Ch. 6 - Three Dimensional structure of Proteins

Basic Themes & Principles:

(1) Protein conformation (3D structure) is described by secondary (2°), tertiary (3°) and quarternary (4°) structure.

- Higher order levels determined by amino acid sequence (primary structure (1°))

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

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(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-turn, collagen helix

2

(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

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

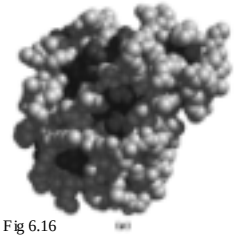

Fig 6.16

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

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

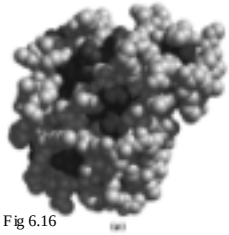

Fig 6.16

Stability = tendency to maintain a native conformation

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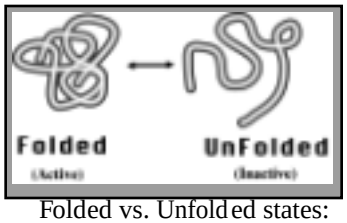
Native Proteins are only marginally stable



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 !

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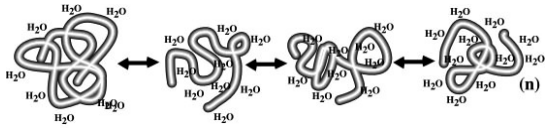
Polypeptide chains can assume countless different conformations (Conformational entropy)



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

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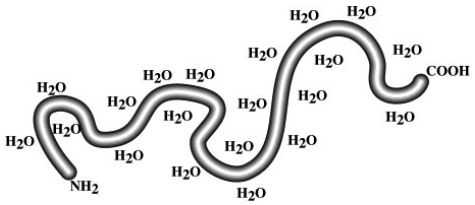
How do proteins maintain a folded state?



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

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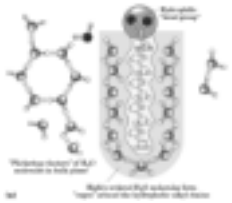
Answer:Look back at H-bonding properties of H_2O



↓S, ↑G

- Pure H_2O: Network of H-bonded H_2O
- No molecule has H_2O's H-bonding potential
  - Solutes (even hydrophilic) disrupt H-bonding capacity to some extent
  - Solvation layers - ordered shells around solutes

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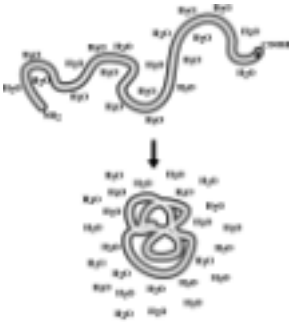
Clustering of nonpolar groups in proteins decreases solvation layer



Result: Favorable increase in Entropy (S)

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

- Polar groups on protein form H-bonds w/H_2O
  - Number of H-bonds/unit mass always greater for pure H_2O
- "Structure" introduced (solvation shell, ↓ S)
- Energetic "gain" from formation of weak, intramolecular bonds cancelled out by elimination of such interactions w/H_2O

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protein folding releases "structured" H_2O - provides entropic driving force for folding

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

(elimination of solvation shells)

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Polar or charged groups in protein interior must have bonding partners

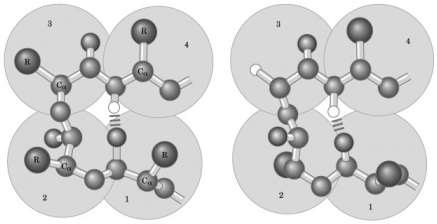

Fig 6.8

Presence of such groups without partners too destabilizing - conformation untenable

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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

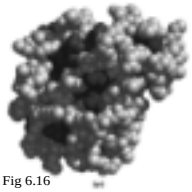

Fig 6.16

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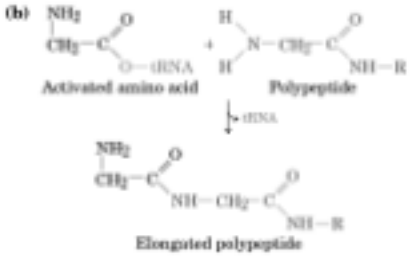
The Peptide bond



Covalent bonds forming polypeptide backbone

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The peptide bond has partial double-bond characteristics

The carbonyl oxygen has a partial negative charge and the amide 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 6–8b.

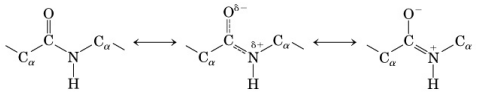

Fig 6.2

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C-N bond partial double-bond "fixes" each peptide group in a planar configuration

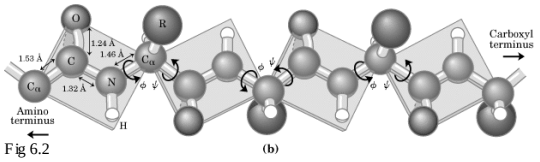

Fig 6.2

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

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Theoretically, N-C_α (ϕ) = C_α-C (Ψ) = 180°

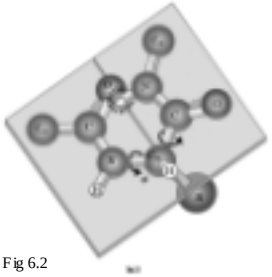

Fig 6.2

Many values for ϕ and Ψ prohibited by steric hindrance (R groups, peptide backbone)

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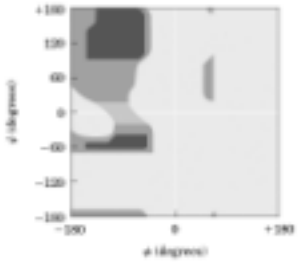
Ramachandran plot - permissible bond angles for N-C_α, C_α-C bonds



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

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Protein 2° - local conformation within polypeptide

Fig 6.16

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

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α-helix: simplest 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

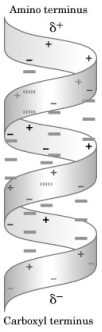

Fig 6.6

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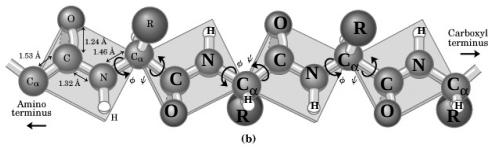
α-helices make optimal use of H-bonds



H-bonds between every 4 th amino acid residue

Each turn of the helix is stabilized by 3-4 H-bonds

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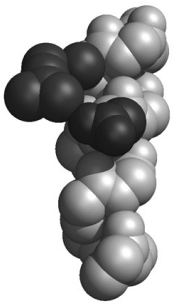
αα sequence affects α-helix stability



Interaction between αα side chains can stabilize or destabilize helix structure

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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

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Stabilizing Forces for α-helices

- (+/-) ionic interactions 3-4 residues apart
  - Ex: Asp (-)/Lys (+)
- Hydrophobic interactions 3-4 residues apart
  - Ex: Phe/ Trp
- Interactions of αα's near terminal ends of helix

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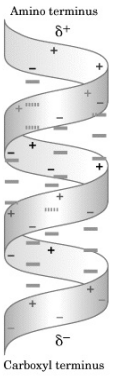
α-helices exhibit polarity

- Electric dipoles in each peptide bond
- Connected via H-bonding
- Net dipole extends down helix
- 4 αα'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



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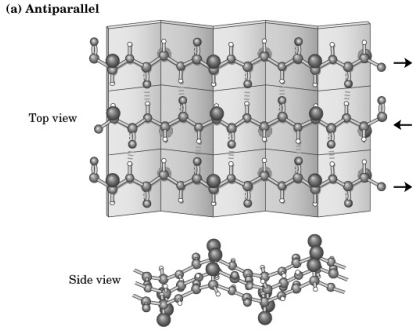
β-conformation organizes polpeptide chains into sheets



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β-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

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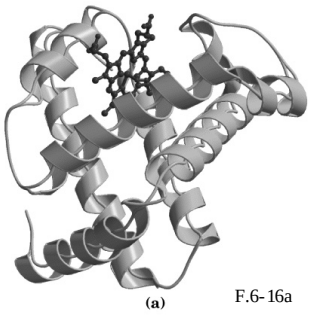
β-turns



F.6-16a

Polpeptide chains must often reverse direction

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β-turns

- Link successive runs of α-helix or β-sheet
- 180° turns
- Consist of 4 or 5 residues
  - Frequently Gly or Pro
  - Small and Flexible
- Often near protein surface
- Form "intra-turn" H-bonds plus H-bond w/ solvent

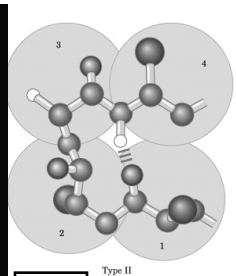

Fig. 6-8a

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2° has characteristic bond angles and αα content

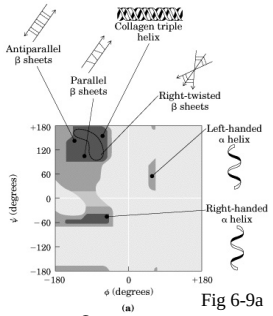


Fig 6-9a

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°

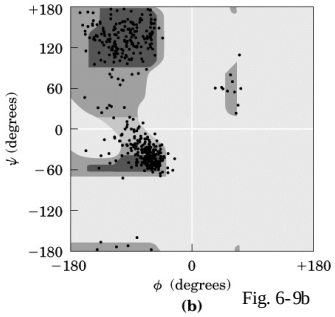
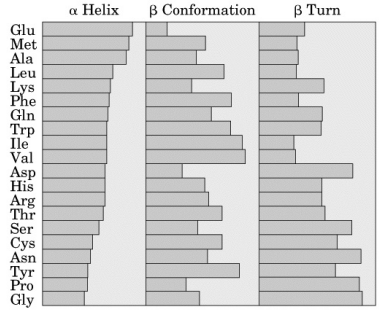


Fig. 6-9b

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°) & Quarternary (4°) Structure

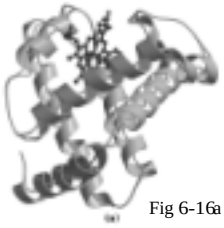
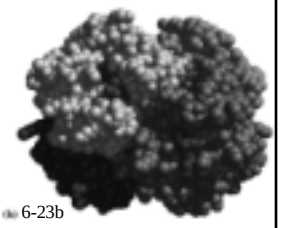


Fig 6-16a

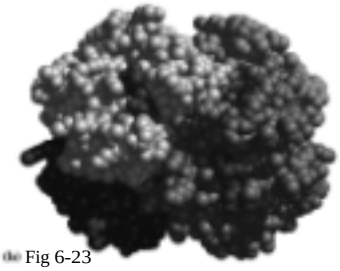


6-23b

Tertiary (3°) Structure

- 3D arrangement of <u>all</u> atoms in a protein
- Includes <u>long range</u> 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 <u>covalent</u> disulfide bonds (-S-S-)

Quarternary (4°) Structure



6-23 Fig 6-23

Arrangement of <u>separate</u> polypeptide subunits in multisubunit proteins

2 major protein groups: Fibrous & Globular

Fibrous Proteins: "Strands" or "Sheets"
Globular Proteins: Compact, spherical

Structurally distinct:
- Fibrous: 1 type of 2°
- Globular: Several types of 2°

Fuctionally distinct:
- Fibrous: Support, shape, external protection
- Globular: enzymes, regulatory proteins

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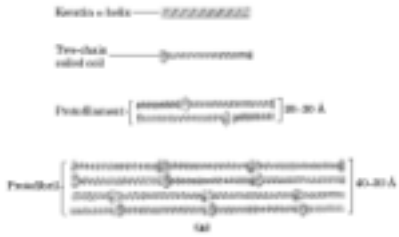
Fibrous Proteins



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

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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

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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)

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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.

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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

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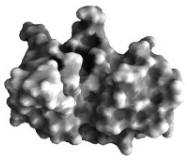
Small proteins have lower surface:volume ratios - fewer buried residues



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

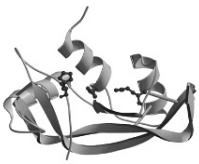


Fig 6.18 Ribonuclease

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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

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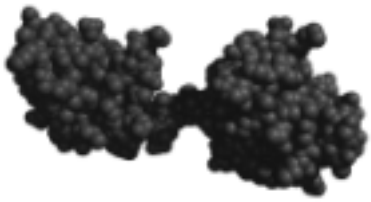
Large proteins (several hundred residues) often fold into two or more stable, globular domains



Domains often have distinct functions:

- Catalysis
- Regulation of Catalytic activity
- Binding of ligands

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2°and Supersecondary Structure Folding Rules :

- Burial of hydrophobic R groups to exclude H_2O
  - 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

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

Usually indicates a strong evolutionary relationship

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Quarternary (4°) Structure

"Multimers" (> 2 subunits) Advantages:

- Binding effects - sum greater than parts
  - Synergism 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)

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Most multimers have identical subunits, or repeating groups of nonidentical subunits

Repeating unit (single or group) = Protomer

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Multimer example = Hemoglobin

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

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Rotational & Helical Symmetry

Subunits can be superimposed by rotation about 1 or more axes

Two types of cyclic symmetry (a)

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Rotational & helical symmetry

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

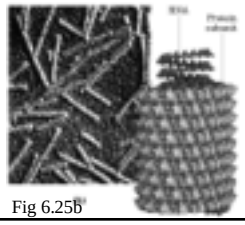

Fig 6.25b

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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

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Protein Folding & Denaturation

Polypeptides must fold during and after synthesis to native conformation

(difference = 3-4 H bonds)

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

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Folding likey a cooperative process

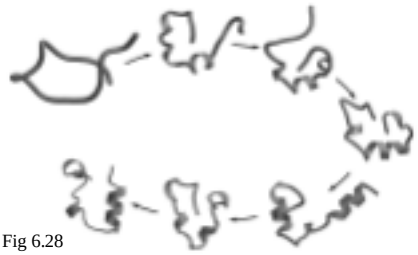

Fig 6.28

Formation of 2° in one part enhances formation in another

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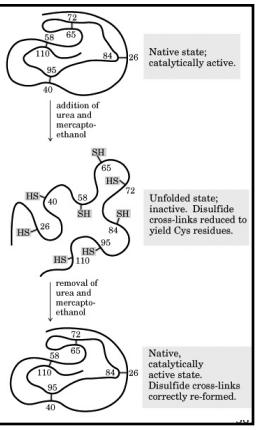
Amino Acid sequence (1°) determines 3 °

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

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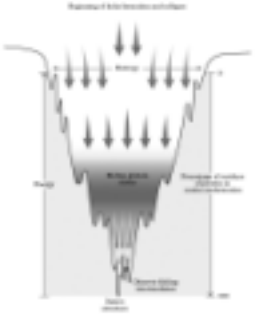
The Folding Process as a Free Energy Funnel

High conformational entropy →

Fully folded, native structure →



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

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Some Proteins Undergo Assisted Folding

Assisted by the action of specialized proteins:

- Molecular Chaperones
- Chaperonins
- Isomerase enzymes
  - Rearrangement of disulfide bonds

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Chaperones & Chaperonins bind to unfolded regions and prevent inappropriate aggregation

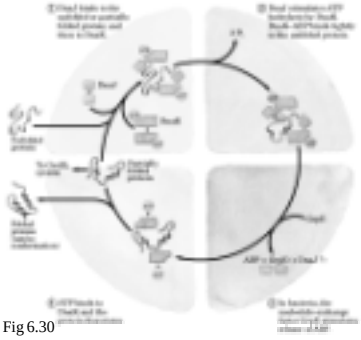

Fig 6.30

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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

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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-C_α, C_α-C specified by Φ & Ψ
   - 2° defined completely if all Φ and Ψ known (Ramachandran plots)

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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

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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)

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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

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Summary (contd.)

8) Quarternary 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

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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)

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