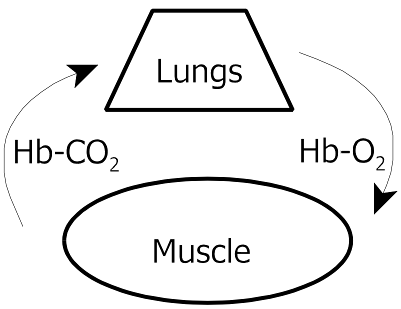
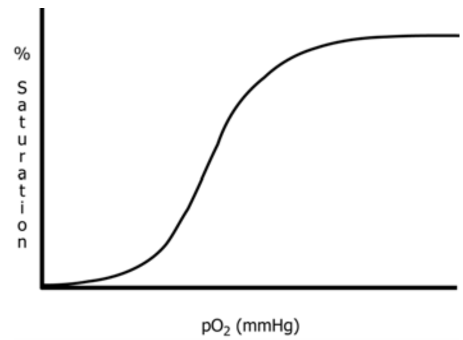
Hemoglobin in a Nutshell

The Raw Facts:

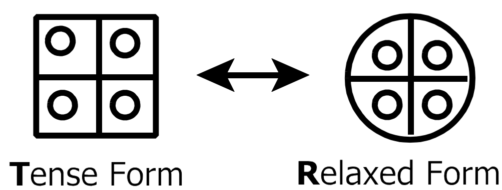
- 4 chains, each has a heme group.
- 2 α chains, 141 AA each
- 2 β chains, 146 AA each
- Each chain resembles a myoglobin chain
- There is high AA conservation in mammals around the heme and the subunit interface sites
- Each heme group consists of 4 pyrole rings surrounding the Fe^{2+} ion.





Special Allostery:

- In order to prove an efficient carrier of substances, Hb exhibits allostery.
- at low partial pressures of gas, it releases its load
- at high partial pressures of gas, it picks up a load
- In the lungs the pO_2 is 100 mmHg, while in the muscles its 20-40 mmHg
- This can be explained by the so called T & R forms:



- In the T form, Hb does not bind readily
  - The iron is stablized by His F8 (penta-coodrinate)
  - 4 unpaired e^- in the d orbitals
- In the R form, Hb does bind readily

What Happens When We Bind Oxygen:

- We “favor” the R form
- The d orbital e^- rearrange
- The iron is now also stabilized by the substrate (hexa-coordinate)
- It is now diamagnetic
- Radius of the Fe^{2+} decreases allowing it to fall
- The $\alpha_1\beta_2$ subunit rotates 15°
- 8 salt bridges are broken
- The hole in the center gets smaller

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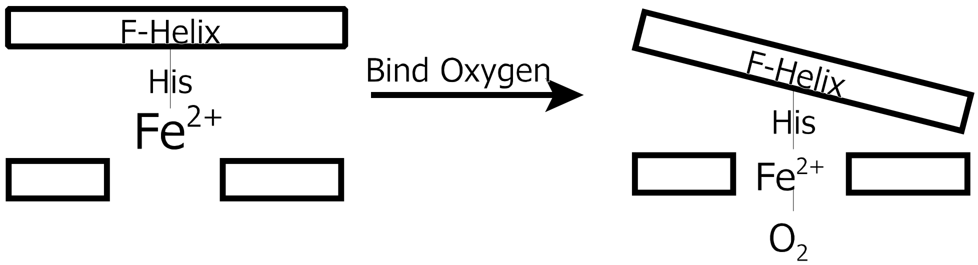
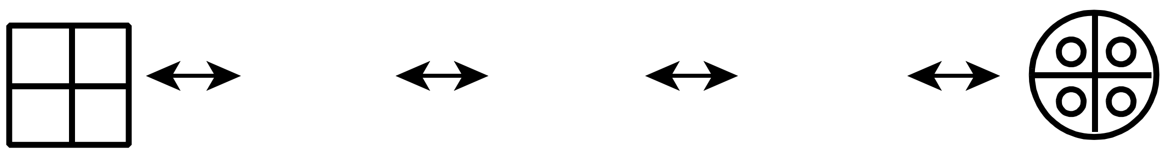


Sequential Model of Coopertivity:

- Each time something binds, we have an effect on the subunits around it.
- Each binding can have either a positive or negative effect
- We have "mixed states"

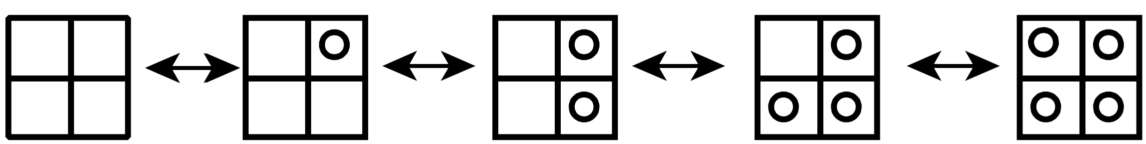
Concerted Model of Coopertivity:

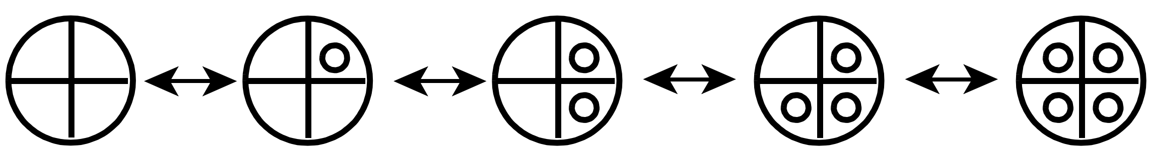


T Form

R Form

- Only two forms, T & R, no intermediates
- The more things bound, the more the equilibrium favors the "R" state





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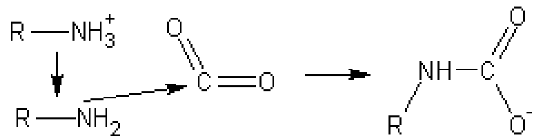
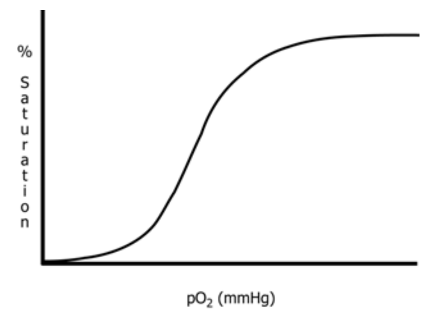
Coopertivity Mathematically:

- Hill Plot
- Review Derivation from Notes
- Y (fraction of Hb bound to O_2) = $(Hb{\cdot}O_2)/[(Hb{\cdot}O_2)(Hb)]$
  - $Y = (pO_2)^n/[(pO_2)^n + (K_D)^n]$
- Plot log Y/(1-Y) vs. log pO_2
- Slope = n, hill coefficient
  - $n < 1$, negative coopertivity
  - $n = 1$, no coopertivity
  - $n > 1$, positive coopertivity

Effects of CO_2 on Hb:

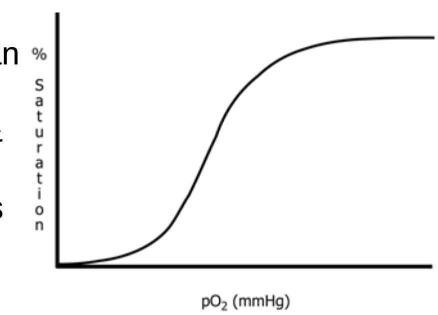
- CO_2 reacts with the terminal NH^{3+} on the Beta Chains to form a carbamate
- (+) going to (-)





Effects of pH on Hb:

- 0.5 H^+ taken up for each O2 released
- deoxygenated form binds more protons than oxygenated form
- due to change in pKa of both the His 146 & α-amino terminus
  - The His 146, when protonated forms a salt bridge with Asp 94



	T form	R form
His 146 β	8.0	7.1
α-amino	7.8	7.0

- remember the golden rule: pH < pKa, then protonate
- so at pH ≈ 7.3, when we raise the pKa above it, we protonate it

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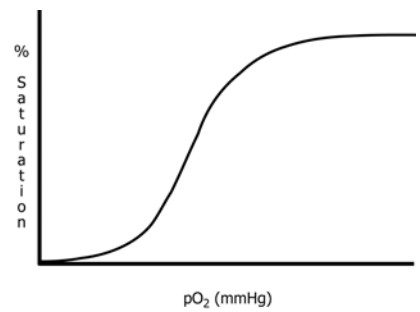
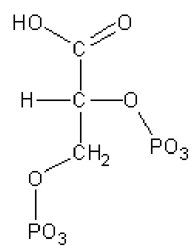
Effects of BPG on Hb:



- Bis phosphoglycertate, or 2,3 diphosphoglycerate
- found in RBC at the same concentration as Hemoglobin
- use 1 molecule per Hb tetramer
- Binds to positively charged Lys, Arg, His, NH^{3+}



Sickle Cell Anemia:

- Caused by a mutation of Gly at 6 to Val
- in the deoxygenated form, the Val6 sticking out can bind to the hydrophobic patch from Phe85 to Leu88.
- This nucleation forms fibers in a "sickle" shape and blocks off the blood vessel