Based on BIOL1007 - Dr Mark Coldwell

**Protein structure**

**Tertiary structure**

Chapter 4, page 52-54

**Summary of previous lecture**

- Polypeptide chains are able to fold because of rotation around the bonds on either side of Cα: phi and psi dihedral angles
- Local structures form via hydrogen bonding between NH of one residue and C=O of another
- The simplest secondary structures are α-helices and β-sheets

**Supersecondary structures**

- Helix-turn-helix
- DNA binding
- Coiled coil (α-keratin) formed from amphipathic helices
- Helix-loop-helix
- EF-hand motif
- Ca²⁺ binding

**Levels of protein structure**

- Tertiary structure is the folding of polypeptide chains, with R-groups, usually far apart in the primary structure, interacting with each other through:
  - Non-covalent forces/bonds:
    - Hydrophobic interactions
    - Hydrogen bonds
    - van der Waal interactions
    - Electrostatic bonds (ionic)
  - Covalent bonds

Chapter 3, page 36-38
**Disrupting protein structure**

- Heat (normal range 20 – 40°C)
- pH (normal intracellular pH 7.2 ± 0.4)
- Ionic strength (normally ~0.1 M KCl)
- Denaturing agents
  - organic solvents
  - chaotropic agents (disrupt H-bonding in water):
    - guanidinium hydrochloride
    - urea
- Proteolytic enzymes (proteases)
- UV/oxidative/radiation damage

**Hydrophobic interactions**

- Thought to be the prime driving force for protein folding – termed 'hydrophobic collapse'

  i) Hydrophobic clusters of non-polar amino acids
  - Disrupted by organic solvents or denaturing agents

  ![Diagram of hydrophobic interactions](http://proteopedia.org/wiki/index.php/Cation-pi_interactions)

  - Hydrophobic core region contains nonpolar side chains
  - Polar side chains on the outside of the molecule can form hydrogen bonds to water

  ![Diagram of protein structure](http://proteopedia.org/wiki/index.php/)

  - Unfolded polypeptide
  - Folded conformation in aqueous environment

**Hydrogen bonds**

- Involve polar non-charged R-groups
- Broken by heat and by denaturing agents (e.g. urea)
- Exposed hydrogen bonds (on the surface of a protein) are also disrupted by water

  ![Diagram of hydrogen bonds](http://proteopedia.org/wiki/index.php/)

  - For example:
    - Gln
    - Asn
    - Ser
    - Thr

  Hydrogen bonds are weak electrostatic interactions between partial positive and negative charges. Because these are at defined locations, hydrogen bonds are highly directional.
van der Waals interactions

- Very short range effects (electron shells of two atoms must be almost touching)
- Fluctuations in electron spatial distribution in one atom induce electron redistribution in adjacent atom
- Weak electrostatic forces
- Disrupted by heat, denaturing agents

Electrostatic bonds

- Ionic interactions between full charges, also called 'salt bridges'
- Between charged residues
  - acidic: Asp, Glu
  - basic: Lys, Arg (& His depending on pH)
  - cysteine and tyrosine are also ionisable (see later)
- Ineffective unless in a hydrophobic environment (for example hydrophobic amino acid side chains)

Electrostatic bonds

- Remember the zwitterionic form? A zwitterionic molecule has charged groups, but its net charge is zero (same number of + and – charges)
- The protonation state of the R-groups can also change according to pH
- The pH where the side chain has no net charge is called the isoelectric point (pI)

Titration curves of amino acids

- Titration curve of aspartic acid
  - measured pH
  - titration of OH⁻
Titration curves of amino acids

Sidechain ionisations:

- Aspartic acid
- Lysine

- The Henderson Hasselbalch equation describes the relationship between pH, pKa and the extent of ionisation of a weak acid:
  \[ \text{HA} \rightleftharpoons A^- + H^+ \]
  \[ \text{pH} = \text{pKa} + \log \left( \frac{[A^-]}{[HA]} \right) \]
- The pKa of the ionising group is the pH where 50% ionisation has occurred

Breaking electrostatic bonds

- Therefore the charge on a protein is determined by the number and type of the amino acids with ionisable sidechains and also by the pH of the solution
- Ionic bonds ('salt bridges') are broken by changes in pH, when these cause the charge on relevant side chains to disappear
- Increasing ionic strength can have the same effect: salt screening of electrostatic charges

Quick recap

- Recall structures of the amino acids to see ionising groups on the various sidechains
- Note that the actual pKa of each amino acid side chain is dependent on its position in the folded protein, so the pKa values shown above are only rough estimates

Figure 3-4 Molecular Biology of the Cell (© Garland Science 2008)
Disulphide bonds

- The -SH group (‘sulphydryl’ or ‘thiol’) of the Cysteine sidechain can form a disulphide bond with another Cysteine residue

- Disulphide bond formation occurs under oxidizing conditions (*), while reducing conditions break the disulphide bond

(*) https://www.chemguide.co.uk/inorganic/redox/definitions.html

Disulphide bonds

- Disulphide bonds are strong (because they are covalent bonds) and make the 3D fold of a protein significantly more stable
- Most proteins with disulphide bridges are extracellular (possibly because the cell interior is a strongly reducing environment)
- Inside the cell special enzymes catalyze S-S bond formation, usually takes place in the endoplasmic reticulum (ER)

Anfinsen experiment on Ribonuclease

1. add 8M urea and excess β-mercaptoethanol
2. remove βME
3. add trace amount of β-ME

105 different disulphide bonding patterns are possible

104 residues (8 Cys) 4 disulphide bonds

H-bond breaking with urea & disulphide bond reduction with excess of β-mercaptoethanol

H-bonds can reform after urea removal & disulphide bond formation by bubbling O₂ (oxidizing)

Native form of ribonuclease

Denatured/unfolded ribonuclease

Mostly correctly refolded ribonuclease (>90% active)

Incorrectly folded ribonuclease

Only 1% of enzymes regain activity

Native ribonuclease (100% active)

Denatured/unfolded ribonuclease (inactive)

Native ribonuclease (100% active)

Denatured/unfolded ribonuclease (inactive)

https://www.youtube.com/watch?v=pZee0XCCqH4

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Anfinsen Experiment on Ribonuclease

- The Anfinsen experiment on ribonuclease shows:
  - The folded, active form of a protein has the lowest free energy (thermodynamically most stable protein structure)
  - All of the information needed by a protein to fold to this structure is encoded in the amino acid sequence (~DNA gene)

- But not all proteins fold as easily (the Anfinsen experiment does not work with all proteins)
- Some proteins require protein disulphide isomerases (PDIs) to (re)fold correctly
- PDI enzymes make and break disulphide bonds until the correct protein conformation (the correct fold) is achieved
- These enzymes are example of “chaperones”

Breaking peptide bonds

- Broken by hydrolysis by boiling in 6M acid (or alkali)

- Protease enzymes can either be indiscriminate or sequence specific
  - Trypsin cuts C-terminal to Arginine (R) and Lysine (K)

- In programmed cell death (apoptosis), caspases cleave after DxxD

Summary

<table>
<thead>
<tr>
<th>Primary</th>
<th>Secondary</th>
<th>Tertiary</th>
<th>Quaternary</th>
</tr>
</thead>
<tbody>
<tr>
<td>(amino acid sequence)</td>
<td>(local structure: helix, sheet, etc)</td>
<td>(global structure, folded protein)</td>
<td>(assembly of multiple proteins)</td>
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</tbody>
</table>

- Primary
  - Different types of R-group (amino acid side chains)

- Secondary
  - Interactions between polypeptide backbone atoms
  - Stable local structures: α-helices, β-sheets
  - Hydrogen bonds only

- Tertiary
  - Interactions between R-groups
  - Weak = hydrogen bonds, hydrophobic interactions
  - Stronger = electrostatic, covalent (disulphide bridges)

- Various agents / environmental changes can break these bonds
- Next: quaternary structures and the addition of extra groups