

## Structure-Function Relationships of Proteins

[Study guide covering chapter 7]

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### Characteristics of 3D Structure.

- 3D conformation and the type of amino acid side chains determine the character and function of the protein.
- Globular proteins usu. water soluble.
- Fibrous proteins are linear, arranged about a single axis, have repeating units.
- Transmembrane proteins that have 1+ regions aligned so they cross the lipid membrane.
- Primary structure. Linear AA residues joined by peptide bonds to form polypeptide chain.
- Secondary structure. Recurring structures that form in short localized regions. Beta pleats, parallel, antiparallel.
- Tertiary structure. 3d globular structure.
- Quaternary structure. 2+ protein structures.

### Requirements for 3D Structure.

- Creating a binding site that's specific for just one molecule or a group of molecules w/similar structural properties/characteristics. Binding sites on a protein define that protein's role.
- Has appropriate rigidity and flexibility allow the protein to fulfill its role.
- Structural flexibility and mobility so that the protein can fold appropriately.
- The external surface of the protein must be appropriate for its environment and aid in the protein's functionality.
- Conformations must be stable.
- When the protein is degraded/damaged, it must be able to be broken down and disposed of or recycled.

### 3D Structure of Peptide Bond.

- When AA are joined to form a polypeptide chain, the peptide bond takes on a "trans" configuration to minimize steric strain between different alpha carbons and side chains.
- The backbone is a resonance structure and has very restricted "bends". The carboxyl and amide groups are planar.

- Torsion angles, are limited allowed rotation about the alpha carbon and alpha amino group around the bond between the alpha carbon and carbonyl group. Limited by steric constraints and favor the max distance between substituents.

## Secondary Structure.

- Recurring localized structures in polypeptide chains are secondary structures.
- Alpha helix: secondary structure of proteins; membrane spanning; rigid, stable conformation; maximize H-bond. Peptide backbone formed by H-bonds betw. carbonyl oxygen and amide hydrogen located 4 residues down. Ea. peptide bond is connected to the other peptide bond +4 and -4 amino residues away. Proline is known as the “helix breaker” because it cannot fit in the alpha helix due to proline’s dual attachment points to a backbone.
- Beta sheets: maximize H-bond betw. peptide backbones; maintain allowed torsional angles; parallel or antiparallel “weaving”; the carbonyl oxygen of one peptide bond is H-bonded to the amide hydrogen of a peptide bond on adjacent strand. Antiparallel is like hairpin turns or the chain folded backwards on itself. Sheets have hydrophilic and hydrophobic sides.
- Nonrepetitive secondary structures: bends, loops, and turns that don’t have the same organization as alpha helixes and beta sheets.
- Patterns of secondary structure.

## Tertiary Structure.

- Secondary elements folded into 3d structure.
- 3d is dynamic and flexible.
- Fluctuating movement of the side chains and domains. Don’t require “unfolding”.
- Maintains appropriate surface residues respective of its environs.
- Flexibility is a key feature.
- Forces: ionic bonds, H-bonds, disulfide deformations, London forces, hydrophobia-hydrophilia.
- Tertiary structural domains: physically indep. regions that fold independently. Domains are fairly obvious to visual inspection.
- Domain: formed from continuous AA sequence in a polypeptide chain that’s folded into 3d indep. of rest of the protein. 2 domains are connected via a loop or similar simple structure.
- Folds in globular proteins: relatively large patterns of 3d structure recognizable in many proteins. Each “fold” hosts a certain kind of activity.
- Actin fold: G-actin.
- Nucleotide binding fold.

- Globular proteins solubility in aqueous environment. Most are soluble in the cell. The core is mostly hydrophobic and is densely packed to maximize London forces.
- Charged/polar parts of the polypeptide chains generally on the surface or close to it.
- Charged side chains bind to inorganic ions to minimize repulsion between like groups.
- Some charged aminos in the interior usu. serve as binding sites.
- Polar uncharged: serine, threonine, asparagine, glutamine, tyrosine, tryptophan.
- Transmembrane proteins have characteristics that allow them to cross membrane structures. They usu. have hydrophobic and hydrophilic components. Transmembrane proteins usu. have many post-translational modifications providing additional chemical groups to interact as needed. Receptors need both flexibility and “rigidity”.
- Binding domain.

### Quaternary Structure.

- Homo-
- Hetero-
- Protomer: an unit made from two identical units.
- Oligomer: multi subunit protein made of identical G-actin subunits.
- Multimer: a complex w/lots of subunits of 1+ types. “Melting/melding pot”.
- The larger a complex protein, the more difficult it is to fold and unfold etc. Requires much larger effort. It also means that it’s more stable, too. It has fewer “options”. It can also operate in more of a coordinated effort due to less subunits “going rogue”.

### Quantification of Ligand Binding.

- $K_a$ . Association constant. Binding affinity of a protein for a ligand. The  $K_{eq}$  for the binding reaction of P protein and L ligand. Used for comparing proteins made by diff. alleles.
- $K_d$ . Dissociation constant for the ligand-protein binding. Is the reciprocal of  $K_a$ .

### Structure-Function in Myoglobin and Hemoglobin.

- Myoglobin and hemoglobin are oxygen-binding proteins similar in structure.

- Myoglobin: globular; simple polypeptide that has one oxygen binding site; 8 alpha helices connected via short coils called globin fold; no beta pleated sheets (unusual); helices create hydrophobic oxygen binding pocket w/tightly bound heme w/an iron atom  $Fe^{2+}$ .
- Hemoglobin: tetramer made up of different subunits (2 alpha and 2 beta polypeptide chains called alpha-beta-protomers). Maximizes oxygen carrying capacity. Planar porphyrin ring of 4 pyrrole rings linked by methenyl bridges lying w/nitrogen atoms in the center, binding  $Fe(II)$  in the center. Negatively charged areas interact w/arginine and histidine side chains from hemoglobin; hydrophobic methyl and vinyl groups interact w/hydrophobic amino acid side chains as well that help position the heme group. There are 16 diff. interactions betw. myoglobin amino acids and diff. groups in porphyrin ring.
- When  $PO_2$  (partial pressure of oxygen) is high (in lungs), myoglobin and hemoglobin are oxygen-saturated.
- When  $PO_2$  is lower (e.g. in tissues), hemoglobin can't bind to oxygen as well as myoglobin can.
- Myoglobin is in heart and skeletal muscle and is able to bind to (and store)  $O_2$  released by hemoglobin.
- Cytochrome oxidase, a heme-containing enZ in ETC( electron transport chain), has higher affinity than myoglobin.
- Prosthetic groups: organic ligands tightly bound to proteins. They are a part of the protein and don't dissociate until the protein is degraded.
- Holoprotein: a protein w/prosthetic groups.
- Apoprotein: a protein w/out its prosthetic group.
- In the binding pocket of myoglobin and hemoglobin, oxygen binds to the  $Fe^{2+}$ . The  $Fe^{2+}$  can chelate (bind to) 6 different ligands (4 ligands are co-planar, 2 ligand positions are perpendicular). 4 ligand positions taken by N, 1 of the perpendicular positions is taken by N on histidine called proximal histidine, the other position taken by  $O_2$ .
- When  $O_2$  binds, conformational changes occur >> Tertiary structure changes from T-state (tense state) with low  $O_2$  affinity >> to R (relaxed) state w/high  $O_2$  affinity. Binding rate for the first  $O_2$  is low but with subsequent  $O_2$ 's the binding rate is higher. This is known as positive cooperativity. The first  $O_2$  is difficult but subsequent binding of  $O_2$ 's gets easier.
- $HbO_2 \rightarrow Hb + O_2$
- Agents that affect  $O_2$  binding: hydrogen ions; 2,3-bisphosphoglycerate; covalent binding of  $CO_2$ .
- 2,3-bisphosphoglycerate (2,3-BPG). Formed in red blood cells. 2,3-BPG binds to hemoglobin increasing energy requirements for the conformational changes that facilitate  $O_2$  binding. Lowers hemoglobin affinity for  $O_2$ . RBC can use

2,3-BPG to modulate affinity to O<sub>2</sub> binding as needed by changing the rates of synthesis/degradation of 2,3-BPG.

- Proton binding, (Bohr Effect). When hemoglobin binds protons, it has less affinity for oxygen. pH decreases in tissues (proton concentration is higher) as metabolic CO<sub>2</sub> is converted to carbonic acid via carbonic anhydrase in RBC's. Dissociated protons react w/AAs >> conformational changes >> promote release of O<sub>2</sub>. In the lungs, this process is reversed. Lungs have high O<sub>2</sub> concentration >> O<sub>2</sub> binds to hemoglobin causing release of protons >> pH of blood rises >> carbonic anhydrase cleaves carbonic acid to H<sub>2</sub>O and CO<sub>2</sub> >> CO<sub>2</sub> exhaled.
- Carbon dioxide. Most of it is from tissue metabolism. CO<sub>2</sub> is carried to lungs as bicarbonate. Some of the CO<sub>2</sub> is covalently bonded to hemoglobin.
- <https://www.khanacademy.org/science/health-and-medicine/advanced-hematologic-system/hematologic-system-introduction/v/bohr-effect-vs-haldane-effect>
- <https://www.khanacademy.org/science/health-and-medicine/advanced-hematologic-system/hematologic-system-introduction/v/hemoglobin-moves-o2-and-co2>
- <http://www.pathwaymedicine.org/bohr-effect>
- <https://youtu.be/FtA4Xy-lMSY>

## Structure-Function Relationships in Immunoglobulin.

- Immunoglobins/antibodies bind to antigens (ligands) on the invaders, like marking them for inactivation/destruction. Marking the invaders as “not self”.
- Immunoglobins have the same structure: ea. antibody molecule has 2 identical polypeptide chains (L light chains) and 2 identical large polypeptide (H heavy) chains. L and H chains are joined via disulfide bonds.
- 5 major classes of immunoglobins.
- IgG gamma (most abundant); 220 aminos in light chain; 440 aminos in heavy chain. L and H chains have immunoglobulin fold (collapsed number of B sheets called Beta-Barrel). Have attached oligosaccharides that help mark “not self”.
- V = variable regions. VL (variable light chain) and VH (variable heavy chain) interact to make one antigen-binding site at each branch of the Y-shaped molecule. V regions have different AA compositions.
- C = constant regions. Form the fragment, crystallizable part of the antibody important for the antigen-antibody complex.
- Antigens bind tightly w/almost no tendency to dissociate. K<sub>d</sub> is betw. 10<sup>-7</sup> to 10<sup>-11</sup> M.

## Protein Folding.

- Peptide bonds may be rigid, but other bonds in the molecule can allow some flexibility.
- Native conformation: every molecule of the same protein has the same stable “native” conformational state.
- Primary structure (sequence of AA side chains) determines folding and assembly of subunits.
- Denaturation can affect protein structure. However, under certain conditions, the denaturation may be reversed.
- Not all proteins fold into their native state by themselves. Sometimes the folding and refolding occurs as the protein searches for its most stable state.
- Kinetic barriers are the higher energy states and conformation the protein may pass through.
- These kinetic barriers may be overcome by heat-shock (chaperonins) which use energy from ATP hydrolysis to help in the folding process.
- Cis-trans isomerase.
- Protein disulfide isomerase.
- Collagen: fibrous protein; made mostly by fibroblasts (cells in interstitial connective tissue), muscle cells, and epithelial cells. Type 1 collagen most abundant in mammals and major component in connective tissue. Found in ECM, loose conn. tissue, bone, tendons, skin, blood vessels, and cornea. 33% glycine, 21% proline, and hydroxyproline.
- Hydroxyproline is an AA made by posttranslational mods of peptidyl proline residues.
- Procollagen 1 is the precursor of collagen 1; triple helix of 3 pro-alpha polypeptide chains twisted around ea. other (ropelike). Interchain H-bonds. Every 3rd residue is a Glycine.
- Collagen 1 polymerizes >> collagen fibrils, great tensile strength.
- Vit C functions as a cofactor of prolyl hydroxylase and lysyl hydroxylase. These hydroxylase aid in H-bond formation >> strength and stability. Vit C deficiency causes the melting point to drop from 42 deg C to 24 deg C.
- Aldehyde residues, allysine, make covalent crosslinkages betw. collagen and other structures >> improve stability and structural support.
- Allysine on one collagen molecule + lysine of another molecule >> Schiff base (N=C double bond).
- Aldol condensation may occur betw. 2 allysine to form lysinonorleucine.
- Protein denaturation via nonenzymatic modification of proteins.
- Protein denaturation via temp, pH, and solvent.
- Protein denaturation via misfolding and prions.

Resources.

- <http://leah4sci.com/mcat/mcat-biochemistry/hemoglobin-and-oxygen-dissociation-curves-on-the-mcat/>
- <https://www.youtube.com/watch?v=57P2Gsdq7is>
- <https://youtu.be/dxCd1yaMi-8>

References.

Lieberman, M., & Peet, A. (2017). *Marks' basic medical biochemistry: A clinical approach*(5th ed.). Philadelphia, PA: LWW.