Section B: Enzymes - Structure & mechanism of action

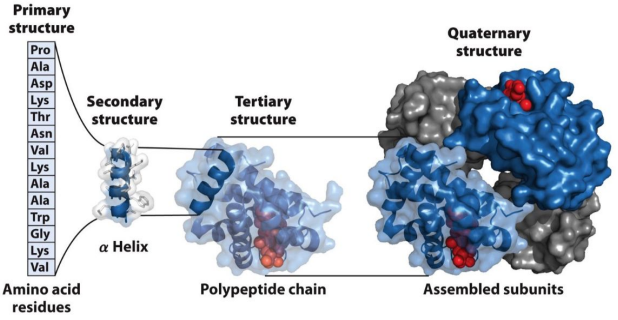
Lecture 1: Protein structure and function I

Learning objectives

- The structure of a protein is dependent on its amino acid sequence
- Proteins have 3D (tertiary) structures containing secondary elements
- Proteins secondary and tertiary structures are mainly held together by numerous non-covalent interactions
- Proteins can spontaneously fold into their native state
- The tertiary structures of proteins are adapted to their function

Proteins contain 4 levels of structure

- Amino acids differ by their side chain functionality
- All have amine and carboxyl group (except for proline which has a different type of amine group)
- 20 different amino acids that can be arranged in numerous ways
- X-ray crystallography shows level of structure
- Protein structure is determined by the amino acid sequence



Non-covalent interactions

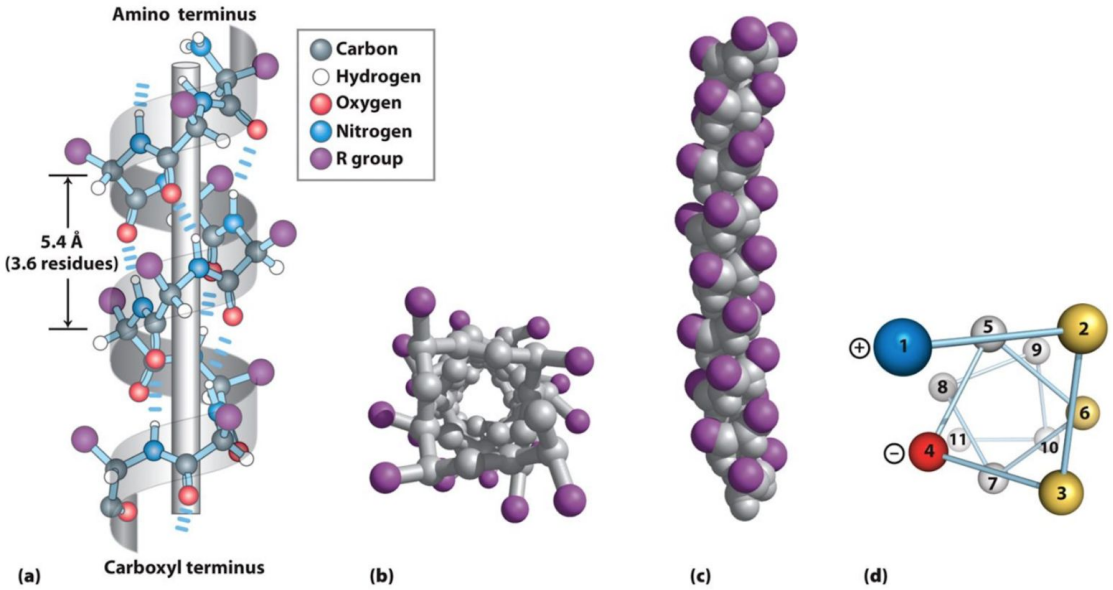
- Primary structure of proteins is defined by covalent interactions
- Other levels of structure are mainly (one important exception) formed from non-covalent interactions
- These are weak forces including:
    - H-bonding: between the O and the H
    - Hydrophobic interactions: water wants to stick together, causes other molecules to also cluster together
    - Van der Waal's forces: any 2 atoms in close proximity
    - Ionic interactions (salt bridges): attraction between opposite charges and repulsions between like charges

Secondary structure

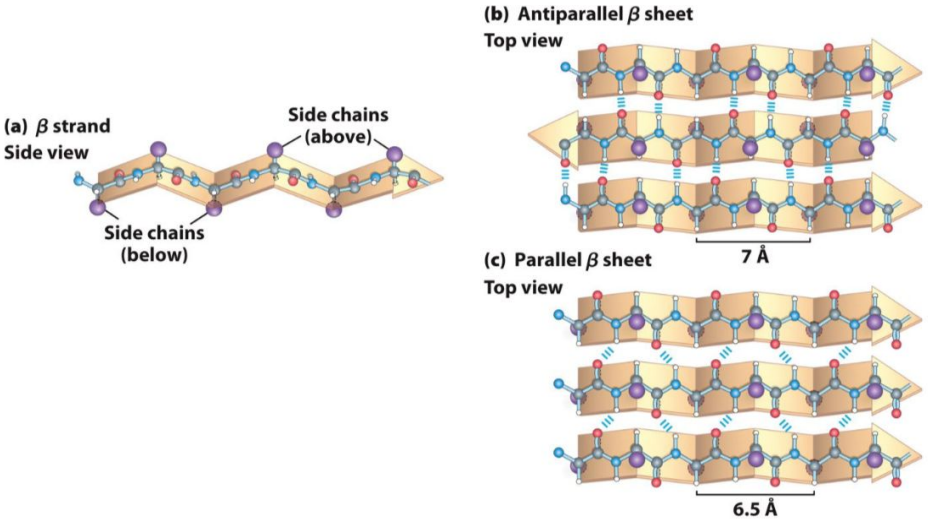
- The regular recurring arrangements at a local level within a protein
- Mainly defined through hydrogen bonding patterns between peptide bonds (see right), but are also stabilised by the other weak forces

- α-Helix:
    - Amino acid chain can cause a local secondary structure (α-helix)
    - All amino acid side chains are facing outwards
    - COOH and NH_2 groups are positioned in a way that allows for H-bonding

- Carbonyl is facing downwards, nitrogen group is facing upwards
- Proline in an α-helix cannot form a H-bond between the COOH and NH_2
- See ‘d’ for ionic interactions that create α-helix shape



- β-strands and β-sheets
  - Amino acids linked by peptide bond - holds itself fairly planar (zigzag effect)
  - Side chains point up and down
  - Carbonyl and amine groups stick out to the sides of the plane
  - β-strands come together (antiparallel/parallel formation) to create β-sheets (done by H-bonding between carbonyl and amine groups)



- β-turn:
  - Turn in the structure stabilised by H-bonds

Tertiary structure

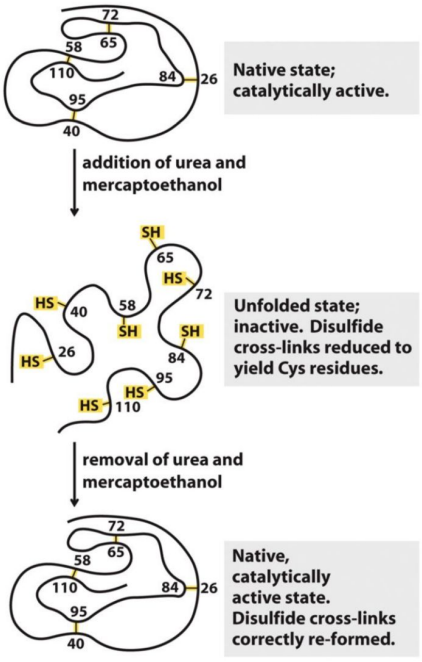
- Spatial conformation of all atoms in a single polypeptide chain - 3D structure of a protein
- Globular proteins are said to have conformation, the functional state (most often found) is said to be the *native conformation*
- This native conformation gives the protein its function

Native conformation of proteins

- Most energetically favourable state (lowest free energy state)
- Native conformation is driven by:
    - Maximisation of H-bonds and electrostatic attractive forces
    - Burying hydrophobic residues (clustering them to make room for more H-bonds)

Proteins can spontaneously fold into their native states

- Mercaptoethanol is a reducing agent, reduces the disulfides (protonated sulfurs)
- Urea is a very strong denaturing agent (has strong groups that can form H-bonds so it can interfere with the H-bonds of the protein - break them apart)
- Addition of urea and mercaptoethanol makes the protein unfold
- Removal of urea and mercaptoethanol leads to the protein spontaneously folding into its original (native) state and it regains its enzymatic abilities
- This happens spontaneously!!

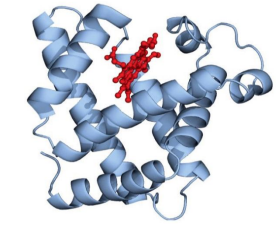


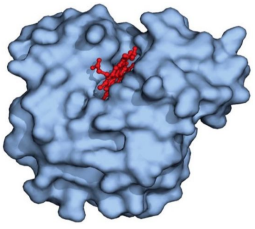
How does this folding occur?

- Folding takes place through intermediate states
- Proteins can fold by different pathways but by exploring conformational space
- When folding is complete, this native form remains

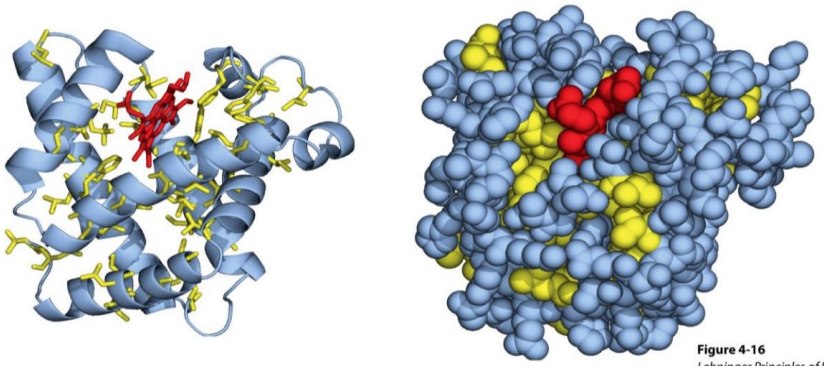
Myoglobin

- Tightly folded protein, mostly consisting of 8 α-helices, found in muscles
- Structure of protein is specially adapted to provide a pocket to bind heme group (red) - shape of pocket is perfectly contoured for heme group
- Binding through non-covalent interactions
- Sections that are hydrophobic and hydrophilic in the pocket correlate with more hydrophobic/philic parts of the heme group
- Iron protoporphyrin (heme) group is where iron can bind, and the iron can bind the oxygen
- Surface contour picture shows the binding pocket for the heme group





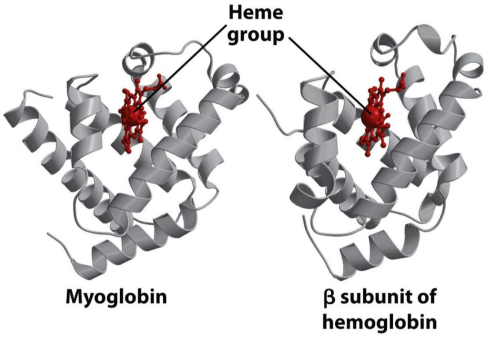
- Hydrophobic groups (yellow) are hidden in the interior - soluble in water
- In the stick representation (below on the left), hydrophobic groups are clearly seen. In the space filling model (below, right), however, the groups are mostly hidden
- Note also how the heme group (red) is buried in its pocket



Quaternary structure

- Structure of proteins made up of more than one subunit
- Subunit - different chains that come together to form a protein (eg. myoglobin is a single subunit, AKA a monomer)
- 2 subunits = dimer; 3 = trimer; many = oligomer
- If the chains are the same or different, they're called homodimer and heterodimer, respectively
- Hemoglobin is a tetramer (4 subunits)

Myoglobin and hemoglobin



- Both contain heme groups; both can bind oxygen; both made of α-helices
- Myoglobin is in muscles, whereas hemoglobin is in red blood cells (transports oxygen from lungs to muscles)
- Myoglobin is a monomer, whereas hemoglobin is a tetramer
- Heme group is a planar ring structure containing an iron (Fe) atom at its centre
- Heme group has some hydrophobic groups and carboxyl groups which makes it a perfect fit for the protein pockets on both myoglobin and hemoglobin
- Fe is bonded to 4 different groups (can bond to a total of 6)
- The 2 vacancies remaining for Fe to bond (see 'd' below)
- 1 vacancy is taken by oxygen, another by histidine
- Therefore Fe has direct contact with oxygen and the protein (see 'edge view' below)