

Chapter Outline

4-1 Protein Structure and Function

4-2 Primary Structure of Proteins

4-3 Secondary Structure of Proteins

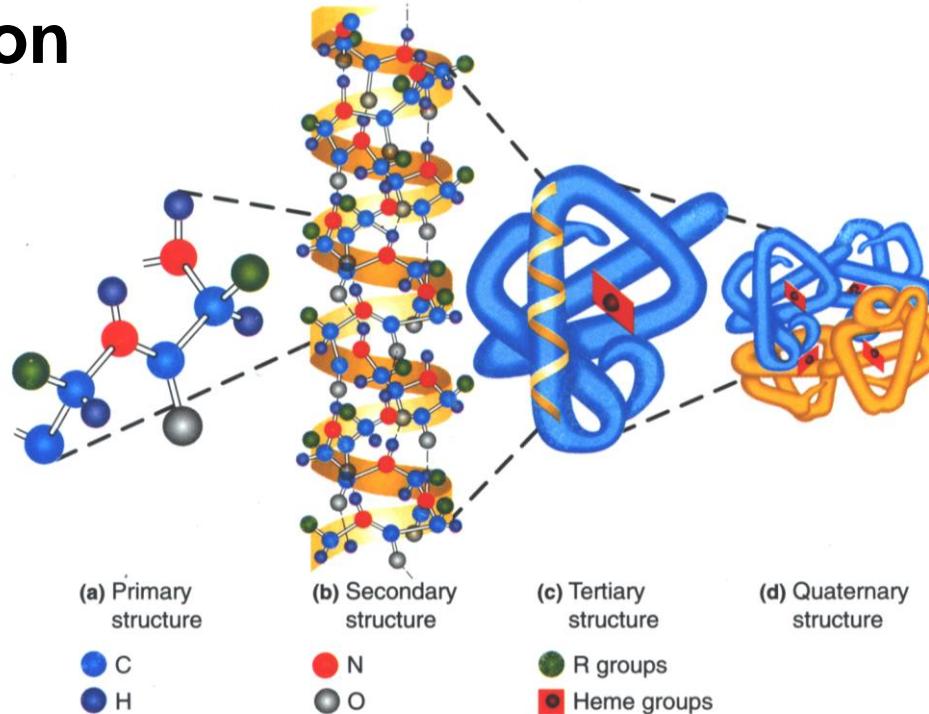
4-4 Tertiary Structure of Proteins

4-5 Quaternary Structure of Proteins

4-6 Ligand Binding and Cooperativity

Protein Structure

- Many conformations are possible for proteins:
 - Due to flexibility of amino acids linked by peptide bonds
 - At least one major conformation has biological activity, and hence is considered the protein's **native conformation**



Levels of Protein Structure

1°structure: the sequence of amino acids in a polypeptide chain, read from the N-terminal end to the C-terminal end

- **2°structure:** the ordered 3-dimensional arrangements (conformations) in localized regions of a polypeptide chain; refers only to interactions of the peptide backbone
 - e. g., α -helix and β -pleated sheet
- **3° structure:** 3-D arrangement of all atoms
- **4° structure:** arrangement of monomer subunits with respect to each other

1° Structure

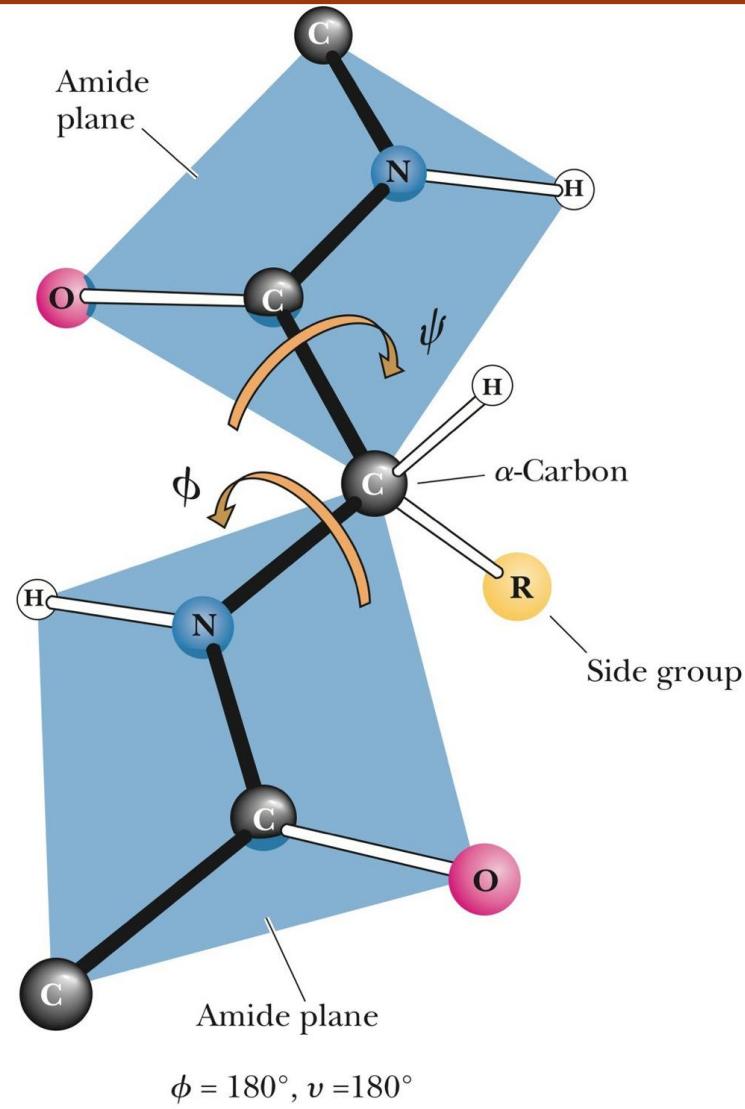
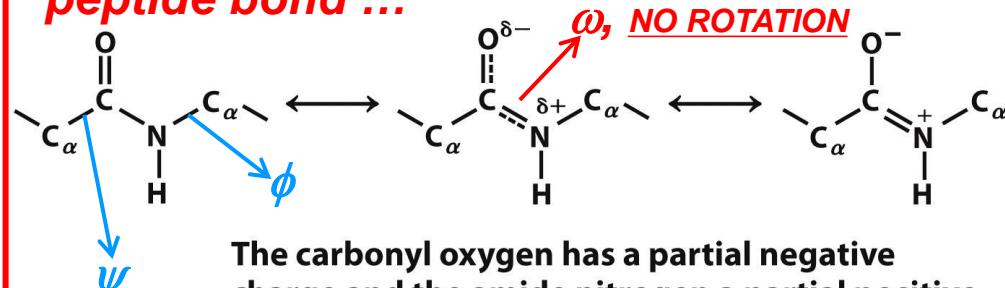
...the one letter notation of a protein...

- The 1° sequence of proteins determines its 3-D conformation
- Changes in just one amino acid in sequence can alter biological function, e.g. hemoglobin associated with sickle-cell anemia
- Determination of 1° sequence is routine biochemistry lab work (See Ch. 5).

2° Structure: Torsion Angles/Ramachandran Angles

- 2° of proteins is hydrogen-bonded arrangement of backbone of the protein

recall resonance structure & planarity of peptide bond ...



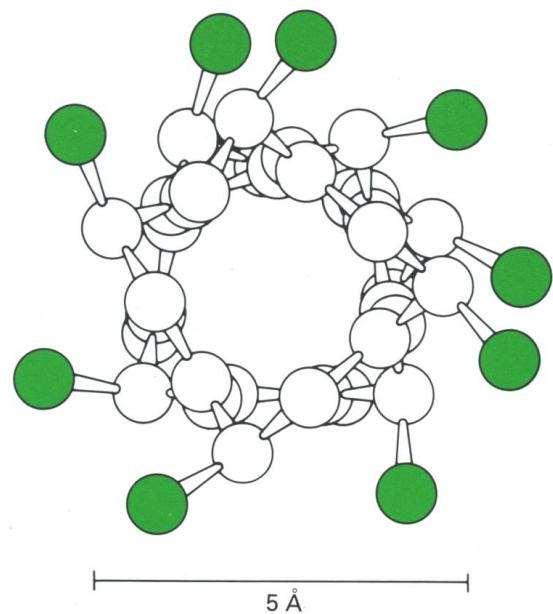
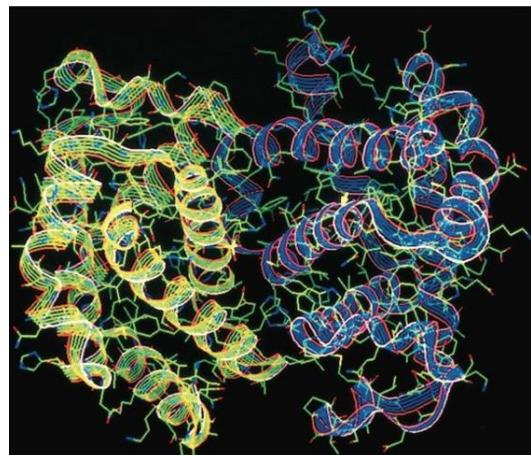
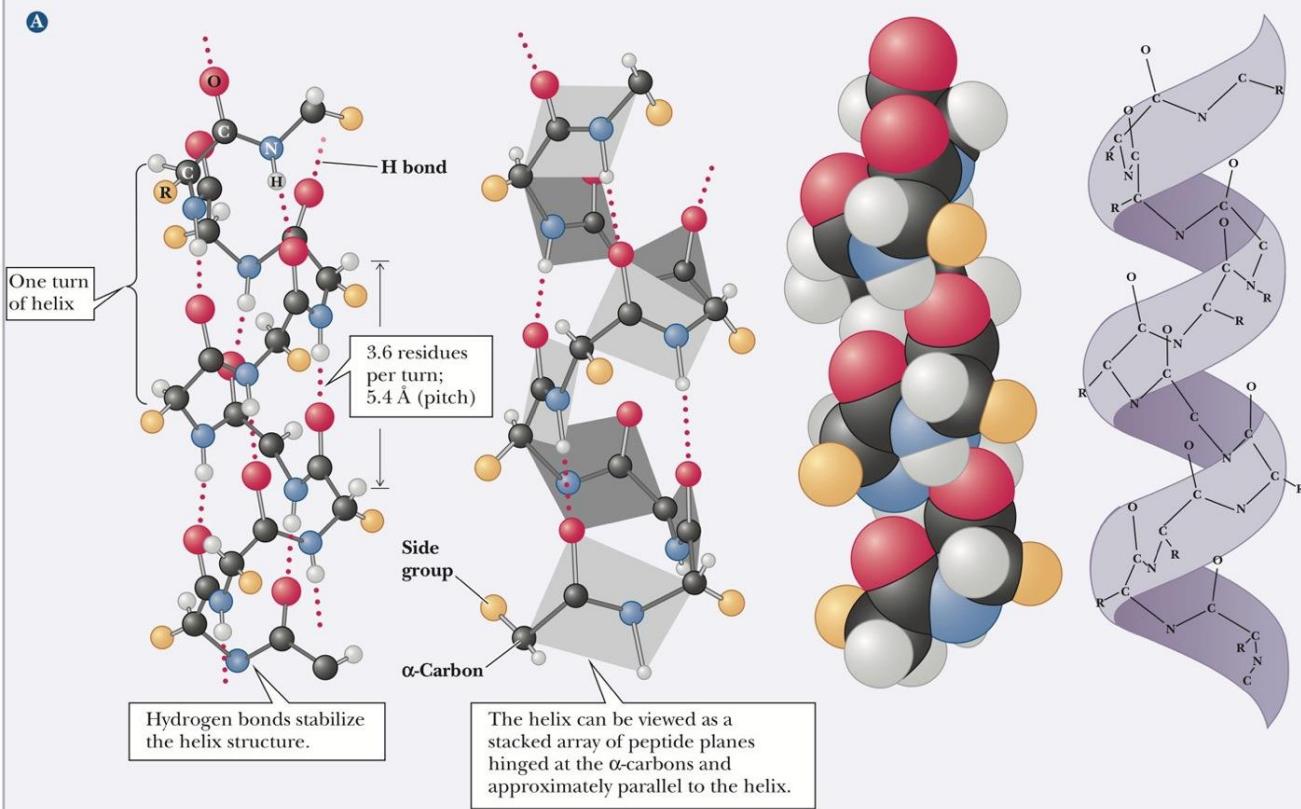
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polypeptides consists of a series of rigid planes, with consecutive planes sharing a common pivot point at Ca

α -Helix

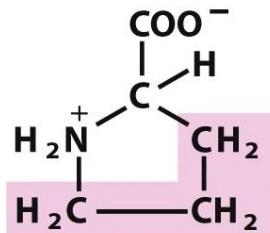
- Coil of the helix is clockwise or right-handed
(... a consequence of L-amino acid incorporation)
- There are 3.6 amino acids per turn
- Repeat distance is 5.4 \AA
- Each peptide bond is s-trans and planar
- C=O of each peptide bond is hydrogen bonded to the N-H of the fourth amino acid away
- C=O----H-N hydrogen bonds are parallel to helical axis
- All R groups point outward from helix

α -Helix (Cont'd)



α -Helix (Cont'd)

- **Several factors can disrupt an α -helix**
 - **proline** creates a bend because of (1) the restricted rotation due to its cyclic structure and (2) its α -amino group has no N-H for hydrogen bonding



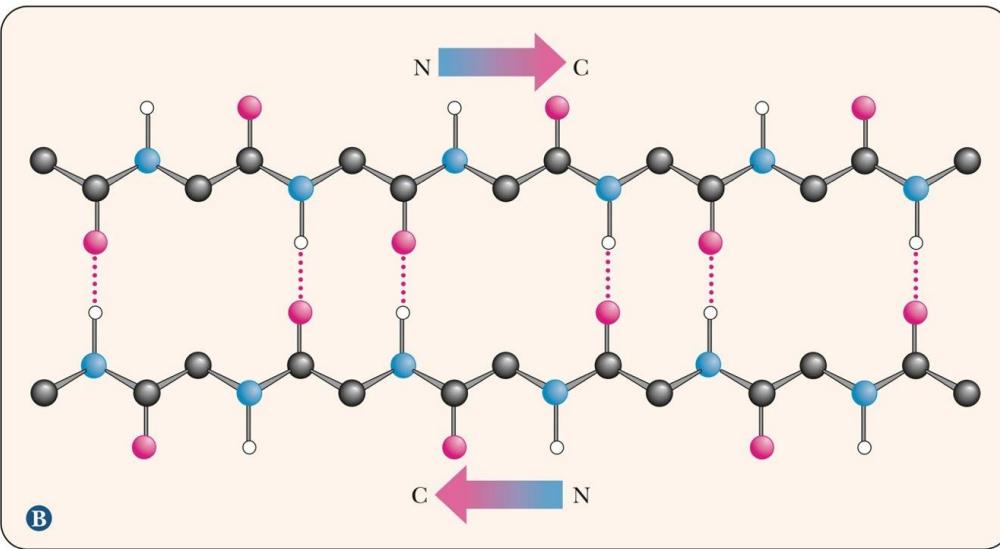
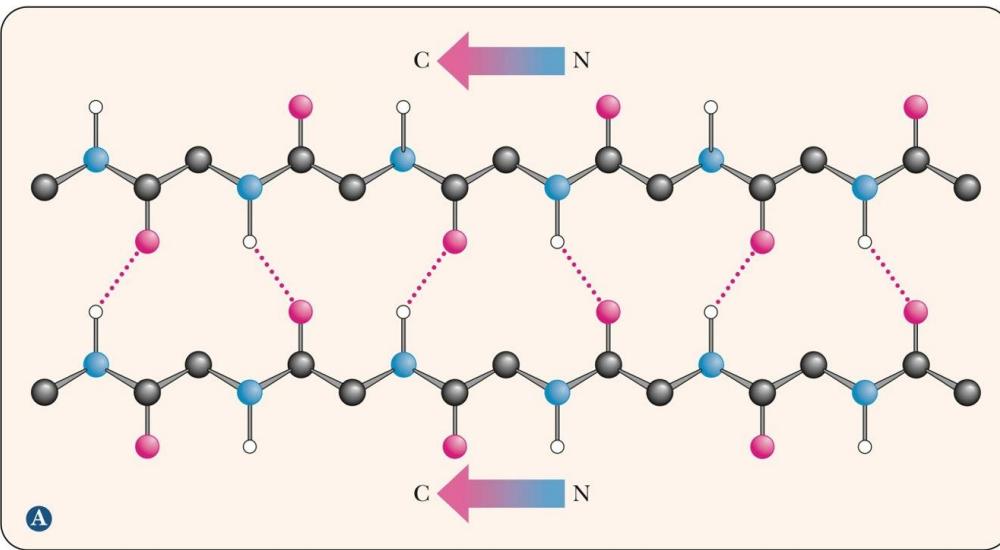
Proline

- strong electrostatic repulsion caused by the proximity of several side chains of like charge, e.g., Lys and Arg or Glu and Asp
- steric crowding caused by the proximity of bulky side chains, e.g., Val, Ile
- Gly provides high conformational flexibility

β -Pleated Sheet

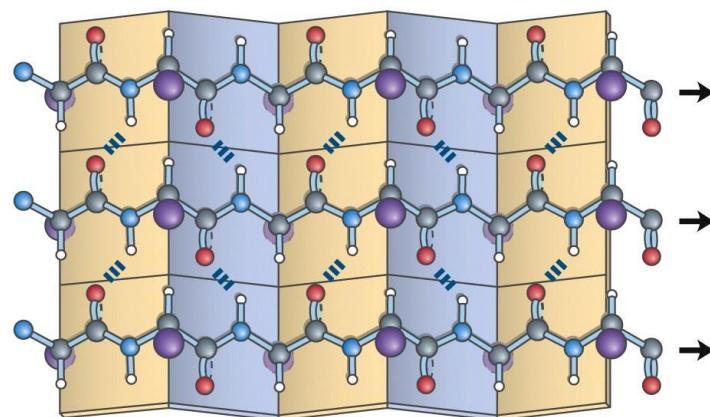
- Polypeptide chains lie adjacent to one another; may be parallel or antiparallel
- R groups alternate, first above and then below plane
- Each peptide bond is s-trans and planar
- C=O and N-H groups of each peptide bond are perpendicular to axis of the sheet
- C=O---H-N hydrogen bonds are between ***adjacent sheets*** and perpendicular to the direction of the sheet

β -Pleated Sheet (Cont'd)



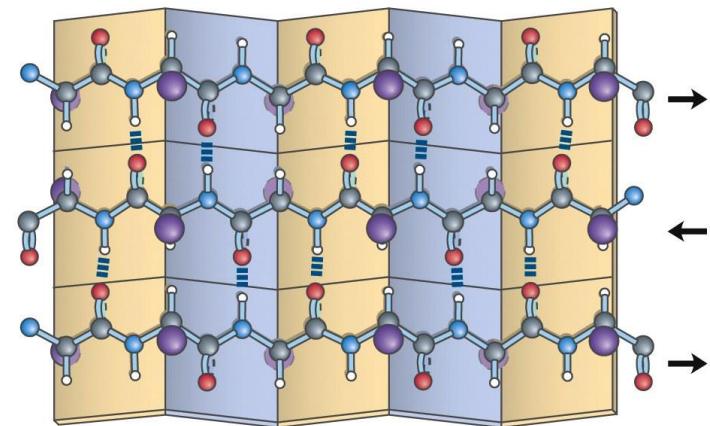
β -Pleated Sheet (Cont'd)

Parallel

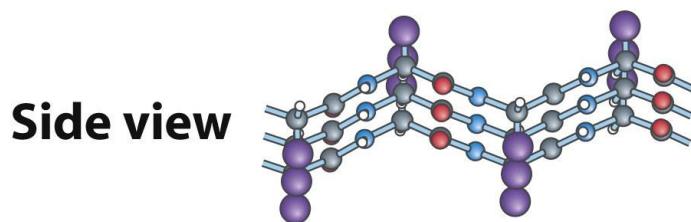


Top view

Antiparallel



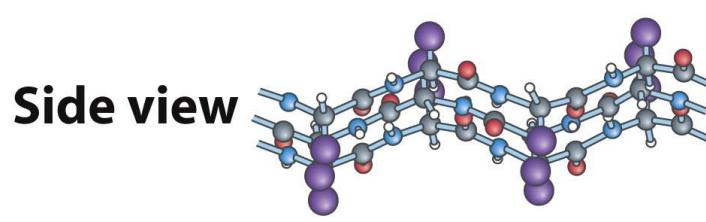
Top view



Side view

nonpolar sidechains are distributed on both sides of the beta sheet

Found within protein core



Side view

nonpolar sidechains can be distributed on one side only

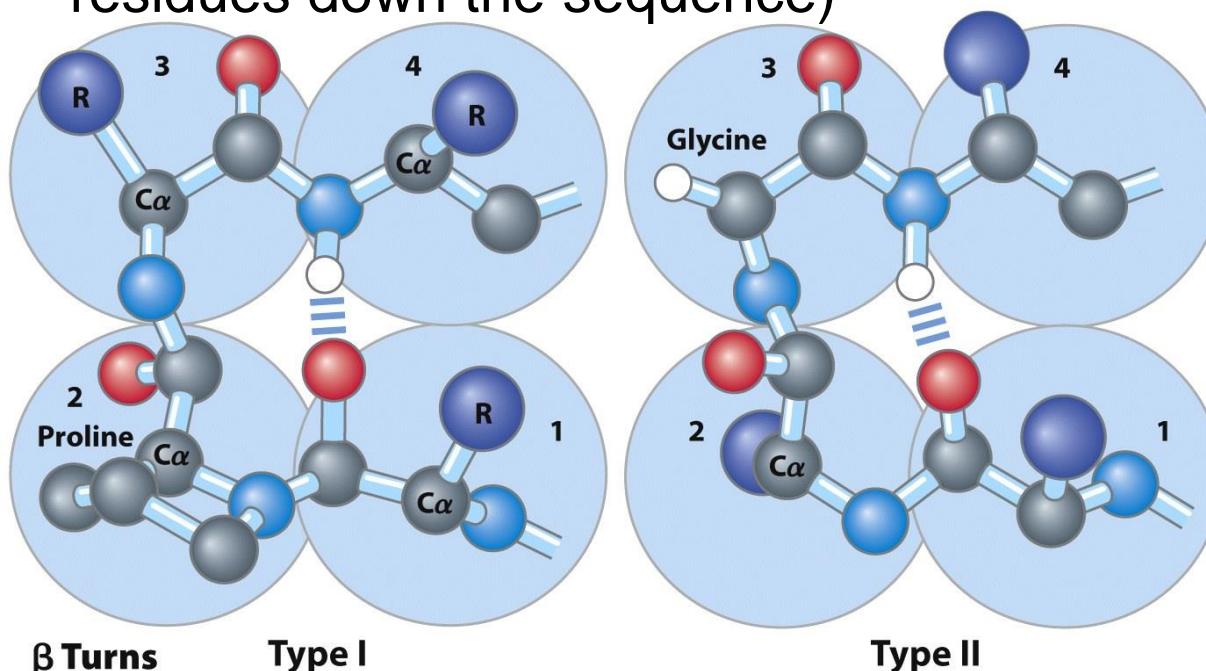
As few as two layers are required. Only one protected from H₂O

Structures of Reverse Turns (β -turns)

Polypeptides change direction in order to FOLD

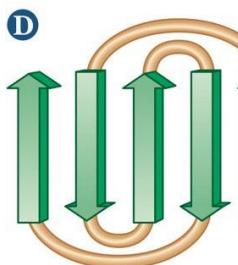
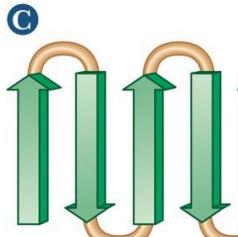
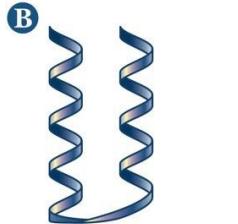
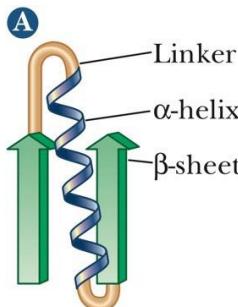
→ stable turn structures (or loops) are required...
commonly formed by 4 amino acids

- Gly (unrestrained) and Pro (cyclic) are found in turns
- Stabilized by H-bonding (a carbonyl oxygen to amide proton three residues down the sequence)

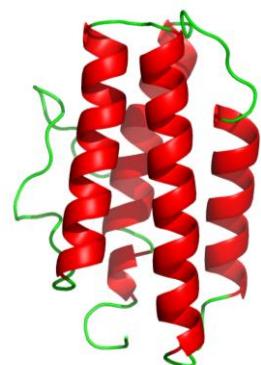


- Type I more common than Type II
- Pro at pos. 2 for Type I and Gly at pos. 3 for Type II

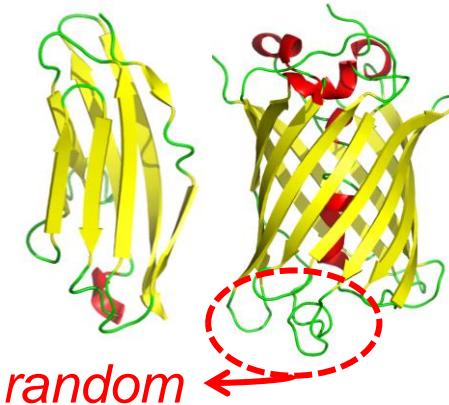
Examples of common structural motifs



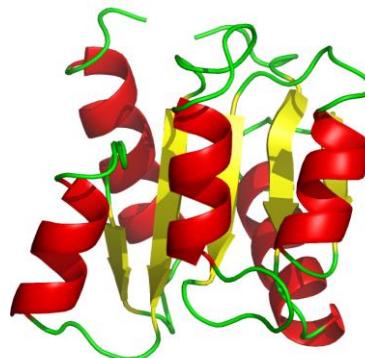
all- α fold



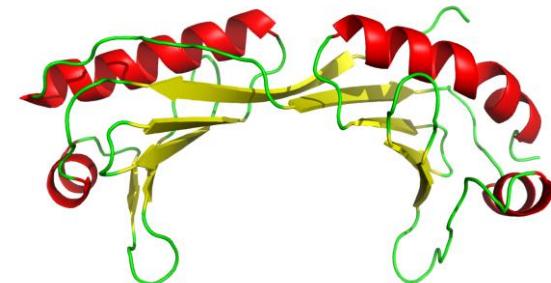
all- β folds



α/β fold



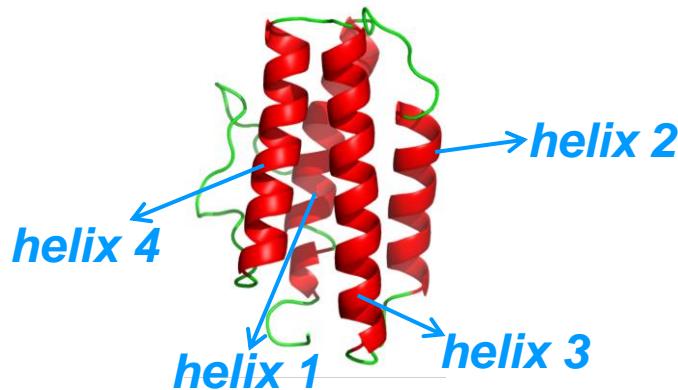
$\alpha + \beta$ fold



and more ...

Tertiary Structure (3°)

- or three dimensional structure or conformation or fold
 - *for most proteins, the active conformation is a compact conformation*
- refers to the overall spatial arrangement of atoms in a polypeptide chain or in a protein
- results from interactions between 2° structural elements.



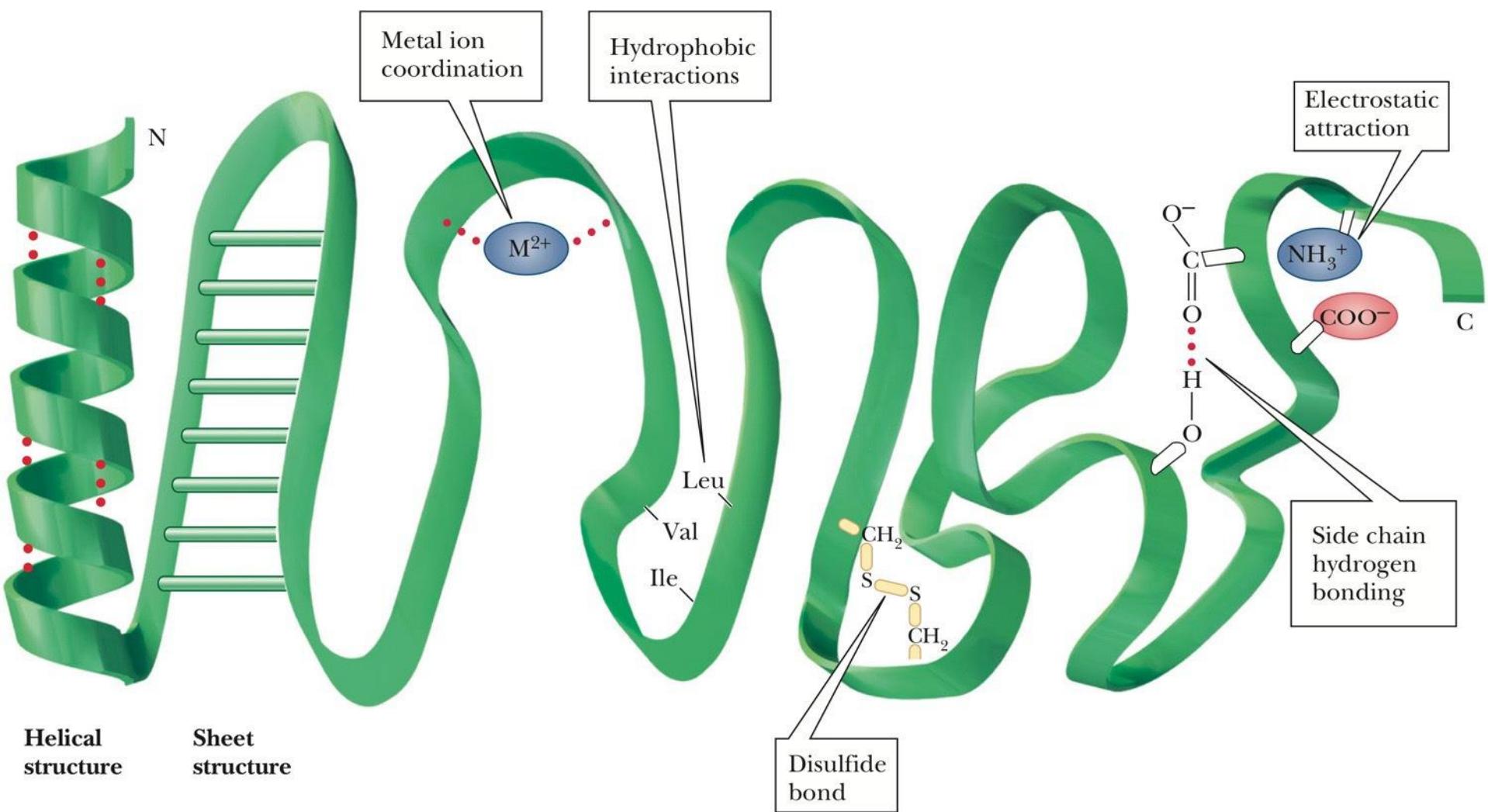
e.g. *in a four-helix bundle, the helices acquire specific interhelical angles. This conformation is stabilized by a hydrophobic core formed by non-polar residues from helices.*

- classified as fibrous or globular

Forces Involved in 3° Structure

- **Noncovalent interactions**, including
 - hydrogen bonding between polar side chains, e.g., Ser and Thr
 - hydrophobic interaction between nonpolar side chains, e.g., Val and Ile
 - electrostatic attraction between side chains of opposite charge, e.g., Lys and Glu
 - electrostatic repulsion between side chains of like charge, e.g., Lys and Arg, Glu and Asp
- **Covalent interactions:** Disulfide (-S-S-) bonds between side chains of cysteines

Forces That Stabilize Protein Structure



Fibrous Proteins

- **Fibrous proteins:** contain polypeptide chains organized approximately parallel along a single axis.

They

- consist of long fibers or large sheets
- tend to be mechanically strong
- are insoluble in water and dilute salt solutions
- play important structural roles in nature

Examples are

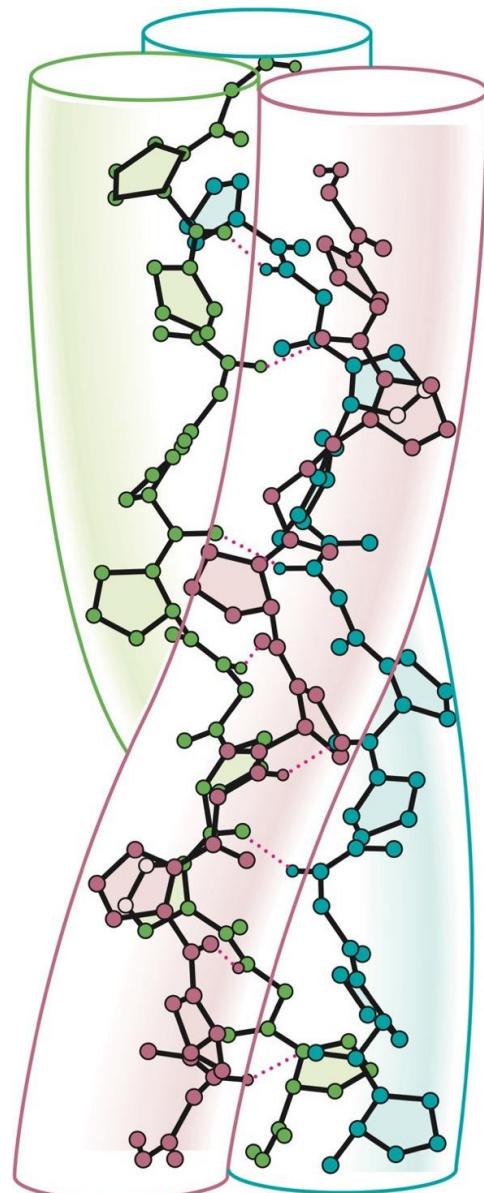
- keratin of hair and wool
- collagen of connective tissue of animals including cartilage, bones, teeth, skin, and blood vessels

Collagen Triple Helix

- Consists of three polypeptide chains wrapped around each other in a ropelike twist to form a triple helix called tropocollagen; MW approx. 300,000
- 30% of amino acids in each chain are Pro and Hyp (hydroxyproline); hydroxylysine also occurs
- Every third position is Gly and repeating sequences are X-Pro-Gly and X-Hyp-Gly
- Each polypeptide chain is a helix but not an α -helix (left-handed helix)
- The three strands are held together by hydrogen bonding involving hydroxyproline and hydroxylysine
- With age, collagen helices become cross linked by covalent bonds formed between Lys and His residues

Collagen Triple Helix

Collagen is an important constituent of connective tissues: tendons, cartilage, bones, cornea of the eye



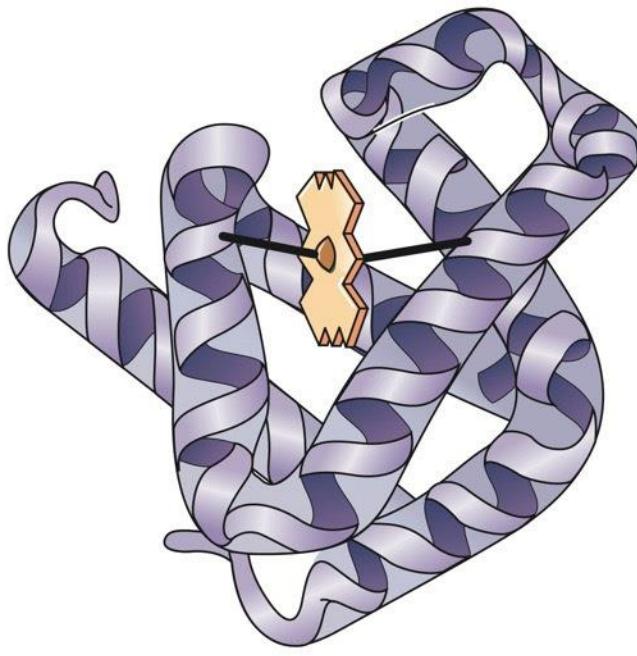
Globular Proteins

- **Globular proteins:** proteins which are folded to a more or less spherical shape
 - they tend to be soluble in water and salt solutions
 - most of their polar side chains are on the outside and interact with the aqueous environment by hydrogen bonding and ion-dipole interactions
 - most of their nonpolar side chains are buried inside
 - nearly all have substantial sections of α -helix and β -sheet

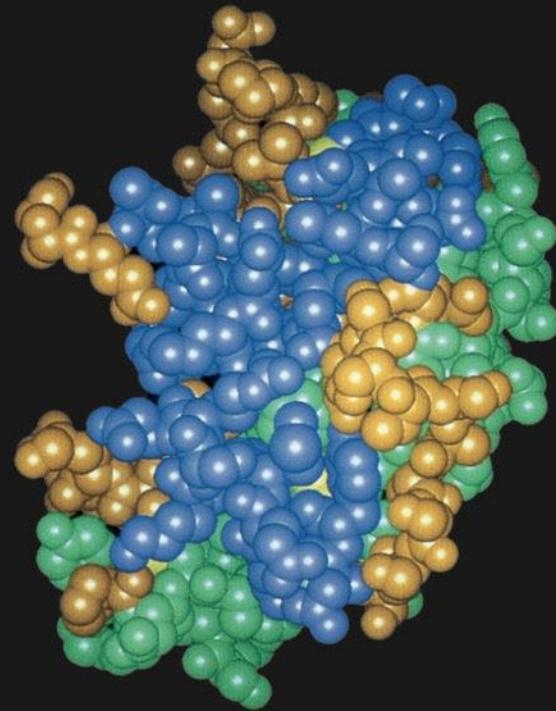
Comparison of Shapes of Fibrous and Globular Proteins



Filament
(four right-hand
twisted protofilaments)



Myoglobin, a globular protein



B Computer-generated model of a globular protein. Alpha-helices are shown in blue, beta-sheets are shown in green, and random coil is gold.

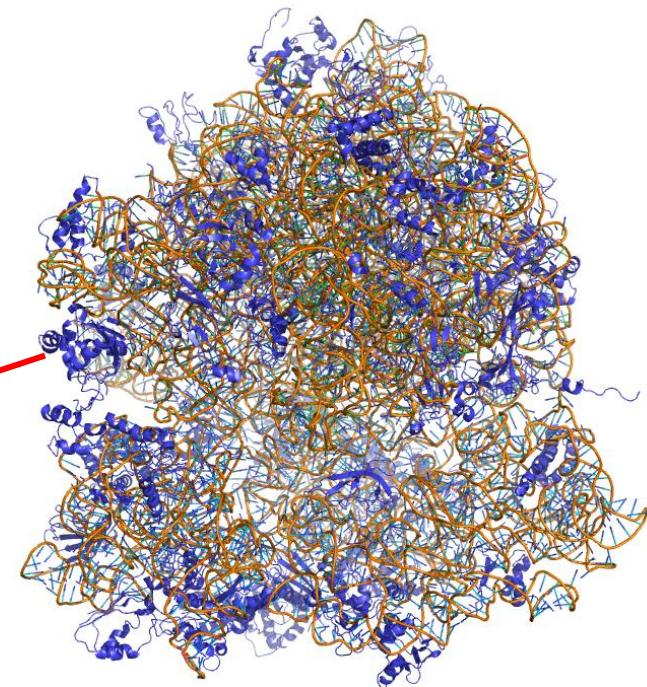
A Schematic diagrams of a portion of a fibrous protein and of a globular protein.

Quaternary Structure of Proteins (4°)

- **Quaternary (4°) structure:** the association of polypeptide monomers into multisubunit proteins. Some common multi-subunits include:

- dimers
- trimers
- tetramers

ribosome is a supramolecular complex of 52 proteins and RNA molecules, held together by non-covalent interactions

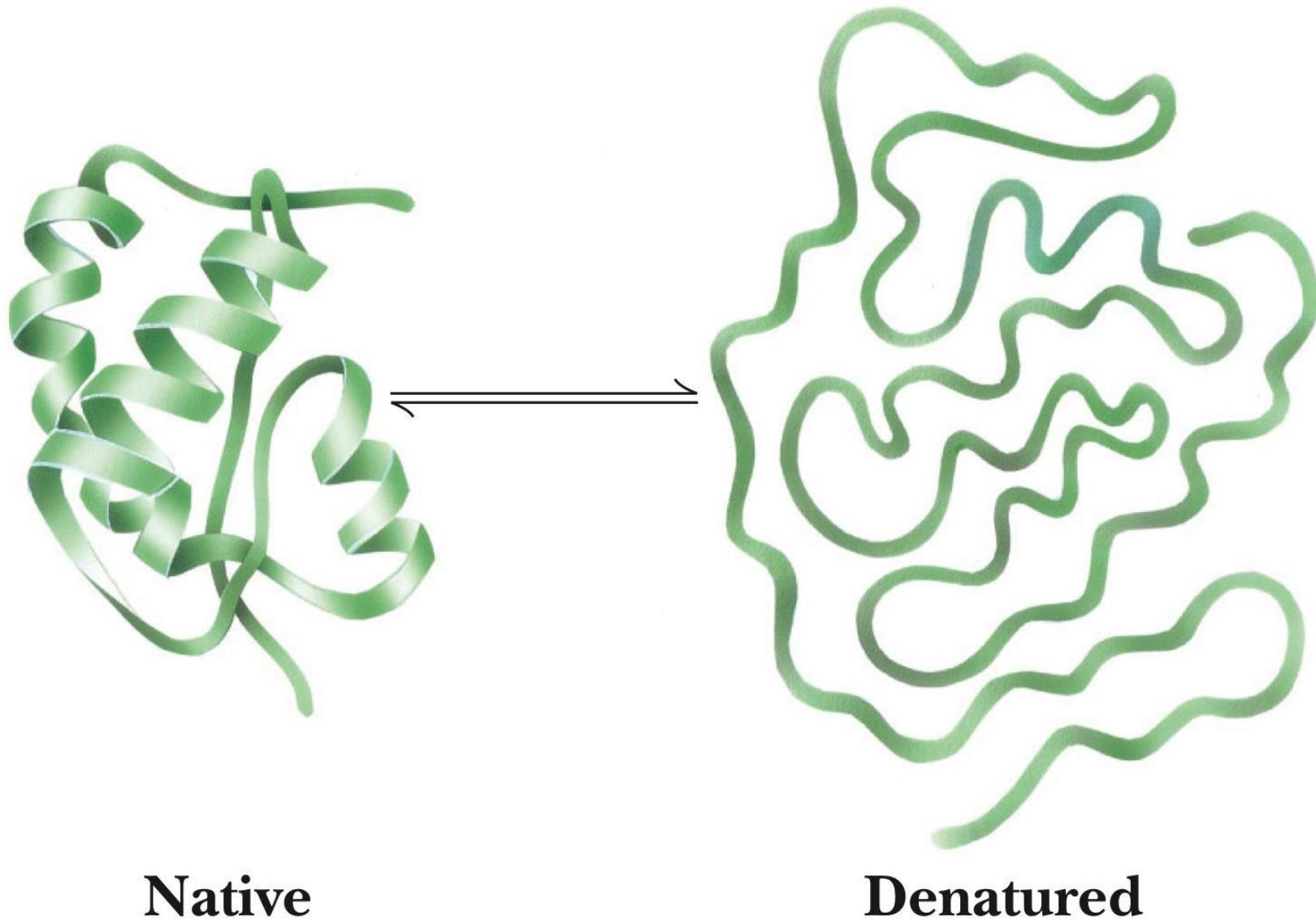


- Noncovalent interactions
 - electrostatics, hydrogen bonds, hydrophobic

Denaturation (or unfolding) and Refolding

- **Noncovalent** interactions that stabilize proteins are weak and can be disrupted.
- **Denaturation:** the loss of structural order (2°, 3°, 4°, or a combination of these) that gives a protein its biological activity; that is, the loss of biological activity
- **Denaturation can be brought about by:**
 - heat
 - large changes in pH, which alter charges on side chains, e.g., -COO^- to -COOH or -NH_3^+ to -NH_2
 - detergents such as sodium dodecyl sulfate (SDS) which disrupt hydrophobic interactions
 - urea or guanidine, which disrupt hydrogen bonding
 - mercaptoethanol, which reduces disulfide bonds

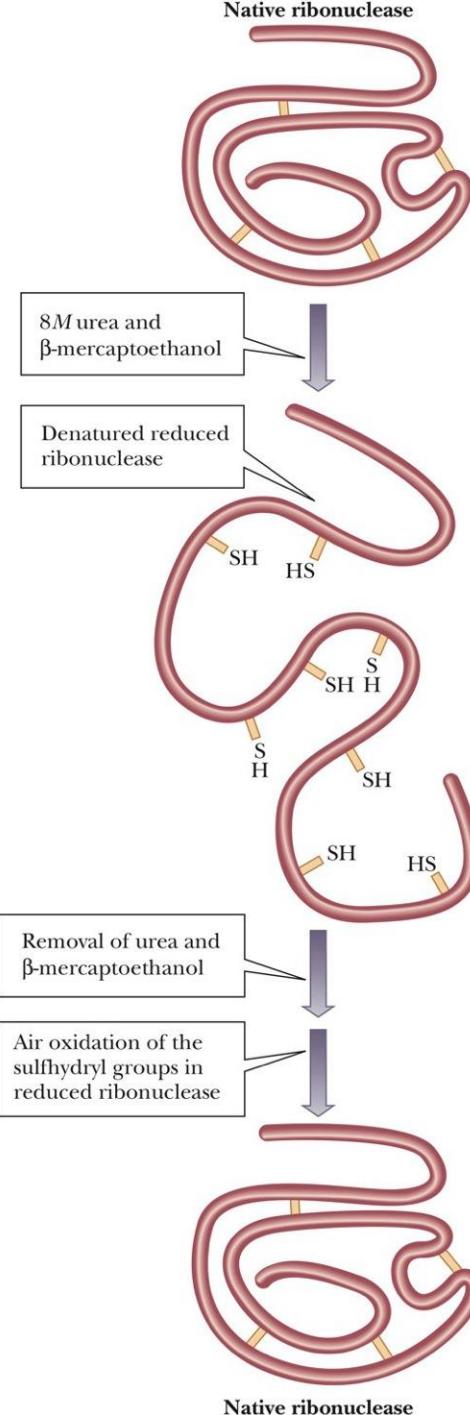
Denaturation of a Protein



Denaturation and Refolding in Ribonuclease

Several ways to denature proteins

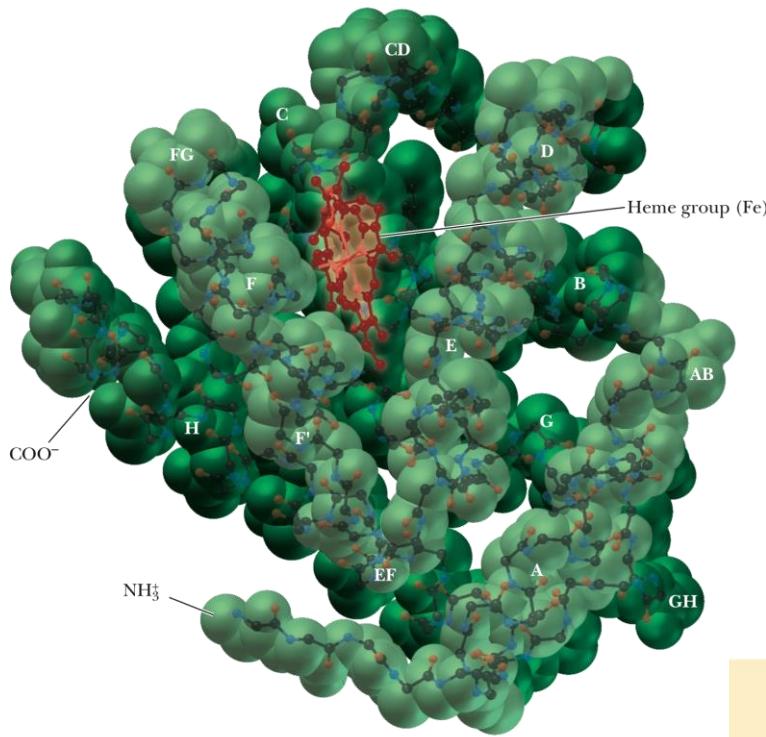
- Heat
- pH
- Detergents
- Urea
- Guanadine hydrochloride



Myoglobin

- A single polypeptide chain of 153 amino acids
- A single heme group in a hydrophobic pocket
- 8 regions of α -helix; no regions of β -sheet
- Most polar side chains are on the surface
- Nonpolar side chains are folded to the interior
- Two His side chains are in the interior, involved with interaction with the heme group
- Fe(II) of heme has 6 coordinates sites; 4 interact with N atoms of heme, 1 with N of a His side chain, and 1 with either an O_2 molecule or an N of the second His side chain

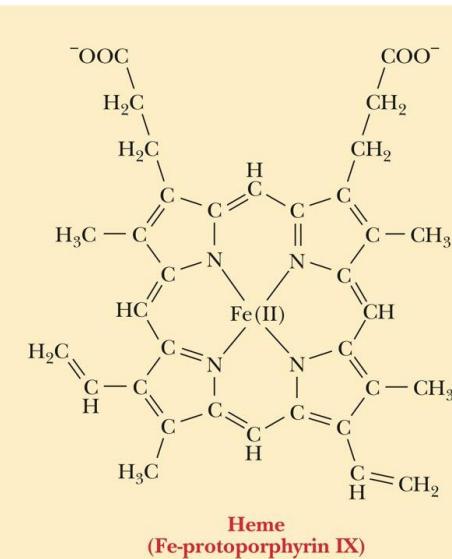
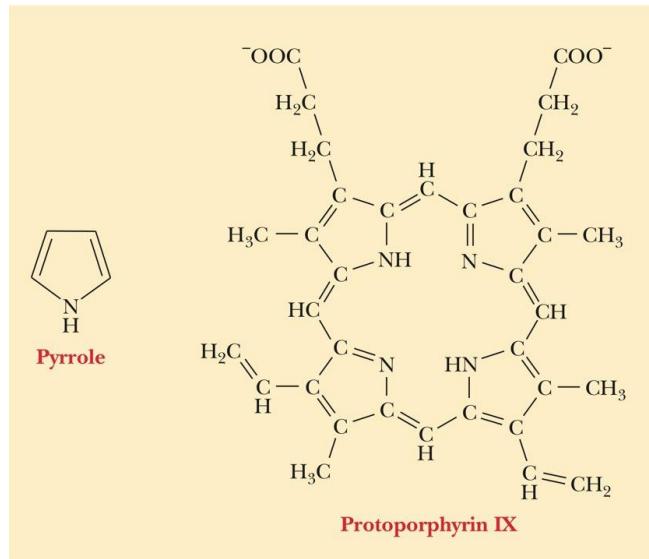
The Structure of Myoglobin



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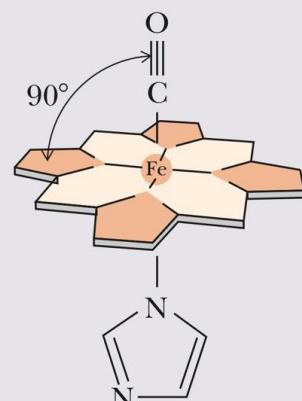
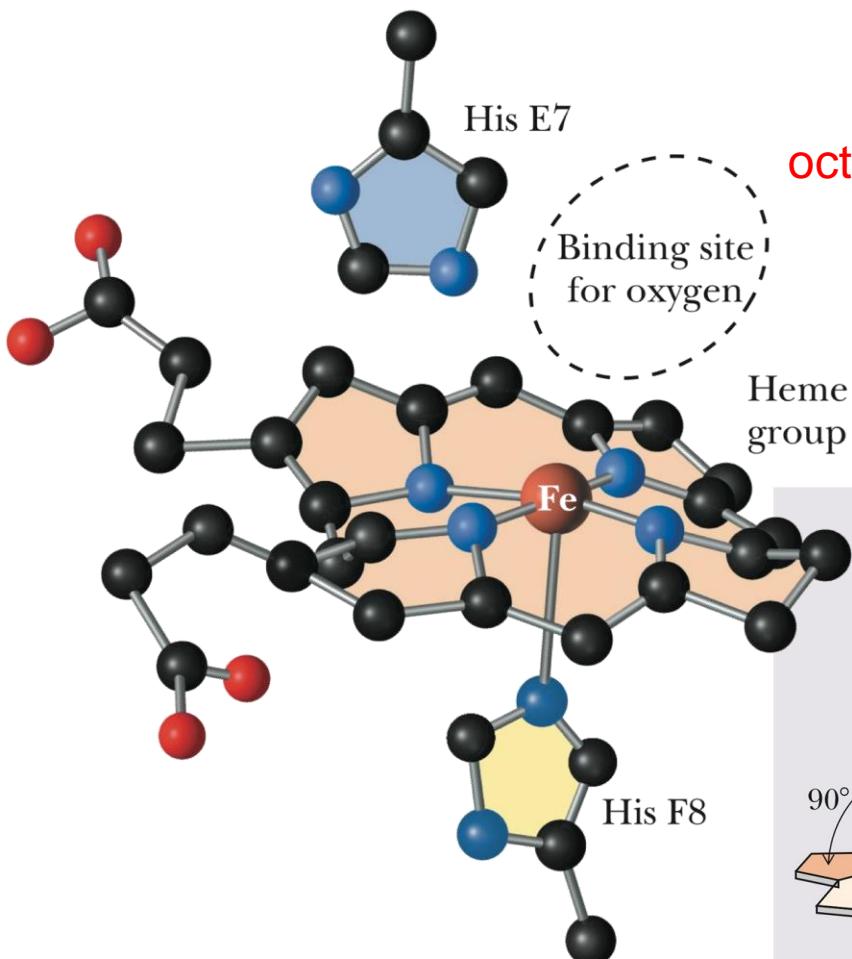
*Myoglobin is an O₂ storage protein
(found in muscles)*

The heme group

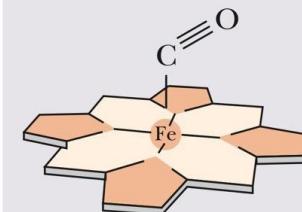
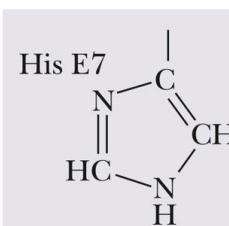


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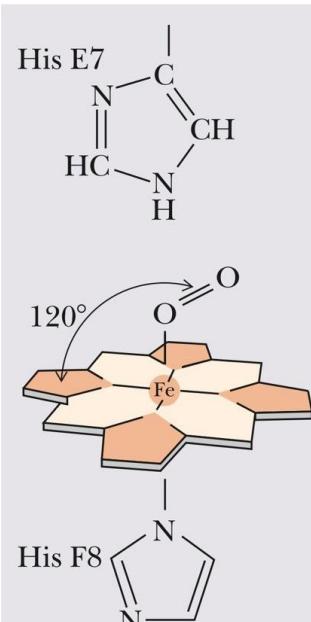
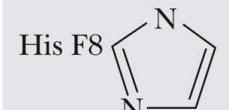
O₂ (and CO) Binding to Myoglobin



A Free heme with imidazole



B Mb:CO complex



C Oxymyoglobin

Protein-“partner” Interactions

- Reversible transient process i.e. a chemical equilibrium



- If partner is a small molecule it's typically called a **ligand**
- The region on the protein where the ligand binds is called the **binding site**
- Define an association and a dissociation constant:

$$K_a = \frac{[PL]}{[P][L]}$$

$$K_d = \frac{[P][L]}{[PL]}$$

$$K_d = \frac{1}{K_a}$$

In biochemistry (and chemistry, mol. biol., pharmacology, medicine etc...) we are interested in the bound fraction of a protein (e.g. a receptor):

$$\theta = \frac{[PL]}{[PL] + [P]} = \text{bound fraction (bound over total)}$$

Protein-“partner” Interactions

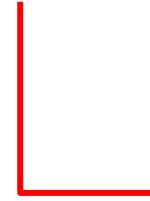


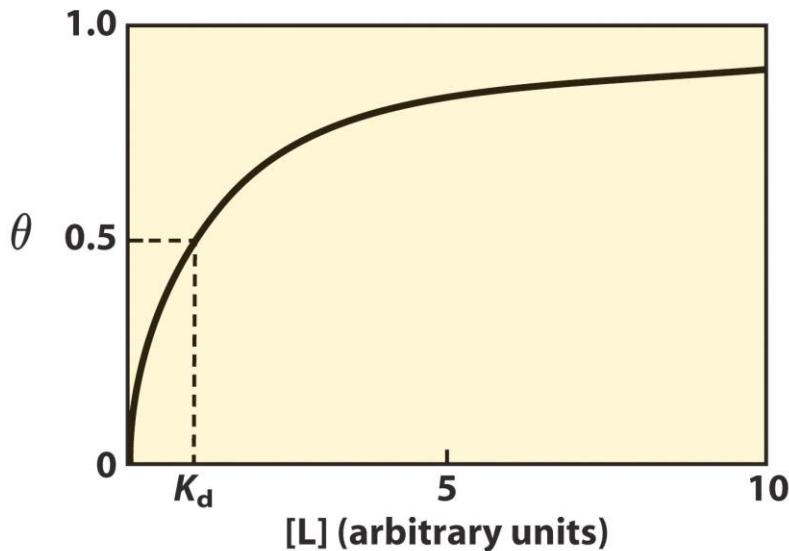
$$\theta = \frac{[PL]}{[PL] + [P]} = \text{bound fraction (bound over total)}$$

substitute $[PL]$ with $K_a[P][L] \Rightarrow \theta = \frac{K_a[P][L]}{K_a[P][L] + [P]}$

which can be rearranged to: $\theta = \frac{[L]}{[L] + \frac{1}{K_a}}$

and finally to:


$$\theta = \frac{[L]}{[L] + K_d}$$



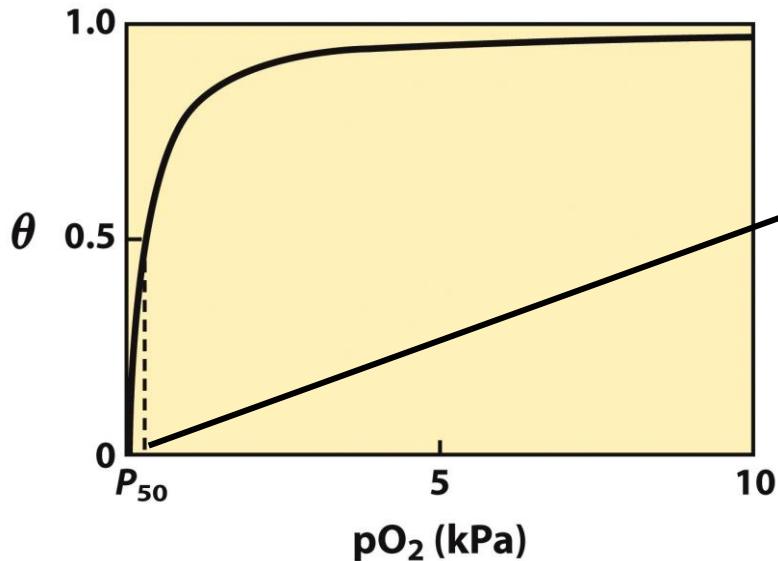
$K_d \Rightarrow$ is the concentration of the free ligand at which half of the protein is bound

O_2 Binding to Myoglobin

Consider the reversible binding of a single O_2 molecule to myoglobin, Mb.



$$\theta = \frac{[O_2]}{[O_2] + K_d} \quad \text{or more accurately: } \theta = \frac{pO_2}{pO_2 + P_{50}}$$



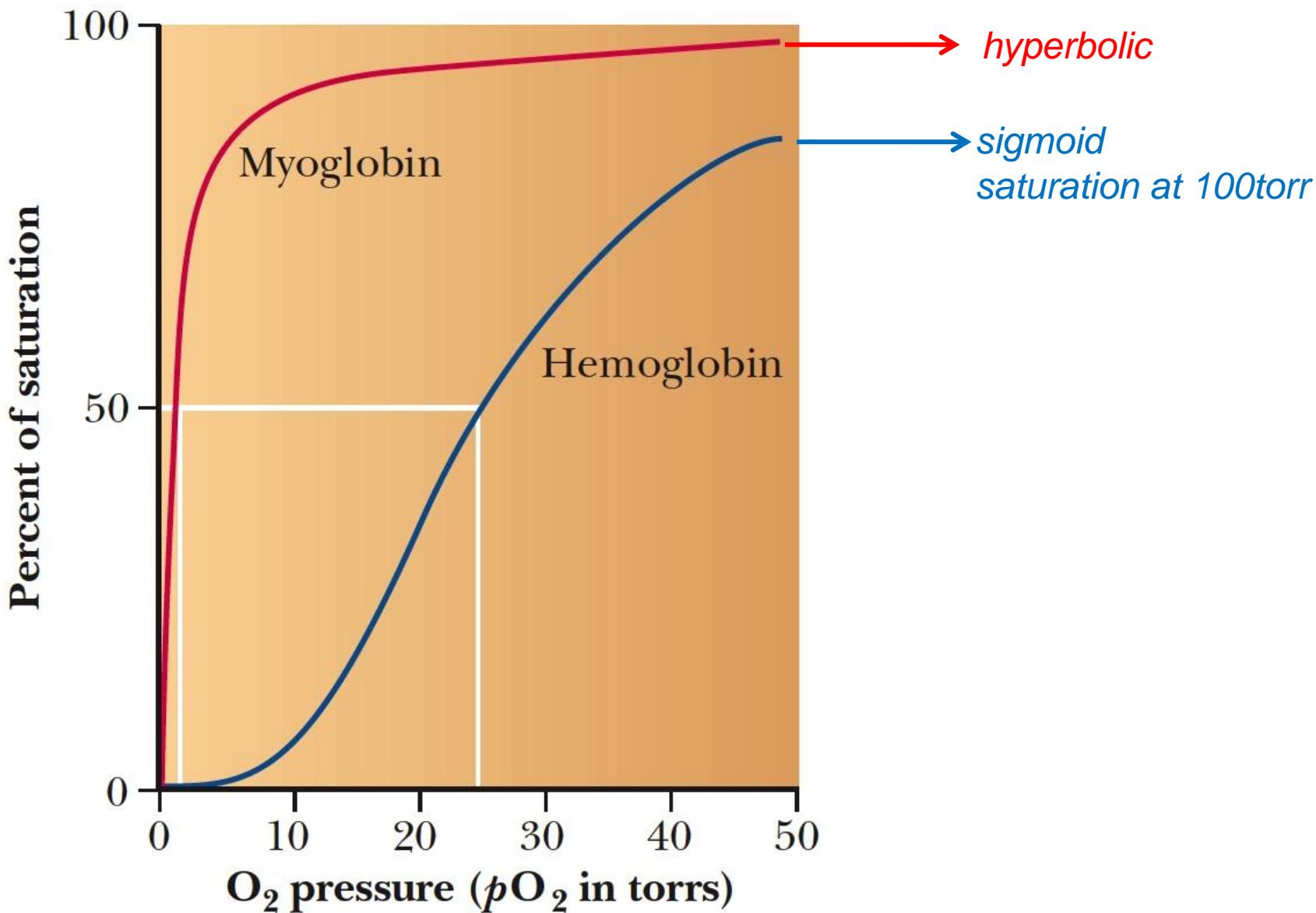
$$P_{50} = 0.26 \text{ kPa of } O_2$$

\Rightarrow Half the myoglobin molecules have an O_2 molecule bound when the partial pressure of $O_2 = 0.26 \text{ kPa}$

Oxygen Binding of Hemoglobin (Hb)

- A tetramer of two α -chains (141 amino acids each) and two β -chains (153 amino acids each); $\alpha_2\beta_2$
- Each chain has 1 heme group; hemoglobin can bind up to 4 molecules of O_2
- Binding of O_2 exhibited by **positive cooperativity**; when one O_2 is bound, it becomes easier for the next O_2 to bind
- The function of hemoglobin is to transport oxygen
- The structure of oxygenated Hb is different from that of unoxygenated Hb
- H^+ , CO_2 , Cl^- , and 2,3-bisphosphoglycerate (BPG) affect the ability of Hb to bind and transport oxygen

Oxygen-Binding to Hb vs. Mb Can Be Shown Graphically



Oxygen Binding of Hemoglobin (Hb)

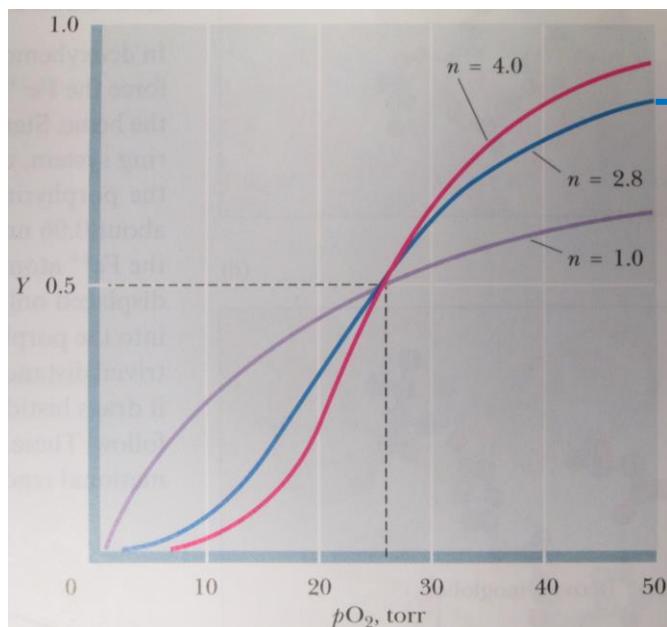
In general, when we have:



then: $K_d = \frac{[P][L]^n}{[PL_n]}$ and

$$\theta = \frac{[L]^n}{[L]^n + K_d}$$

for O_2 binding to Hb: $\theta = \frac{[O_2]^n}{[O_2]^n + K_d} = \frac{p_{O_2}^n}{p_{O_2}^n + P_{50}}$



→ *experimental*

$$\Rightarrow n = 2.8 \text{ for Hb}$$

different than the total number of binding sites. We call it, n_H

n_H = Hill number or coefficient

O_2 binding to Hb can be explained by cooperativity; the phenomenon that multiple binding sites of a protein “interact” or “communicate” or “affect” one another.

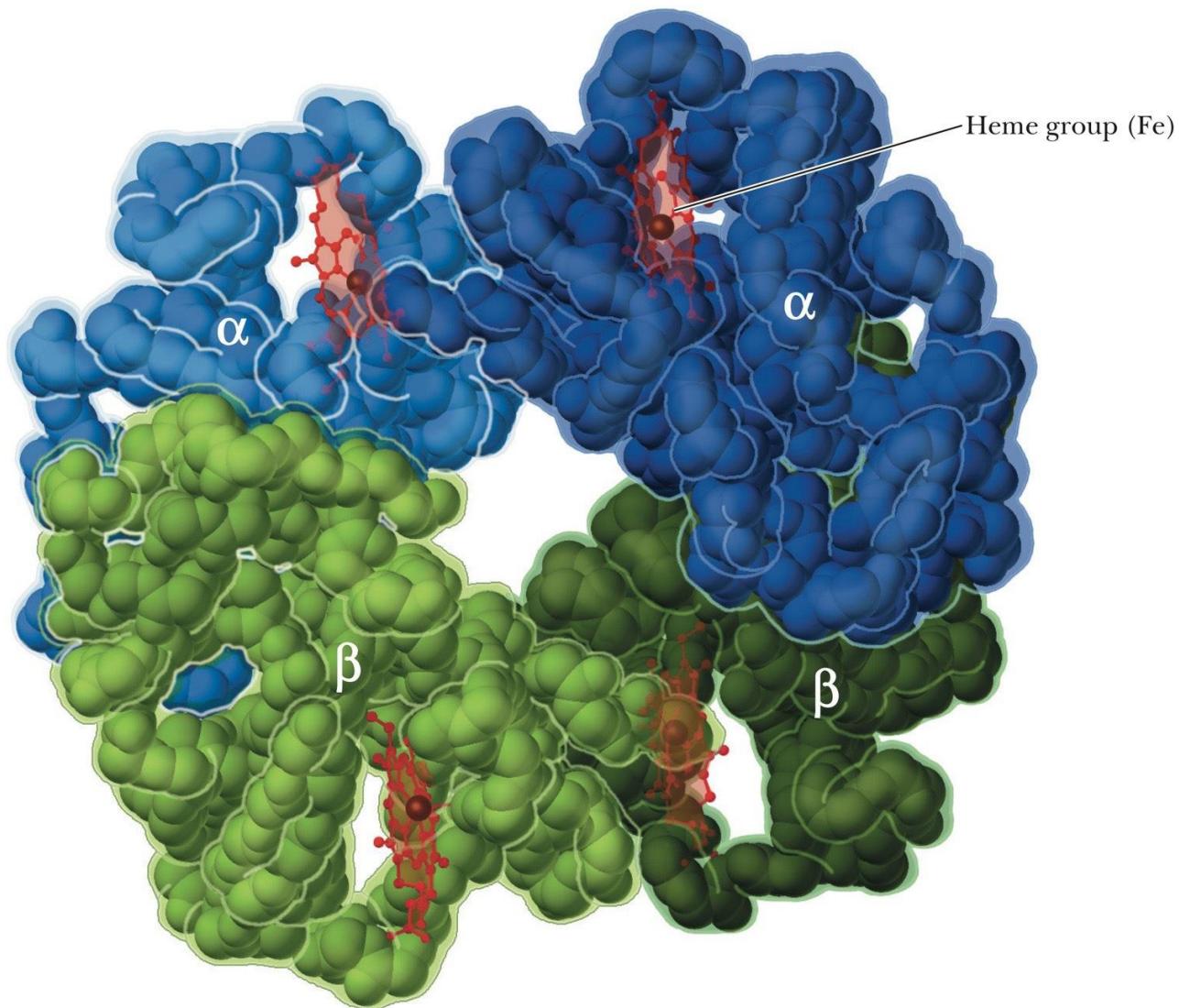
Hill Coefficient

n_H provides a degree of cooperativity (interaction) between binding sites. $n = \text{number of binding sites}$

- $n_H = 1 \Rightarrow$ No cooperativity, e.g. Mb and O₂ (single binding site). Occurs also in proteins with multiple binding sites when the sites do not “communicate”/ “interact” with one another.
- $1 < n_H < n \Rightarrow$ Positive cooperativity, e.g. Hb and O₂. Typical for a cooperatively binding protein. Ligand binding *increases* the affinity for further ligand binding.
- $n_H = n \Rightarrow$ All-or-non binding (fully cooperative). Hypothetically, one Hb molecule would fill up its four sites before any other Hb molecule had taken oxygen. Thus, only fully-unliganded and fully-liganded Hb can be present at any point in the binding process.
- $0 < n_H < 1 \Rightarrow$ Negative cooperativity. Ligand binding to one site *decreases* the affinity for further ligand binding to another site.

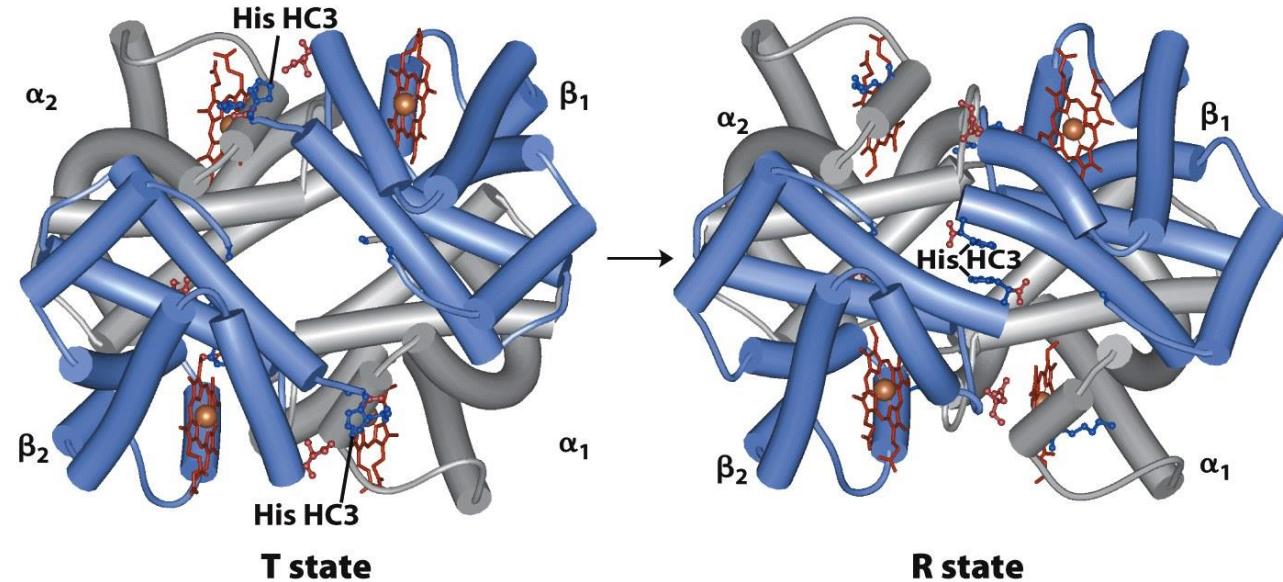
+ve cooperativity is recognized by sigmoidal binding curves

Structure of Hemoglobin



Conformation Changes That Accompany Hb Function: The T and R states

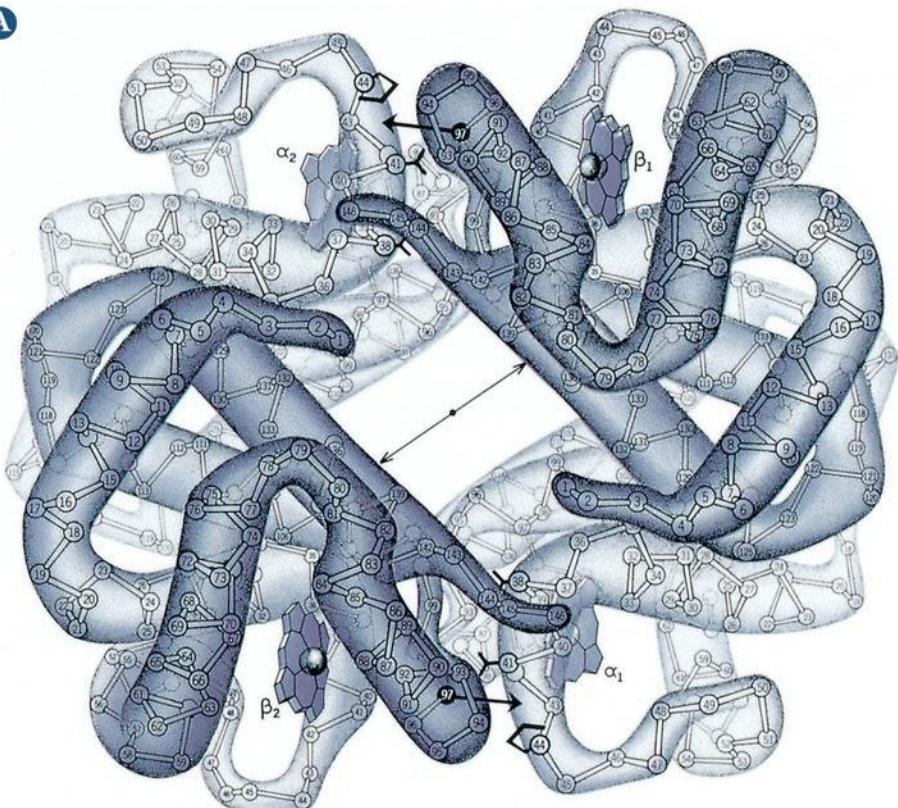
- Hb exists in either T or R states (T=Tense & R=relaxed)
- Deoxyhemoglobin subunits are more stable in the T state
- R state has higher affinity for O₂ than the T state
- O₂ binding triggers a T → R conformational change that involves breaking ion pairs between the α₁-β₂ interface
- Characteristic of **allosteric** behavior
- Hb exhibits different 4° structure in the bound and unbound oxygenated forms
- Other ligands are involved in cooperative effect of Hb can affect protein's affinity for O₂ by altering structure



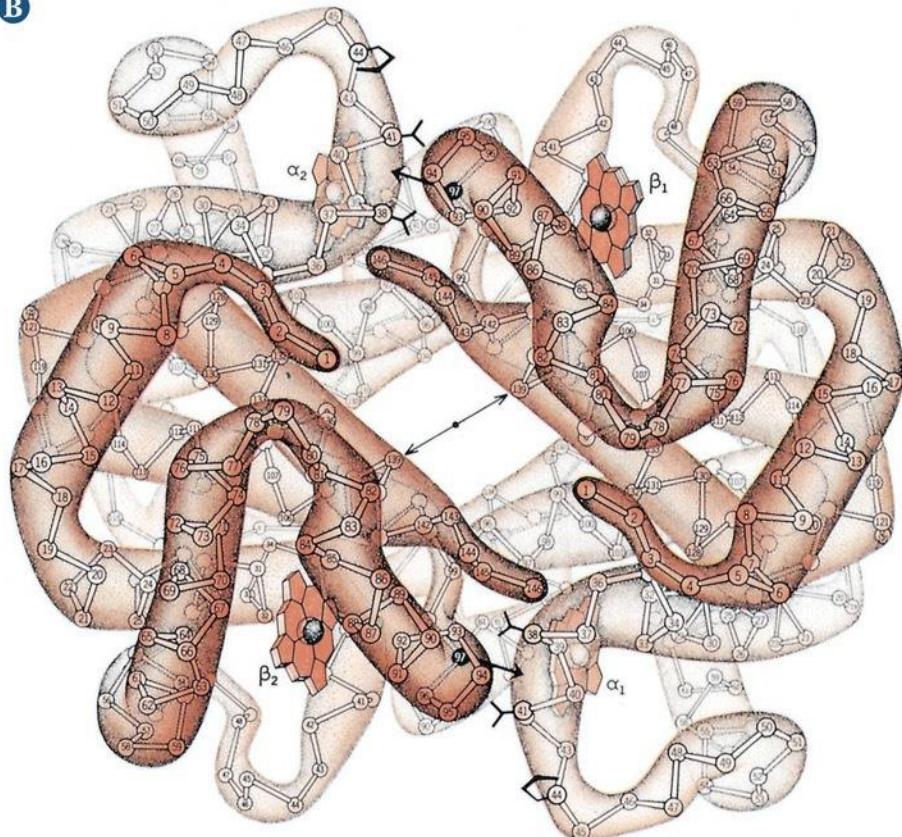
allosterism refers to communication between distal sites

The Structures of Oxy- and Deoxyhemoglobin

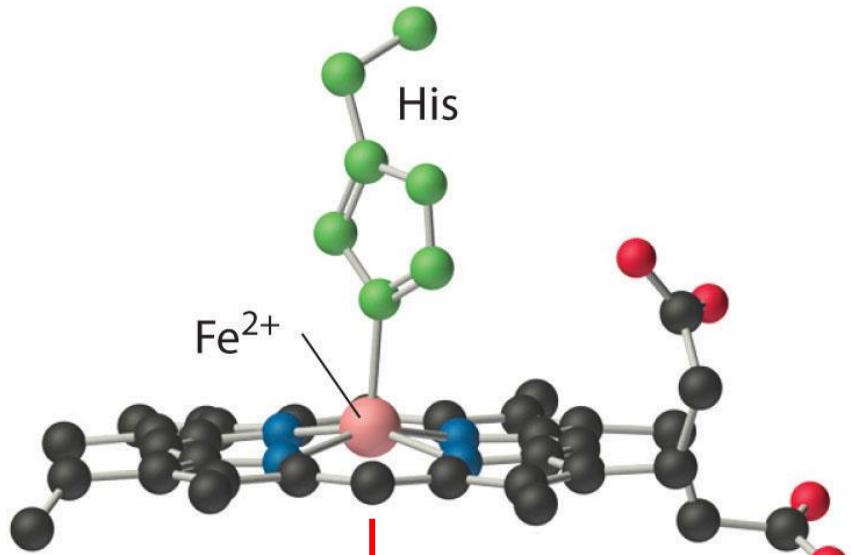
A



B

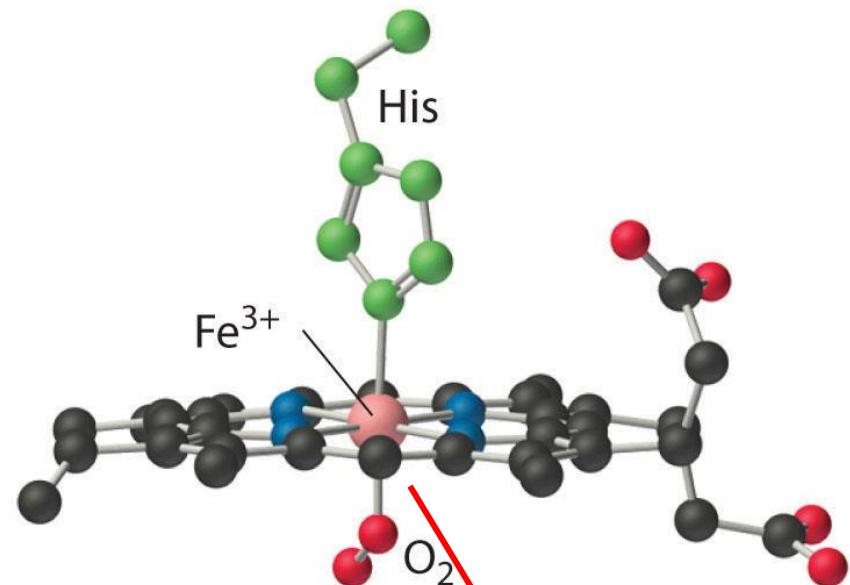


A closer look into O₂ binding by Mb and Hb



(a) Deoxymyoglobin

Fe^{2+} lies above the
porphyrin plan
(0.055 nm)

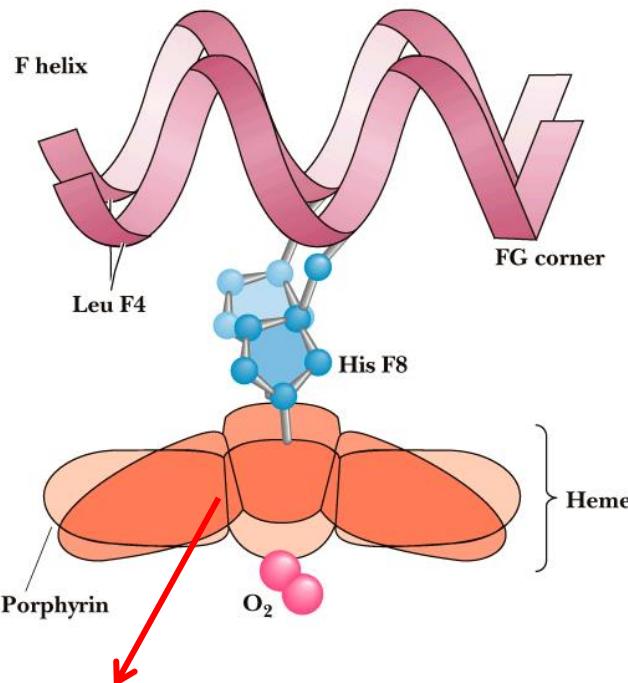


(b) Oxymyoglobin

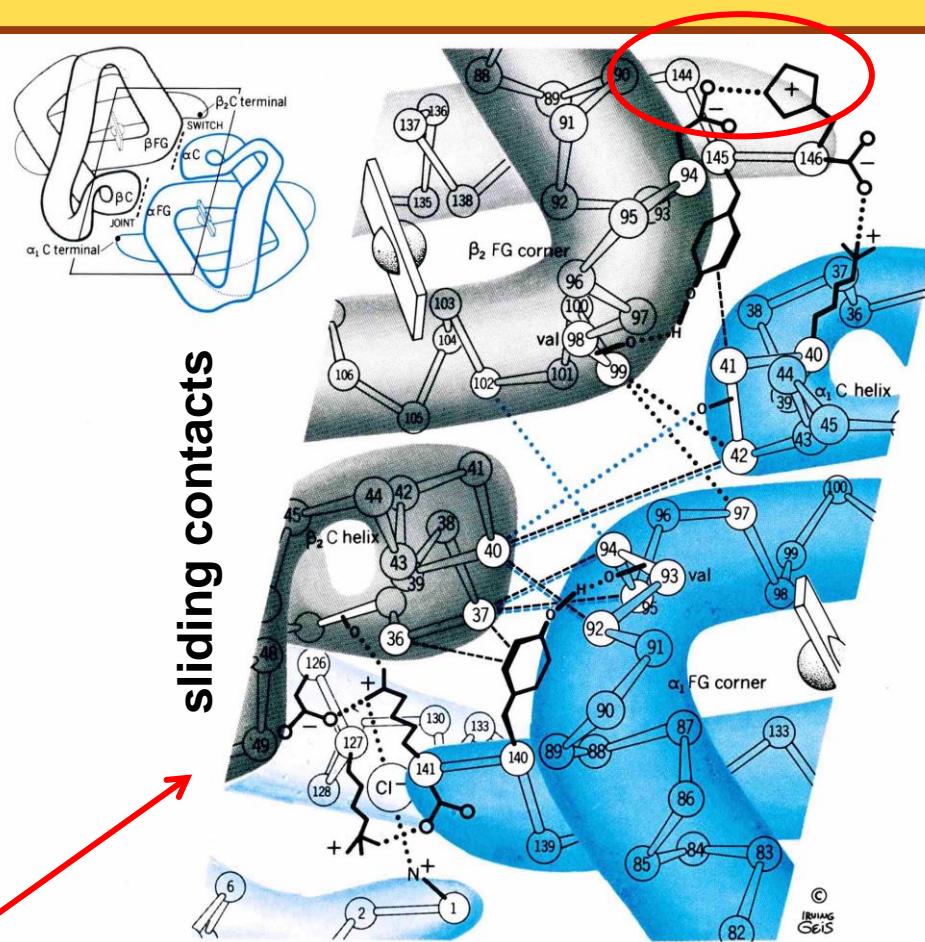
Fe^{3+} lies closer to
the porphyrin plan
(0.025 nm)

Changes associated with the iron binding site geometry have no functional implications for Mb

A closer look into O₂ binding by Mb and Hb

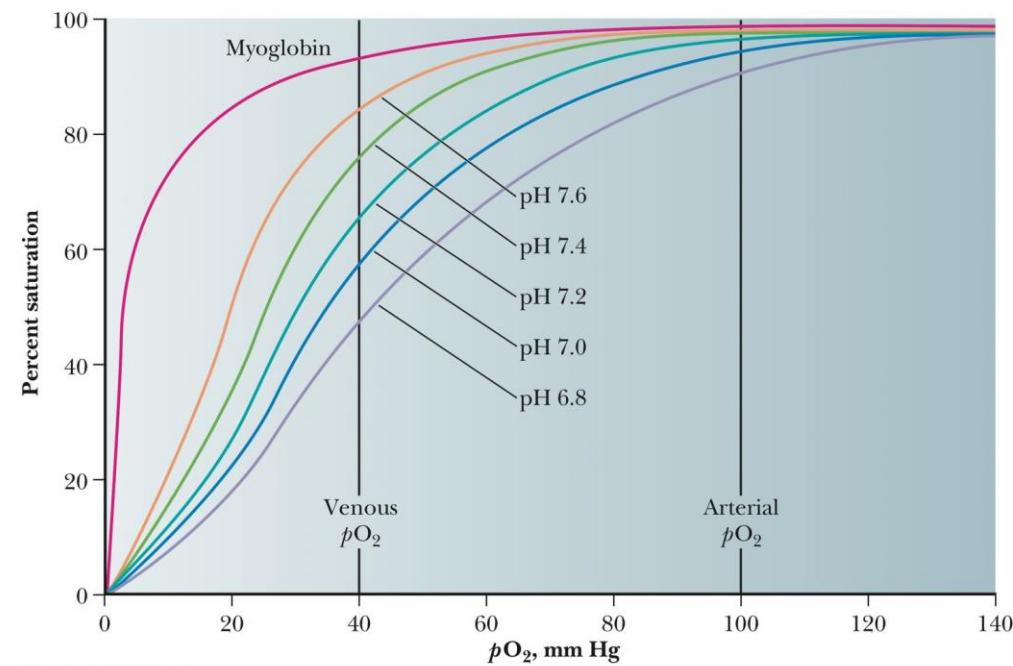


similar local conformational changes occur to the heme group of Hb

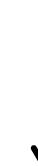


However, they are propagated to distal regions of the protein, affecting intersubunit interactions and subsequent O₂ binding

The Bohr effect: H⁺ as an antagonist of O₂ binding



protonation of functional groups (N-termini and His sidechains)



at the protonated state, the deoxygenated form is favored

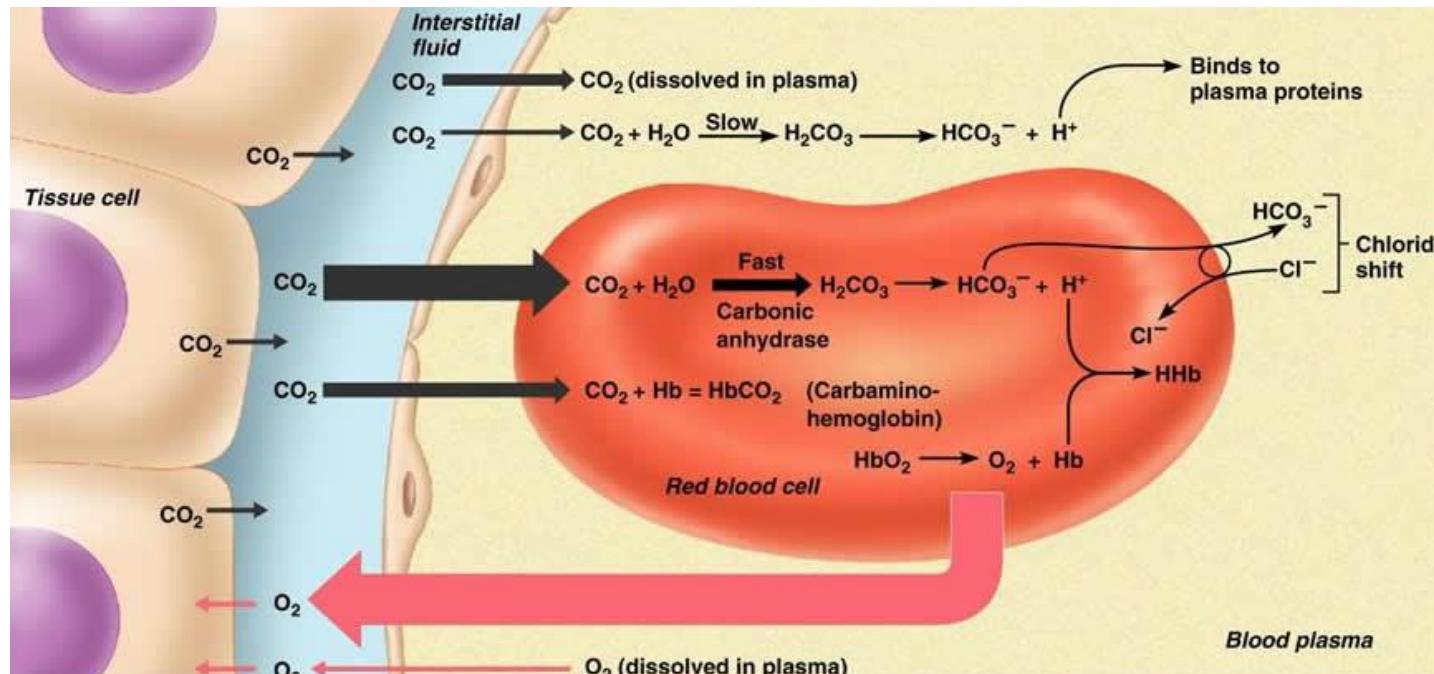


the saturation curve of Hb is shifted to the right as pH decreases

H⁺ binding to Hb, affects its structure and thus its O₂ binding properties (promotes O₂ release)

(and vice versa): O₂ binding to Hb modulates its “buffering” properties

The Bohr effect: CO_2 produces H^+ when dissolved in blood



(a) Oxygen release and carbon dioxide pickup at the tissues

CO_2 is produced in actively metabolizing tissues → converted to H_2CO_3 in blood cells

→ H_2CO_3 is dissociated producing H^+ → H^+ favors the deoxygenated Hb state →

→ O_2 is released to fuel metabolism

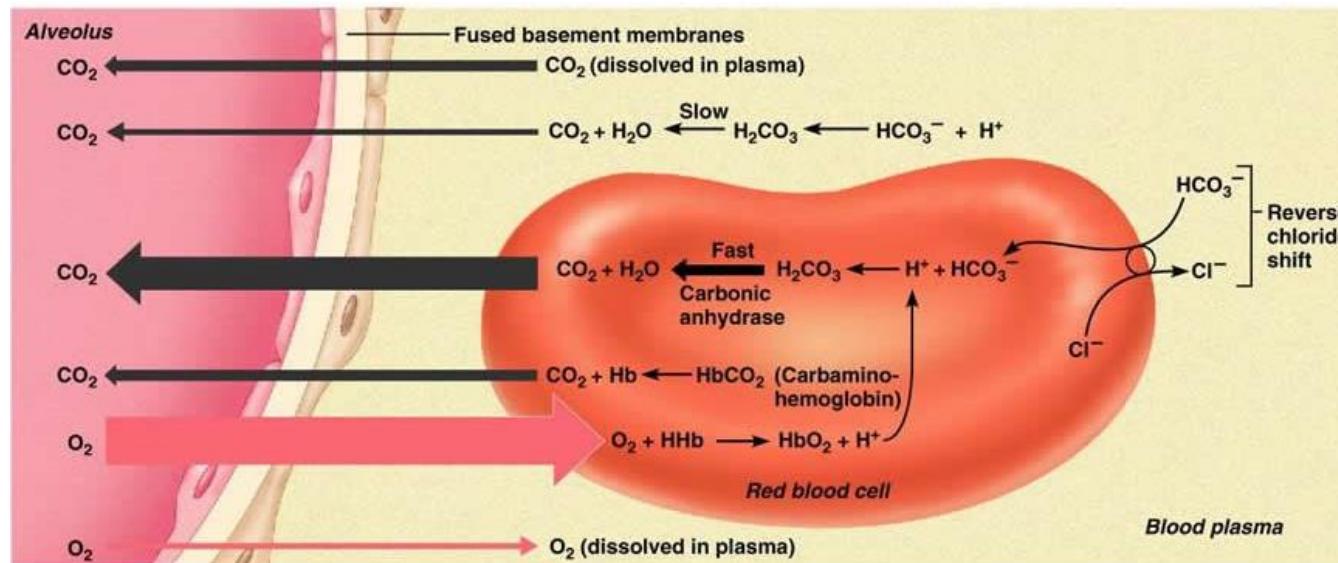
$$\text{blood pH} = 7.4$$

$$\text{H}_2\text{CO}_3 \text{ pKa} = 6.35$$



90% dissociated

The Bohr effect: CO_2 produces H^+ when dissolved in blood



(b) Oxygen pickup and carbon dioxide release in the lungs

In the lungs Hb shifts from the deoxygenated to the oxygenated state $\rightarrow \text{H}$ is released

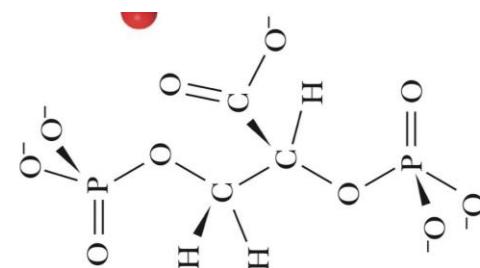
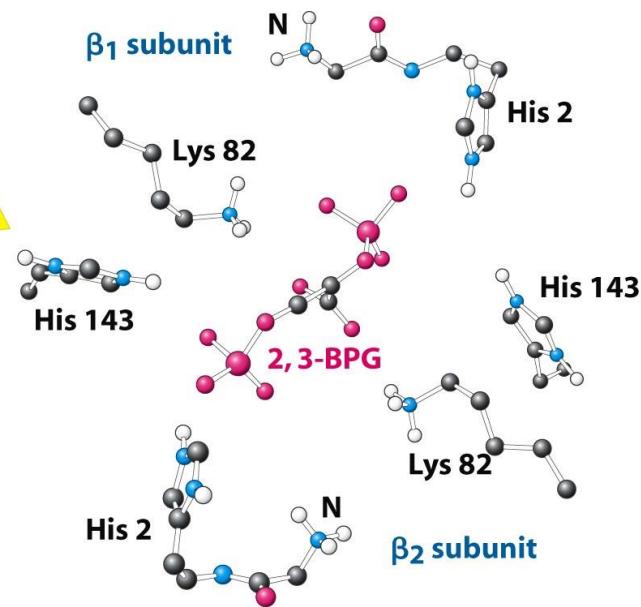
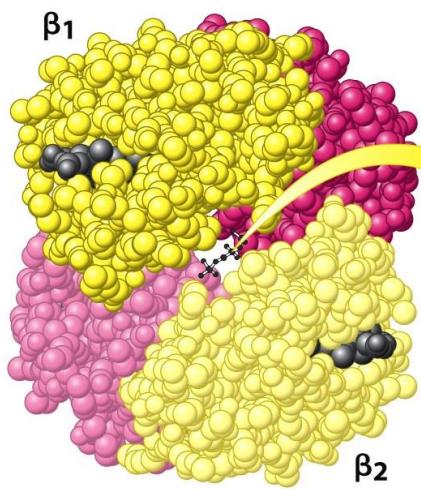
$\rightarrow \text{H}^+$ reacts with $\text{HCO}_3^- \rightarrow \text{H}_2\text{CO}_3$ liberates $\text{CO}_2 \rightarrow \text{CO}_2$ is exhaled as gas



Hb can also transport CO_2 by reacting with free alpha-amino groups:



2,3-Biphosphoglycerate (BPG) is an allosteric effector

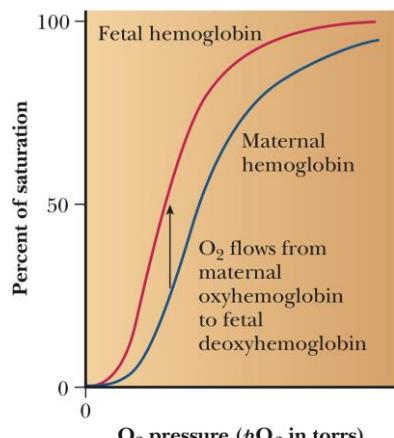


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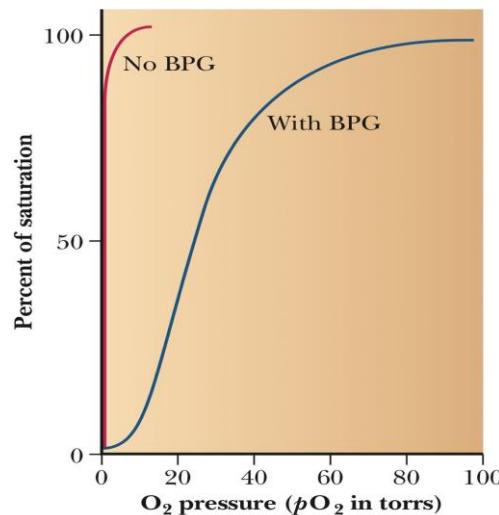
BPG “crosslinks” two β -subunits

stabilizes the deoxy form

favors O_2 release

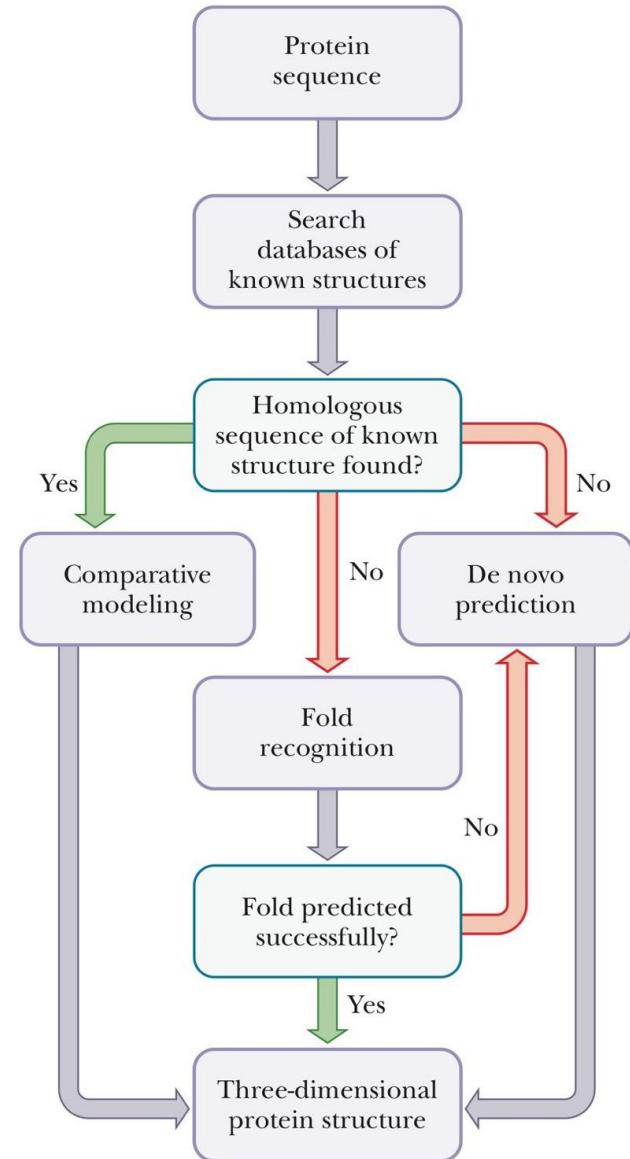
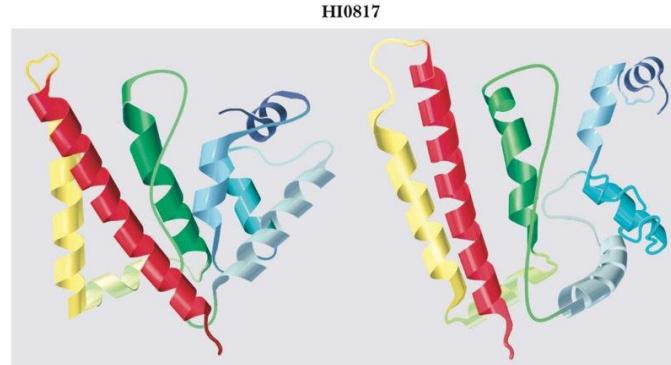
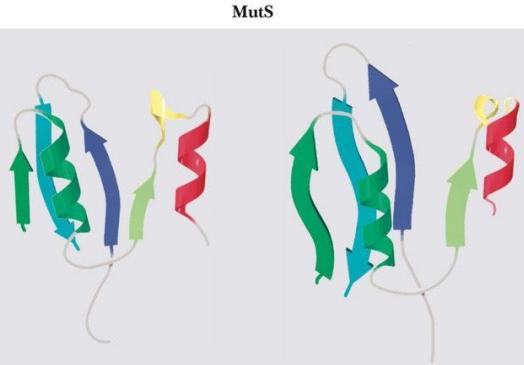


H143S substitution



Predicting Protein Structure

- Biochemistry and computing together gave rise to the field of bioinformatics
- Predicting protein architecture is a search of databases of known structures for sequence homology
- Homology- Refers to similarity



The Hydrophobic Effect (on protein folding)

- The hydrophobic effect is the determining factor in protein folding
- Folding occurs so that: (a) nonpolar hydrophobic side chains become buried in the protein core, and (b) polar side chains are exposed to the aqueous environment
- Protein folding is spontaneous process
- An introduction to this concept has been described in the description of the formation of **liposomes**

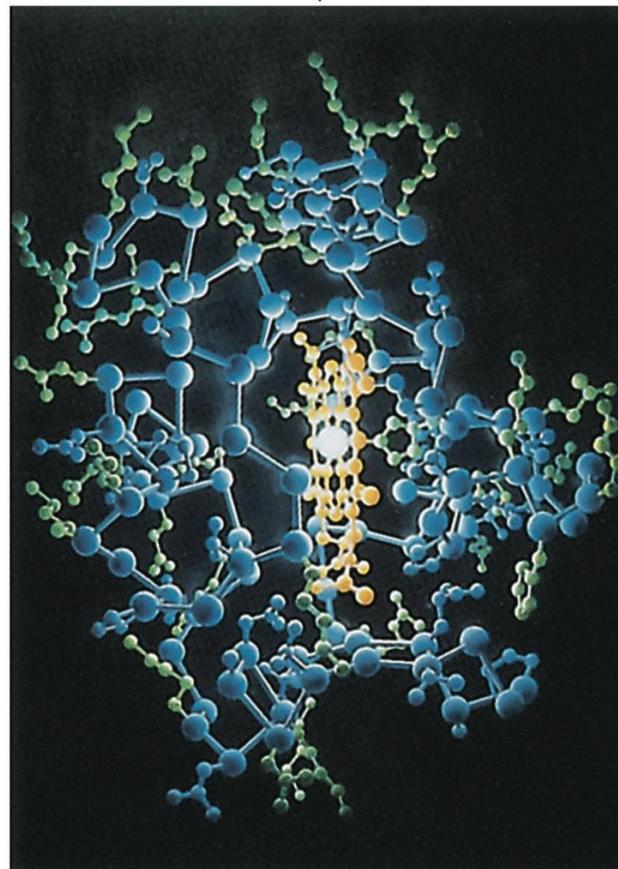
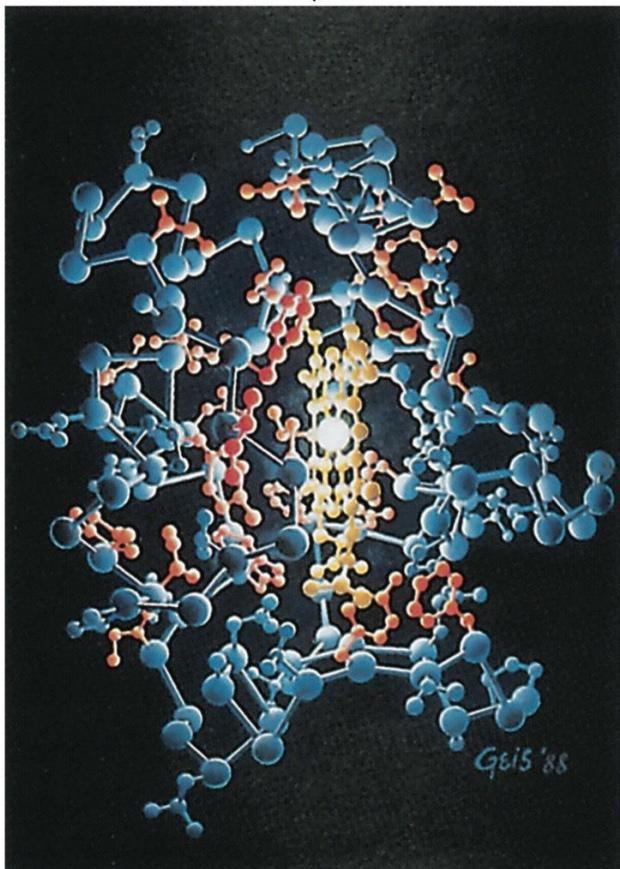
Hydrophobic and Hydrophilic Interactions in Proteins

A

The hydrophobic side chains (shown in red) are found in the interior of the molecule.

B

The hydrophilic side chains (shown in green) are found on the exterior of the molecule.



Tertiary Structure

DNA → RNA → PROTEIN

Bacillus amyloliquefaciens ribonuclease
BARNASE

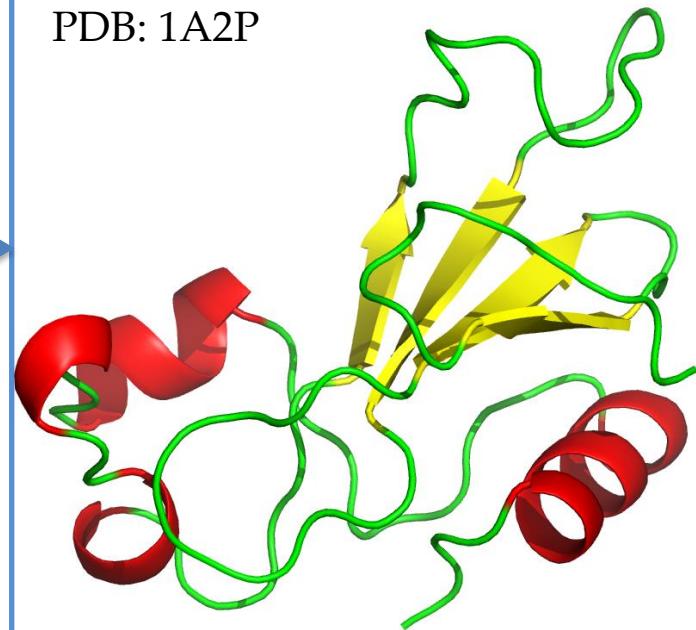
AQVINTFDGVADYLQTYHKLPDNYITKSEAQALGWVA
SKGNLADVAPGKSIGGDIIFSNREGKLPGKSGRTWREA
DINYTSGFRNSDRILYSSDWLIYKTTDHYQTFTKIR

→ folding means arriving at the right angles
for every residues in the sequence

→ amino-acid sequence of a protein
determines its 3D shape

Journal of Biological Chemistry (1954) 207, 201-210
Nobel Prize in Chemistry, 1972

PDB: 1A2P

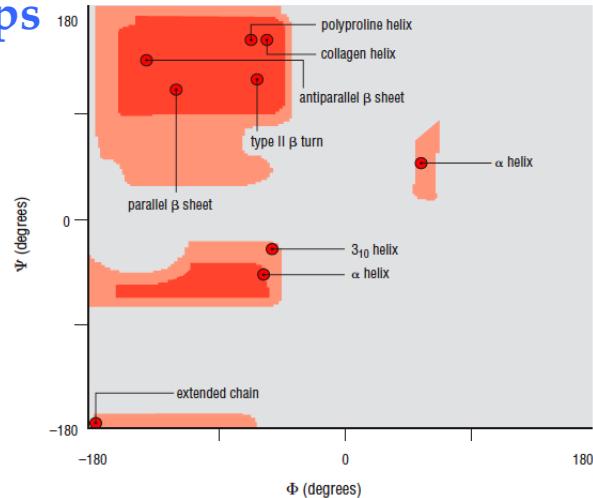


FUNCTIONAL,
FOLDED
PROTEIN

Tertiary Structure

Levinthal's paradox

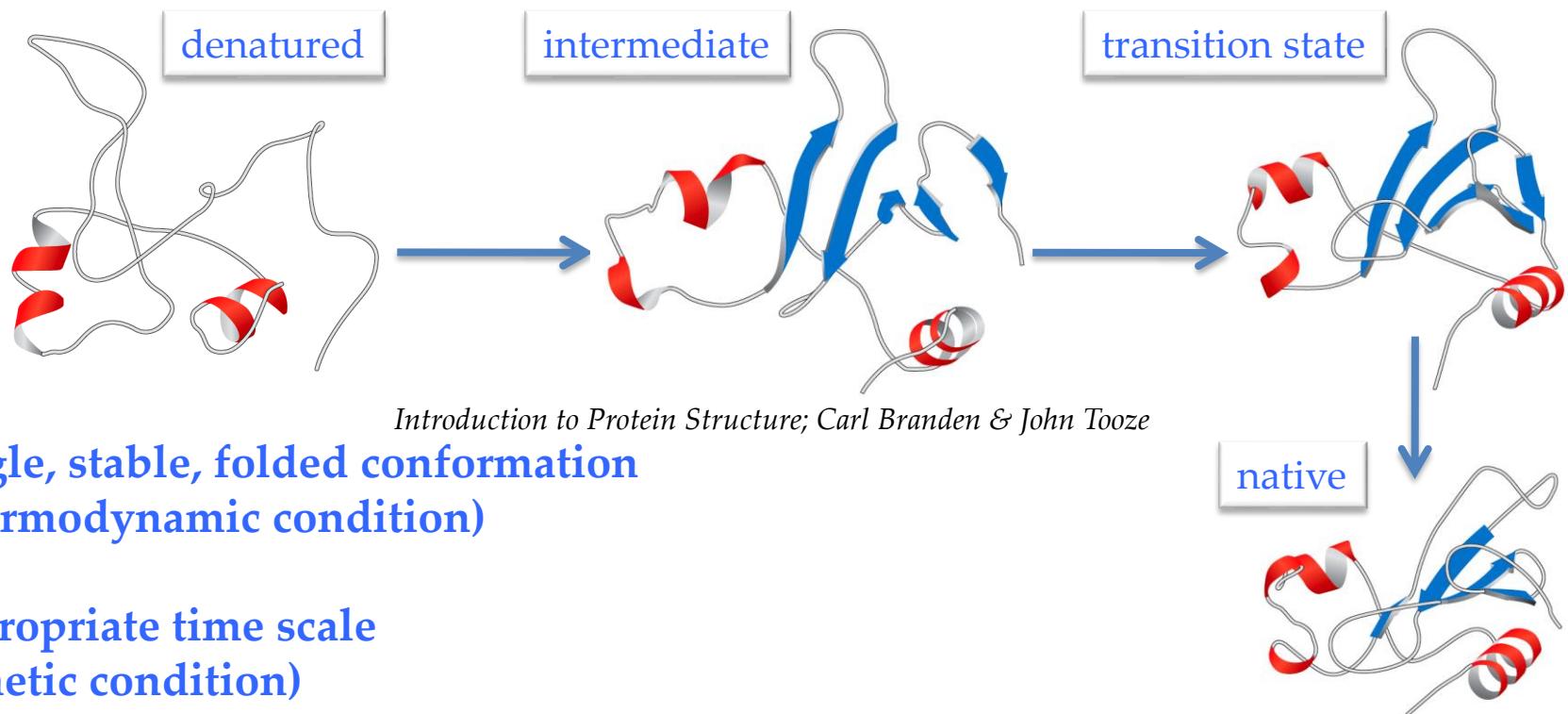
- each amino-acid can adopt one the three discrete groups from the Ramachandran plot
- conformational changes occur with a rate of 10^{-12} seconds
- ⇒ a protein with 150 residues would need to explore 3^{150} possible states (10^{71})
- ⇒ $\sim 10^{50}$ years to reach the lowest energy conformation
 - *Proteins fold between 0.1s and 1000s*



Tertiary Structure

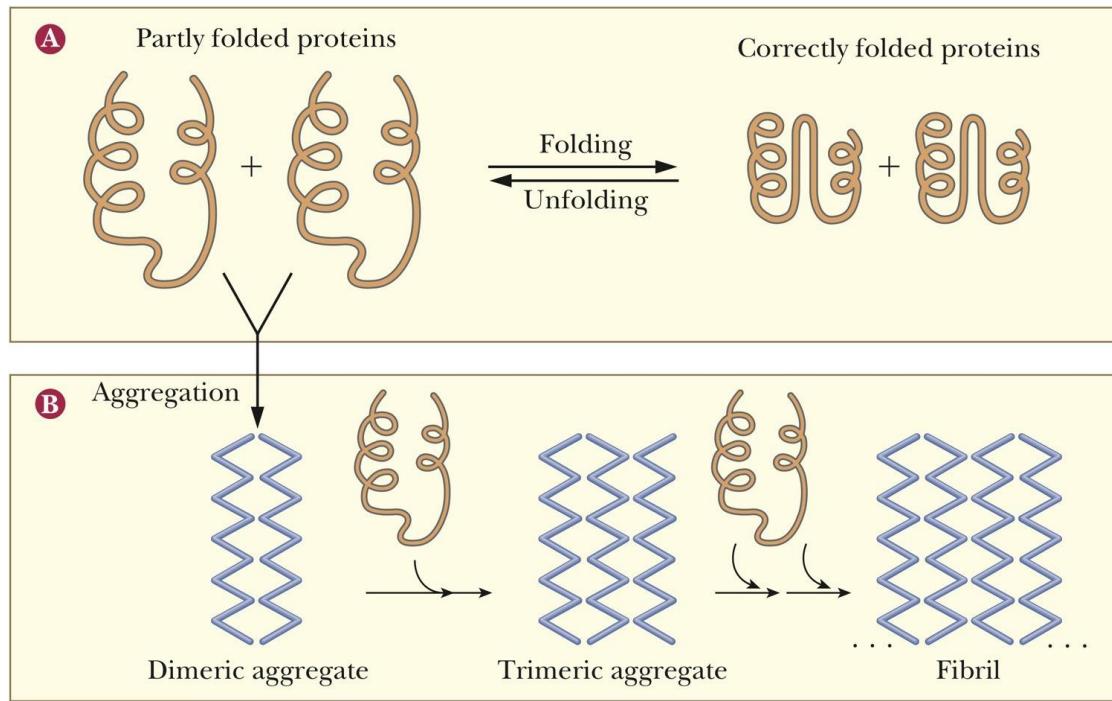
Levinthal's paradox

- proteins fold on a defined pathway (or a small number of alternative pathways)
- no search randomly all possible conformations until they arrive the most stable (lowest free energy) structure



The Importance of Correct Folding

- Proteins that do not fold correctly may interact with other proteins in an undesired manner and aggregates may result.



Protein Folding Chaperones

- In the protein-dense environment of a cell, proteins may begin to fold incorrectly or may associate with other proteins before folding is completed
- Special proteins called **chaperones** aid in the correct and timely folding of many proteins
- hsp70 were the first chaperone proteins discovered
- Chaperones exist in organisms from prokaryotes to humans

Suggested problems

1-7, 9, 10, 12, 13, 15, 16, 17, 19-32, 34, 39, 41, 49, 55, 56