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CHAPTER 6

Proteins: Secondary, Tertiary, and Quaternary Structure

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Levels of Protein Structure

- Primary (sequence)
- Secondary (ordered structure along peptide bond)
- Tertiary (3-dimensional overall)
- Quaternary (subunit relationships)

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Forces Contributing to Overall Structure

- Strong (peptide bond, disulfide bond)
- Weak
  - Hydrophobic (40 kJ/mol)
  - Ionic bonds (~20 kJ/mol)
    - Figure 6.1
  - Hydrogen bonds (~12-30 kJ/mol)
  - Dispersion (van der Waals) (0.4-4 kJ/mol)

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Effect of Sequence on Structure

- Sufficient information for folding into correct 3-dimensional structure is in the sequence (primary structure) of the protein
  - Experiments of Anfinsen and White on Ribonuclease
- However—the "folding problem" is one of the major unsolved problems of biochemistry and structural biology

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Secondary Structure

- Folding probably begins with nucleation sites along the peptide chain assuming certain stable secondary structures.
- Planarity of the peptide bond restricts the number of conformations of the peptide chain. Rotation is only possible about the
  - C(alpha)-N bond (the Φ (phi) angle)
  - C(alpha)-C bond (the Ψ (psi) angle)
    - See Figure 6.2

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Steric Constraints on Φ and ΨAngles

- Examine the effects of rotation about the Φ and Ψ angles using Kinemage
  - Download Kinemage
  - Download Peptide file
- Note that some angles are precluded by orbital overlap:
  - Figure 6.3

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Ramachandran Map

- Plot of Φ versus Ψ angle for a peptide bond is called a Ramachandran Map
- Ordered secondary structures have repeats of the Φ and Ψ angles along the chain.
  - See Figure 6.4

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Some Common Secondary Structures

- Alpha Helix (Figure 6.6)
  - Residues per turn: 3.6
    - 13 atoms in a turn (3.6_{13} helix)
  - Rise per residue: 1.5 Angstroms
  - Rise per turn (pitch): 3.6 x 1.5 A = 5.4 A
  - Φ = -60 degrees; Ψ = -45 degrees
- Discuss polyglutamate and polylysine
- Two proteins with substantial alpha helix structure (Figure 6.7)
- Other helix structures (3_{10} and 4.4_{16}helices)

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Common Secondary Structures, con't.

- Beta Sheet (or "pleated sheet")
  - See Figure 6.10
- Can be Parallel or Antiparallel
  - See Figure 6.11
- Parallel sheets usually large structures
  - Hydrophobic side chains on both sides
- Antiparallel sheets often smaller
  - Hydrophobic side chains on one side

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Common Secondary Structures, con't.

- Beta-Turn
  - See Figure 6.12
- Beta-Bulge
  - See Figure 6.13Tertiary Structure
- Secondary structures form first, then pack together in tight structures called **motifs**
  - Beta-alpha-beta, beta hairpin, alpha-alpha,
  - Greek key, beta barrel, alpha/beta barrel
    - (your text doesn't use these terms)
- Motifs might be considered "supersecondary structure. They associate into **domains** (discrete, independently folding globular units)

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Fibrous Proteins

- Organized parallel to an axis
- Mechanically strong
- Usually insoluble
- Structural roles in nature

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Alpha-Keratin

- Hair, fingernails, claws, horns, beaks
- Rods of 311-314 residues with non-helical N- and C- termini
- Non-polar residues every fourth position form a "stripe" twisting around helix.
- Coiling of two helices stabilized by the "stripe" interactions
- Overall filament is a coil of coils of coils
  - See Figure 6.14

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Beta-Keratin

- Silk fibroin, bird feathers
- Antiparallel beta sheets, alternating glycine (one side of sheet) and glycine or serine (other side of sheet)
- Sheets stack with like surfaces interacting
- Fibroin also has regions of disorder surrounding "microcrystalline" regions
  - See Page 175 "Charlotte's Web"

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Collagen

- Connective tissue (tendons, cartilage, bones, teeth, skin, blood vessels)
- Tropocollagen is basic unit
  - Three intertwined chains, ~1000 residues each
  - MW~285,000
  - 300 nm long, 1.4 nm diameter
  - Unique amino acid composition

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Collagen, con't

- Sequence is $(gly\text{-}X\text{-}Y)_n$, where X is usually proline, and Y is usually hydroxyproline
- Find both 3- and 4- hydroxyproline as well as 5-hydroxylysine
  - See Figure 6.16
- Hydroxylation is a **posttranslational modification**
  - See Figure 6.17
- Crosslinking occurs between chains
  - See Figure 6.21 and 6.22

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Collagen, con't.

- Some collagen related diseases
  - Lathyrism (seeds of sweet pea contain beta amino propionitrile, inhibitor of lysyl oxidase
  - Scurvy (vitamin C—ascorbic acid– is required as a cofactor in prolyl hydroxylase)
  - Marfan's syndrome, Ehlers-Danlos syndrome are rare genetic disorders

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Globular Proteins

- Polar residues out, non-polar residues in
  - Helix orientation depends on environment
    - (See Figure 6.24)
- Residue packing close—ratio of amino acid van der Waals volume to protein volume about 0.72 to 0.77
- Empty space primarily small cavities
- Majority of peptide chain in alpha helix or beta sheet structure, but some ordered, non-repetitive structure

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Globular Proteins, con't.

- Some disordered segments may not show in x-ray structures
- Possible fluctuations of atoms, residues, and chains suggest proteins should be viewed as **dynamic structures**
- "Layered" structures –backbones joined by hydrophobic cores (See Figure 6.28)
- Coiled-Coil Motifs (Deeper Look, page 188)

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Classes of Globular Proteins

- One type of classification (Jane Richardson)
  - Antiparallel alpha helix (includes globins)
    - (Figure 6.29)
  - Parallel or mixed beta sheet
    - Figures 6.30 and 6.31
  - Antiparallel beta sheet
    - Figure 6.32, 6.33 and 6.34
  - Metal and disulfide-rich
    - Figure 6.35

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Thermodynamics of Folding

- Consider separately enthalpy and entropy terms for peptide chain and for solvent
- Largest contribution from entropy of interaction of non-polar residues with the solvent (See Box, page 192)

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Protein Folding

- Levinthal's paradox
  - 100 amino acid protein, 2 conformations/AA
  - $2^{100} = 1.27 \times 10^{30}$ possible conformational isomers
  - At 10^{-13}sec for each, time to search all conformations is 4×10^{9} years
- Predictive algorithms, based on propensities of amino acids to be found in certain structures

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Protein Folding, con't.

- Role of **molecular chaperones**
  - Originally identified as "Heat Shock" proteins
- Model for steps in folding
  - See Figure 6.36
- Some diseases related to improper folding
  - (See essay on course links page)

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Mosaic Proteins

- Many proteins share common **modules** or **domains**, even if function is quite different
- Suggests evolution occurred by shuffling domains around
  - See Figure 6.38

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Quaternary Structure

- Typical dissociation for two subunits is 10^{-8} to 10^{-16} M—energies of 50-100 kJ/mol
- Entropy loss due to association is unfavorable
- Entropy gain due to burying hydrophobic groups is favorable
- **Symmetry** of subunit interactions is an important structural feature
  - (See Figure 6.44)

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Advantages of Quaternary Association

- Stability (reduction in surface/volume ratio)
- Genetic economy and efficiency—in relation to size of overall protein
- Bringing together catalytic sites
- Cooperativity between binding of ligands provides regulatory mechanisms