Review I: Protein Structure

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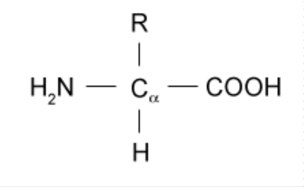
Amino Acids

- Building blocks of proteins
- 20 amino acids
- Linear chain of amino acids form a peptide/protein
- α–carbon = central carbon
- α–carbon = chiral carbon, i.e. mirror images: L and D isomers
- Only L isomers found in proteins
- General structure:

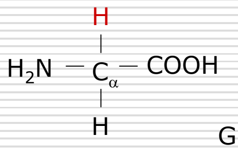


$$\mathrm{H_2N} - \overset{\displaystyle \mathrm{R}}{\underset{\displaystyle \mathrm{H}}{\mathrm{C}_\alpha}} - \mathrm{COOH}$$

(R = side chain)

Amino Acids (contd.)

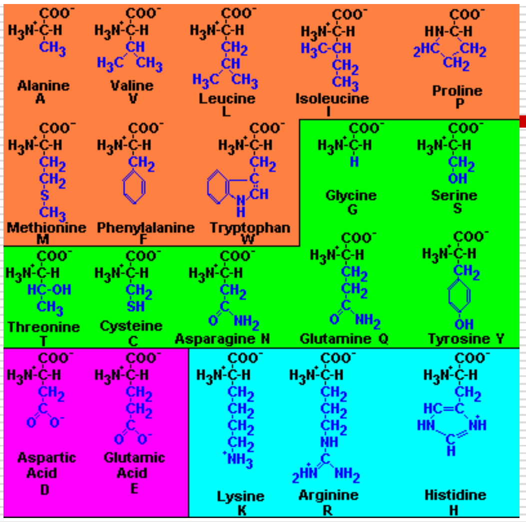
- R group varies
- Thus, can be classified based on R group
- Glycine: simplest amino acid
- Side chain R = H
- Unique because Gly α carbon is *achiral*



$$H_2N - \overset{H}{\underset{H}{C_{\alpha}}} - COOH$$

Glycine, Gly, G

Amino Acids: Structures



blue = R (side chain)

orange = non-polar, hydrophobic neutral (uncharged)

green = polar, hydrophillic, neutral (uncharged)

magenta = polar, hydrophillic, acidic (- charged)

light blue = polar, hydrophillic, basic (+ charged)

Amino Acids: Classification

Non-polar, hydrophobic, neutral (uncharged)

Alanine, Ala, A
Valine, Val, V
Leucine, Leu, L
Isoleucine, Ile, I
Proline, Pro, P
Methionine, Met, M
Phenylalanine, Phe, F
Tryptophan, Trp, W

Polar, hydrophillic, neutral (uncharged)

Glycine, Gly, G
Serine, Ser, S
Threonine, Thr, T
Cysteine, Cys, C
Asparagine, Asn, N
Glutamine, Gln, Q
Tyrosine, Tyr, Y

Polar, hydrophillic, Acidic (negatively charged)

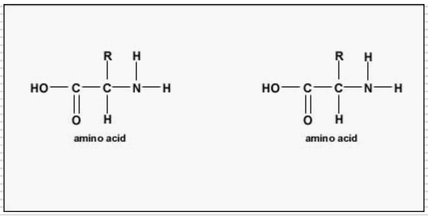
Aspartic acid, Asp, D
Glutamic acid, Glu, E

Polar, hydrophillic, basic (positively charged)

Lysine, Lys, K
Arginine, Arg, R
Histidine, His, H

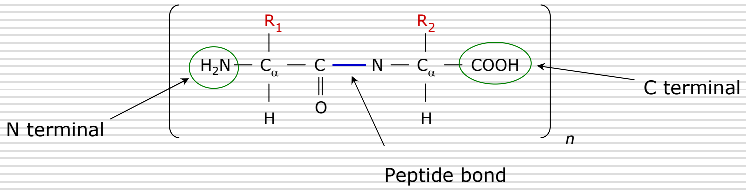
Peptide Bond Formation

- ❑ Condensation reaction
- ❑ Between –NH_2 of *n* residue and –COOH of *n+1* residue
- ❑ Loss of 1 water molecule
- ❑ Rigid, inflexible



Peptides/Proteins

- Linear arrangement of *n* amino acid residues linked by peptide bonds
- $n < 25$, generally termed a peptide
- $n > 25$, generally termed a protein
- Peptides have *directionality, i.e. N terminal ⟶ C-terminal*



$$\left[H_2N-\underset{H}{\overset{R_1}{C_\alpha}}-\underset{O}{\underset{\|}{C}}-N-\underset{H}{\overset{R_2}{C_\alpha}}-COOH\right]_n$$

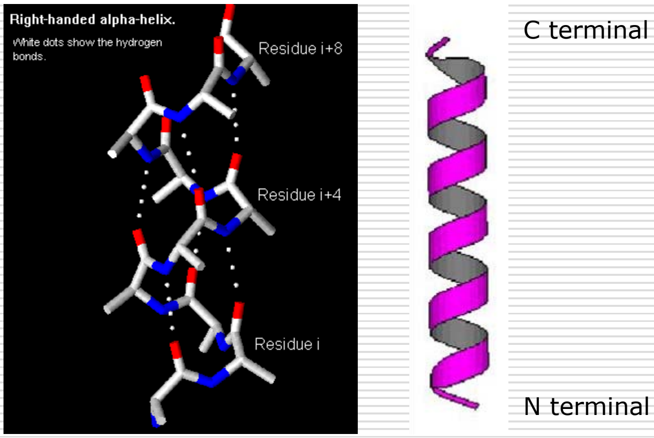
Hierarchy of Protein Structure

- Four levels of hierarchy
- Primary, secondary, tertiary, quarternary
- **Primary structure**: Linear sequence of residues
- e.g: MSNKLVLVLNCGSSSLKFAV …
- e.g: MCNTPTYCDLGKAAKDVFNK …
- **Secondary Structure**: Local conformation of the polypeptide backbone
- α-helix, β-strand (sheets), turns, other

Secondary Structure: α-helix

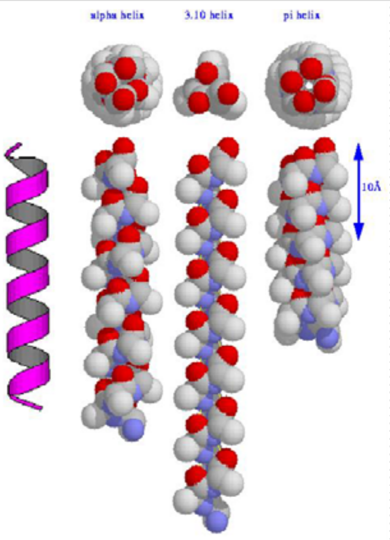
- Most abundant; ~35% of residues in a protein
- *Repetitive secondary structure*
- 3.6 residues per turn; pitch (rise per turn) = 5.4 Å
- C′=O of *i* forms H bonds with NH of residue *i+4*
- *Intra-strand* H bonding
- C′=O groups are parallel to the axis; side chains point away from the axis
- All NH and C′O are H-bonded, except first NH and last C′O
- Hence, polar ends; present at surfaces
- Amphipathic

α-helix (contd.)



α–helix Variations

- Chain is more loosely or tightly coiled
- 3_{10}-*helix*: very tightly packed
- *π–helix*: very loosely packed
- Both structures occur rarely
- Occur only at the ends or as single turns



Hemoglobin (PDB 1A3N)

β–sheets

- Other major structural element
- Basic unit is a β-strand
- Usually 5-10 residues
- Can be parallel or anti-parallel based on the relative directions of interacting β-strands
- “Pleated” appearance

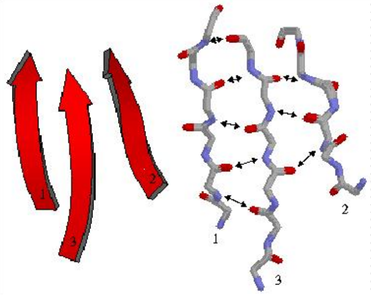
β–sheets

- ***Unlike* α-helices:**
- Are formed with different parts of the sequence
- H-bonding is *inter-strand* (opposed to intra-strand)
- Side chains from adjacent residues are on opposite sides of the sheet and do not interact with one another

- ***Like* α-helices:**
- Repeating secondary structure (2 residues per turn)
- Can be amphipathic

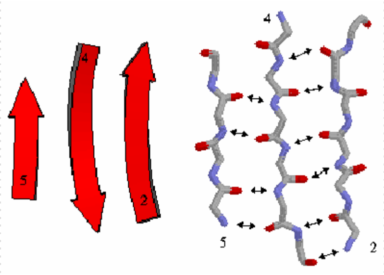
Parallel β–sheets

- The aligned amino acids in the β-strand all run in the same biochemical direction, N- to C-terminal

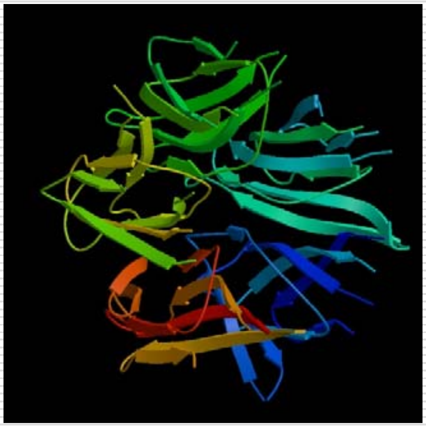


Anti-parallel β–sheets

- The amino acids in successive strands have alternating directions, N-terminal to C-terminal



Nucleoplasmin (PDB 1K5J)



Amino Acid Preferences (1)

- **α-helix forming**
- The amino acid side chain should *cover and protect the backbone H-bonds* in the core of the helix
- Ala, Leu, Met, Glu, Arg, Lys: good helix formers
- Pro, Gly, Tyr, Ser: very poor helix formers

- **β-strand forming**
- Amino acids with large bulky side chains prefer to form β-sheet structures
- Tyr, Trp, Ile, Val, Thr, Cys, Phe

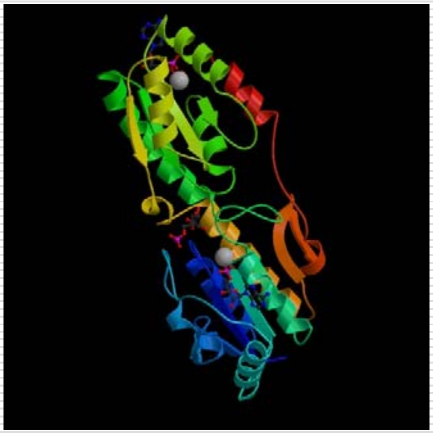
Amino Acid Preferences (2)

- **Secondary structure disruptors**
- Gly: side chain too small
- Pro: side chain linked to α-N, has no N-H to H-bond; rigid structure due to ring
- Asp, Asn, Ser: H-bonding side chains compete directly with backbone H-bonds

Turns/Loops

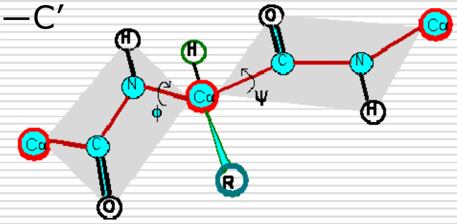
- Third "classical" secondary structure
- Reverses the direction of the polypeptide chain
- Located primarily on protein surface
- Contain polar and charged residues
- Three types: I, II, III

Phosphofructokinase (PDB 4PFK)



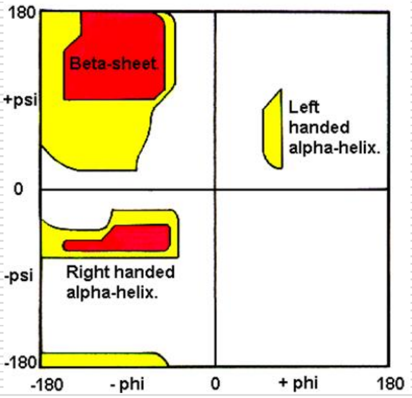
The Torsional Angles: ϕ and ψ

- Each amino acid in a peptide has two degrees of backbone freedom
- These are defined by the ϕ and ψ angles
- ϕ = angle between C_α—N
- ψ = angle between C_α—C′



The Ramachandran Plot

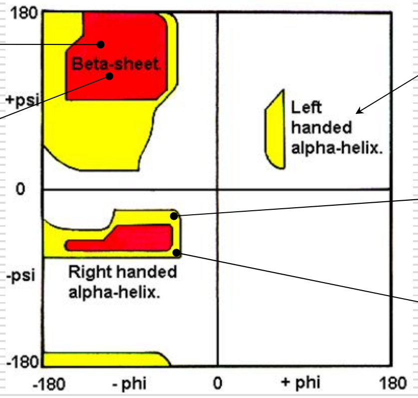
- Plot of allowable ϕ and ψ angles
- ϕ and ψ refer to rotations of two rigid peptide units around C_α
- Most combinations produce steric collisions
- Disallowed regions generally involve steric hindrance between side chain C_β methylene group and main chain atoms



The Ramachandran Plot (contd.)

Anti-parallel β-sheet

Parallel β-sheet



Theoretically possible; energetically unstable

3_{10}-helix

π-helix

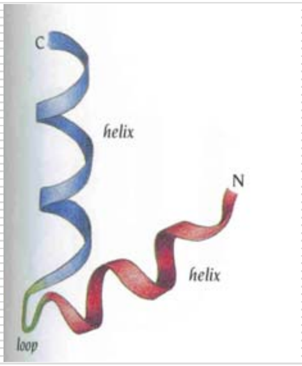
- White: sterically disallowed (except Gly)
- Red: no steric clashes
- Yellow: “allowable” steric clashes

Protein Motifs

- (Simple) combinations of secondary structure elements with a specific geometric arrangement
- "Super-secondary structures"
- Can be associated with a specific function, e.g. DNA binding, or metal ion binding
- Can be part of a larger functional and/or structural assembly ("domain")

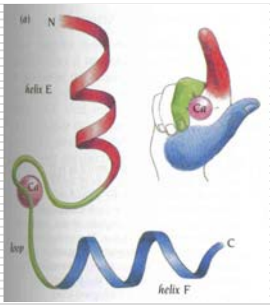
Helix-Turn-Helix (HTH)

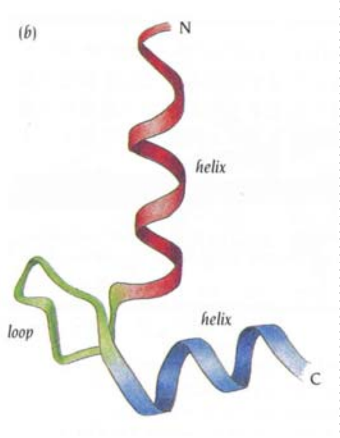
- Simplest α-helix motif: 2 α helices joined by a loop
- Also called *Helix-Loop-Helix*, *HLH*
- Common structural motif for DNA binding proteins
- One helix recognizes specific sequence of nucleotides and fits into the groove in the DNA double helix; the other stabilizes the bound configuration



EF Hand

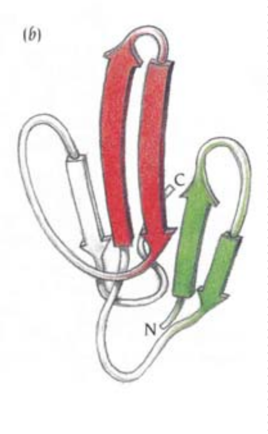
- ❑ Specific for several different calcium-binding proteins
- ❑ E.g. calmodulin, Troponin-C





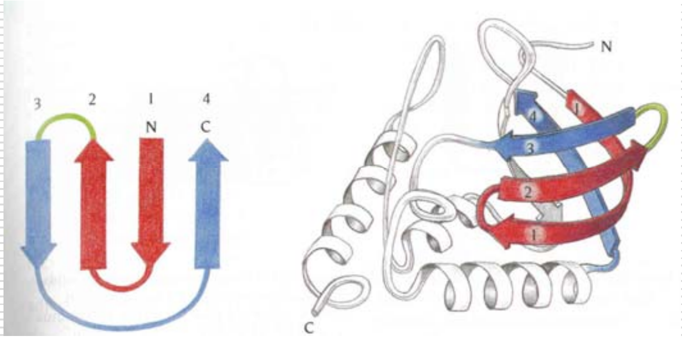
Hairpin β-motif

- ❑ Also called *β-hairpin*
- ❑ 2 adjacent anti-parallel strands joined by a loop



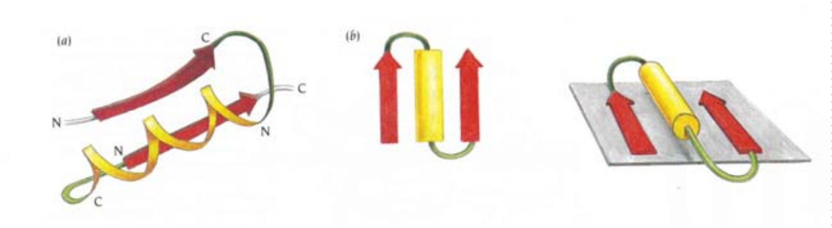
Greek Key motif

- 4 adjacent anti-parallel β-strands



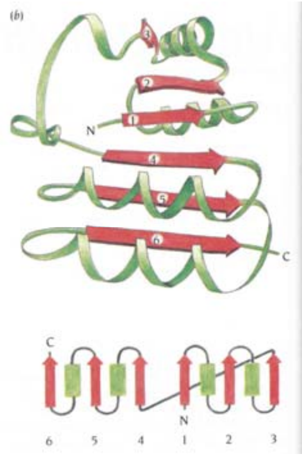
β-α-β Fold

- Parallel β-strands connect by an α helix
- Found in most proteins with parallel β strands. E.g. Triose phosphate isomerase



Rossman Fold

- Unique example of a β–α–β fold
- 3 parallel β-sheets with 2 linking α helices
- Often seen in nucleotide-binding proteins



Domains

- ✓ Primary structure
- ✓ Secondary structure
- ✓ Super-secondary structure
- *Domains*
- Fundamental unit of tertiary structure
- (Part of a) polypeptide chain that can fold independently into a stable tertiary structure
- E.g. catalytic domain of protein kinase; binding pocket of a ligand

3D Structure of a Protein Kinase Domain

N-terminal

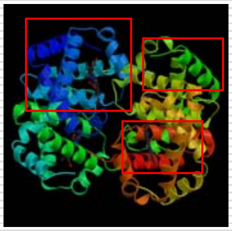
C-terminal

phosphate binding loop

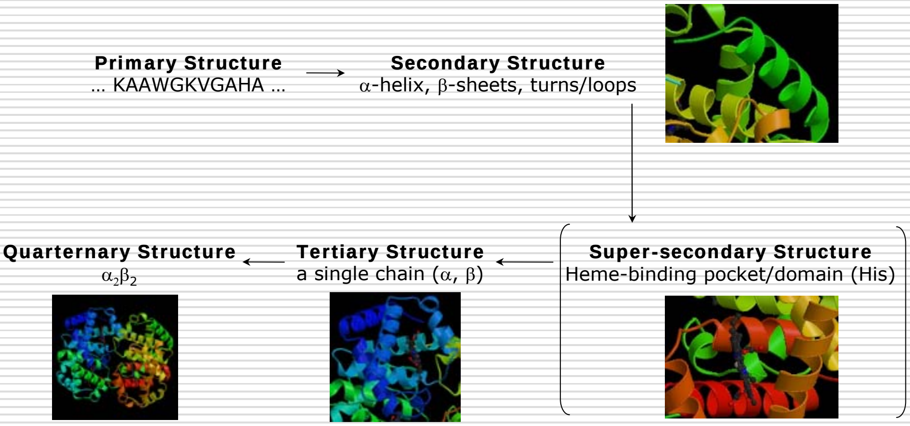
catalytic loop

Quarternary Structure

- Spatial organization of subunits to form functional protein
- E.g. Hemoglobin
- 2 α chains, 2 β chains
- Each chain binds heme (Fe)
- Forms an $\alpha_2\beta_2$ tetramer



Putting it all together



Primary Structure
… KAAWGKVGAHA …

→

Secondary Structure
α-helix, β-sheets, turns/loops

↓

Super-secondary Structure
Heme-binding pocket/domain (His)

←

Tertiary Structure
a single chain (α, β)

←

Quarternary Structure
$\alpha_2\beta_2$

Additional Reading

- **General information**
- Biochemistry, 5th ed., Berg, Tymoczko, Stryer
- Biochemistry, 3rd ed., Voet & Voet

- **Detailed information**
- Proteins, 2nd ed., Creighton
- Introduction to Protein Structure, 2nd ed., Branden & Tooze

- **Internet**
- Images: Protein Data Bank (PDB): www.rcsb.org/pdb
- Numerous wesbites (Google *protein secondary structure)*