Biochemistry I: Proteins, Lipids and Metabolism

Tutorial #2

Sept 14th, 2018

Welcome to BCH210

Chloe Mitchell

chloe.mitchell@mail.utoronto.ca

- Tutorial notes, discussion board

  Quercus via Portal

  Tutorial notes will be posted by 4:00 pm day before

- Course information

  http://biochemistry.utoronto.ca/courses/bch210h-fall-biochem-1-proteins-lipids-metabolism/

Take away Points

Driving forces involved in protein stabilization

- Hydrophobic effect, Ionic interactions, Hydrogen bonds, Van der Waals

Proteins have four structural hierarchies

- Primary, Secondary, Tertiary and Quaternary
- Can describe the entire protein structure using the dihedral angles

Sequence – structure – function relationships

- Fibrous, globular, membrane, transport, receptors, channels

Protein Folding

What forces secondary, tertiary and quaternary structure?

Interactions that stabilize protein structure:

- Electrostatic
- Hydrogen bonds
- Hydrophobicity
- Van der Waals

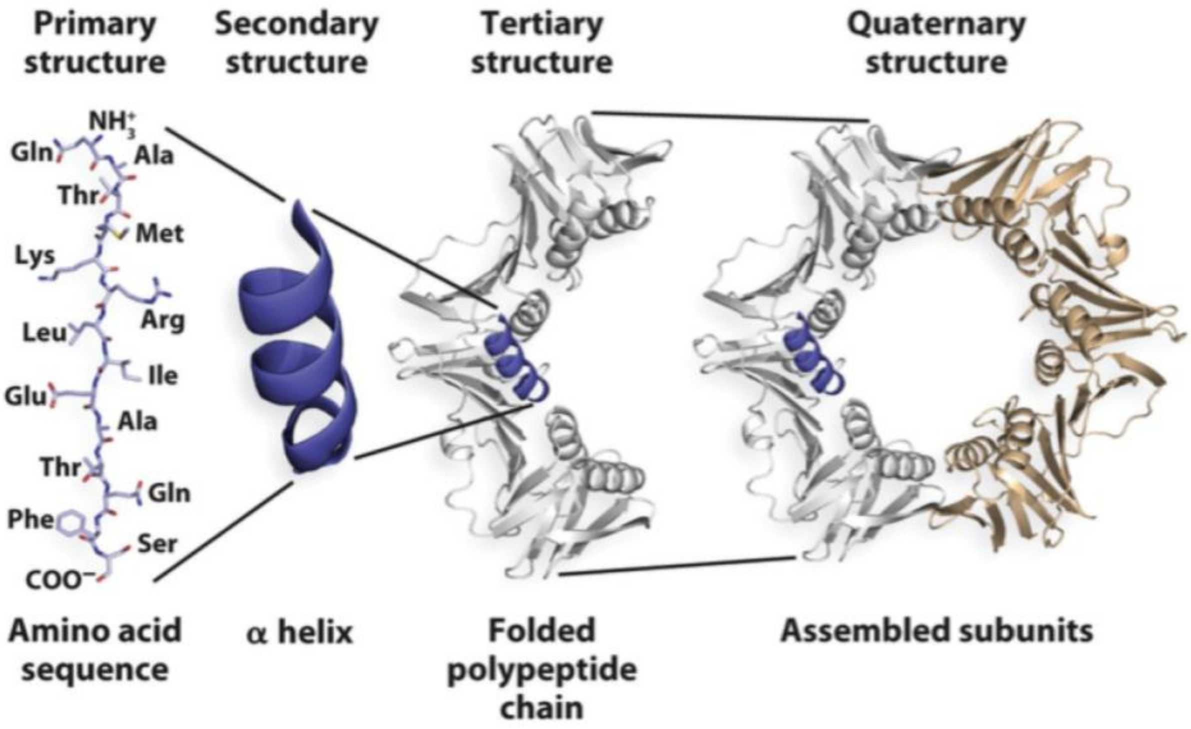


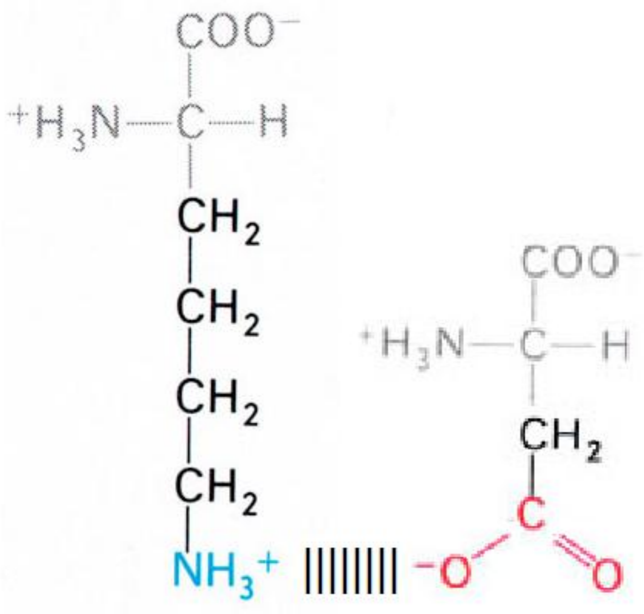
Figure 4-1
Molecular Biology: Principles and Practice
© 2012 W. H. Freeman and Company

Electrostatic Interactions

Occurs between positive and negatively charged species.

Opposites attract!

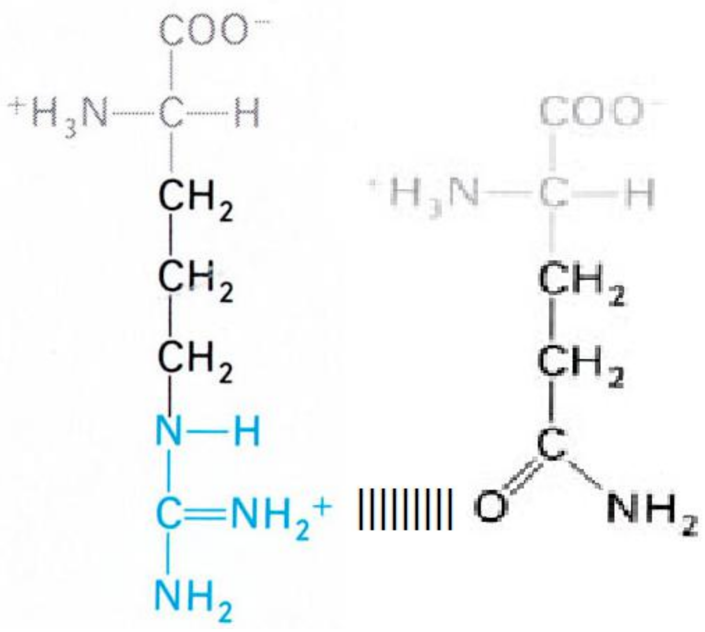
Two Charged Species



Lysine

Aspartic Acid

Charged and Polar Species



Arginine

Glutamine

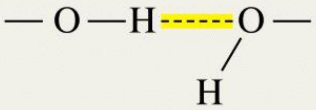
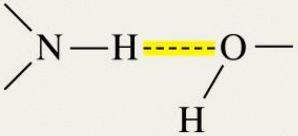
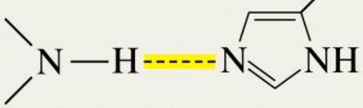
Question

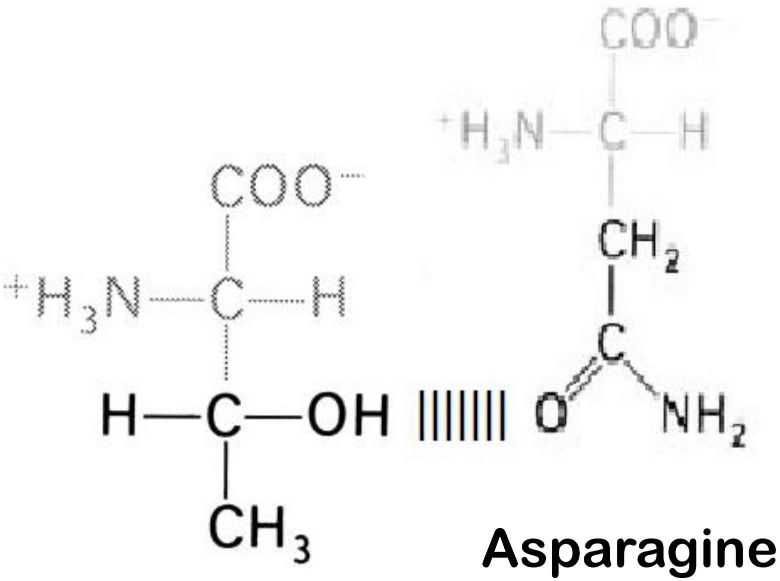
Electrostatic interaction can happen between the R-groups of:

~~A~~. Glycine and Aspartate
~~B~~. Leucine and Isoleucine
[✓ C.] Lysine and Aspartate
D. Arginine and Lysine
E. Lysine and Lysine

Hydrogen Bonds

Occurs between a proton (-OH, -SH, -NH) and an electron donating species (C=O).

Type of hydrogen bond	
Hydroxyl-hydroxyl	
Hydroxyl-carbonyl	
Amide-carbonyl	
Amide-hydroxyl	
Amide-imidazole nitrogen	



Asparagine

Threonine

Question

Which statement(s) apply to Hydrogen bonds?

~~A)~~ Non-covalent interaction between electroneutral atoms of different molecules.

~~B)~~ Non-covalent interaction between two electroneutral atoms of the same molecule.

[✓ C)] Partially electropositive hydrogen atom of one molecule and partially electronegative atom of another molecule.

[✓ D)] Partially electropositive hydrogen atom of one molecule and partially electronegative atom of the same molecule.

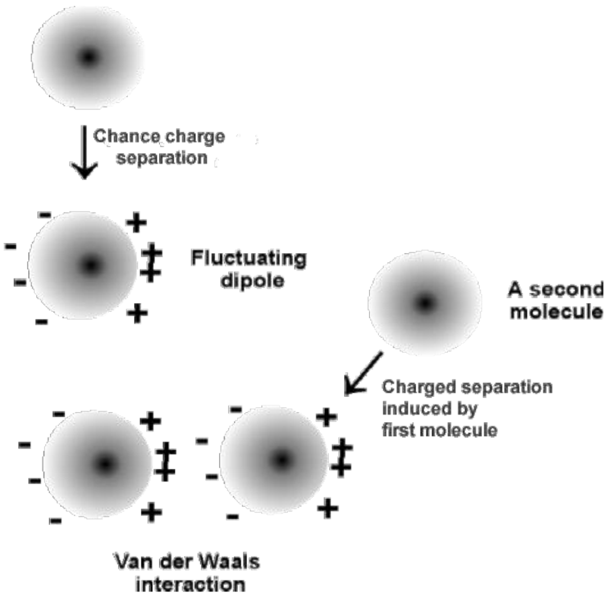
Van der Waals Interactions

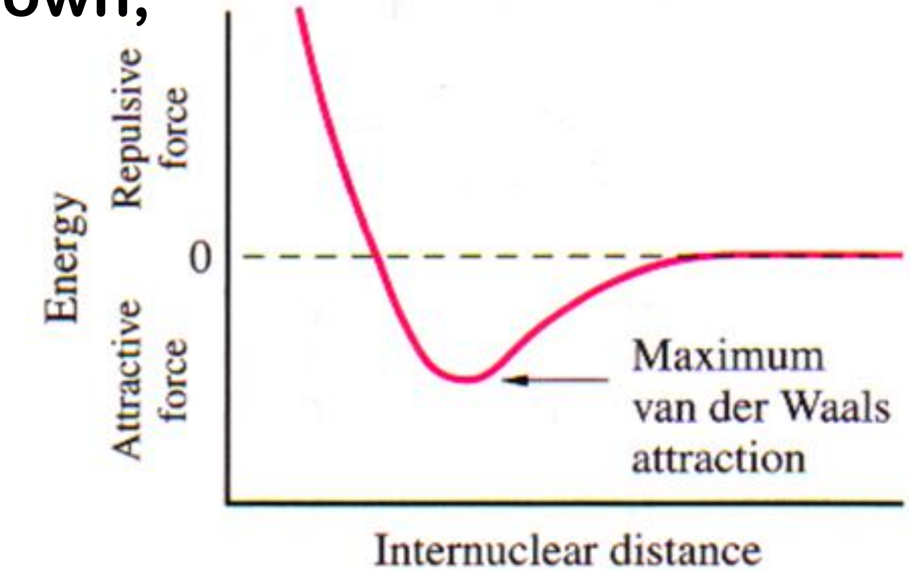
Weak interactions between close molecules.

- Can not be closer than the van der Waals contact distance (radii) or else positively charged nuclei will repel!

Weak interactions on their own,

but strength in numbers!



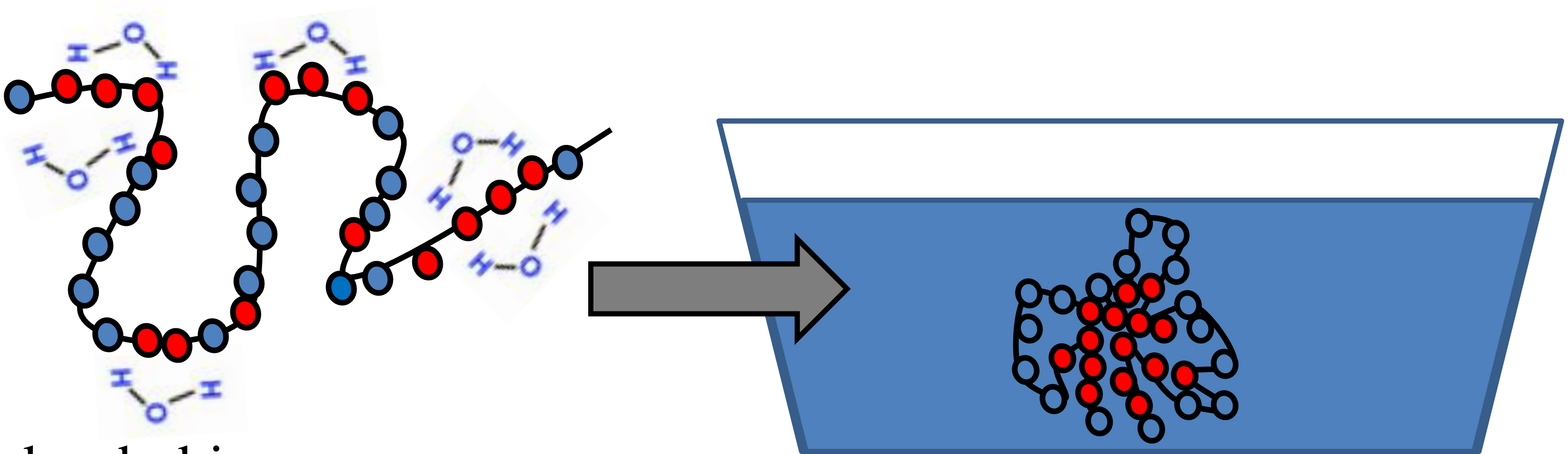


Similar to a temporary electrostatic interaction (induced dipole)

Hydrophobic Interactions

Association of non-polar residues driven by the HYDROPHOBIC EFFECT. Hydrophobic side chain exposure causes unfavourable water molecule 'ordering', reduced entropy! Water also has a high affinity for itself, forming H-Bonds!

The burial of hydrophobic residues is the main driving force for protein folding!



Red = hydrophobic a.a.

Question

Which of the stabilizing forces on a single molecule basics is the weakest?

A. Hydrogen Bonds
B. Electrostatics
C. Hydrophobic affect
[✓ D.] Van der Waals

How would having many interacting molecules affect this force?

strengthen

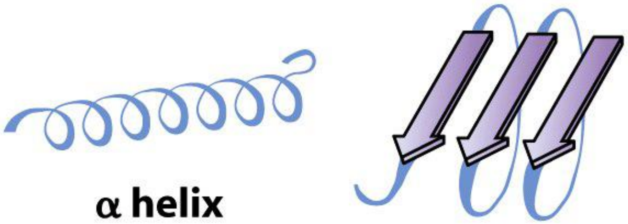
Levels of Protein Folding

(a)Primary structure

–Ala–Glu–Val–Thr–Asp–Pro–Gly–

Order of the amino acids

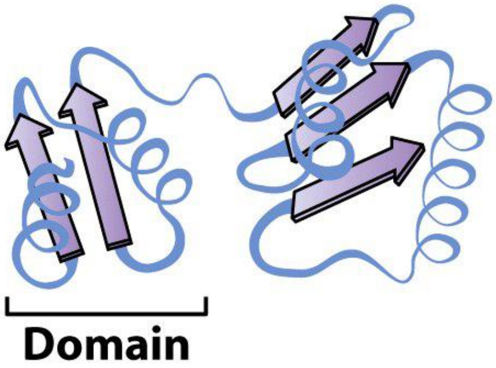
(b)Secondary structure



β sheet

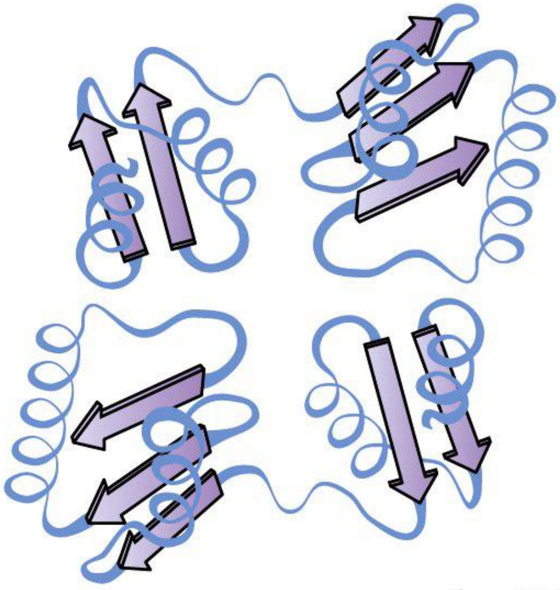
Periodic, repetitive

(c)Tertiary structure



Single molecule/subunit folded into 3D Structure

(d)Quaternary structure



Multiple molecules/subunits

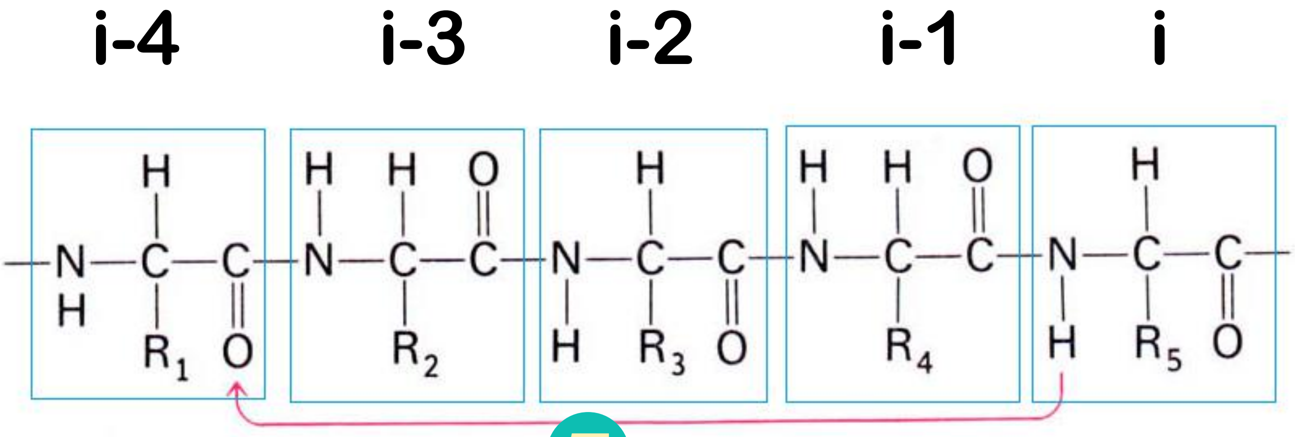
Figure 4-1 Principles of Biochemistry, 4/e

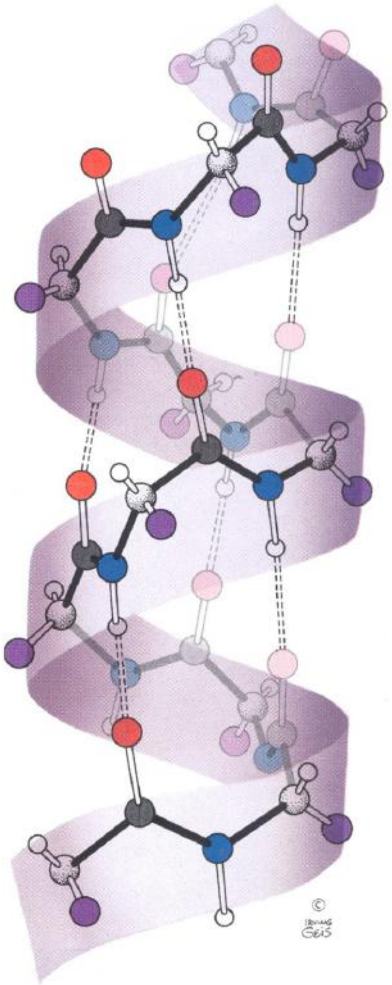
Figure 4-1 Principles of Biochemistry, 4/e

α-helix

Hydrogen bonds between i (N-H) and i-4 (C=O) residues in the peptide backbone.

Hydrogen bonds are intrastrand (within the same molecule) ________.

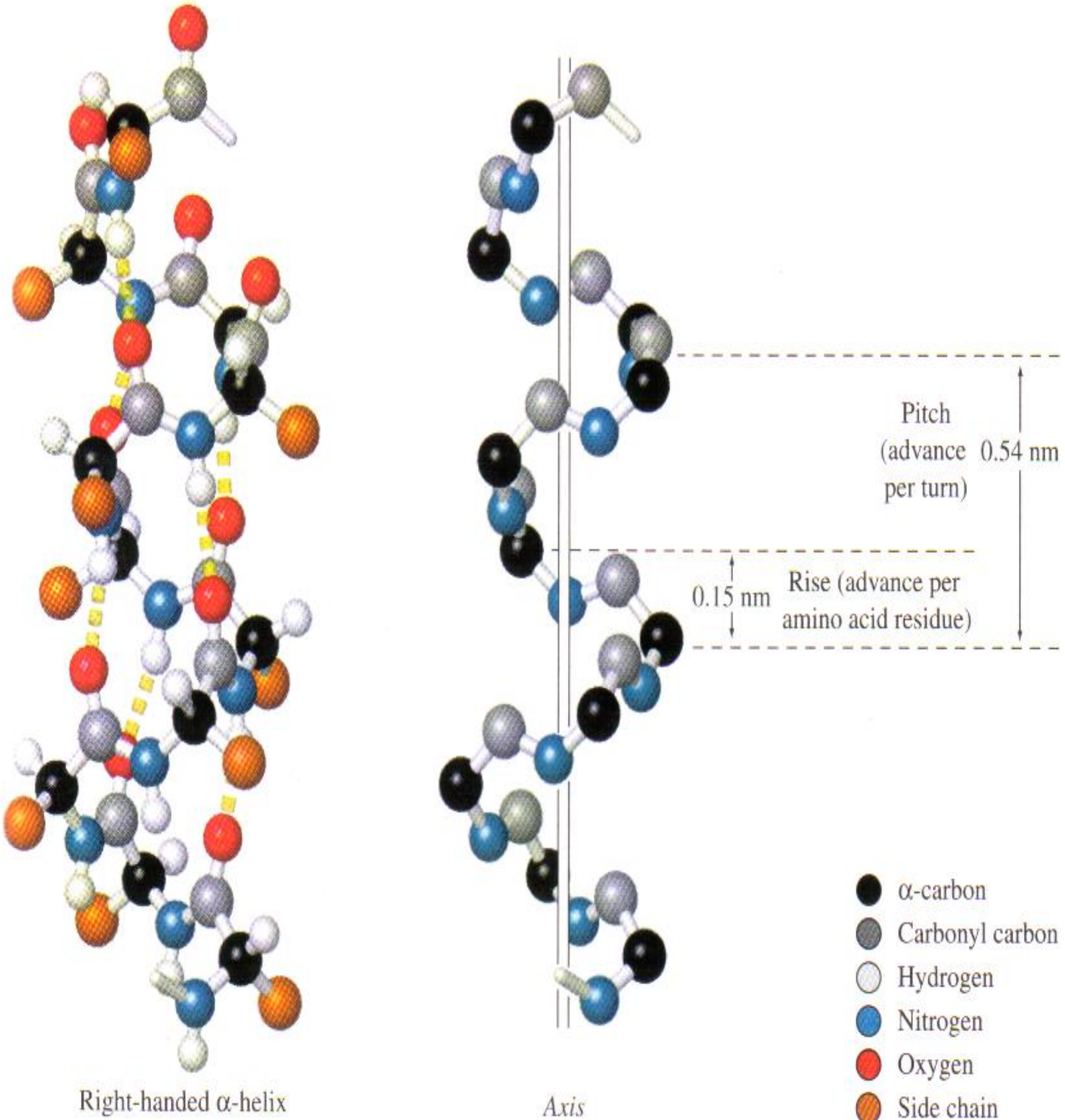




Dimensions of the α-helix

The math....

- Each amino acid contributes to 100° turn
- A complete turn of the helix (360°), requires 3.6 amino acids.
- Each amino acid is 1.5 Å high, 3.6 amino acids per turn gives us (3.6 x 1.5 =) 5.4 Å per turn.



Question

How many amino acids are needed to make an alpha helix 42 Å long?

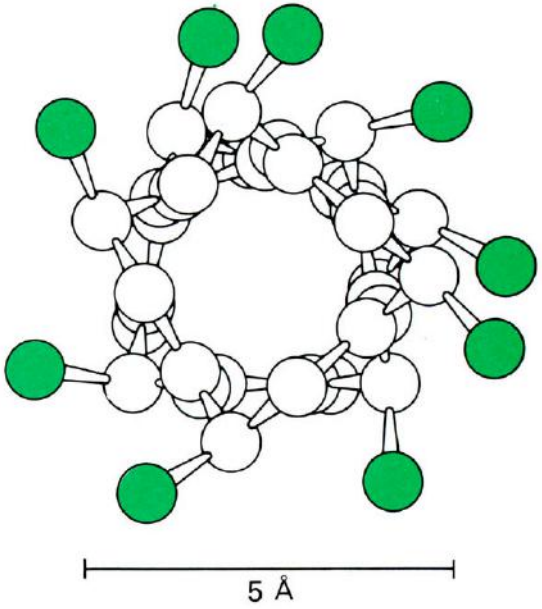
42 A / 1.5 A = 28 amino acids

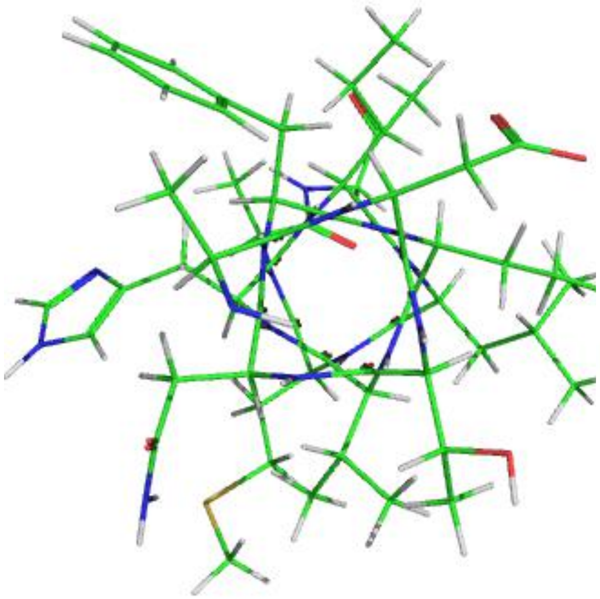
How many turns in a 42 Å long alpha helix?

8 turns (42/5.4)

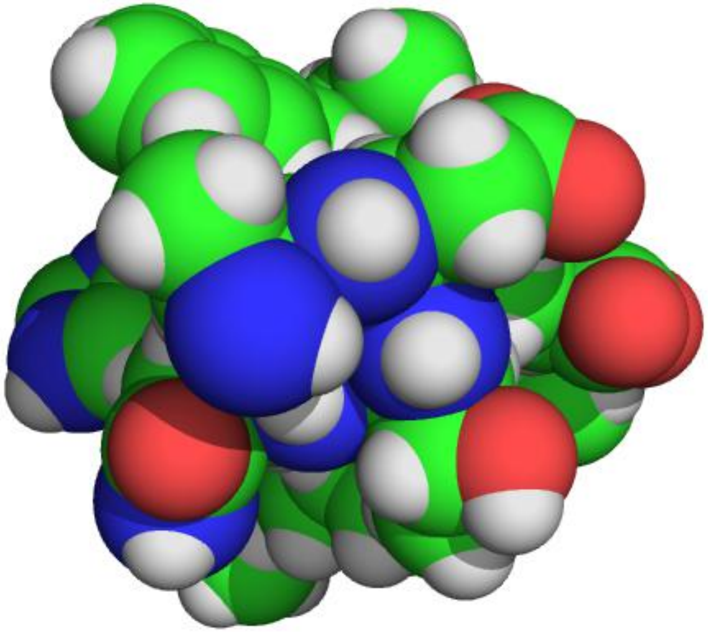
α-helix

Amino acid side chains stick out from helix center.





alpha helix: ADTNFEVHILMAS
Stick Model
rendered in Pymol



alpha helix: ADTNFEVHILMAS
Space Filling Model
rendered in Pymol

β-sheet

Extended chains of amino acids: β strand

intermolecular

Hydrogen Bonds are __________.

Parallel **Anti-parallel**

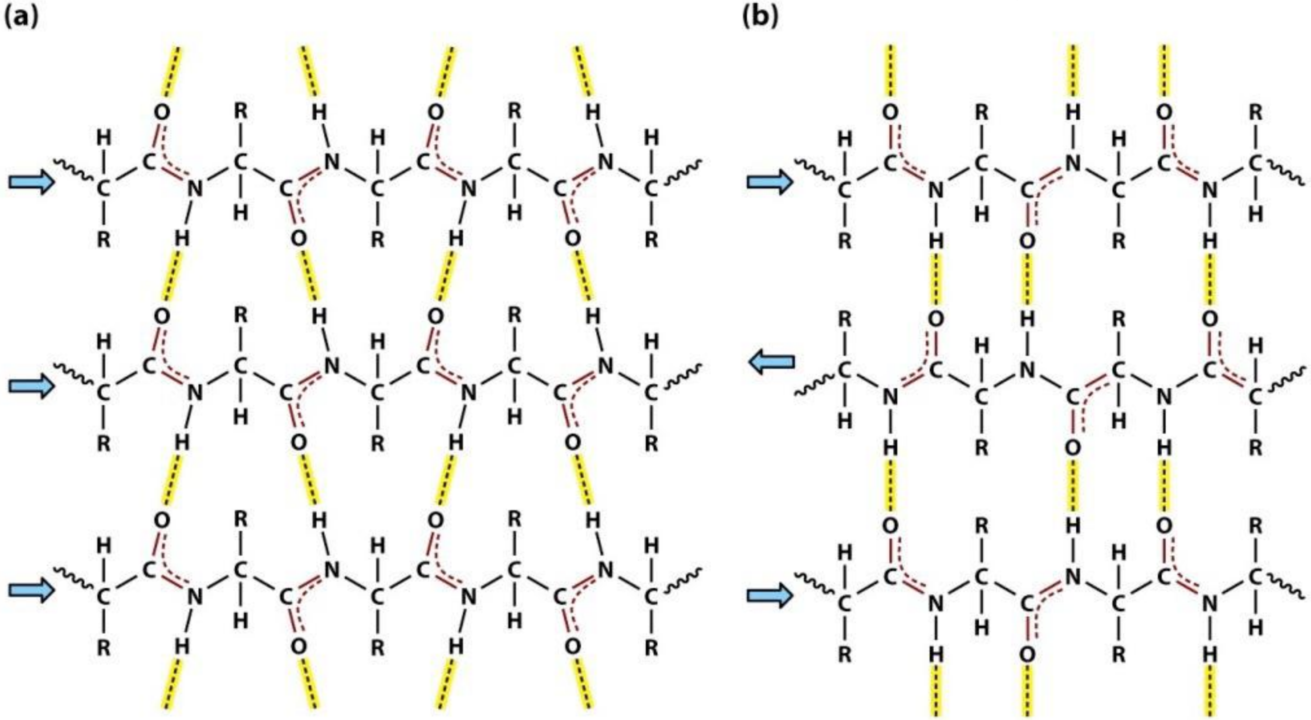
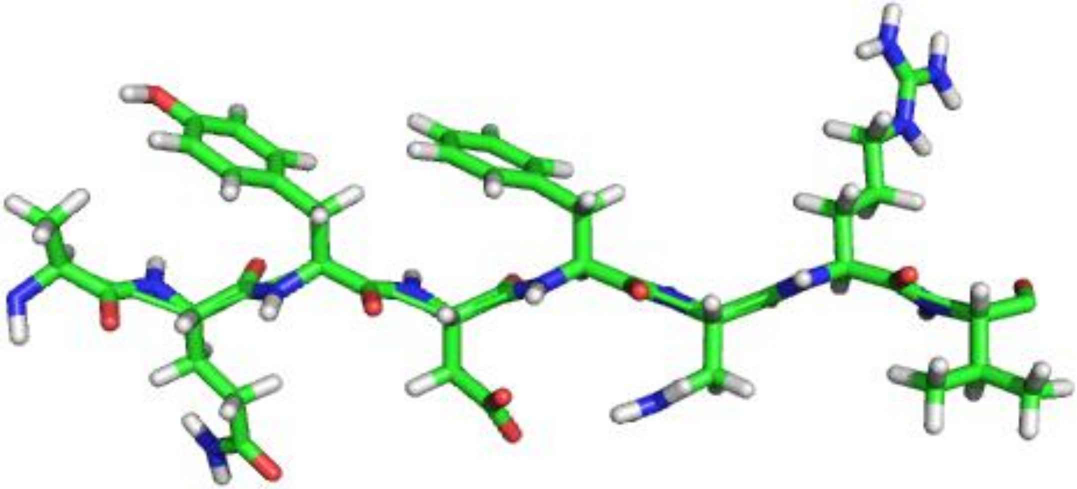


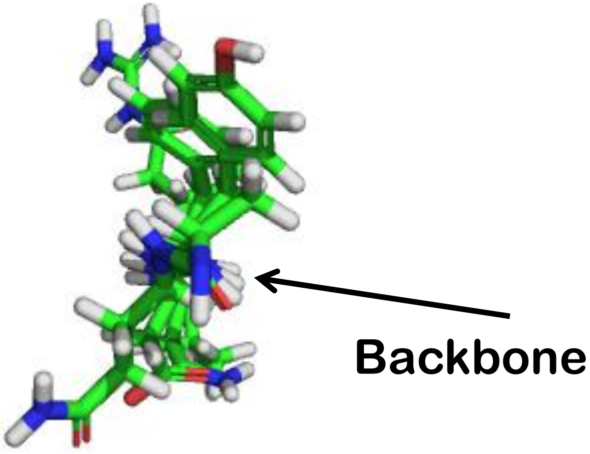
Figure 4-15 Principles of Biochemistry, 4/e
© 2006 Pearson Prentice Hall, Inc.

β-sheet

R groups stick above and below plane of strand.



Beta strand: AQYDFNRV
Stick Model
Rendered in Pymol



Rotate 90° towards yourself

Question

How many amino acids are needed to make a beta strand 42 Å long?

β-turn

Consists of 4 residues which form a 180° turn through

intracellular

i and I-3

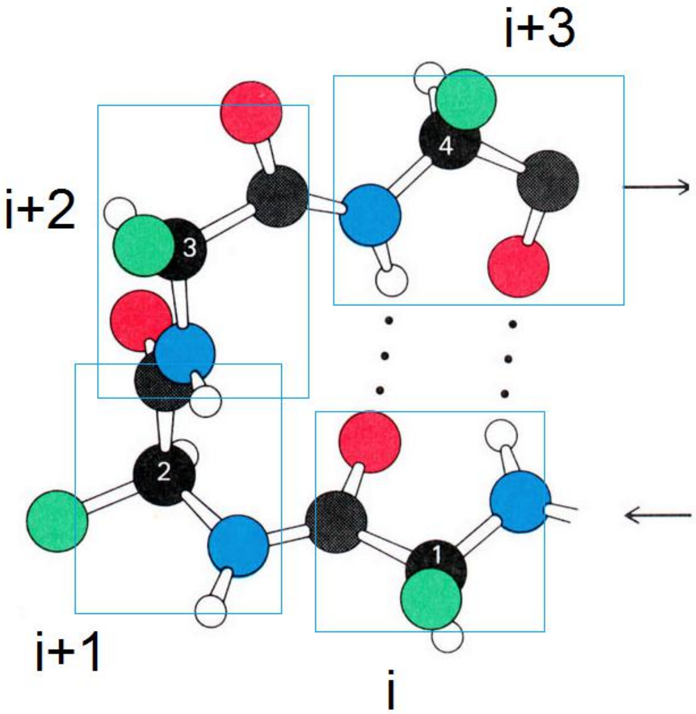
__________________ H-bonding

Typically occur at surface of globular proteins.

Composition:

high in Ser, Gly, Pro, Asn,

Low in Phe, Val



Why?

sidechains of phenylalanine and valine are sterically bulky.

Beta Turns

The main structural difference between the two types of turns is the direction of the peptide bond between positions i + 1 and i + 2.

Type I

Proline generally at position 3 (I+2)

small residues such as glycine at position 2 (I+1)

Type II

glycine must be at position 3 (I+2)
proline preferred in position 2 (I+1)

Secondary structure summary

(Great for studying ☺)

- α-helix
  - (i) H-bond to (i-4)
  - Intrastrand H-bonds
  - 3.6 residues/turn
  - 100°/residue
  - 1.5 Å rise/residue, 5.4 Å rise/turn

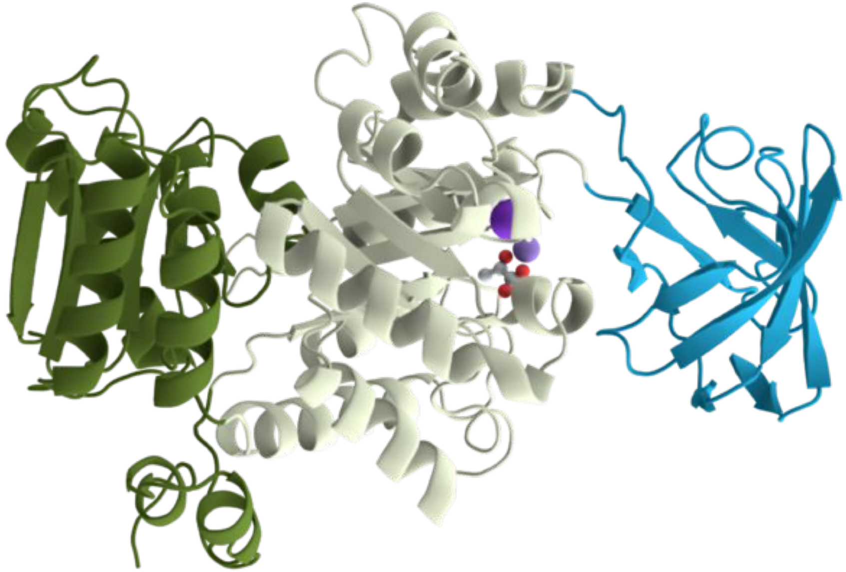
- β-sheet
  - Interstrand H-bonds
  - 2 residues/repeat
  - 3 Å length/residue

- β-turn: (i) H-bonds to (i-3)

Tertiary structure

Assembly of the polypeptide into a completely folded functional protein

Proper arrangement of secondary structures, intervening segments and disulfide bonds.



Pyruvate kinase PDB 1PKN

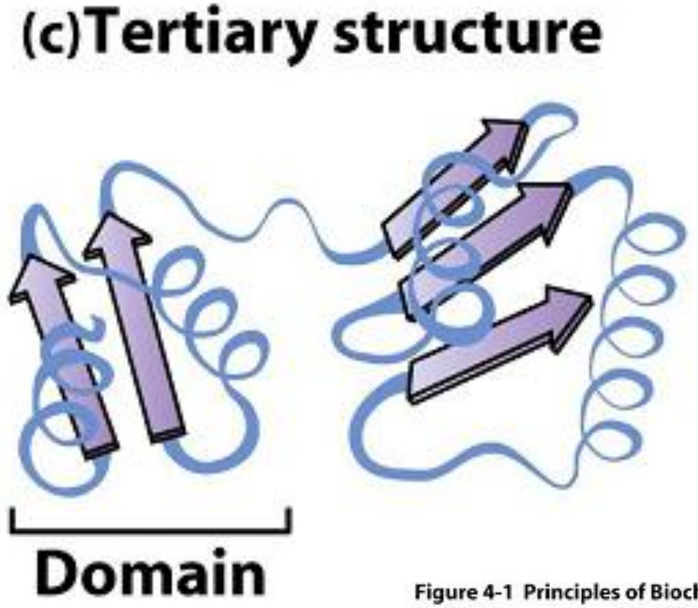
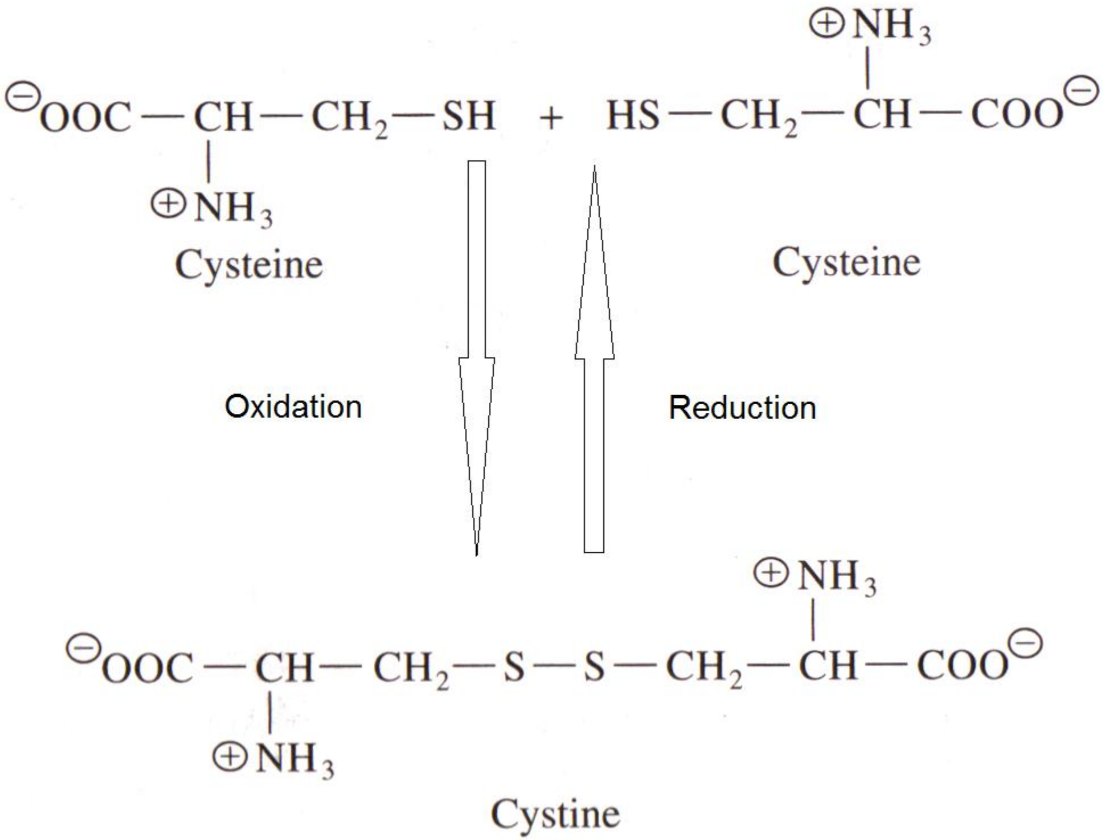


Figure 4-1 Principles of Biochemistry, 4/e
© 2006 Pearson Prentice Hall, Inc.

Disulfide bridges

Disulfide bridges can bring distant parts of the same molecule close together (_____________) and individual polypeptide chains together (____________)



$$^{\ominus}OOC-\underset{\underset{\oplus NH_3}{|}}{CH}-CH_2-SH + HS-CH_2-\overset{\overset{\oplus NH_3}{|}}{CH}-COO^{\ominus}$$

$$^{\ominus}OOC-\underset{\underset{\oplus NH_3}{|}}{CH}-CH_2-S-S-CH_2-\overset{\overset{\oplus NH_3}{|}}{CH}-COO^{\ominus}$$

Reversible

Reaction!

Question

Which of the following has the longest end to end distance?

- A 10 residue α-helix
- [✓ A 10 residue β-strand]
- A β-turn
- A disulfide bridge

Quaternary structure

Association of two or more individually folded polypeptide chains

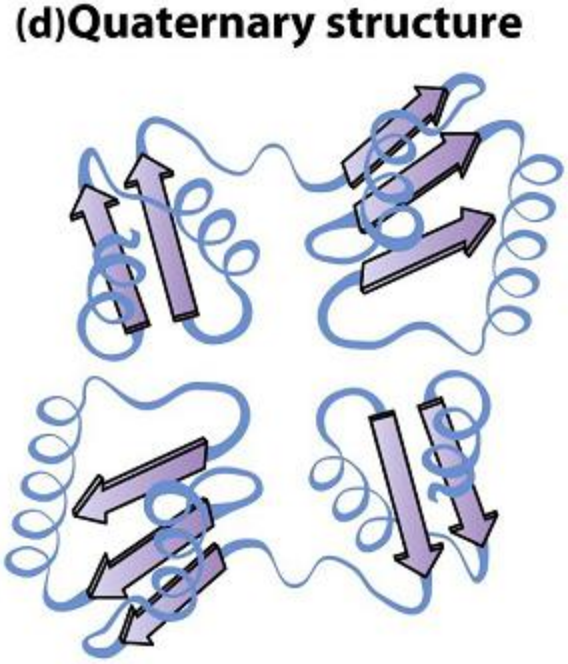
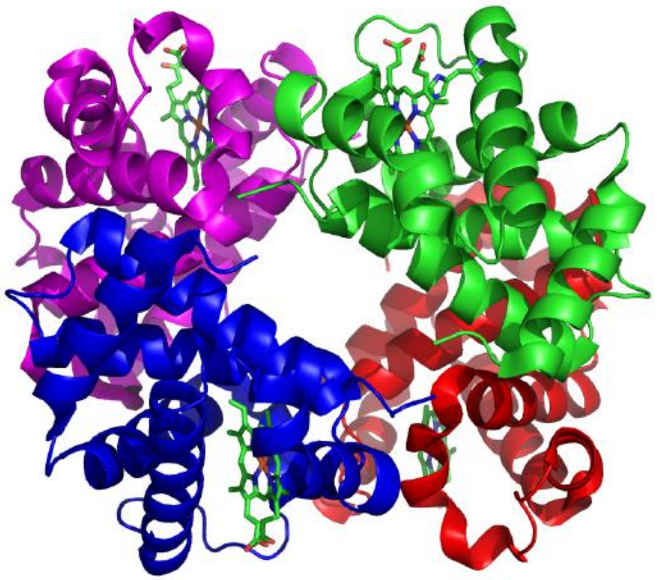

(d)Quaternary structure

Figure 4-1 Principles of Biochemistry, 4/e
© 2006 Pearson Prentice Hall, Inc.



2 α chains

2 β chains

Human Haemoglobin
PDB ID 1GZX
Rendered in PyMol 0.99rc6

Question

Protein quaternary structure may be held together by:

A) Hydrogen bonding and van der Waals

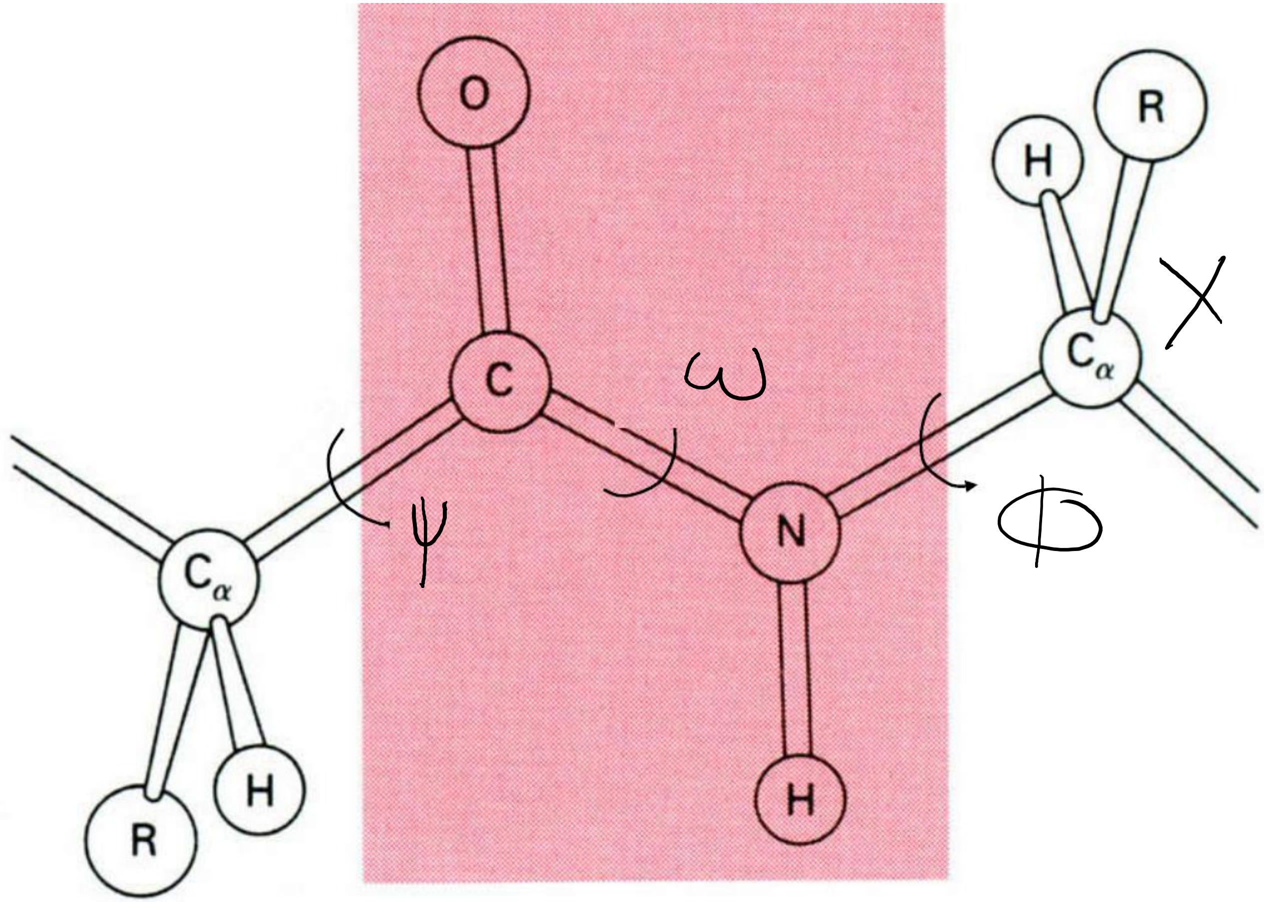
B) Disulfide bonds

C) Electrostatic forces and hydrophobic interactions

D) A and C

[✓ E)] All of the above

How do proteins adopt structure?

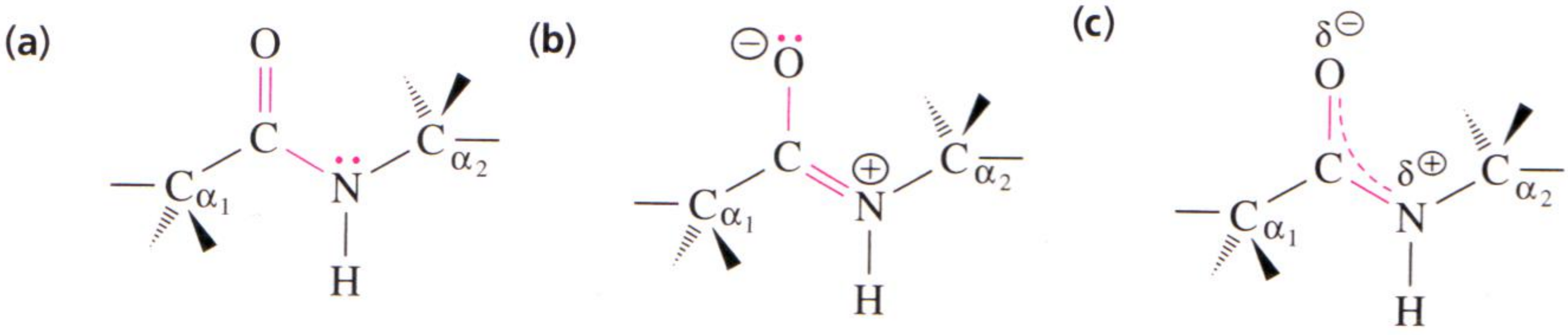


Can describe the entire protein structure using the dihedral angles

Amide Bond (Omega ω)

The angle between C=O and NH in the peptide bond.

The peptide bond exhibits double bond character due to resonance. What are the implications of this?



Question

Which of the following is TRUE about dihedral angles?

a) Phi, psi, and omega (ϕ, ψ, ω) allow one to completely describe a protein's structure χ angle

b) Omega (ω) does not vary much from 90° due to its partial double bond character

c) Amino acids that are next to Gly have increased flexibility, thus they can access ϕ, ψ values that they normally cannot

[✓ d)] Residues in the same secondary structure of a protein all have roughly the same ϕ, ψ values

Question

Which linkage has two bonds with free rotation?

a) $C_{\alpha}\text{- CO – NH}_2$

b) $\text{CO – NH}_2\text{ – C}_{\alpha}$

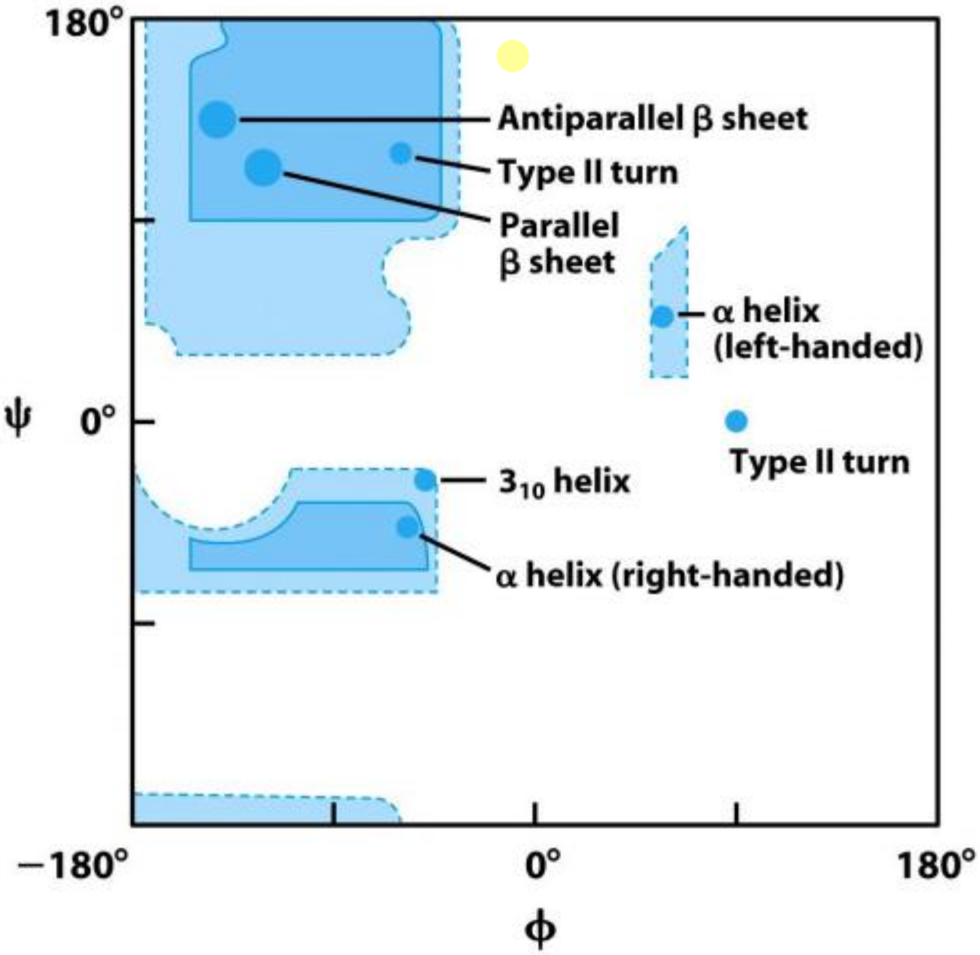
[✓ c)] $\text{NH}_2\text{ – C}_{\alpha}\text{ – CO}$

d) O = C – NH_2

Which linkage has no rotation?

d

Ramachandran plot

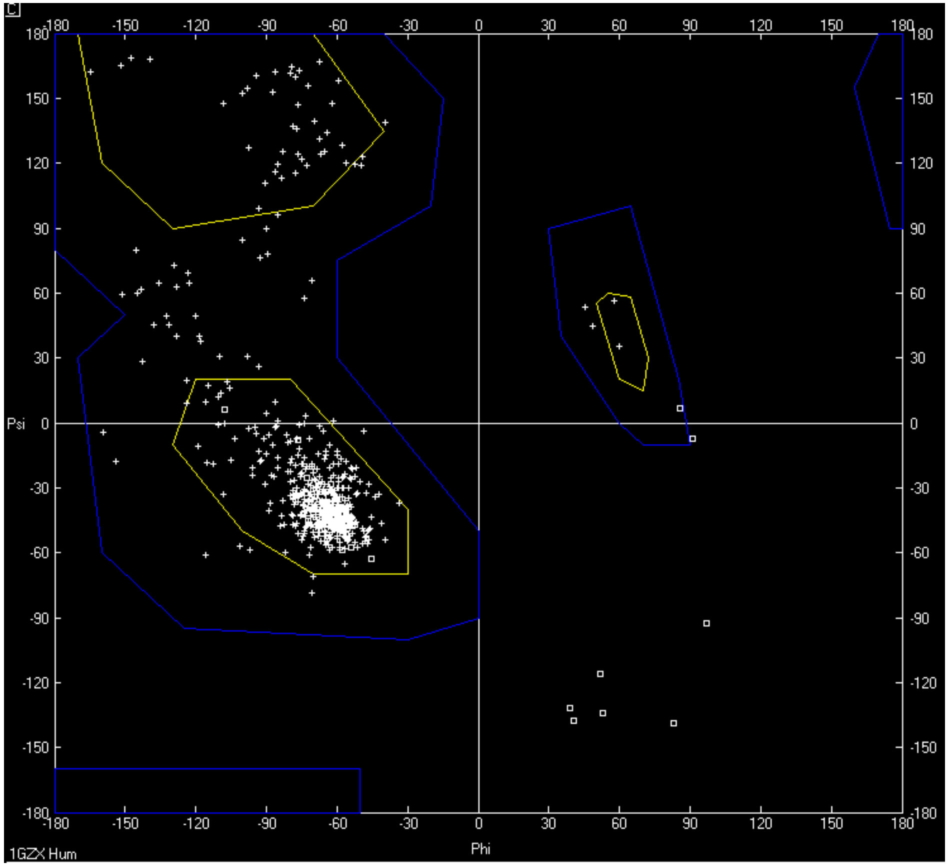


α -helix: (ϕ,ψ)
=(-60°, -50°)

β-sheet: (ϕ,ψ)
=(-140°, +135°)

Poly-pro: (ϕ,ψ)
=(-60°, +135°)

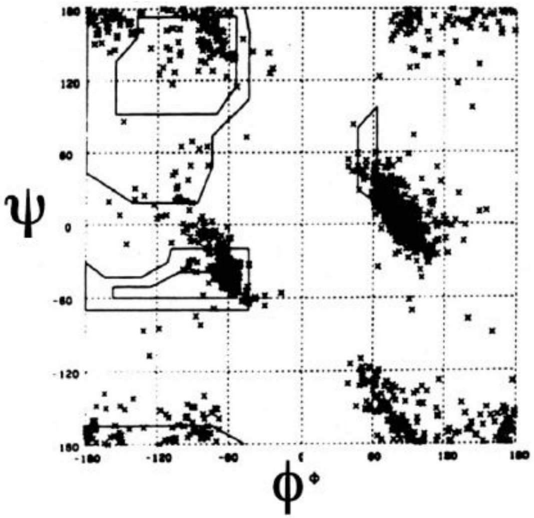
Ramachandran Plot

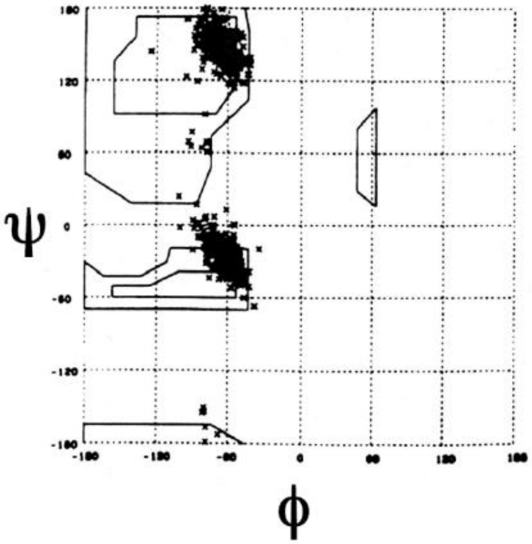


What type of secondary structure is more abundant in in this protein?

Ramachandran Plots

Which amino acids are represented by these plots?





Question

Which of the following CAN be determined from a Ramachandran plot?

a) The total % of β-strands in the protein structure

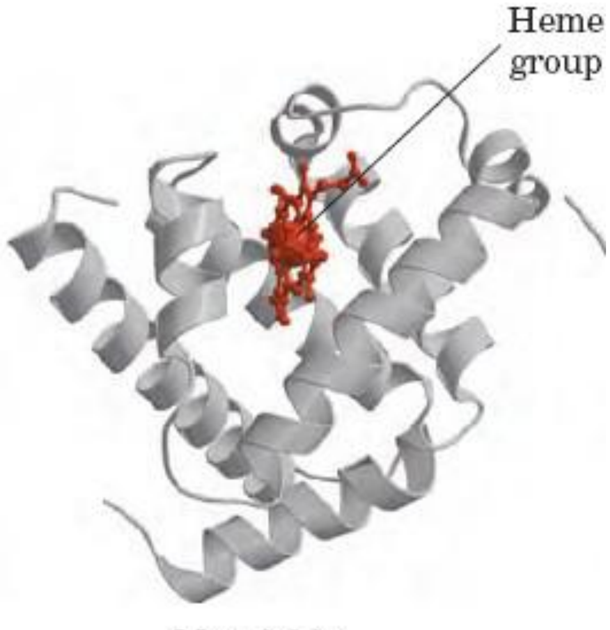
b) The number of α-helices and β-sheets in the structure

c) The number of amino acid residues in an α-helical structure

[✓ d)] The number of amino acids that have phi/psi angles compatible with an α-helical structure

Question

[✓ True] or False: An alpha helix in myoglobin will have the same phi and psi angles as an alpha helix in bacteriorhodopsin.



Myoglobin

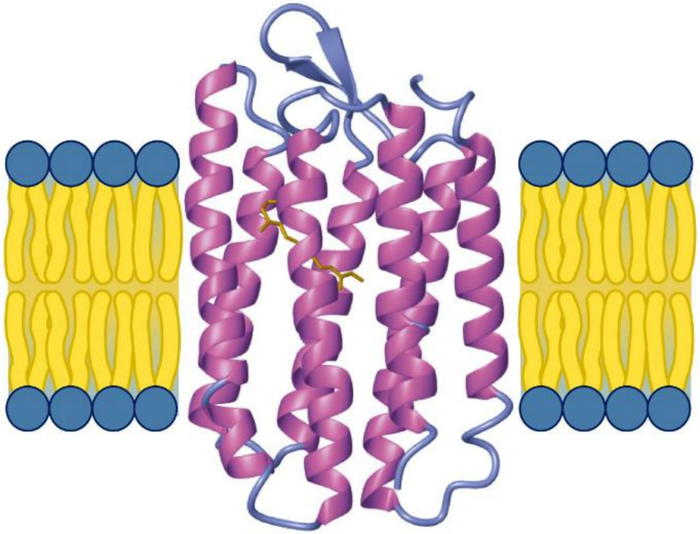


Figure 9-27 Principles of Biochemistry, 4/e
© 2006 Pearson Prentice Hall, Inc.

Fibrous Proteins

Proteins that form fibrils and fibres

- Silk: Stacked Beta sheets
- Keratin: Coiled coil alpha helices
- Collagen: Coiled coil polyproline helices (not alpha-helix!)

Note the differences!!

Silk

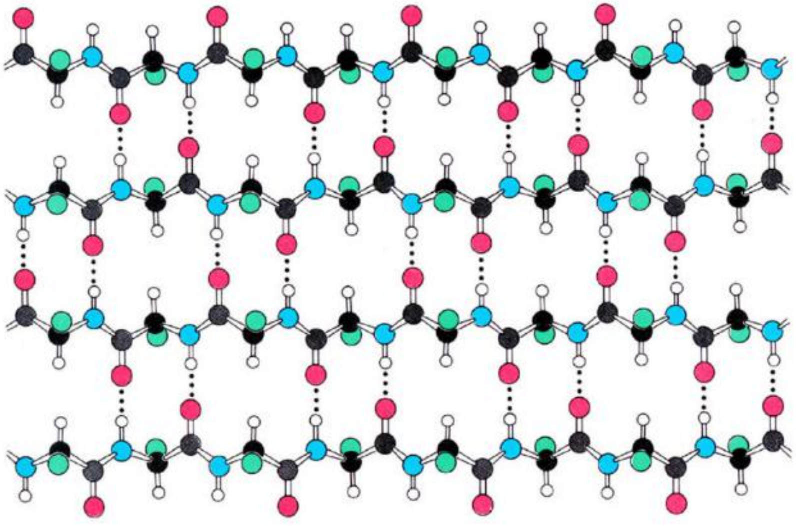
Consists of stacked β-sheets

Sequence:

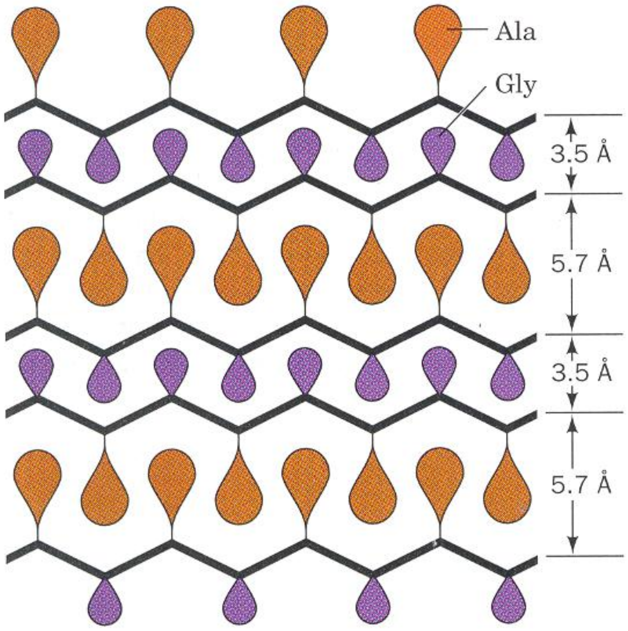
Composition: 44.6% Gly, 29.5% Ala, 12.2% Ser

Glycine's small side chain allows close packing of strands

Fibers are strong because already in the most extended form (Beta sheet!)



Rotate 90°



Question

The composition of natural silk is about 80% Gly + Ala residues, with Gly occurring at every second position in a natural silk. What is the most likely structural implication if Ser was replaced by Proline:

a. Increase the probability that Gly residues would occur every third residue in strand of the non-natural silk.

b. Increase the number of inter-molecular H-bonds between two non-natural strand vs. the number of inter-molecular H-bonds normally found between two natural silk strands.

[✓ c.] Increase the probability that β-turns would occur in a strand of the non-natural silk.

d. Convert the non-natural silk strand into a collagen triple helix.

Keratin: skin, hair, wool

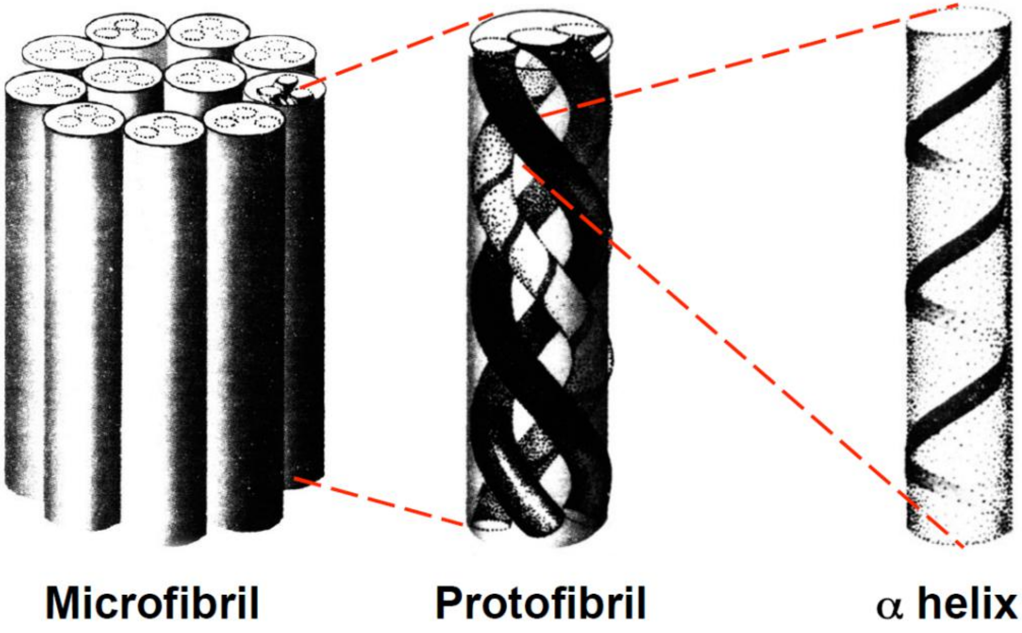
Consists of α-helices (coiled coil)

Sequence: ____________________

Composition: 11.2% Cys, amount of disulfide bonds determines hardness.

(ie. low disulfide – hair

high disulfide – horns)



Keratin: Wool

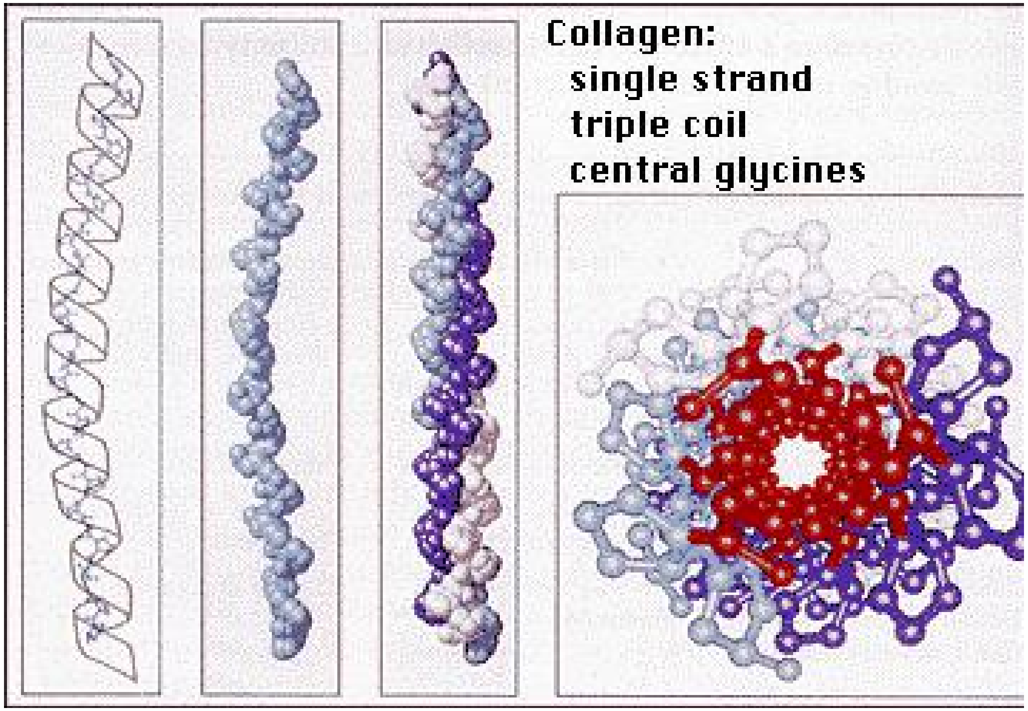
Wool can be stretched (unlike silk), why?

Collagen: skin, tendons, connective tissue

Consists of 3 left handed polyproline helices in a right handed coiled coil. **(Note: These are not alpha helices!)**

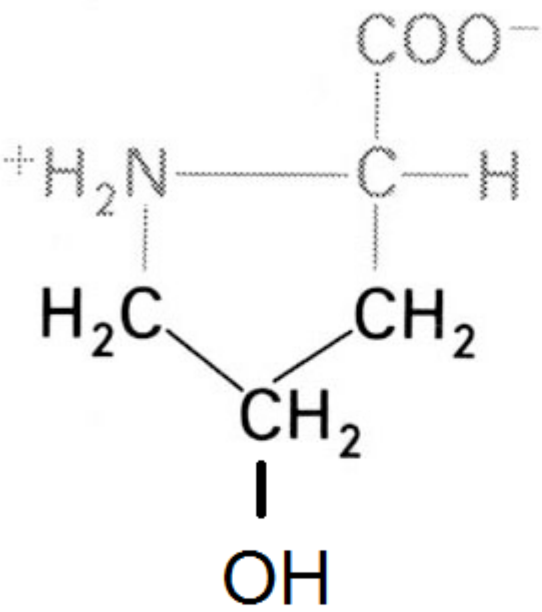
Sequence:

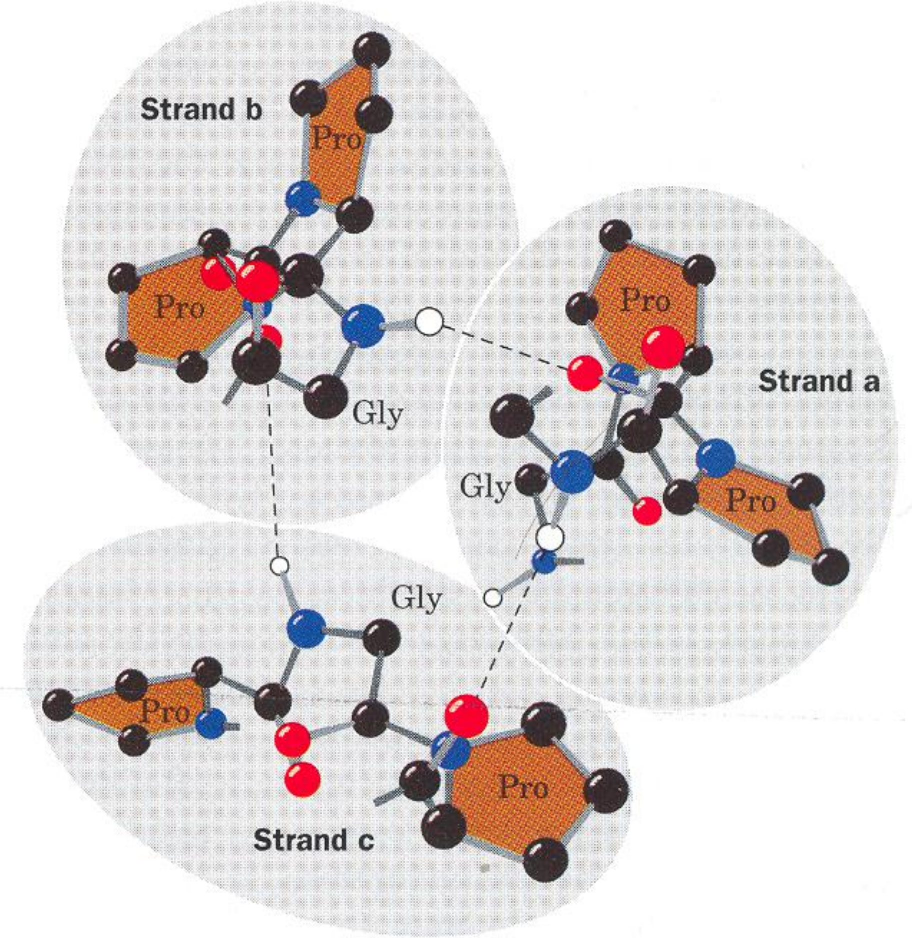
Composition:
33% Gly, 22% Pro/Hypro



Collagen: Hydroxyproline

Hydroxyproline allows inter-strand H-bonding





Question

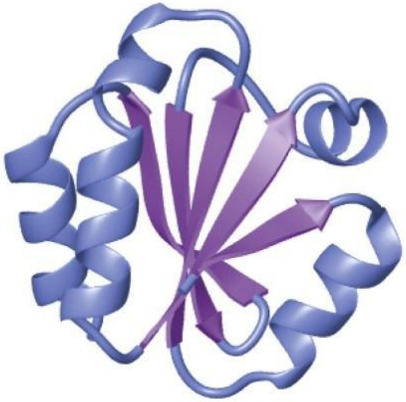
Could helices made of only proline form a collagen like triple helix?

Globular proteins

Typically spherical in shape and contain a combination of secondary structures (helices, sheets, turns, loops)

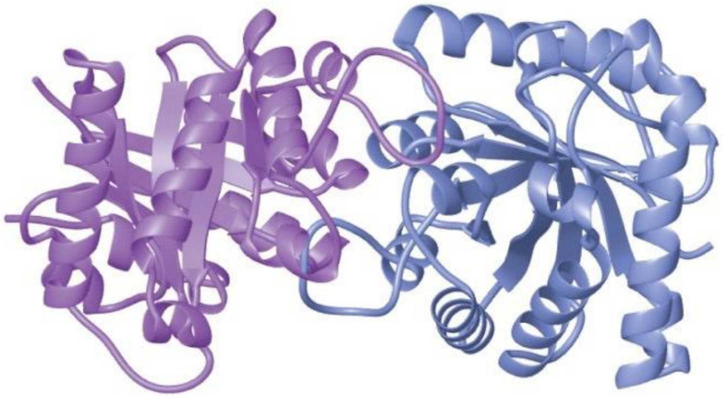
Perform specialized functions in the cell

-Function as enzymes or transporters within the cell.



Human thioredoxin

Figure 4-23m Principles of Biochemistry, 4/e
© 2006 Pearson Prentice Hall, Inc.



Chicken triose phosphate isomerase

Figure 4-25a Principles of Biochemistry, 4/e
© 2006 Pearson Prentice Hall, Inc.

Question

Consider the statement: “All molecules of a given globular protein have the same 3° structure”.

This statement is true because:

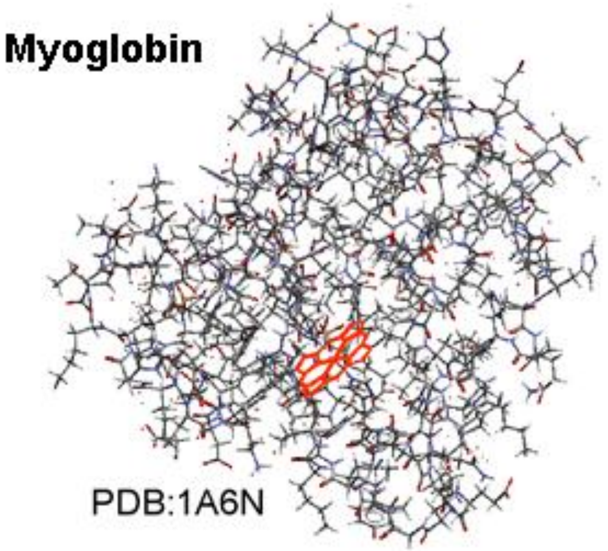
[✓ A)] The biological function of the protein requires that all its amino acids be arranged in a geometrically correct manner in every molecule of that protein.

B) Helix and β-sheet strands in a protein can only be arranged in one way.

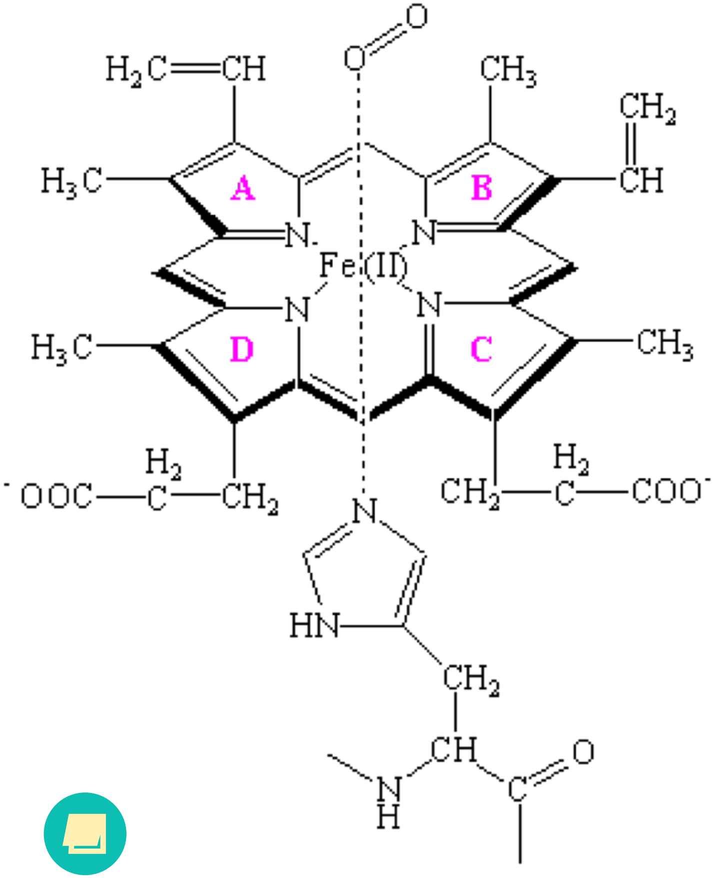
C) The unstructured regions of the 3° structure would change back and forth to helix region.

D) A change in any amino acid residue in the native primary structure will result in considerable loss in protein function.

Myoglobin: Heme group



The heme group in myoglobin is a prosthetic group that contains a protoporphyrin (4 pyrolle rings) and an Iron atom.

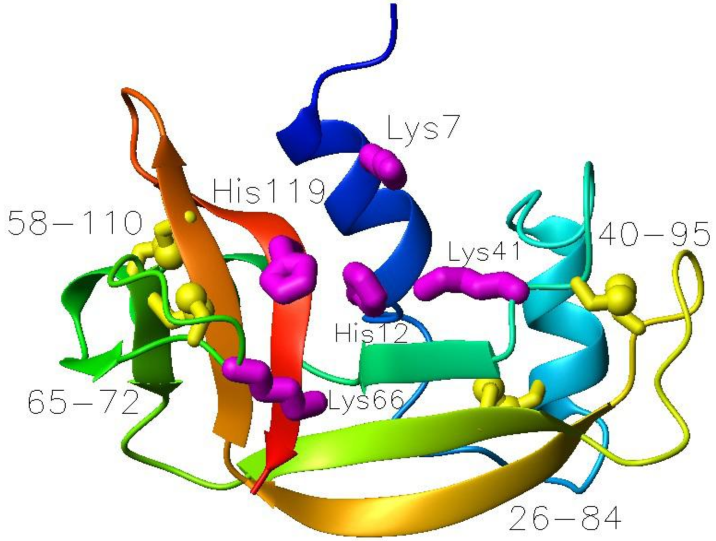


What amino acid helps coordinate the Fe(II)?

RNase A

Function: Cleaves RNA

Active site located within crevice and consists of residues: His12, His119, Lys41.



RNAse A is whole protein, RNAse S has first 20 a.a. cleaved (S-peptide)

Question

Which of the following statements about RNase A is correct?

a) RNase A cleaves double stranded DNA through the use of a positively charged active site.

[✓ b)] RNase A cleaves single stranded RNA through the use of a positively charged active site.

c) RNase A cleaves double stranded RNA through the use of a negatively charged active site.

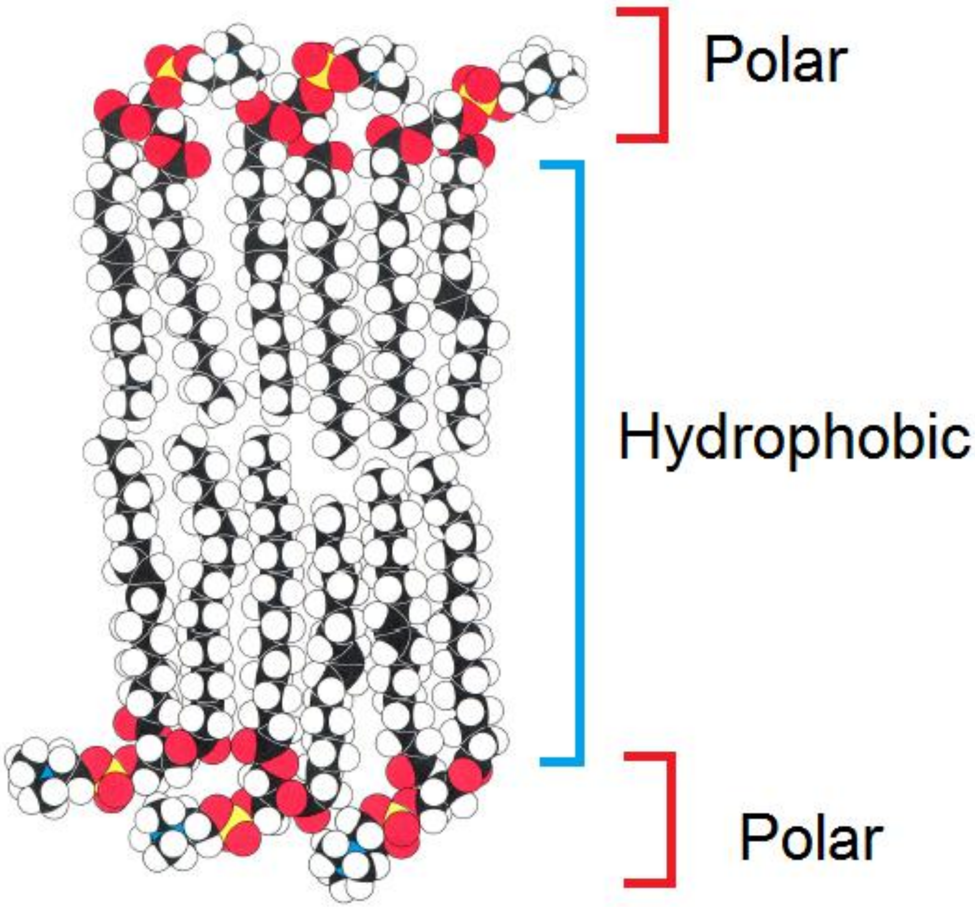
d) RNase A cleaves single stranded RNA through the use of a hydrophobic active site.

Membrane phospholipid bilayer

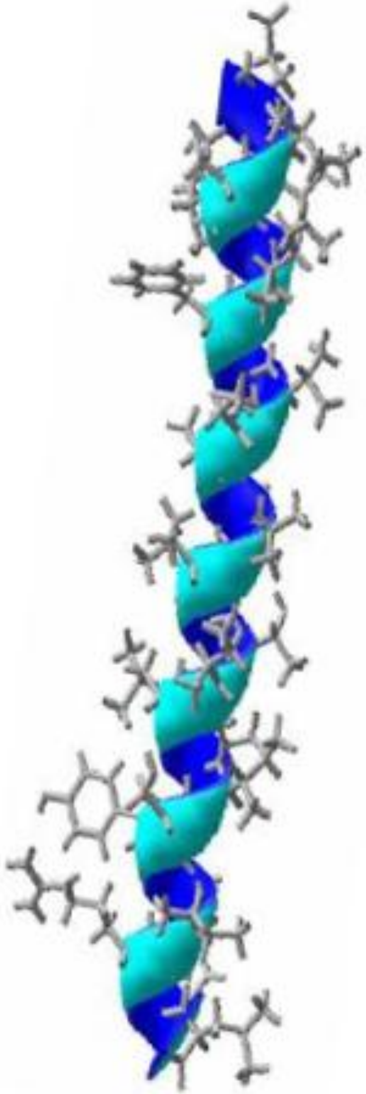
A typical bilayer is 30Å width

How will this affect folding?

Can this prevent molecules from passing in/out of the cell?



Membrane proteins



Composed of alpha helical segments (why not beta sheets?)

Membrane proteins are the link to the outside world!

Function in nutrient transport and signal transduction

Largely composed of hydrophobic amino acids (~80%)

Approximately 20% polar amino acids.

What are the likely roles of polar amino acids in the membrane?

Question

Approximately how many amino acids does an alpha helical TM require to span a typical membrane?

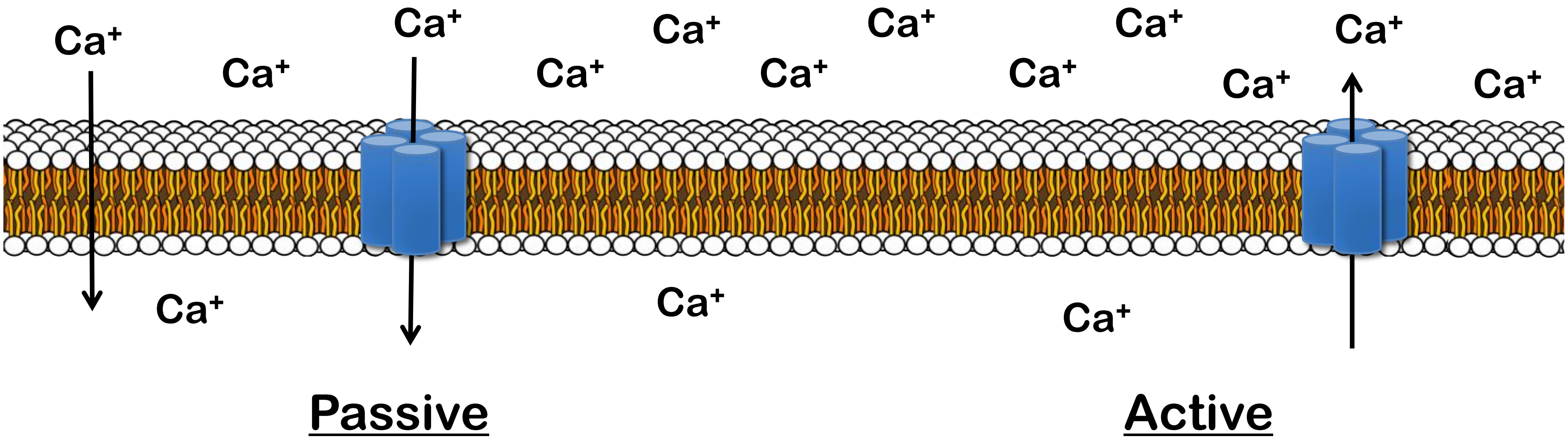
[✓ A)] 15

B) 47

C) 5

[✓ D)] 20

Types of Transporters



- Does not require energy!
- Substances move along a concentration gradient.
- Equilibrate gradients across the membrane of the substance they transport.
- Ex: Glucose Transporter

- Requires energy! (i.e. ATP)
- Substances move against a concentration gradient.
- Transport is coupled with a conformational change.
- Ex: Sarcoplasmic reticulum Ca^{2+} ATPase

Question

The sarcoplasmic reticulum Ca^{2+} ATPase (example given in class) transports calcium across the sarcoplasmic reticulum....

a) Actively, against a concentration gradient using the power of ATP.

b) Actively, against a concentration gradient using the power from a proton gradient.

c) Passively, along a concentration gradient using the power of ATP

d) Passively, along a concentration gradient

Signal Transduction: Receptors

Transmission of extracellular signals (no substance transportation!)

Link between the environment and the inner cell.

Activated by ligand binding which causes an intracellular signal transmission via conformational change (oligomerization, dissociation, phosphorylation)

Single spanning and multi-spanning

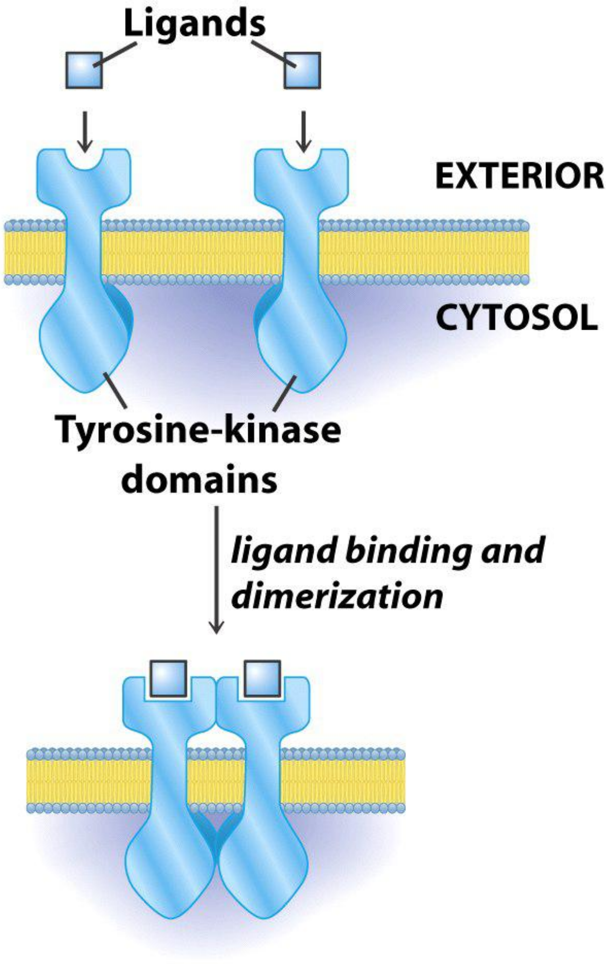


Figure 9-46 part 1 Principles of Biochemistry, 4/e
© 2006 Pearson Prentice Hall, Inc.

Question

Which of the following peptide sequences would likely be found in the membrane?

a) **K-G-H-L-P-G-A-I-L-E-D**
b) **G-I-L-M-S-I-V-A-V-F-Y-Q**
c) **H-P-K-D-T-S-W-E-C-G-L**
d) **G-S-G-A-G-A-G-S-G-A-G**

Which sequence could form a disulfide bond?

Does any sequence here look familiar….

Take away Points

Driving forces involved in protein stabilization

- Hydrophobic effect, Ionic interactions, Hydrogen bonds, Van der Waals

Proteins have four structural hierarchies

- Primary, Secondary, Tertiary and Quaternary
- Can describe the entire protein structure using the dihedral angles

Types of defining characteristic's of proteins classes

- Fibrous, globular, membrane, transport