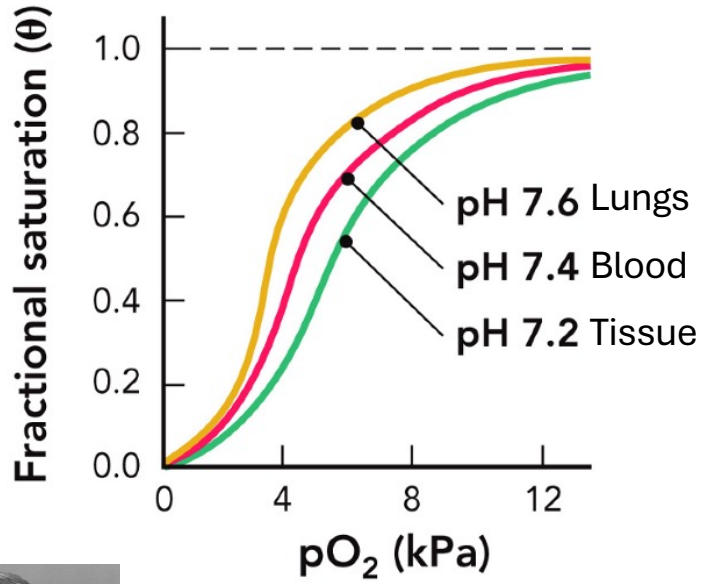
BIOC 384: M04.T06-Miesfeld

Assigned Reading: *Biochemistry* Chapter 6.2c

Allosteric Control of O_2 Transport by Hemoglobin

The Big Picture

- Oxygen, CO_2, H^+, and 2,3-BPG interact with hemoglobin to shift its structure between R and T states.
- These molecular interactions allow hemoglobin to match O_2 delivery with the metabolic demands of tissues.

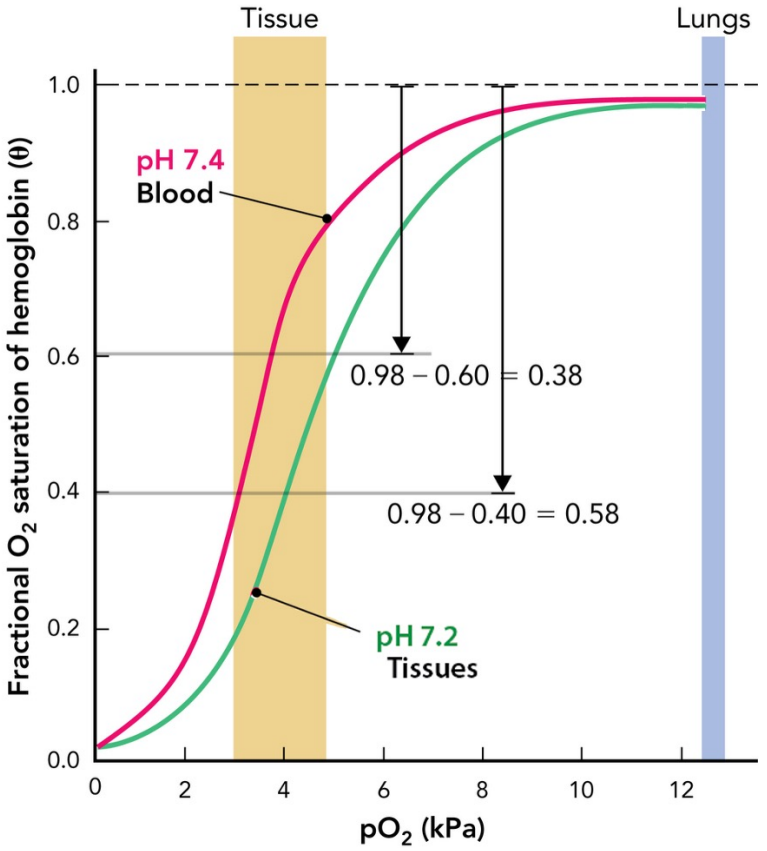


"The Bohr effect"
pH affects O_2 affinity

Christian Bohr

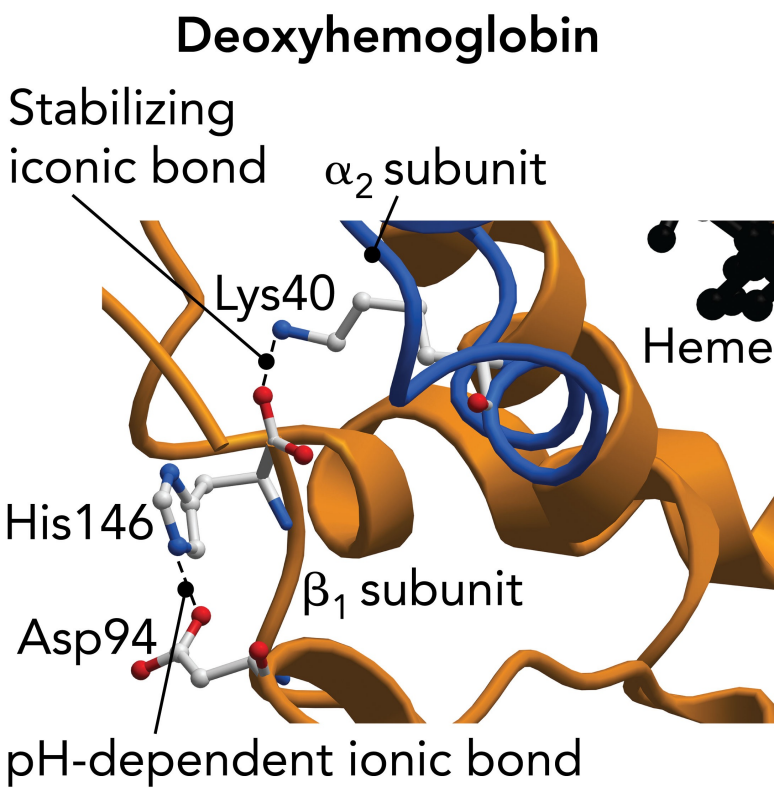
pH Dependence of O_2 Binding: Bohr Effect

- The Bohr effect describes how in addition to a drop in O_2 concentration in tissues compared to lungs, there is also a decrease in pH in the tissue (7.2) compared to the lungs (7.6).
- Comparing O_2 saturation in lung versus blood there is a 40% reduction due primarily to a drop in O_2 concentration from 12 kPa to 4 kPa.
- There is another drop of 20% in O_2 saturation at 4 kPa when comparing blood at pH 7.4 to tissue at pH 7.2, which illustrates the Bohr effect.



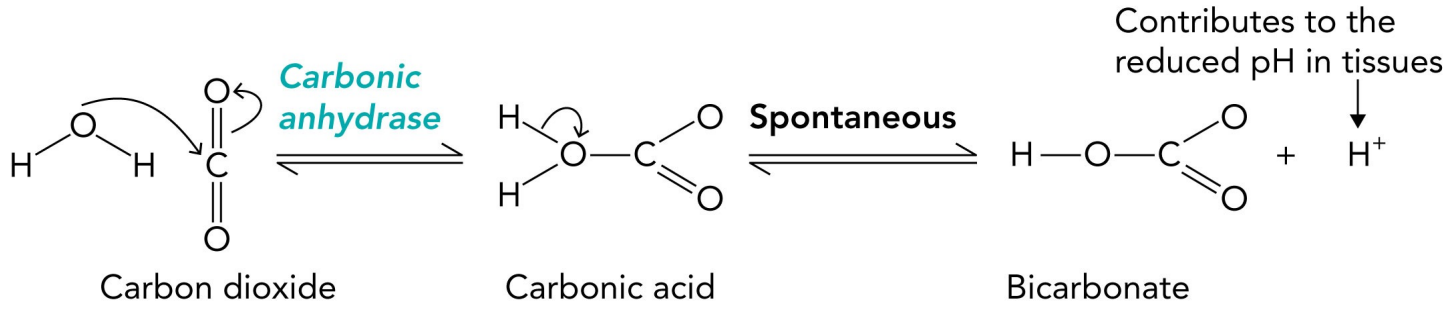
Molecular Basis of the Bohr Effect

- Lower pH leads to protonation of His146 in the β_1 subunit, which forms an ionic bond with Asp94, stabilizing the T state.
- A second ionic bond with Lys40 in the α_2 subunit positions His146 properly, which contributes significantly to the pH-sensitive conformational change.



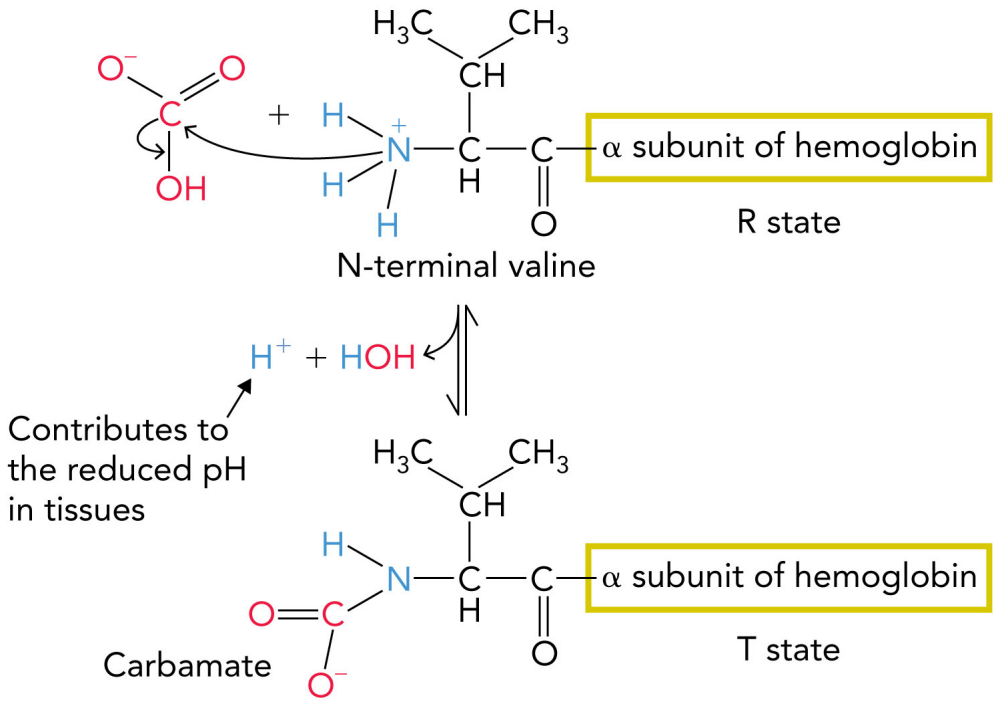
CO_2 Transport and Tissue pH

- A primary by-product of aerobic respiration is CO_2, which is hydrated by carbonic anhydrase to form highly soluble bicarbonate (HCO_3^-).
- In addition to forming bicarbonate from $CO_2 + H_2O$, carbonic anhydrase produces H^+, which helps reduce the pH in the tissues.



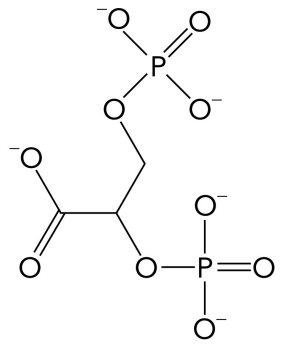
Carbamate Formation on N-term Valine

- Bicarbonate generates a carbamate group on the N-terminal residue (Val) of all four hemoglobin subunits.
- This reversible reaction releases H^+ that contributes to the Bohr effect and stabilizes the T state in tissues.

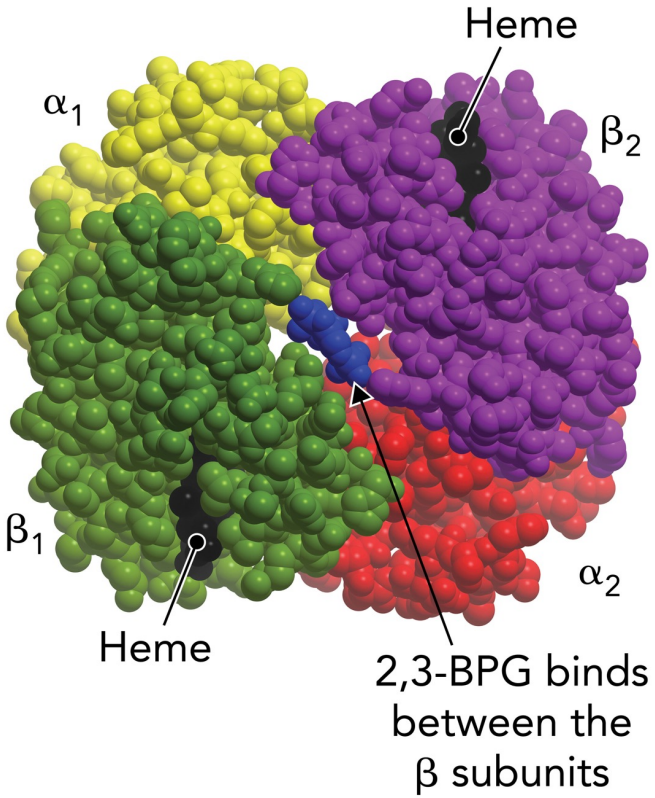


2,3-BPG Stabilizes the T State Conformation

- 2,3-BPG is a negative heterotropic regulator that binds the central cavity of deoxyhemoglobin, stabilizing the T state.
- Only one 2,3-BPG molecule binds per tetramer, but its effect is sufficient to reduce O_2 binding to all subunits.

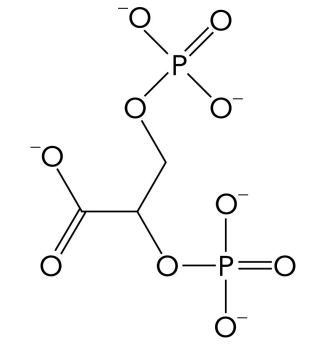


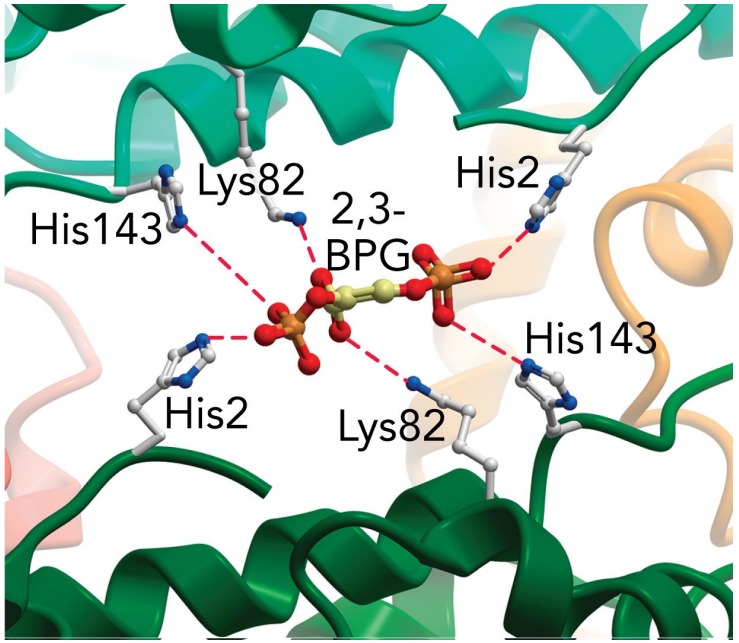
2,3-Bisphosphoglycerate
(2,3-BPG)



Structural Basis for 2,3-BPG Binding

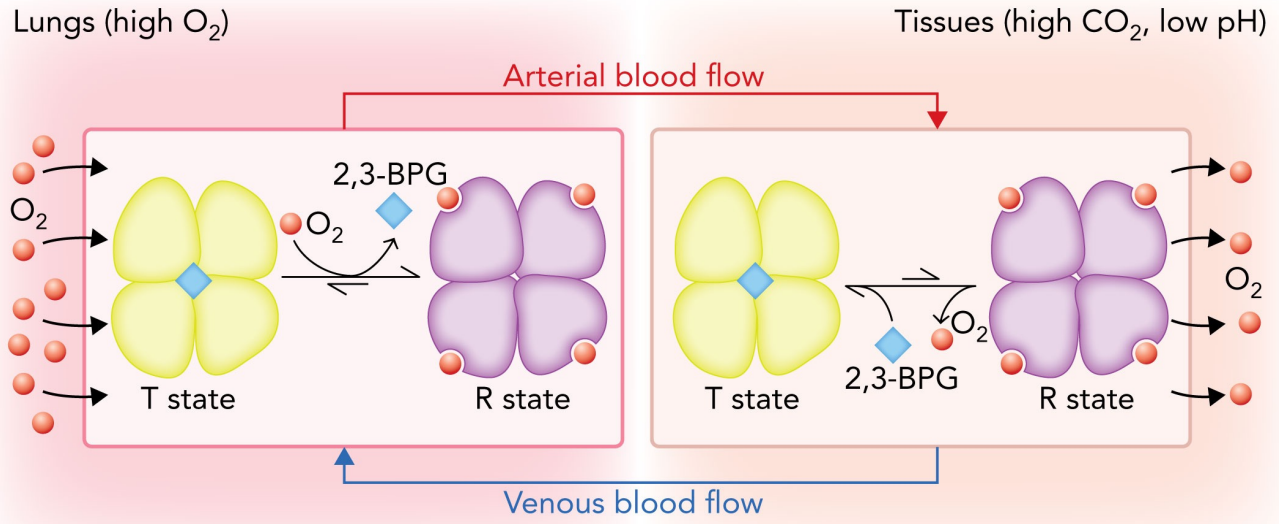
- 2,3-BPG is negatively charged and interacts with His2, Lys82, and His143 on both β subunits.
- These ionic interactions stabilize the T state, lower O_2 affinity, and promote O_2 unloading in tissues, especially under low O_2 conditions.





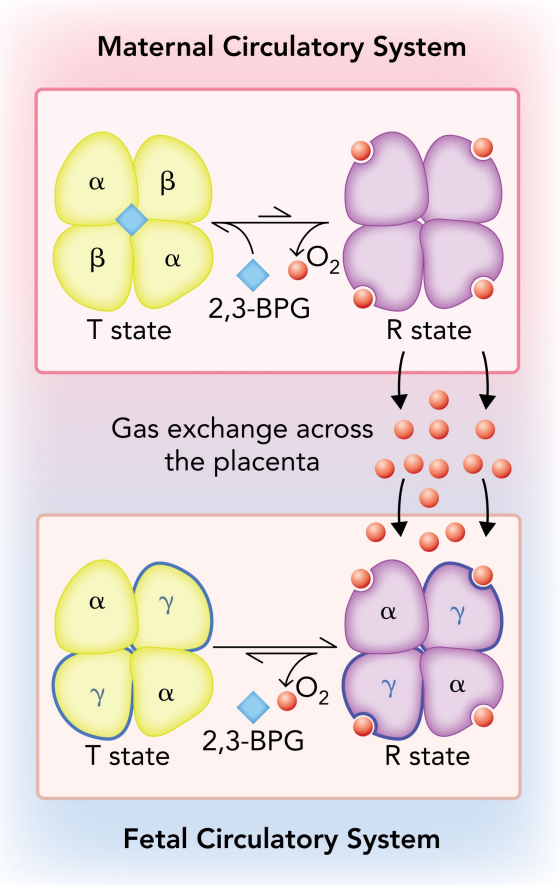
Changes in O_2 Affinity Mediate O_2 Delivery

- 2,3-BPG levels remain constant in tissues and lungs; O_2 affinity is lower in tissues due to low O_2 concentration and the pH dependence of the Bohr effect.
- The function of 2,3-BPG is to maintain T state stability, maximizing O_2 release when hemoglobin encounters low pO_2 and high CO_2/H^+ in tissues.



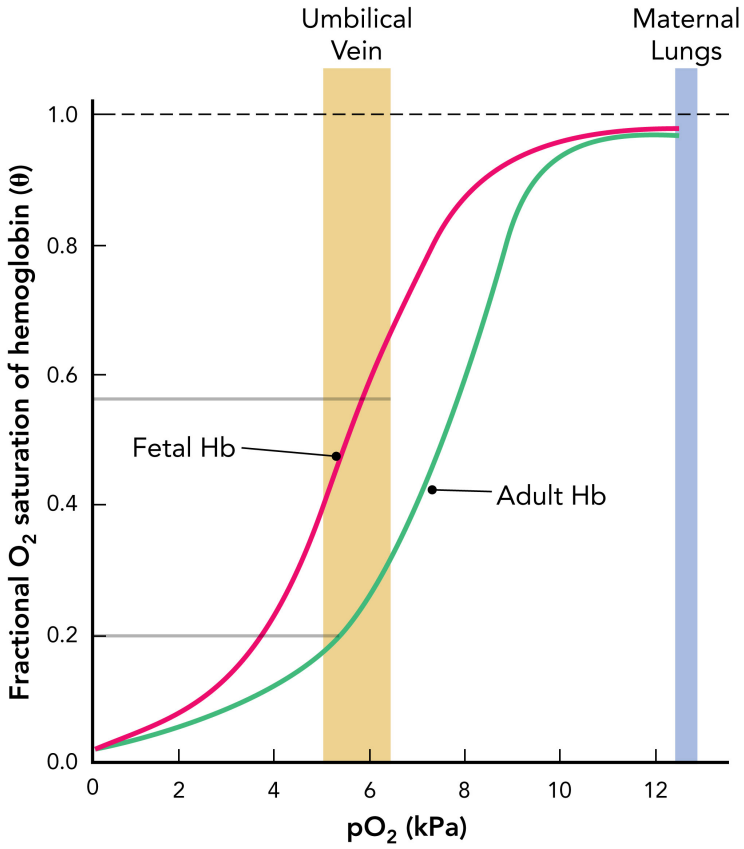
Fetal Hemoglobin has a Higher O_2 Affinity

- Fetal hemoglobin ($\alpha_2\gamma_2$) has reduced 2,3-BPG affinity because His143 → Ser143 in γ subunits eliminates two positive charges.
- This promotes more R state hemoglobin in the fetus, enhancing O_2 transfer from mother to fetus across the placenta.



O_2 Saturation Curves: Fetal vs. Adult Hb

- At pO_2 = 5.7 kPa (umbilical vein), fetal hemoglobin exhibits higher O_2 saturation than adult hemoglobin.
- This differential O_2 affinity facilitates efficient maternal-to-fetal O_2 delivery, a critical adaptation during gestation.



Key Concepts to Guide Your Learning

- Allosteric mechanisms control the ratio of T and R conformational states by shifting the conformational equilibrium, with O_2 being a positive allosteric regulator and CO_2, H^+, and 2,3-BPG being negative allosteric regulators.
- The Bohr effect describes the effect of reduced pH on O_2 saturation, which can best be seen in reduced O_2 saturation between blood (pH 7.4) and tissues (pH 7.2) at the same O_2 concentration of 4 kPa.
- Increased CO_2 levels in tissues also contributes to decreased O_2 saturation by shifting Hb to the T state through carbamate formation of the N terminal Val of hemoglobin subunits; this also increases H^+ concentration to reduce pH.
- 2,3-BPG is negatively charged and binds to Lys and His residues in β subunits in deoxyhemoglobin, which stabilizes the T-state conformation; fetal hemoglobin ($\alpha_2\gamma_2$) does not bind 2,3-BPG thereby favoring the R state conformation.