

Chem*3560 Lecture 1:

Structure and Function in Biochemistry

How does structure of proteins and enzymes control the way they work?

How are proteins and enzymes regulated so they act efficiently and effectively, and don't conflict with each other?

How do all the biochemical pathways in a cell work as an integrated whole?

Review of protein structure:

review weeks 1-4 of Chem*2580

A protein is a chain of amino acids linked by peptide bonds.

Important properties of amino acids:

Non polar	Ala Val Leu Ile Met Phe	} hydrophobic interaction
Borderline	Gly Pro Cys Trp Tyr	
Polar uncharged	Ser Thr Asn Gln	} hydrogen bonding, ion pairs
Positive	His Lys Arg	
Negative	Asp Glu	

Amino acids that have side chain pKa values:

Amino acid	pKa	State at low pH	at pH 7	at high pH
Asp	4.0	neutral	deprotonated and negative	negative
Glu	5.0	neutral	deprotonated and negative	negative
His	6.5	positive	mostly deprotonated, neutral	neutral
Cys	8.5	neutral	protonated and neutral	negative
Tyr	10	neutral	protonated and neutral	negative
Lys	10.5	neutral	protonated and positive	neutral
Arg	12.5	neutral	protonated and positive	neutral

Protein structure is organized in four levels:

Primary structure the **sequence** of amino acids in the polypeptide chain.
Amino acids in a protein are normally numbered consecutively starting from the N-terminus.

Secondary structure regular repetitive patterns such as **alpha helix** and **beta sheet** held together by **backbone hydrogen bonds**

Alpha helix is the default preference

Helix of 3.6 AAs per turn, H-bonds from CO group i to NH group $i + 4$

Ala, Leu, Met, (Phe), Glu, Gln, His, Lys, Arg

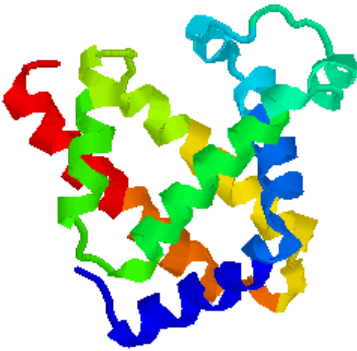
Extended strands may be arranged as parallel or antiparallel beta sheets, with H-bonds between CO and NH in adjacent strands.

Extended polypeptide leaves the maximum room for the side chain, hence bulky amino acids prefer beta sheet

Val, Ile, Thr, Cys, Trp, Tyr, (Phe)

Amino acids that disrupt secondary structure and give rise to turns or loops
Gly, Pro, Asp, Asn, Ser

Tertiary structure The overall pattern of folding, determined by the distribution of secondary structure:



mostly α
mostly β
alternating α and β

**alpha-helix bundles,
antiparallel beta barrels,
parallel beta/alpha barrels
alpha-beta sandwich**

Folded pattern is held together by

**hydrophobic effect,
van der Waals effect,
side-chain H-bonds
ion pairs**

Myoglobin structure shown is about 75% alpha helix in a chain of 153 amino acids. See Lehninger p.203.

Quarternary structure

Association of multiple protein subunits into a **larger complex**. The different subunits **act cooperatively** to enhance the function of the protein

Hemoglobin, right, is a tetramer of four myoglobin-like subunits, Lehninger p.210.



Many proteins bind and recognize other molecules

Ligand: any substance bound by another molecule (Lehninger p. 203-204)

Ligands may bind to proteins, and may be described according to specific functions:

A ligand that binds to an enzyme and participates in a catalytic reaction may be called a **substrate**.

A ligand that binds to an enzyme and does not participate in reaction, but slows down catalysis of other substrates may be called an **inhibitor**.

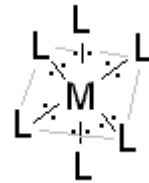
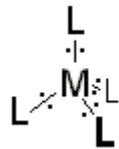
A ligand that binds to an enzyme and does not participate in reaction, but regulates catalytic activity may be called an **effector**.

The term ligand is also applied to small molecules that bind to metal ions to form **complex ions**, e.g. H_2O in $[\text{Fe}(\text{H}_2\text{O})_6]^{2+}$, NH_3 in $[\text{Ni}(\text{NH}_3)_6]^{3+}$

Ligands bind to metals by donating lone pairs.

Particular metal ions have specific patterns of ligands

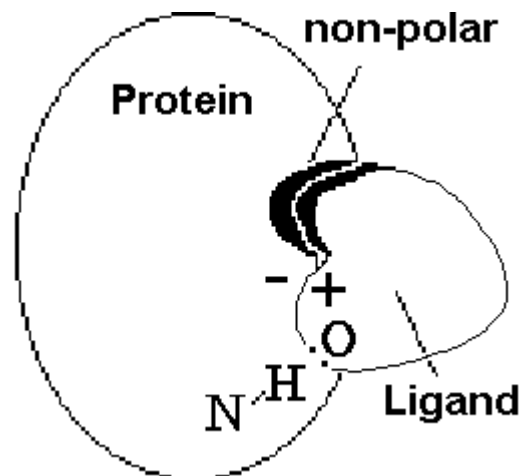
4 ligands, **tetrahedral** or **square planar** 6 ligands **octahedral**,



Proteins may change shape when they bind a ligand

Ligands bind to a protein which has a binding site which:

- matches **hydrophobic regions** in protein and ligand;
- is **complementary in shape**;
- matches up **pairs of opposite charges**;
- matches up **H-bond donors with H-bond acceptors**.



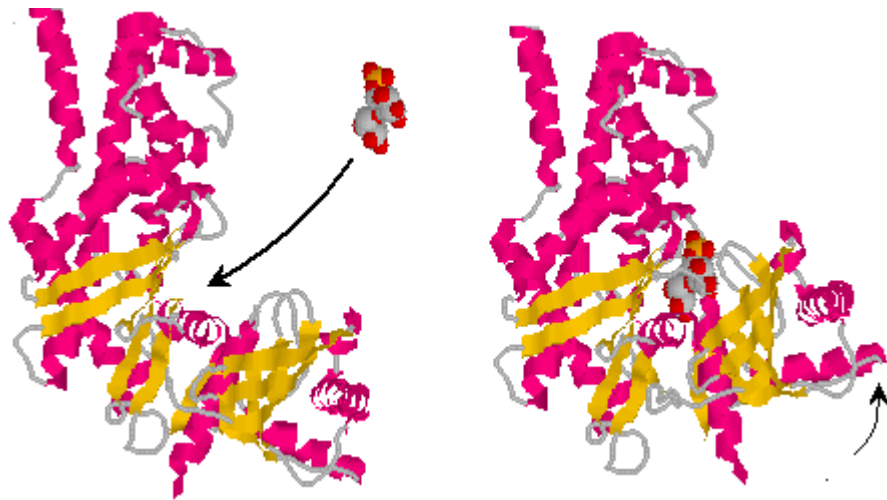
When a ligand binds to a protein, it **alters the balance of internal forces** in the protein.

Since protein structure is based on relatively weak forces, proteins have a degree of **flexibility**, and the protein will adjust its overall shape or **conformation** when a ligand such as a **substrate** or **effector** binds. This kind of change is known as **induced fit**.

In many cases, induced fit causes an enzyme to **close up around the bound substrate**, in particular, the second substrate of a two substrate reaction. This is well illustrated by **hexokinase**, which must bind both ATP and glucose for catalysis to happen:



When both substrates are present, hexokinase **wraps around the bound glucose**, and only then does the enzyme adopt the correct alignment of substrate and catalytic amino acids. (Lehninger p.275-276.)



The problem that this solves is that the mechanism which activates the **glucose-6-OH** group to act as a phosphate acceptor, could also activate an H_2O molecule. Since the reaction is conducted in aqueous solution, if glucose is not yet present, H_2O molecules will occupy the acceptor site.

If induced fit was not part of the mechanism, hexokinase could **catalyse reaction of ATP with H_2O** , or in other words, bring about **undesired hydrolysis of ATP**. Instead, the enzyme does not adopt the ideal catalytic conformation until glucose is present, preventing reaction with wrong substrates.

As a further illustration, **D-xylose** is the pentose sugar that has the identical configuration to the first 5 carbon atoms of glucose. Hexokinase can bind D-xylose, leaving the room for H_2O in the gap left by the missing 6th carbon. D-xylose triggers the induced fit effect, so that the catalytic amino acids become aligned, and as a result ATP hydrolysis occurs.

Oxygen binding proteins

(Lehninger p.204)

Myoglobin is an **O₂-binding protein** for **storing** oxygen in **muscle** (particularly abundant in diving animals such as whales and seals).

Hemoglobin is an **O₂-binding protein** present in red blood cells. **Hemoglobin transports O₂** from lungs to peripheral tissues.

Both hemoglobin and myoglobin are composed of closely related **globin proteins** (see Lehninger p. 210-211 and Fig. 7.7) and **heme pigment** (Lehninger p.205), which gives blood its characteristic red colour.

Since amino acids don't possess O₂-binding ability, globins recruit **heme** to act as a **prosthetic group** to carry out the O₂-binding.

An **apoprotein** refers to a polypeptide that requires an additional non-amino acid component to carry out its function.

A **prosthetic group** is a molecule that carries out some function that amino acids can't perform.

A **holoprotein** refers to the complete functional unit of **apoprotein + prosthetic group**.

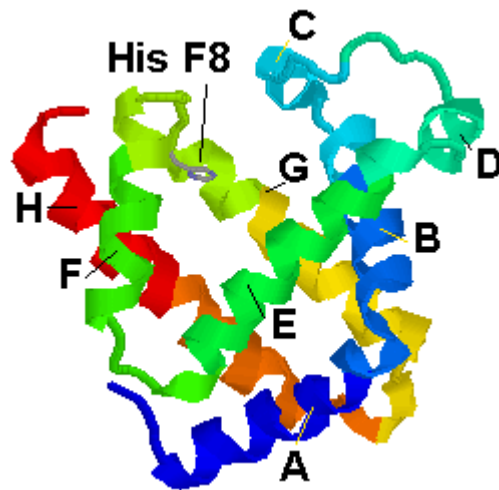
Also: apoenzyme + prosthetic group = holoenzyme for proteins with a catalytic role.

Globin proteins are designed to bind heme:

Globin consists of a bundle of **8 helical segments**, labelled **A-H** (Helix A is closest to N-terminus, then in order through to H at the C-terminus).

Different globins have different chain lengths (Lehninger p. 211). To relate different locations in the structure it is common to refer to **position within each helical segment** rather than position in the whole sequence.

Thus **His F8** is the **8th amino acid in helix F**, and plays **identical roles** in different globins.

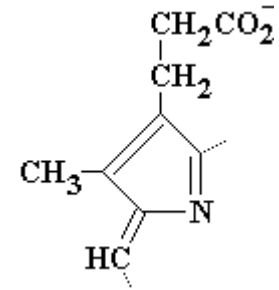


O₂ binds to Fe²⁺ within the heme

Both globin and heme play important roles that allow Fe²⁺ to bind O₂ **reversibly**, so that O₂ can be released when needed. **O₂ normally oxidizes Fe²⁺ to Fe³⁺**, and this releases reactive and toxic oxygen derivatives such as **•OH free radicals**. This oxidation affects both **free aqueous Fe²⁺ ions** and **Fe²⁺ in heme**. **The role of globin is to protect against oxidation.**

Heme is protoporphyrin IX containing bound Fe^{2+}

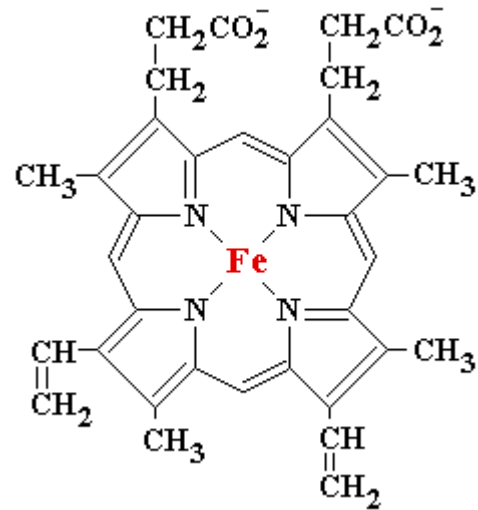
Porphyrins are molecules made up from **four** repeating units containing the **pyrrole** ring, a 5-member ring with one N atom. Each pyrrole carries one methyl group and a propionate side chain.



The pyrrole rings are linked together through a bridging $-\text{CH}=\text{C}-$ group.

Molecules of this type are described as **tetrapyrroles**, and may be linear or cyclic. Protoporphyrin IX is cyclic, and two of the propionate side chains have been decarboxylated to form vinyl side chains $-\text{CH}=\text{CH}_2$.

The four pyrrole N atoms form a **square planar array** at ideal spacing to act as **ligands for metal ions** such as Fe^{2+} . This locks Fe^{2+} in place with high affinity, so all cellular Fe^{2+} will effectively be sequestered in the form of heme.



Heme can be bound by various kinds of protein, and the function of the heme depends on the organization within the protein:

Globins bind heme so as to prevent oxidation to Fe^{3+} , so O_2 can be bound reversibly.

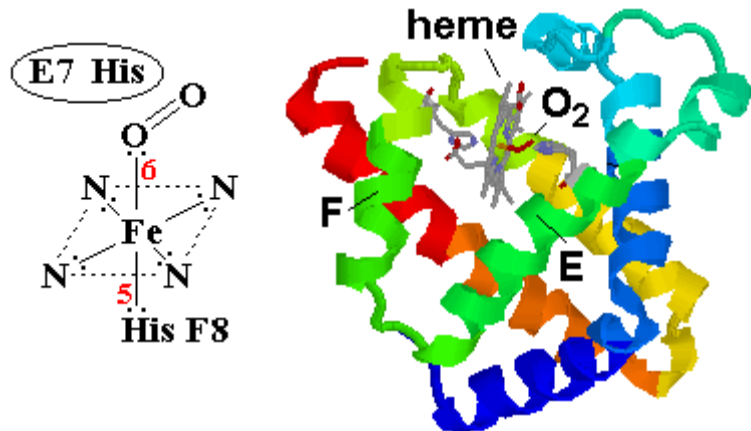
Various **cytochromes** also bind heme, but allow the heme to be reversibly **oxidized to ferriheme containing Fe^{3+}** . The cytochromes do not bind O_2 , and the oxidations and reductions are part of the electron transport process of oxidative phosphorylation and photosynthesis.

Heme binds to globin in a slot between helix E and Helix F

The N atoms of heme form a square planar array of ligands for the Fe^{2+} , which actually prefers 6 ligands in an **octahedral** complex.

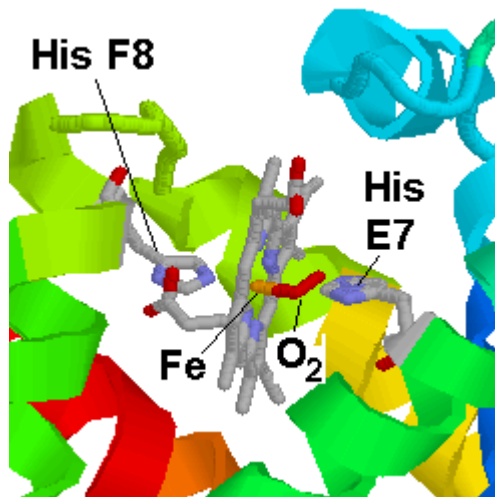
Ligand 5 is provided by **His F8** of the globin.

Ligand 6 is free to be occupied by a molecule of O_2 .



The position of **His E7** next to ligand 6 forces a diatomic ligand to bind to Fe^{2+} **at an angle**. This **favours $\text{O}=\text{O}$, but disfavors $\text{C}\equiv\text{O}$** , which binds **straight on** (remember Lewis structures and the orientation of lone pairs from first year Chemistry). **Position 7 in helix E is consistently His** in different forms of globin.

This effect is important, because the affinity of $\text{C}\equiv\text{O}$ for Fe^{2+} is normally **20,000 times stronger** than O_2 . Affinity for $\text{C}\equiv\text{O}$ in myoglobin is still about 20 times stronger than O_2 , but the difference in affinities is less pronounced. Since $\text{C}\equiv\text{O}$ readily displaces O_2 , but O_2 has difficulty displacing $\text{C}\equiv\text{O}$, this accounts for the **toxic effects of carbon monoxide**.



Globin protects Heme- Fe^{2+} from oxidation to Fe^{3+}

Simple aqueous Fe^{2+} ion and soluble heme- Fe^{2+} are both **oxidized to Fe^{3+}** in the presence of O_2 . The oxidation mechanism requires two heme- Fe^{2+} molecules to **sandwich** a single O_2 .

Oxidation converts heme to **ferriheme** which contains Fe^{3+} , and **changes the ligand preferences** of the metal ion. **Fe^{3+} binds H_2O as ligand 6, so the O_2 binding capability is lost.**

When heme is **embedded in globin**, the presence of protein surrounding the heme **physically obstructs the close approach of two hemes**, so that the sandwich arrangement that is necessary for oxidation can't form.

As a result, heme- Fe^{2+} within a globin protein is **not easily oxidized**, and instead can **bind and release O_2 in a reversible manner**. This is key to the function of these proteins.

