# Oxygen-Binding Proteins

- Myoglobin, Hemoglobin, Cytochromes bind $O_2$.
- Oxygen is transported from lungs to various tissues via blood in association with hemoglobin
- In muscle, hemoglobin gives up $O_2$ to myoglobin which has a higher affinity for $O_2$ than hemoglobin.
- Oxygen-binding curve for hemoglobin is sigmoidal whereas for myoglobin it is hyperbolic. This facilitates transfer of $O_2$ to myoglobin.
- Cytochromes participate is redox reactions and are components of the electron transport chain.

# Hemoglobin Structure

- Hemoglobin is a $O_2$ transport protein found in the RBCs
- Hemoglobin is an oligomeric protein made up of 2 $\alpha\beta$ dimers, a total of 4 polypeptide chains: $\alpha_1\beta_1\alpha_2\beta_2$.
- Total $M_r$ of hemoglobin is 64,500.
- The $\alpha$ (141 aa) and $\beta$ (146 aa) subunits have < 50 % identity.
- The 3D- structures of $\alpha$ (141 aa) and $\beta$ (146 aa) subunits of hemoglobin and the single polypeptide of myoglobin are very similar; all three are members of the globin family.
- Each Hb subunit consists of 7 ($\alpha$) or 8 ($\beta$) alpha helices and several bends and loops folded into a single globin domain.
- Each subunit has a heme-binding pocket.

# The Prosthetic Heme Group

- The heme group is responsible for the $O_2$-binding capacity of hemoglobin.
- The heme group consists of the planar aromatic protoporphyrin made up of four pyrrole rings linked by methane bridges.
- A Fe atom in its ferrous state ($Fe^{+2}$) is at the center of protoporphyrin.
- $Fe^{+2}$ has 6 coordination bonds, four bonded to the 4 pyrrole N atoms.  The nucleophilic N prevent oxidation of $Fe^{+2}$.
- The two additional binding sites are one on either side of the heme plane.
- One of these is occupied by the imidazole group of His.
- The second site can be reversibly occupied by $O_2$, which is hydrogen-bonded to another His.

# Different forms of Hemoglobin

- When hemoglobin is bound to $O_2$, it is called oxyhemoglobin. This is the relaxed (R ) state.
- The form with a vacant $O_2$ binding site is called deoxy-hemoglobin and corresponds to the tense (T) state.
- If iron is in the oxidized state as $Fe^{+3}$, it is unable to bind $O_2$ and this form is called as methemoglobin
- CO and NO have higher affinity for heme $Fe^{+2}$ than $O_2$ and can displace $O_2$ from Hb, accounting for their toxicity.

# T and R states of Hemoglobin

- Hemoglobin exists in two major conformational states: Relaxed (R ) and Tense (T)
- R state has a higher affinity for $O_2$.
- In the absence of $O_2$, T state is more stable; when $O_2$ binds, R state is more stable, so hemoglobin undergoes a conformational change to the R state.
- The structural change involves readjustment of interactions between subunits.

# Changes Induced by $O_2$ Binding

- $O_2$ binding rearranges electrons within $Fe^{+2}$ making it more compact so that it fits snugly within the plane of porphyrin.
- Since Fe is bound to histidine of the globin domain, when Fe moves, the entire subunit undergoes a conformational change.
- This causes hemoglobin to transition from the tense (T) state to the relaxed (R) state.
- The $\alpha_1\beta_1$ and $\alpha_2\beta_2$ dimers rearrange and rotate approximately 15 degrees with respect to each other
- Inter-subunit interactions influence $O_2$ binding to all 4 subunits resulting in cooperativity.

# $O_2$-binding kinetics

- Four subunits, so four $O_2$-binding sites
- $O_2$ binding is cooperative meaning that each subsequent $O_2$ binds with a higher affinity than the previous one
- Similarly, when one $O_2$ is dissociated, the other three will dissociate at a sequentially faster rate.
- Due to positive cooperativity, a single molecule is very rarely partially oxygenated.
- There is always a combination of oxygenated and deoxygenated hemoglobin molecules.  The percentage of hemoglobin molecules that remain oxygenated is represented by its oxygen saturation.
- $O_2$-binding curves show hemoglobin saturation as a function of the partial pressure for $O_2$.

# Oxygen Saturation Curve

- Saturation is maximum at very high $O_2$ pressure in the lungs ($pO_2$ = ~ 100 torr).
- As hemoglobin moves to peripheral organs and the $O_2$ pressure drops ($pO_2$ = ~20 torr), saturation also drops allowing $O_2$ to be supplied to the tissues.
- Due to co-operative binding of $O_2$ to hemoglobin, its oxygen saturation curve is sigmoid.
- Such a curve ensures that at lower $pO_2$, small differences in $O_2$ pressure result in big changes in $O_2$ saturation of hemoglobin. This facilitates dissociation of $O_2$ in peripheral tissues.

# Effectors of $O_2$ binding

- Small molecules that influence the $O_2$-binding capacity of hemoglobin are called effectors (allosteric regulation).
- Effectors may be positive or negative; homotropic or heterotropic effectors.
- Oxygen is a homotropic positive effector.
- Positive effectors shift the $O_2$-binding curve to the left, negative effectors shift the curve to the right.
- From a physiological view, negative effectors are beneficial since they increase the supply of oxygen to the tissues.

# The Bohr Effect

- The regulation of $O_2$-binding to hemoglobin by $H^+$ and $CO_2$ is called the Bohr effect
- Both $H^+$ and $CO_2$ are negative effectors of $O_2$-binding.
- Addition of a proton to His imidazole group at C-terminus of β-subunit facilitates formation of salt bridge between His and Asp and stabilization of the T quaternary structure of deoxyhemoglobin.
- $CO_2$ reduces $O_2$ affinity by reacting with terminal $–NH_2$ to form negatively charged carbamate groups
- Metabolically active tissues need more $O_2$; they generate more $CO_2$ and $H^+$ which causes hemoglobin to release its $O_2$.

# 2,3-Bisphosphoglycerate

- 2,3-Bisphosphoglycerate is a negative effector.
- A single 2,3-BPG binds to a central pocket of deoxyhemoglobin and stabilizes it by interacting with three positively charged aa of each β-chain.
- 2,3-BPG is normally present in RBCs and shifts the $O_2$-saturation curve to the right
- Thus, 2,3-BPG favors oxygen dissociation and therefore its supply to tissues
- In the event of hypoxia, the body adapts by increasing the concentration of 2,3-BPG in the RBC

# Fetal Hemoglobin

- Fetal hemoglobin has 2 $\alpha$ and 2 $\gamma$ chains
- The g chain is 72% identical to the b chain.
- A His involved in binding to 2,3-BPG is replaced with Ser. Thus, fetal Hb has two less + charge than adult Hb.
- The binding affinity of fetal hemoglobin for 2,3-BPG is significantly lower than that of adult hemoglobin
- Thus, the $O_2$ saturation capacity of fetal hemoglobin is greater than that of adult hemoglobin
- This allows for the transfer of maternal $O_2$ to the developing fetus