Ch. 6 - Three Dimensional structure of Proteins

Basic Themes & Principles:

(1) Protein conformation (3D structure) is described by secondary (2°), tertiary (3°) and quaternary (4°) structure.
   - Higher order levels determined by amino acid sequence (primary structure (1°))

(2) The function of a protein depends on its structure

(3) The most important forces stabilizing protein structure are noncovalent interactions

(4) Peptide bonds connect amino acid residues
   - N-C bonds have double bond character
   - Limits possible no. of conformations

(5) 2°: arrangement of amino acids in regular, recurring patterns.
   - α-helix, β-conformation, β-tum, collagen helix

(6) 3°: global 3D arrangement of polypeptide chain
   - Determined by 1°
   - Stabilized by weak, noncovalent interactions

(7) 4°: 3D arrangement of subunits in multi-subunit proteins
   - Stabilized by weak, noncovalent interactions

Protein conformation - any structural state achieved without breaking covalent (peptide) bonds

A protein’s conformation is usually the one that is most stable, thermodynamically

Conformation stabilized largely by weak interactions (hydrophobic, H-bonds, ionic, etc.)

Stability = tendency to maintain a native conformation

Native Proteins are only marginally stable

Folded vs. Unfolded states:

ΔG = 20 - 65 kJ/mole
   - Recall, H-bonds: 5-20 kJ/mole
   - Folded vs. Unfolded states differ by the equivalent of ~ 3-5 H-bonds
Polypeptide chains can assume countless different conformations (Conformational entropy)

Conformational entropy and H-bonding with water drives polypeptides toward unfolded state

How do proteins maintain a folded state?

Every H-bonding group within protein was H-bonded to water prior to folding

Answer: Look back at H-bonding properties of H₂O

- Pure H₂O: Network of H-bonded H₂O
- No molecule has H₂O’s H-bonding potential
  - Solutes (even hydrophilic) disrupt H-bonding capacity to some extent
  - Solvation layers - ordered shells around solutes

Concentration of nonpolar groups in proteins decreases solvation layer

Result: Favorable increase in Entropy (S)

Formation of H-bonds & ionic interactions driven largely by same entropic effect

- Polar groups on protein form H-bonds w/H₂O
  - Number of H-bonds/unit mass always greater for pure H₂O
- “Structure” introduced (solvation shell, ↓S)
- Energetic “gain” from formation of weak, intramolecular bonds cancelled out by elimination of such interactions w/H₂O

Protein folding releases “structured” H₂O - provides entropic driving force for folding

Net change in free energy between unfolded and folded states derived from increased entropy in surrounding aqueous solution

(elimination of solvation shells)
Polar or charged groups in protein interior must have bonding partners.

Presence of such groups without partners too destabilizing - conformation untenable.

Protein structure:
- Hydrophobic residues are buried
- H-bonds within proteins are maximized
  - H-bond formation is cooperative
- Interior H-bonding or ionic groups are paired with bonding partners.

Protein structure:

The Peptide bond

Covalent bonds forming polypeptide backbone.

The peptide bond has partial double-bond characteristics

The peptide bond has partial double-bond characteristics:

The carbonyl oxygen has a partial negative charge and the amido nitrogen a partial positive charge, setting up a small electric dipole. Virtually all peptide-bonds in proteins occur in this trans configuration, an exception is noted in Figure 6.8.

Theoretical, $N-C_\alpha(\phi) = C_\alpha-C(\Psi) = 180^\circ$

The peptide bond has partial double-bond characteristics:

Theorically, $N-C_\alpha(\phi) = C_\alpha-C(\Psi) = 180^\circ$

Limits ability of peptide group to rotate - limiting possible protein conformations.

Theorically, $N-C_\alpha(\phi) = C_\alpha-C(\Psi) = 180^\circ$

Many values for $\phi$ and $\Psi$ prohibited by steric hindrance (R groups, peptide backbone).
**Ramachandran plot - permissible bond angles for N-Cα, Cα-C bonds**

- Dark Blue - fully allowed
- Med. Blue - unfavorable
- Lt. Blue - unlikely

**Protein 2° - local conformation within polypeptide**

- Common patterns: i) α-helix,
  - ii) β-conformations (sheet, turn)

**α-helix: simple**

- Arrangement for rigid peptide bonds
  - Backbone wrapped around an imaginary axis
  - Amino acid side chains project out
  - Repeating unit = 1 turn (360°) of helix
  - 1 turn extends 5.4 angstroms along axis
  - 3.6 residues/turn
  - Helical twist is “right-handed”
  - 1/4 of all amino acid residues in α-helices

**α-helices make optimal use of H-bonds**

- H-bonds between every 4th amino acid residue
- Each turn of the helix is stabilized by 3-4 H-bonds

**Destabilizing forces**

- Blocks of (+) or (-) charges (repulsion)
  - Glu, Lys, Arg, etc. side chains
- Large, bulky side chains in near proximity
  - Asn, Ser, Thr, Leu
- Constraint of Pro or Gly residues
  - N in proline ring rigid, no H-bond, makes kinks
  - Gly - small, takes up unique conformation

**αα sequence affects α-helix stability**

- Interaction between αα side chains can stabilize or destabilize helix structure
Stabilizing Forces for $\alpha$-helices
- $(+/\pm)$ ionic interactions 3-4 residues apart
  - Ex: Asp (-)/Lys (+)
- Hydrophobic interactions 3-4 residues apart
  - Ex: Phe/Trp
- Interactions of $\alpha\alpha$'s near terminal ends of helix

$\alpha$-helices exhibit polarity
- Electric dipoles in each peptide bond
- Connected via H-bonding
- Net dipole extends down helix
- 4 $\alpha\alpha$'s near terminal ends don’t fully participate in H-bonding
- Creates partial $(+)$ and $(-)$ charge at N- and C-terminal ends of helix
- $(+)$ or $(-)$ side chains near ends bond w/dipoles and stabilize helix

$\beta$-conformation organizes polypeptide chains into sheets
- Backbone in “zig-zag” vs. helical structure
- zig-zag chains arranged side by side (sheets)
  - H-bonds form between adjacent chains
  - R-groups protrude from sheets in opposite directions
- Chains can be parallel or anti-parallel
  - Same or opposite N- and C- termini
- Favors small side chains

$\beta$-turns
- Link successive runs of $\alpha$-helix or $\beta$-sheet
- 180° turns
- Consist of 4 $\alpha$ residues
  - Frequently Gly or Pro
  - Small and flexible
- Often near protein surface
- Form “turn-turn” H-bonds plus H-bonds w/solvent
2° has characteristic bond angles and αα content

α-helices and β-sheets can be described by φ and Ψ at each residue.

If bond angles are allowed for a given αα, it can be found in such 2°

Ex: φ and Ψ for the αα’s in pyruvate kinase

Some αα’s are found more frequently in specific types of 2°

Ex: Pro & Gly (β-turns), Glu & Met (α-helices)

Tertiary (3°) & Quaternary (4°) Structure

Tertiary (3°) Structure
- 3D arrangement of all atoms in a protein
- Includes long range aspects of αα sequence
  - Interactions between atoms in different sections of 2°
- Segments of polypeptide chains held in 3° position by weak bonding interactions and covalent disulfide bonds (-S-S-)

Quaternary (4°) Structure
- Arrangement of separate polypeptide subunits in multisubunit proteins
2 major protein groups: Fibrous & Globular

**Fibrous Proteins**: “Strands” or “Sheets”
- **Structurally distinct**:
  - Fibrous: 1 type of 2°
  - Globular: Several types of 2°
- **Functionally distinct**:
  - Fibrous: Support, shape, external protection
  - Globular: enzymes, regulatory proteins

**Globular Proteins**
- Variety of 2°
  - Combination of α-helices, β-helices and turns
- Polypeptide chains fold back on each other
  - Provides structural diversity
  - Hydrophobic residues buried
  - Hydrophilic residues exposed or paired
- Include enzymes, transport proteins, regulatory proteins, immunoglobulins, etc.

**Fibrous Proteins**

- High conc. of hydrophobic residues (F, W, I)
- Insoluble in Water
- Often associate to form supramolecular complexes (hair, wool, etc.)

Fibrous proteins: α-Keratin
- Hair, wool, nails, horns, hooves, etc.
  - Evolved for tensile strength
- Structure: right-handed α-helix
- α-helices form coiled coils
  - Supertwisting amplifies strength (“rope”)
- Surfaces where 2 helices coil made up of hydrophobic residues - interlocking pattern
- Coiled coils enhanced by covalent X-links
  - Disulfide bonds, non-standard α α’s

Fibrous Proteins: Collagen
- Function = Tensile strength (> steel wire!)
- Tendons, cartilage, cornea
- Unique 2°: Collagen helix
  - Left-handed α-helix
  - 3 vs. 3.6 α α’s / turn
  - Coiled coils from 3 supertwisted chains
- Unique αα composition
  - High levels of Gly, Ala, Pro
- Chains X-linked by covalent bonds (His, Lys)

Globular Proteins
- Variety of 2°
- Polypeptide chains fold back on each other
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- Include enzymes, transport proteins, regulatory proteins, immunoglobulins, etc.

Protein interiors are densely packed
- Liquid packing density 0.4 - 0.6
- Crystals 0.7-0.8
- Proteins ~0.75

Tight packing reinforces weak interactions
van der Waals forces become significant
Small proteins have lower surface:volume ratios - fewer buried residues

Often stabilized by covalent bonds: disulfides (-S-S-), links to prosthetic groups

Fig. 6.18: ribosome

Supersecondary structure & Domains

An intermediate stage of structure ("motifs", "folds", "Supersecondary structure") are stable arrangements of 2° and their connections

Examples: β-α-β loops
- α-α corner
- α/β Barrel

Large proteins (several hundred residues) often fold into two or more stable, globular domains

Domains often have distinct functions:
- Catalysis
- Regulation of Catalytic activity
- Binding of ligands

2° and Supersecondary Structure: Folding Rules:
- Burial of hydrophobic R groups to exclude H₂O
  - 2 layers of 2°
- α-helices and β-sheets generally found in different layers (H-bonding problems)
- Protein segment adjacent in 1° usually stacked together in folded structure
- Connections b/w segments of 2° can’t cross or form knots
- β-conformation most stable when twisted slightly in right-hand sense

Protein Families: Proteins with significant 1°, structural or functional similarity

Usually indicates a strong evolutionary relationship

Quaternary (4°) Structure

"Multimers" ( > 2 subunits) Advantages:
- Binding effects - sum greater than parts
- Synergy b/w subunits (Hemoglobin)
- Functional differences among subunits
- Catalytic vs. Regulatory roles
- Structural enhancement
  - Coiled coils, etc. - increase tensile strength
- Multi-step catalytic reactions
  - "Assembly line biosynthesis" (Fatty acids)
Most multimers have identical subunits, or repeating groups of nonidentical subunits

Repeating unit (single or group) = Protomer

Multimer example = Hemoglobin

- 4 protein subunits: 2 α chains, 2 β chains
- 4 Heme groups
- Structural subunit: αβ protomer

Rotational & Helical Symmetry

Subunits can be superimposed by rotation about 1 or more axes

- Cyclic symmetry: single axis
  - \( C_n; n = \) no. subunits
- Dihedral symmetry: 3D symmetry (x,y,z axes)
- Helical symmetry: symmetry about axis
  - Viral capsids

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Limits to Protein Size

- Genetic coding capacity
  - Use of many smaller polypeptides vs. 1 gigantic protein conserves genomic space (ex: viruses)
- Accuracy of Protein Biosynthesis
  - Error frequency = 1 mistake/10,000 residues
  - Implications for protein stability, catalysis, etc.
  - Probability of introducing errors increases with size

Protein Folding & Denaturation

Polypeptides must fold during and after synthesis to native conformation

- Loss of native conformation (denaturation) = loss of function
- Does not require complete unfolding
- Unfolding induced w/mild treatment
  - pH, heat, organic solvents, etc.
Folding like a cooperative process

Formation of 2° in one part enhances formation in another

Fig 6.28

Amino Acid sequence (1°) determines 3°

Classic example: Denaturation & Refolding of ribonuclease

Conclusion: Folding not a “trial and error” process

Models for protein folding:
(1) Cooperative folding
1° → local 2° → super 2° → 3°

(2) Molten Globule:
Hydrophobic “Collapse” followed by interaction among non-polar residues

Actual process probably incorporates features of both models

Some Proteins Undergo Assisted Folding

Assisted by the action of specialized proteins:
- Molecular Chaperones
- Chaperonins
- Isomerase enzymes
  - Rearrangement of disulfide bonds

The Folding Process as a Free Energy Funnel

High conformational entropy →

Fully folded, native structure

Partially folded states are energetically favorable and promote advancement to fully folded state

Chaperones & Chaperonins bind to unfolded regions and prevent inappropriate aggregation

Fig 6.30
Chapter 6 - Summary

1) Protein structure is stabilized by multiple weak interactions
   - Hydrophobic interactions major contributions
   - H-bonds & ionic interactions optimized in most stable structure
   - Dense packing in protein interior allows for significant van der Waals interactions

Summary (contd.)

2) Nature of peptide bond places constraints on structure
   - Exhibits partial double-bond characteristics
   - Keeps peptide group in rigid planar config
   - Rotation about N-Cα, Cα-C specified by Φ & Ψ
   - 2° defined completely if all Φ and Ψ known (Ramachandran plots)

Summary (contd.)

3) 3 major types of 2°:
   - α-helix
   - β-conformation (sheets)
   - β-turns
α-helix and β-conformation characterized by optimal H-bonding b/w peptide bonds in protein backbone

Summary (contd.)

4) Stable segments of 2° are variably called supersecondary structure, motifs or folds

5) 3°, the complete 3D structure of a polypeptide chain, is the association of secondary structure

6) In very large proteins, stable and independently folding regions are called domains
   - Often have discrete functions (catalysis, regulatory)

Summary (contd.)

7) 2 general classes of proteins:
   - Fibrous:
     - Primarily structural roles (skin, hair, nails, etc.)
     - Single type of 2° predominates
     - Often combine to form superstructures
   - Globular:
     - Enzymes, transporters, regulatory proteins, etc.
     - Several types of 2°
     - Often multimers arranged as symmetric associations of subunits

Summary (contd.)

8) Quaternary structure (4°):
   - Interactions b/w subunits of multimeric proteins
   - Consist of units of groups of different subunits (protomers)
   - Protomers usually related by rotational or helical symmetry

9) Amino Acid Sequence determines 3°
   - Proteins fold (probably) in a series of steps, along an energetically favorable pathway
Summary (contd.)

- Protein folding is cooperative; folding within localized regions promotes folding in other areas.

- Amino Acid sequence provides sufficient information for most proteins to fold correctly, including placement of disulfide bonds.

- Folding is assisted for some proteins by other proteins: molecular chaperones, chaperonins and isomerases (disulfide bond placement).