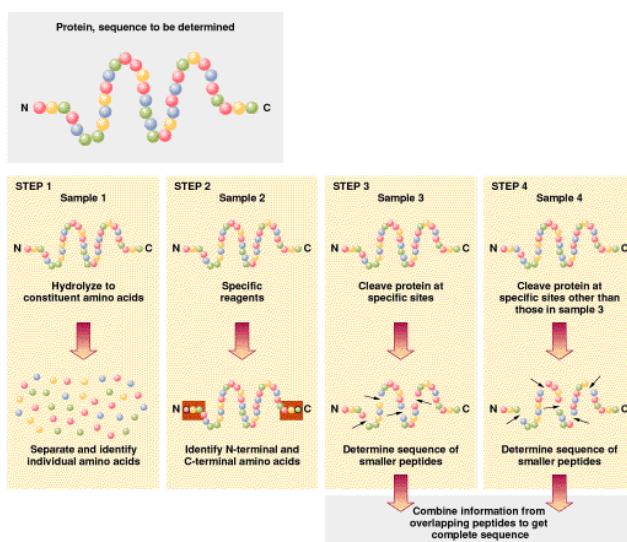


Levels of Protein Structure:

PRIMARY STRUCTURE (1°)

- Defined, non-random **sequence of amino acids** along the peptide backbone
 - o Described in two ways:
 - Amino acid **composition**
 - Amino acid **sequence**
 - M-L-D-G-C-G Peptide A
 - M-L-C-D-G-G Peptide B
 - Composition is **IDENTICAL**; Sequence is **DIFFERENT**

Campbell, Biochemistry, 3/e
Text Figure 04.01



- How to determine the **COMPOSITION**
 - o Purify the protein of interest – separate away from all other types of proteins and biomolecules
 - o Estimate the molecular weight of the protein
 - o Establish the composition by complete hydrolysis of the protein under acidic conditions
 - Treat with 6M HCl at 110°C; 12-36 hours
 - Each peptide bond is broken and products are all of the free amino acids
 - Each amino acid is separated, identified and quantified
 - Final result: Know **HOW MANY** of each amino acid present in the original

- How to determine the **ORDER**

- o Determine the C-terminal amino acid

- Use carboxypeptidase – enzyme that removes the last (C-terminal) amino acid in a free form by breaking the peptide bond

- Hydrolyzes the peptide bond nearest the C-terminus

- o Identify the N-terminal amino acids in order

- Process called **SEQUENCING**

- Often difficult to characterize an intact protein

- Instead, employ a “divide and conquer” approach to analyze peptide fragments of the intact protein

- Cut large proteins into smaller parts

- Use enzymes called **PROTEASES**

- Cleave peptide bond in a specific way

- TWO Examples:

- o **Trypsin** – Cleaves on the C-terminal side of **Lys** and **Arg** residues

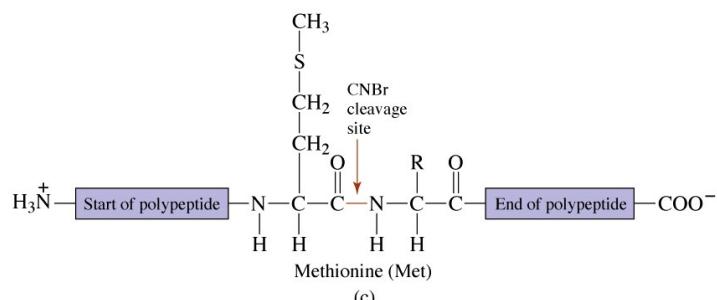
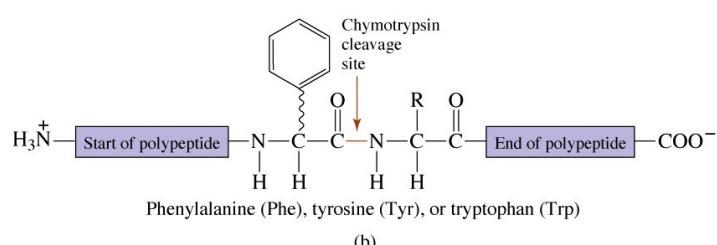
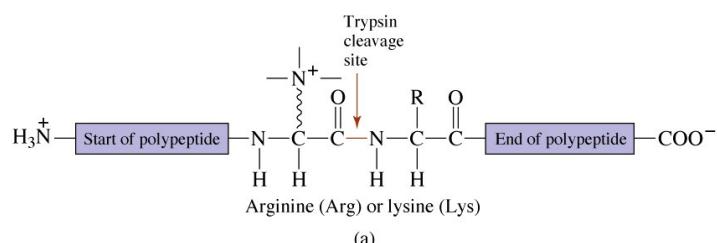
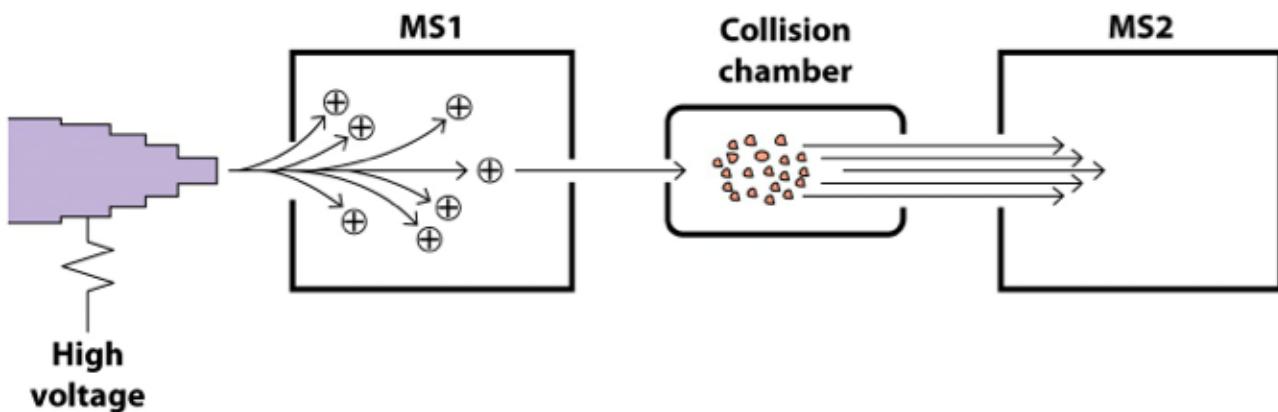
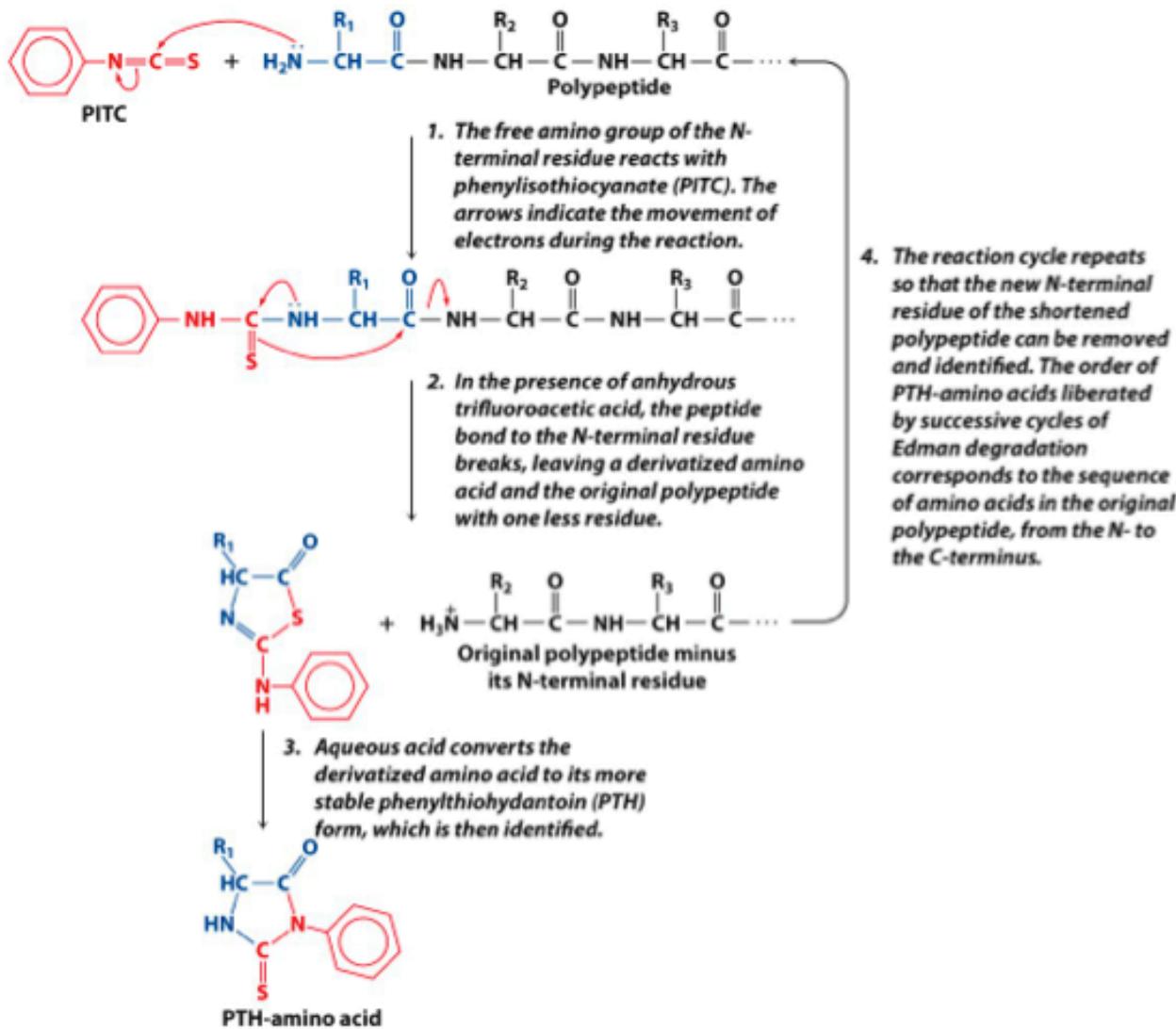


Figure 3-17 Concepts in Biochemistry, 3/e
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Sequencing of the peptides generated by proteases

- Procedure called **Edman Degradation**
- React the N-terminal amino acid with phenylisothiocyanate
- Derivatized amino acid released as PTH – phenylthiohydantoin
- Each PTH amino acid derivative is identified by chromatography
- Newly exposed N-terminal residue can be derivatized, removed and identified sequentially
- Useful up to 25-50 amino acids



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SECONDARY STRUCTURE (2°): HYDROGEN BONDING IS KEY!

- Three-dimensional structure of the **peptide backbone**
- 3 major classes of **secondary structure** are dictated by the **RIGIDITY** and **PLANARITY** of the peptide bond and the nature of the side chains
 - o α -helix
 - o β -sheet
 - o turns and random coil

1) α -helix

- Rod-like structure (phone cord)
- Involves only one polypeptide chain
- Main chain atoms on the **INSIDE**
- R-group side chains on the outside – stick out
- Stabilized by **HYDROGEN BONDS**
- Carbonyl (C = O) of each amino acid is H-bonded to the amide (N-H) of the amino acid that is 4 amino acids further toward the C-terminus
- **n+4** (e.g. amino acid 1 is H-bonded to amino acid 5 – see model)
- α -helices have sidedness: n+4 on the same side of the helix
- 3.6 aa's per turn (about 4aa)
- Pitch of the helix (i.e. one turn = 5.4 Å)
- Overall dipole moment – positively charged N-terminus \rightarrow negatively charged C-terminus
- Helices can be right or left handed
 - PROTEINS ARE RIGHT HANDED



Toilet roll representation of the main chain hydrogen bonding in an alpha-helix.

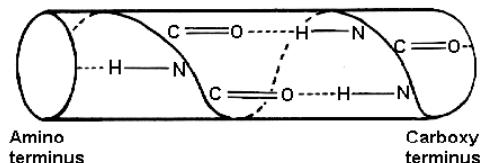
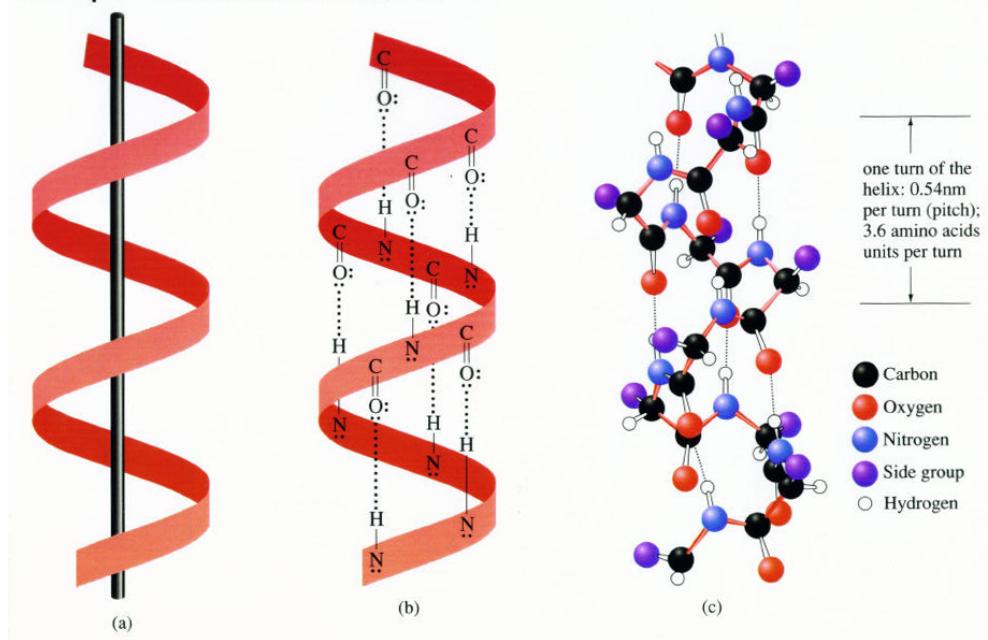


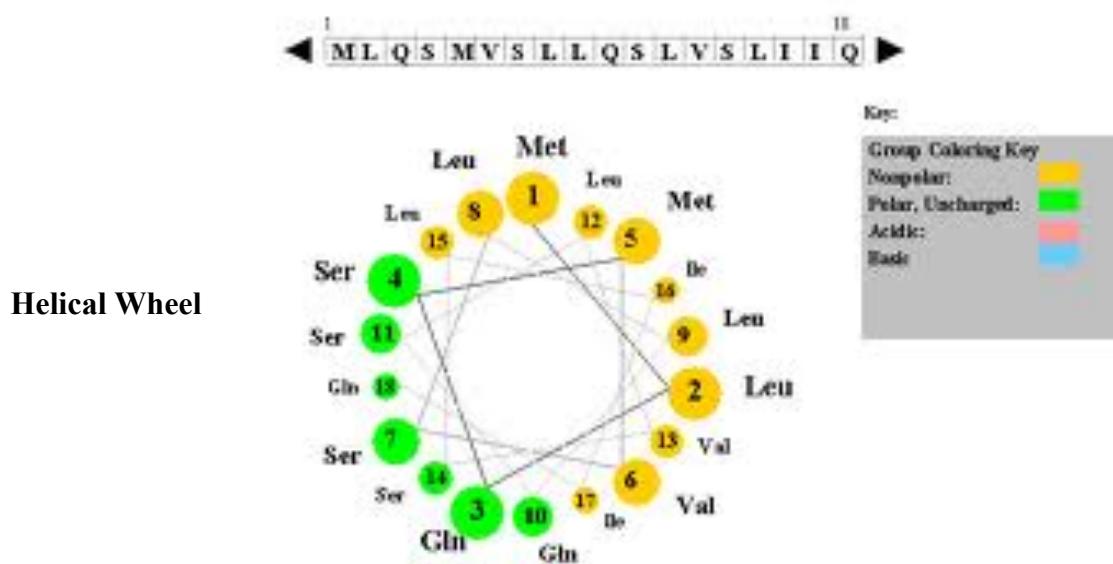
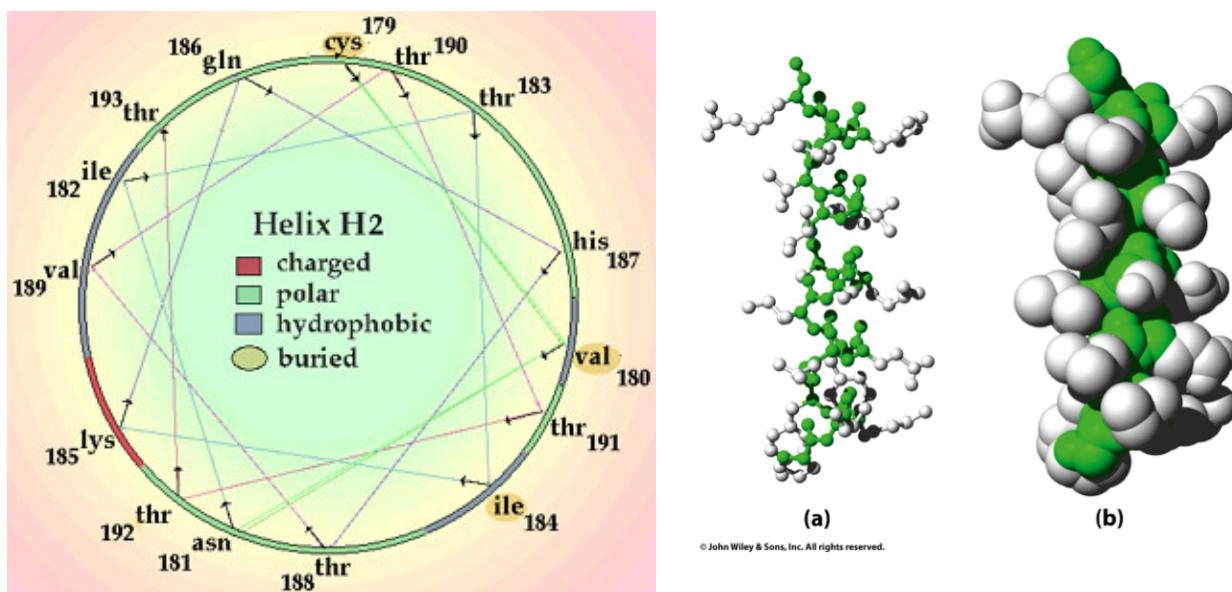
Figure 5.5 a-c, page 117
The alpha-helix structure shown in three levels of detail

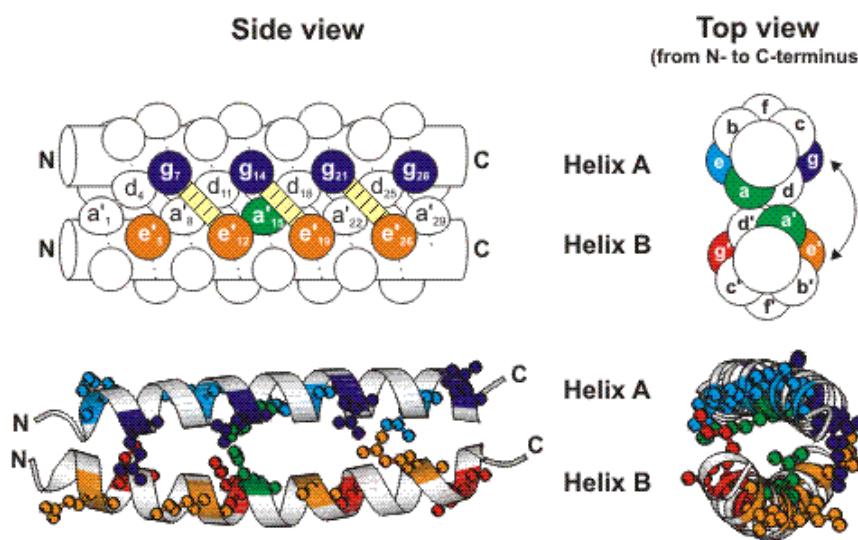


- Factors that affect stability of an α -helix

- o Although the helix is defined by the H-bonding of the peptide backbone, the nature of the side chains can affect overall stability
 - Adjacent bulky amino acids unfavorable (steric hindrance)
 - Proline unfavorable – creates bends; helix-breaker
 - Glycine unfavorable – too mobile (no side chain but H)
 - Too many + or – charged groups near each other in space are unfavorable – electrostatic repulsion
- o <http://www.wiley.com/college/fob/anim/index.html> Fig. 6-9 – Alpha Helix

- An **amphipathic α -helix** has the hydrophobic amino acids on one side of the helix and the polar/charged amino acids on the other side of the helix.





Coiled – Coil Structure:

- Coiled coils consist of two or more α -helices that wrap around one another
- Exist in skin – springy
- Exist in hair and claws – cysteines disulfide link and give rigidity
- α -keratin is the major protein in hair.
-

- Can rearrange disulfide bonds in hair – **PERMANENT WAVE!**
 - In the permanent wave process, a basic reducing substance (usually ammonium thioglycolate) is first added to reduce and rupture some of the disulfide cross-links.
 - The hair is put on rollers or curlers. Since the alpha-helices are no longer tightly cross-linked to each other, the α -helices can shift positions in relation to each other. An oxidizing agent, usually a dilute solution of hydrogen peroxide, (also called the neutralizer) is added to reform the disulfide bonds in their new positions.

2) β -sheet

Proteins with major β -pleated sheet secondary structure are generally fibrous, such as silk, but pleated sheet is observed as a significant part of secondary stucture in other proteins.

- Generally have rod-like shapes and are not so soluble in water.
- **β sheets** consist of **β strands** connected laterally by at least two or three backbone **hydrogen bonds**, forming a generally twisted, pleated sheet.
- Unlike the α -helix, β -sheets can involve one or more polypeptide chains – interchain or intrachain interactions
- A β -strand is a stretch of **polypeptide** chain typically 3 to 10 **amino acids** long with the peptide backbone almost **completely extended**
- R-groups stick UP and DOWN from β -sheets – alternating on either side of the strand
 - Usually small compact side chains like Gly, Ser, Ala
 - In an **amphipathic β -sheet** the amino acids alternate with polar/charged and non-polar (hydrophobic) amino acids. Thus, the hydrophobic residues are on one side and the polar/charged are on the other.
 - Stabilized by Hydrogen bonds (near perpendicular to direction of peptide backbone)
 - Carbonyl of each amino acid is H-bonded to the NH of another amino acid

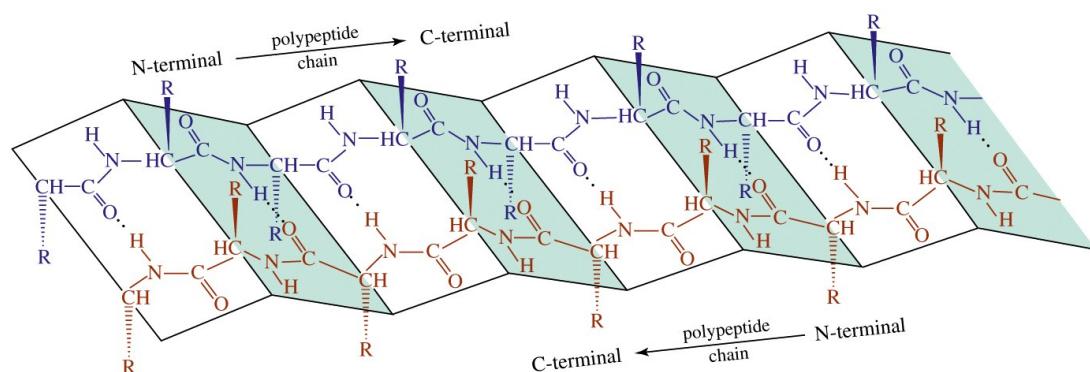
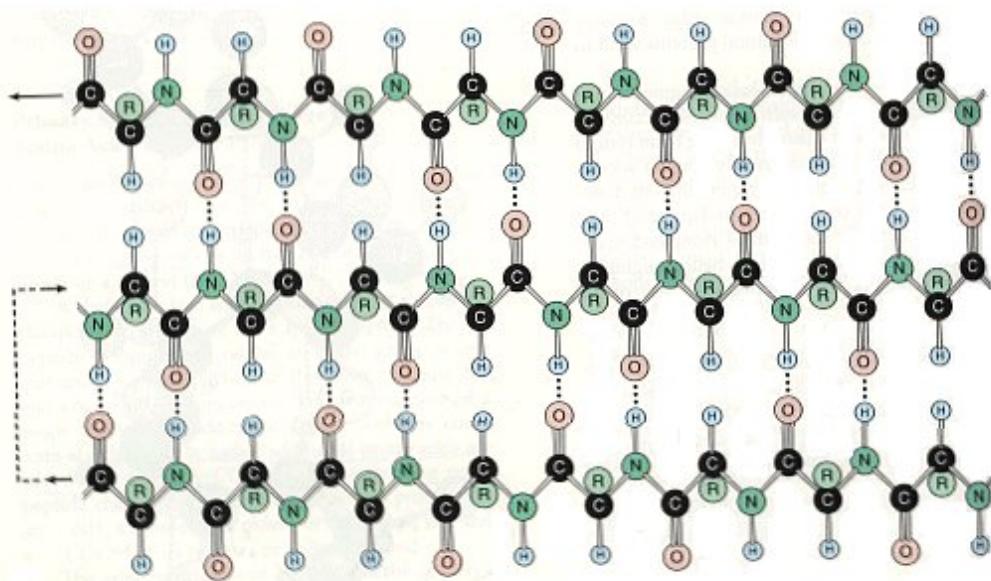


Figure 4-7 Concepts in Biochemistry, 3/e
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<http://www.wiley.com/college/fob/anim/index.html> Fig. 6-9 -- Beta Sheet

Parallel and Anti-Parallel β -sheets

- Adjacent chains can be PARALLEL or ANTI-PARALLEL
- **Parallel β -sheet:** H-bonds between 2 β -strands running in the same direction
- **Anti-parallel β -sheet:** H-bonds between 2 β -strands running in opposite directions



ANTI-PARALLEL BETA SHEET

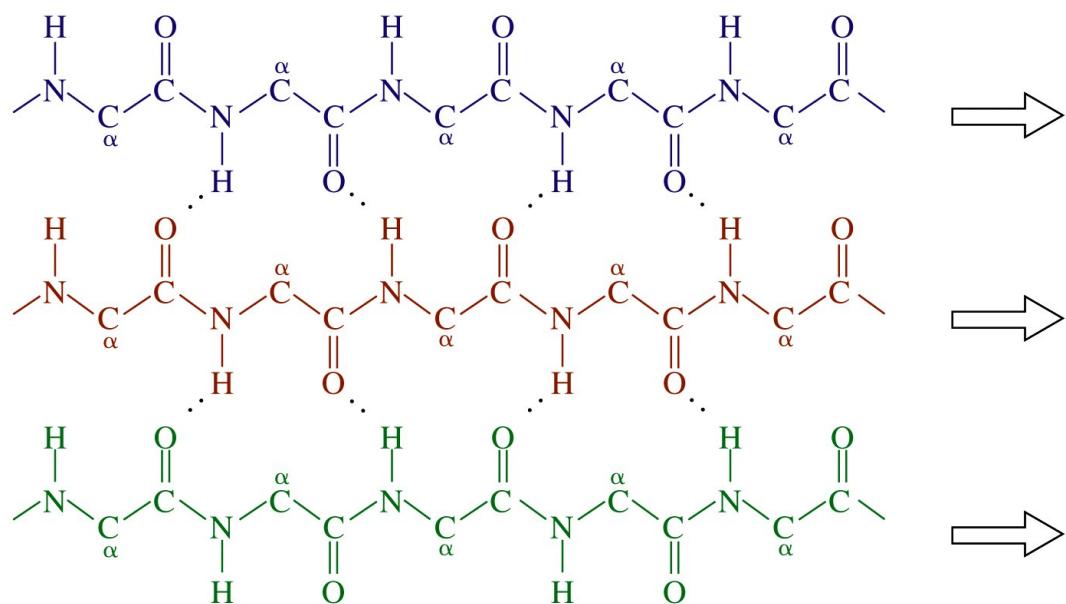


Figure 4-6b Concepts in Biochemistry, 3/e
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PARALLEL BETA SHEET

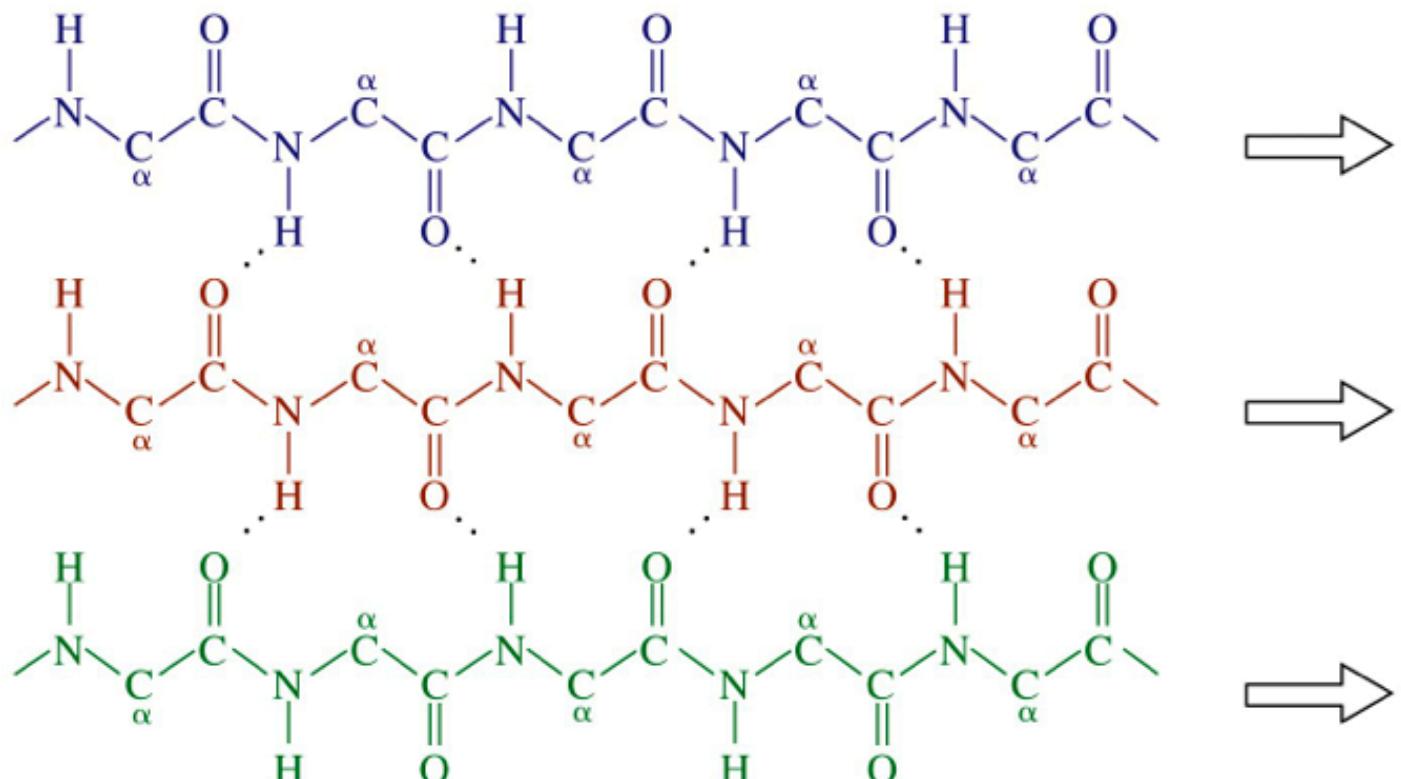
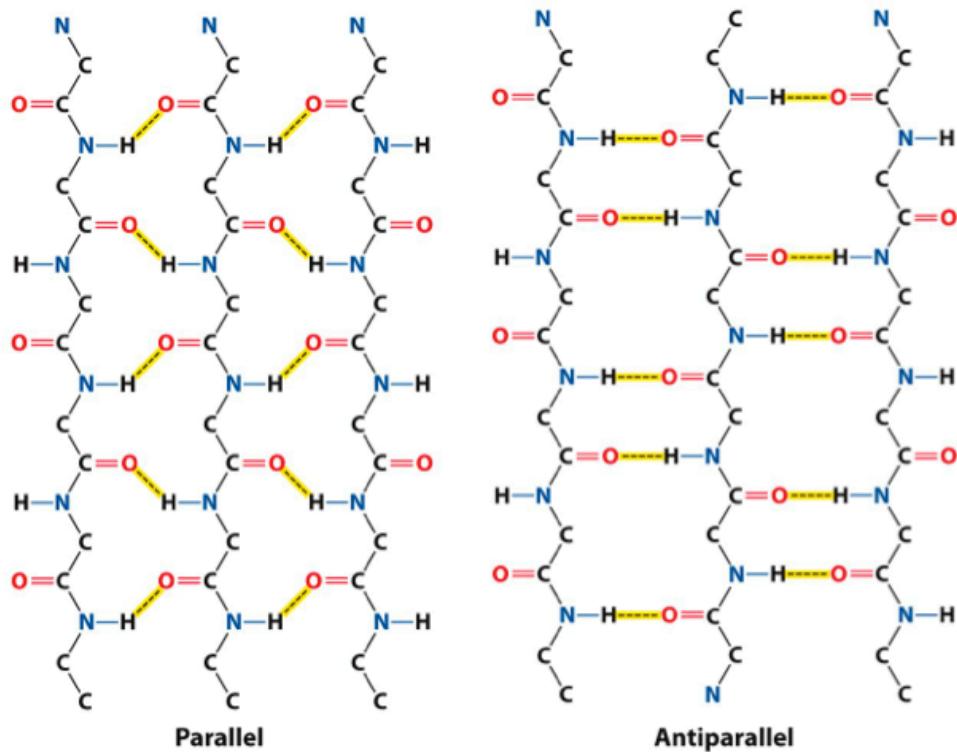


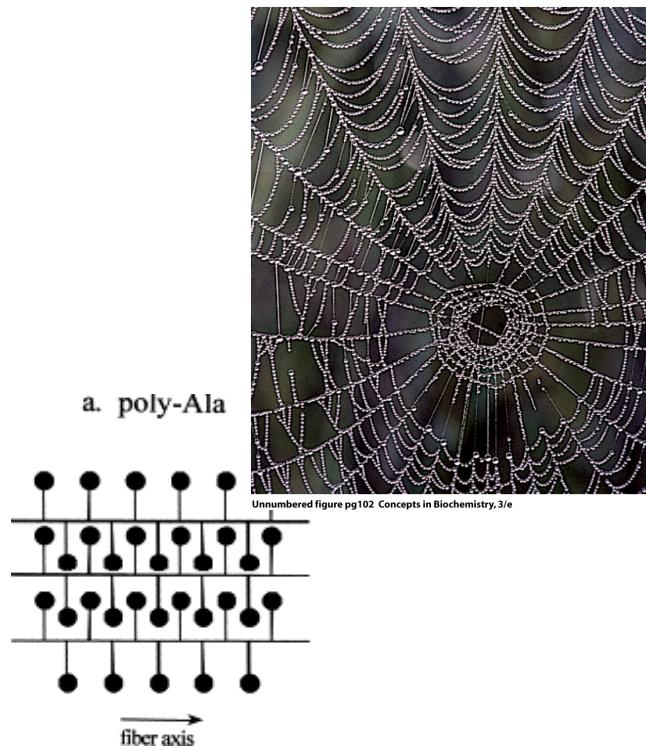
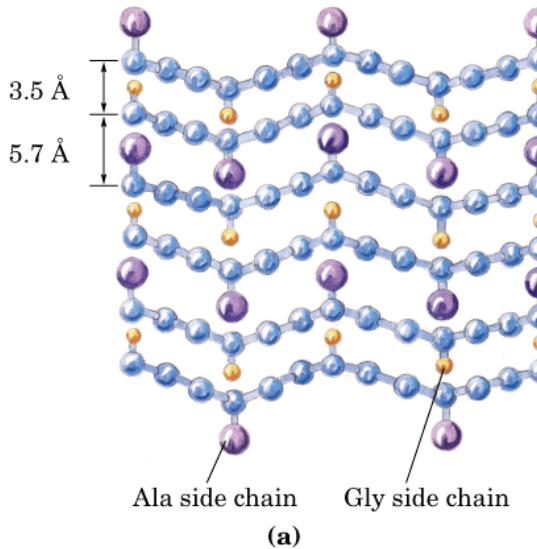
Figure 4-6b Concepts in Biochemistry, 3/e
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Silk is made from a β -pleated sheet.

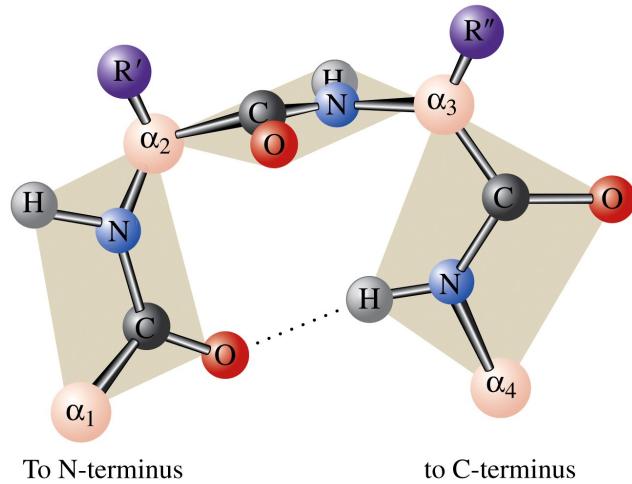
Gly-Ser-Gly-Ala-Gly-Ala
repeats



Strength along the fiber results from extended, covalent peptide chain along the fiber axis.

2) Loops or Turns

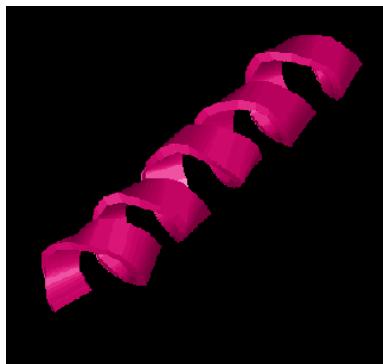
- Small regions of peptide backbone that can form small loops
- Often contain **glycine** (small and mobile) and **proline** (causes kinks)
- Reverse direction of the main polypeptide chain
- Connects regions of more regular secondary structure
- Not periodic; irregular
- Extended bend = loop and contains 6 -16 amino acids; ~10 Å long



β -bend or hairpin turn – connects anti-parallel β -sheets. Example below. Forms a loop to change direction in the polypeptide chain.

MOTIFS:

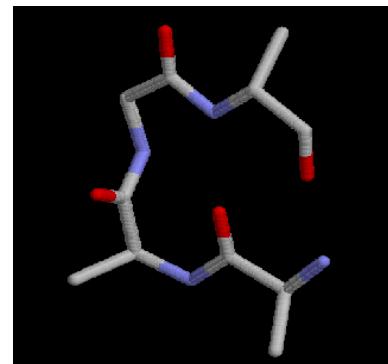
- Patterns of arrangements of α -helices and/or β -sheets
- Arranged in stable geometries – visualized as **RIBBON DIAGRAMS**
 - Ribbon diagrams don't show atomic detail
 - Held together by non-covalent interactions
 - Show elements of secondary structure and the outline of the general directions of the protein chain
 - Cylinders or coils = α -helices
 - Arrows = β -strands (direction shown by arrow) – together make **β -sheets**
 - Ribbons = bends, loops and random



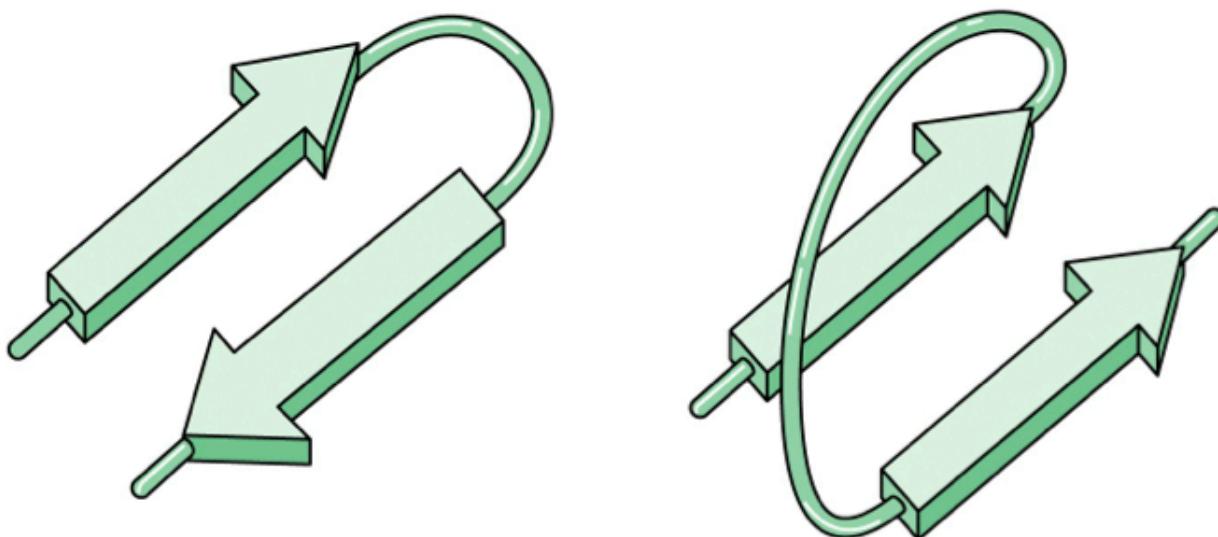
α -helix



β -sheet



Turn



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β -sheets (each arrow is a β -strand)

EXAMPLES:

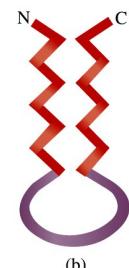
a: $\alpha\alpha$ motif (helix-loop-helix motif)\

Helix – loop – helix

- Called the E-F hand
- Calcium binding motif
- Bound to three Asp side chains



(a)

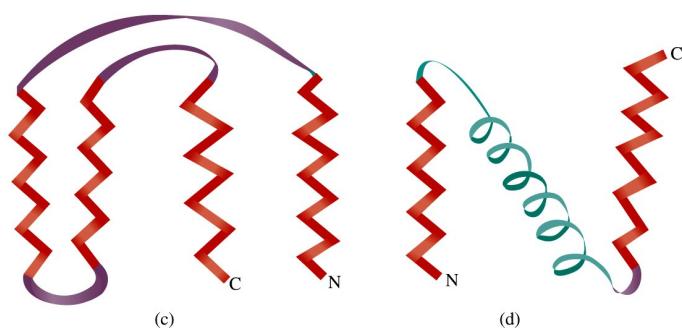


(b)

b: $\beta\beta$ motif antiparallel

c: Greek Key ($\beta\beta\beta\beta$ motif)

d: $\beta\alpha\beta$ motif parallel (Note that the beta strands are parallel (direction of the arrows))



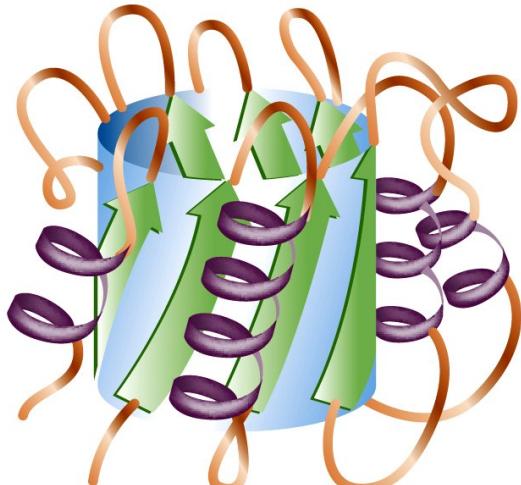
(c)

(d)

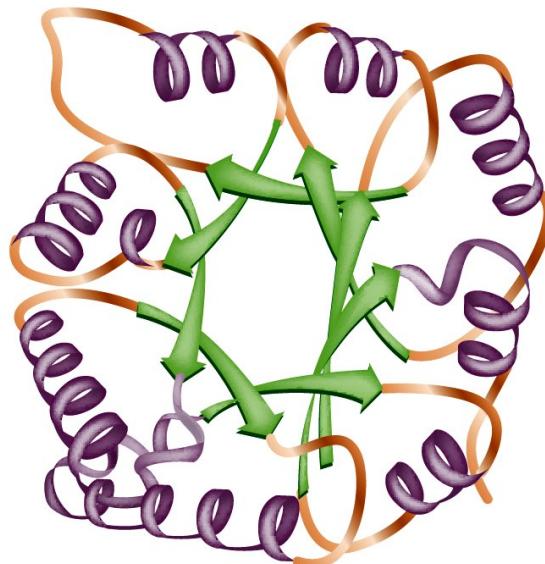
Figure 4-9 Concepts in Biochemistry, 3/e
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Triose Phosphate Isomerase

a – side view; b – top view



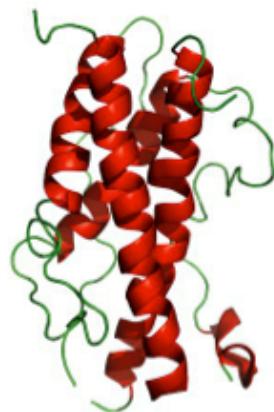
(a)



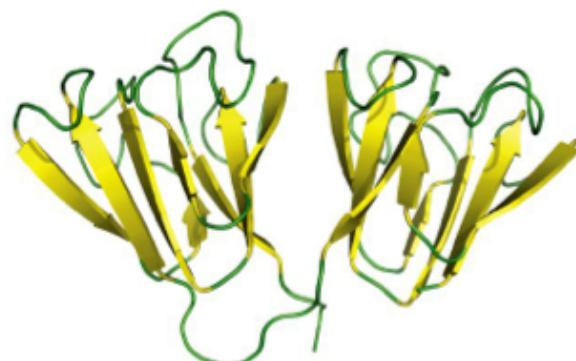
(b)

Figure 4-10 Concepts in Biochemistry, 3/e
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Several $\beta\alpha\beta$ motifs combine to form a **β -barrel** or **superbarrel** in this enzyme involved in glycolysis; Note helices, beta sheets and turns



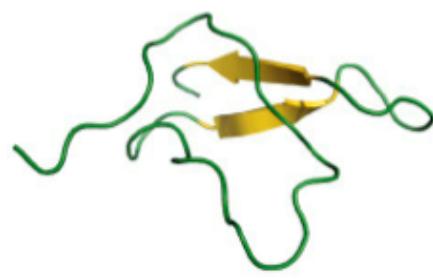
(a)



(b)



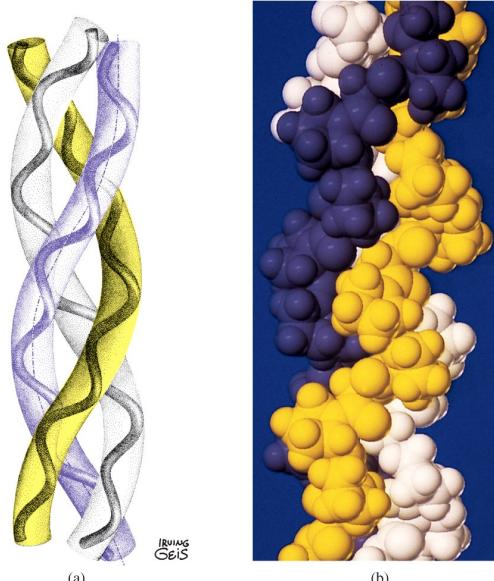
(c)



(d)

Fibrous Proteins:

- Usually perform a structural role
 - Most prominent structural protein = **collagen** (major protein in skin, bone & tendons)
 - Collagen contains repeating units **Pro – Gly – X OR Hyp** (hydroxyproline) – **Gly – X**
 - Rich in proline, therefore unable to fold into α -helices or β -sheets
 - Form a **triple helix** – three extended helical chains wrapped together
 - Rope-like structure
 - Helices held together by hydrogen bonding and covalent cross-links
 - High tensile strength

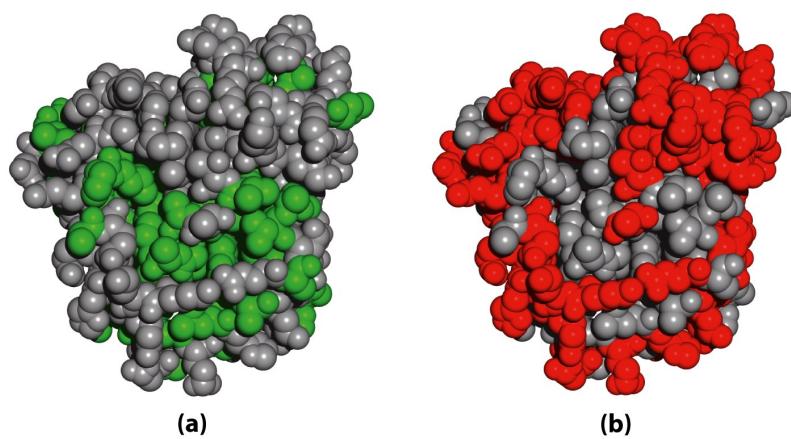
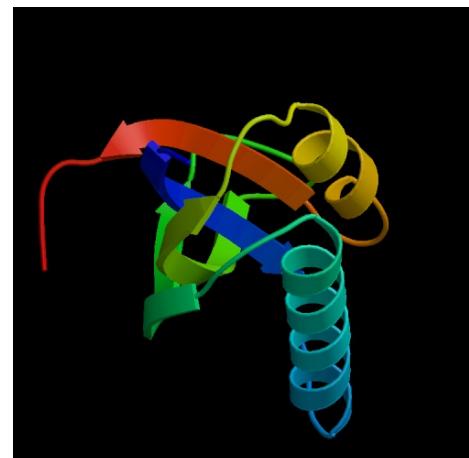


(a) Figure 4-11 Concepts in Biochemistry, 3/e

TERTIARY STRUCTURE (3°)

Global 3-dimensional arrangement of ALL atoms in a protein

- Includes:
 - 2° structural elements (alpha helices and beta sheets)
 - Amino acid side chains
 - Prosthetic groups
 - Small organic molecule or metal ion associated with a protein
 - Regions of **SECONDARY** structure **INTERACT** to give a protein its **TERTIARY** structure
 - Major forces stabilizing tertiary structure are hydrophobic interactions among nonpolar side chains in the compact core of the proteins.

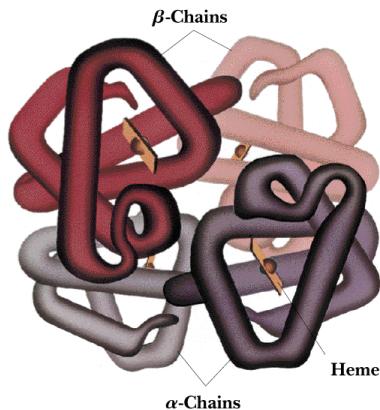


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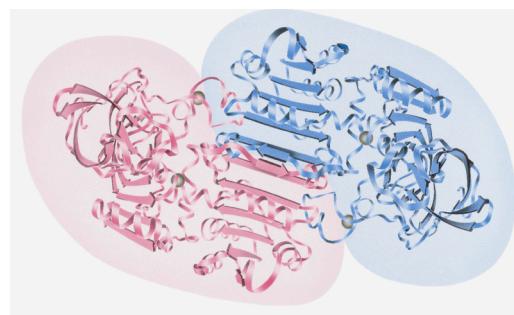
QUATERNARY STRUCTURE (4°)

- Arrangement of multiple protein molecules into COMPLEXES
- The three dimensional structure of a protein made of >1 polypeptide
- Complexes of 2, 3, 4 etc... protein molecules are called dimers, trimers, tetramers...oligomers
- Oligomers may be:
 - Formed with **identical** protein monomers = **HOMOOLIGOMER**
 - Formed with **different** protein monomers = **HETEROOLIGOMERS**
 - Example: Hemoglobin:
 - 2 alpha subunits
 - 2 beta subunits
- Protein subunits of oligomers interact through **NON-COVALENT** interactions

Garrett & Grisham: Biochemistry, 2/e
Figure 5.10



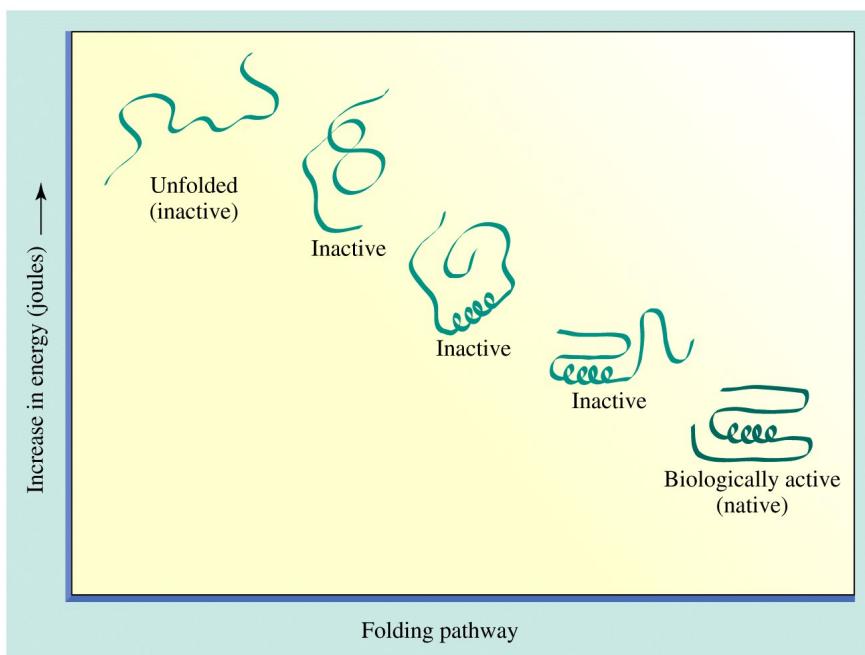
Hemoglobin (4 subunits)
Heterotetramer



Liver Alcohol Dehydrogenase (2 subunits)
Can be either a homo- or heterodimer

PROTEIN FOLDING

- Goal: To achieve the **LOWEST** energy state



- Formation of hydrophobic domains often the primary driving force for tertiary structure

The hydrophobic effect

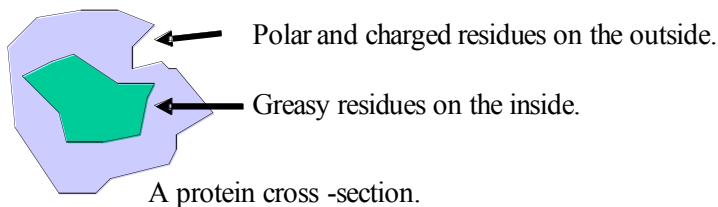
Water and oil: They don't like each other.

When you drop oil into water, it tends to glob up into little droplets.

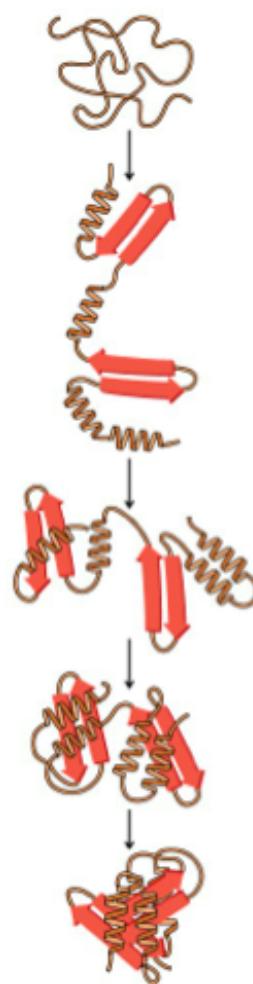
Proteins act the same way.

All the 'greasy' hydrophobic residues tend to end up in the middle of the protein making a 'hydrophobic core'.

The polar and charged residues tend to line the outside of the protein as they are happy interacting with water.



- **Hydrogen bonding stabilizes interactions between regions of polypeptide chain**
 - o Secondary structural elements have H-bonds to stabilize the peptide backbone – 2° structure does not directly involve side chains



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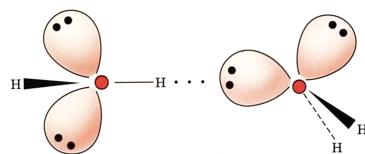
Steps in protein folding:

1. Rapid & reversible formation of SECONDARY STRUCTURAL elements.
2. Formation of domains through cooperative aggregation of folding nuclei (set of contacts made during folding process)
3. “Molten globule” formation of assembled domains
4. Adjustments in the conformations of domains
5. Final protein monomer formation

Molten globules are compact, partially folded conformations of proteins that have near-native compactness, substantial secondary structure, little tertiary structure and more water-exposed hydrophobic surface area relative to the native protein.

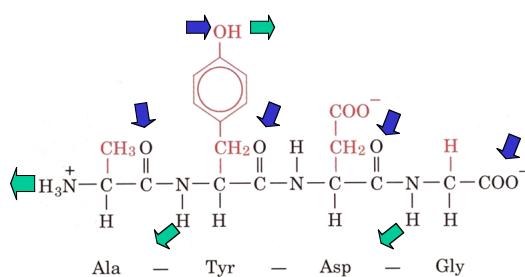
Hydrogen bonds

Hydrogen bonds occur when a proton (hydrogen) is shared between a donor group and the unpaired electrons of an acceptor oxygen.

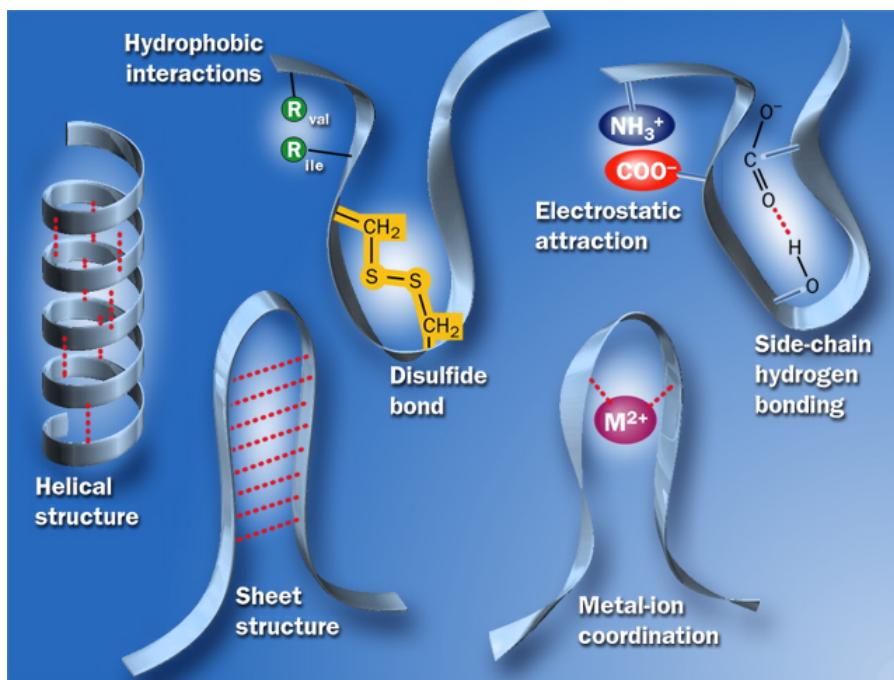


Proteins fold such that all hydrogen bonding groups participate in a hydrogen bond.

Donors: Acceptor:
N-H → O
O-H



- Other **forces** involving side chains that influence how proteins fold:
 - **Metal ion coordination** to negatively charged amino acid side chains
 - **Hydrophobic interactions** between NON-POLAR side chains
 - Favored on interior – not exposed to water
 - **Ionic/Electrostatic Interactions** between charged side chains
 - Favored on the outside
 - Sometimes on inside if near opposite charge
 - **Hydrogen bonding among side chains** of polar amino acids
 - **Disulfide bridges** between Cys amino acids stabilize tertiary structure COVALENTLY (**only** covalent interaction – rest are non-covalent)
- Note: Once folded, proteins are not rigid; highly dynamic



FORCES THAT STABILIZE STRUCTURE OF PROTEINS

How do we determine the 3-D structure?

1. **X-ray crystallography** – use crystal of pure protein
2. **NMR (2-D NMR)** – measures magnetic characteristics of each atom
 - Both methods are extremely difficult and require lots of computer power to make sense of data

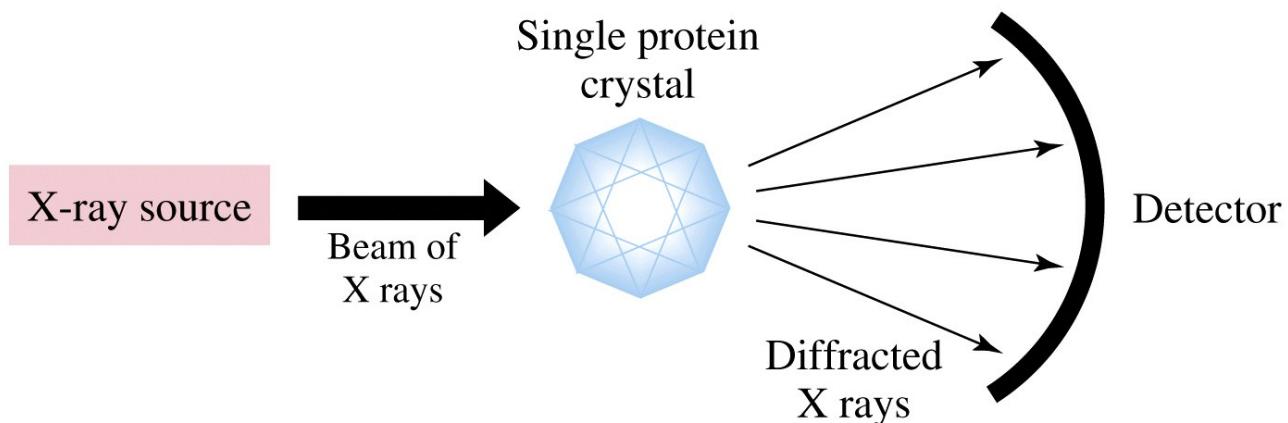


Figure 4-15 Concepts in Biochemistry, 3/e
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Protein Folding Interactive Animation:

<http://www.wiley.com/legacy/college/boyer/0470003790/animations/animations.htm>

CAN WE UNFOLD PROTEINS ONCE THEY ARE FOLDED? YES!

- Proteins can be unfolded = **DENATURED**
 - o Lose most levels of structure
 - o Protein adopts a random coil conformation
 - o Primary amino acid sequence is maintained
 - o Loss of protein function – enzymatic etc...
 - Go from **NATIVE** (correctly folded, biologically active state) to **DENATURED** and **UNFOLDED** (loss of organized structure and function)
- Use **denaturing agents**: Interfere with the forces that stabilize protein folding

<u>DENATURING AGENT</u>	<u>TARGET</u>
heat	H-bonds, hydrophobic interactions
agitation	H-bonds, hydrophobic interactions
pH	salt bridges
mercaptoethanol	disulfide bridges
detergents (SDS)	hydrophobic interactions
urea, guanidine HCl	H-bonds, hydrophobic interactions

Is this process **REVERSIBLE**? – i.e. can we restore a protein, once denatured to its original configuration and restore function?

- Yes – Denaturation CAN BE reversible
 - o Heat treatment usually is not reversible
- The **renaturation of the protein RIBONUCLEASE A** (an enzyme that cleaves DNA) won Christian Anfinsen the Nobel Prize in 1972
- Experiment:
 1. Denatured pure Ribonuclease A by treatment with **UREA** and **β -mercaptoethanol** to give a completely unfolded, denatured protein
 - o β -mercaptoethanol used to reduce disulfide bonds
 - o Urea breaks H-bonds and hydrophobic interactions
 2. Then he removed the denaturants and exposed the protein to air
 3. The protein had folded back into its original 3-D shape and activity was restored!!

This experiment suggested that the unfolded polypeptide refolded by itself in the test tube

Further experiments determined that it DID refold back to its original state

CONCLUSION: ALL THE NECESSARY INFORMATION AS TO HOW A PROTEIN FOLDS IS ENCODED INTO THE PRIMARY SEQUENCE!

1° SEQUENCE DICTATES 2° AND 3° STRUCTURE!

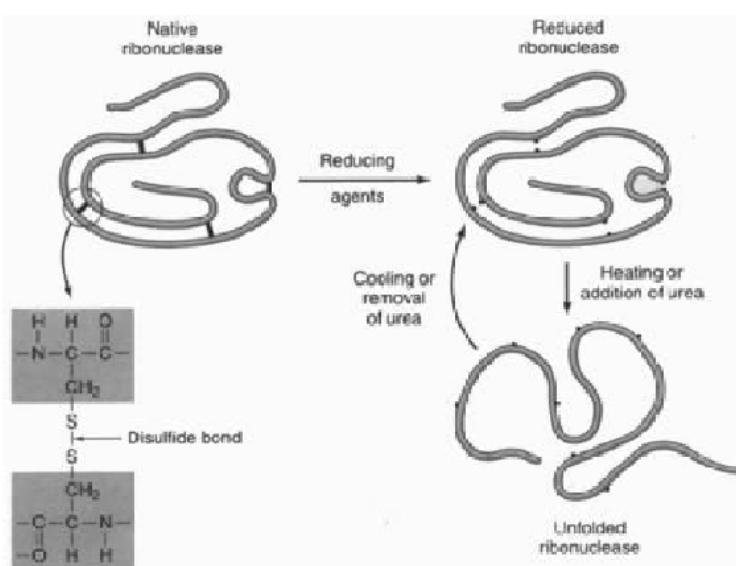


Figure 6.1
Anfinsen's experiment.

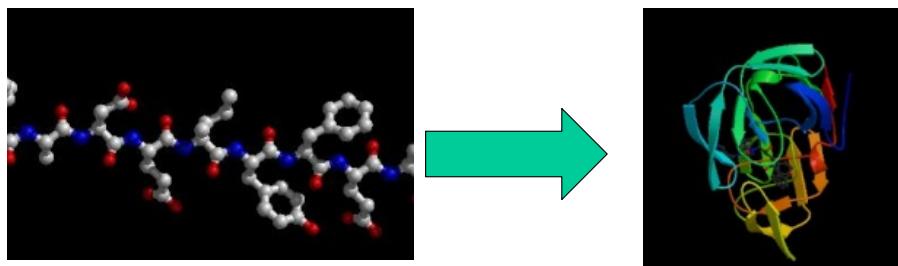
Anfinsen's Ribonuclease A Denaturation and Renaturation Experiment

ANFINSEN:

**AMINO ACID
SEQUENCE
DETERMINES
PROTEIN SHAPE**

Primary Sequence = Structure

-Leu - Arg - Asp - Asp - Ser - Leu - Ala - Asp - Glu - Leu - Tyr - Phe - Glu -



Proteins can self-assemble!

All the information needed to make a working 3-D machine is encoded in the amino acid sequence!

Unfortunately, we haven't figured out the code yet. We can't effectively predict 3-D structure of a protein from looking at the primary amino acid sequence.

Diseases Associated with Defects in Primary Structure:

1. Cystic Fibrosis (CF)

- b. Inherited disease that affects breathing, digestion, reproduction and other functions
- c. 1000 cases/year in the US
- d. Symptoms:
 - i. Chronic cough, wheezing and breathing problems
 - ii. Frequent sinus and respiratory infections
 - iii. Excessive mucous production
 - iv. Recurrent pneumonia
 - v. Salty skin
 - vi. Sterility in males
- e. CF attacks endocrine (outwardly secreting) glands, preventing them from functioning normally
- f. In CF, exocrine glands produce thick, sticky mucous secretions that plug up the body's ducts and passages
- g. When mucous clogs the respiratory system, bacteria and microorganisms can grow and impair body's defenses
- h. Sweat glands affected: Abnormal amount of chloride in sweat
 - i. Use "sweat test" to identify CF patients

i. In CF patients, Cl^- ions don't move properly resulting in reduced or eliminated chloride transport.

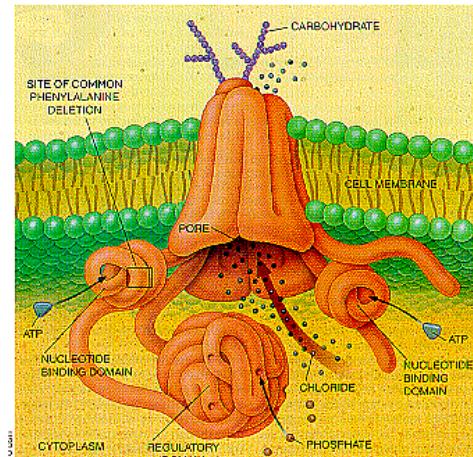
j. Salt stays in sweat and doesn't escape into epithelium. Cells don't secrete normal mucous

k. Also causes deficiency in WATER transport

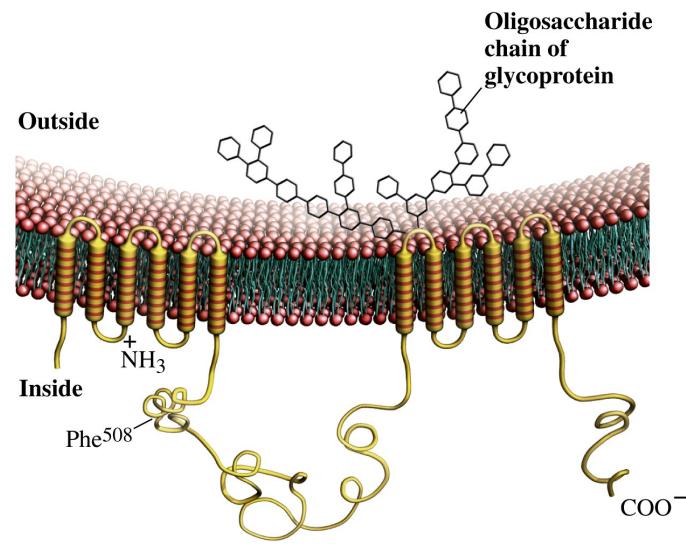
Not enough water to wash away mucous from surface and consequently is abnormally sticky.

l. Leads to obstruction and inflammation in glands/ducts and ultimately tissue damage and death

m. Disease caused by mutations in CFTR gene – both alleles must be mutated otherwise "carriers"



Intact CFTR protein forms a chloride-permeable channel in the outer membrane of many cells. The precise structure has yet to be determined, but movement of chloride through the pore is known to be regulated by three cytoplasmic domains of the protein. Passage is allowed only when the two nucleotide binding domains dock with and cleave adenosine triphosphate (ATP) and when the regulatory domain becomes studded with phosphate groups.



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CFTR = cystic fibrosis transmembrane regulator

- i. Protein expressed at the plasma membrane of epithelial cells
- ii. Acts as a chloride channel
- iii. Way the salt component enters and leaves cells
 - 1. Deficiency in chloride transport is basis for the symptoms

Most severe mutation is deletion of amino acid 508 – Phenylalanine

- Mutation causes the protein to get stuck in the endoplasmic reticulum on its way to the plasma membrane
- Other mutations (over hundreds identified) have varying effects and affect severity of the disease.

Treatments:

- Pancreatic enzymes to aid in digestion – pancreatic ducts get clogged
- Aerosols to help breathing
- Antibiotics to help respiratory infections
- Exercise
- Chest physical therapy
- Proper nutrition and vitamins
- Gene therapy – introduce “good” copy of the gene into the genome

Sickled and Normal Red Blood Cells**2. Sickle Cell Anemia**

- a. Inherited blood disorder
- b. Chronic anemia and periodic episodes of pain
- c. Defective **hemoglobin** in red blood cells – has consequences in oxygen transport in blood
- d. After hemoglobin is **deoxygenated**, hemoglobin clusters together forming rod-like structures
- e. Cause red blood cells to become stiff and assume a **sickle shape**
- f. Get trapped in capillaries and block circulation to organs, producing pain along with many other problems.
- g. Sickle cells are more fragile because their membranes are stretched – break and lyse easily
- h. Red blood cells only live 10-20 days versus 120 days (normal)

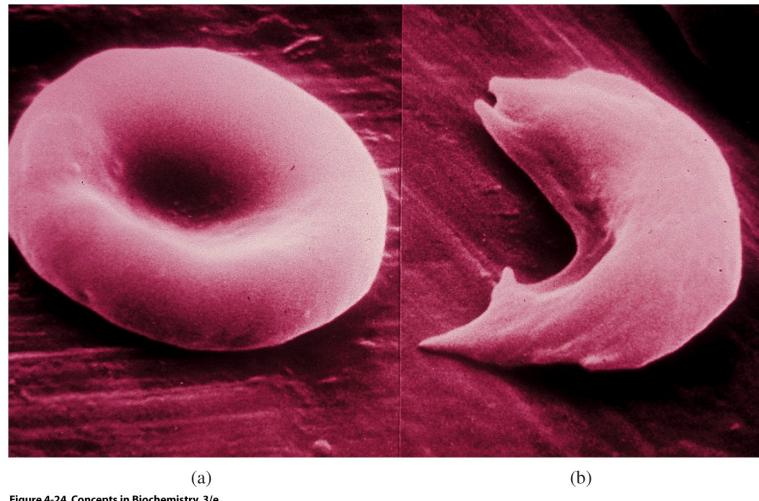
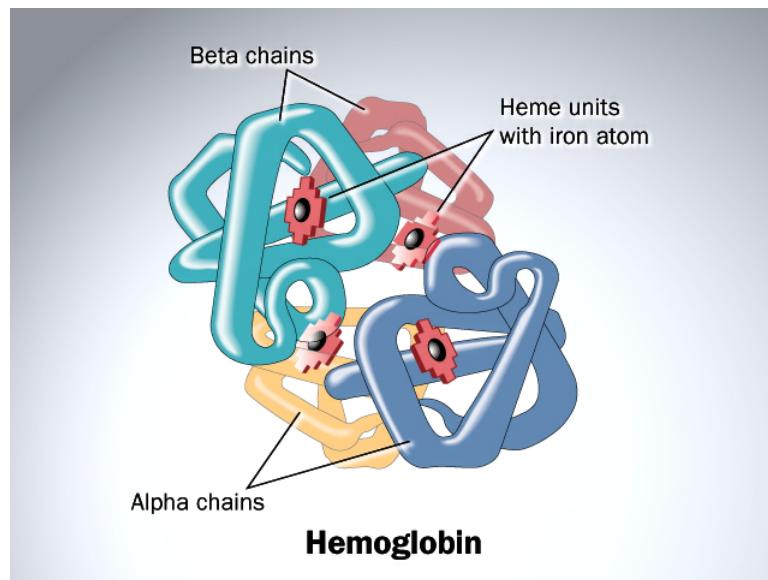
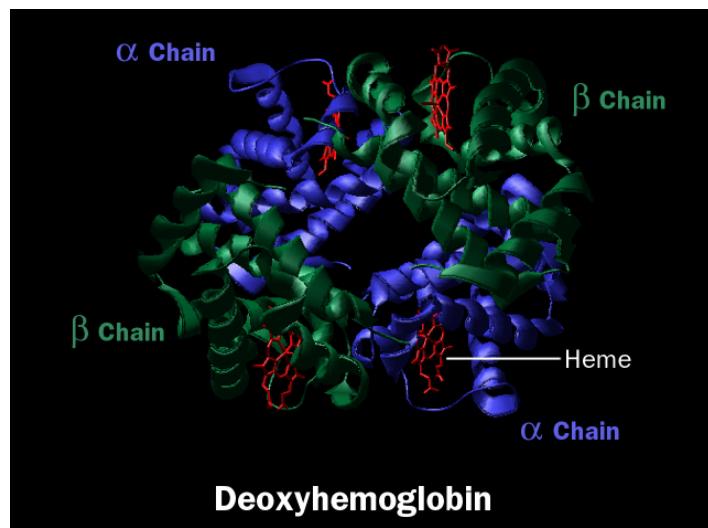
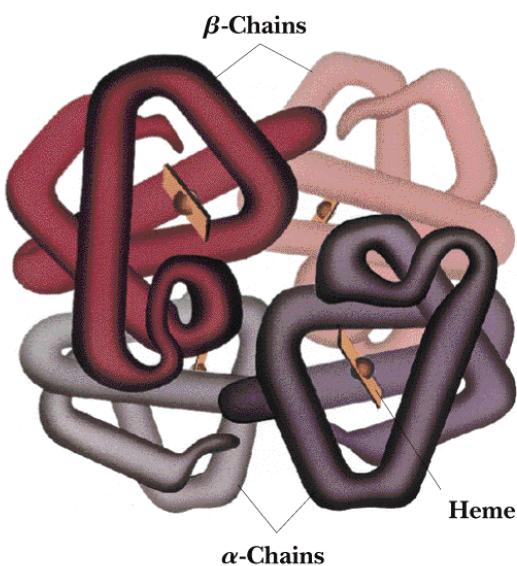


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STRUCTURE OF HEMOGLOBIN:

- Tetramer (4 subunits)
 - o 2 alpha (α) subunits
 - o 2 beta (β) subunits
 - o Mutation occurs in the beta subunit (Glu \rightarrow Val; position 6)
 - o Sickle cell has 2 abnormal β -chains and 2 normal α -chains

**Quaternary Structure of Hemoglobin**

See:

CHIME Models of Hemoglobin and Sickle Hemoglobin

<http://www.umass.edu/microbio/chime/hemoglob/index.htm>

Electron microscopy picture of Fibrils:

<http://dwb4.unl.edu/Chem/CHEM869K/CHEM869KLinks/gingi.uchicago.edu/hbs.html>

Mutations:

- Most common
 - o Single amino acid change from **Glu → Val at position 6**
 - o Places **hydrophobic** side chain on surface of the protein
 - o When deoxygenated, having this hydrophobic group on the surface causes a decrease in **protein solubility** and rod-like structure production
- **Heterozygotes**
 - o Carriers without symptoms
 - o Selective advantage
 - Survive malarial outbreaks
 - **Homozygotes** – have the disease

Table 4.1
Amino acid substitutions in mutant hemoglobins

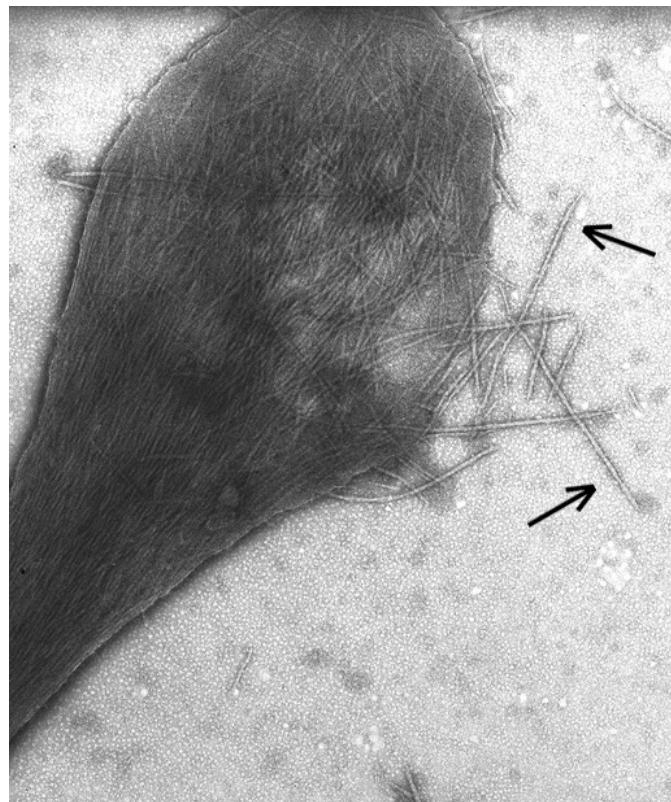
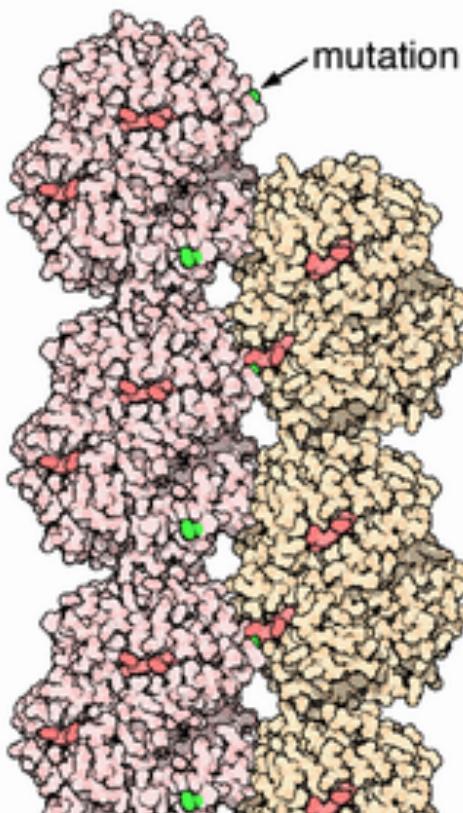
Mutant Hemoglobin ^a	Position Number ^b	Normal Residue	Substitution
α Chain			
G _{Honolulu}	30	Glu	Gln
G _{Philadelphia}	68	Asn	Lys
I	16	Lys	Glu
M _{Boston}	58	His	Tyr
Norfolk	57	Gly	Asp
O _{Indonesia}	116	Glu	Lys
β Chain			
C	6	Glu	Lys
D _{Punjab}	121	Glu	Gln
G _{San Jose}	7	Glu	Gly
E	26	Glu	Lys
S	6	Glu	Val
Zurich	63	His	Arg

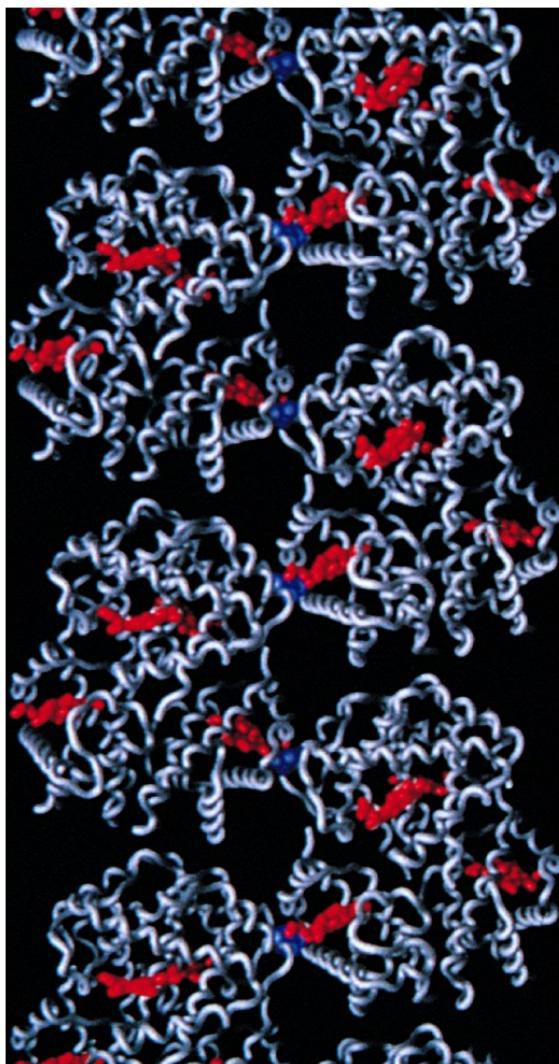
^aThe hemoglobins are often named for the cities where they were first discovered.

^bThe numbering for an amino acid position begins at the N-terminus.

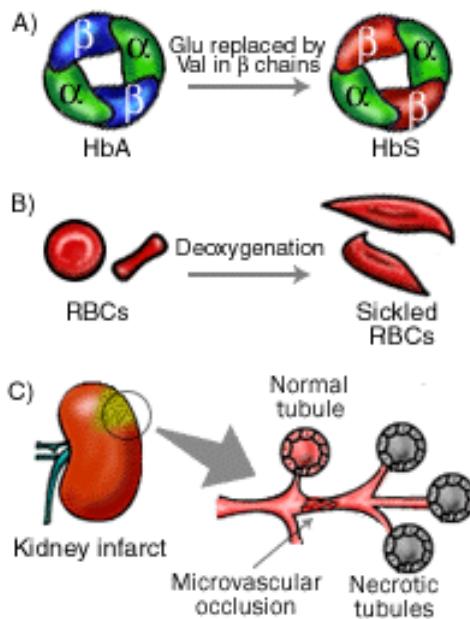
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A sickled red cell filled with sickle hemoglobin fibers





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A) Hemoglobin is made up of 4 chains: 2 α and 2 β . In SCA, a point mutation causes the amino acid glutamic acid (Glu) to be replaced by valine (Val) in the β chains of HbA, resulting in the abnormal HbS. B) Under certain conditions, such as low oxygen levels, RBCs with HbS distort sickled shapes. C) These sickled cells can block small vessels producing microvascular occlusions which may cause necrosis (death) of the tissue.

Model of Polymerized Hemoglobin

- Therapies:

- Pain Killers
- Prevent cell dehydration
 - o Use of *clotrimazole* – drug that prevents loss of water
- Gene therapy with fetal hemoglobin or induce fetal hemoglobin expression
 - o Fetal hemoglobin seems to prevent sickling of red cells and cells containing fetal hemoglobin tend to survive longer in the bloodstream
 - Hydroxyurea stimulates production of fetal hemoglobin
- Blood transfusions
- Antibiotics