Protein Folding – Tertiary and Quarternary Structures

Lecture 7

Acknowledgements:
Alberts - Molecular Biology of the Cell
Scitable by Nature Education
Nature Resources
Internet resources

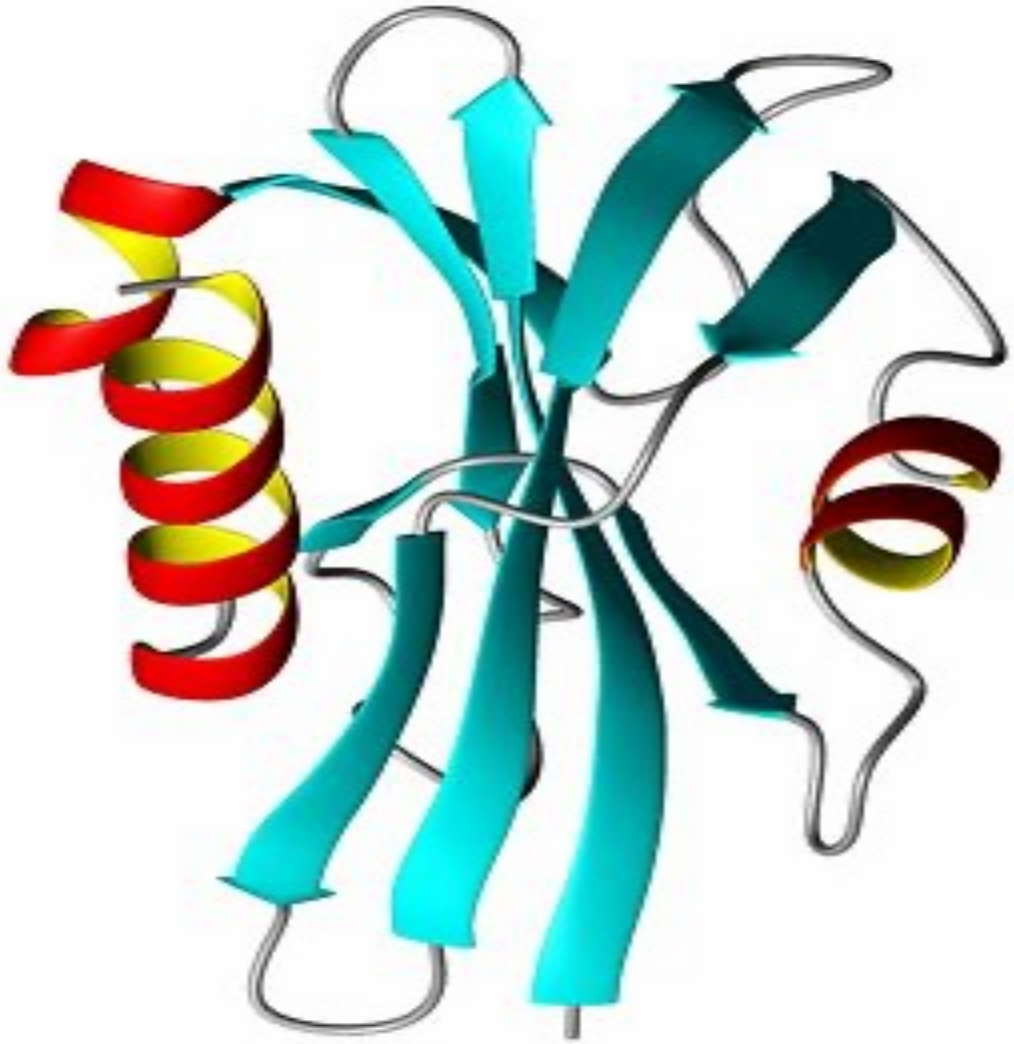
OBJECTIVE OF THE LECTURE

1. 3-D conformation – structure and function
2. Tertiary structures and fold symmetry
3. Protein Denaturation and re-folding
4. Thermodynamic of Protein Folding
5. Chaperone Proteins

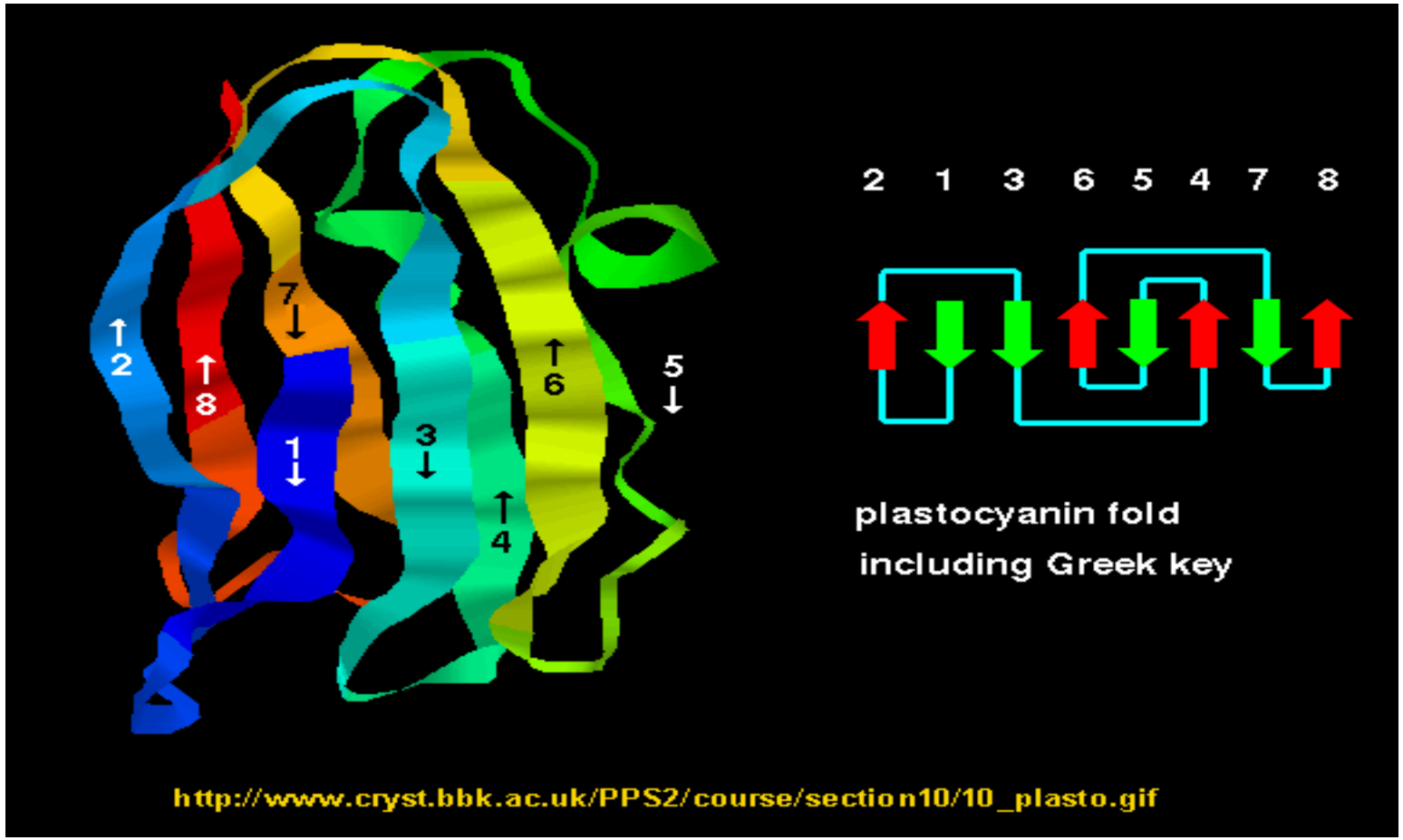
3-D structure of Proteins

1. the three-dimensional structure of a protein is determined by its amino acid sequence
2. the function of a protein depends on its structure
3. an isolated protein usually exists in one or a small number of stable structural forms
4. the most important forces stabilizing the specific structures maintained by a given protein are noncovalent interactions

Protein Structure of Beta Sheets and Alpha Helices

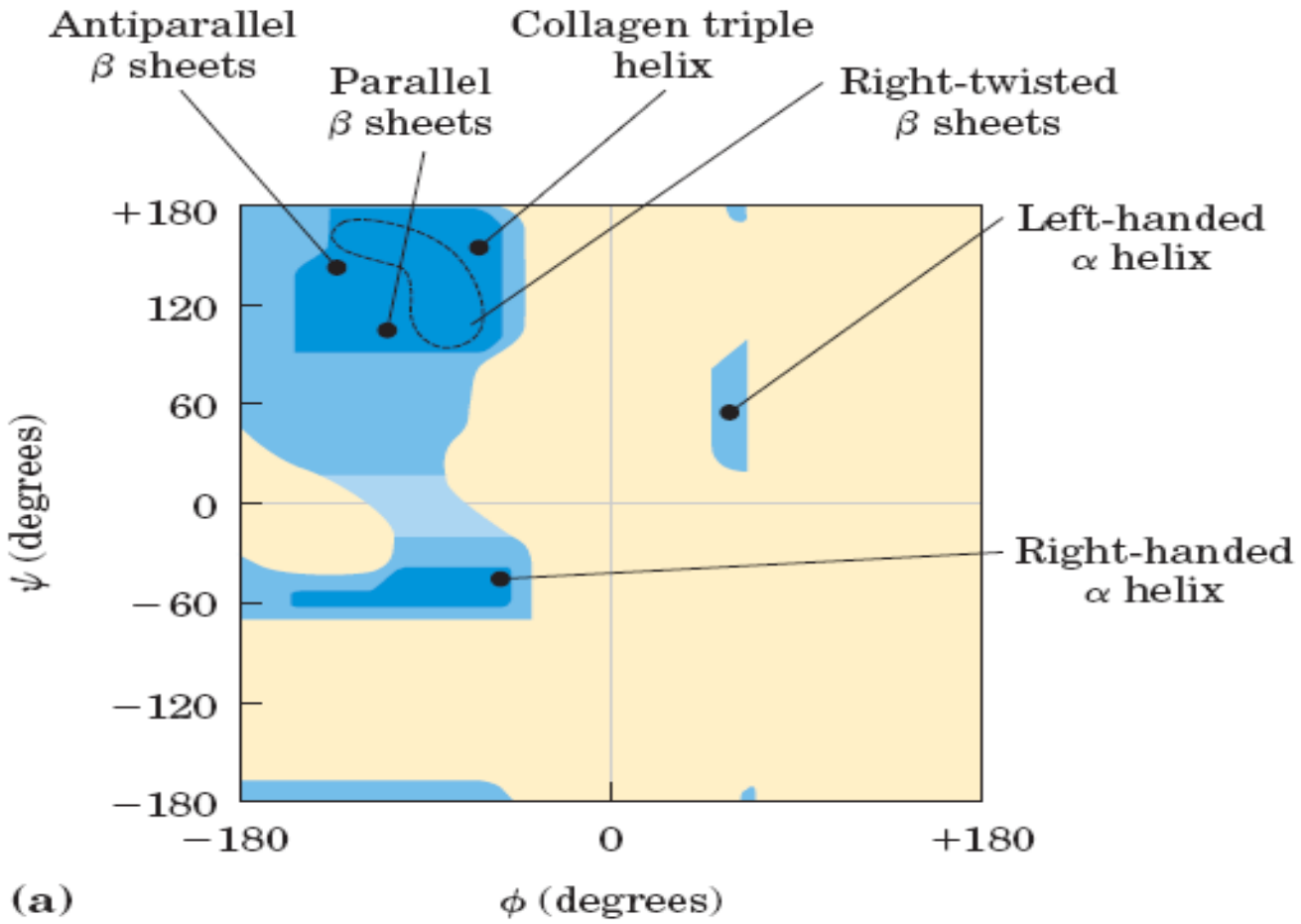


Greek Key Motif

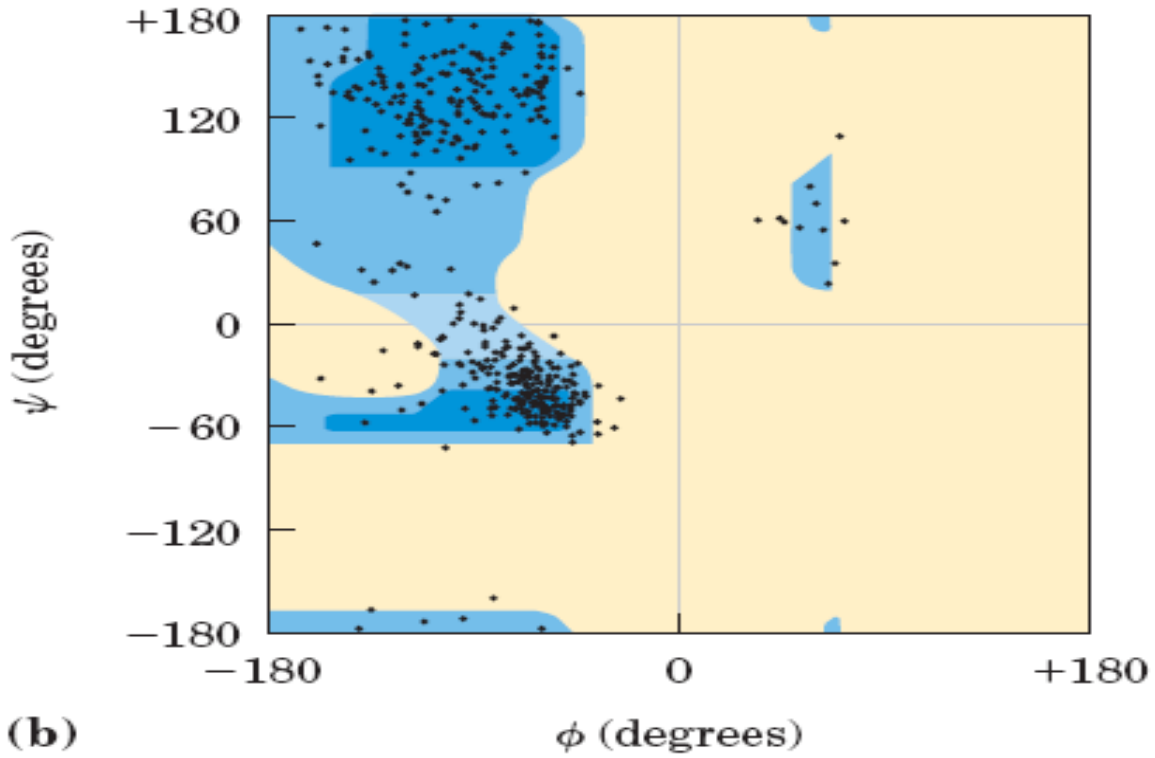


Secondary Protein Structure

- Secondary structure is the regular arrangement of amino acid residues in a segment of a polypeptide chain, in which each residue is spatially related to its neighbors in the same way
- The most common secondary structures are the alpha helix, the beta conformation, and beta turns
- The secondary structure of a polypeptide segment can be completely defined if the phi and psi angles are known for all amino acid residues in that segment



- The values of ϕ and ψ for various allowed secondary structures are overlaid
  - Although left-handed helices extending over several amino acid residues are theoretically possible, they have not been observed in proteins



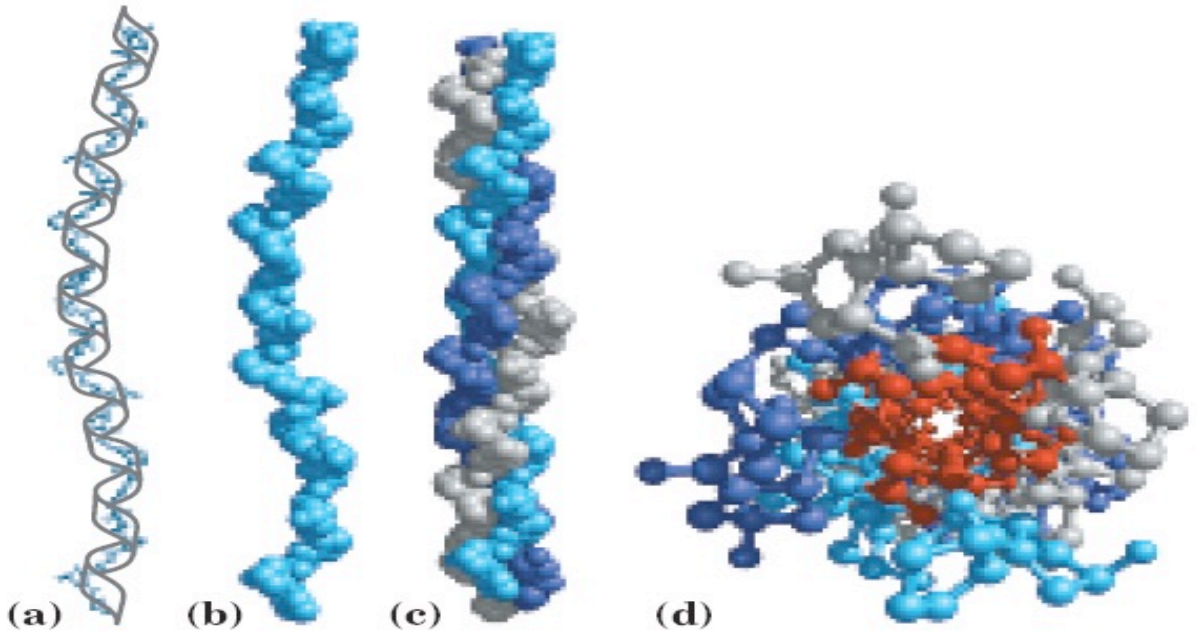
- All the amino acid residues except Gly in the enzyme pyruvate kinase (isolated from rabbit) are overlaid on the plot of theoretically allowed conformations. The small, flexible Gly residues were excluded because they frequently fall outside the expected ranges

Tertiary and Quaternary Structures

- The overall three-dimensional arrangement of all atoms in a protein is referred to as the protein's **tertiary structure**
- Some proteins contain two or more separate polypeptide chains, or subunits, which may be identical or different. The arrangement of these protein subunits in three-dimensional complexes constitutes **quaternary Structure**
  - In considering these higher levels of structure, it is useful to classify proteins into two major groups:
    - **fibrous proteins,** having polypeptide chains arranged in long strands or sheets, and
    - **globular proteins,** having polypeptide chains folded into a spherical or globular shape.

Fibrous Proteins: α-keratin, collagen

- Collagen: has evolved to provide strength. It is found in connective tissue such as tendons, cartilage, the organic matrix of bone, and the cornea of the eye
- Collagen is also a coiled coil, but one with distinct tertiary and quaternary structures: three separate polypeptides, called α-chains (not to be confused with α-helices), are supertwisted about each other
- The tight wrapping of the chains in the collagen triple α-helix provides tensile strength greater than that of a steel wire of equal cross-section



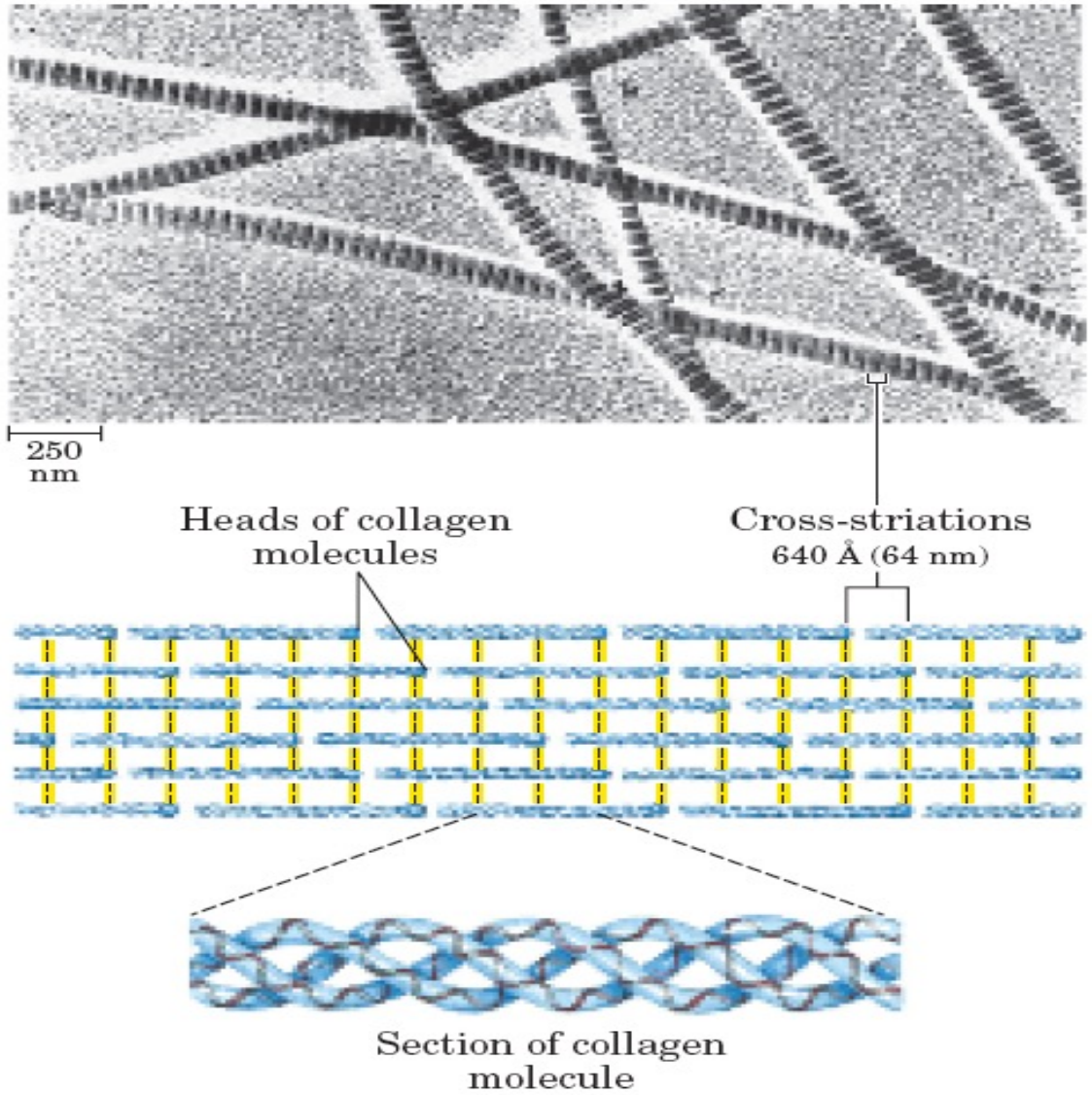
a) The repeating tripeptide sequence Gly–X–Pro or Gly–X–4-Hyp adopts a left-handed helical structure with three residues per turn. The repeating sequence used to generate this model is Gly–Pro–4-Hyp

b) Space-filling model of the same chain

c) Three of these helices (shown here in gray, blue, and purple) wrap around one another with a right-handed twist

d) The three-stranded collagen superhelix shown from one end, in a ball-and-stick representation. Gly residues are shown in red

Structure of Collagen



Collagen arrangement in a fish scale

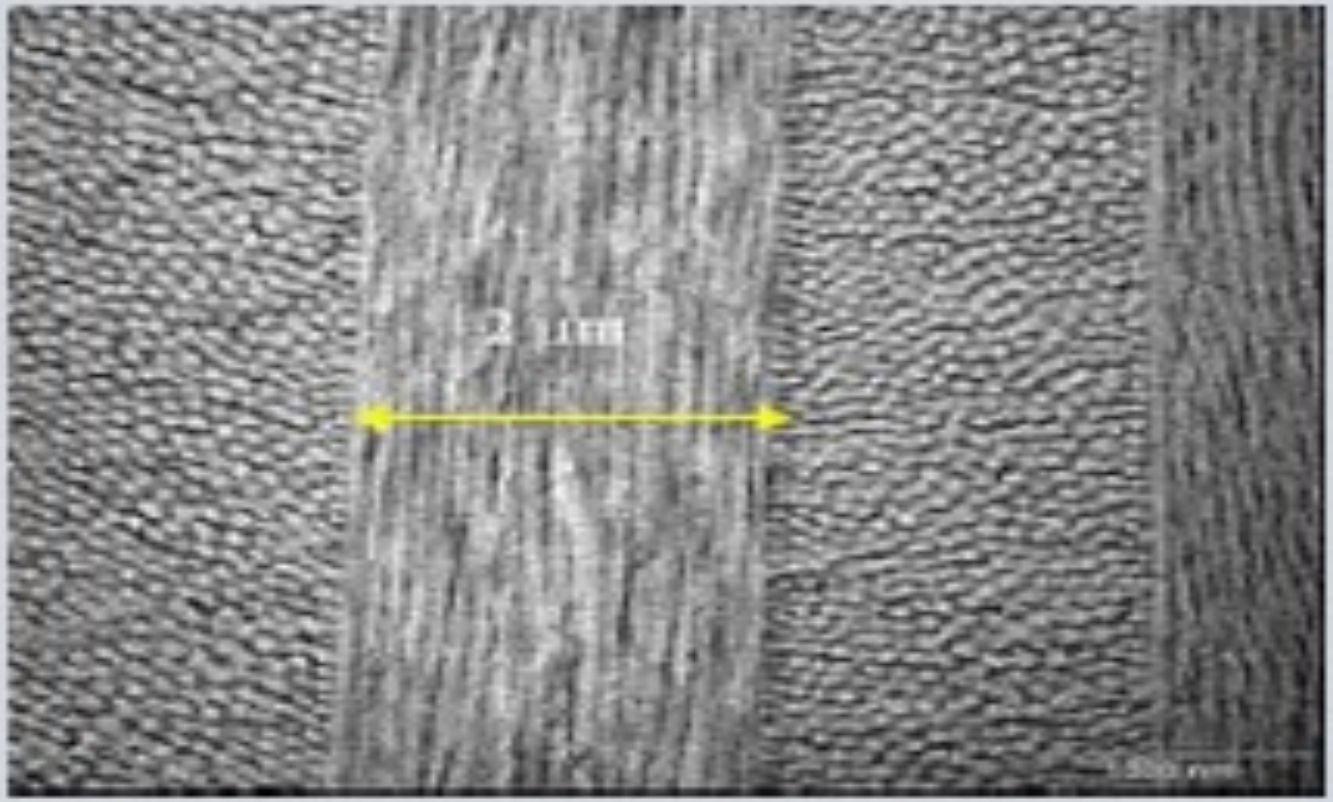


Fig.1: Transmission electron microscopic image of structure of the inside of fish scale. The lining-up structure of collagen-fibril sheets with alternate rotation of 90 degrees was observed.

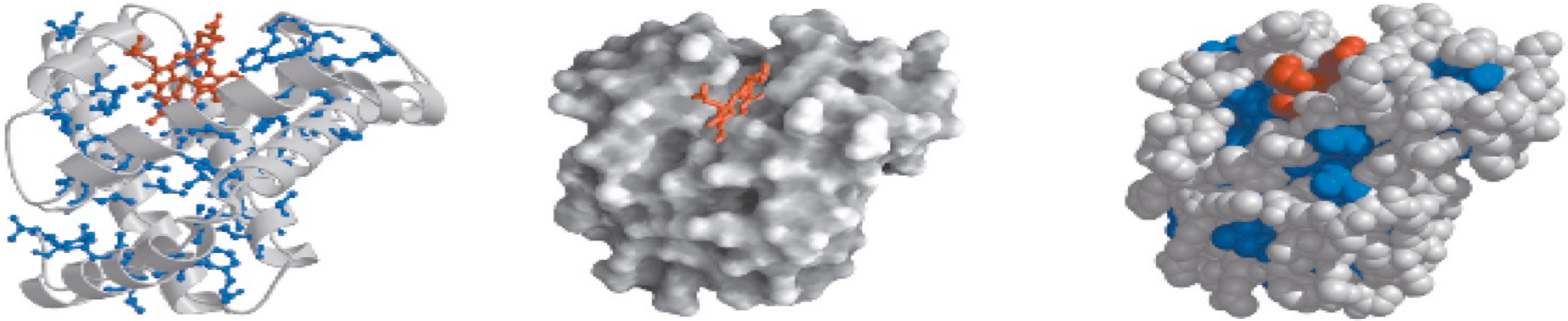
Globular Proteins

- Folding generates a compact form relative to polypeptides in a fully extended conformation
- The folding also provides the structural diversity necessary for proteins to carry out a wide array of biological functions
- Globular proteins include enzymes, transport proteins, motor proteins, regulatory proteins, immunoglobulins, and proteins with many other functions.

Globular Protein Structure - Myoglobin

- The first breakthrough in understanding the three-dimensional structure of a globular protein came from x-ray diffraction studies of myoglobin carried out by John Kendrew and his colleagues in the 1950s
- Myoglobin contains a single polypeptide chain of 153 amino acid residues of known sequence and a single iron protoporphyrin, or heme, group
  - The same heme group is found in hemoglobin, the oxygen-binding protein of erythrocytes, and is responsible for the deep red-brown color of both myoglobin and hemoglobin.

Different visualizations of Myoglobin

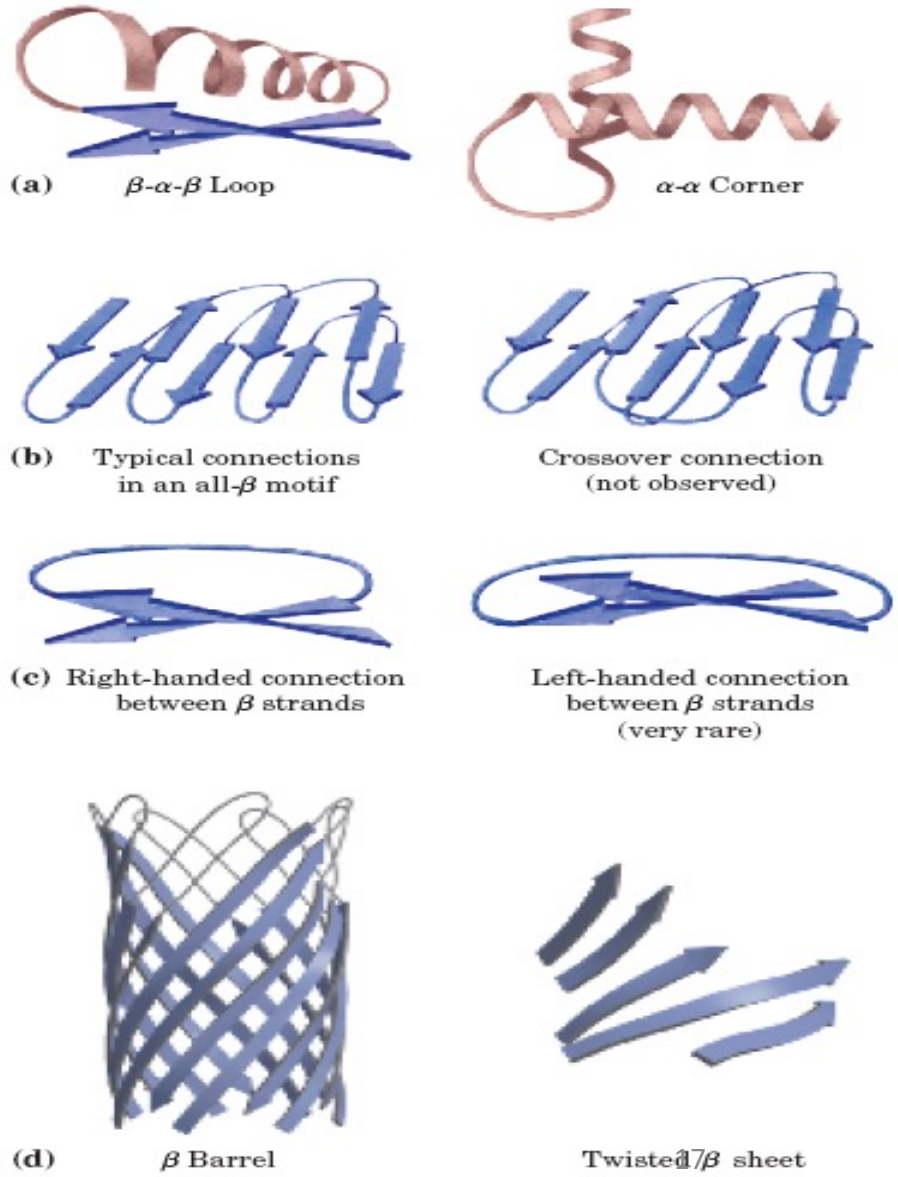


Tertiary structure of sperm whale myoglobin

- A ribbon representation, including side chains (blue) for the hydrophobic residues Leu, Ile, Val and Phe
- A surface contour image is useful for visualizing pockets in the protein where other molecules might bind
- A space-filling model with all amino acid side chains. Each atom is represented by a sphere encompassing its van der Waals radius. The hydrophobic residues are again shown in blue; most are not visible, because they are buried in the interior of the protein

Common Structural Patterns

- **Supersecondary structures,** also called **motifs** or simply **folds,** are particularly stable arrangements of several elements of secondary structure and the connections between them
- A single large motif may comprise the entire protein
  - coil of –keratin
- Motifs of different types occur based on structural constraints



Rules for Folding

1. Hydrophobic interactions make a large contribution to the stability of protein structures. Burial of hydrophobic amino acid R groups so as to exclude water requires at least two layers of secondary structure. Two simple motifs, the β-α-β **loop** and the α-α **corner**, create two layers.
2. Where they occur together in proteins, α-helices and β-sheets generally are found in different structural layers. This is because the backbone of a polypeptide segment in the β conformation cannot readily hydrogen-bond to an α-helix aligned with it

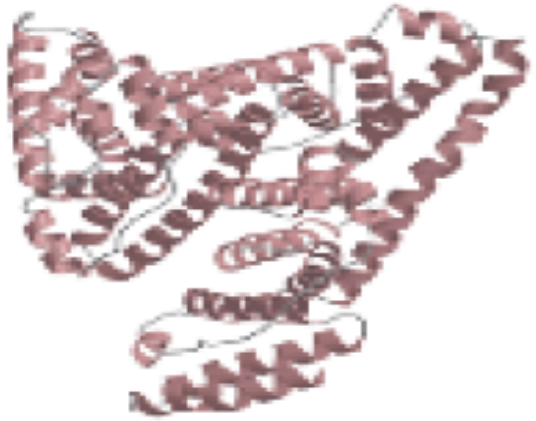
3. Polypeptide segments adjacent to each other in the primary sequence are usually stacked adjacent to each other in the folded structure. Although distant segments of a polypeptide may come together in the tertiary structure, this is not the norm.
4. Connections between elements of secondary structure cannot cross or form knots
5. The β conformation is most stable when the individual segments are twisted slightly in a right handed sense. This influences both the arrangement of β sheets relative to one another and the path of the polypeptide connection between them.

α-Helix and β-Sheet content in different proteins vary

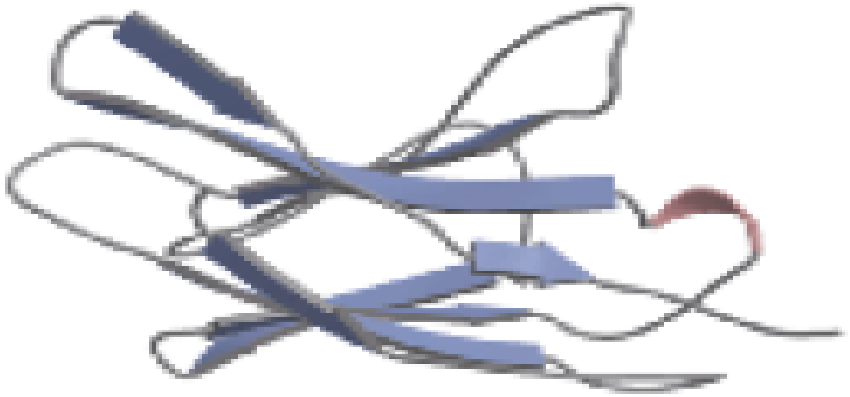
Protein (total residues)	Residues (%)*	
	α Helix	β Conformation
Chymotrypsin (247)	14	45
Ribonuclease (124)	26	35
Carboxypeptidase (307)	38	17
Cytochrome *c* (104)	39	0
Lysozyme (129)	40	12
Myoglobin (153)	78	0

Structural Classification of Proteins

- The Structural Classification of Proteins (SCOP) database offers a good example of this very important trend in biochemistry
  - At the highest level of classification, the SCOP database (http://scop.mrc-lmb.cam.ac.uk/scop) borrows a scheme already in common use
  - all α
  - all β
  - α/β (in which the α and β segments are interspersed or alternate)
  - α and β (in which the and regions are somewhat segregated)

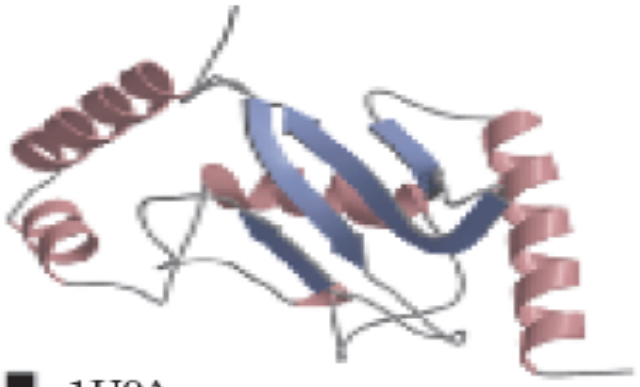


1A06
Serum albumin
Serum albumin
Serum albumin
Serum albumin
Human (*Homo sapiens*)



1CD8
Immunoglobulin-like β sandwich
Immunoglobulin
V set domains (antibody variable domain-like)
CD8
Human (*Homo sapiens*)

1DUB
ClpP/crotonase
ClpP/crotonase
Crotonase-like
Enoyl-CoA hydratase (crotonase)
Rat (*Rattus norvegicus*)



1U9A
UBC-like
UBC-like
Ubibuitin-conjugating enzyme, UBC
Ubiquitin-conjugating enzyme, UBC
Human (*Homo sapiens*) ubc9

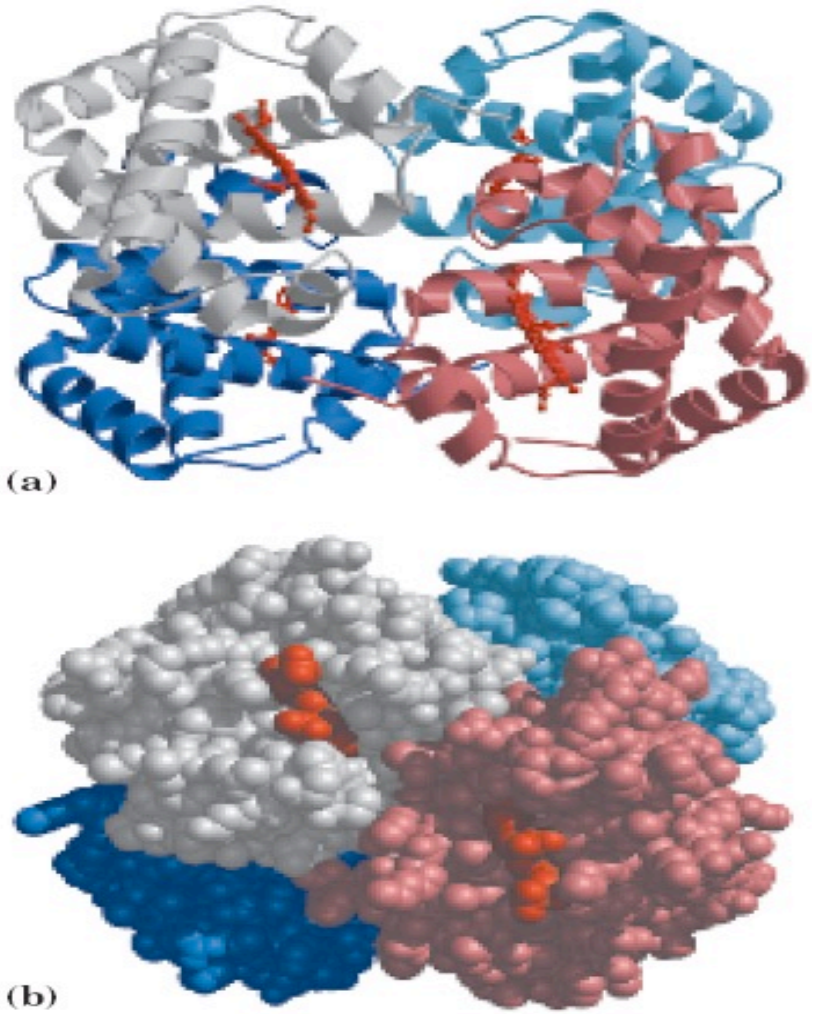
PDB identifier
Fold
Superfamily
Family
Protein
Species

Quarternary Protein Structures

- Many proteins have multiple polypeptide subunits. The association of polypeptide chains can serve a variety of functions.
  - Many multisubunit proteins have regulatory roles
    - The binding of small molecules may affect the interaction between subunits, causing large changes in the protein's activity in response to small changes in the concentration of substrate or regulatory molecules
- A multisubunit protein is also referred to as a **multimer**
- **Few subunits – Oligomers**
- **Repeating subunits - protomers**

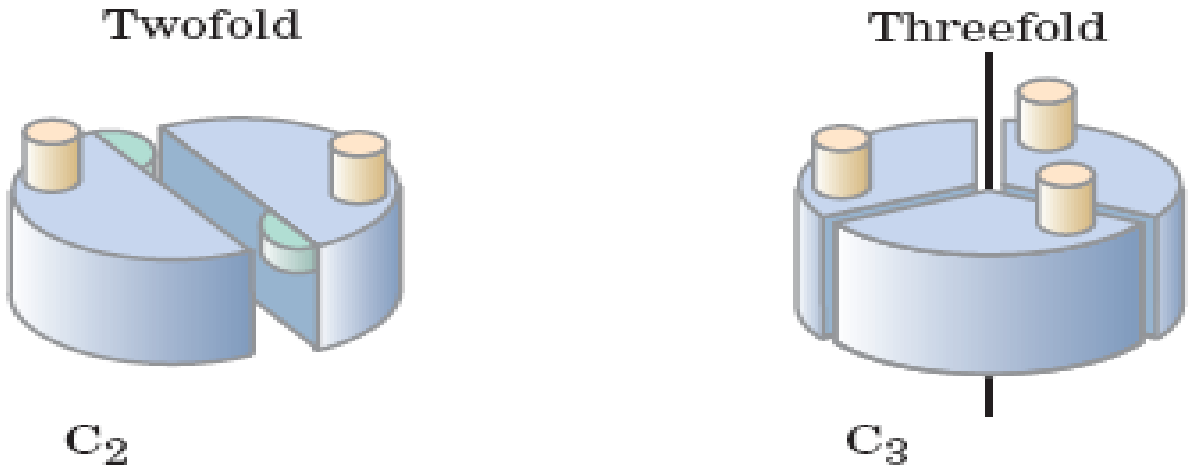
Hemoglobin

- X-ray diffraction analysis of deoxyhemoglobin (hemoglobin without oxygen molecules bound to the heme groups) shows how the four polypeptide subunits are packed together
- A ribbon representation.
- A space-filling model. The α subunits are shown in gray and light blue; the β subunits in pink and dark blue
- the heme groups (red) are relatively far apart



- Identical subunits of multimeric proteins are generally arranged in one or a limited set of symmetric patterns. A description of the structure of these proteins requires an understanding of conventions used to define symmetries.
- Oligomers can have either **rotational symmetry** or **helical symmetry**

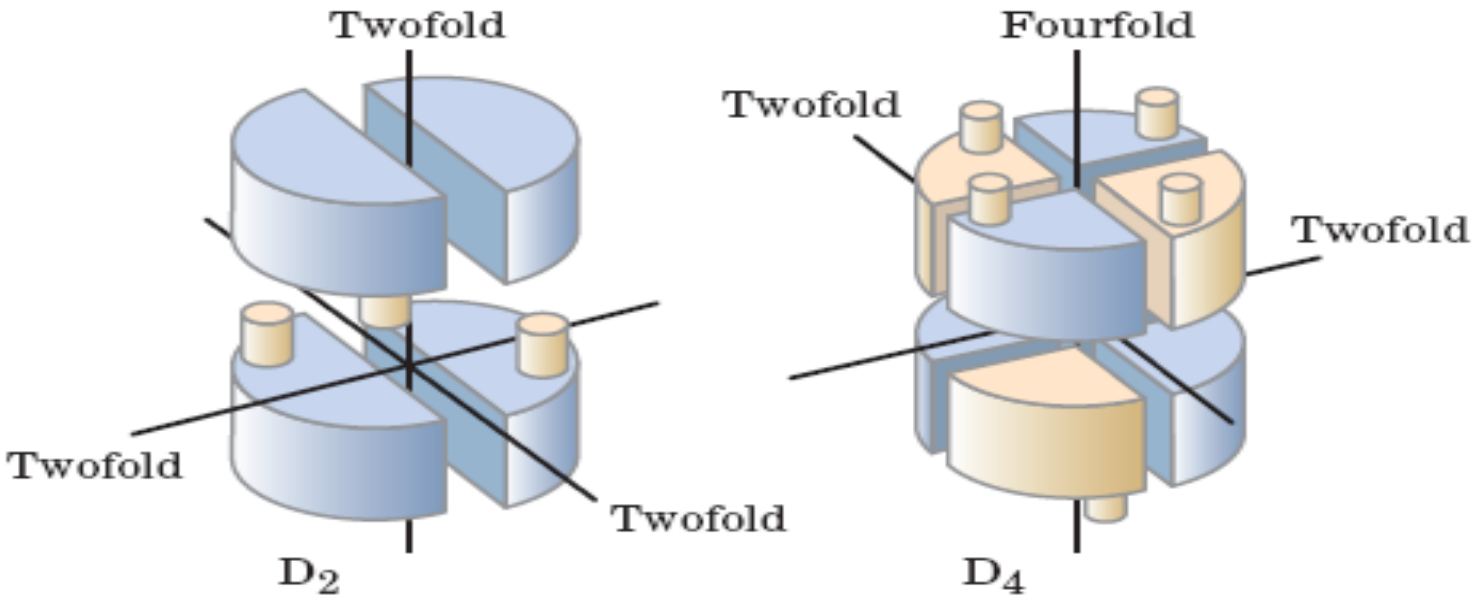
Cyclic Symmetry



Two types of cyclic symmetry

- In cyclic symmetry, subunits are related by rotation about a single *n*-fold axis, where *n* is the number of subunits so related. The axes are shown as black lines; the numbers are values of *n*. Only two of many possible C_n arrangements are shown

Dihedral Symmetry

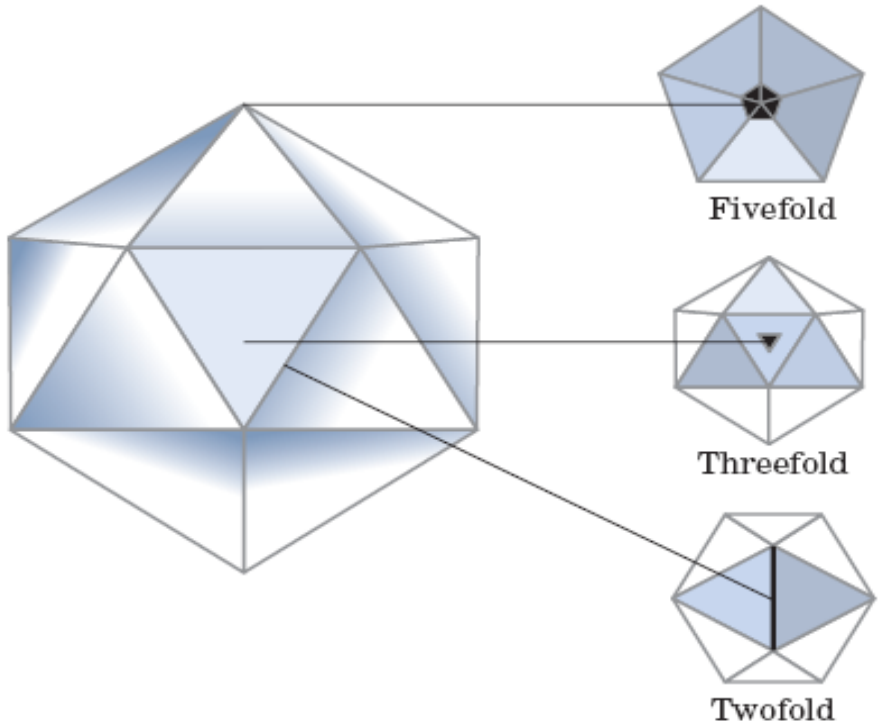


Two types of dihedral symmetry

- In dihedral symmetry, all subunits can be related by rotation about one or both of two axes, one of which is twofold. D_2 symmetry is most common.

Icosahedral Symmetry

- Icosahedral symmetry. Relating all 20 triangular faces of an icosahedron requires rotation about one or more of three separate rotational axes: twofold, threefold, and fivefold.



Icosahedral symmetry

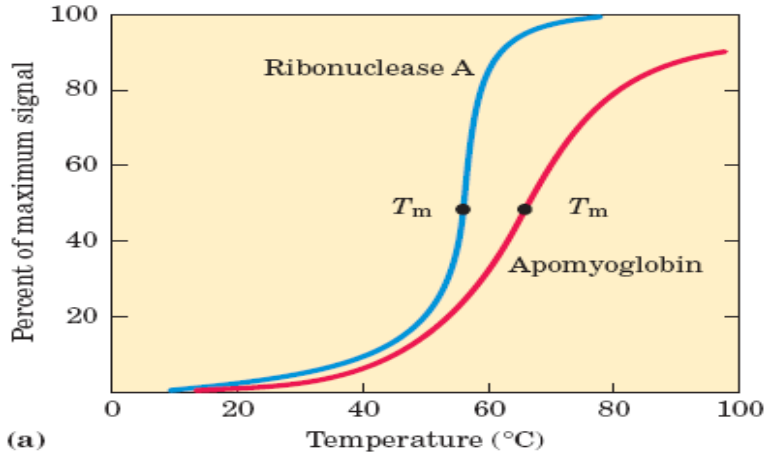
Tertiary and Quarternary Structure

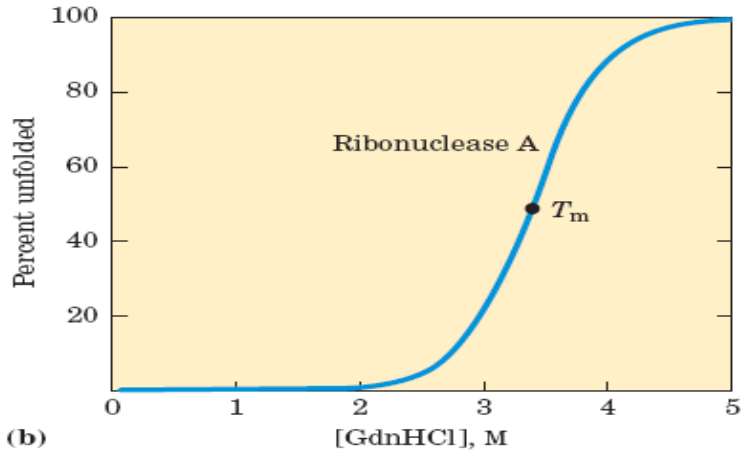
- Tertiary structure is the complete 3-D structure of a polypeptide chain. There are two general classes of proteins based on tertiary structure: fibrous and globular.
- Fibrous proteins, which serve mainly structural roles, have simple repeating elements of secondary structure.
- Globular proteins have more complicated tertiary structures, often containing several types of secondary structure in the same polypeptide chain. The first globular protein structure to be determined, using x-ray diffraction methods, was that of myoglobin.
- The complex structures of globular proteins can be analyzed by examining stable substructures called supersecondary structures motifs, or folds. The thousands of known protein structures are generally assembled from a repertoire of only a few hundred motifs. Regions of a polypeptide chain that can fold stably and independently are called domains.
- Quaternary structure results from interactions between the subunits of multisubunit (multimeric) proteins or large protein assemblies. Some multimeric proteins have a repeated unit consisting of a single subunit or a group of subunits referred to as a protomer. Protomers are usually related by rotational or helical symmetry

Denaturation and folding

- Proteins are marginally stable
  - Changes in the environmental conditions affects structure and function
- Denaturation is the process in which change in the 3-D structure of the protein is sufficient to cause a loss of function
- Factors:
  - Temperature
  - pH
  - Solvents (acetone, alcohol)
  - Solutes such as urea, guanidine hydrochloride
  - Detergents

Protein Denaturation

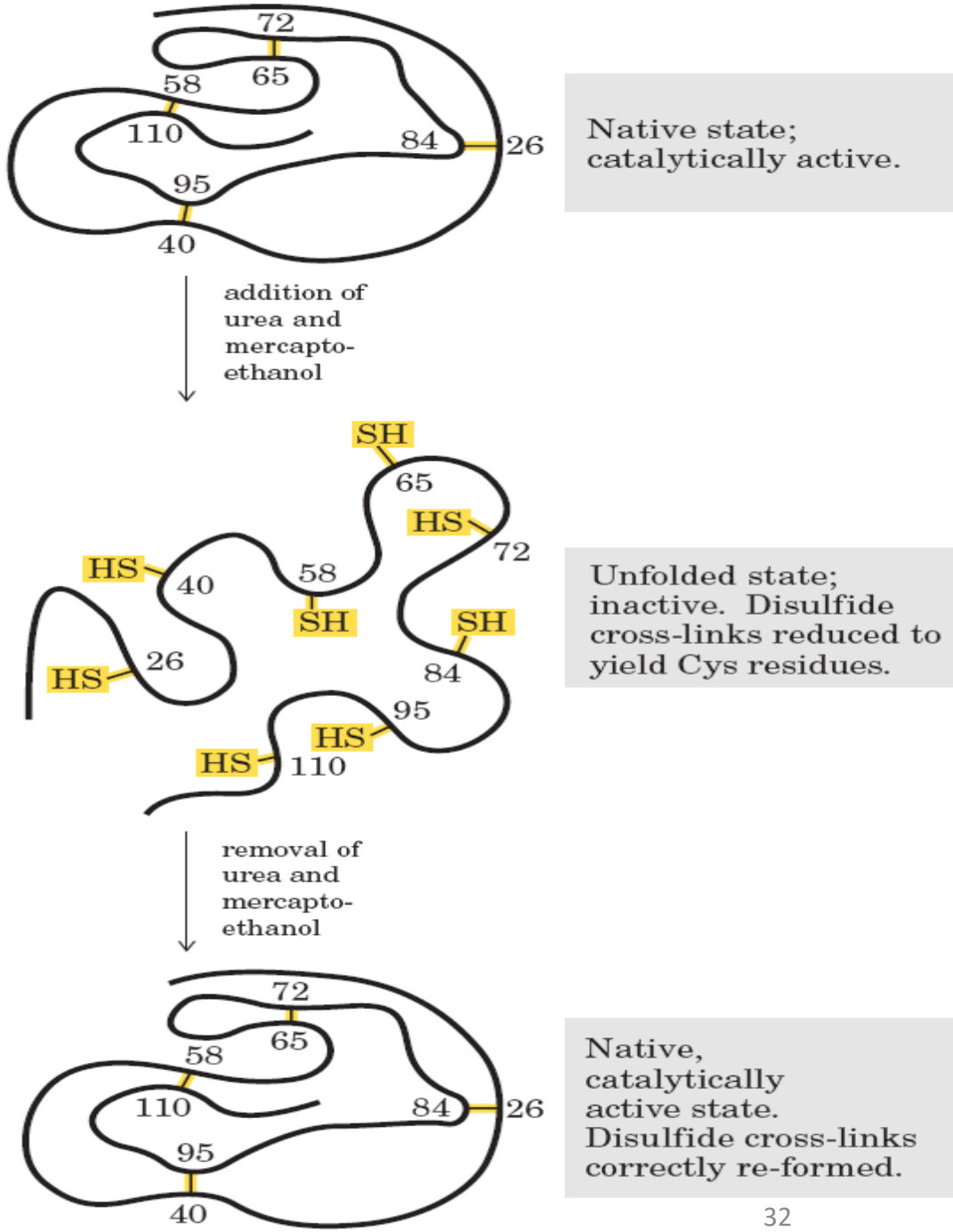




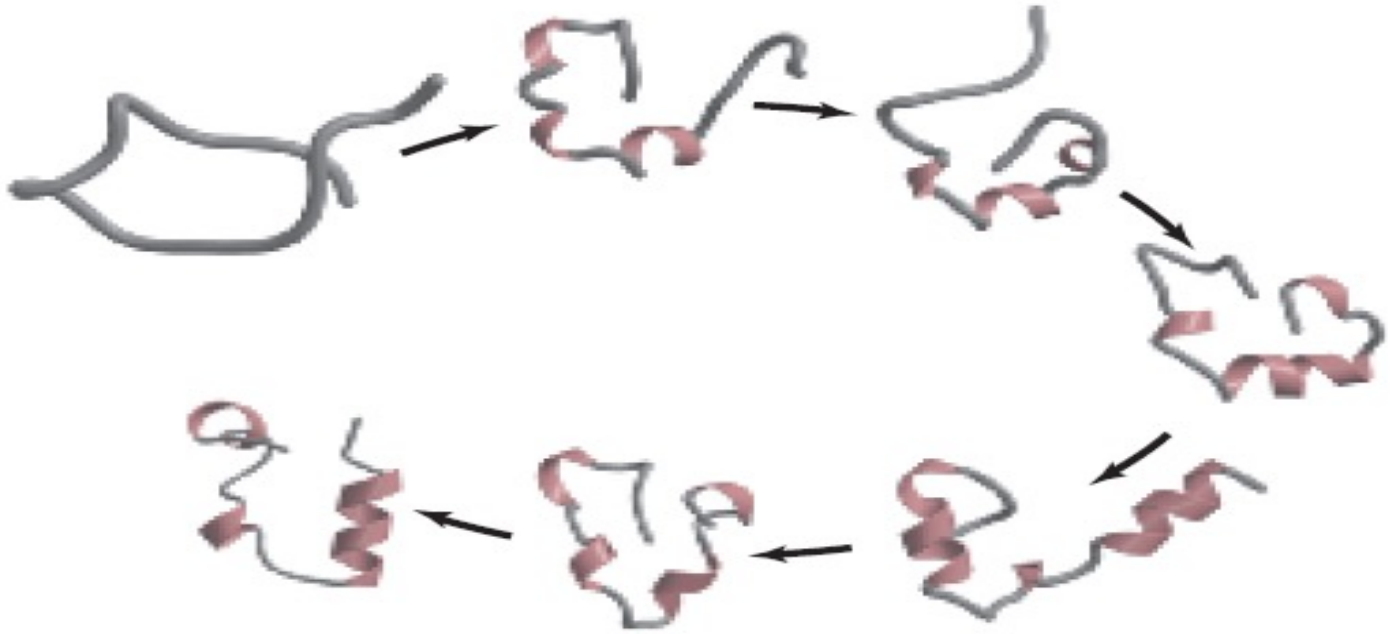
- Denaturation (a) by temperature change (b) by addition of guanidine HCl monitored using Circular Dichroism

Renaturation of unfolded Protein

- Urea is used to denature ribonuclease, and mercaptoethanol ($HOCH_2CH_2SH$) to reduce and thus cleave the disulfide bonds to yield eight Cys residues. Renaturation involves reestablishment of the correct disulfide cross-links.



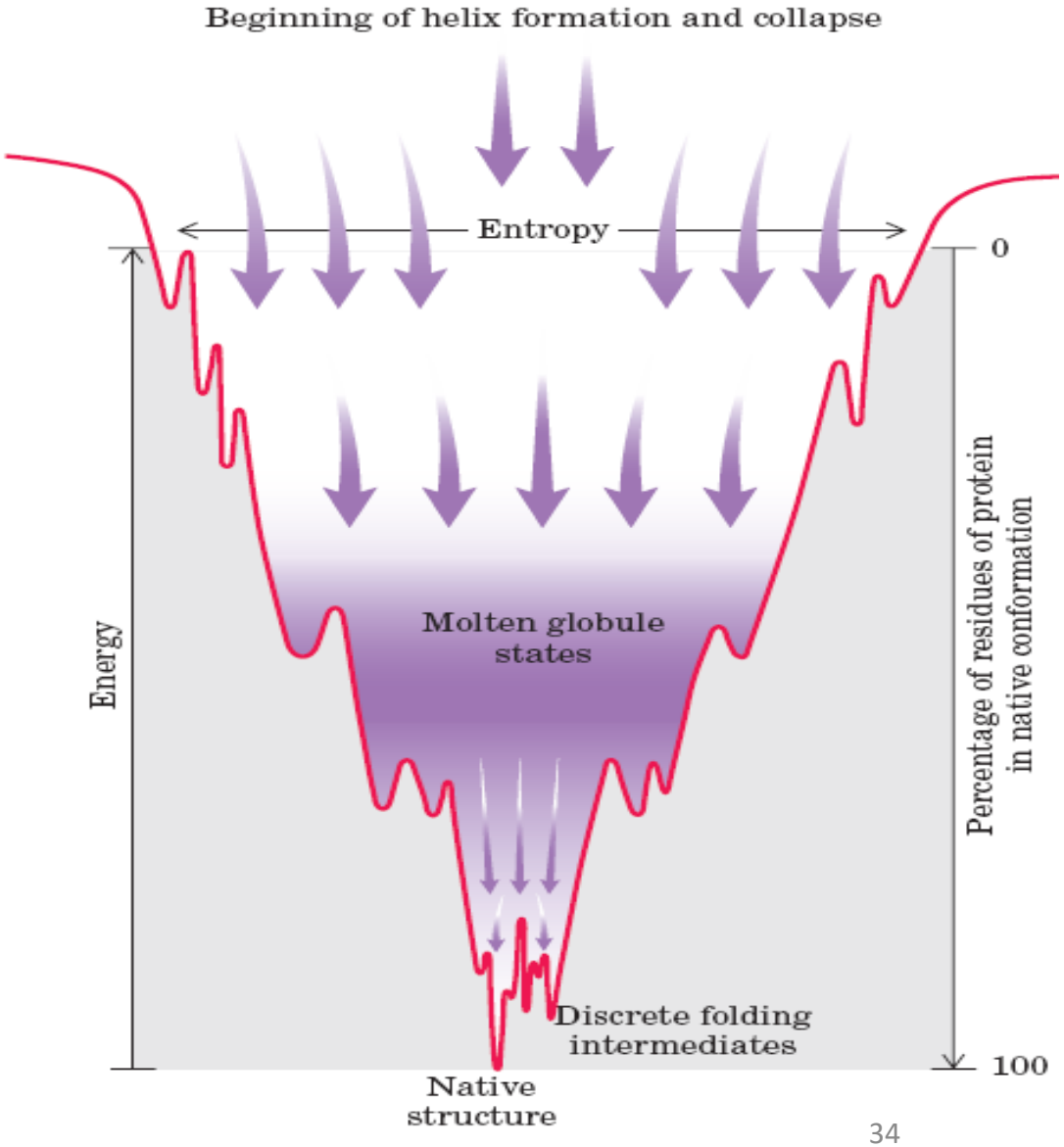
Protein folding simulation



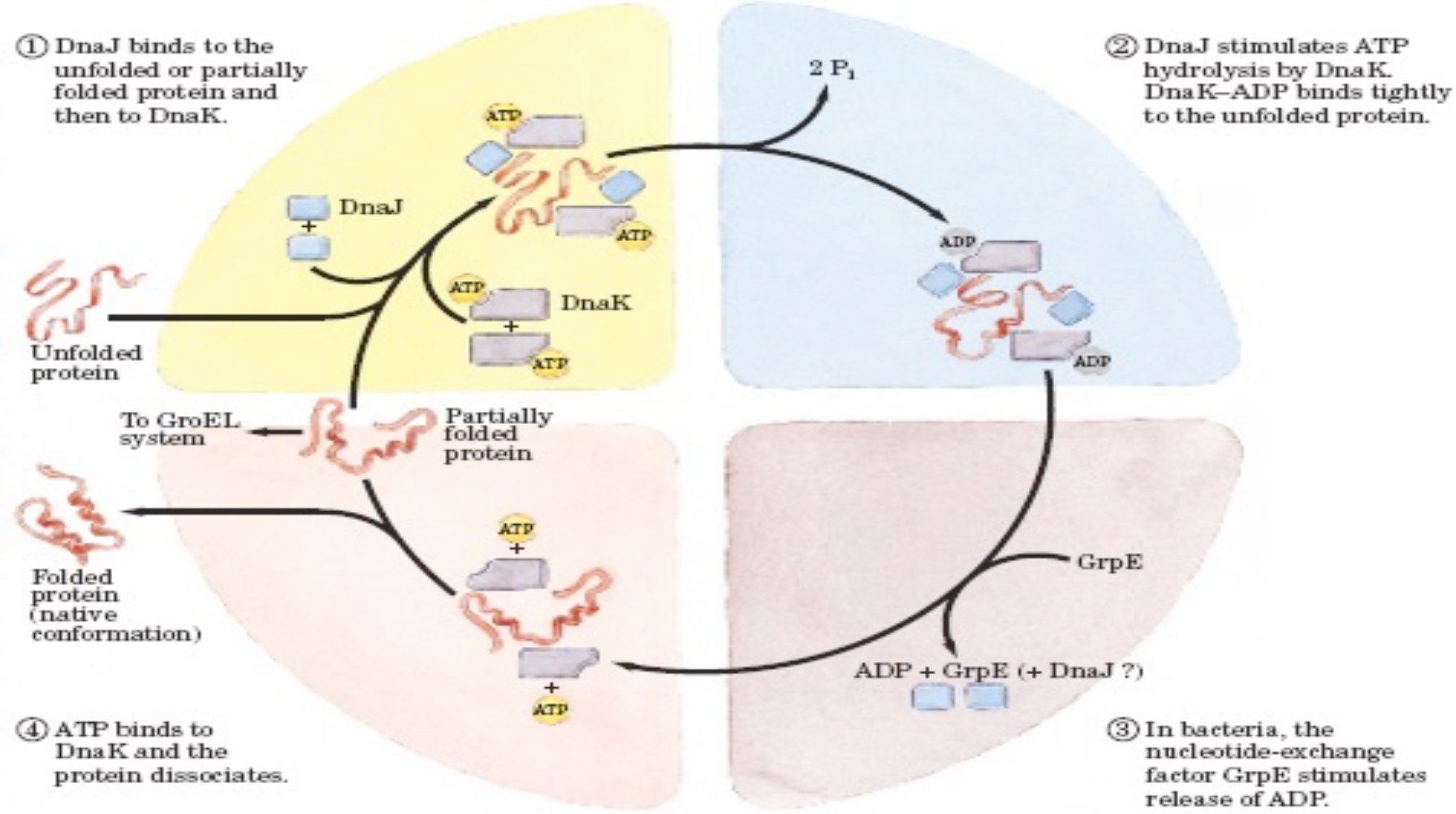
- The process started with the randomly coiled peptide and 3,000 surrounding water molecules in a virtual “water box.” The molecular motions of the peptide and the effects of the water molecules were taken into account in mapping the most likely paths to the final structure among the countless alternatives.

Thermodynamics of Protein Folding

- The number of conformations, and hence the conformational entropy, is large. Only a small fraction of the intramolecular interactions that will exist in the native conformation are present.
- As folding progresses, the thermodynamic path down the funnel reduces the number of states present (decreases entropy), increases the amount of protein in the native conformation, and decreases the free energy.
- Depressions on the sides of the funnel represent semistable folding intermediates, which may, in some cases, slow the folding process.



Chaprones in Protein Folding



GroEL-GroES – member of HSP 60 family