Bio-Systems Modeling and Control

Lecture 22
Enzymes
Example: Hemoglobin, Myoglobin and O_2 Transport in the Blood

Ideal Gas Law

$$PV = nKT$$

- Number of molecules of gas = n
- K=Boltzmann's constant
- T=temperature [^{0}K]
- P,V are the pressure and volume of the gas.

Ideal Gas Law Scaled

$$PV = nKT = \frac{n}{N_A}(N_A K)T$$

- $N_A \approx 6 \cdot 10^{23}$ = Avogadro's number. This is the number of molecules in <u>one mole</u> of the substance (i.e. the weight in grams which is equal numerically to the molecular weight of the molecule)
- Scaling is done because n is very large and K is very small.

Scaled Ideal Gas Law Definitions

$$PV = nKT = \frac{n}{N_A}(N_A K)T = mRT$$

- Number of moles = m = n/N_A.
- Gas Constant = R = $N_A K$
- Now the numbers are “less extreme”.
- However, we shall use more often the original form (with n), to be able to refer to the specific number of molecules.

Meaning of the Ideal Gas Law

$$PV = nKT$$

- In a fixed volume, at a fixed temperature, pressure is proportional to n, regardless if the molecule is heavy or light! Only the number of molecules matters in determining the pressure.
- We also neglect internal interaction of the gas molecules.

Partial Pressure of a Gas

$$PV = KT\sum_{j=1}^{N} n_j \Rightarrow P_j V = KTn_j \Leftrightarrow P = \sum_{j=1}^{N} P_j$$

- If we have a mix of N gases in a chamber, let n_j denote the number of molecules of gas j.
- P_j is the <u>partial pressure</u> of gas j.
- The total pressure equals the sum of all partial pressures.

Partial Pressure is a measure of the gas concentration in the chamber!

$$P_j V = KTn_j \Rightarrow P_j = \frac{n_j}{V} KT = c_j KT$$

- The concentration of the gas, c_j ,equals the number of molecules per volume.
- We see that, at a given temperature, c_j is proportional to P_j.

Partial Pressure is also a measure of the gas concentration in a solution

$$P_j V = KTn_j \Rightarrow P_j = \frac{n_j}{V} KT = c_j KT$$

- If liquid and gas are in contact across the surface of the liquid, molecules of the gas can become dissolved in the liquid, forming a <u>solution</u>.
- Likewise, gas molecules in the liquid can leave the liquid at the surface.

Gas-Liquid at Equilibrium

$$P_j V = KTn_j \Rightarrow P_j = \frac{n_j}{V} KT = c_j KT$$

- At steady-state , the rate of gas molecules leaving the liquid equals the rate of gas molecules entering the liquid.
- Concentration of gas in the liquid is related to the gas partial pressure (in the chamber containing the gas).

Simple Solutions

$$P_j = \frac{c_j}{\sigma_j}$$

- In a <u>simple solution</u> the gas concentration is linearly proportional to the gas partial pressure (in the gaseous part of the compartment).
- Constant of proportionality σ is called <u>solubility</u>

Meaning of partial pressure as measure of concentration of gas in a solution

$$P_j = \frac{c_j}{\sigma_j}$$

Given a simple solution in which concentration of gas j is c_j, P_j (above) is the partial pressure of gas j that is needed at equilibrium to maintain that concentration in the fluid. As a shortcut, we say that gas j in the solution "has partial pressure P_j"

Non-simple Solutions

$$c_j = C_j(P_j) \xleftrightarrow{?} P_j = C_j^{-1}(c_j)$$

- In a non-simple solution c_j and P_j are related via a nonlinear curve $C_j()$.
- Important example to a non-simple solution: O_2 in the blood. Here C_j is monotone increasing→ $P_j=C_j^{-1}(c_j)$.
- Many anesthetics, on the other hand, form simple solutions in the blood.

Preliminary facts about transport of oxygen in the blood

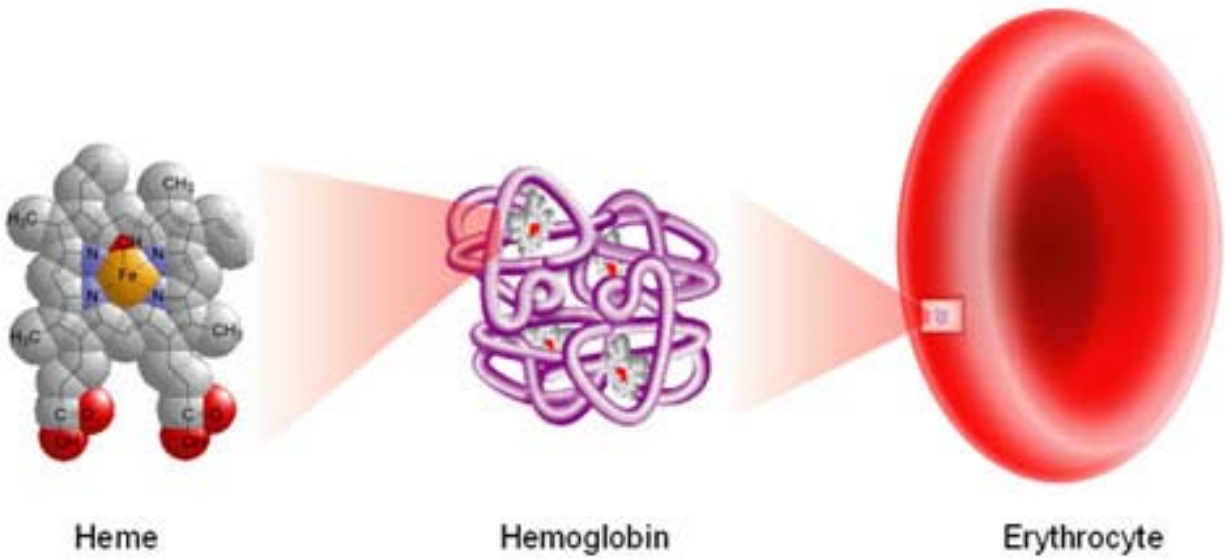
- The oxygen rich arterial blood transports the O_2 primarily in combination with hemoglobin (97%, compared with 3% carried dissolved in the blood).
- Hemoglobin molecules carry almost 100% of the O_2 needed at the tissues, where it is released for use by the cells.
- The presence of hemoglobin in the red blood cells allows the blood to transport 30-100 times more O_2, compared to O_2 molecules directly being dissolved in the water of the blood.

Red Blood Cells facts

Red Blood Cells

- Red blood cells (Erythrocytes) are small biconcave discs (diameter≈8μm).
- They are flexible→ can change shape to pass without breaking through blood vessels with diameter as small as 3μm!

Red Blood Cells and Hemoglobin

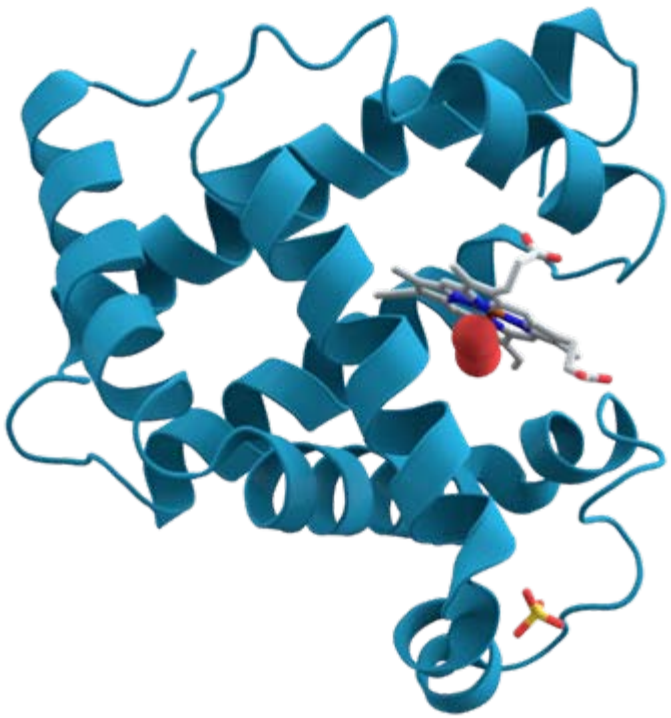


- Average volume is 90-95$(\mu m)^3$.
- Red blood cells contain the large protein molecules of hemoglobin (~340 [g/l])

Other functions of Red Blood Cells

- Red blood cells contain also large quantity of the enzyme carbonic anhydrase that catalyzes the reversible reaction between CO_2 and water.
- It makes it possible for the blood water to transport enormous quantities of CO_2 from the tissues to the lungs, in the form of bicarbonate ion.
- Hemoglobin plays an important role (second to carbonic acid bi-carbonate) acid-base buffering of the blood.

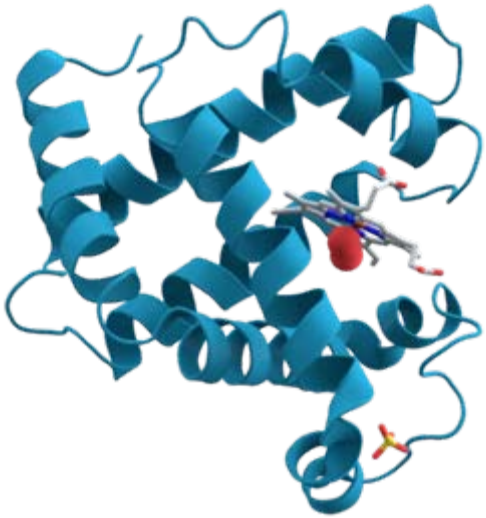
Myoglobin

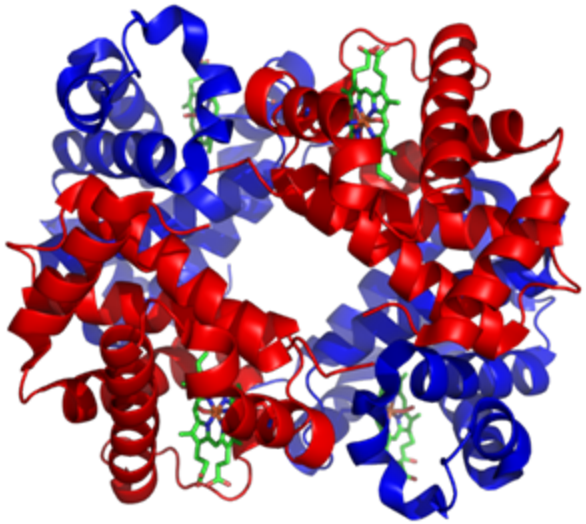


- Myoglobin is a single-chain protein used to store and transport oxygen within the muscles.
- Deep-diving mammals, such as whales and seals, have skeletal muscle that is specially rich in myoglobin.

Myoglobin vs. Hemoglobin

- Myoglobin molecule can only bind one oxygen molecule
- Hemoglobin (a four-chain protein) can bind four O_2 molecules, one to each “heme” binding site (where the Fe atom is).





Enzyme Kinetics Theory applied to Myoglobin (enzyme) and O_2 (substrate and product)

$$O_2 + Mb \underset{k_-}{\overset{k_+}{\rightleftharpoons}} MbO_2$$

Formation of MbO_2 complexes (in the lungs capillaries) is fast, and then subsequently the slower release of O_2 (in the tissues capillaries) obey the rules of simple enzyme kinetics: we have simple hyperbolic Michaelis-Menten curves that describe aspects of the process (to be discussed next)

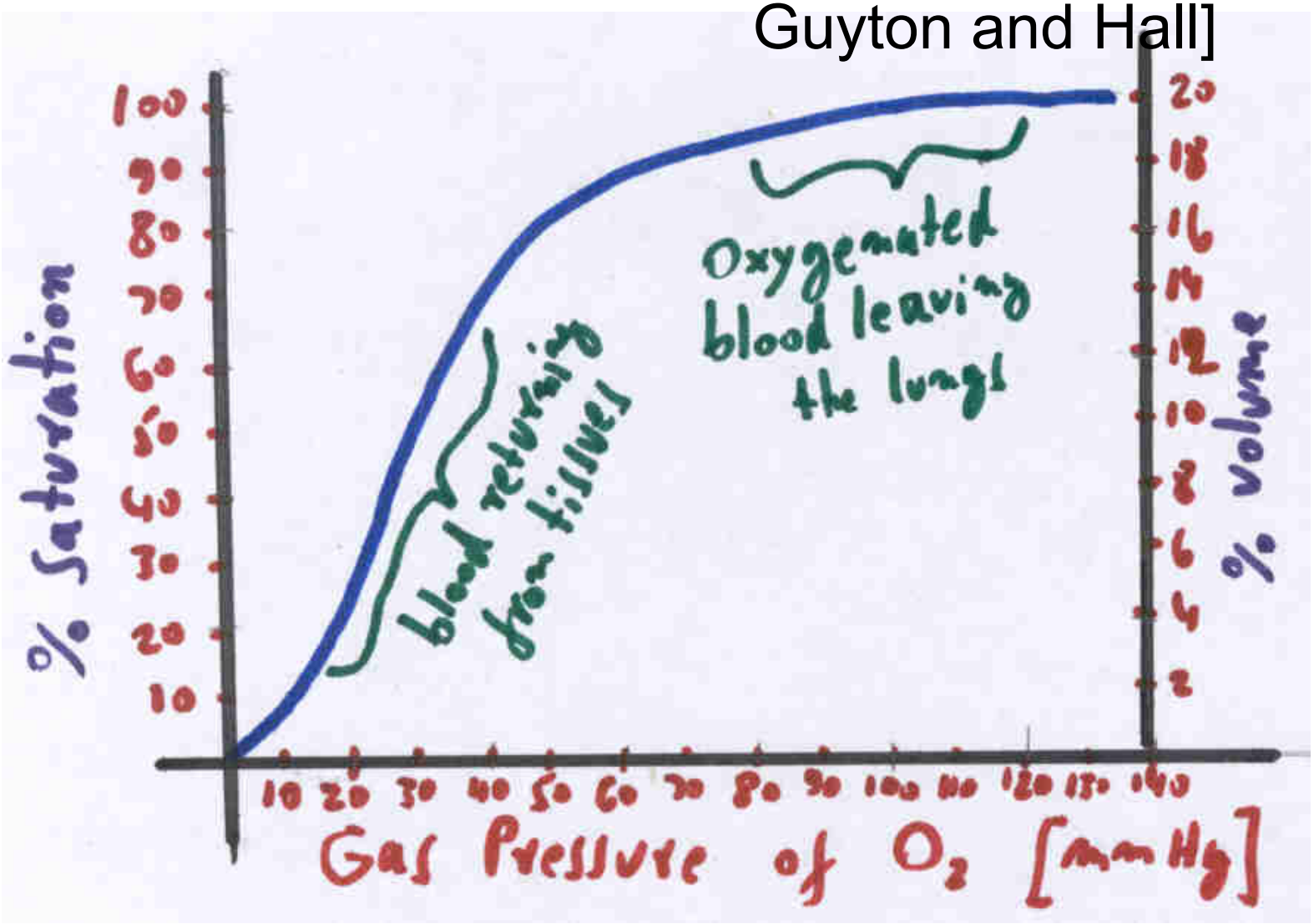
Enzyme Kinetics Theory applied to Hemoglobin (enzyme) and O_2 (substrate and product)

$$4O_2 + Hb \underset{k_-}{\overset{k_+}{\rightleftharpoons}} Hb(O_2)_4$$

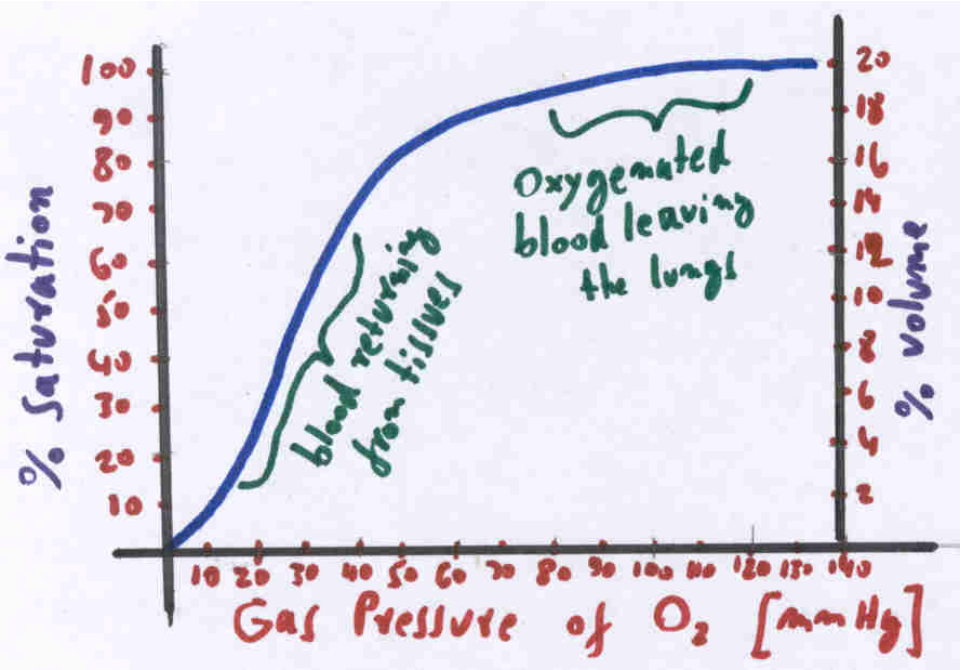
- This process has "cooperation" → Sigmoid shape input-output curves.
- There is a greatly enhanced affinity of Hb to O_2 if three Hb active sites are already filled up → Positive cooperativity.

Oxygen-Hemoglobin Dissociation Curve

[Sketched based on Guyton and Hall]

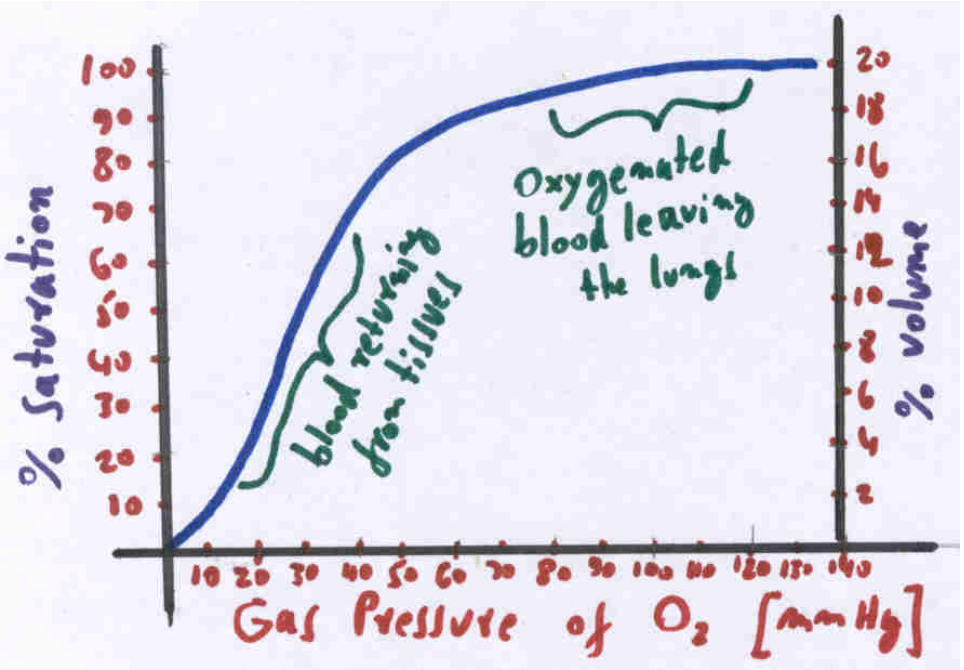


Oxygen-Hemoglobin Dissociation Curve: Systemic arteries



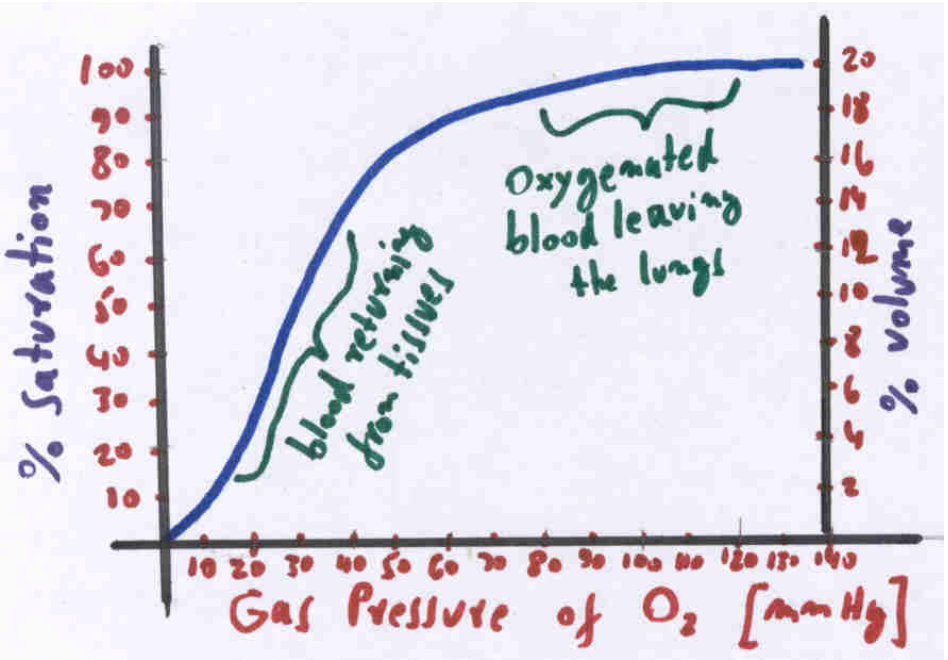
- Curve shows the % of Hb bound with O_2 as the blood partial pressure of O_2 increases.
- At PO_2=95mmHg, which is the partial pressure at the systemic arteries, about 97% of Hb is saturated with O_2.

Oxygen-Hemoglobin Dissociation Curve: Systemic Veins



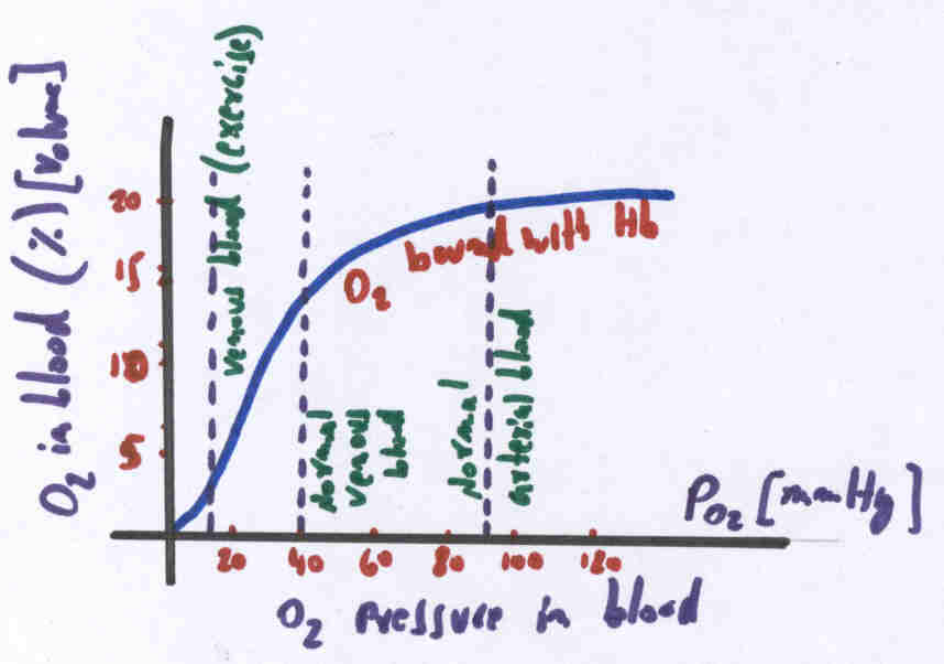
- In normal venous blood PO_2 is around 40mmHg.
- Consequently, saturation of Hb is about 75%.

Oxygen-Hemoglobin Dissociation Curve: What is "Volume percent"?



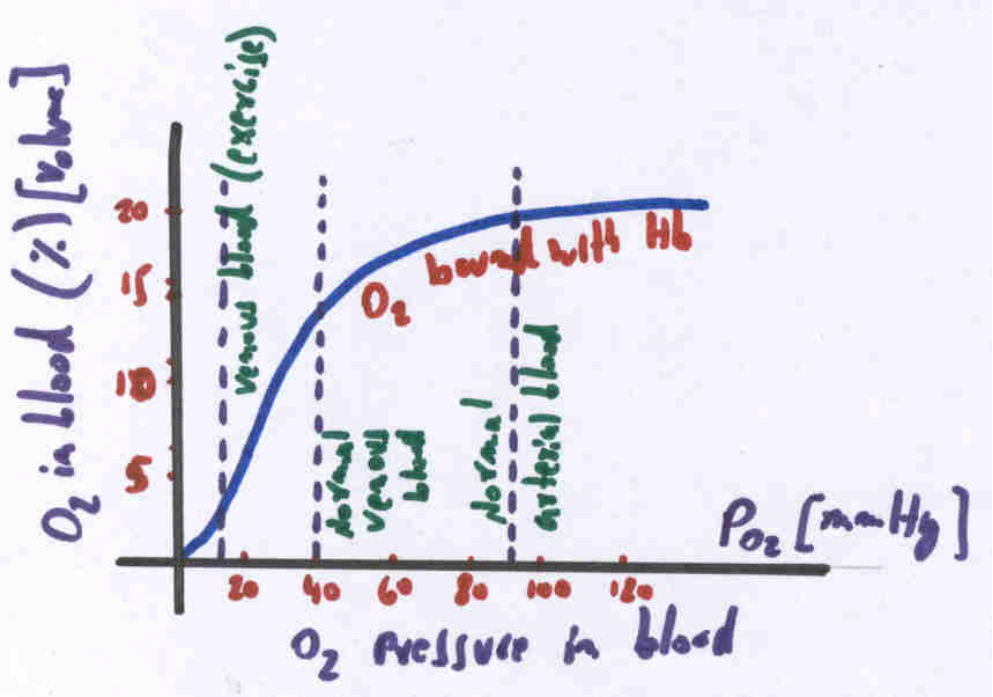
- Normal blood contains about 15g of Hb in each 100ml of blood.
- 1g of Hb can bind with at most 1.34ml of O_2 → On the average, Hb in 100ml of blood can carry about 20ml of O_2 ("20 volumes percent")

Oxygen-Hemoglobin Dissociation Curve: O_2 delivered to tissues



- On passing through the tissue capillaries, the amount of O_2 is reduced from 19.4ml O_2 ,carried by 100ml of arterial blood, to 14.4ml (corresponds to PO_2=40mmHg and 75% saturated Hb).
- Every 100ml of blood delivers ~5ml O_2 to tissues.

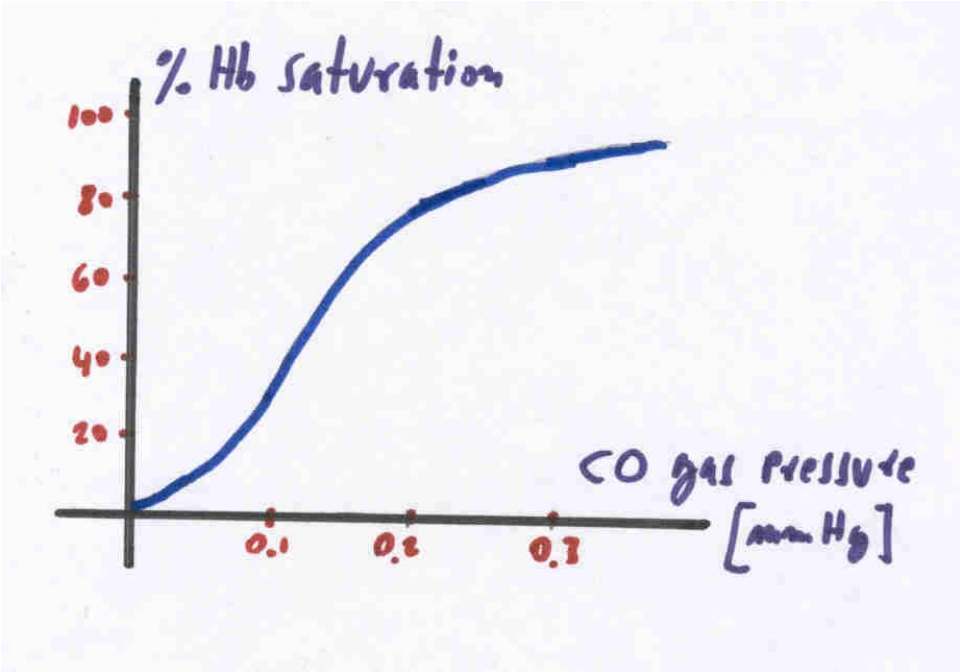
Oxygen-Hemoglobin Dissociation Curve: O_2 delivery during exercise



- During heavy exercise, muscle cells use O_2 at higher rate. PO_2 may fall to 15mmHg, amounting to 4.4ml O_2 bound to Hb, and a net delivery of 15ml O_2 to tissues.

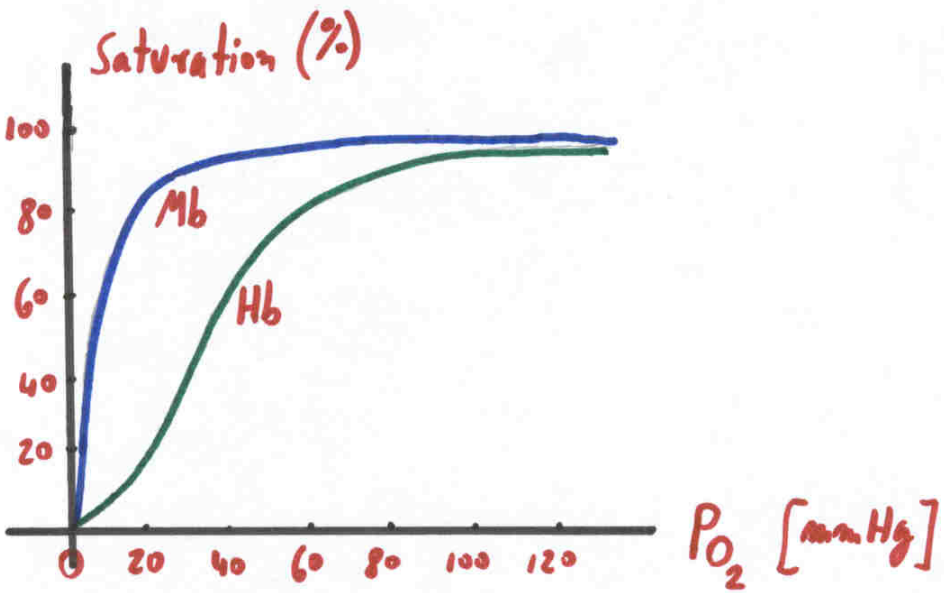
Carbon Monoxide – Hemoglobin Dissociation Curve

- The poison gas CO combines with Hb at same sites as O_2, displacing O_2.
- CO pressure of only 0.4mmHg allows CO to compete equally with O_2 (occupying about 50% of Hb). CO Pressure of 0.6mmHg (0.1% of air) is lethal.



Uptake of O_2 by Mb and Hb

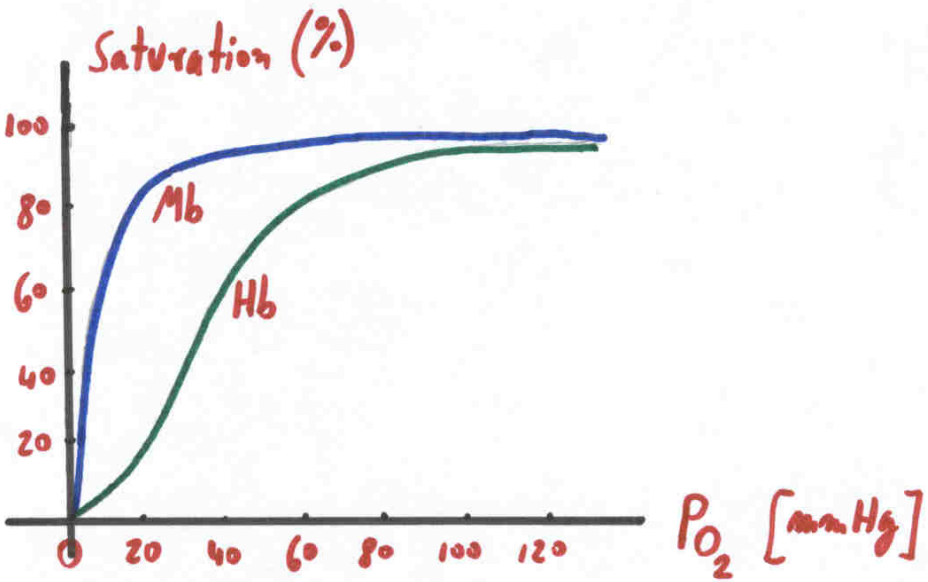
- When PO_2 = 100 mmHg (as in the lungs), Hb is 97% saturated. This varies very little as PO_2 changes in that region.
- At PO_2=40mmHg, we are on the steeper part of the Hb curve.
- What does it mean?



[Sketch based on Keener and Sneyd]

Release of O_2 by Mb and Hb

- When PO_2 = 20 mmHg Hb gives up its O_2 readily.
- At PO_2=20mmHg, Mb is still at 90% saturation.
- In muscle tissue O_2 is transferred from Hb to Mb, as Mb affinity to O_2 is greater than Hb's affinity.



Mb-O_2 dissociation curve

$$O_2 + Mb \underset{k_-}{\overset{k_+}{\rightleftharpoons}} MbO_2$$

$$equilibrium: _ k_+[Mb][O_2] = k_-[MbO_2]$$

$$\%_occupied_sites = Y = \frac{[MbO_2]}{[Mb]+[MbO_2]} = \frac{[O_2]}{K+[O_2]}$$

$$K = \frac{k_-}{k_+}$$

Simple Michaelis-Menten curve

Mb-O_2 dissociation curve with linear solubility model

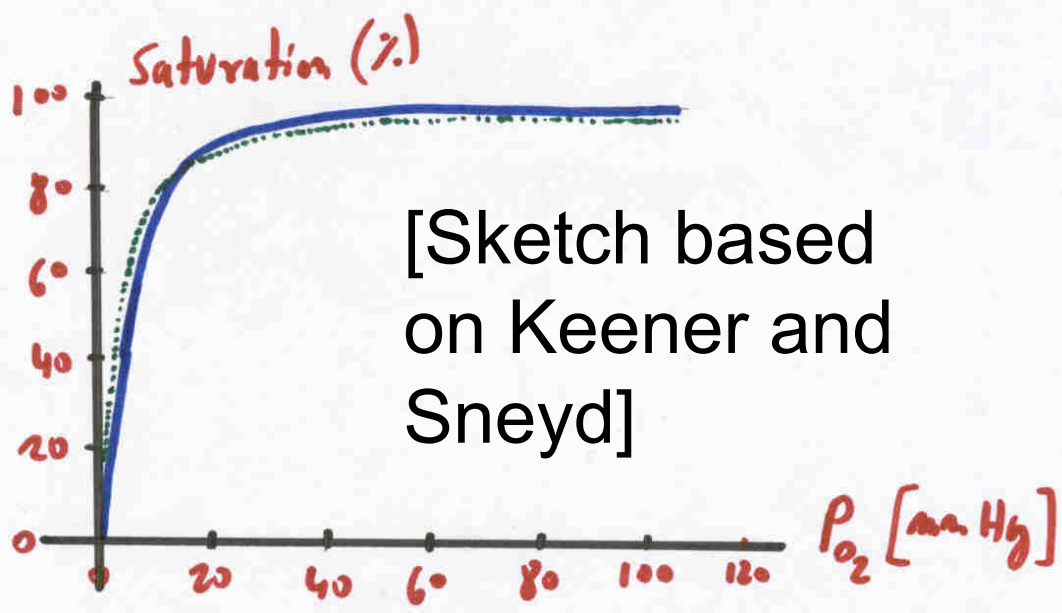
$$O_2 + Mb \underset{k_-}{\overset{k_+}{\rightleftharpoons}} MbO_2$$

$$\%_occupied_sites = Y = \frac{[MbO_2]}{[Mb]+[MbO_2]} = \frac{[O_2]}{K+[O_2]}$$

$$K = \frac{k_-}{k_+}$$

$$[O_2] = \sigma P_{O_2} \Rightarrow Y = \frac{P_{O_2}}{\frac{K}{\sigma} + P_{O_2}} = \frac{P_{O_2}}{K_P + P_{O_2}}$$

Parameter fitting for the Mb-O_2 dissociation curve



[Sketch based on Keener and Sneyd]

$$Y = \frac{P_{O_2}}{K_P + P_{O_2}}$$

- A good fit constant is K_P=2.6mmHg
- Solubility of O_2 in blood plasma = $\sigma = 1.4 \cdot 10^{-6}$ [M/mmHg].
- A good fit for K is K=2.6σ.

Hb-O_2 dissociation curve

$$4O_2 + Hb \underset{k_-}{\overset{k_+}{\rightleftharpoons}} Hb(O_2)_4$$

$$\frac{d[Hb]}{dt} \approx k_-[Hb(O_2)_4] - k_+[Hb][O_2]^4$$

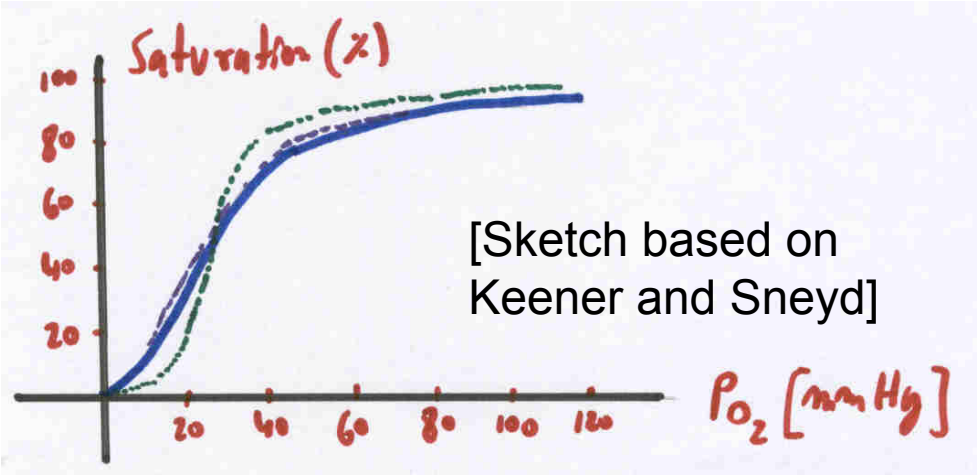
$$\%_occupied_sites = Y \approx \frac{[Hb(O_2)_4]}{[Hb]+[Hb(O_2)_4]} = \frac{[O_2]^4}{K^4+[O_2]^4}$$

$$K^4 = \frac{k_-}{k_+}$$

Cooperation type Michaelis-Menten curve

Parameter fit for Hb-O_2 dissociation curve

$$Y \approx \frac{[O_2]^4}{K^4 + [O_2]^4}$$

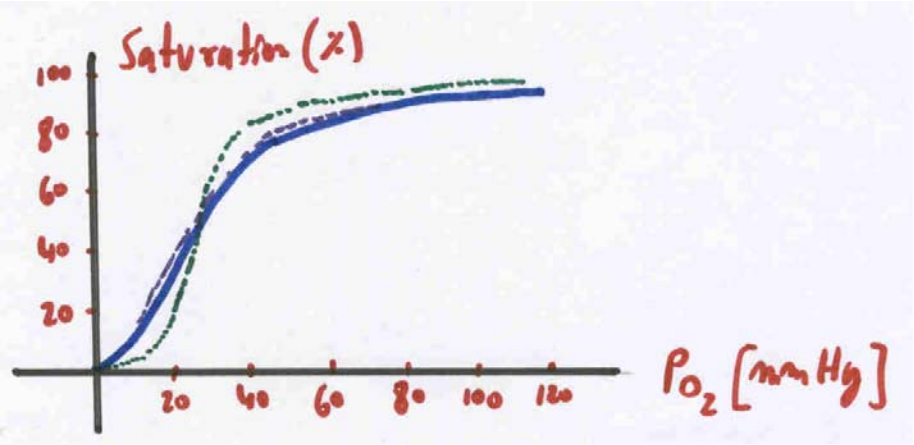


[Sketch based on Keener and Sneyd]

- Here a good fit comes with K=26σ mmHg → the green broken-dot curve.
- A better fit arises, if power 4 is replaced by a Hill curve with n=2.5, keeping the same value of K.

Hb-O_2 dissociation curve using Hill's curve

$$Y \approx \frac{[O_2]^n}{K^n + [O_2]^n}$$



- Here a good fit comes with K=26σ mmHg.
- A better fit arises, if power 4 is replaced by a Hill curve with n=2.5, keeping the same K.
- Due to cooperation, n is no longer integer → fitted curve very close to measured curve