**Protein Function**

Function relies on interactions with other molecules
Binding of molecules to proteins is reversible

Ligand = molecule bound reversibly by a protein
Binding site = site on protein where ligand binds

HIV protease + drug inhibitor
Protein Function

Induced fit = conformational change on binding of protein and ligand, makes binding site more complementary to ligand

Enzymes are Proteins

Enzymes have a special kind of protein function  
Enzymes bind and chemically transform molecules - catalyze reactions  
Enzymes bind and act on substrates (instead of ligand)  
Enzymes have catalytic or active sites (instead of binding site)
Protein Function

Myoglobin
Protein Function

Myoglobin (also hemoglobin)

Protein can carry and store oxygen
Oxygen poorly soluble in aqueous solution (cannot be carried by blood)
How does protein bind oxygen? Uses heme (Fe$^{2+}$)
Myoglobin/hemoglobin - Found in almost all higher organisms

Protoporphyrin IX

Fe$^{2+}$
**Protein Function**

**Myoglobin (also hemoglobin)**

Iron in Fe$^{2+}$ state binds oxygen reversibly.

O$_2$ bound - electronic properties of heme change
Purple O$_2$-depleted blood --> red O$_2$-rich blood

Other small molecules can bind to heme:
CO and NO can also coordinate to heme (toxic)
Oxygen/Carbon Monoxide Binding to Heme

(a)

(b)

(c)
Protein Function
Myoglobin

Single binding site for \( O_2 \)
Measure binding

**Protein** \( \text{Ligand} \)

\[
\text{Mb} + \quad O_2 \quad \rightleftharpoons \quad \text{Mb-O}_2
\]

Equilibrium expression:

**Association Constant**

\[
K_a = \frac{[\text{Mb-O}_2]}{[\text{Mb}] \cdot [O_2]} \quad \left\{ \frac{[\text{PL}]}{[P] \cdot [L]} \right\}
\]

\[
[L] \quad K_a = \frac{[\text{PL}]}{[P]}
\]

Ratio of bound-free protein \( \propto [L] \)

**Saturation**

\[
\theta = \frac{\text{binding site occupied}}{\text{total binding sites}} = \frac{[\text{PL}]}{[\text{PL}] + [P]}
\]

\[
\theta = \frac{K_a [P][L]}{K_a [P][L] + [P]} = \frac{K_a [L]}{K_a [L] + 1} = \frac{[L]}{[L] + \frac{1}{K_a}}
\]
Protein Function
Myoglobin

\[ \theta = \frac{[L]}{[L] + \frac{1}{K_a}} \]

Equation describes a hyperbola

\[ \frac{1}{K_a} (K_d) \text{ is equal to: } [L] \text{ where half of the available ligand-binding sites are occupied (} \theta = 0.5) \]

Substitute \([O_2]\) for \(L\)
\([O_2] \propto \text{partial pressure } O_2\)
Protein Function
Myoglobin vs. Hemoglobin

Differences in protein structure
Hb - 4 subunits, 2α and 2β (better suited for O₂ transport); 4 heme groups
Mb - 1 subunit (O₂ storage) so 1 heme group
Protein Function
Myoglobin vs. Hemoglobin

Differences in binding of oxygen
Mb - hyperbolic binding curve for $O_2$, insensitive to small changes in $[O_2]$
Hb - sigmoidal binding curve for $O_2$, highly sensitive response to changes in $[O_2]$
Protein Function
Hemoglobin

Quartenary structure
Protein Function
Hemoglobin

Structural states of hemoglobin before and after $O_2$ binding

T (tense) state  deoxyHb, no $O_2$ bound
stabilized by ion pairs between subunits

R (relaxed) state  oxyHb, $O_2$ bound, higher affinity for $O_2$
destabilize some ion pairs, form some new ones
Protein Function
Hemoglobin

Hb must bind O$_2$ efficiently in lungs (pO$_2$ ~13.3 kPa) and release it in tissues (pO$_2$ ~4 kPa)

Cooperative binding (O$_2$ to Hb) is a type of allosteric binding

**Allosteric proteins**
1. binding of ligand to one site affects binding properties of another site on same protein (Hb)
2. have “other shapes” induced by binding of ligands
Carbon Monoxide Binding to Heme

CO - colorless, odorless gas
250-fold greater affinity for Hb
High levels a result of incomplete combustion of fossil fuels
Tight binding of CO to Hb

Healthy person 1% CO-Hb, Smokers 3-8% CO-Hb
Chain smokers 15% CO-Hb

<10% few symptoms, 15% mild headaches
20-30% severe headache, nausea, dizziness, etc.
30-50% severe neurological symptoms, unconscious, coma
60% death

Greater risk - smokers, heart/lung/blood disease, fetus
**Hemoglobin**

Fetal vs. Maternal

Fetal Hb must have greater affinity for $O_2$ than maternal Hb. In fetus, no $\beta$ subunits but $\gamma$ instead ($\alpha_2\gamma_2$) ($\alpha_2\gamma_2$) tetramer has lower affinity for BPG, so higher affinity for $O_2$. After birth, $\gamma$ subunits no longer made, $\beta$ subunits synthesized.
Hemoglobin Effectors

1. $H^+$

2. $CO_2$

$$CO_2 + H_2O \leftrightarrow HCO_3^- + H^+$$

3. 2,3-bisphosphoglycerate (BPG)

All these effectors lower hemoglobin’s affinity for oxygen.
Hemoglobin Effectors

Hb transports ~40% of total H+, rest of H+ absorbed by plasma bicarbonate buffer
Hb transports ~20% of the CO₂ to lungs and kidneys, rest of CO₂ transported as dissolved HCO₃⁻ and CO₂

binding of H+ and CO₂ is inversely related to binding of O₂

BOHR EFFECT

Effect of pH ([H⁺]) and [CO₂] on binding and release of O₂ by Hb
Hemoglobin Effectors

Blood

Tissues

Lungs

Tissues - lower pH and higher [CO₂], affinity of Hb for O₂ decreases as H⁺ and CO₂ are bound, O₂ released to tissues

Lung - higher pH and CO₂ excreted, affinity of Hb for O₂ increases and Hb binds more O₂ and releases H⁺

\[
\text{CO}_2 + \text{H}_2\text{O} \quad \leftrightarrow \quad \text{HCO}_3^- + \text{H}^+ \text{ (lower pH)}
\]

Hb - communicates ligand binding information throughout subunits to integrate transport of CO₂, O₂, H⁺ by blood
2,3-bisphosphoglycerate (BPG)
BPG lowers affinity of Hb for O\textsubscript{2}
[BPG] high in red blood cells

BPG binds at different site than O\textsubscript{2}-binding site and regulates O\textsubscript{2}-binding affinity of Hb
What is BPG? Allosteric effector

\[
\text{HbBPG} + \text{O}_2 \rightleftharpoons \text{HbO}_2 + \text{BPG}
\]
**Hemoglobin**

**Sickle Cell Anemia**

Genetic disease in which person inherits gene for sickle-cell Hb from both parents

\[
\begin{align*}
\text{Hb} & : \text{V H L T P E E K} \\
\text{Hb-sickle} & : \text{V H L T P V E K}
\end{align*}
\]

Hb-sickle is deoxygenated, insoluble and forms polymers that aggregate
Valine has hydrophobic side chain, glutamate has negative charge
Valine creates sticky hydrophobic contact point where deoxy-Hb-sickle molecules associate forming long, fibrous aggregates

Symptoms:
- weak, dizzy, short of breath, heart murmurs
- sickle cells fragile - anemia
- capillaries blocked - abnormal organ function

Patients with sickle cell anemia have to have inherited 2 copies of mutant gene
Inherit only 1 copy - resistance to malaria