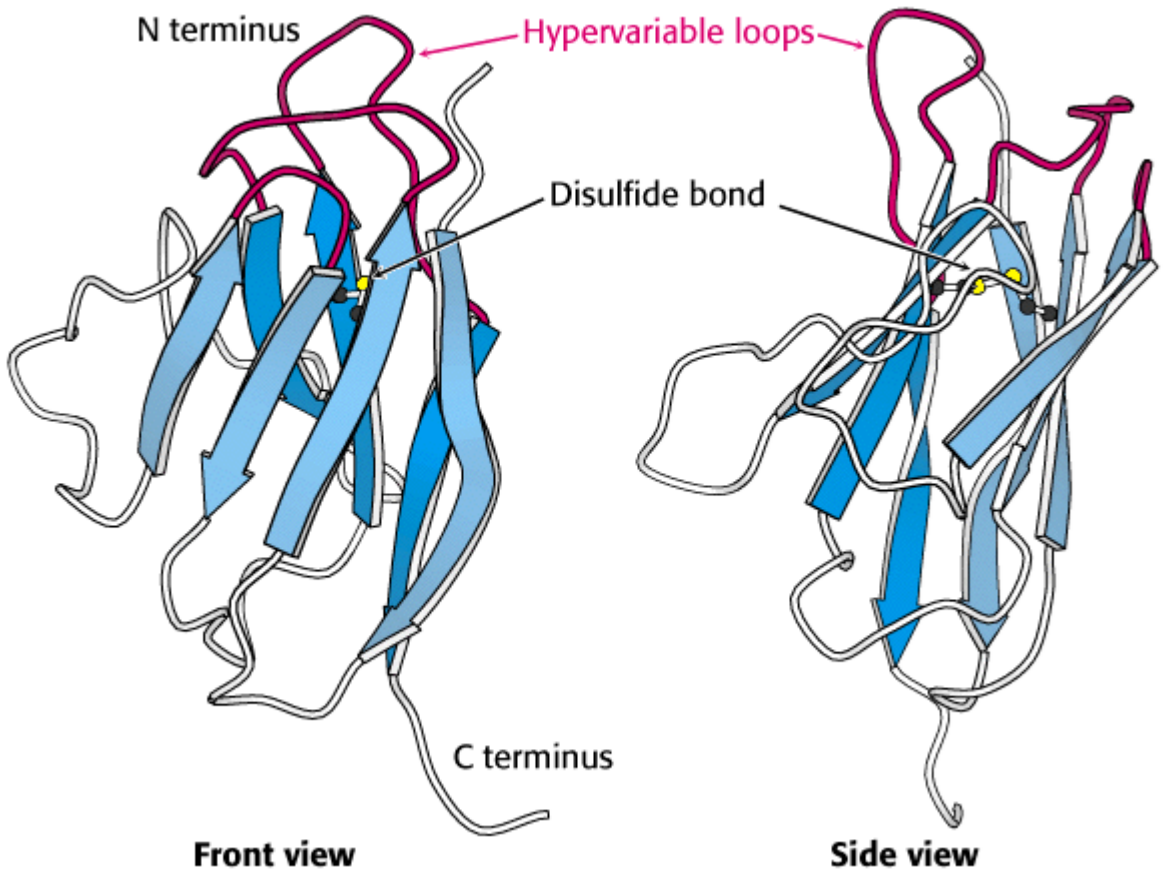
Antiparallel sheet with helices on one side



Aldolase

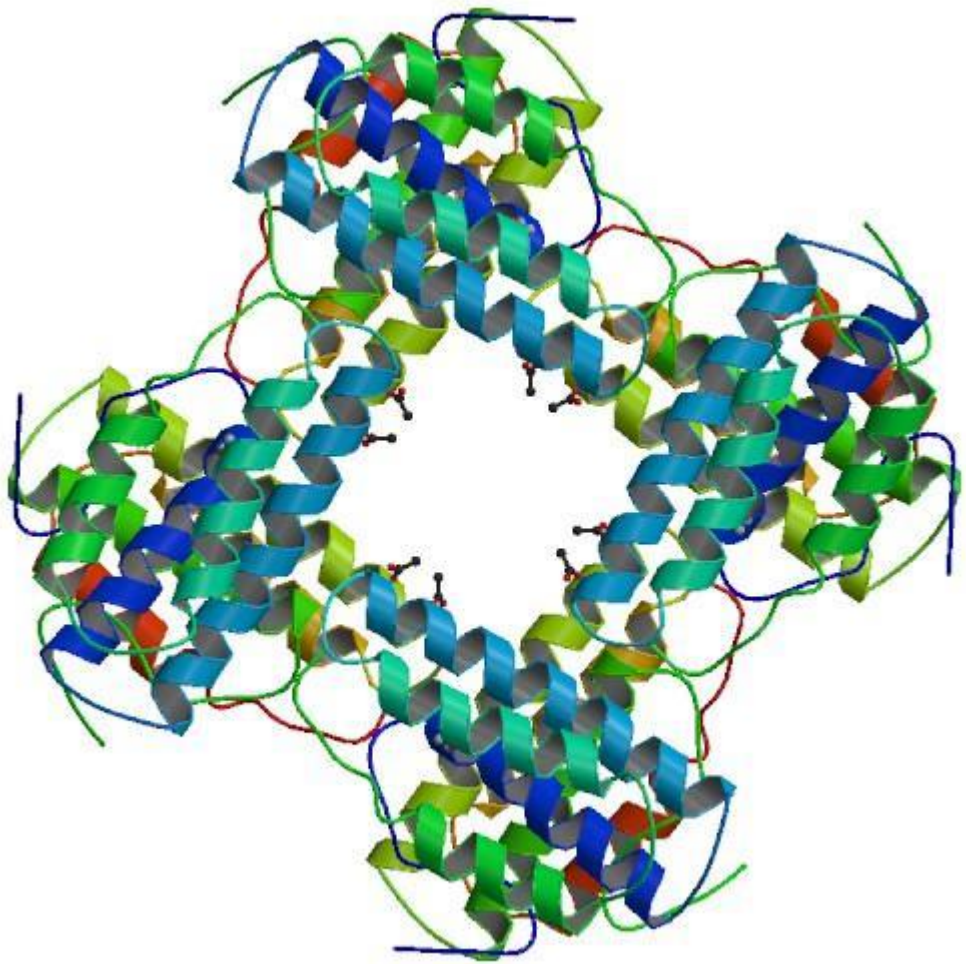
Immunoglobulin

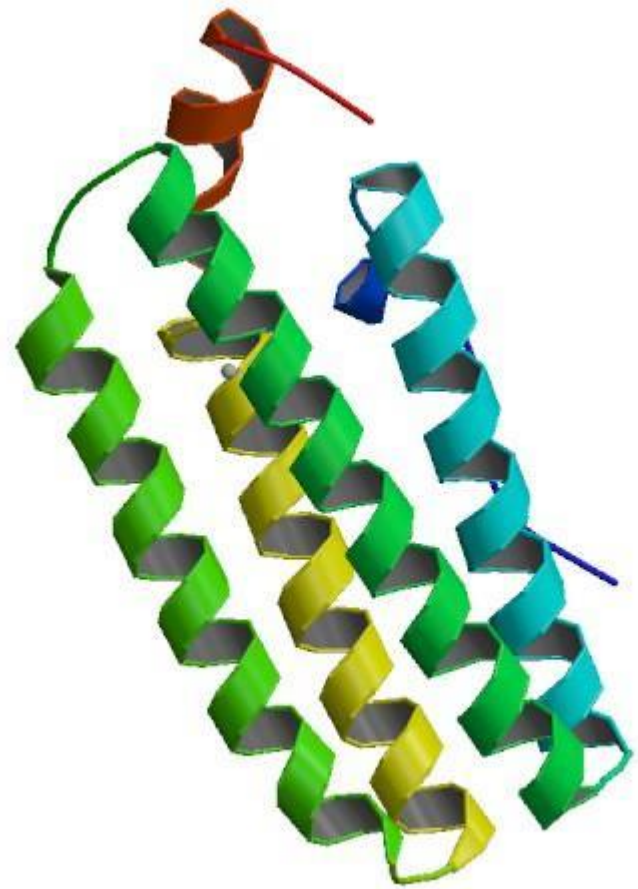
Beta-sandwich



α up and down

Four pairwise antiparallel helices

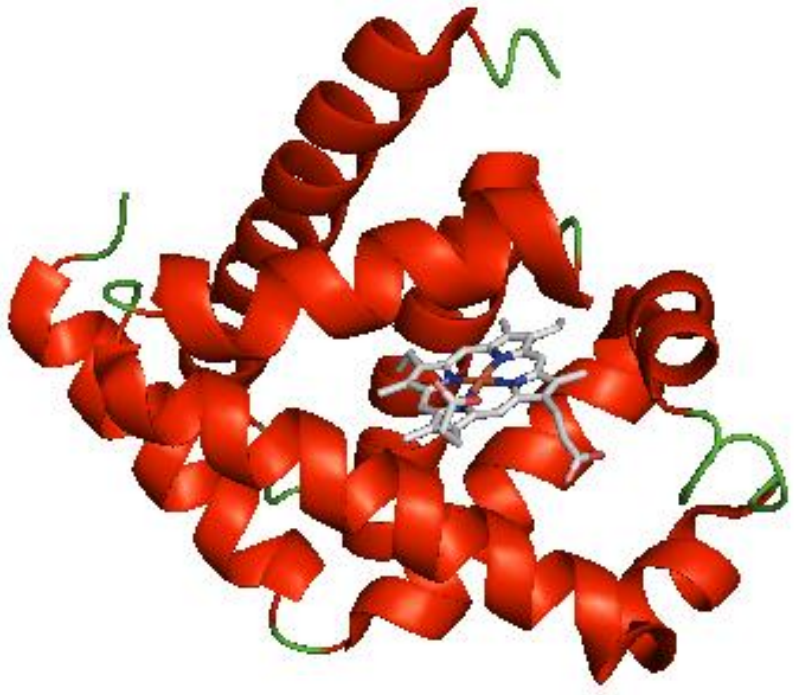


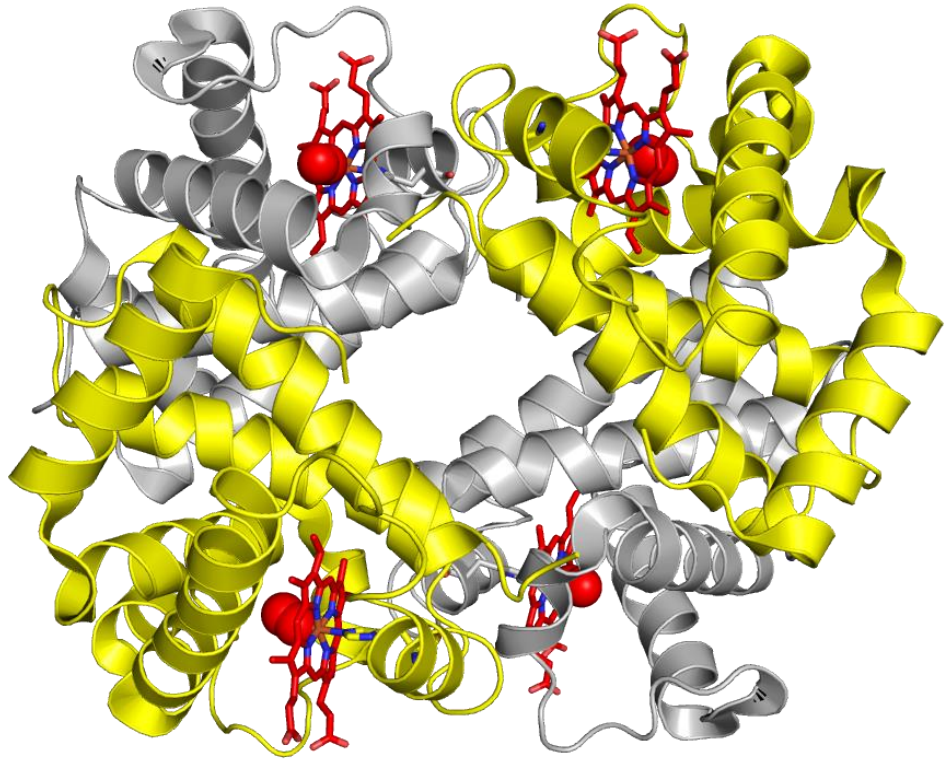


Hemerythrin

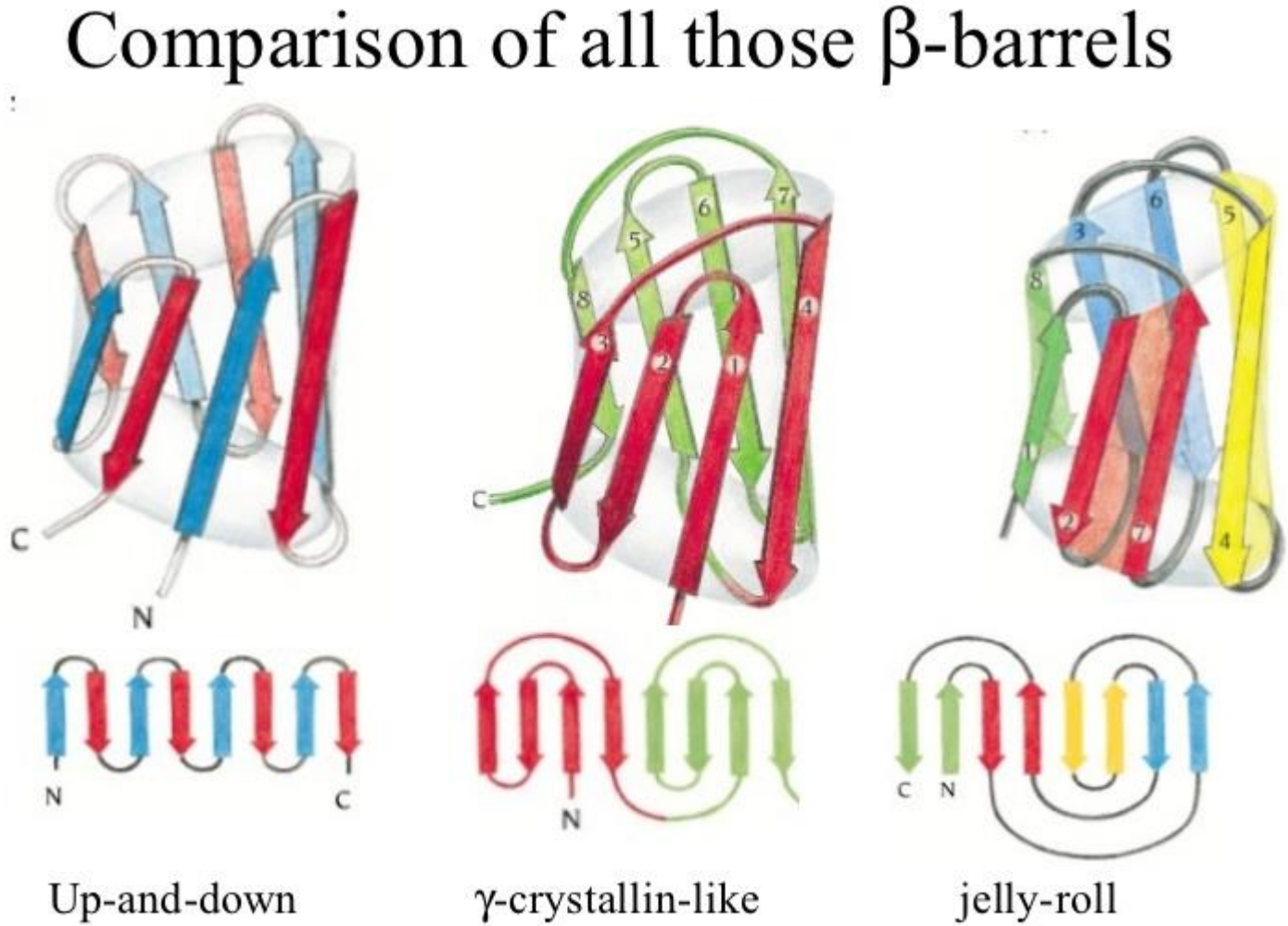
Globin

Two layers of non-parallel helices



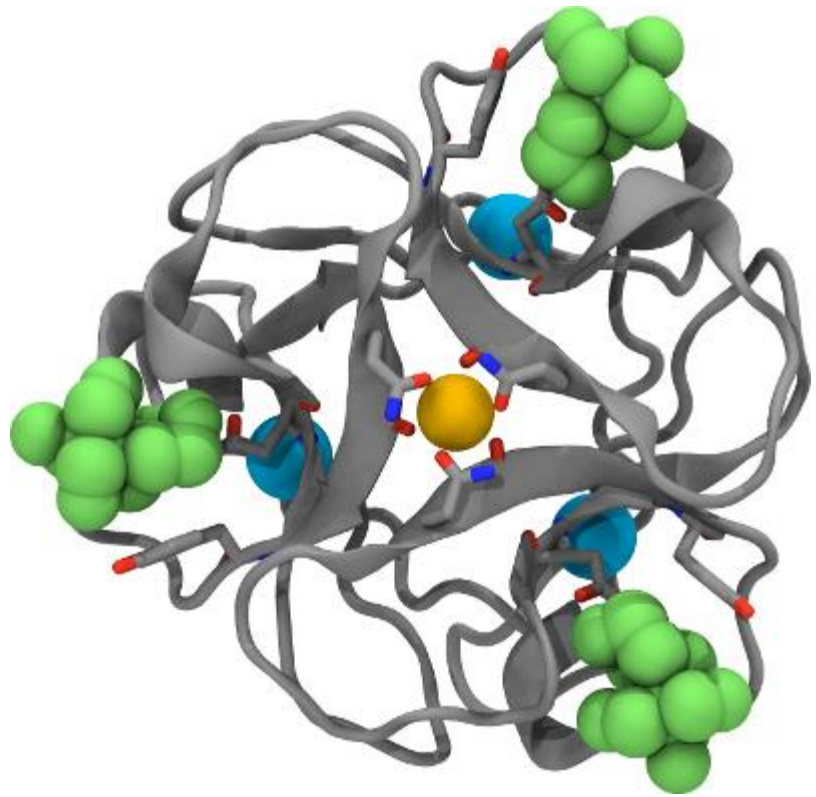


Jellyroll and related β-sandwich

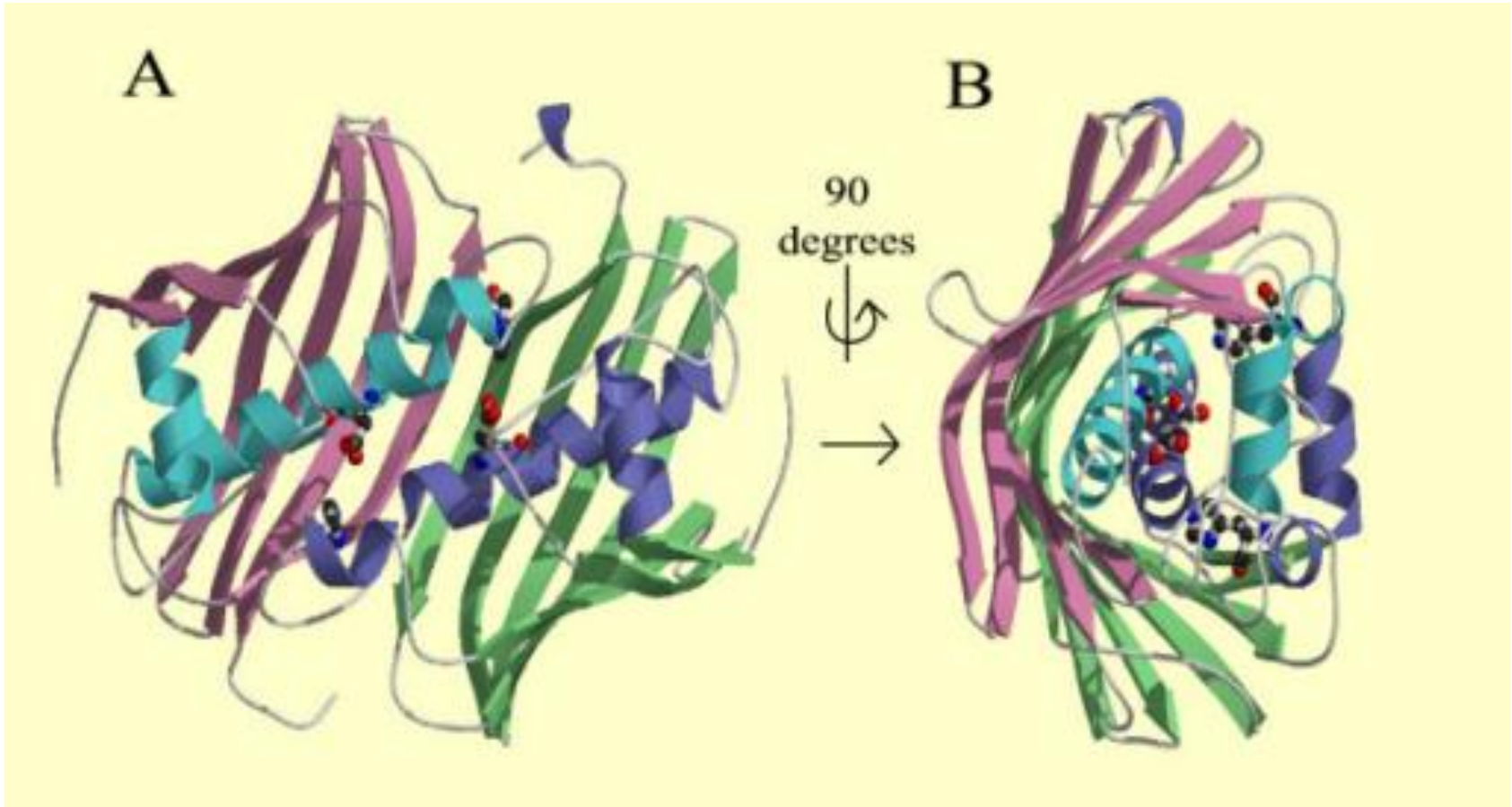


Trefoil

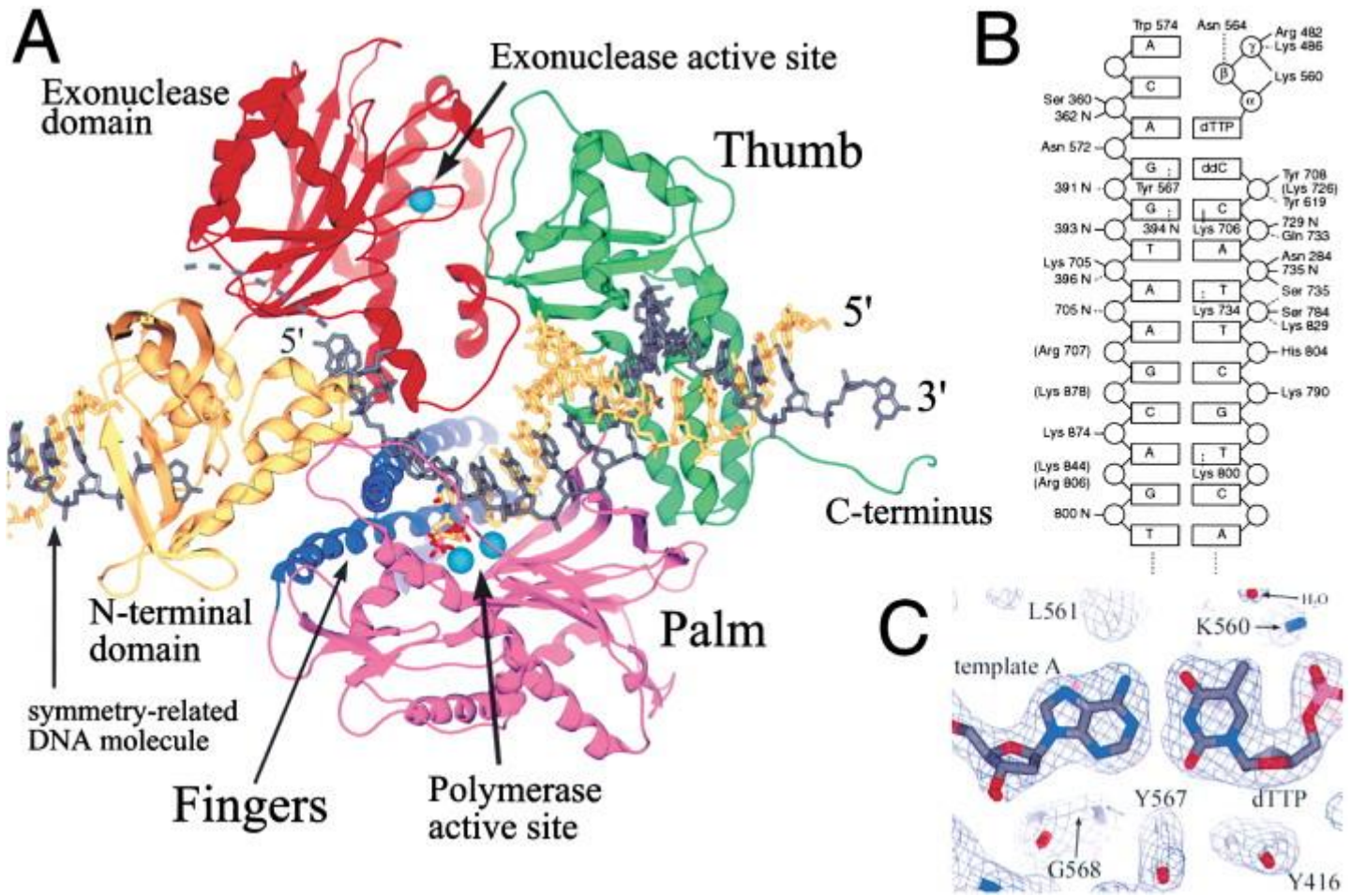
Cylinder formed by three sheets

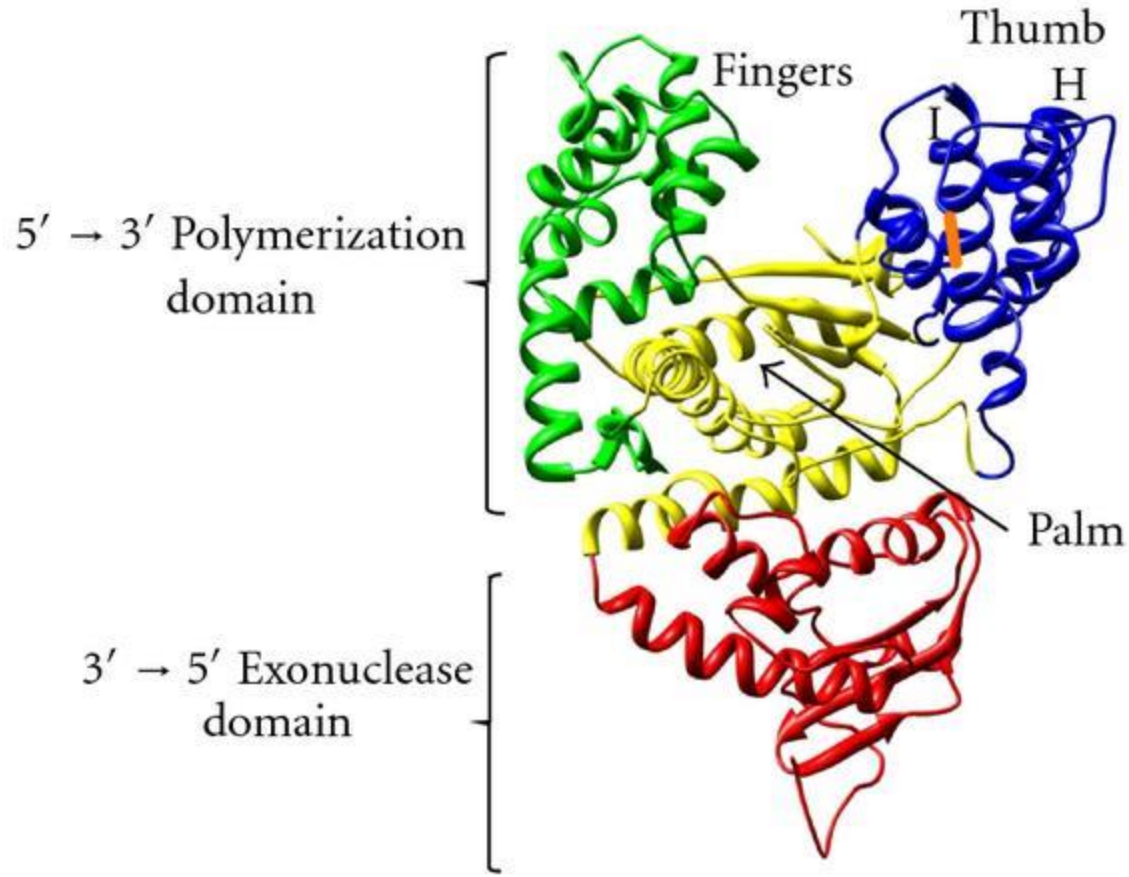


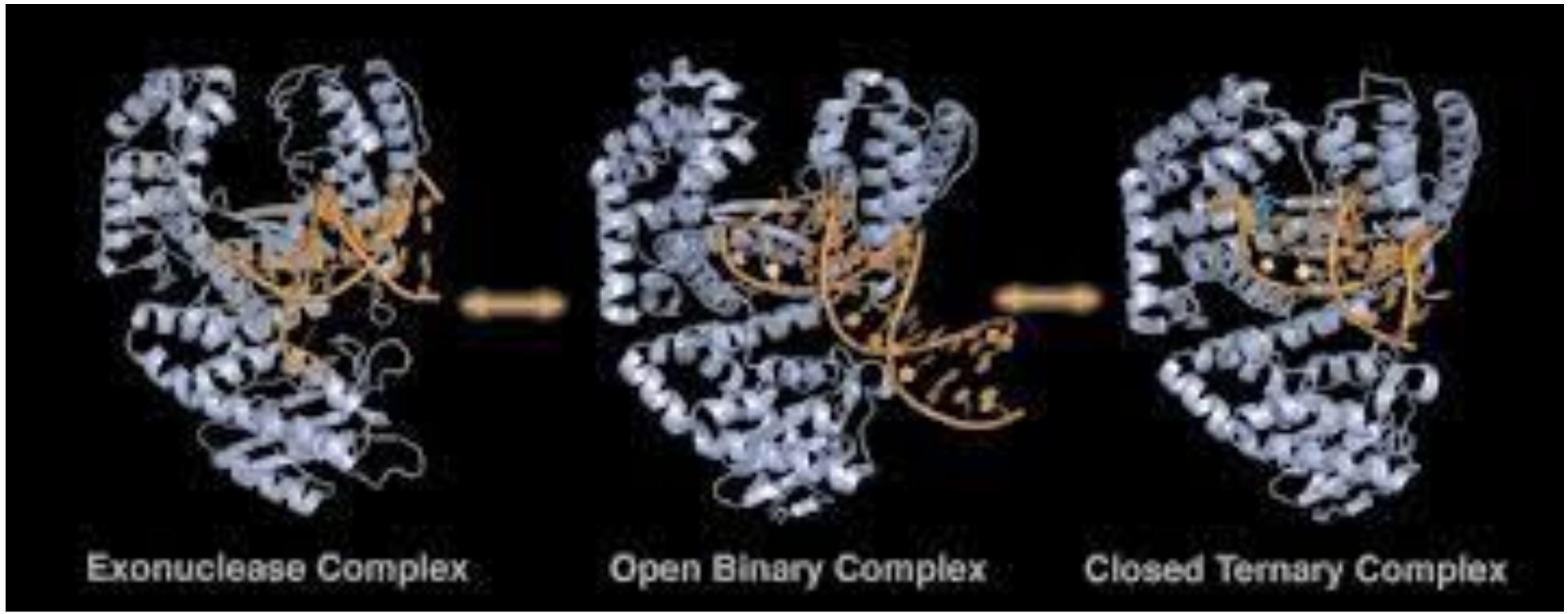
Hot dog fold



DNA polymerase




Thumb
Fingers
H
I
5′ → 3′ Polymerization domain
Palm
3′ → 5′ Exonuclease domain


Exonuclease Complex
Open Binary Complex
Closed Ternary Complex

Qual a importância dos domínios ou a formação de complexos proteicos para a replicação do DNA? Como as mudanças conformacionais contribuem para o processo de realização das atividades de replicação?