

Proteins: Three-Dimensional Structure

1. Protein Structure - 5 themes

- Primary structure determines 3D structure
- Function of protein depends on 3D structure
- Unique primary structure (Covalent bonds) => Unique 3D structure
- 3D structure stabilized mostly by noncovalent interactions
- Many proteins share common structural elements or structural “motifs”

2. Proteins: Biological Function depends on conformation

- Globular Proteins: water soluble, compact, hydrophobic interior / hydrophilic surface
enzymes, receptors, carriers, hormones, etc. (dynamic agents)
- Fibrous Proteins: water insoluble, structural roles, extended structure
collagen, α -keratin, etc. (static agents)

3. Four Levels of Description of (Native) Protein Structure

- Primary Structure: linear sequence of amino acid residues, covalent bonding including -SS-
- Secondary Structure: local conformation of backbone, maintained by hydrogen bonds
- Tertiary Structure: 3D structure of a subunit (one polypeptide chain) in its native state
- Quaternary Structure: Spatial arrangement of subunits in oligomeric proteins
- Denaturation: Partial to complete unfolding of native conformation
- Denatured Protein: Protein that has lost its native conformation

4. Peptide Bonds / Peptide Conformation

- Peptide bond: ~ double bond character; planar group, *trans* conformation (*cis* ? proline)
- Peptide conformations: Phi / Psi (ϕ / ψ) angles; Ramachandran plots - “allowed” angles
 - $\phi = \psi = 180^\circ$ (fully extended, planar conformation)
 - $\phi = -57^\circ$; $\psi = -47^\circ$ (right handed α helix)
 - $\phi = -139^\circ$; $\psi = +135^\circ$ (antiparallel β sheet) / $\phi = -119^\circ$; $\psi = +113^\circ$ (parallel β sheet)

5. The α -helix and the β -sheets (Secondary structures)

- α -helix : (Linus Pauling & Robert Corey - 1951; Diff. α -keratin; hair)
pitch = 0.54 nm; rise 0.15 nm; 3.6 residues / turn; right-handed; electric dipole
helix formers: helix breakers: Pro, Gly
helical wheels / amphipathic helices: (n, n + 4, n + 7, etc.)
- β -sheets : (Linus Pauling & Robert Corey - 1951)(silk / spider webs)
antiparallel β -sheet / parallel β -sheet:

6. Coils / Turns / Bends / Loops (~ 50% of the residues of an average protein; surface regions)

- β -turns : tight turns (x-P-G-x) connecting two adjacent antiparallel β -strands (type I, type II)
- Loops : 6-16 residues

7. Structural Motifs - supersecondary structures

- Greek key motif : $\downarrow \uparrow \downarrow \uparrow$
- Hairpin loop: $\uparrow \downarrow$ (antiparallel); Cross-overs $\uparrow \backslash \uparrow$ \uparrow / \uparrow
- $\beta\alpha\beta$ loop / helix-loop-helix :

8. Fibrous Proteins -

- α -keratin (hair / r.h. α helices / l.h. superhelix / hydrophobic residues)
- Collagen (connective tissue, ~30% total mammalian protein)
 - Sequence (Gly-X-X)_n and often (Gly-Pro-Hyp)_n
 - Left-handed helical chains (3 residues / turn)
 - Triple helix - r.-h. supercoil (~rope) \rightarrow Fibrils
 - Cross links (Lys \rightarrow Allysine / Lys + Allysine form Schiff base) = tensile strength
- Silk Fibroin - β conformation

9. Tertiary Structure of Globular Proteins

- Domains : Combination of motifs - 25 to 300 a.a. / function
 - β meander - antiparallel β -sheets / β sandwich
 - β barrel / α/β barrel
 - helical bundle
- Functional units
 - Nucleotide binding domain $\beta\alpha\beta\alpha\beta$ / dinucleotide binding domain = Rossmann Fold
 - Zn finger

10. Methods of Determining Protein Structure

- X-ray Crystallography (Protein Crystallography) Resolution $< 3\text{\AA}$ ($\lambda = 2d\sin\theta$)
Crystals - Diffraction Pattern - Phases - Electron Density map - Model - Refinement
(1962 - Mb / Kendrew ; Hb / Perutz ; 1970 ~ 6 protein structures ; 1997 ~ 6500)
RASMOL (Chime / Netscape) - Ribbon representations, $C\alpha$ backbone, Space fill, etc.
- NMR Spectroscopy - for proteins with subunit sizes < 20 kDa (Ensemble of Structures)
NOSEY (Nuclear Overhauser Effect Spectroscopy) - interproton dist. thru space $< 5\text{\AA}$
COSY (Correlated Spectroscopy) - Interproton dist. thru bonds $< 5\text{\AA}$

11. Quaternary Structure - Arrangements of subunits in "oligomers" - α_4 ; α_{12} ; $(\alpha\beta)_2$; $(\alpha\beta)_6$

- oligomer / (multimer / protomer) / rotational vs. dihedral symmetry

12. Protein Denaturation and Renaturation

- Denaturation : T_m (melting temperature)
 - 8M Urea ; 5M guanidinium chloride ; 1% SDS
- Renaturation : Chris Anfinsen - Folding of Ribonuclease (4 disulfides)

13. Protein Folding and Stability

- $\Delta G = G_f - G_u = \Delta H_{\text{prot}} + \Delta H_{\text{solv}} - T\Delta S_{\text{prot}} - T\Delta S_{\text{solv}}$ (largest - $T\Delta S_{\text{solv}}$ for nonpolar R)
- Folding as a cooperative, sequential process : Local sec. st. / Domains / Molten globules
- Molecular chaperones (GroEL , GroES / Hsp70) assist with folding / (Prions - misfolding)
- Isomerases (PDI, protein disulfide isomerase; PPI, peptide prolyl cis-trans isomerase)