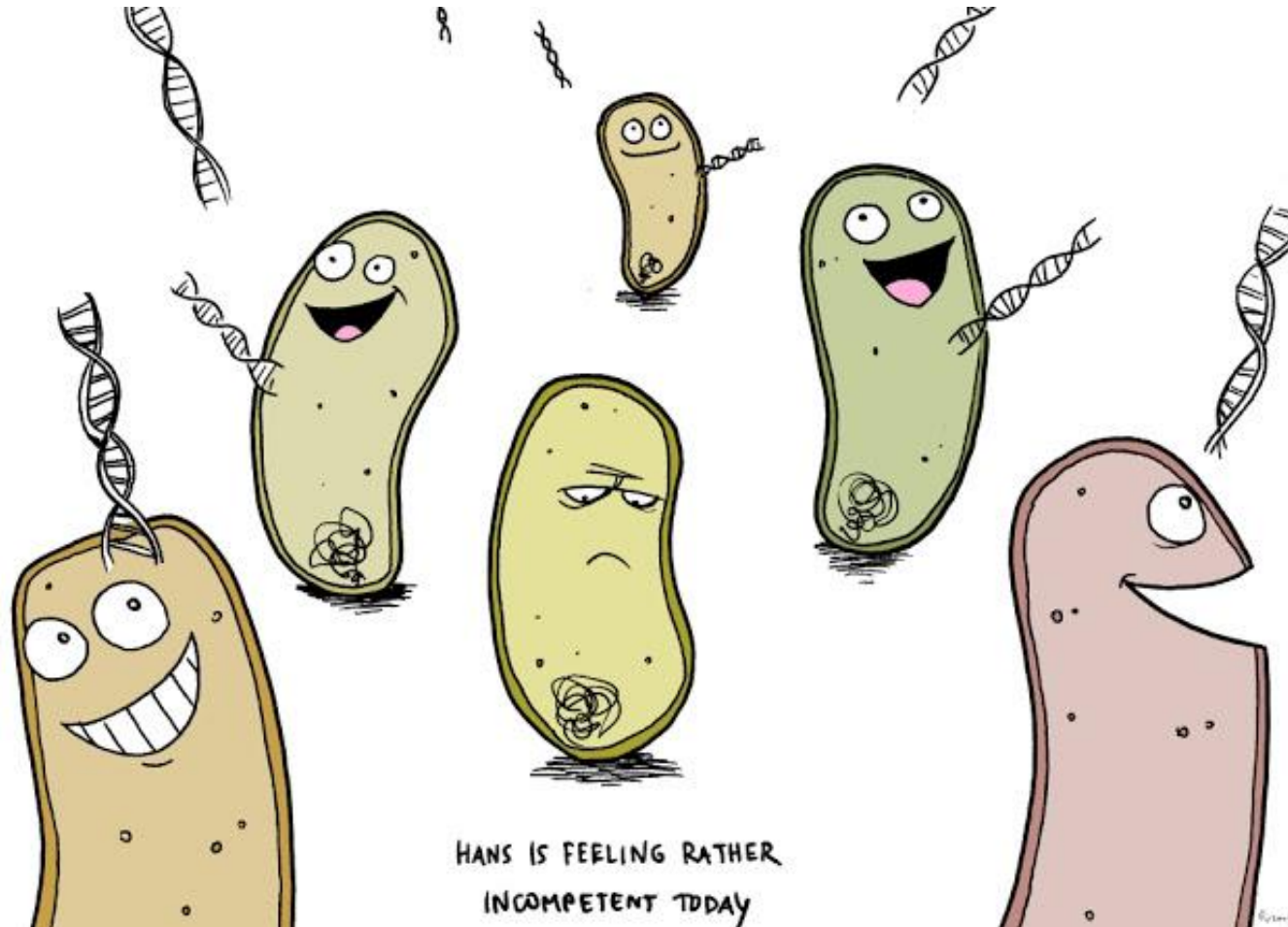


Recitation #2 Proteins: Structure, Function, Folding



CHAPTER 4

Proteins: Structure, Function, Folding

Learning goals:

- Structure and properties of the peptide bond
- Structural hierarchy in proteins
- Structure and function of fibrous proteins
- Structure analysis of globular proteins
- Protein folding and denaturation

Structure of Proteins

- Unlike most organic polymers, protein molecules adopt a specific **three-dimensional conformation**.
- This structure is able to fulfill a specific **biological function**
- This structure is called the **native fold**
- The native fold has **a large number of favorable interactions** within the protein
- There is a **cost in conformational entropy** of folding the protein into one specific native fold

Favorable Interactions in Proteins

- **Hydrophobic effect**

- Release of water molecules from the structured solvation layer around the molecule as protein folds increases the net entropy

- **Hydrogen bonds**

- Interaction of N-H and C=O of the peptide bond leads to local regular structures such as α -helices and β -sheets

- **London dispersion**

- Medium-range weak attraction between all atoms contributes significantly to the stability in the interior of the protein

- **Electrostatic interactions**

- Long-range strong interactions between permanently charged groups
- Salt-bridges, esp. buried in the hydrophobic environment strongly stabilize the protein

Primary structure

Pro
Ala
Asp
Lys
Thr
Asn
Val
Lys
Ala
Ala
Trp
Gly
Lys
Val

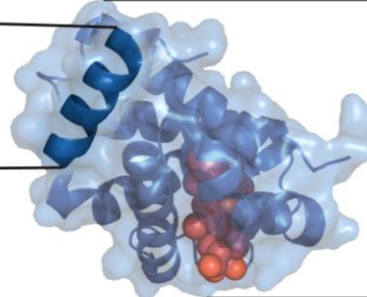
Amino acid residues

Secondary structure



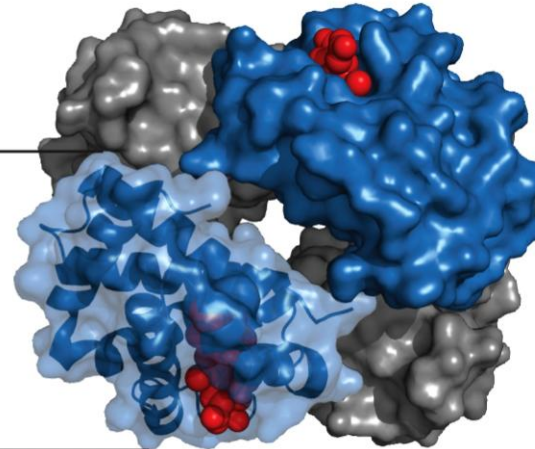
α Helix

Tertiary structure



Polypeptide chain

Quaternary structure



Assembled subunits

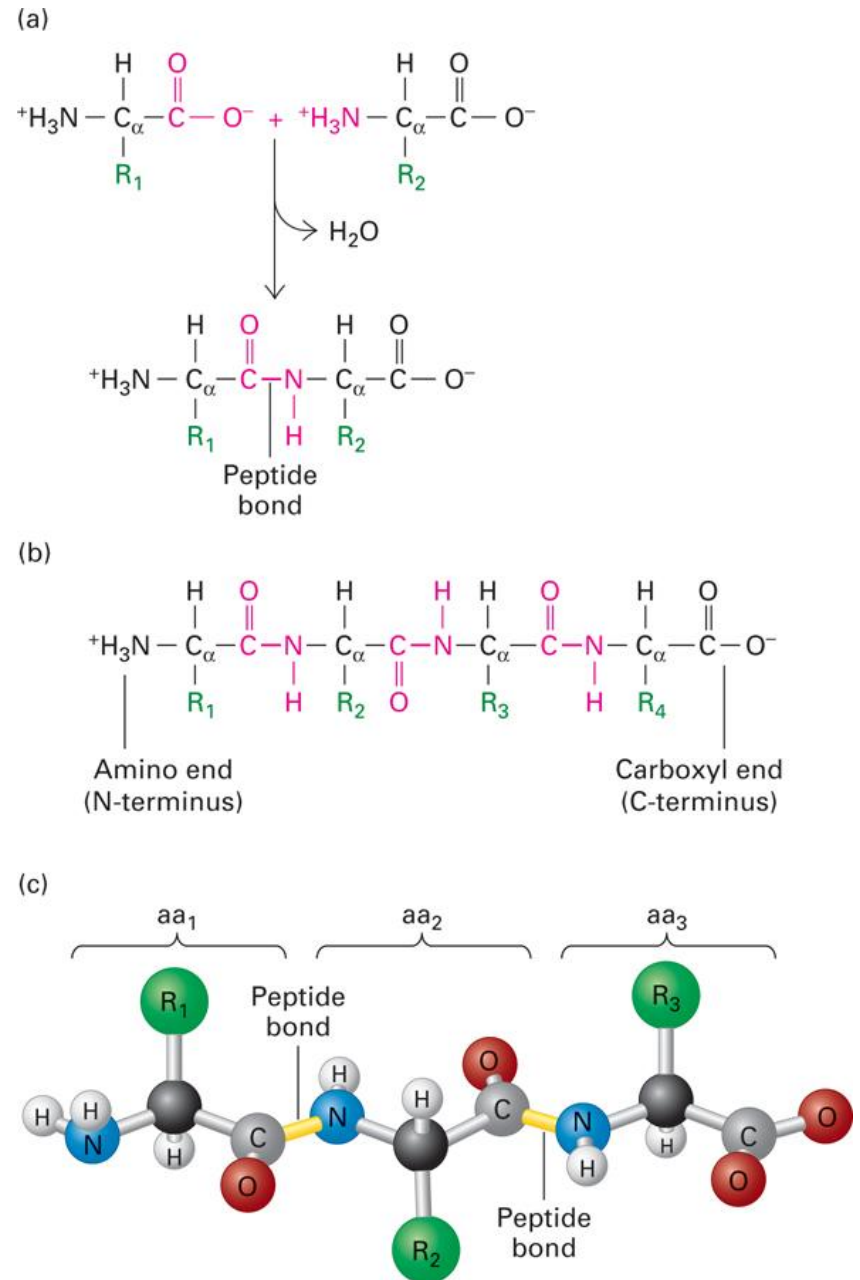
Figure 3-23

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- **Levels of structure in proteins.** The *primary structure* consists of a sequence of amino acids linked together by peptide bonds
- and includes any disulfide bonds. The resulting polypeptide can be arranged into units of *secondary structure*, such as an α -helix. The helix is a part of the *tertiary structure* of the folded polypeptide, which is itself one of the subunits that make up the *quaternary structure* of the multi-subunit protein, in this case hemoglobin.

- The formation of the peptide bond is a dehydration synthesis with the release of H₂O, the breaking of the bond then is a hydrolysis with the addition of a water molecule across the bond.



Structure of the Peptide Bond

- Structure of the protein is partially dictated by the properties of the peptide bond
- The peptide bond is a resonance hybrid of two canonical structures
- The resonance causes the peptide bonds
 - to be less reactive compared to esters, for example
 - to be quite **rigid** and nearly **planar**
 - to exhibit a large dipole moment in the favored trans configuration

The Rigid Peptide Plane and the Partially Free Rotations

- Rotation around the peptide bond is not permitted
- Rotation around bonds connected to the alpha carbon is permitted
- ϕ (phi): angle around the α -carbon—amide nitrogen bond
- ψ (psi): angle around the α -carbon—carbonyl carbon bond
- In a fully extended polypeptide, both ψ and ϕ are 180°

The polypeptide is made up of a series of planes linked at α carbons

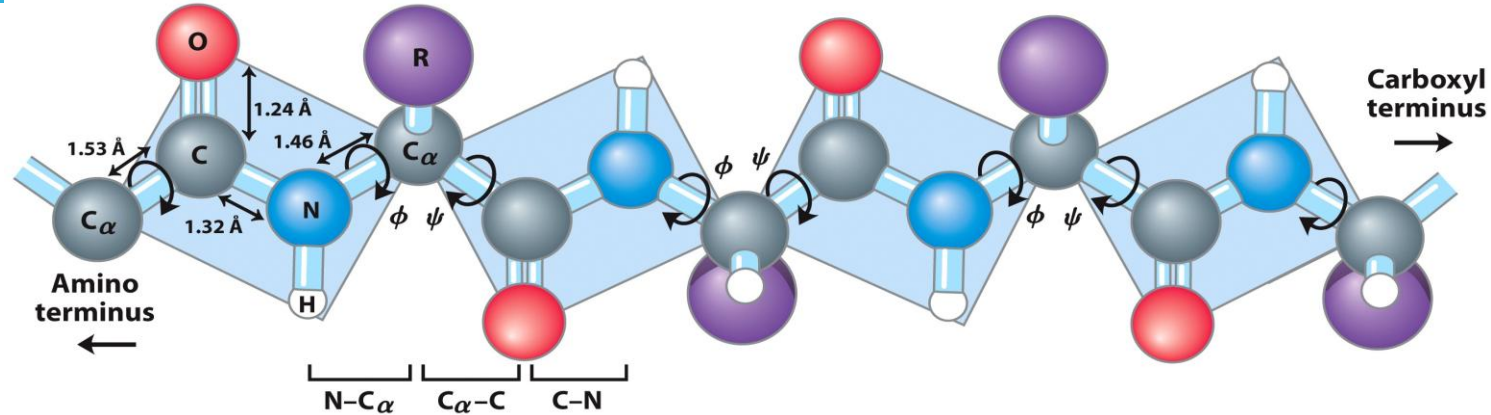


Figure 4-2b
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The planar peptide group. (b) Three bonds separate sequential α carbons in a polypeptide chain. The $\text{N}-\text{C}_\alpha$ and $\text{C}_\alpha-\text{C}$ bonds can rotate, described by dihedral angles designated ϕ and ψ , respectively. The peptide $\text{C}-\text{N}$ bond is not free to rotate. Other single bonds in the backbone may also be rotationally hindered, depending on the size and charge of the R groups.

Distribution of ϕ and ψ Dihedral Angles

- Some ϕ and ψ combinations are very unfavorable because of **steric crowding** of backbone atoms with other atoms in the backbone or side chains
- Some ϕ and ψ combinations are more favorable because of chance to **form favorable H-bonding interactions** along the backbone
- A Ramachandran plot shows the distribution of ϕ and ψ dihedral angles that are found in a protein
 - shows the common secondary structure elements
 - reveals regions with unusual backbone structure

Ramachandran plots showing a variety of structures.

(a) The values of Φ and Ψ for various allowed secondary structures are overlaid on the plot from Figure 4-3. Although left-handed α helices extending over several amino acid residues are theoretically possible, they have not been observed in proteins.

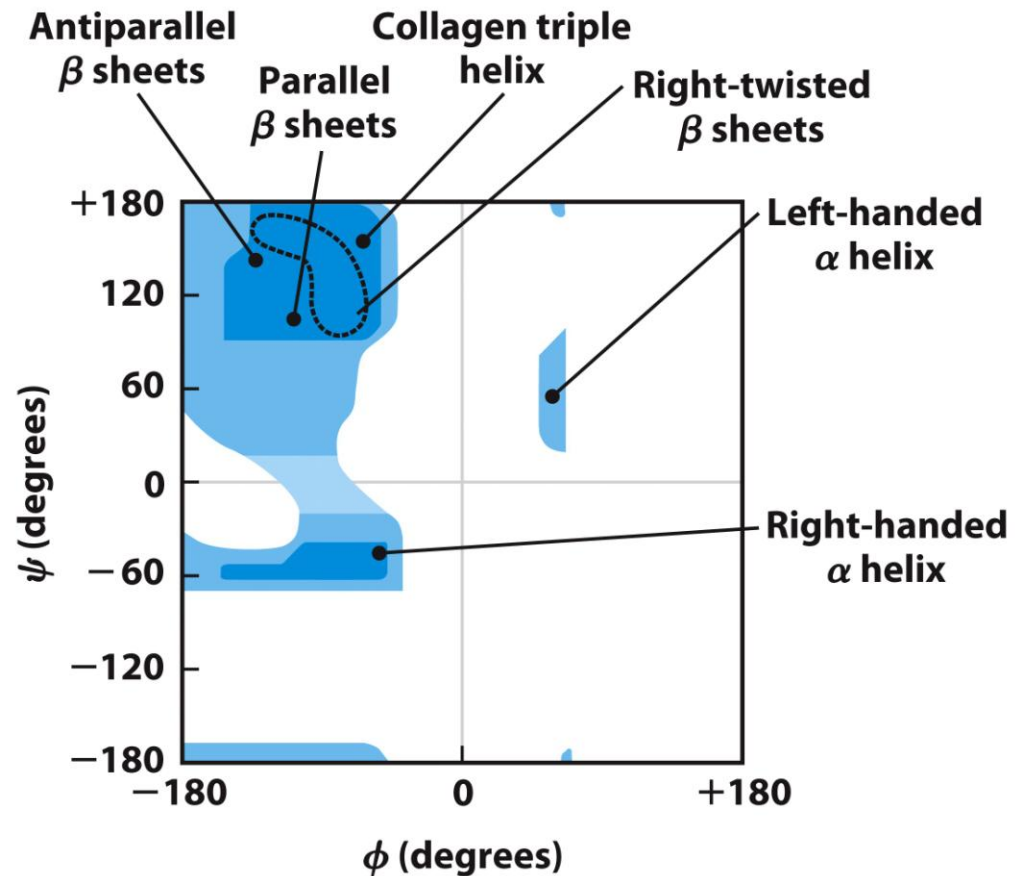


Figure 4-9a
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Secondary Structures

- Secondary structure refers to a local spatial arrangement of the polypeptide backbone
- Two regular arrangements are common:
- The α helix
 - stabilized by hydrogen bonds between nearby residues
- The β sheet
 - stabilized by hydrogen bonds between adjacent segments that may not be nearby
- Irregular arrangement of the polypeptide chain is called the random coil or extended chain.

The α Helix

- Helical backbone is held together by hydrogen bonds between the backbone amides of an n and $n+4$ amino acids
- **Right-handed helix** with 3.6 residues (5.4 \AA) per turn
- Peptide bonds are aligned roughly parallel with the helical axis
- Side chains point out and are roughly perpendicular with the helical axis

4 Models of the α helix, showing different aspects of its structure

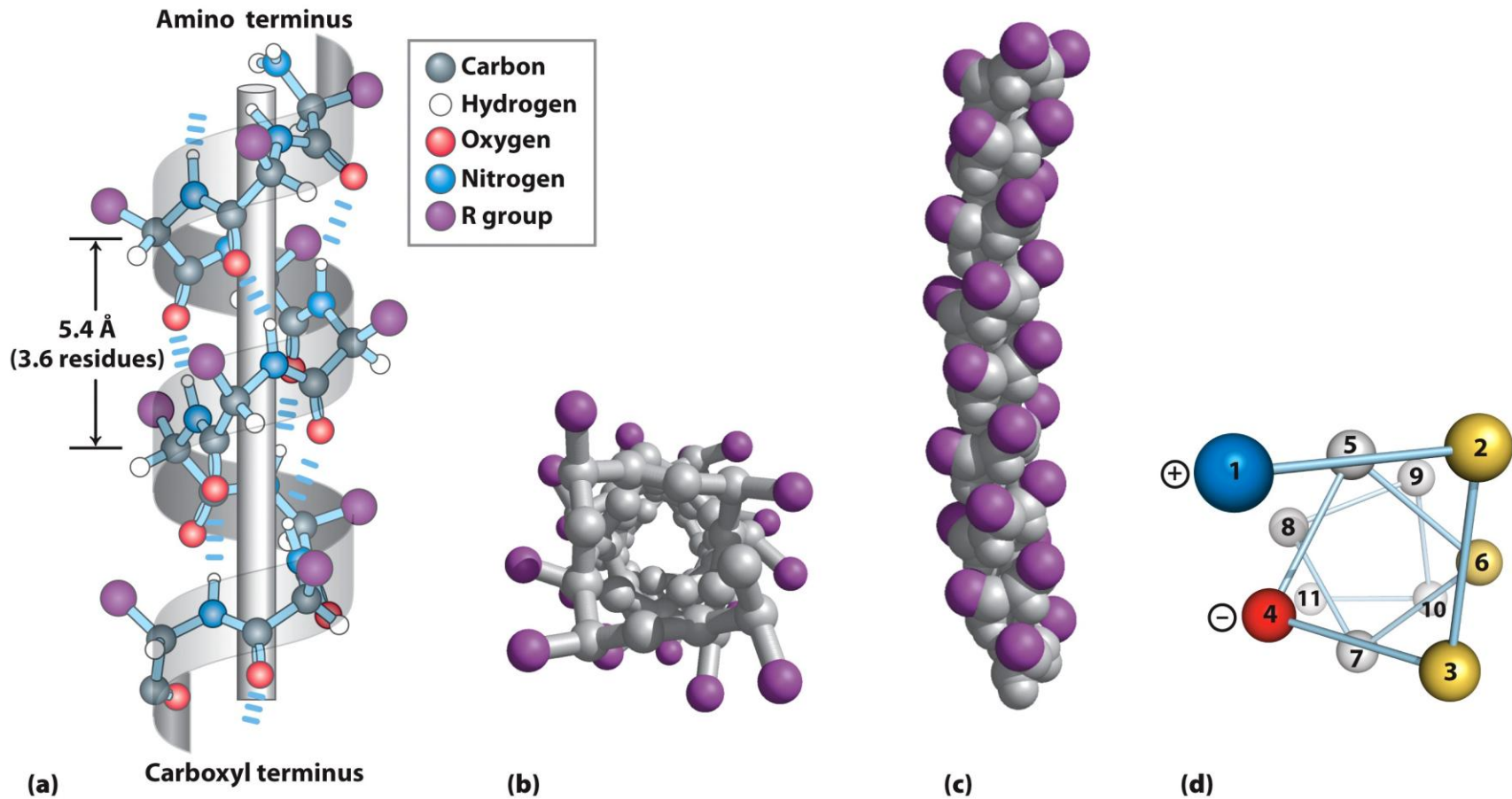


Figure 4-4
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4 Models of the α helix, showing different aspects of its structure

(a) showing the intrachain hydrogen bonds. The repeat unit is a single turn of the helix, 3.6 residues. (b) The α helix viewed from one end, looking down the longitudinal axis. Note the positions of the R groups, represented by purple spheres. This ball-and-stick model, which emphasizes the helical arrangement, gives the false impression that the helix is hollow, because the balls do not represent the van der Waals radii of the individual atoms. (c) As this space-filling model shows, the atoms in the center of the α helix are in very close contact. (d) Helical wheel projection of an α helix. This representation can be colored to identify surfaces with particular properties. The yellow residues, for example, could be hydrophobic and conform to an interface between the helix shown here and another part of the same or another polypeptide. The red and blue residues illustrate the potential for interaction of negatively and positively charged side-chains separated by two residues in the helix.

TABLE 4-2**Propensity of Amino Acid Residues to Take Up an α -Helical Conformation**

Amino acid	$\Delta\Delta G^\circ$ (kJ/mol)*	Amino acid	$\Delta\Delta G^\circ$ (kJ/mol)*
Ala	0	Leu	0.79
Arg	0.3	Lys	0.63
Asn	3	Met	0.88
Asp	2.5	Phe	2.0
Cys	3	Pro	>4
Gln	1.3	Ser	2.2
Glu	1.4	Thr	2.4
Gly	4.6	Tyr	2.0
His	2.6	Trp	2.0
Ile	1.4	Val	2.1

Sources: Data (except proline) from Bryson, J.W., Betz, S.F., Lu, H.S., Suich, D.J., Zhou, H.X., O'Neil, K.T., & DeGrado, W.F. (1995) Protein design: a hierarchic approach. *Science* 270, 935. Proline data from Myers, J.K., Pace, C.N., & Scholtz, J.M. (1997) Helix propensities are identical in proteins and peptides. *Biochemistry* 36, 10,926.

* $\Delta\Delta G^\circ$ is the difference in free-energy change, relative to that for alanine, required for the amino acid residue to take up the α -helical conformation. Larger numbers reflect greater difficulty taking up the α -helical structure. Data are a composite derived from multiple experiments and experimental systems.

Table 4-2

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Helix dipole

The electric dipole of a peptide bond (see Figure 4-2a) is transmitted along an α -helical segment through the intrachain hydrogen bonds, resulting in an overall helix dipole. In this illustration, the amino and carbonyl constituents of each peptide bond are indicated by + and - symbols, respectively. Non-hydrogen-bonded amino and carbonyl constituents of the peptide bonds near each end of the α -helical region are shown in red.

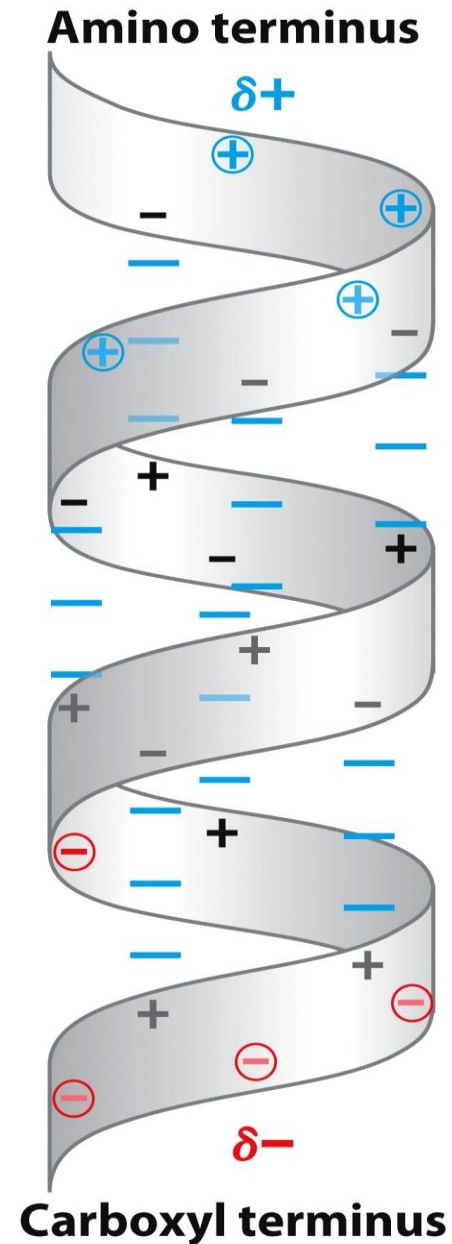


Figure 4-5

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β Sheets

- The planarity of the peptide bond and tetrahedral geometry of the α -carbon create a **pleated sheet-like** structure
- Sheet-like arrangement of backbone is held together by hydrogen bonds between the backbone amides in different strands
- Side chains protrude from the sheet alternating in up and down direction

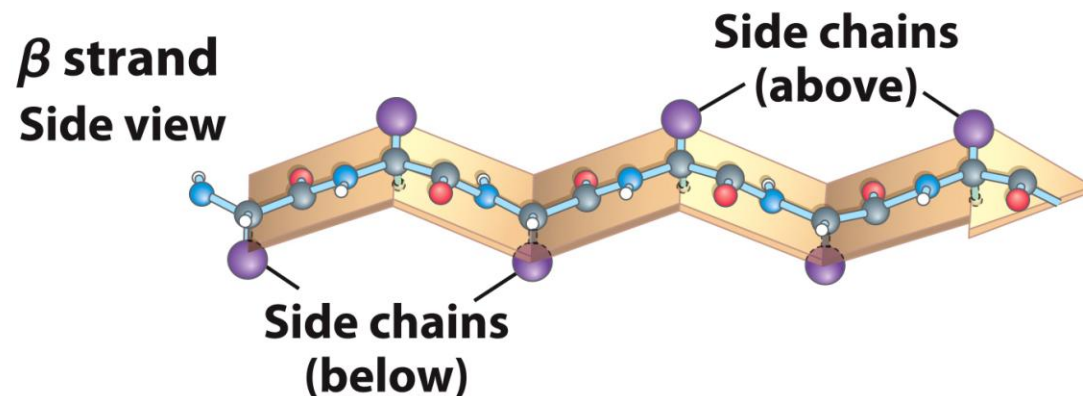


Figure 4-6a
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Parallel and Antiparallel β Sheets

- Parallel or antiparallel orientation of two chains within a sheet are possible
- In parallel β sheets the H-bonded strands run in the same direction
 - Resulting in bent H-bonds (weaker)
- In antiparallel β sheets the H-bonded strands run in opposite directions
 - Resulting in linear H-bonds (stronger)

Antiparallel β sheet

Top view

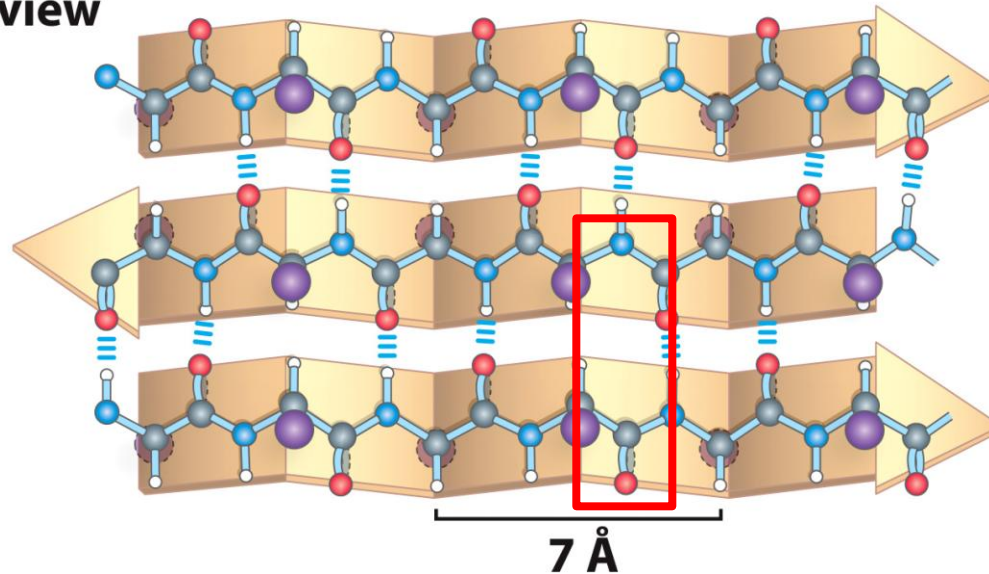
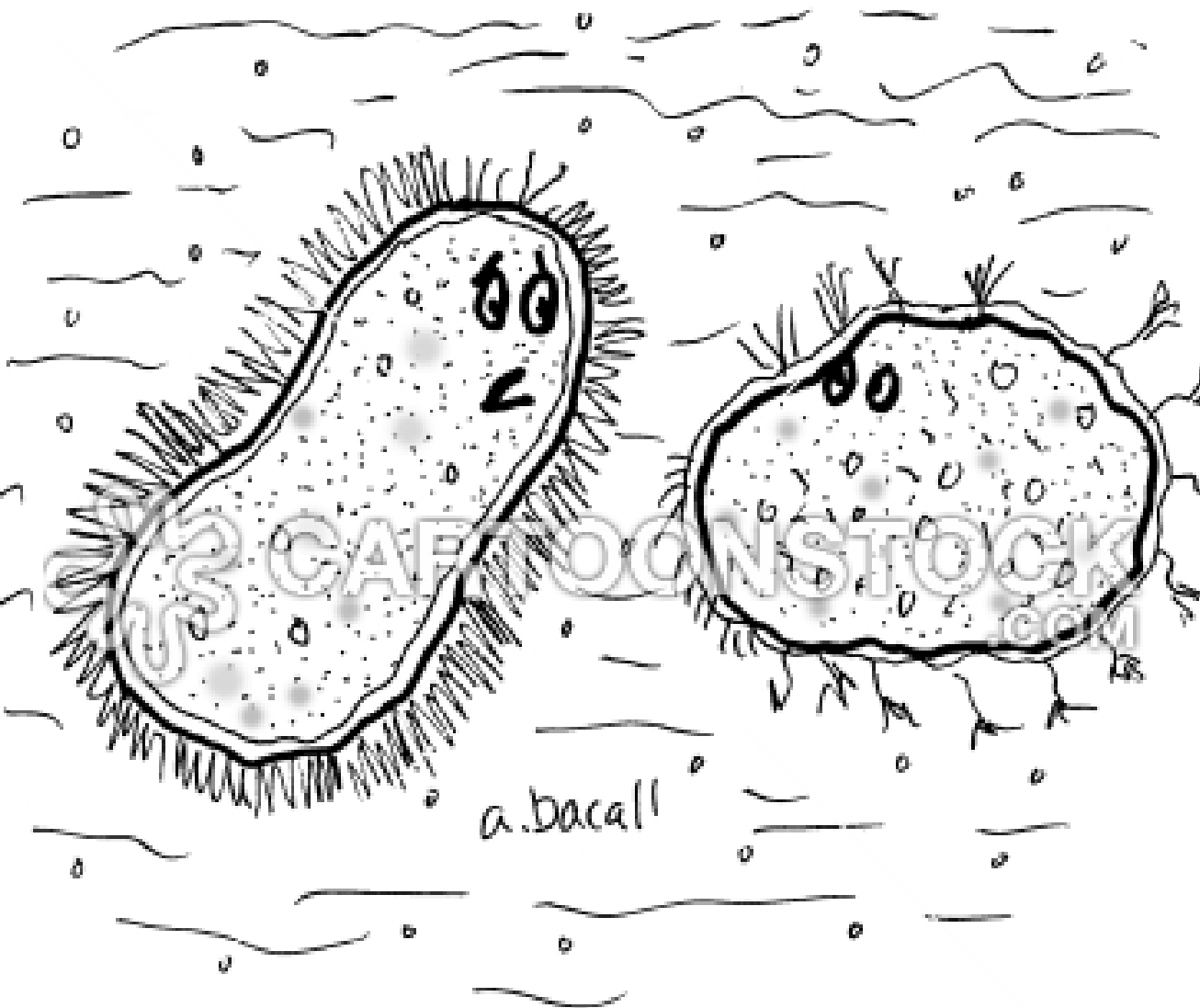


Figure 4-6b
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- **The β conformation of polypeptide chains.** These top and side views reveal the R groups extending out from the β sheet and emphasize the pleated shape described by the planes of the peptide bonds. (An alternative name for this structure is β -pleated sheet.) Hydrogen-bond cross-links between adjacent chains are also shown. The amino-terminal to carboxyl-terminal orientations of adjacent chains (arrows) can be the same or opposite, forming (a) an antiparallel β sheet or (b) a parallel β sheet.

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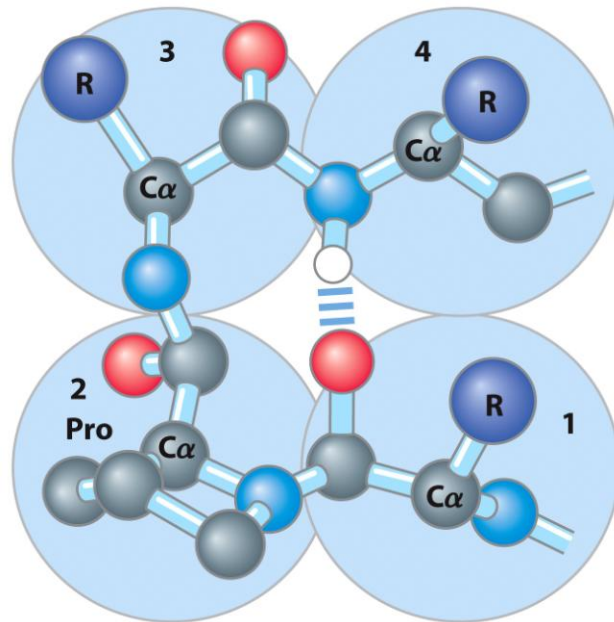


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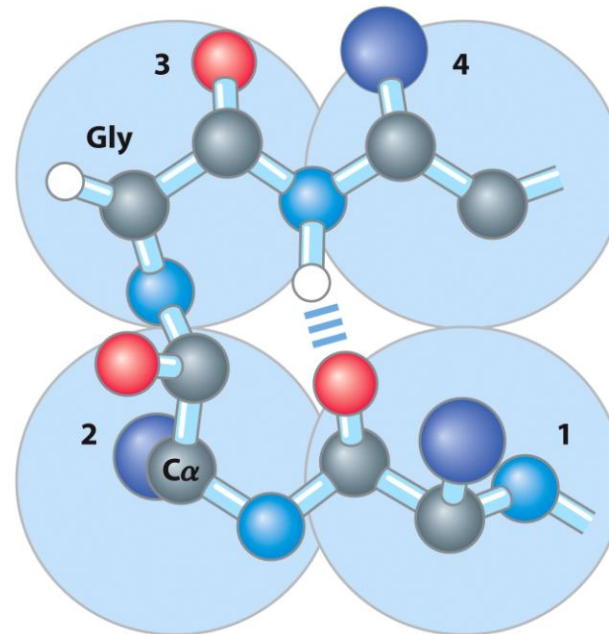
**"A geneticist laid bare my genetic blueprint, and now
I feel violated."**

β Turns

- β turns occur frequently whenever strands in β sheets change the direction
- The 180° turn is accomplished over four amino acids
- The turn is stabilized by a hydrogen bond from a carbonyl oxygen to amide proton three residues down the sequence
- **Proline** in position 2 or **glycine** in position 3 are common in β turns



Type I β turn



Type II β turn

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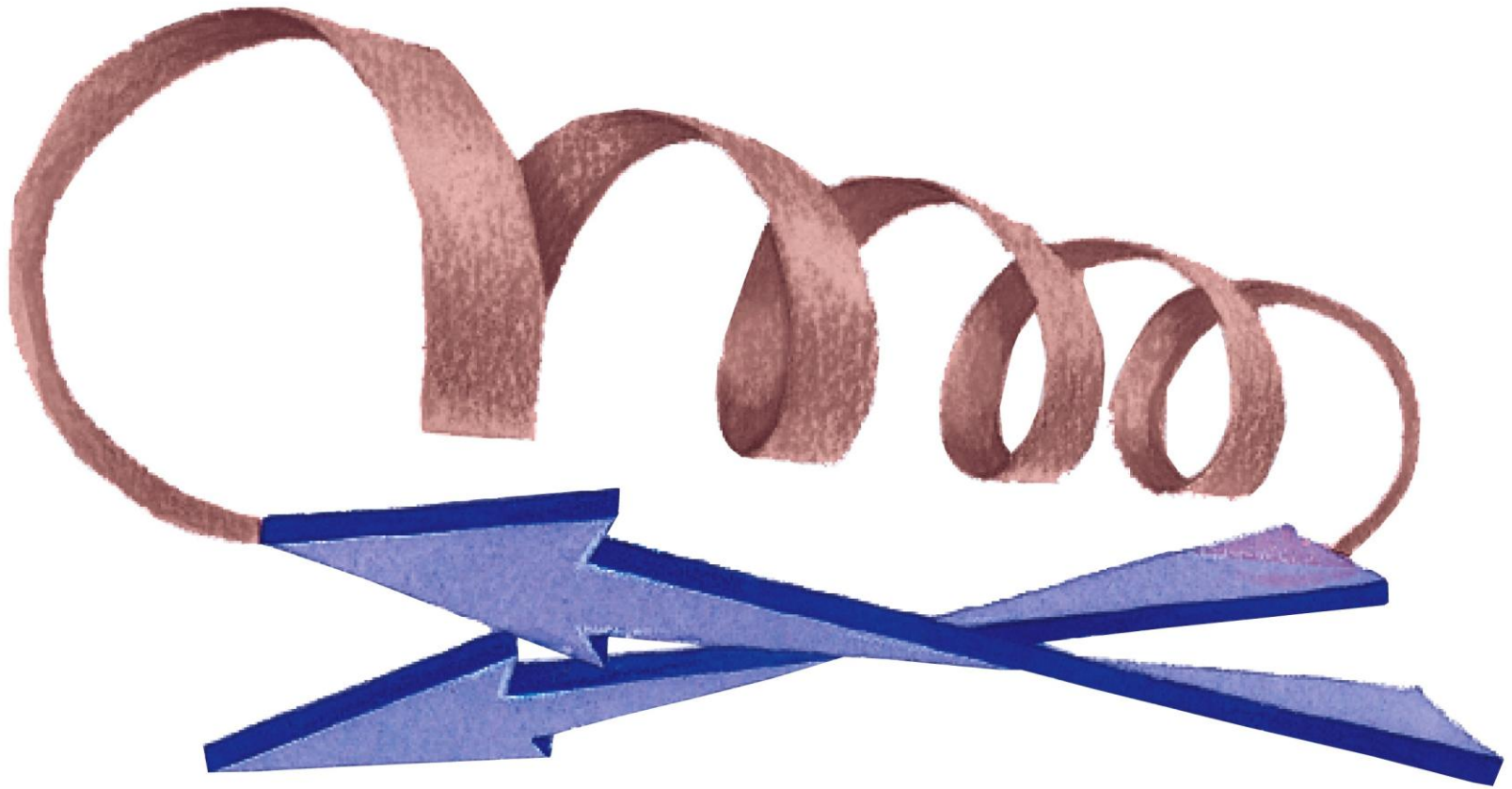
Structures of β turns. (a) Type I and type II β turns are most common; type I turns occur more than twice as frequently as type II. Type II β turns usually have Gly as the third residue. Note the hydrogen bond between the peptide groups of the first and fourth residues of the bends. (Individual amino acid residues are framed by large blue

Protein Tertiary Structure

- Tertiary structure refers to the overall spatial arrangement of atoms in a protein
- Stabilized by numerous weak interactions between amino acid side chains.
 - Largely hydrophobic and polar interactions
 - Can be stabilized by disulfide bonds
- Interacting amino acids are not necessarily next to each other in the primary sequence.
- Two major classes
 - Fibrous and globular (water or lipid soluble)

Motifs (folds)

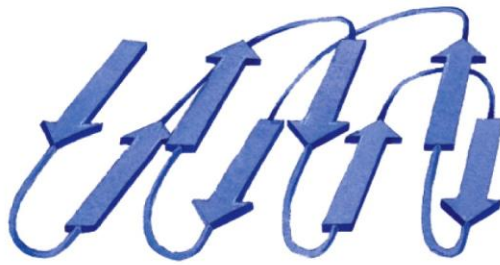
- Specific arrangement of several secondary structure elements
 - All alpha-helix
 - All beta-sheet
 - Both
- Motifs can be found as reoccurring structures in numerous proteins
- Proteins are made of different motifs folded together



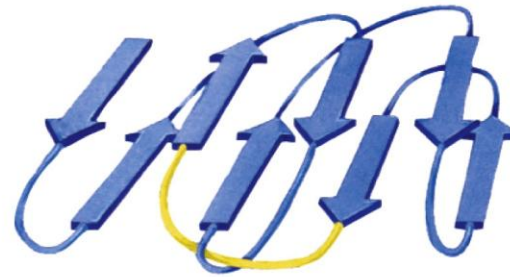
β - α - β Loop

Figure 4-18a

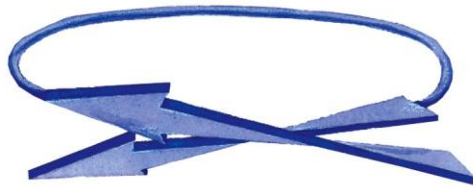
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(a) Typical connections
in an all- β motif



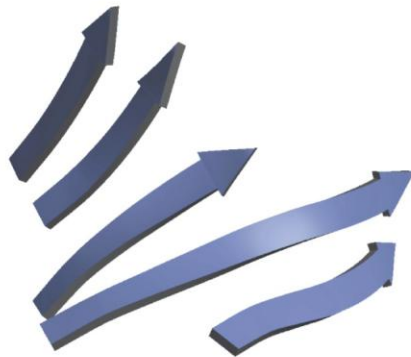
Crossover connection
(rarely observed)



(b) Right-handed connection
between β strands



Left-handed connection
between β strands
(very rare)



(c) Twisted β sheet

Figure 4-20

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- **Stable folding patterns in proteins.** (a) Connections between β strands in layered β sheets. The strands here are viewed from one end, with no twisting. Thick lines represent connections at the ends nearest the viewer; thin lines are connections at the far ends of the β strands. The connections at a given end (e.g., near the viewer) rarely cross one another. An example of such a rare crossover is illustrated by the yellow strand in the structure on the right.
- (b) Because of the right handed twist in β strands, connections between strands are generally right-handed. Left-handed connections must traverse sharper angles and are harder to form.
- (c) This twisted β sheet is from a domain of photolyase (a protein that repairs certain types of DNA damage) from *E. coli* (derived from PDB ID 1DNP). Connecting loops have been removed so as to focus on the folding of the β sheet.

- Constructing large motifs from smaller ones. The α/β barrel is a commonly occurring motif constructed from repetitions of the
- β - α - β loop motif. This α/β barrel is a domain of pyruvate kinase (a glycolytic enzyme) from rabbit (derived from PDB ID 1PKN).

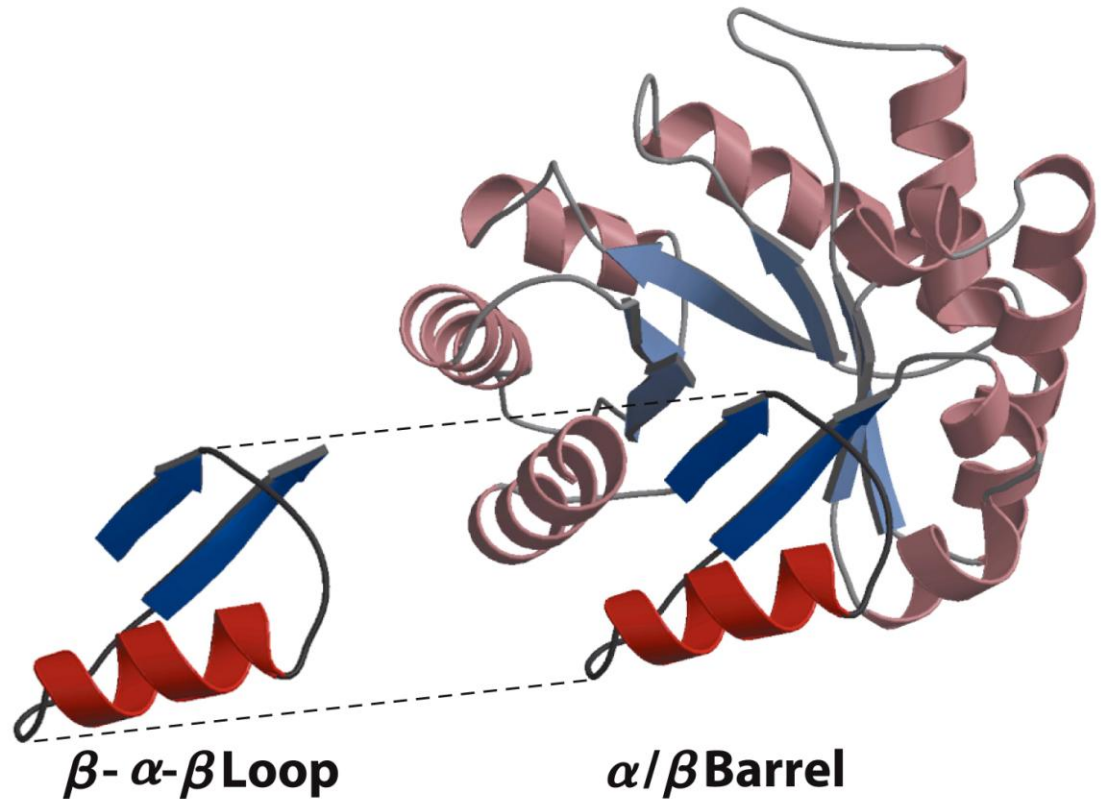


Figure 4-21
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Quaternary Structure

- **Quaternary structure** is formed by the assembly of individual polypeptides into a larger functional cluster

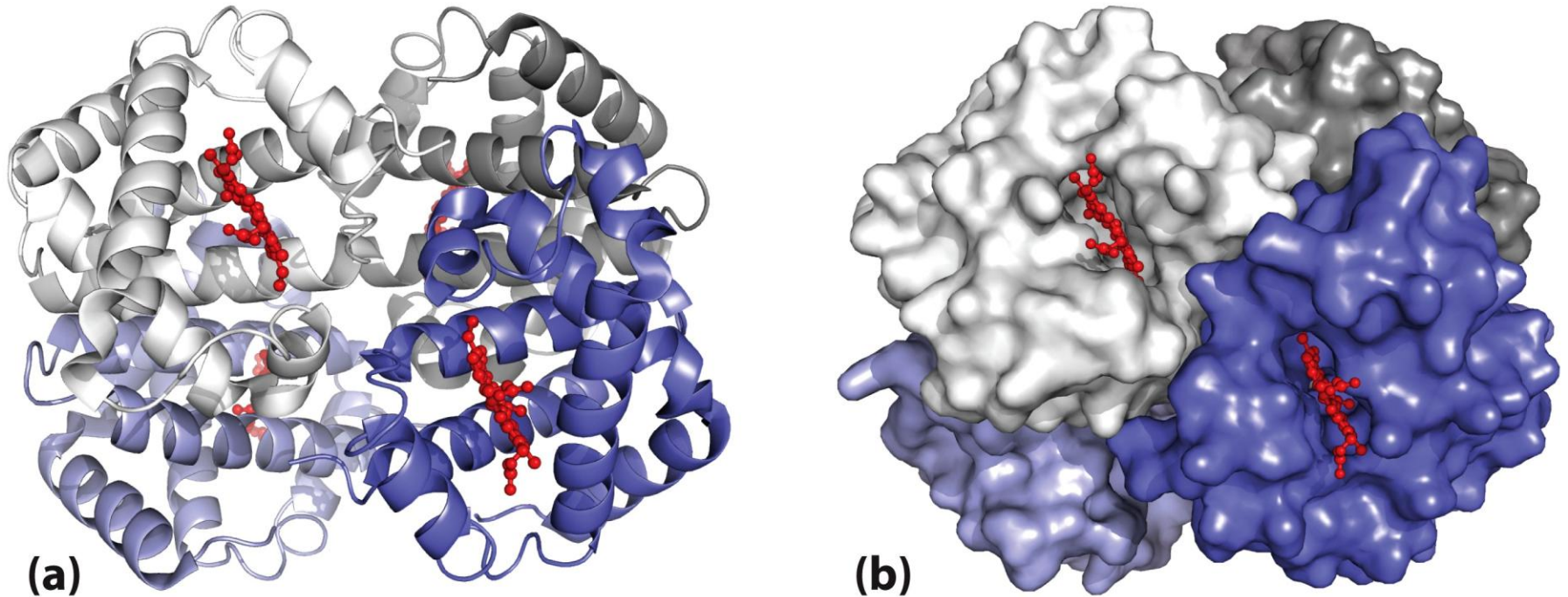


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Protein Structure Methods: X-Ray Crystallography

Steps needed

- Purify the protein
- **Crystallize the protein**
- Collect diffraction data
- Calculate electron density
- Fit residues into density

Pros

- No size limits
- Well-established

Cons

- Difficult for membrane proteins
- Cannot see hydrogens

Protein Stability and Folding

- A protein's function depends on its 3D-structure
- Loss of structural integrity with accompanying loss of activity is called **denaturation**
- Proteins can be denatured by:
 - heat or cold
 - pH extremes
 - organic solvents
 - chaotropic agents: urea and guanidinium hydrochloride

Ribonuclease Refolding Experiment

- Ribonuclease is a small protein that contains 8 cysteines linked via four disulfide bonds
- Urea in the presence of 2-mercaptoethanol fully denatures ribonuclease
- When urea and 2-mercaptoethanol are removed, the protein spontaneously refolds, and the correct disulfide bonds are reformed
- The sequence alone determines the native conformation

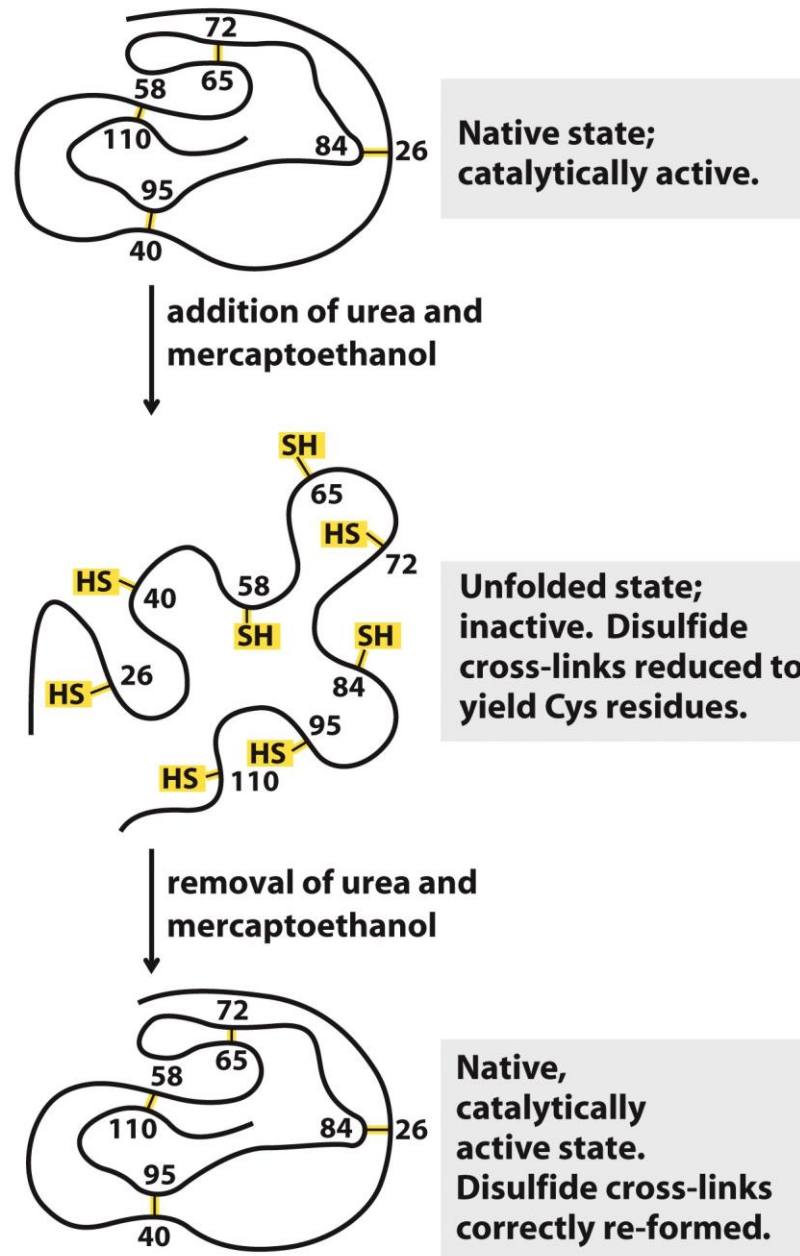


Figure 4-27

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How can proteins fold so fast?

- Proteins fold to the lowest-energy fold in the microsecond to second time scales. How can they find the right fold so fast?
- It is mathematically impossible for protein folding to occur by randomly trying every conformation until the lowest-energy one is found (Levinthal' s paradox)
- Search for the minimum is not random because the direction toward the native structure is thermodynamically most favorable

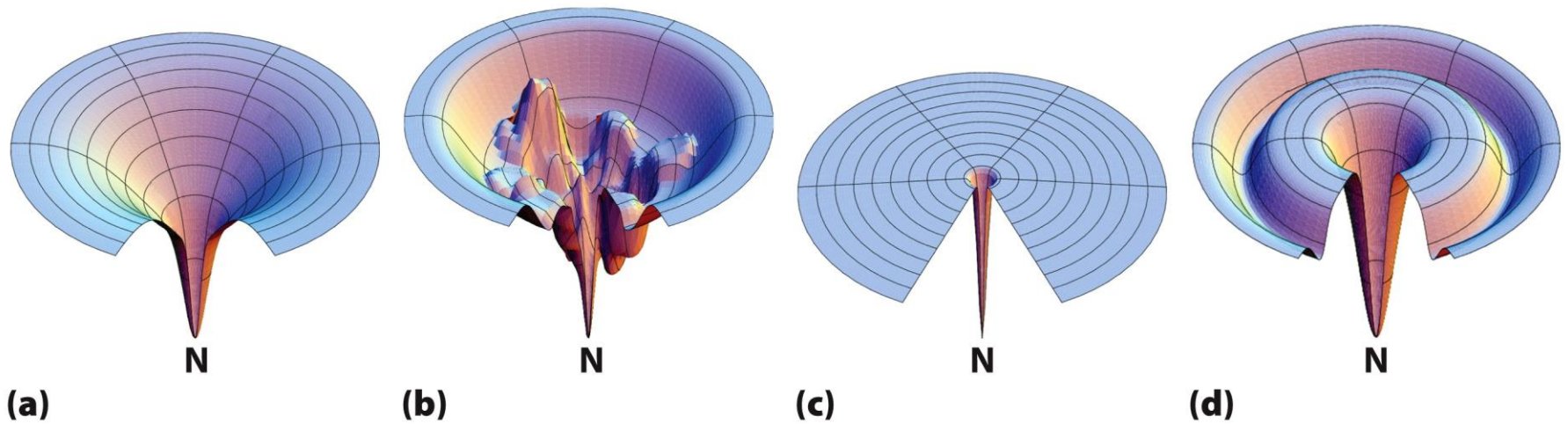


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- **The thermodynamics of protein folding depicted as a free-energy funnel.** At the top, the number of conformations, and hence the conformational entropy, is large. Only a small fraction of the intramolecular interactions that will exist in the native 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 in some cases may slow the folding process.

Proteins folding follow a distinct path

A protein-folding pathway as defined for a small protein. A hierarchical pathway is shown, based on computer modeling. Small regions of secondary structure are assembled first and then gradually incorporated into larger structures. The program used for this model has been highly successful in predicting the three-dimensional structure of small proteins from their amino acid sequence. The numbers indicate the amino acid residues in this 56 residue peptide that have acquired their final structure in each of the steps shown.

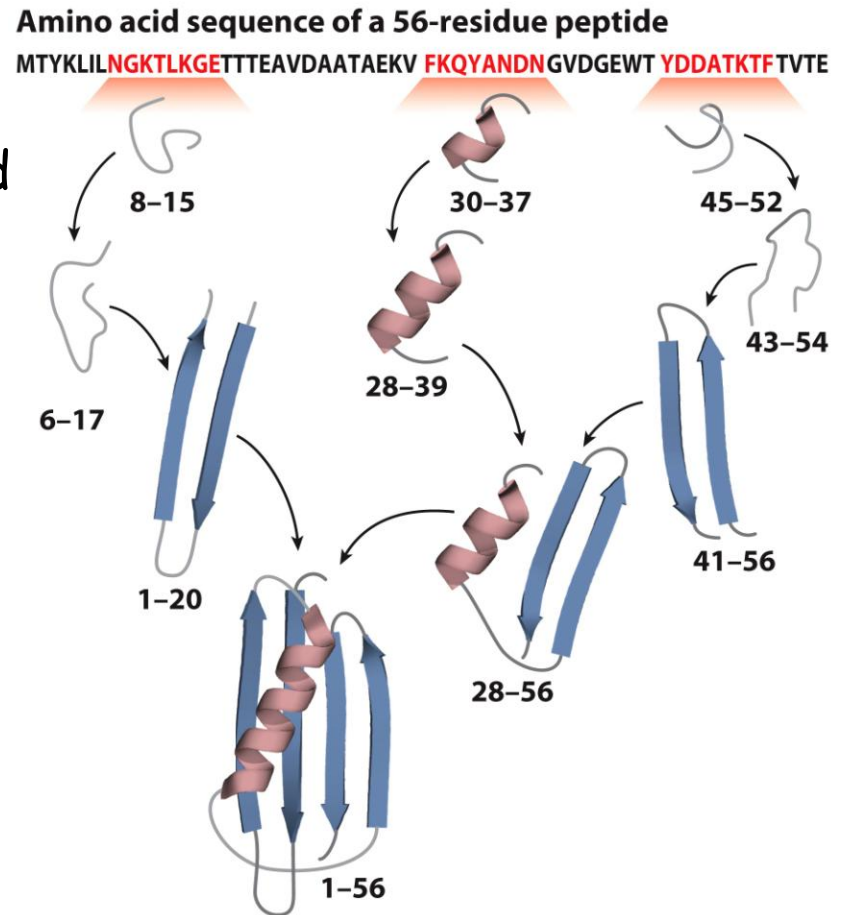


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Chaperones prevent misfolding

The cyclic pathway by which chaperones bind and release polypeptides is illustrated for the *E. coli* chaperone proteins DnaK and DnaJ, homologs of the eukaryotic chaperones Hsp70 and Hsp40. The chaperones do not actively promote the folding of the substrate protein, but instead prevent aggregation of unfolded peptides. For a population of polypeptide molecules, some fraction of the molecules released at the end of the cycle are in the native conformation. The remainder are rebound by DnaK or diverted to the chaperonin system. In bacteria, a protein called GrpE interacts transiently with DnaK late in the cycle, promoting dissociation of ADP and possibly DnaJ. No eukaryotic analog of GrpE is known.

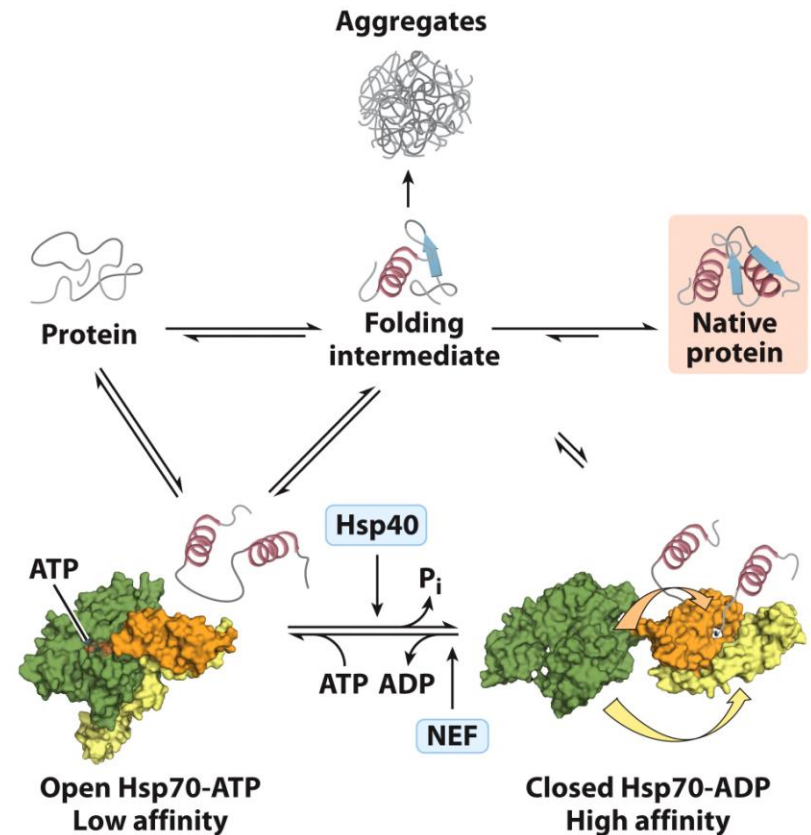


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Chaperonins facilitate folding

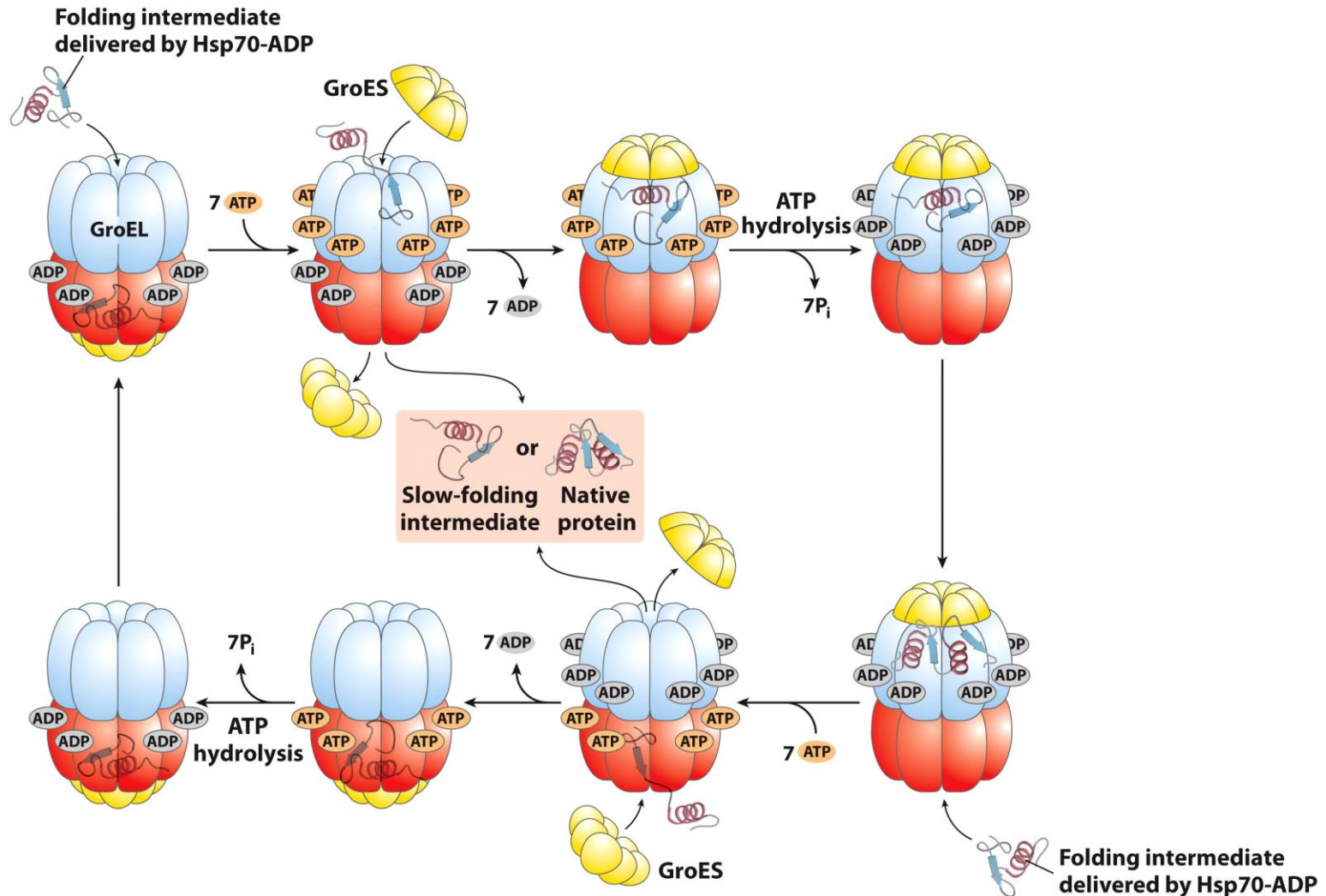


Figure 4-31a

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(In previous slide) A proposed pathway for the action of the *E. coli* chaperonins GroEL (a member of the Hsp60 protein family) and GroES. Each GroEL complex consists of two large chambers formed by two heptameric rings (each subunit M_r 57,000). GroES, also a heptamer (subunit M_r 10,000), blocks one of the GroEL chambers after an unfolded protein is bound inside. The chamber with the unfolded protein is referred to as cis; the opposite one is trans. Folding occurs within the cis chamber, during the time it takes to hydrolyze the 7 ATP bound to the subunits in the heptameric ring. The GroES and the ADP molecules then dissociate, and the protein is released. The two chambers of the GroEL/Hsp60 systems alternate in the binding and facilitated folding of client proteins.

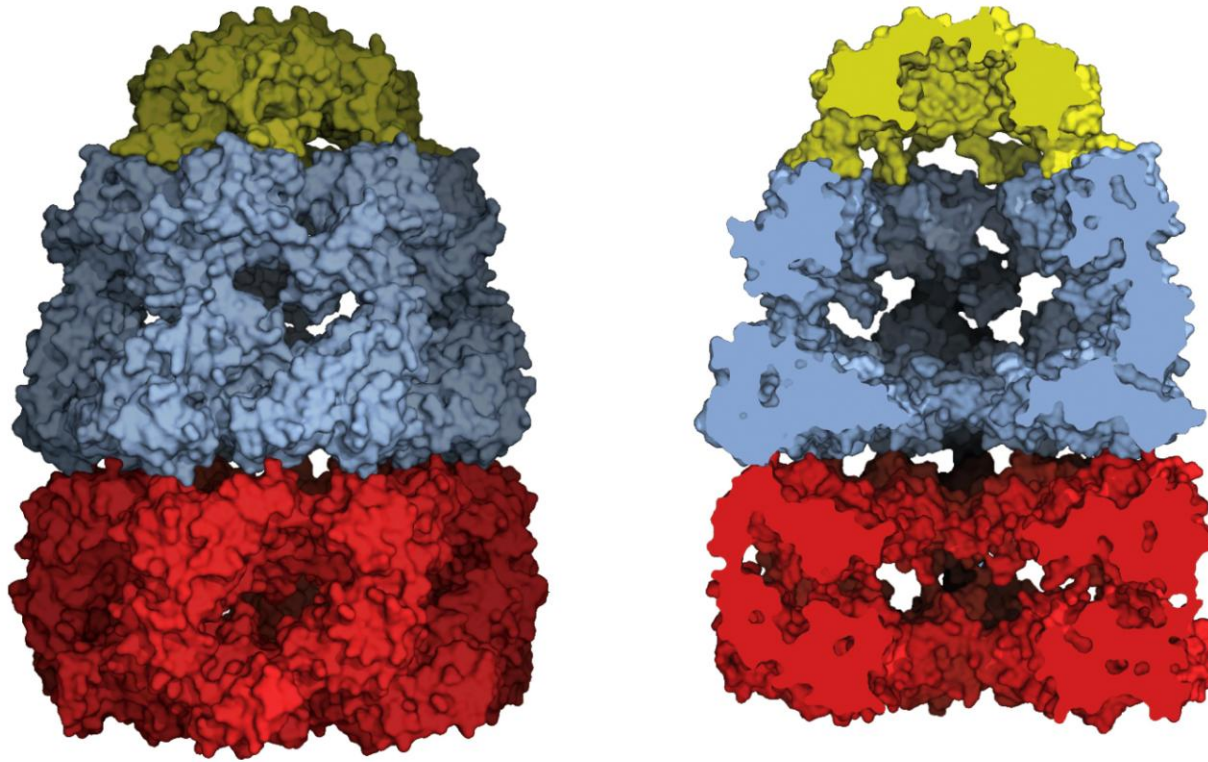


Figure 4-31b
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Chaperonins in protein folding

(b) Surface and cut-away images of the GroEL/GroES complex (PDB ID 1AON). The cutaway (right) illustrates the large interior space within which other proteins are bound.