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

- 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

Campbell, Biochemistry, 3e
Text Figure 04.01
How to determine the ORDER

- **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
- **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:
      - **Trypsin** – Cleaves on the C-terminal side of Lys and Arg residues
      - **Chymotrypsin** – Cleaves on the C-terminal side of Tyr, Phe, and Trp

- Chemical proteases also can cleave proteins
- **Cyanogen Bromide (CNBr)** – cleaves on the C-terminal side of Met
- Agents used to generate an overlapping set of peptides
- If the sequence of each peptide is determined, the entire protein sequence can be reassembled from the fragments

![Figure 3-17 Concepts in Biochemistry, 3/e](https://via.placeholder.com/150)
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
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
  - α-helix
  - β-sheet
  - 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
Factors that affect stability of an α-helix

- 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


Fig. 6-9 – Alpha 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!**
  o 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.
  o 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 beta-pleated sheet secondary structure are generally fibrous, such as silk, but pleated sheet is observed as a significant part of secondary structure in other proteins.
- Generally have rod-like shapes and are not so soluble in water.
- Unlike the α-helix, β-sheets can involve one or more polypeptide chains – interchain or intrachain interactions
- In β-sheets, peptide backbone is almost **completely extended**
- R-groups stick UP and DOWN from β-sheets
  o Usually small compact side chains like Gly, Ser, Ala
- Stabilized by Hydrogen bonds (near perpendicular to direction of peptide backbone)
  o Carbonyl of each amino acid is H-bonded to the NH of another amino acid
- Adjacent chains can be **PARALLEL** or **ANTI-PARALLEL**
- **Parallel β−sheet**: H-bonds between 2 chains running in the same direction
- **Anti-parallel β−sheet**: H-bonds between 2 chains running in opposite directions

**Parallel and Anti-Parallel β-sheets**
ANTI-PARALLEL BETA SHEET

PARALLEL BETA SHEET
Silk is made from a β-pleated sheet.

Gly-Ser-Gly-Ala-Gly-Ala
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
  o Cylinders or coils = α-helices
  o Arrows = β−sheets (direction shown by arrow)
  o Ribbons = bends, loops and random
**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

b: \( \beta \beta \) motif antiparallel

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

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

**Triose Phosphate Isomerase**

- a – side view; b – top view

Several \( \beta \alpha \beta \) motifs combine to form a **\( \beta \)-barrel** or **superbarrel** in this enzyme involved in glycolysis; Note helices, beta sheets and turns
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