Globular proteins :

Different segments of the same polypeptide chain / multiple polypeptide chains fold back on each other.

Globular proteins include:

Enzymes

Transport proteins

Immunoglobulins

Motor proteins

Globular protein structures are compact relative to other extended conformation.

Approximate dimensions a single polypeptide chain would have if it occurred entirely in one conformation

Secondary structure:

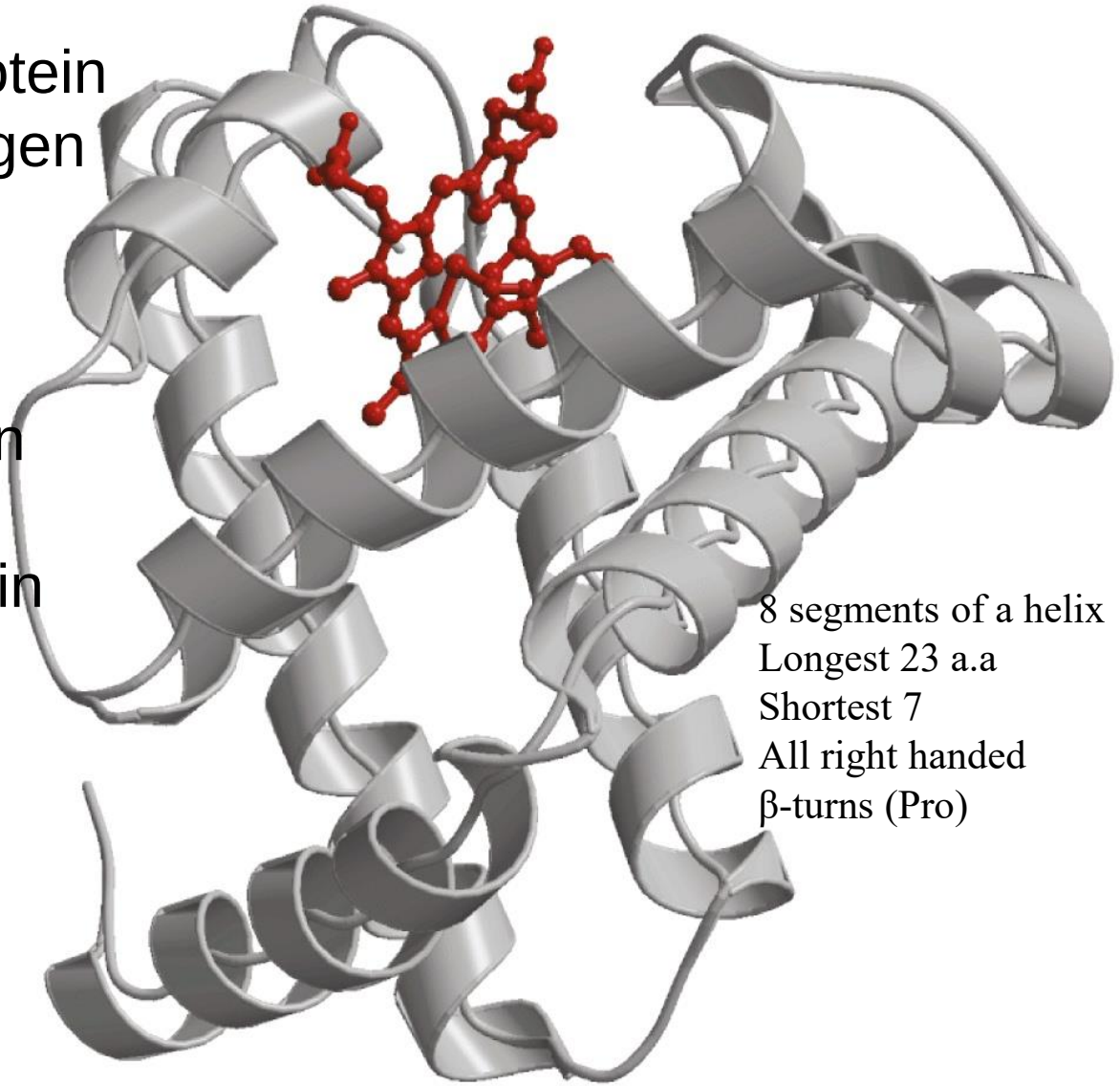
Tertiary structure:

Tertiary structure of myoglobin (PDB ID 1MBO):

The polypeptide backbone (ribbon representation).

Small oxygen-binding protein of muscle cells.store oxygen and facilitate oxygen diffusion in muscle.

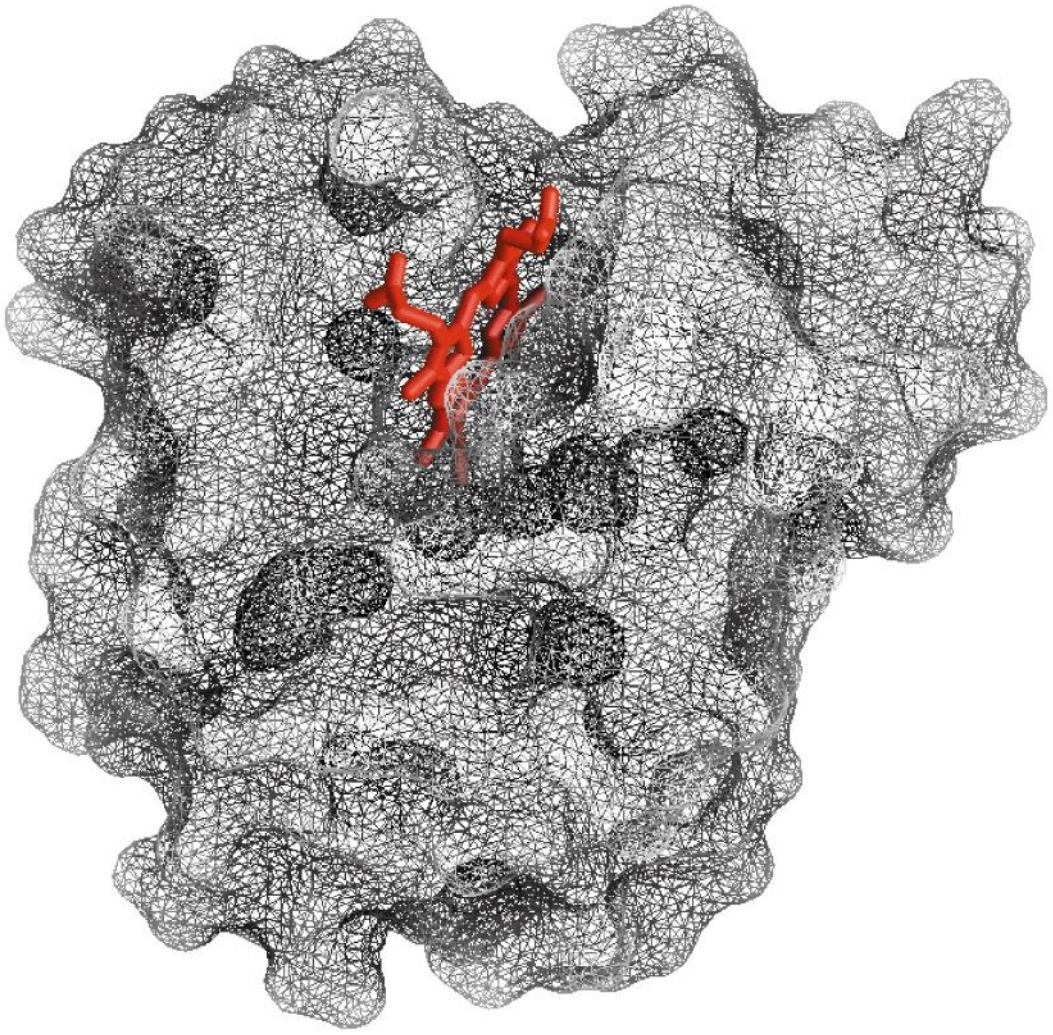
A single polypeptide chain
153 a.a residues
+single iron protoporphyrin
= heme
(Deep red brown color
also in hemoglobin)



8 segments of a helix
Longest 23 a.a
Shortest 7
All right handed
β-turns (Pro)

(a)

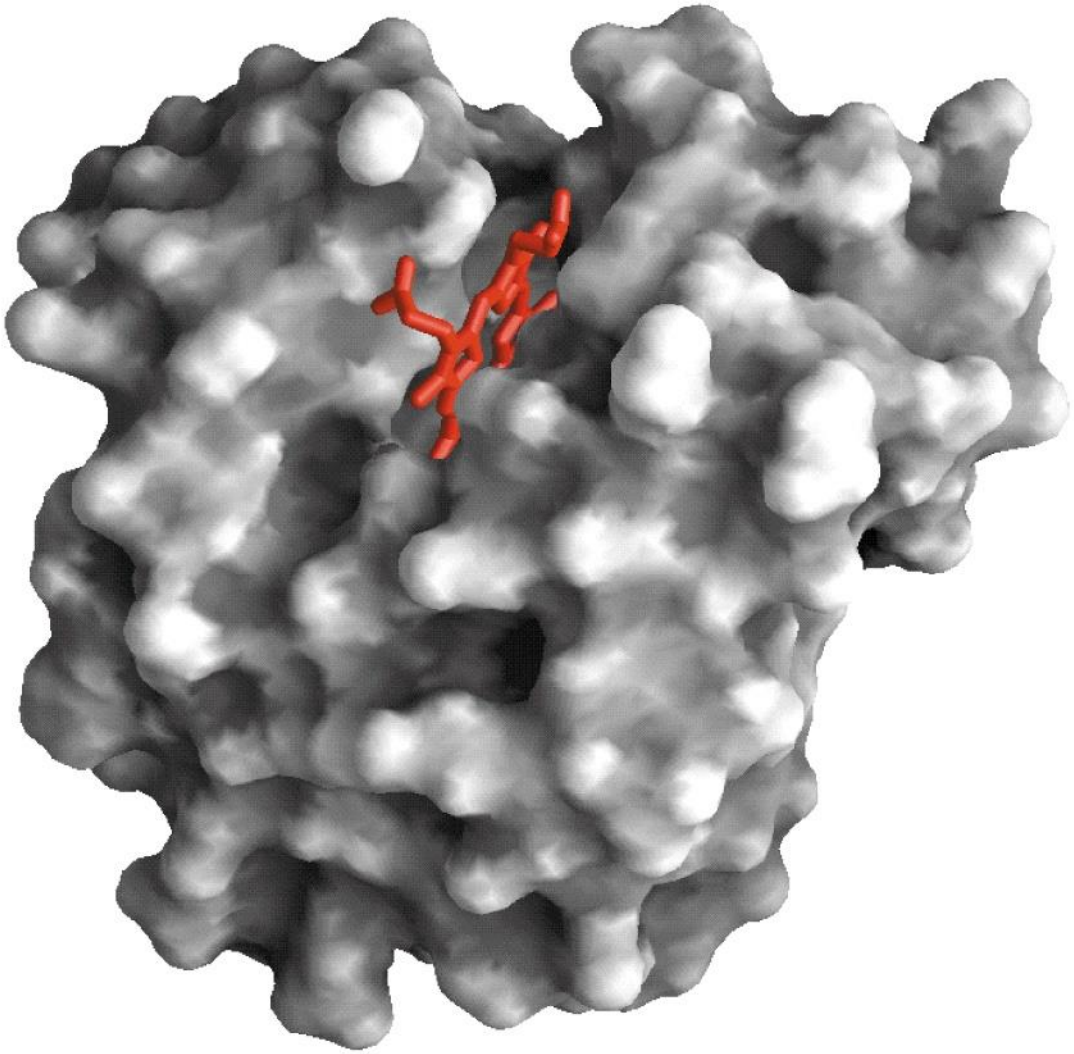
A mesh image emphasizes the protein surface :



(b)

A surface contour image useful for visualizing pockets in protein where other molecules might bind.

Flat Heme group rests in a pocket



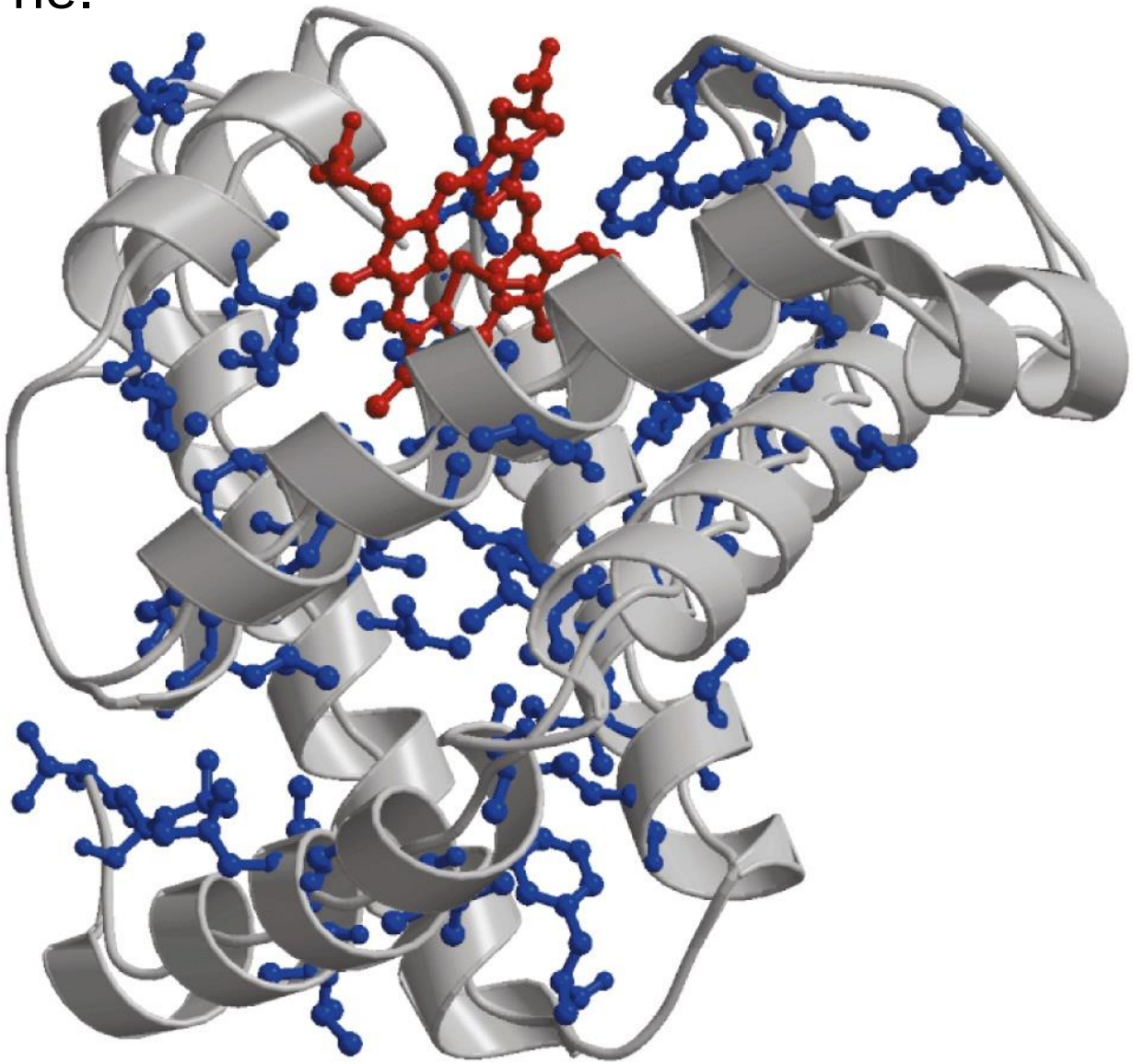
(c)

Ribbon representation including side chains for hydrophobic residues Leu, Ile, Val, Phe.

Dense hydrophobic

core typical for globular

Proteins.

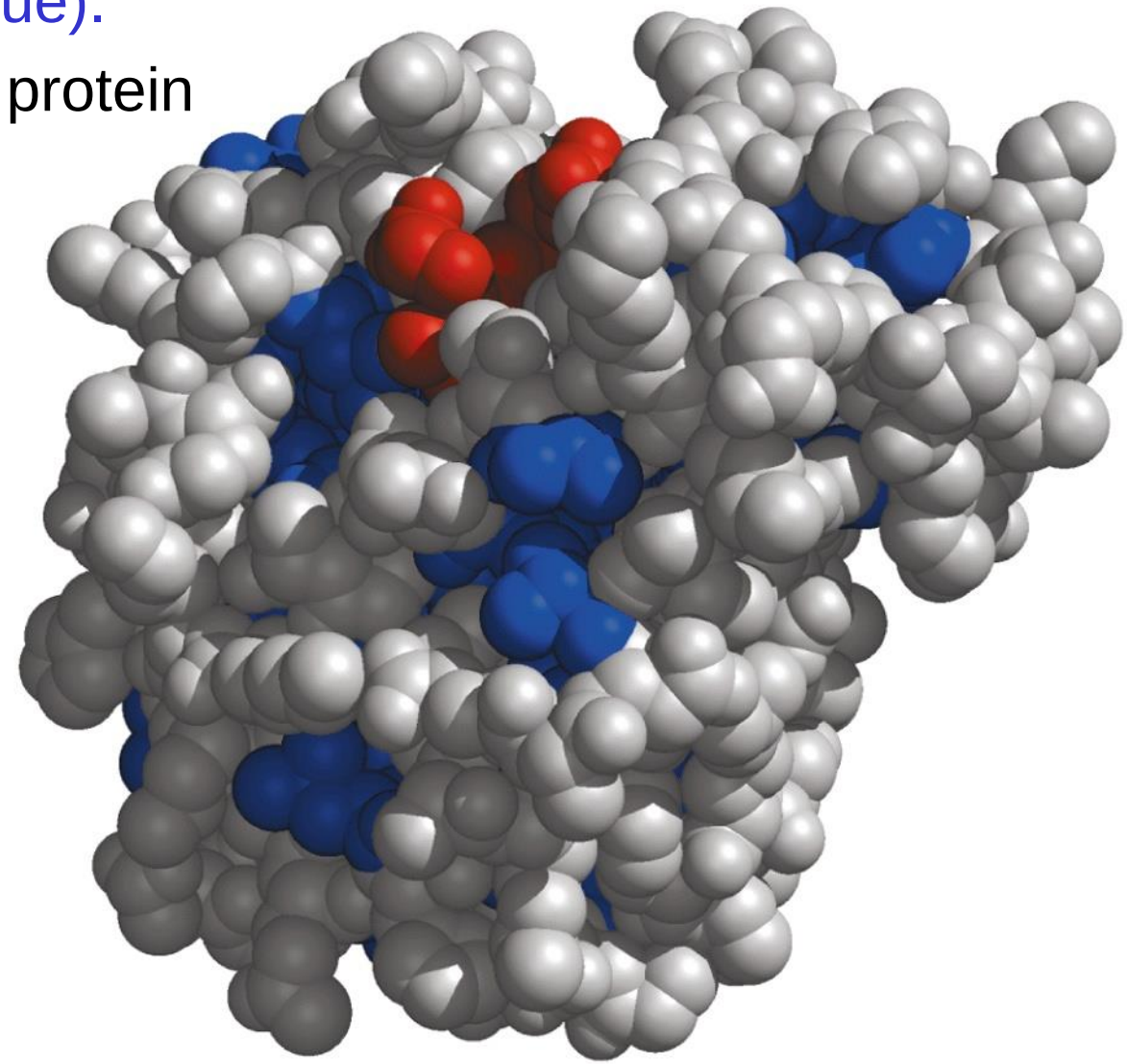


(d)

A space filling model with all a.a chains. Each atom represented by a sphere encompassing van der waals radius.

Hydrophobic residues (blue).

Buried /hidden interior of protein from water.



(e)

The heme group:

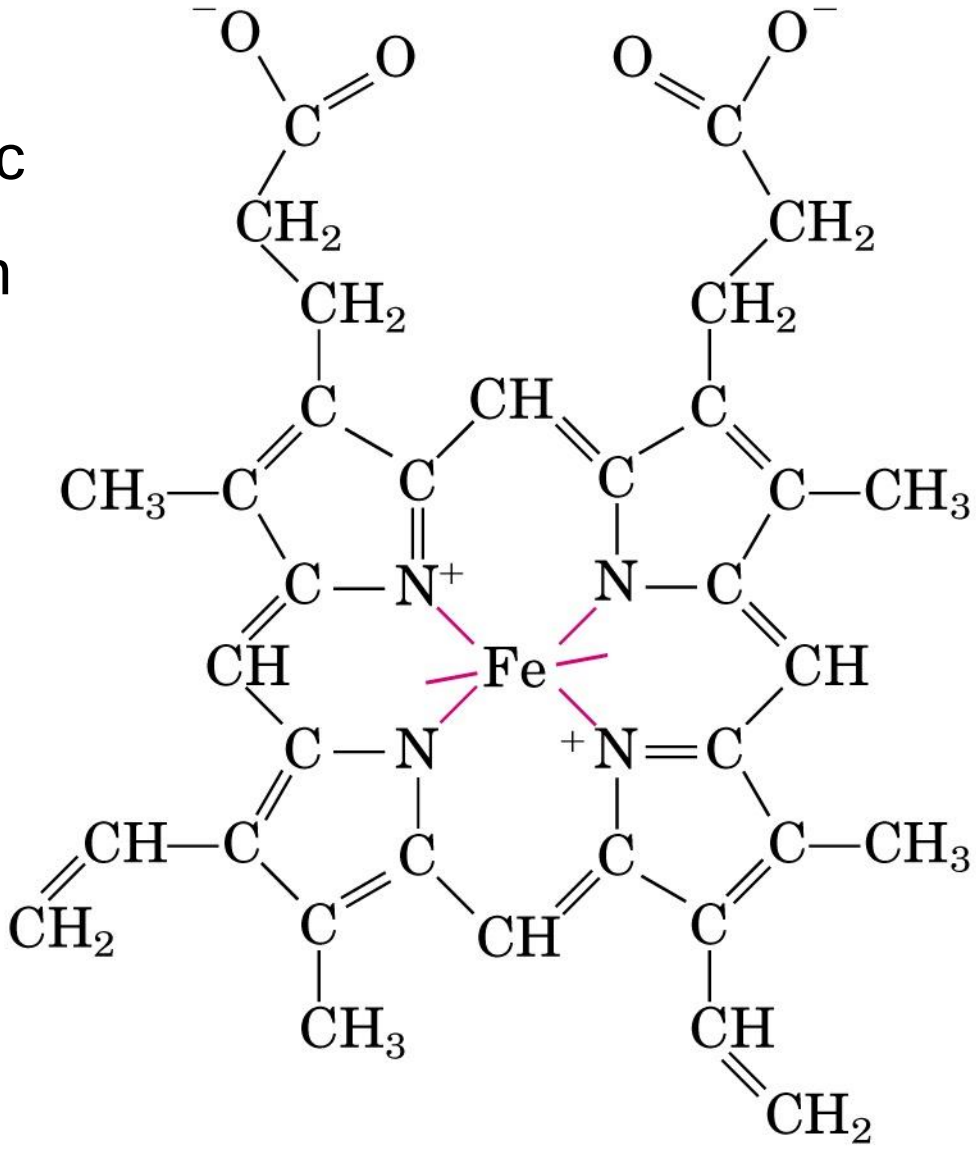
- Present in myoglobin, hemoglobin, cytochromes…etc.

Heme consist of complex organic ring protoporphyrin bound to iron

Ferrouus +2

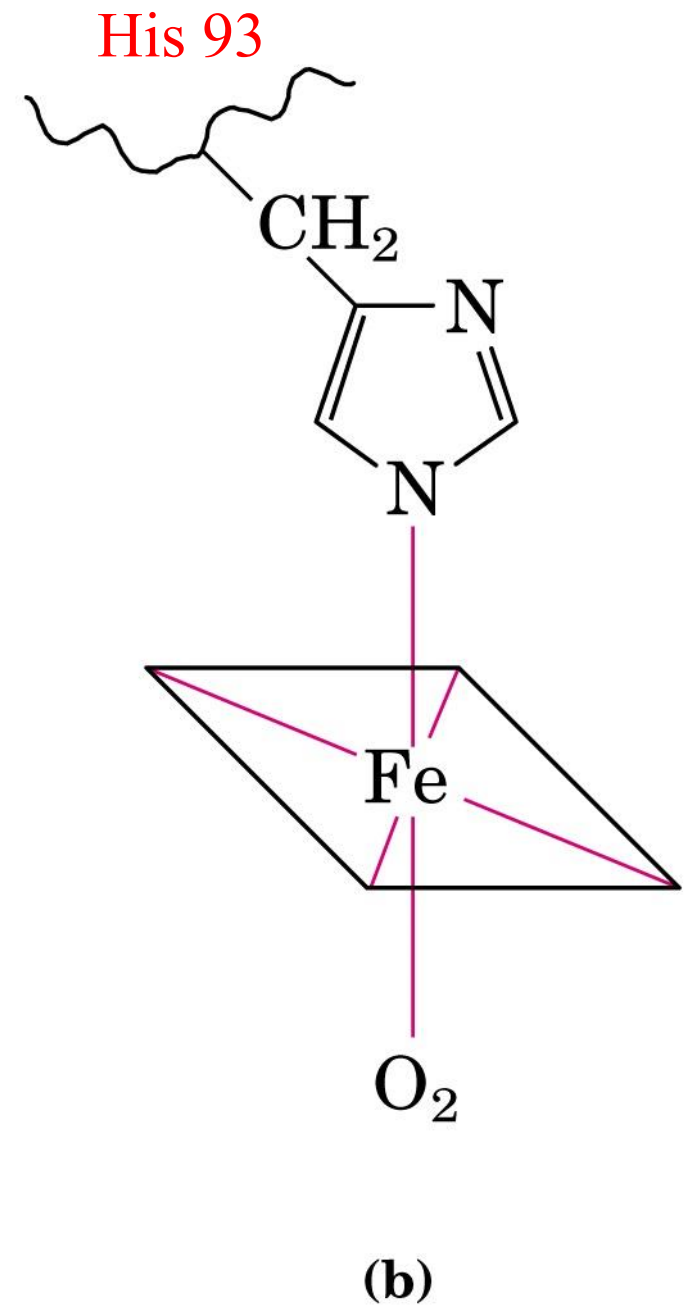
Iron at center of heme has 2 coordination

One with heme.



(a)

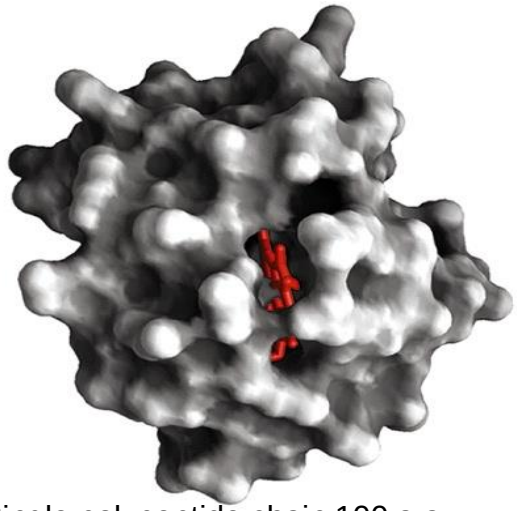
In myoglobin and hemoglobin one of the coordination bonds is bound to a nitrogen of a His residue at position 93. The other is open and serves as O_2 binding site



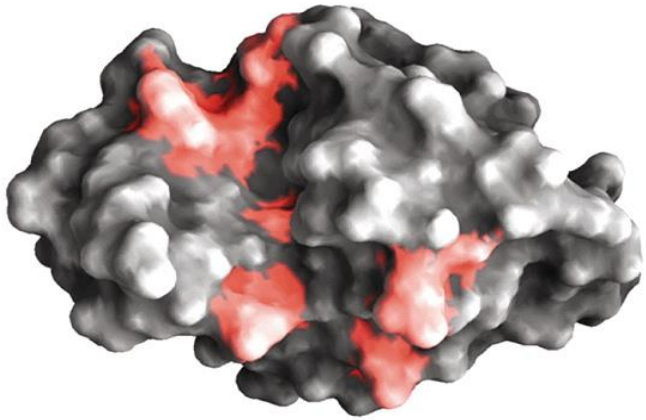
(b)

Three dimensional structure of some small proteins:

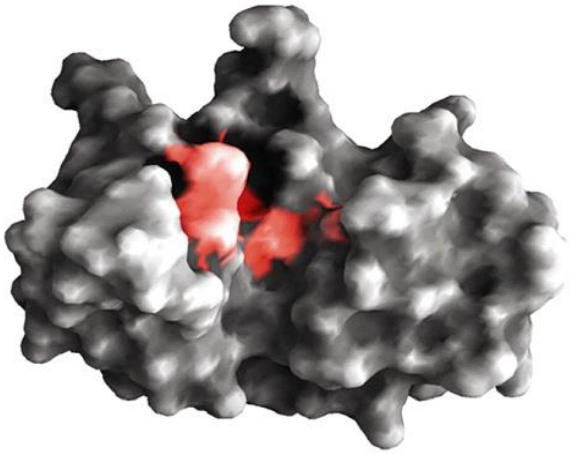
Surface contour + ribbon representation . Flat arrows β-sheets, Spiral ribbon α-helices disulfide bonds yellow, functional groups (heme, a.a in E active site) red.



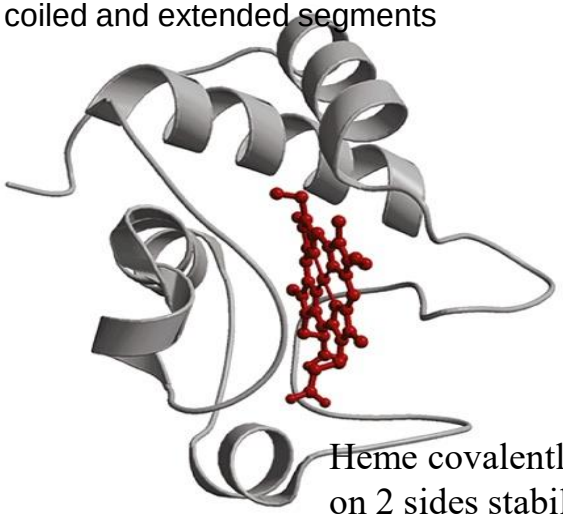
Single polypeptide chain 100 a.a
40% a helical rest turns bents irregular coiled and extended segments



129 a.a 40% a helix + b sheet
4 disulfide bonds stabilize.

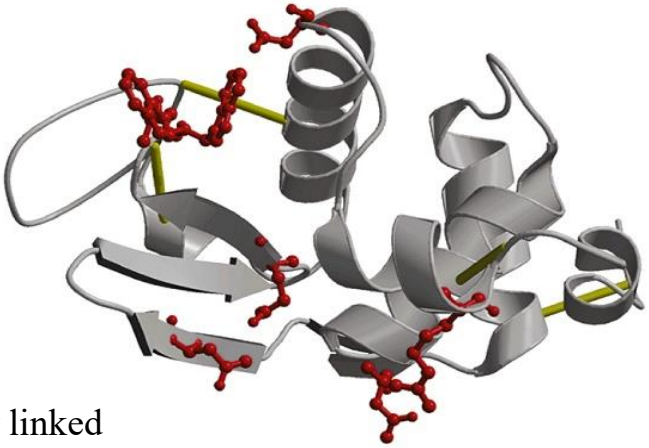


124 a.a , 4 disulfide bonds stabilize.

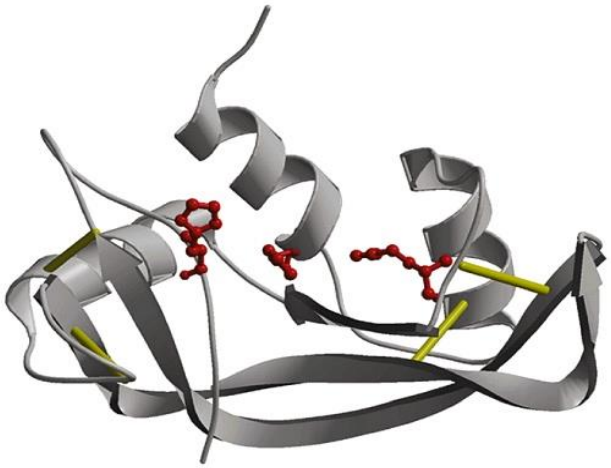


Heme covalently linked on 2 sides stabilize

Cytochrome *c*



Lysozyme



Ribonuclease

Proportions of α-helix and ß-conformation in globular proteins:

table 6–2

Approximate Amounts of α Helix and β Conformation in Some Single-Chain Proteins*

Protein (total residues)	Residues (%)	
	α Helix	β Conformation
Chymotrypsin (247)	14	45
Ribonuclease (124)	26	35
Carboxypeptidase (307)	38	17
Cytochrome *c* (104)	39	0
Lysozyme (129)	40	12
Myoglobin (153)	78	0

Source: Data from Cantor, C.R. & Schimmel, P.R. (1980) *Biophysical Chemistry,* Part I: *The Conformation of Biological Macromolecules,* p. 100, W.H. Freeman and Company, New York.

*Portions of the polypeptide chains that are not accounted for by α helix or β conformation consist of bends and irregularly coiled or extended stretches. Segments of α helix and β conformation sometimes deviate slightly from their normal dimensions and geometry.

Larger globular proteins more complex tertiary structure .

Studied by focusing on structural patterns.

Three dimensional structure of globular protein considered an assemblage of polypeptide segments of α-helix and ß-conformation (stacked on each other) and linked by connecting segments.

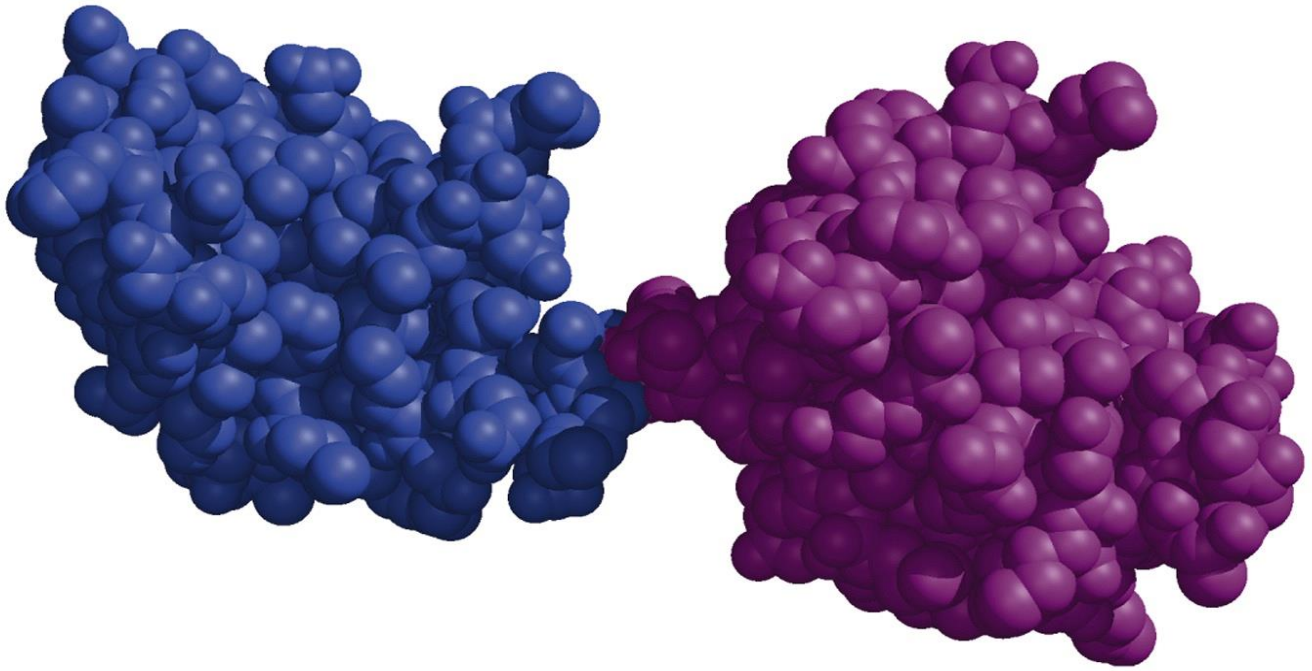
Supersecondary structures / motifs :

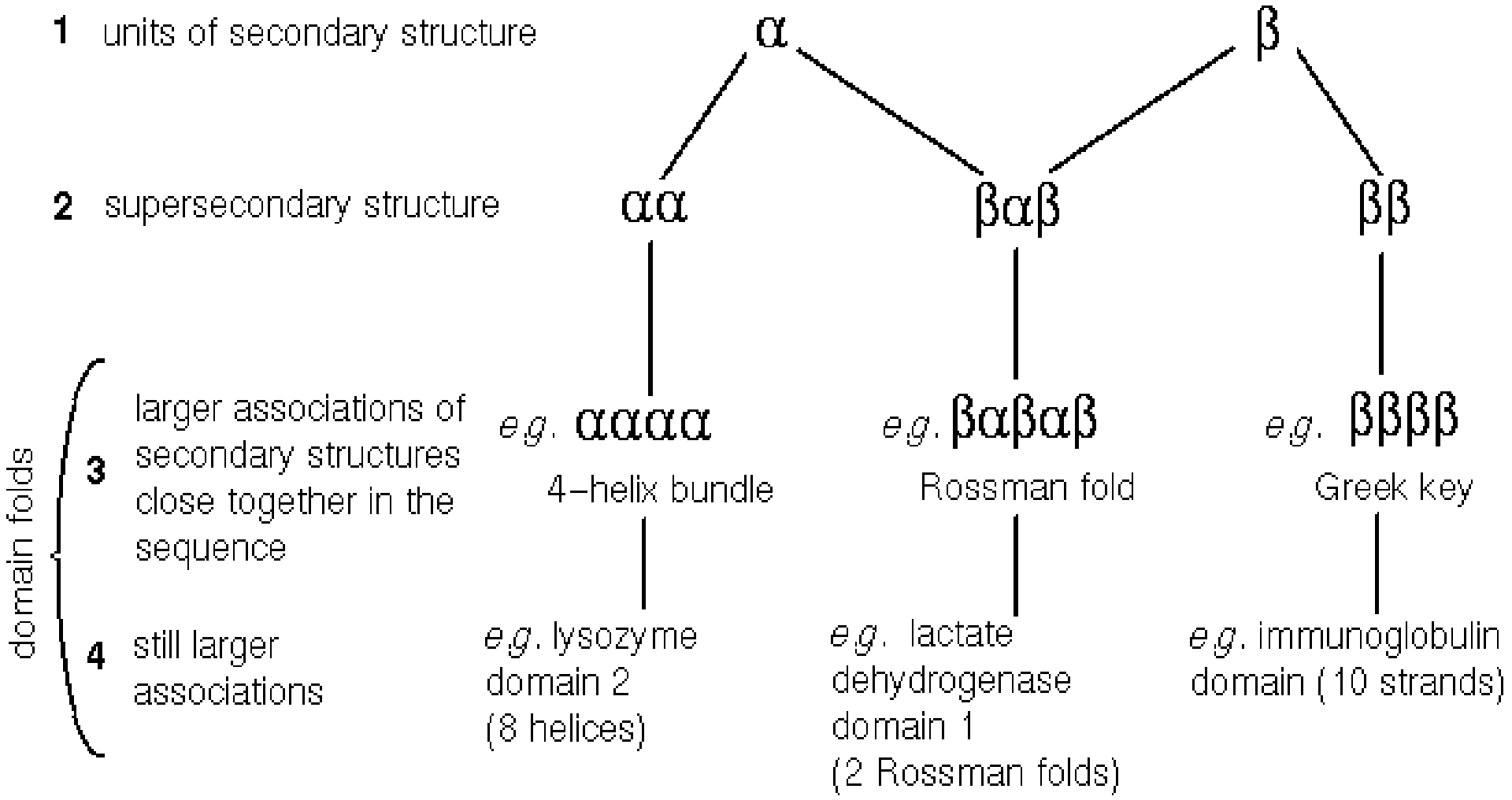
Stable arrangements of several elements of secondary structure and the connections bw them.

Domains: regions of a polypeptide chain that can fold stably into globular units. (retained even after cleavage)

Structural domains in troponin C PDB ID 4TNC

Ca^{2+} binding protein associated with muscle. (2 separate Ca^{2+} binding domains).



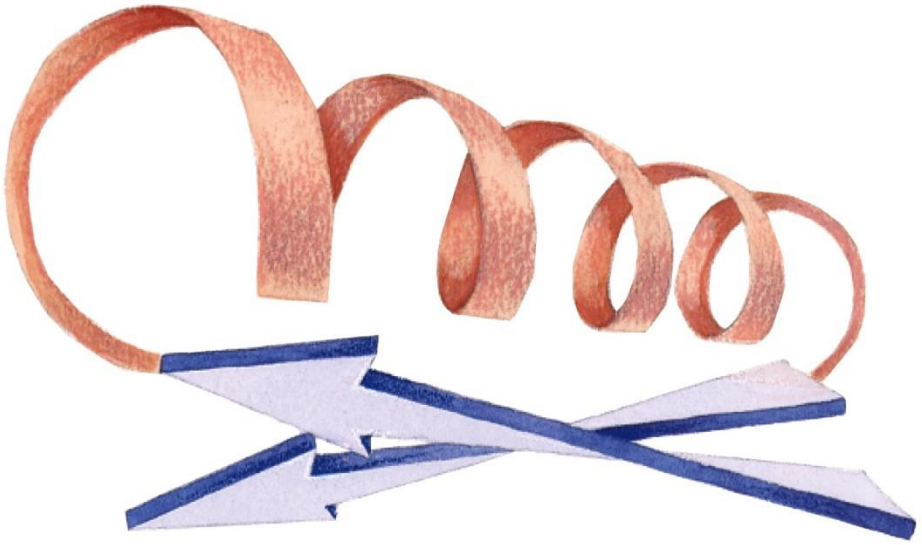

1 units of secondary structure
α
β
2 supersecondary structure
αα
βαβ
ββ
domain folds
3 larger associations of secondary structures close together in the sequence
4 still larger associations
e.g. αααα
4–helix bundle
e.g. lysozyme domain 2 (8 helices)
e.g. βαβαβ
Rossman fold
e.g. lactate dehydrogenase domain 1 (2 Rossman folds)
e.g. ββββ
Greek key
e.g. immunoglobulin domain (10 strands)

Different supersecondary structures/motifs of protein folding:

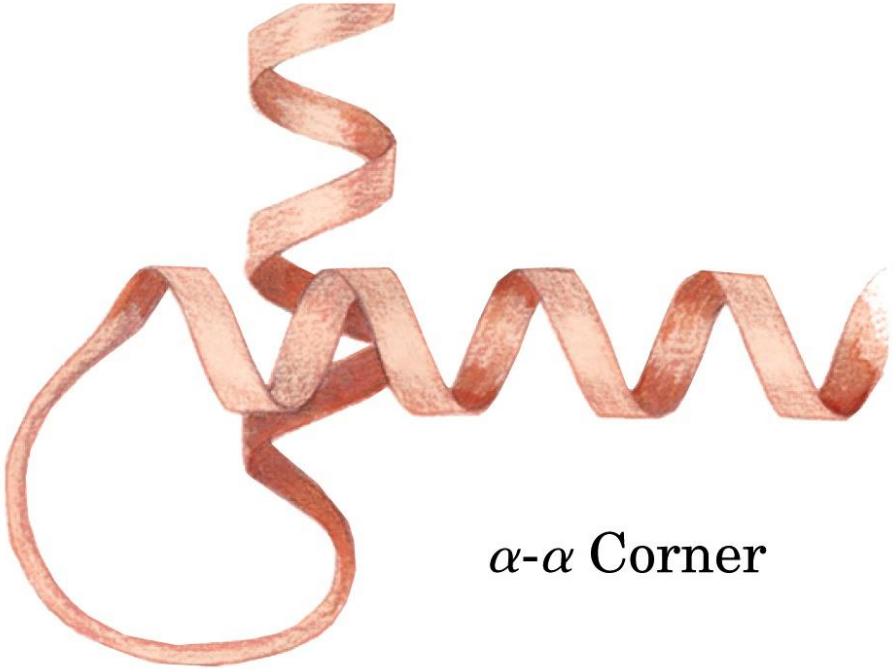
Stable folding pattern in proteins:

Two simple and common motifs.

Two layers of secondary structure.



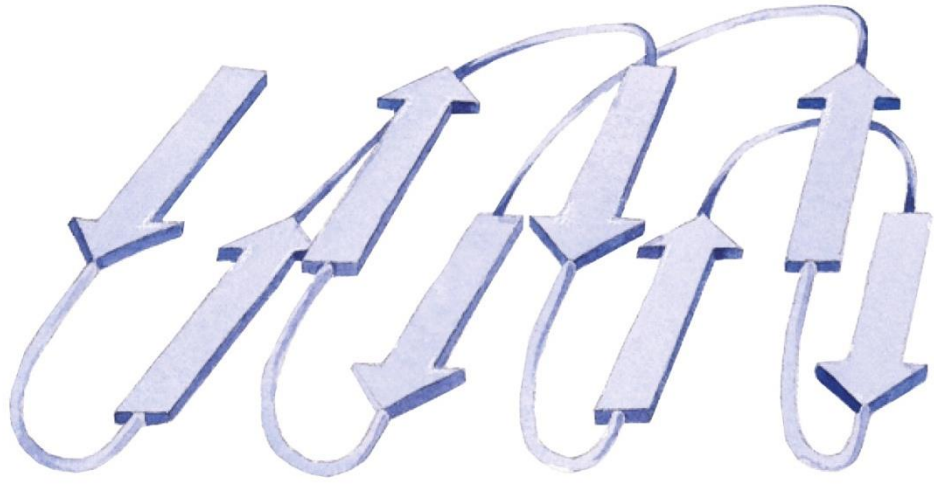
(a) β-α-β Loop



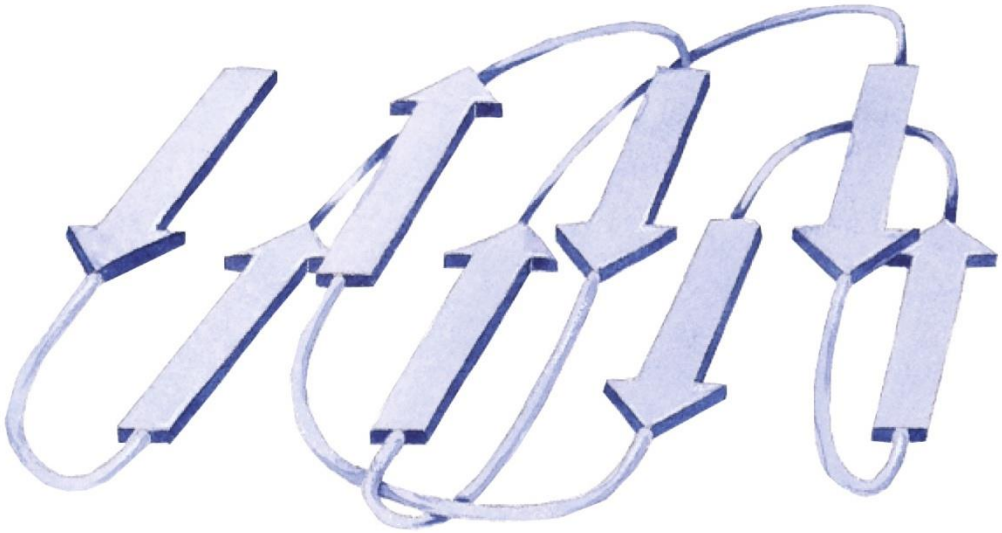
α-α Corner

Connections bw β-strands in layered β sheets

Connections cant cross or form knots.



(b) Typical connections in an all-β motif

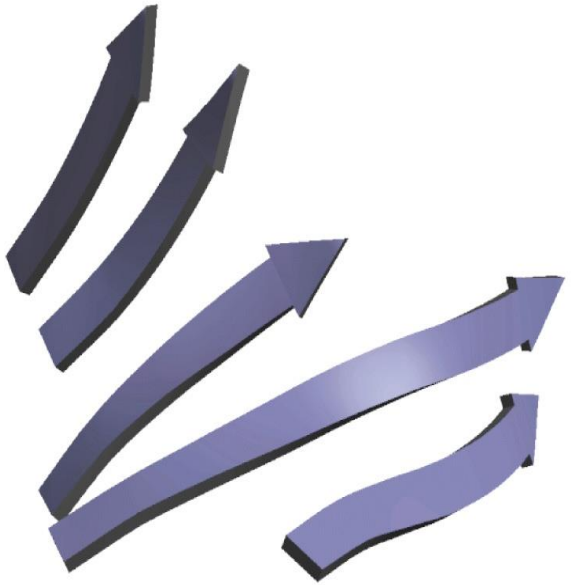


Crossover connection (not observed)

Two arrangements of β strands stabilized by tendency of the strands to twist. (most stable right-handed twist).
Twisting of ß sheet leads to twisting of the structure (when the segments are put together.

Both form the core of many proteins.

(d) β Barrel

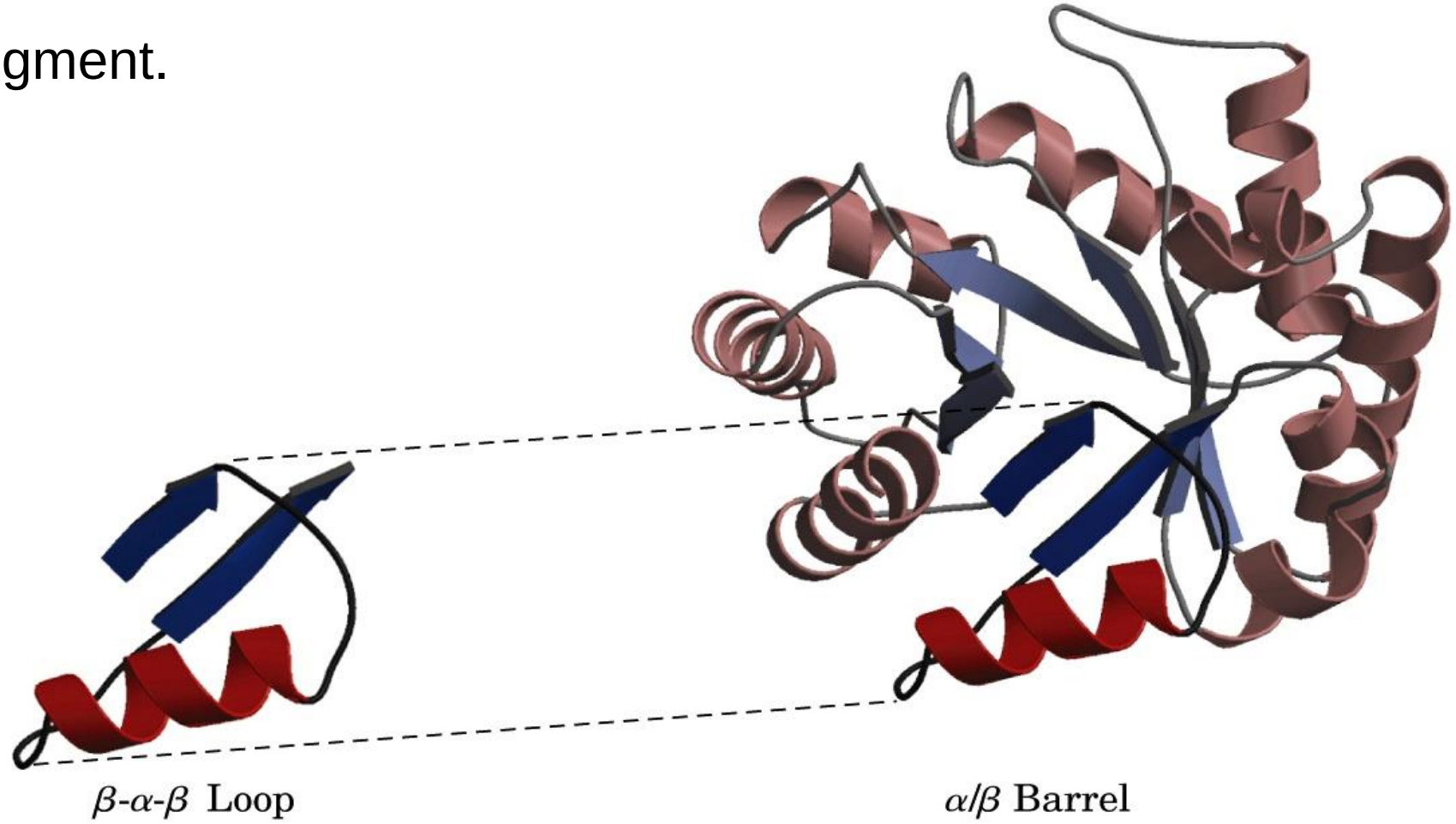


Twisted β sheet

Constructing large motifs from smaller ones:

The α/β barrel (of pyruvate kinase) is a common motif constructed from repetitions of the simpler β-α-β domain.

Each parallel ß segment attached to its neighbor by an α helical segment.



Structural classification of proteins (SCOP) database.

Protein Family:
Proteins with significant sequence similarity, and/or similar structure / function.

Protein superfamilies:
Little primary sequence similarity, but have the same major motifs/ structural similarities and some functional similarities.

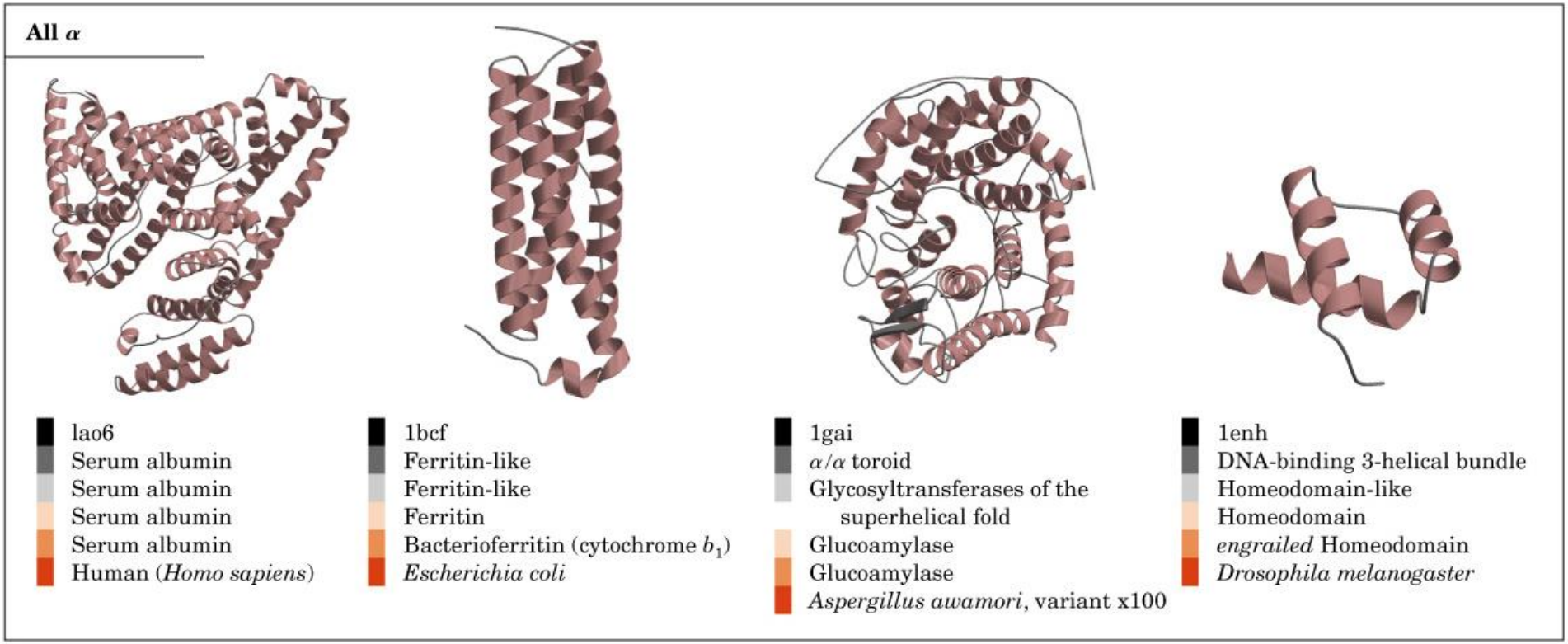
Protein structures divided into **4** classes:

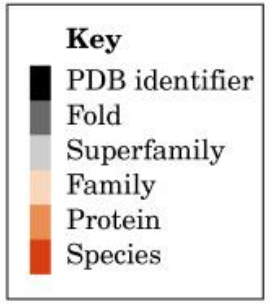
1) All α
2) All ß
3) α/ß
4) α+ß

Organization of proteins based on motifs:

Hundreds of known stable motifs divided into four classes:

1)

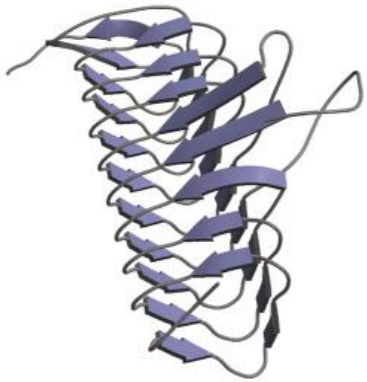




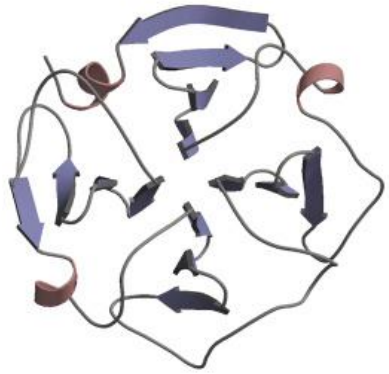
2)

All β

1hoe
α-Amylase inhibitor
α-Amylase inhibitor
α-Amylase inhibitor
HOE-467A
Streptomyces tendae 4158

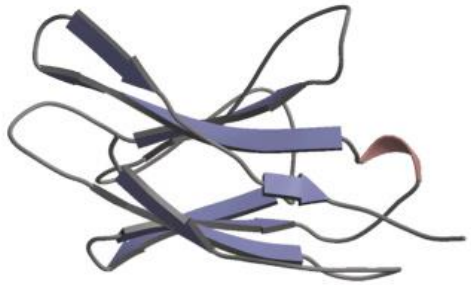


1lxa
Single-stranded left-handed β helix
Trimeric LpxA-like enzymes
UDP *N*-acetylglucosamine acyltransferase
UDP *N*-acetylglucosamine acyltransferase
Escherichia coli



1pex
Four-bladed β propeller
Hemopexin-like domain
Hemopexin-like domain
Collagenase-3 (MMP-13), carboxyl-terminal domain
Human (*Homo sapiens*)

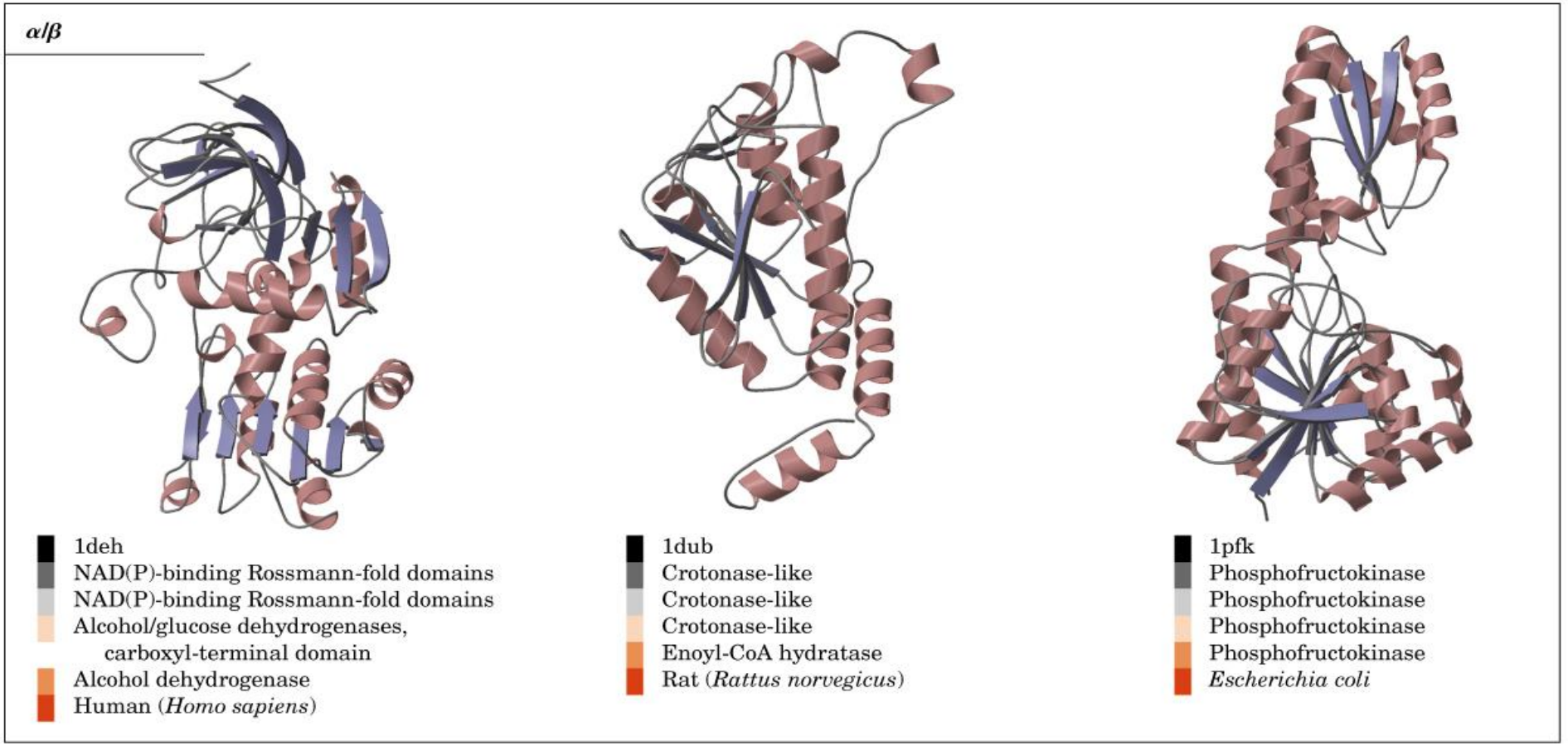
1jpc
β-Prism II
α-D-Mannose-specific plant lectins
α-D-Mannose-specific plant lectins
Lectin (agglutinin)
Snowdrop (*Galanthus nivalis*)



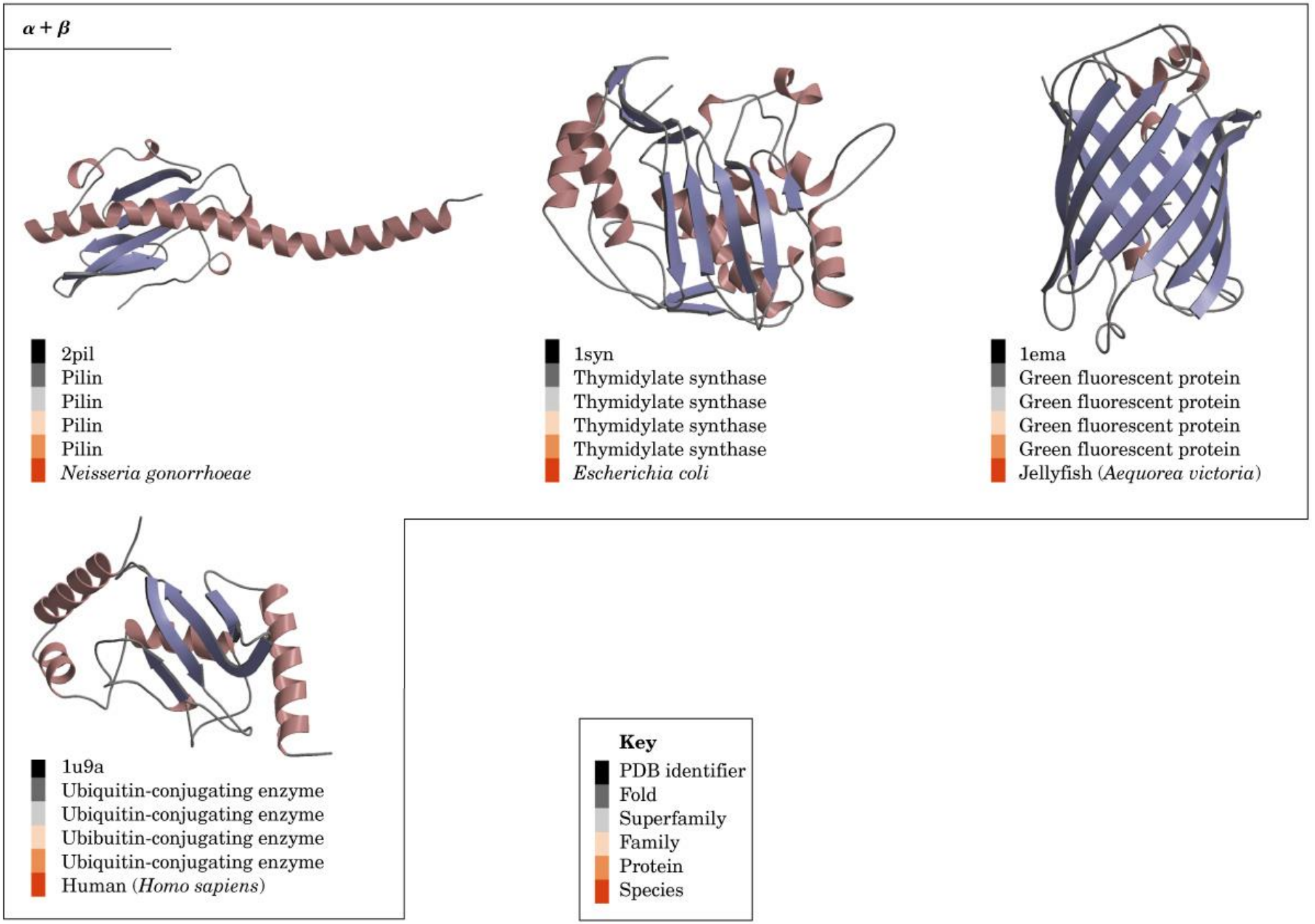
1cd8
Immunoglobulin-like β sandwich
Immunoglobulin
Antibody variable domain-like
CD8
Human (*Homo sapiens*)

Key
PDB identifier
Fold
Superfamily
Family
Protein
Species

3) Alpha, beta are alternate. ßαßα



4) Alpha and beta regions are restricted:



Quaternary Structure:

Association of many polypeptide chains.

Separate subunits can have separate but related function.

Catalysis + regulation.

Quaternary structure of hemoglobin:

Space filling model and Ribbon representation.

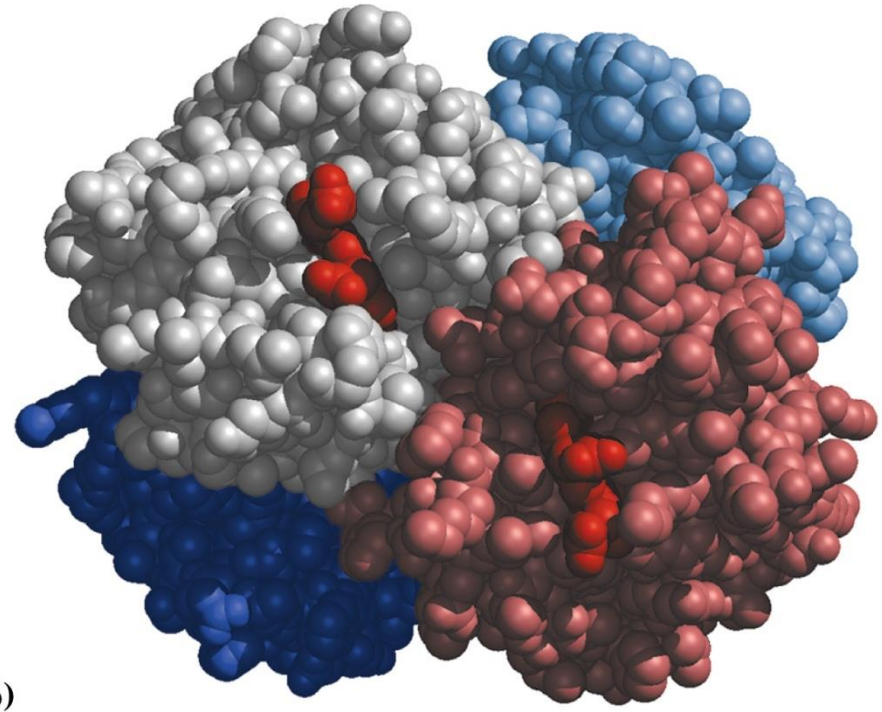
α subunits (141 residues each) (gray +light blue)

β subunits (146 residues each) (pink +dark blue)

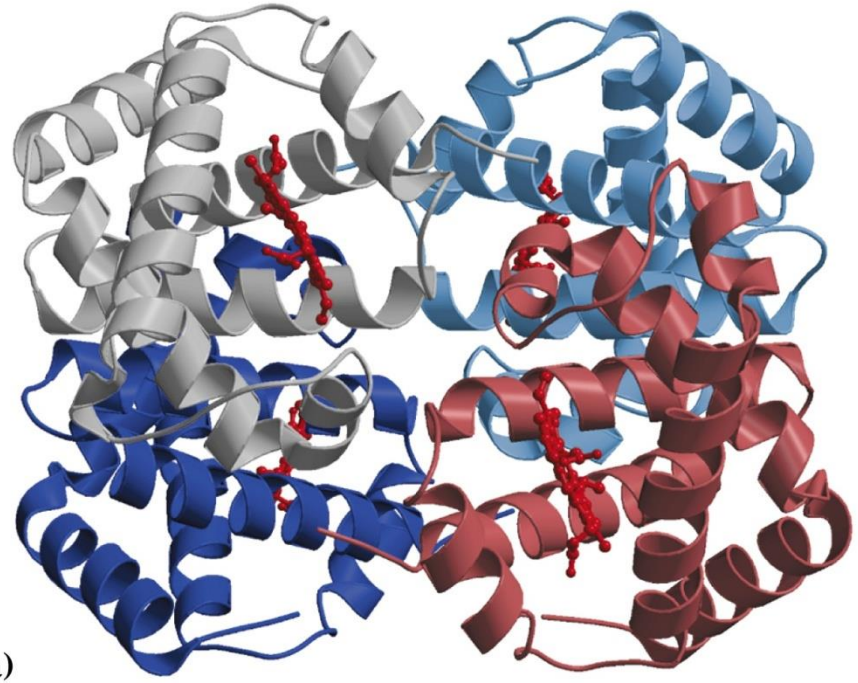
4 Heme (red) + globin

Tetramer. symmetric pairs, dimer of α β protomers

4 times > myoglobin



(b)



(a)

Multisubunit protein = Multimer 2- hundreds of subunits.

Few subunits = oligomer.

Repeating subunit = protomer.

Nonidentical subumits = asymmetric.

Identical subunits = symmetric.

Multisubunit proteins can have:

Rotational symmetry / helical symmetry.

Individual subunits can be superimposed on others by rotation on one/ more rotational axis or by helical rotation.

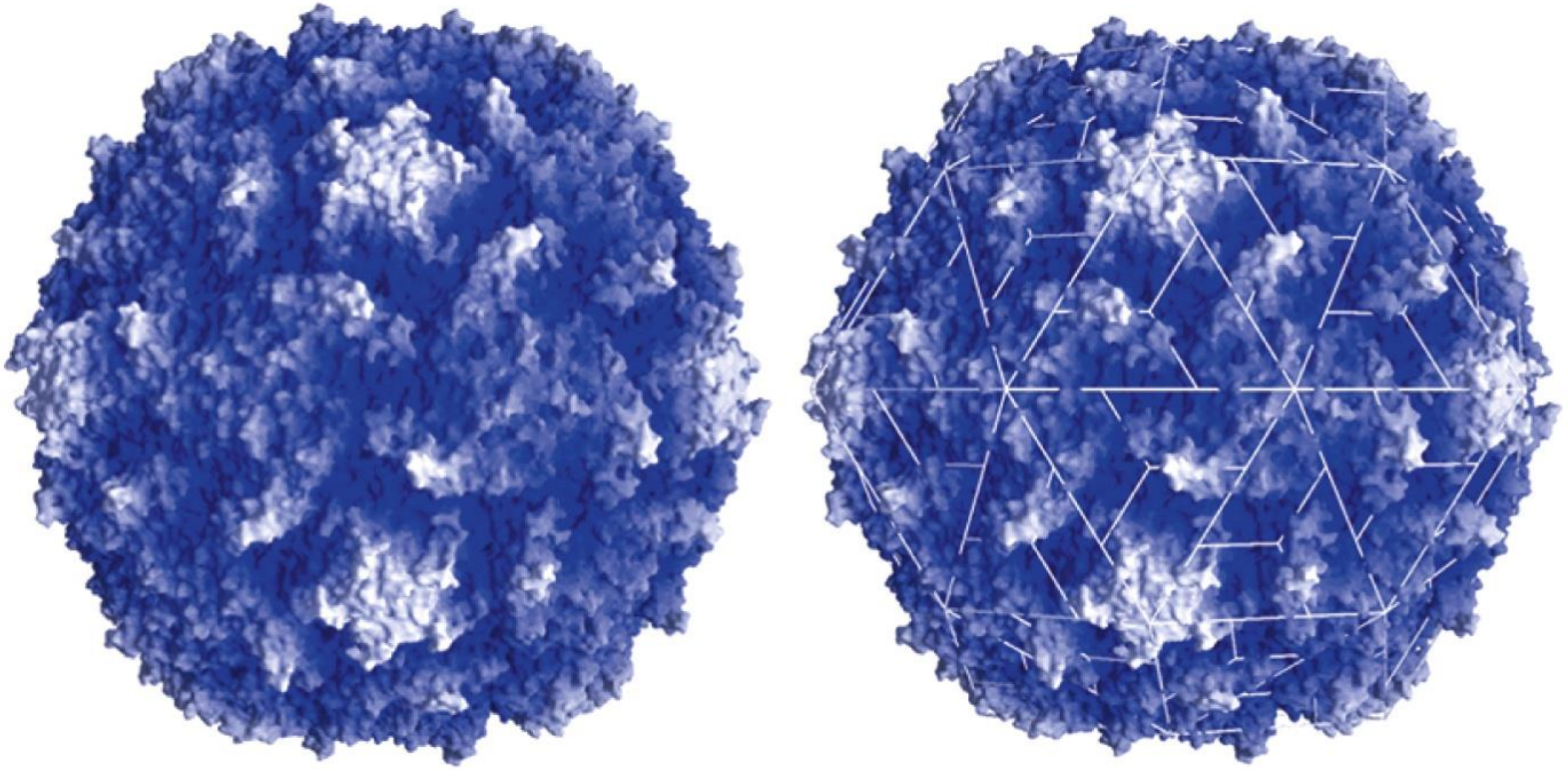
Helical symmetry:

Viral capsids:

Poliovirus (PDB ID 2PLV)

Left: surface contour image.

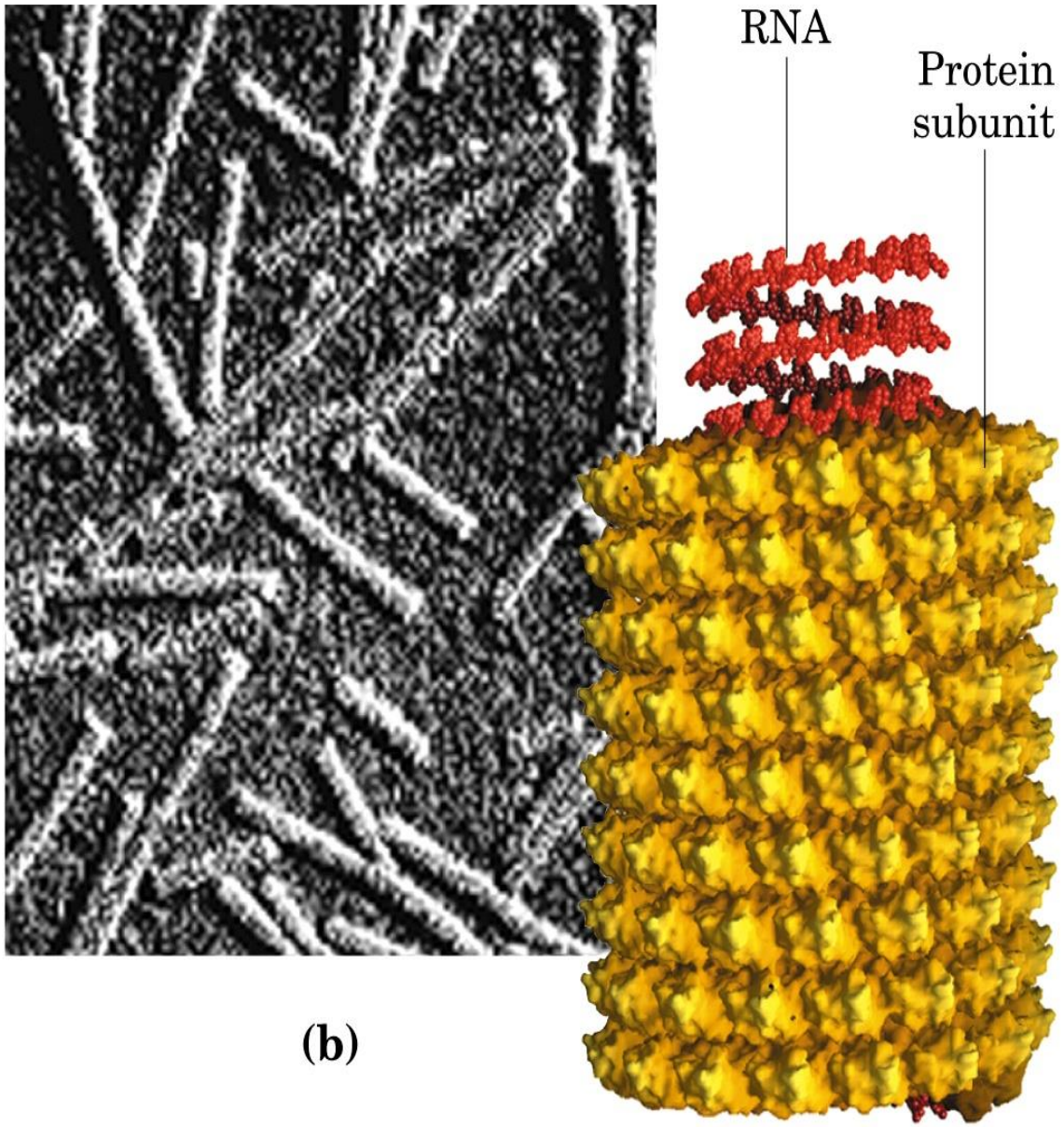
Right: lines superimposed to show axis of symmetry.



(a)

Rotational symmetry: proteins form structures that are open-ended with subunits added in spiraling array.

Tobaco mosaic virus: PDB ID 1VTM rod shaped virus with Right-handed helical symmetry.



Protein denaturation and folding:

Denaturation: loss of three-dimensional structure sufficient to cause loss of function. Mild treatment no covalent bond breaking.

Denaturation by:

1) heat → weak interactions e.g. H bond . Cooperative effect:

Loss of function of one part → destabilizes other parts.

2) pH →alter net charge of protein →electrostatic repulsion+ disrupt H-bond

3) Organic solvents alcohol, acetone.

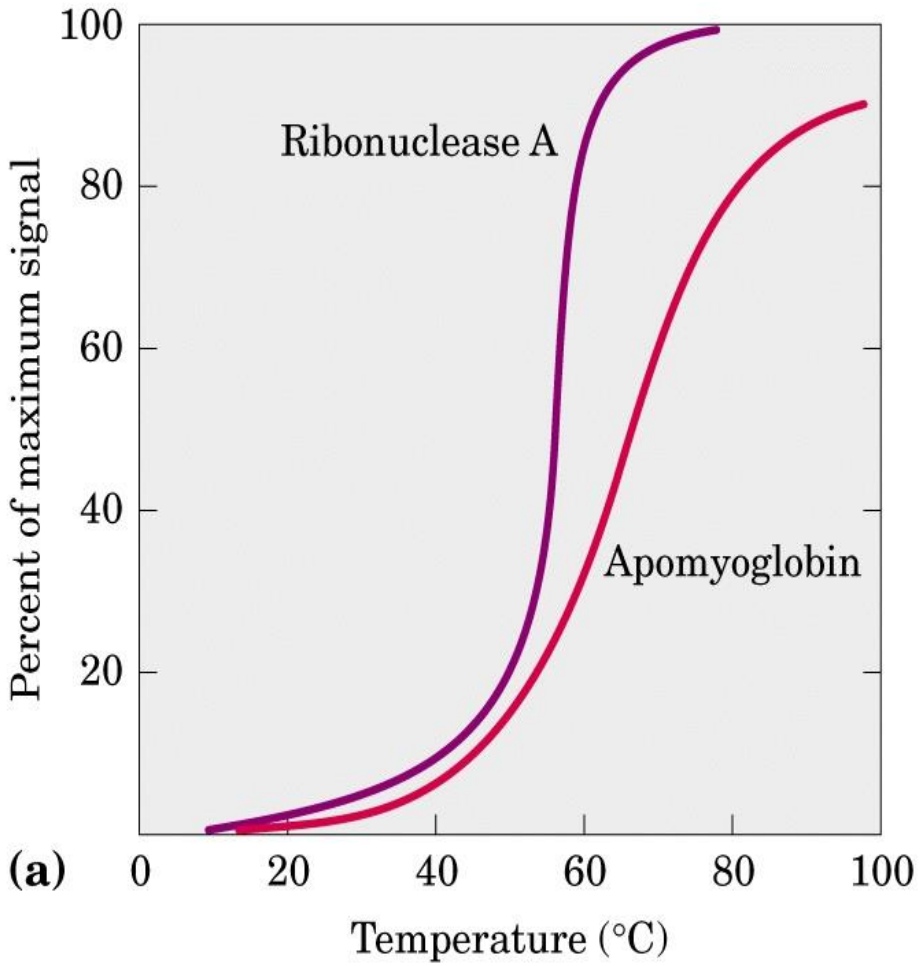
4) Solutes urea, guanidine hydrochloride, detergents.

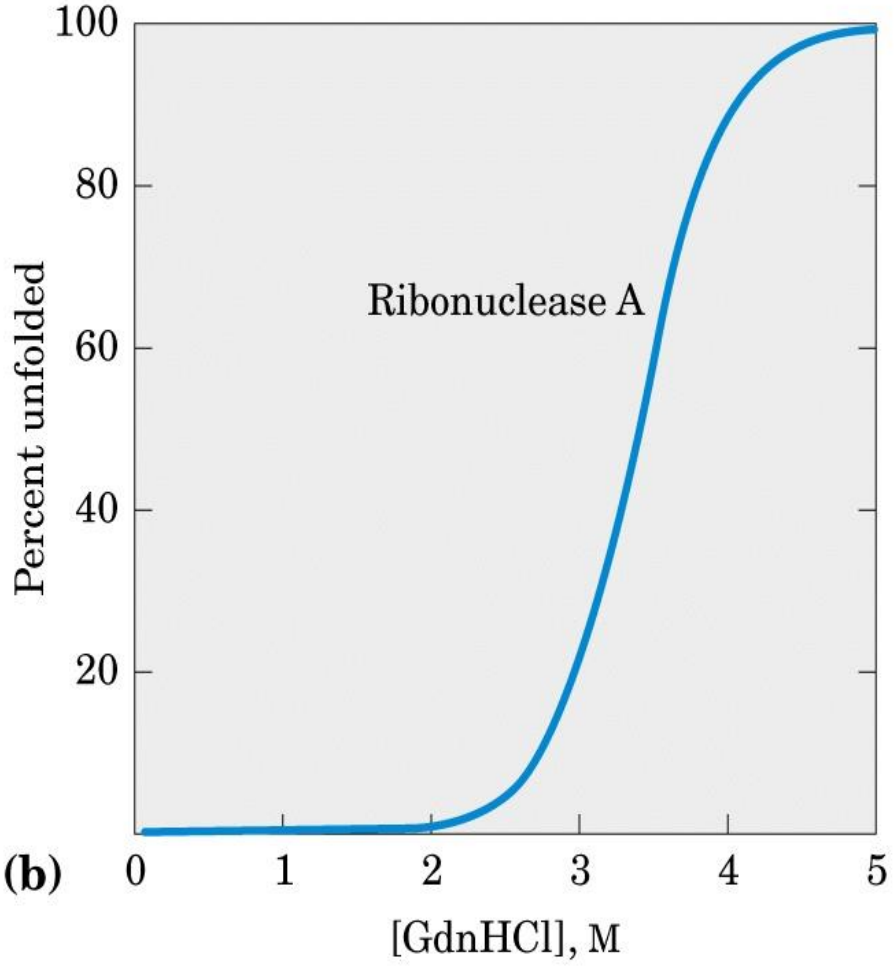
Disrupts hydrophobic interactions that make up stable core of globular proteins.

Protein denaturation:

Apomyoglobin (myoglobin – heme).

Denaturation of disulfide bond by guanidine hydrochloride.





Renaturation of unfolded denatured ribonuclase:

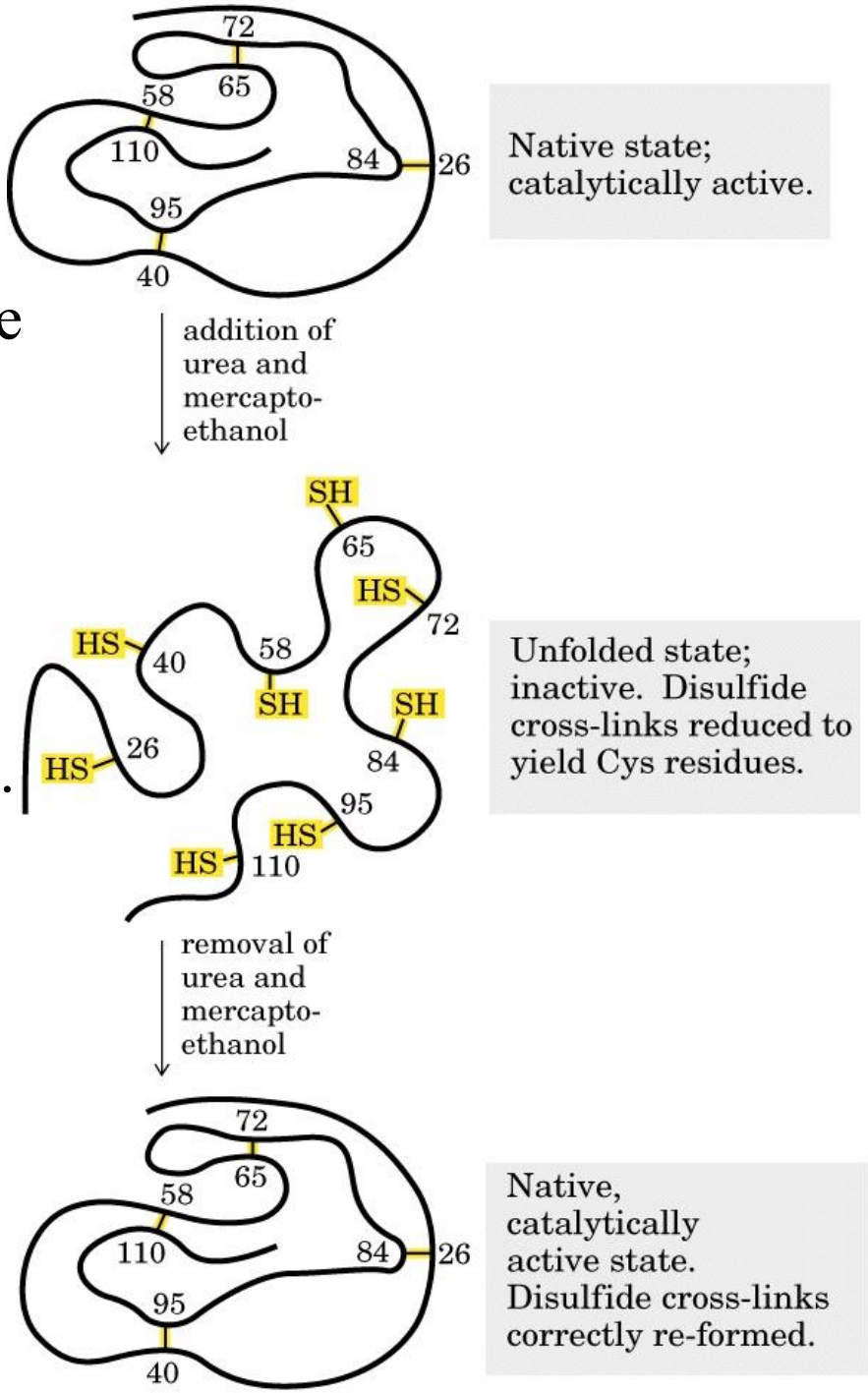
Renaturation: maintain/regain the native structure + biological activity.

Urea used to denature ribonuclease.

Mercaptoethanol reduce and cleave disulfide bond. Yielding 8 Cys residues.

Renaturation →reestablishement of the correct disulfide bond.

Proof primary structure determine tertiary structure.



In living cells folding very fast.

In E coli 100 a.a in 5 sec at 37^{0}C.

Levinthals paradox :

Protein folding cant be random / trial –and-error process.

1st Local secondary structures form:

Certain a.a fold into α helices / ß sheets guided by constrains

2nd Local supersecondary structures :

e.g 2 α helices.

Defects in protein folding :

1) Cystic fibrosis genetic defect in cystic fibrosis transmembrane regulator (CFTR)= a channel for Cl ions.

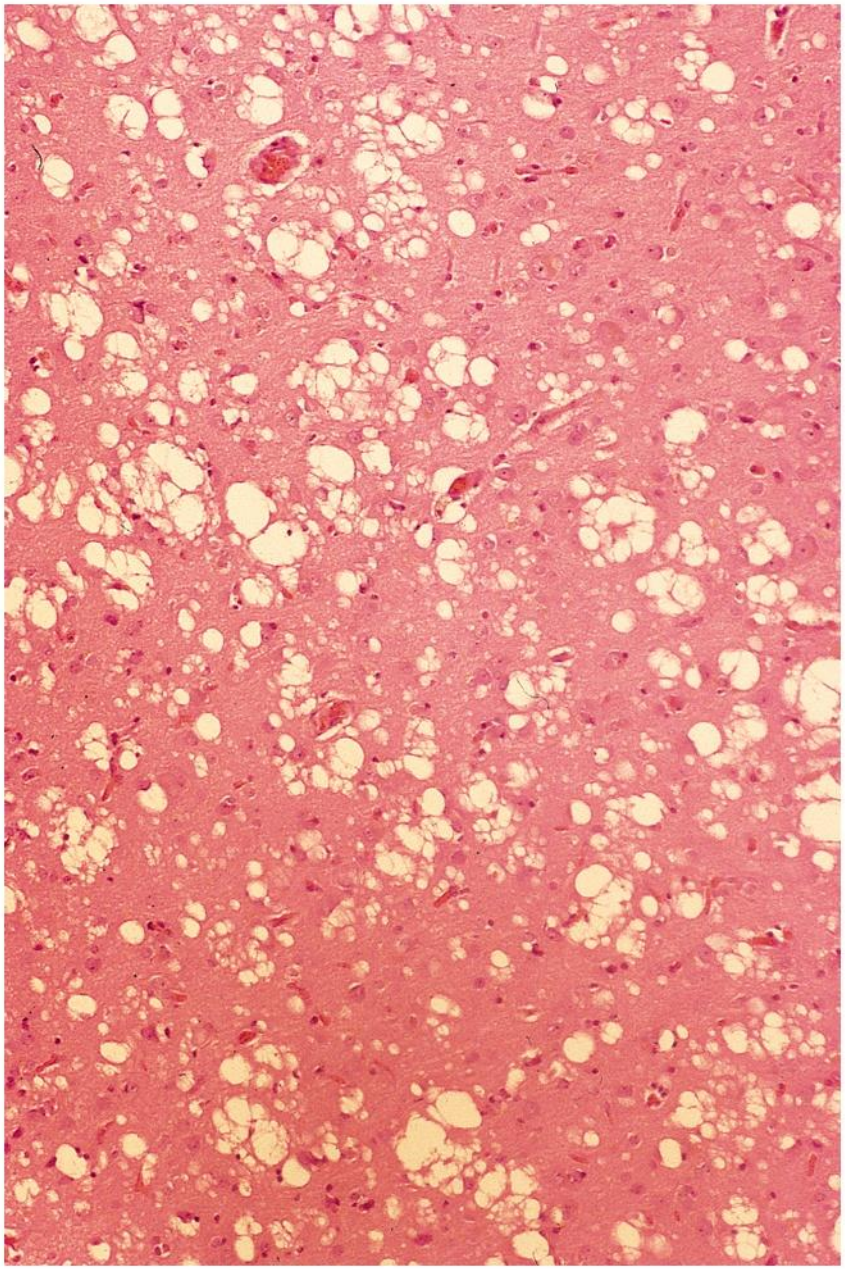
Mutation = deletion in Phe at position 508 → improper protein folding.

2) Osteogenesis imperfecta

Misfolded protein→ causative agent of a number of rare degenerative brain disease in mammals. Mad cow disease.

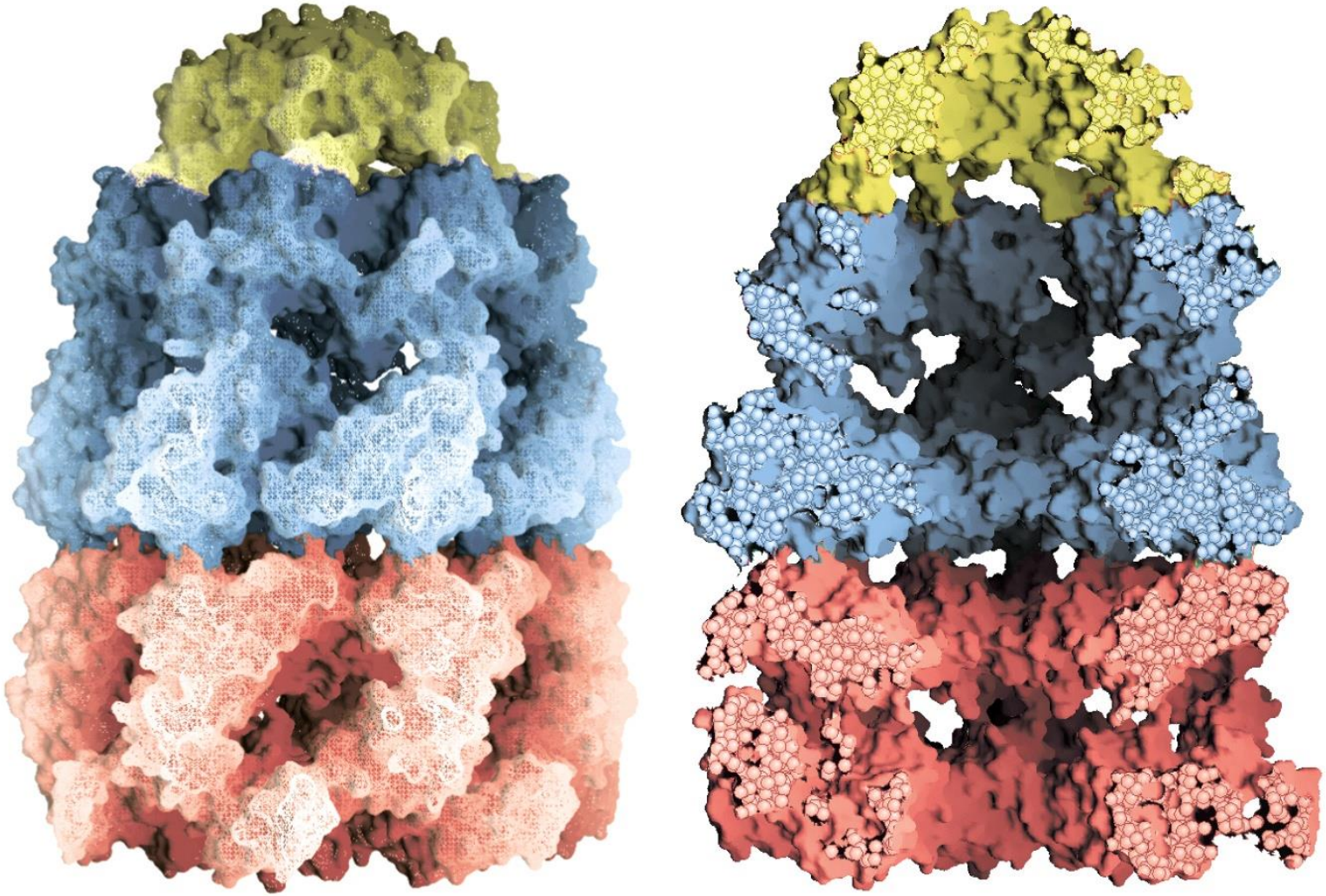
Stained section of a patient with Creutzfeldt-jakob disease.
(riddled with holes).
Prion protein in brain tissue.

Spongiform encephalopathy.

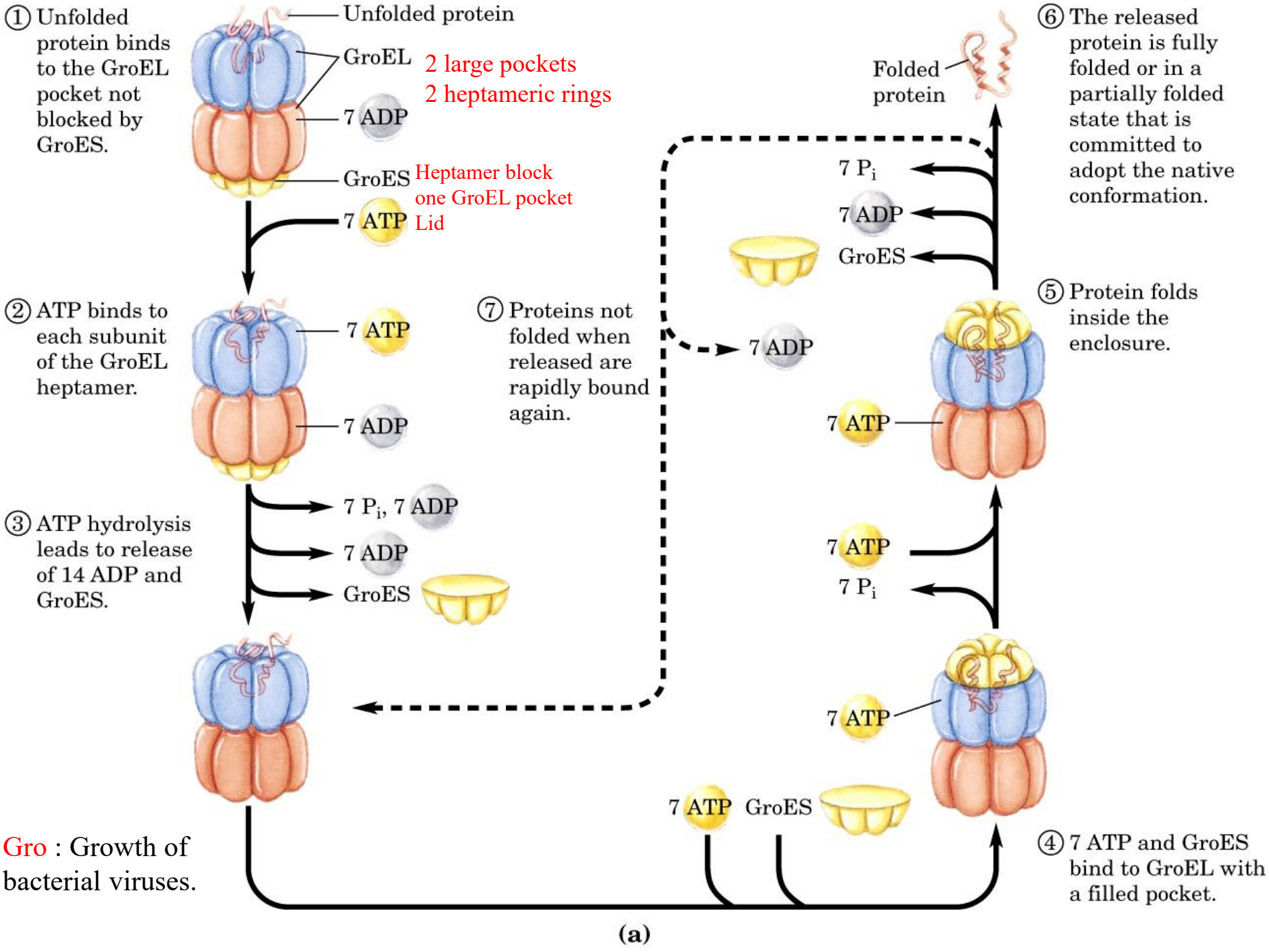


Chaperonins in protein folding:

Surface and cut away images.



(b)


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

Proper folding require 2 enzymes:

1) Protein disulfide isomerase PDI :

   Disulfide bond formation shuffling until reach native conformation + elimination of inappropriate disulfide cross- links.

2) Peptide prolyl cis-trans isomerase:

   interconversion of cis-trans Pro peptide bonds.