Biology of Cells & Tissues

Lecture 2: Protein Structure & Function I

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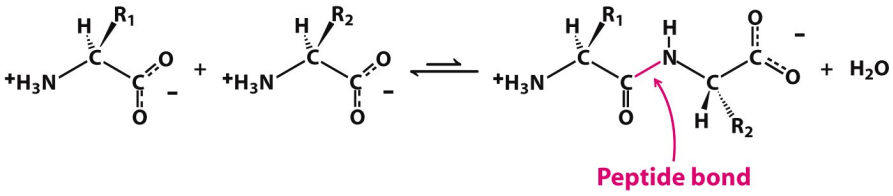
Lecture Plan

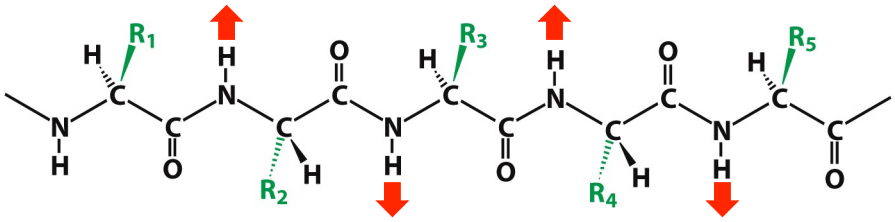
1. Hierarchy of protein structure
2. Elements of primary structure
3. Three types of secondary structure
4. Protein tertiary and quaternary structure

Proteins

The peptide bond is an amide bond between the α-carboxyl of one amino acid and the α-amino of another

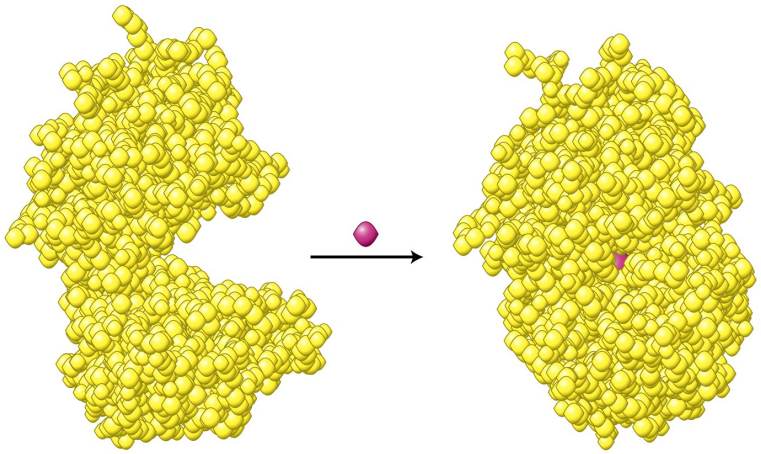


Note the alternation of side chain and peptide bond orientation



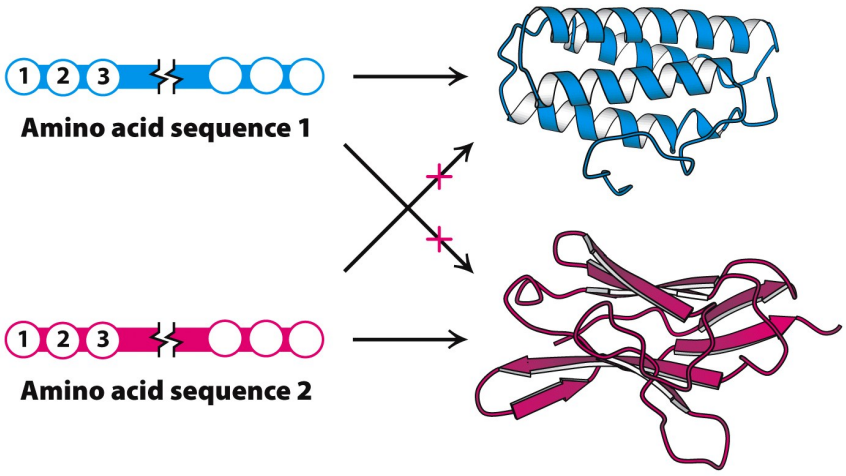
Protein structure

Conformational change is important for the function of many proteins



Protein structure = function

Amino acid sequence dictates protein structure



Hierarchy of protein structure

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4. Quaternary structure: the non-covalent association of multiple protein subunits into a larger, multimeric complex

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   b. Disulfides
   c. Proteolytic cleavages
   d. Post-translational modifications

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Note that the potential variation in protein primary structure is unlimited.

Consider variation in sequence alone: 70 aa polypeptide

Hierarchy of protein structure

2. Secondary structure: polypeptide strand structures dictated by hydrogen bonding between the carbonyl-O and amido-H of peptide bonds
   a. α-helix
   b. β-sheet (parallel and anti-parallel)
   c. β-turn

In contrast to primary structure, there are only a few types of secondary structure.

Hierarchy of protein structure

3. Tertiary structure: the association and arrangement of secondary structural elements into a larger, three dimensional architecture
   a. Stabilized by non-covalent interactions as well as disulfide bonds between amino acid side chains
   b. Hydrophobic side chains typically buried in the interior of the protein
   c. Charged and hydrophilic moieties are solvent-exposed

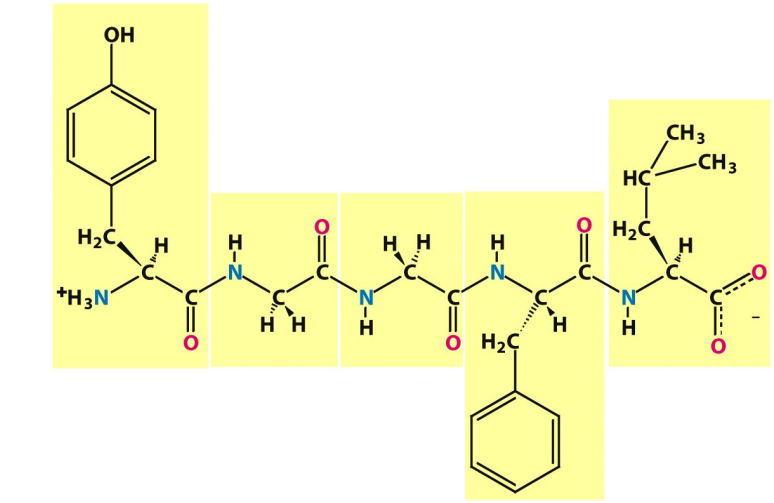
Note that there is also enormous variability in tertiary structure.

Hierarchy of protein structure

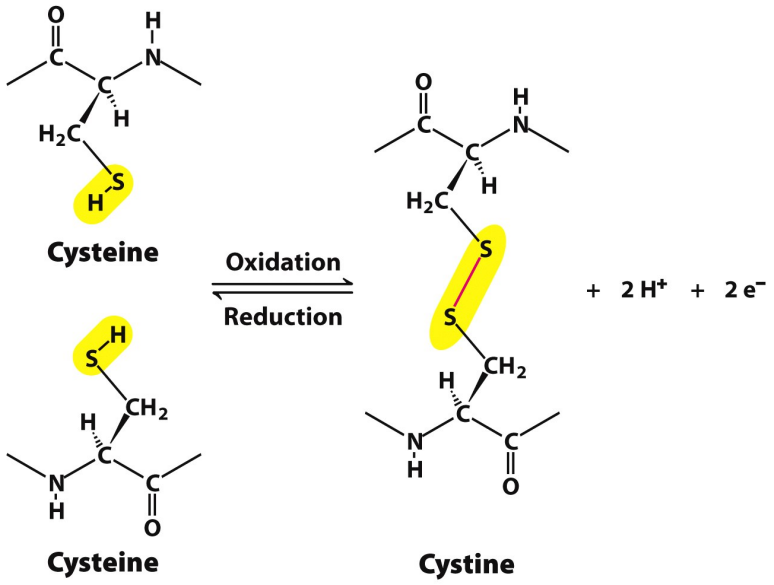
4. Quaternary structure: the non-covalent association of multiple protein subunits into a larger, multimeric complex
   a. Subunits can be identical or different
   b. Required for cooperativity and allosteric regulation
   c. Complexes can range in size from relatively small (hemoglobin) to enormous (ribosome, proteasome, virus capsid)
   d. Many proteins have NO quaternary structure (single polypeptide chains)

Primary structure

Primary structure: Amino acid sequence

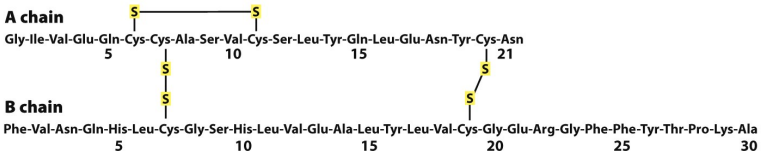


Primary structure: Disulfide bonds



Primary structure: Proteolytic cleavages

Sequence, disulfide-bonding, and two chain primary structure of insulin



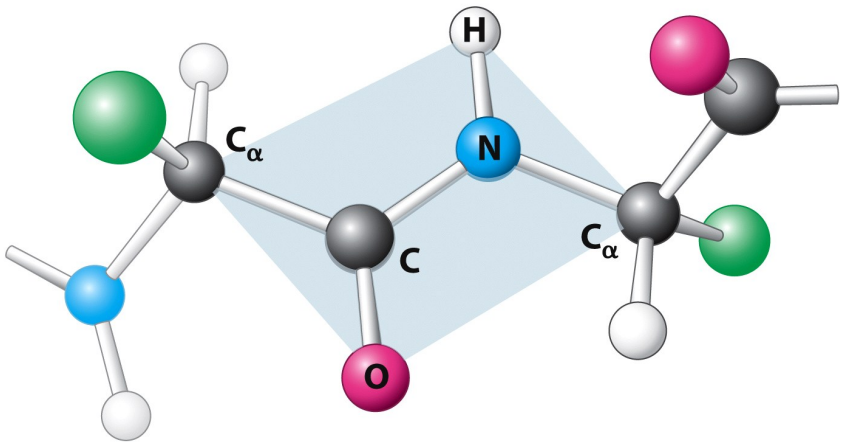
preproinsulin $\xrightarrow[\text{peptidase}]{\text{signal}}$ proinsulin $\xrightarrow[\text{convertase}]{\text{prohormone}}$ $\xrightarrow[\text{peptidase}]{\text{carboxy-}}$ insulin

Primary structure: Post-translational modifications

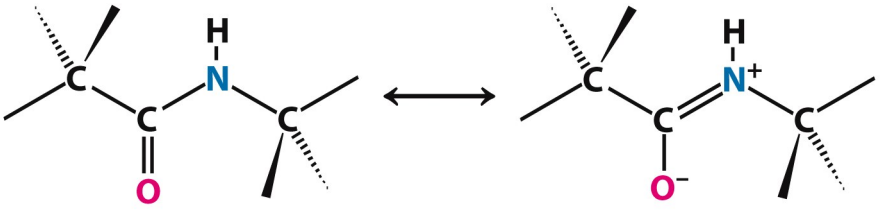
1. More than 17 unique modifications known
2. Modifications to N- and C-termini and to side chains
3. Many functions including protection, destruction, localization, and activity regulation
4. Major significance:
   a. Phosphorylation of S, T, Y
   b. Ubiquitination of K
   c. Glycosylation of S, T, N
   d. Acetylation of K

Secondary structure

Secondary structure: the peptide bond is rigid and planar...

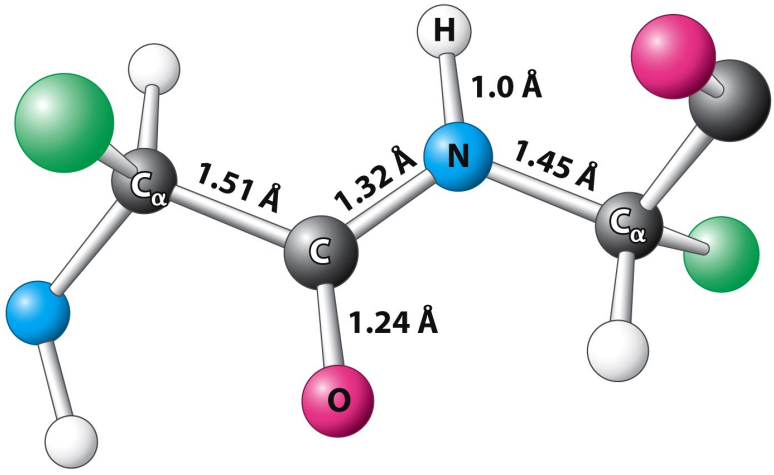


...because of peptide bond resonance...

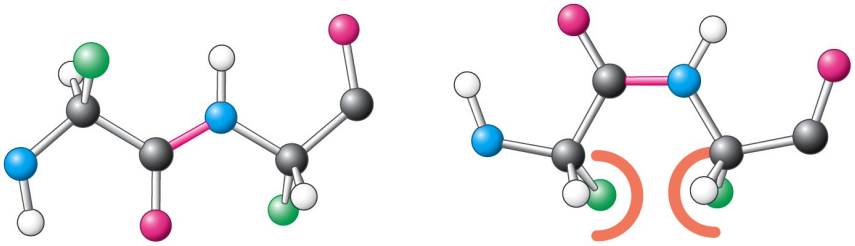


Peptide-bond resonance structures

...that shortens the C-N bond.

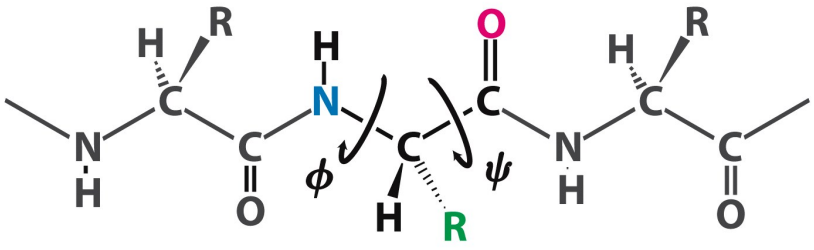


Secondary structure: Cis configuration of the peptide bond is prohibited



Trans Cis

Secondary structure: steric hindrance also limits rotation of α-carbon bonds



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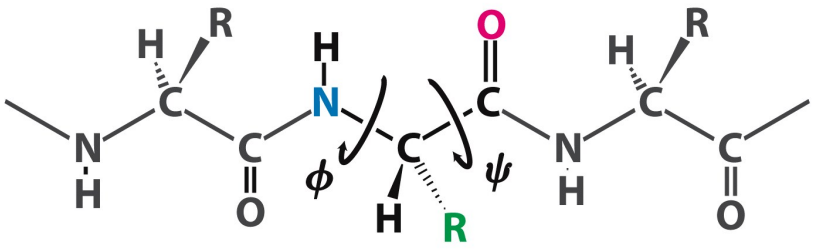
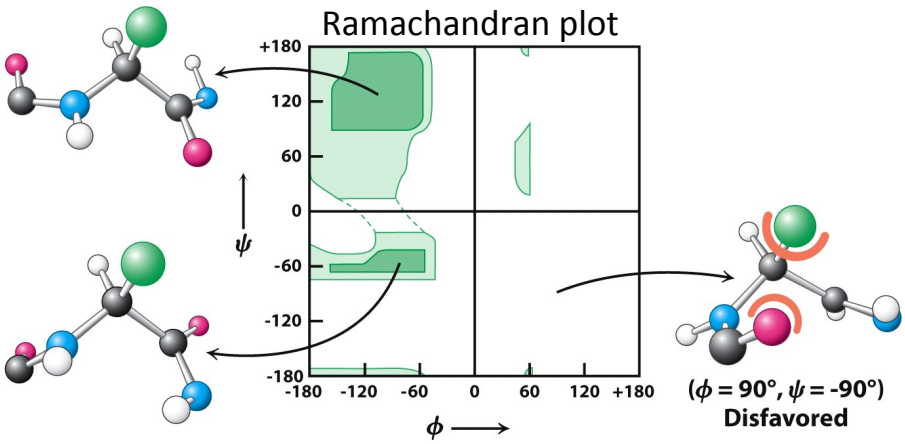
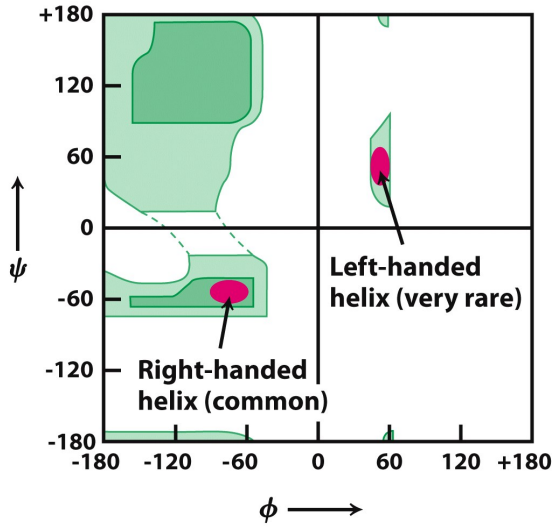


It is the limitation in allowed orientations resulting from rotation of α-carbon bonds that limits the number of possible secondary structures.

Secondary structure: steric hindrance also limits rotation of α-carbon bonds



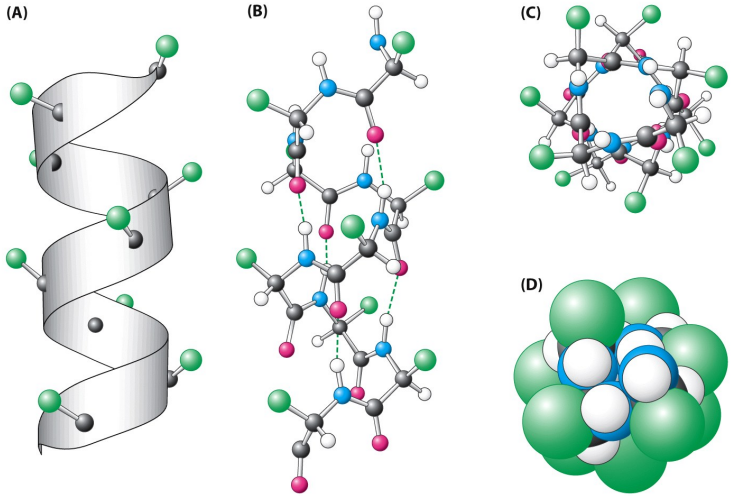
Secondary structure: the α-helix



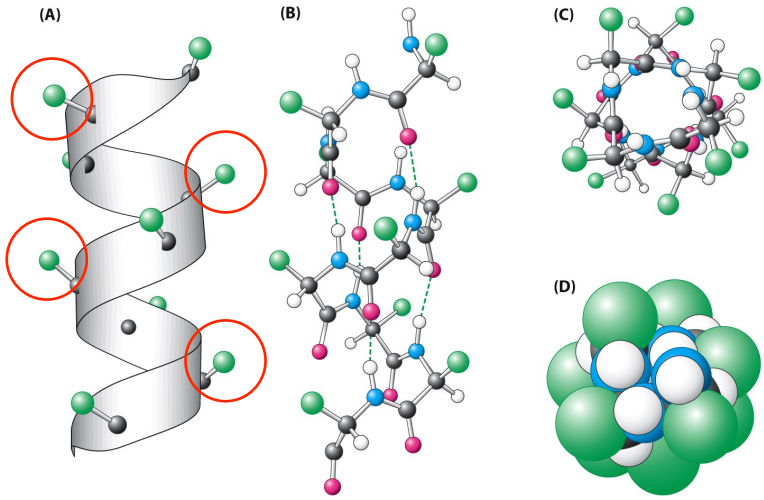
The α-helix is a right-handed helix.

The pitch of the helix is 5.4 Å with 3.6 amino acids per turn.

Secondary structure: the α-helix

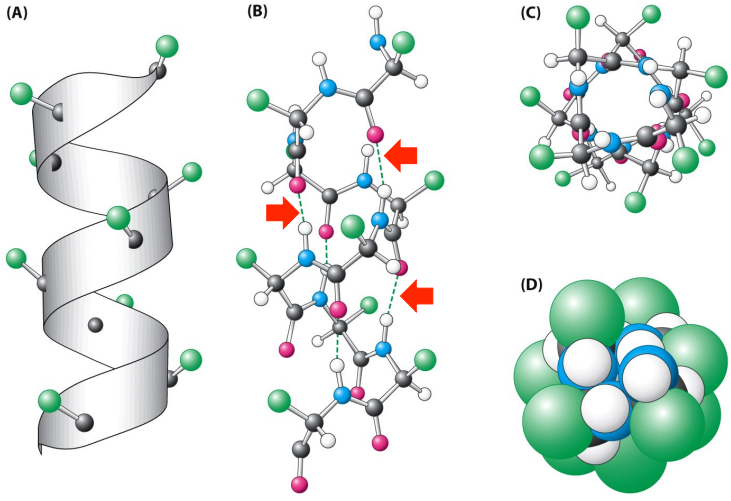


Secondary structure: the α-helix



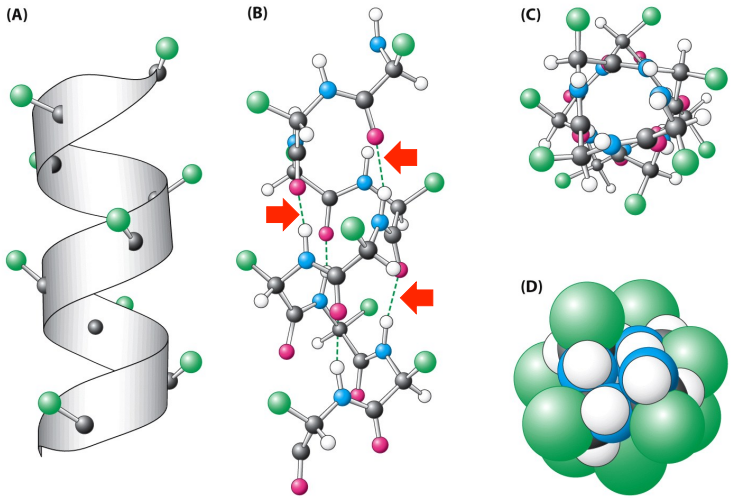
Side chains extend away from the axis of the helix...

Secondary structure: the α-helix



..and H-bonds run roughly parallel to the axis of the helix...

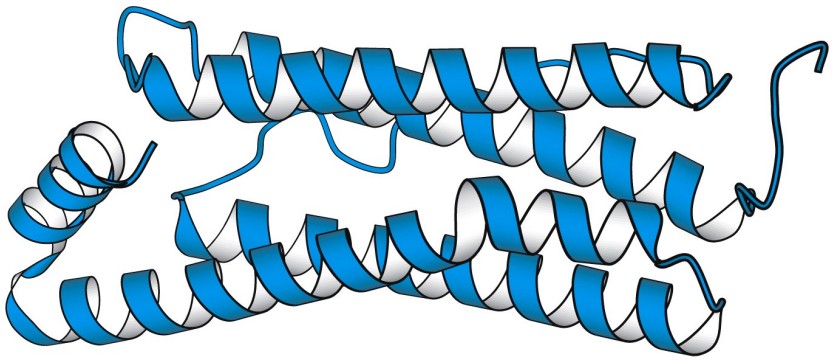
Secondary structure: the α-helix



... consistent with the i + 4 rule.

Secondary structure: the α-helix

An α-helix can have a "kink"...



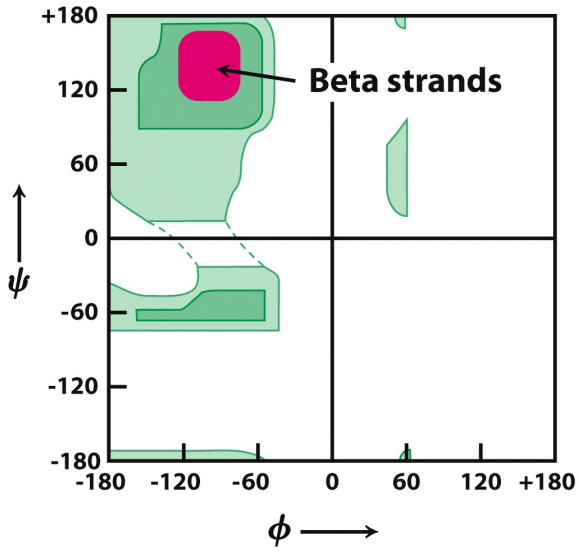
...and several helices can bundle together to produce a **tertiary structure**.

Question

If a lipid bilayer is about 30 Å thick, how many contiguous hydrophobic amino acids would be required to form a membrane-spanning α-helix?

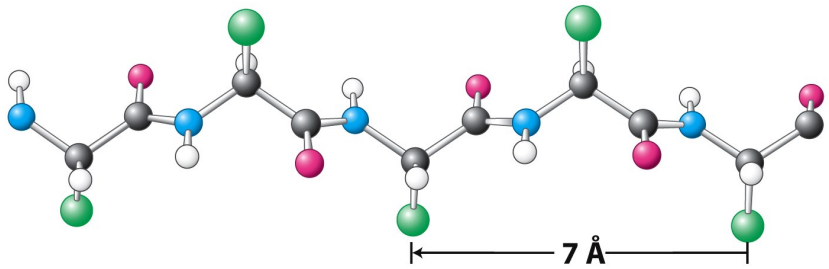
A. 5
B. 10
C. 15
D. 20
E. α-helices cannot span lipid bilayers

Secondary structure: β-sheets



Secondary structure: β-sheets

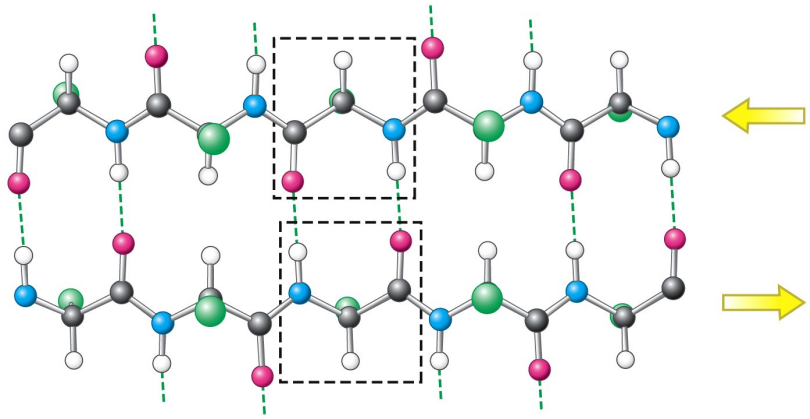
The β-strand has an extended configuration, with the peptide bonds all lying roughly in a single plane, ...



...and with the side chains oriented so they extend above and below the plane.

Secondary structure: β-sheets

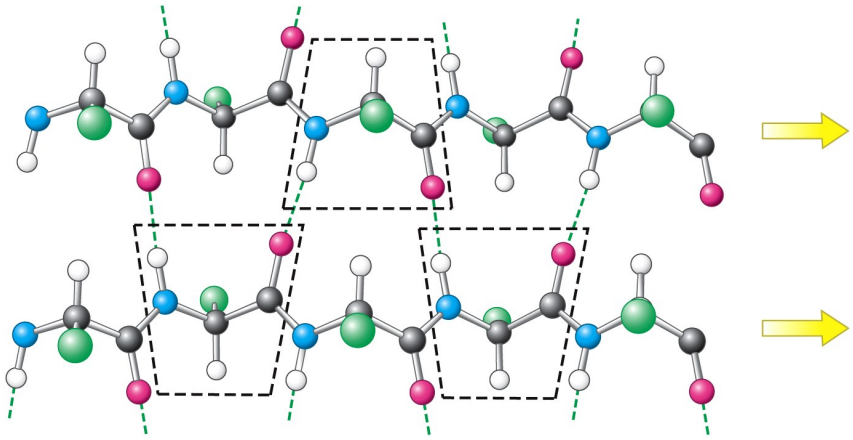
Antiparallel strands



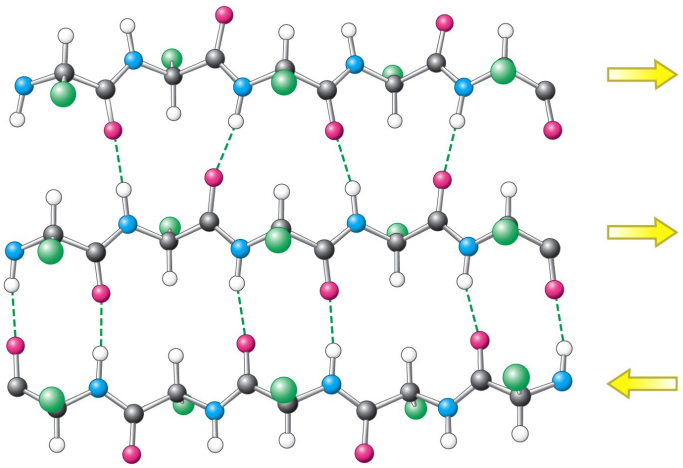
Note that in β-sheets the H-bond acceptor is always in a different strand than the donor (inter-strand bonds).

Secondary structure: β-sheets

Parallel strands

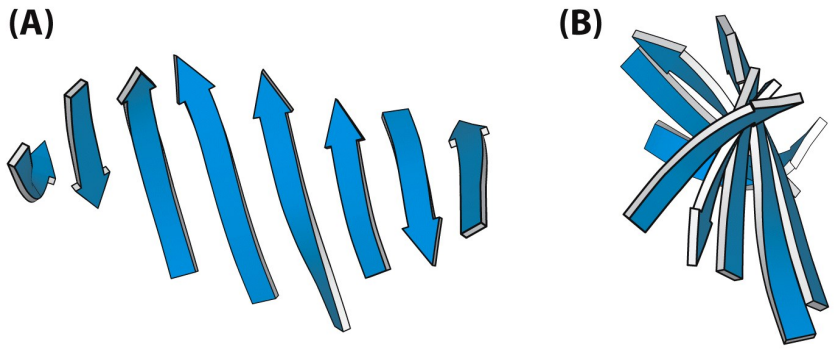


Secondary structure: β-sheets



β-sheets can include both antiparallel and parallel strands...

Secondary structure: β-sheets



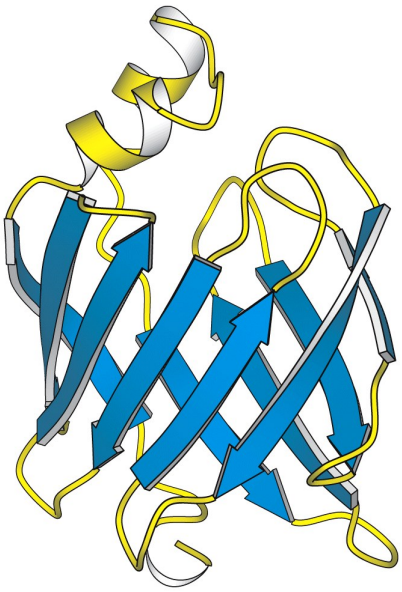
Front view

End view

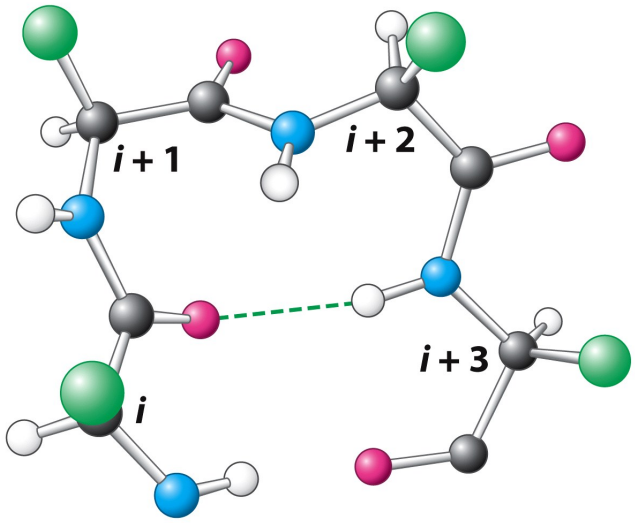
...and can twist and turn...

Secondary structure: β-sheets

...for example to form a β barrel, a **tertiary structure**.

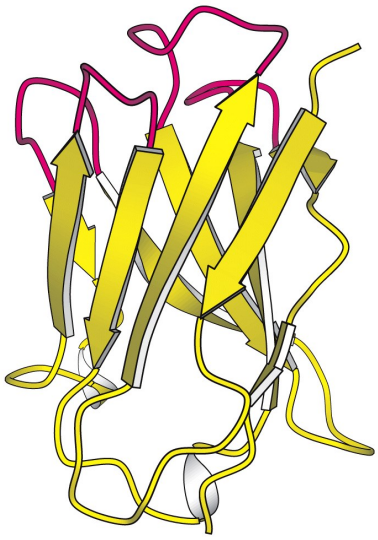


Secondary structure: the β-turn



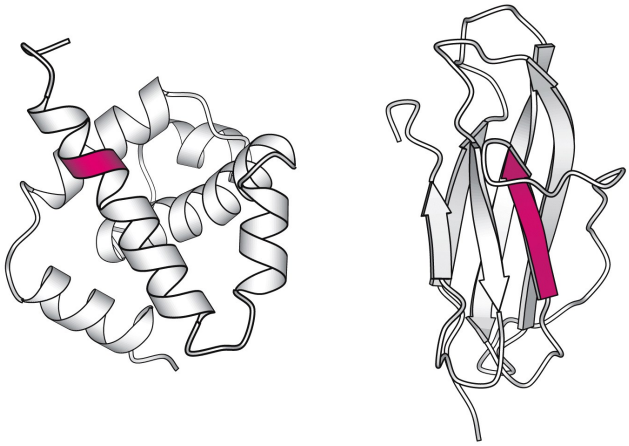
Secondary structure: the β-turn

β-turns often join β-strands in sheets or α-helices in bundles, again to form a **tertiary structure**.



What determines the secondary structure a polypeptide will form?

Short sequences can form different secondary structures...



The peptide **VDLLKN** in an α-helix of one protein and in a β-strand of another

...and there is only a modest preference for certain amino acids in secondary structures.

Amino acid	α helix	β sheet	Reverse turn
Glu	1.59	0.52	1.01
Ala	1.41	0.72	0.82
Leu	1.34	1.22	0.57
Met	1.30	1.14	0.52
Gln	1.27	0.98	0.84
Lys	1.23	0.69	1.07
Arg	1.21	0.84	0.90
His	1.05	0.80	0.81
Val	0.90	1.87	0.41
Ile	1.09	1.67	0.47
Tyr	0.74	1.45	0.76
Cys	0.66	1.40	0.54
Trp	1.02	1.35	0.65
Phe	1.16	1.33	0.59
Thr	0.76	1.17	0.96
Gly	0.43	0.58	1.77
Asn	0.76	0.48	1.34
Pro	0.34	0.31	1.32
Ser	0.57	0.96	1.22
Asp	0.99	0.39	1.24

Note: The amino acids are grouped according to their preference for α helices (top group), β sheets (middle group), or turns (bottom group).
Source: T. E. Creighton, *Proteins: Structures and Molecular Properties*, 2d ed. (W. H. Freeman and Company, 1992), p. 256.

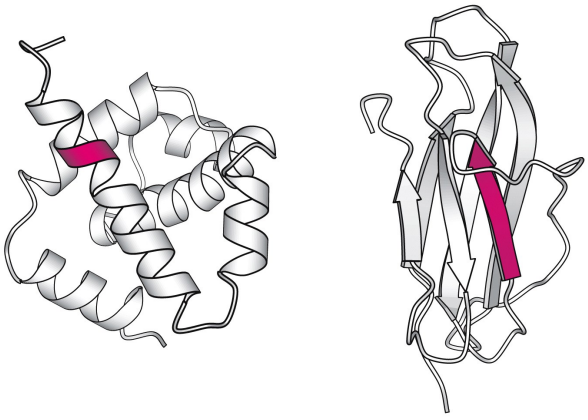
What determines the secondary structure a polypeptide will form?

Pro and Gly ("kinky" and "flexible") are most likely to be in β-turns, and unlikely to be in α-helices or β-sheets, but otherwise correlations are not very strong.

Algorithms for predicting secondary structure from sequence are therefore notoriously inaccurate.

Secondary structure paradox: sequence (i.e. order of side chains) dictates structure even though side chains themselves don't participate in the H-bonding that stabilizes secondary structure

What determines the secondary structure a polypeptide will form?



It is the full sequence of a polypeptide that specifies its native overall structure.

Tertiary structure

Tertiary structure

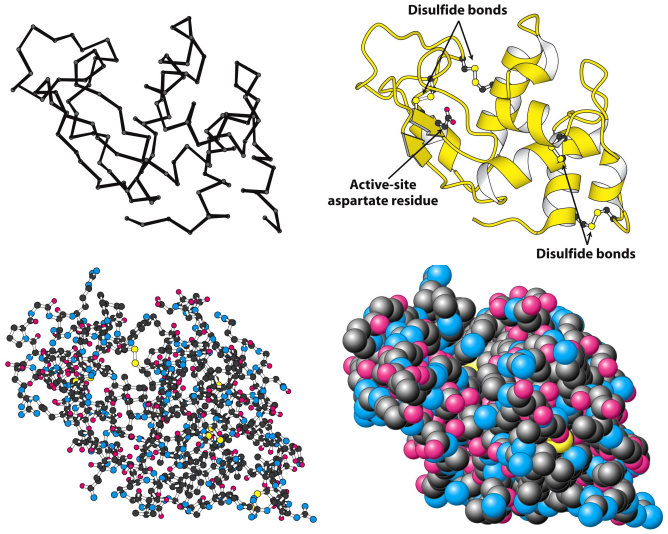
Proteins are generally either globular or fibrous, and composed of virtually any combination of secondary structures.

Globular proteins are generally not very elongated (i.e. they are shaped like a glob); most non-structural proteins, including enzymes, are globular.

Fibrous proteins have very elongated structures. They typically are major constituents of larger physical structures (e.g. keratin in hair and skin, collagen in connective tissue).

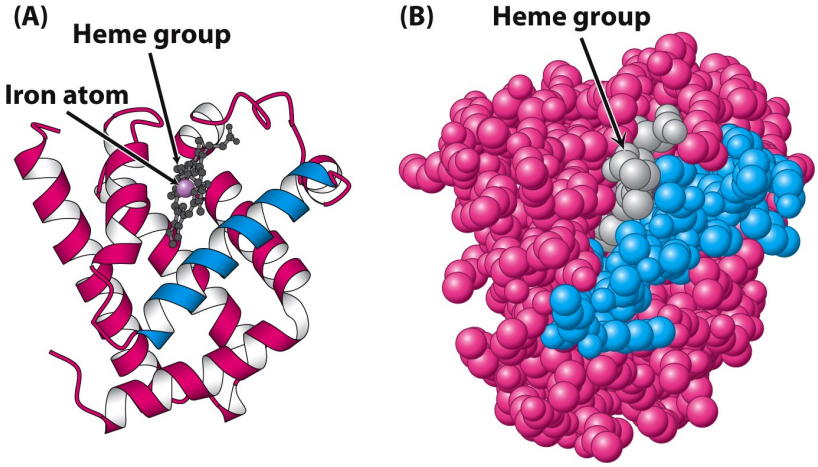
Tertiary structure: globular proteins

Four views of lysozyme



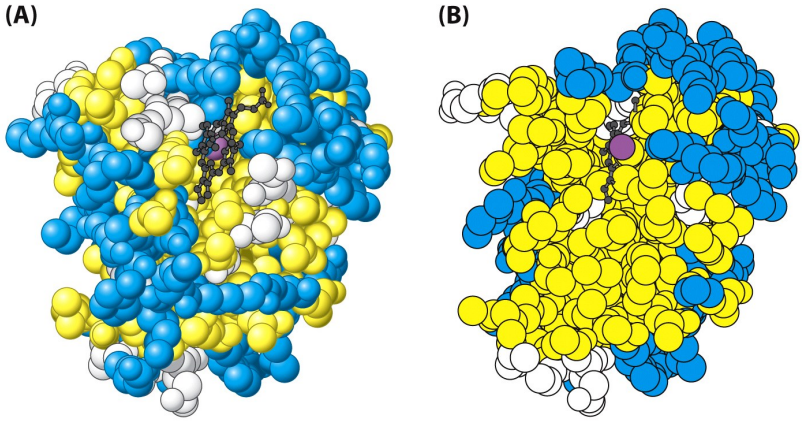
Tertiary structure: globular proteins

Myogobin is mostly α-helical, and it has a heme prosthetic group.



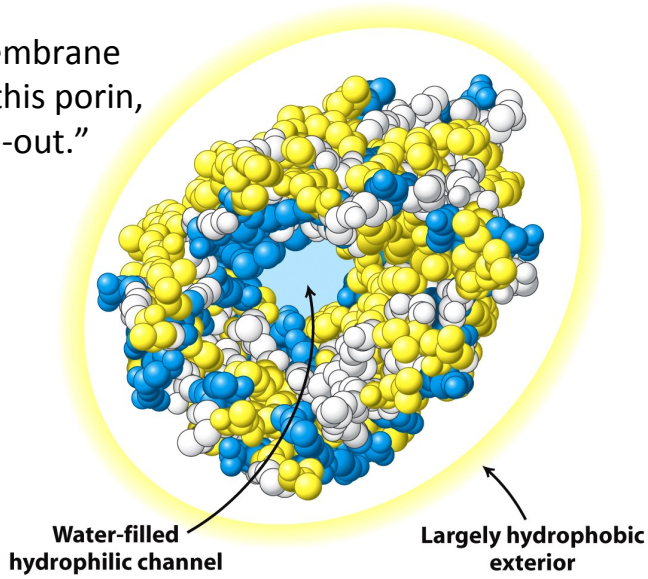
Tertiary structure: globular proteins

Hydrophilic side chains of globular proteins are generally solvent-accessible, whereas hydrophobic ones are buried in the protein's interior



Tertiary structure: globular proteins

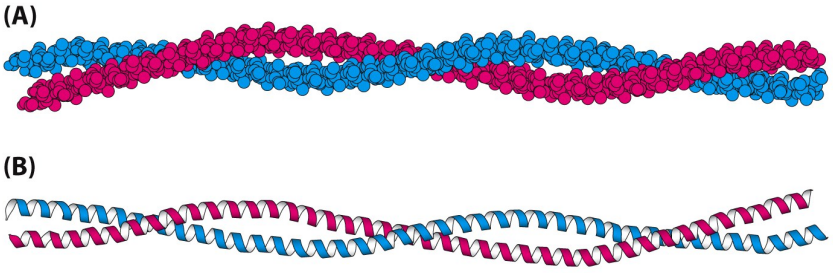
Some globular membrane proteins, such as this porin, are turned "inside-out."



Tertiary structure: fibrous proteins

The alpha helical coiled coil is a common tertiary structure in fibrous proteins.

e.g. keratin:



Defects in keratin proteins cause keratinopathies, such as epidermolysis bulbosa simplex (R125C mutation in K14)

Tertiary structure: fibrous proteins

In collagens, every third amino acid is glycine. Proline content is also high.

```
13
-Gly-Pro-Met-Gly-Pro-Ser-Gly-Pro-Arg-
22
-Gly-Leu-Hyp-Gly-Pro-Hyp-Gly-Ala-Hyp-
31
-Gly-Pro-Gln-Gly-Phe-Gln-Gly-Pro-Hyp-
40
-Gly-Glu-Hyp-Gly-Glu-Hyp-Gly-Ala-Ser-
49
-Gly-Pro-Met-Gly-Pro-Arg-Gly-Pro-Hyp-
58
-Gly-Pro-Hyp-Gly-Lys-Asn-Gly-Asp-Asp-
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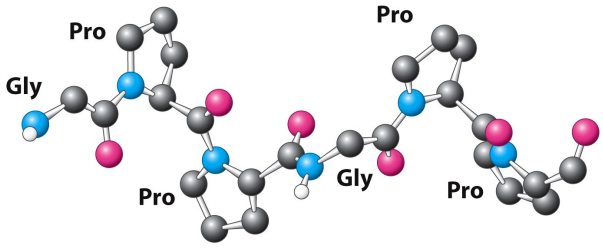
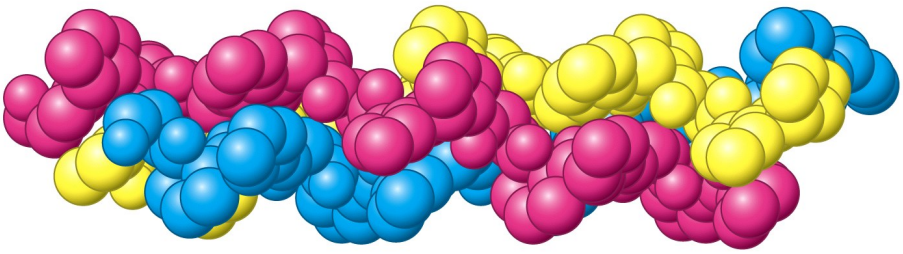


Tertiary structure: fibrous proteins

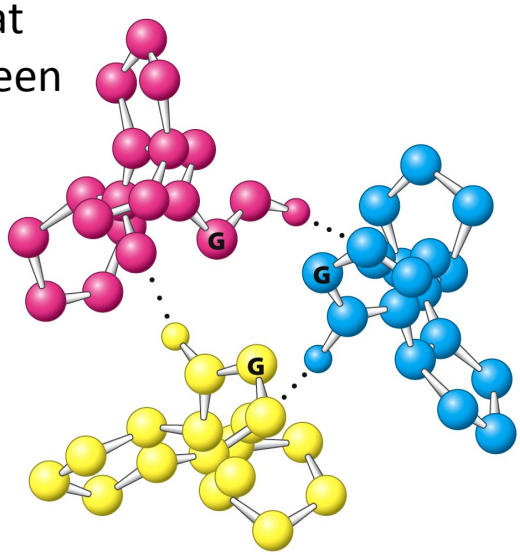
The abundant prolines impart a twist to the collagen strands...



...which intertwine to form a triple helix...

Tertiary structure: fibrous proteins

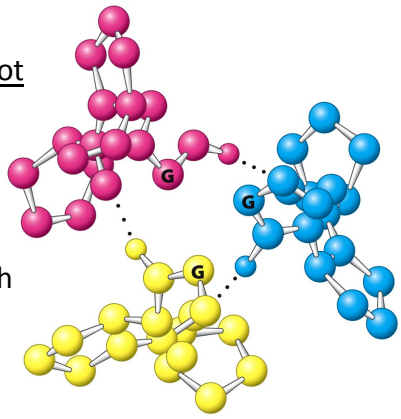
...with the glycines at the interfaces between the strands.



Tertiary structure: fibrous proteins

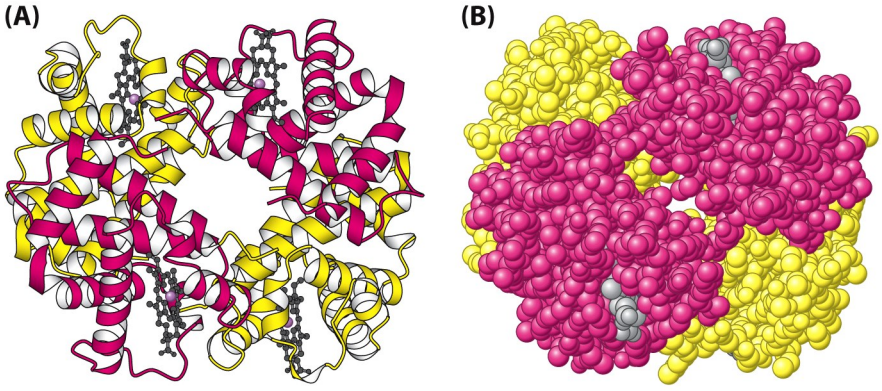
Note that the strands of collagen <u>are not α-helices</u>.

The twisting of the strands brings the glycines into the right orientation to pack tightly in the interior of the helix. There is no room for a side chain, which if present would disrupt the packing of the structure.



Mutations that change G to another amino acid cause the connective tissue disease **osteogenesis imperfecta**.

Quaternary structure: Hemoglobin



3HHB.pdb

Quaternary structure

Same forces as those that stabilize tertiary structure

Quaternary structures can be homomeric or heteromeric

Association of multiple subunits confers capacity for sophisticated regulation of activity (allosterism)

Association of enzymes catalyzing sequential reactions into multienzyme complexes accelerates flux of metabolites through pathways

Lecture 2 Recap

1. Protein primary structure—four elements
2. Protein secondary structure—three types
3. Protein tertiary and quaternary structure—interactions between secondary structures