Hemoglobin

James Hebda

9/28/11

Another look at binding

- $Mb\text{-}O_2 \underset{k_{on}}{\overset{k_{off}}{\rightleftharpoons}} Mb + O_2$

- K_d is a result of two rate constants
  - Rate of dissociation, k_{off}
  - Rate of association, k_{on}
- k_{on} is the result of a collision
  - k_{on} for myoglobin relies on the presence of O_2

- k_{on} = # per mole per second
- k_{off} = # per second

What is K_d ?

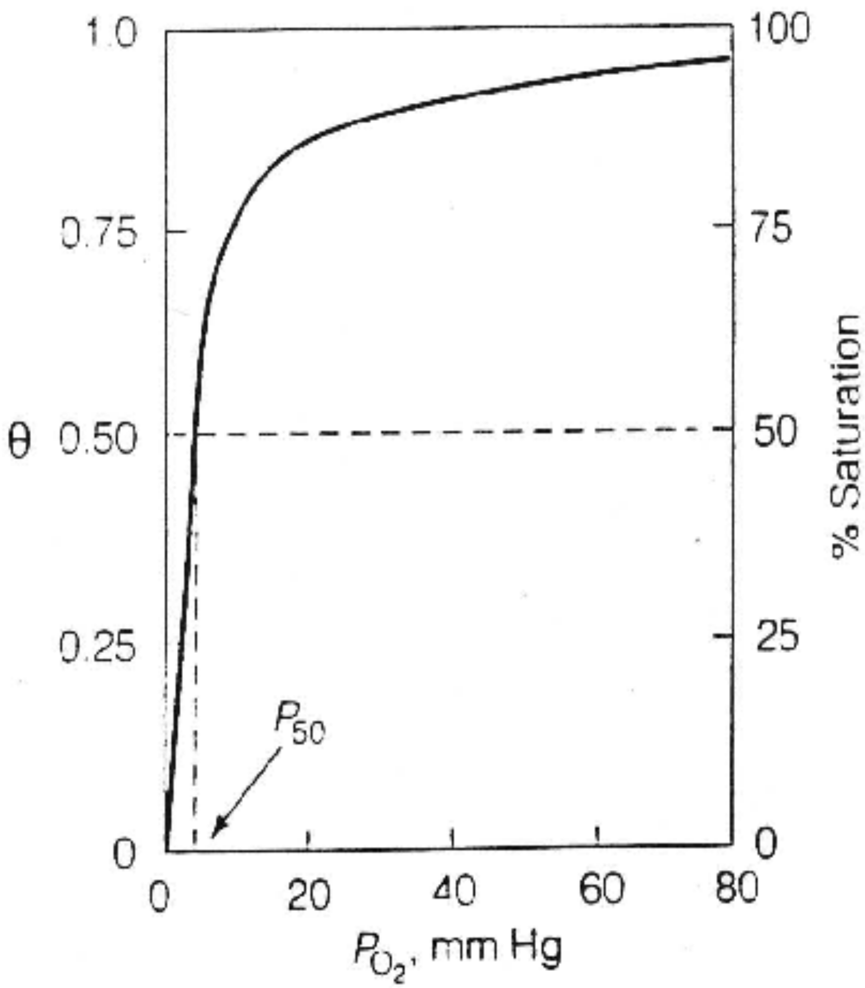
- On and off rates are defined by their rate constants

- k_{on} = # mole^{-1} sec^{-1}
- k_{off} = # sec^{-1}

- $K_a = \frac{k_{on}}{k_{off}} = \frac{\#\ M^{-1}\ sec^{-1}}{\#\ sec^{-1}} = M^{-1}$ = “per molar?”

- $K_d = \frac{k_{off}}{k_{on}} = \frac{\#\ sec^{-1}}{\#\ M^{-1}\ sec^{-1}} = M$ = Useful !

Why K_d is very useful



$$Mb\text{-}O_2 \rightleftharpoons Mb_{free} + O_2$$

$$K_d = \frac{([Mb_{free}][O_2]}{[Mb\text{-}O_2]}$$

$$Mb_{free} = [Mb_T] - [Mb\text{-}O_2]$$

We can make a substitution and rearrangement to get something very useful!

Y = fractional saturation

$Y = [Mb\text{-}O_2] / [Mb]_T$

$Y = [O_2] / (K_d + [O_2])$

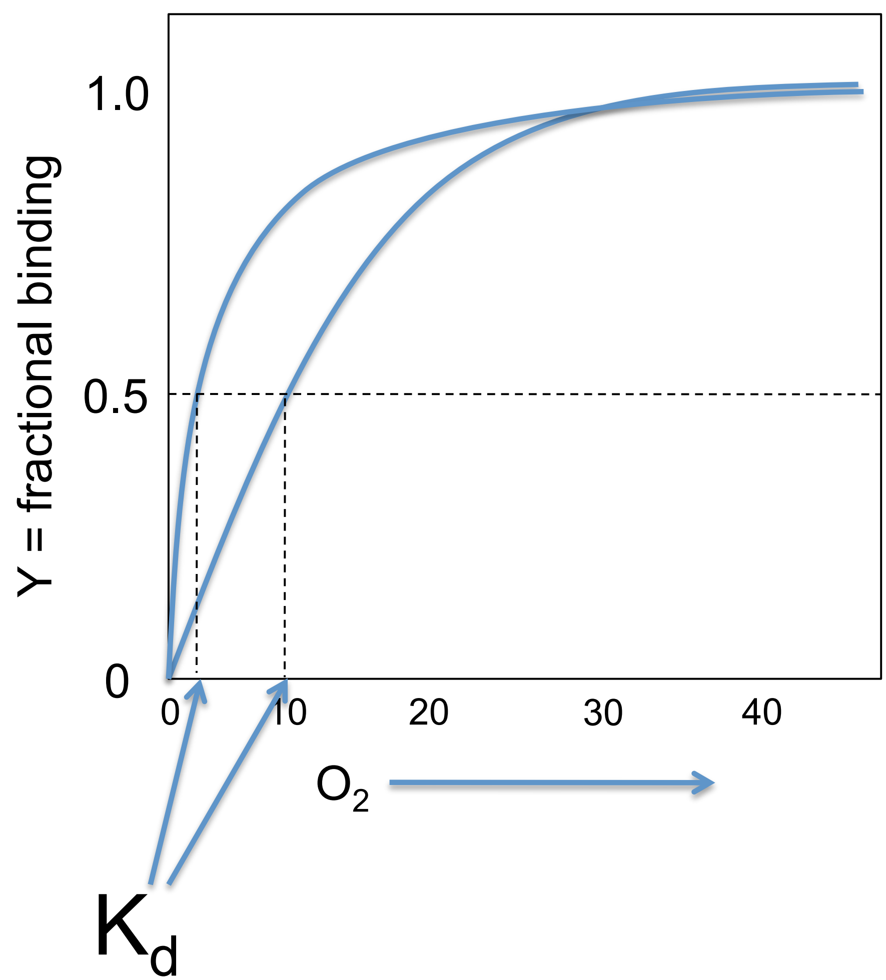
* Now we can use K_d to know the fraction of protein bound at ANY O_2 concentration!

What does a change in K_d look like?

- Fractional Binding curves

- $K_d = \frac{([Mb_{free}][O_2])}{[Mb\text{-}O_2]}$
  - $Mb_{free} = [Mb_T] - [Mb\text{-}O_2]$

- $Y = \frac{[O_2]}{(K_d + [O_2])}$



Thinking some more.

- Constant Kd.
  - Increase the amount of Oxygen.
  - What happens?

- At the same oxygen concentration.
  - You measured more Mb-O_2 for Myoglobin A than for mutant Myoglobin B?
  - MyoA Mb-O_2 > MyoB Mb-O_2
  - Does Mb_{free} or Mb_T change? How?
  - MyoA K_d ? MyoB K_d

Now On to Hemoglobin!

- Background
- Structure
- Cooperative binding.

Hemoglobin

- Oxygen carrying protein in your blood.
  - Oxygen picked up in lungs and transported to tissue throughout the body.
  - Oxygen gets transferred to Myoglobin in muscle

- There are 2.3×10^{8} hemoglobin molecules in a single red blood cell.
- Hemoglobin looks different and binds oxygen differently than Myoglobin

Hemoglobin is “related” to Myoglobin

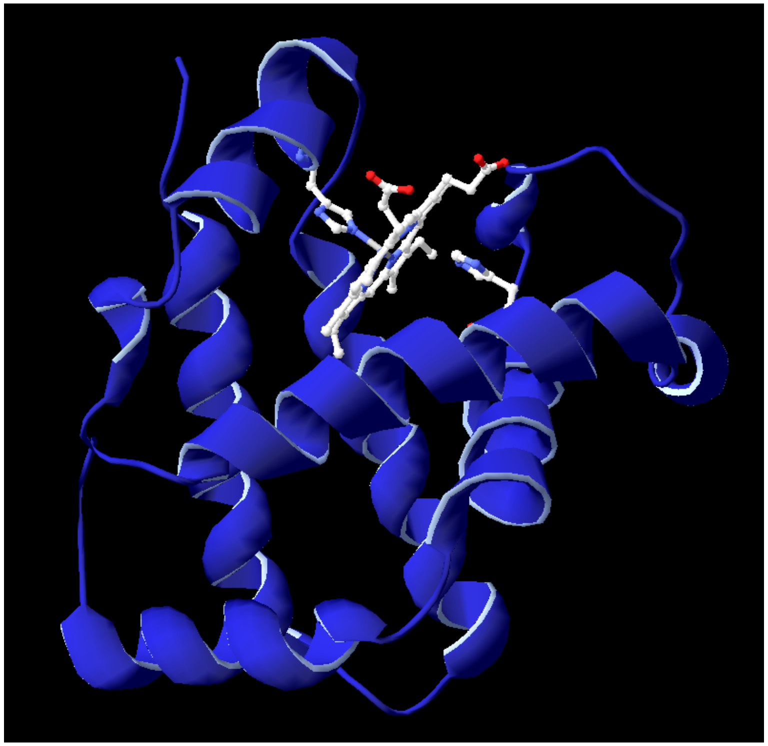
- But:
  - Hemoglobin is a tetrameric protein
  - Two different isoforms α and β
  - Each subunit has a very similar “fold” to myoglobin
    - Yet only 27% of the residues are identical

Primary Sequence Alignment

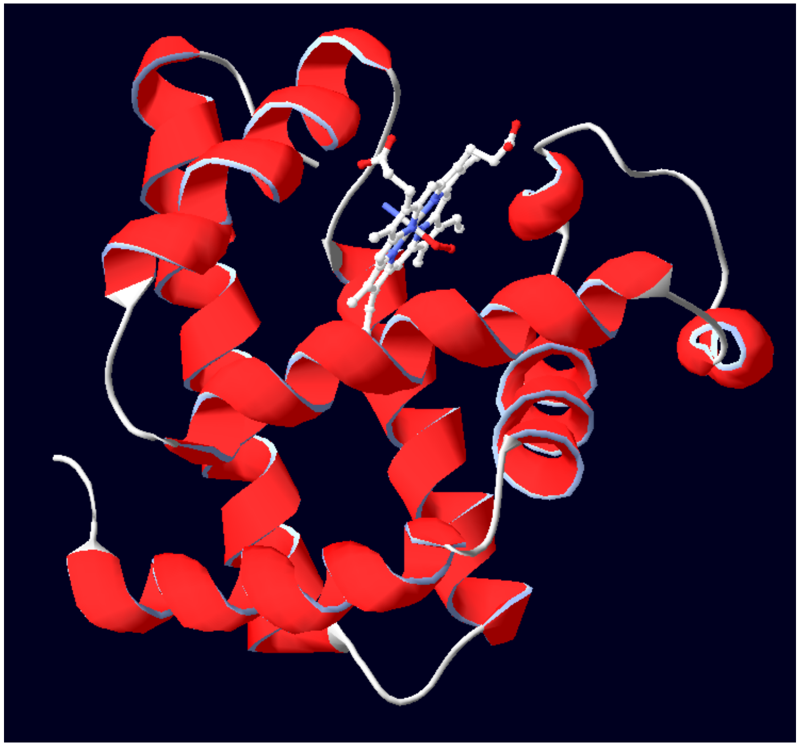
```
sp|P68871|HBB_HUMAN      MVHLTPEEKSAVTALWGKVNV--DEVGGEALGRLLVVYPWTQRFFESFGDLSTPDAVMGN
sp|P69905|HBA_HUMAN      -MVLSPADKTNVKAAWGKVGAHAGEYGAEALERMFLSFPTTKTYFPHFD------LSHGS
sp|P02144|MYG_HUMAN      -MGLSDGEWQLVLNVWGKVEADIPGHGQEVLIRLFKGHPETLEKFDKFKHLKSEDEMKAS
                          : *:  :   *   **** .     * *.* *::  .* *   *  *          ..

sp|P68871|HBB_HUMAN      PKVKAHGKKVLGAFSDGLAHLDNLKGTFATLSELHCDKLHVDPENFRLLGNVLVCVLAHH
sp|P69905|HBA_HUMAN      AQVKGHGKKVADALTNAVAHVDDMPNALSALSDLHAHKLRVDPVNFKLLSHCLLVTLAAH
sp|P02144|MYG_HUMAN      EDLKKHGATVLTALGGILKKKGHHEAEIKPLAQSHATKHKIPVKYLEFISECIIQVLQSK
                          .:* ** .*  *:   : :  .    :  *:: *. * ::    :.::.. :: .*  :

sp|P68871|HBB_HUMAN      FGKEFTPPVQAAYQKVVAGVANALAHKYH------
sp|P69905|HBA_HUMAN      LPAEFTPAVHASLDKFLASVSTVLTSKYR------
sp|P02144|MYG_HUMAN      HPGDFGADAQGAMNKALELFRKDMASNYKELGFQG
                            :*   .:.: :* :  . . :: :*:
```

One dot?
Two dots?
Star?
Colors?

Hemoglobin

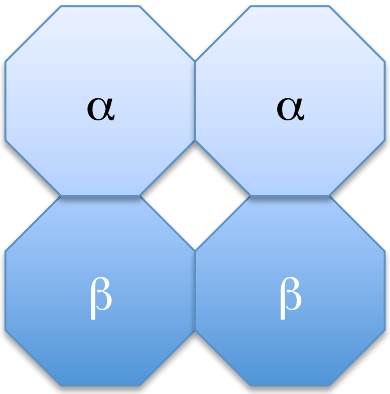


Hemoglobin β



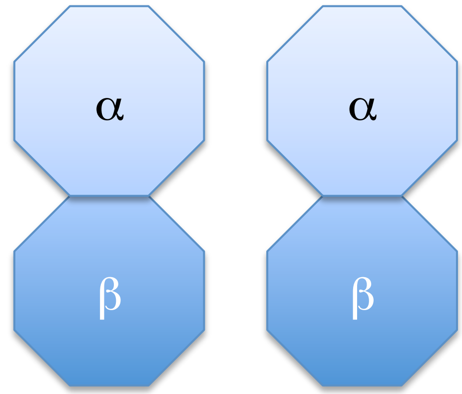
Myoglobin

Hemoglobin is a tetramer



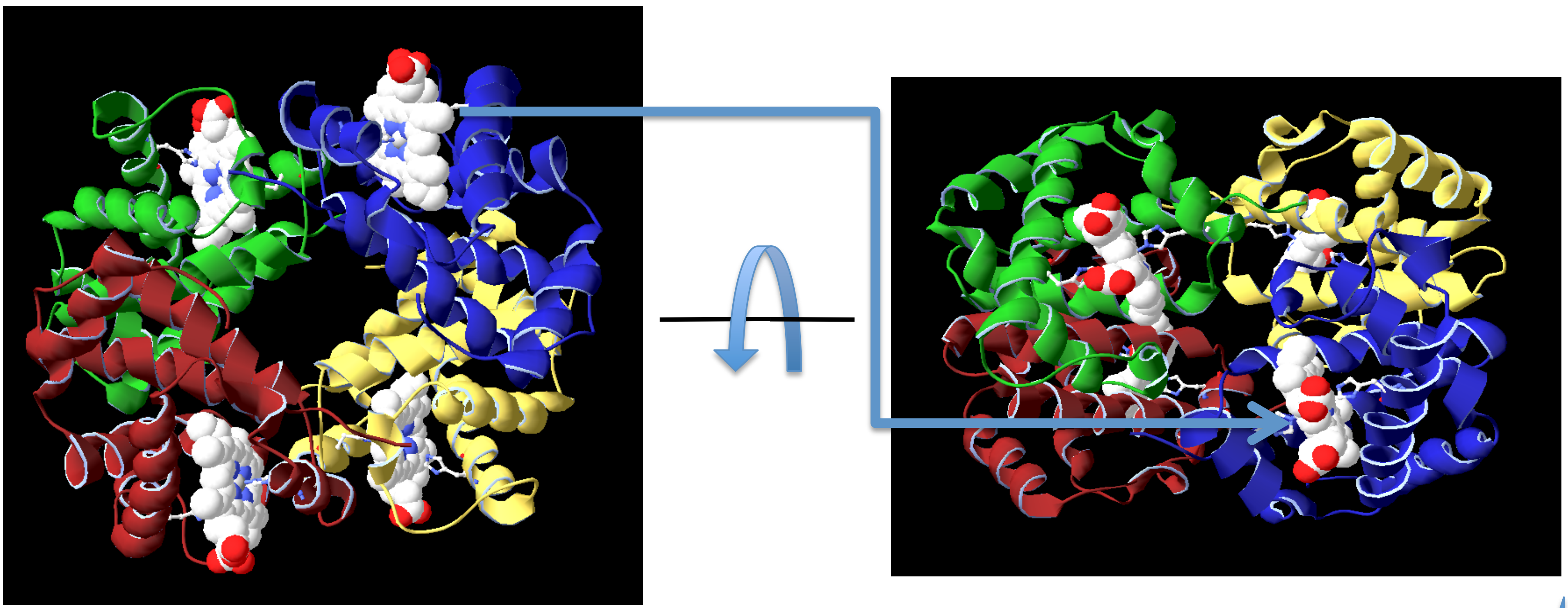
$2\alpha 2\beta$ units
4 peptide chains
Tetramer
Binds four Hemes
Binds four O_2

"Dimer of Dimers"

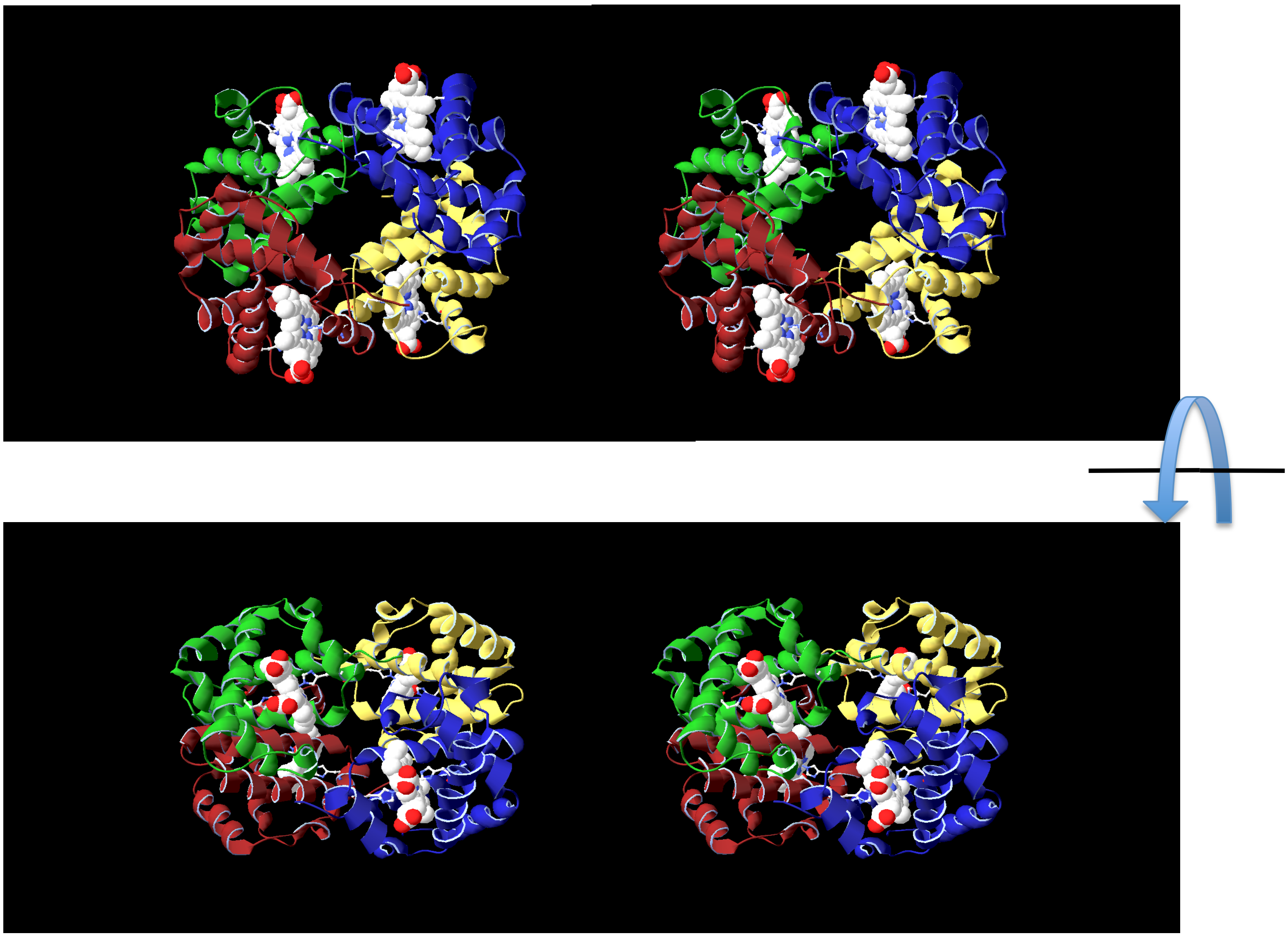


Single unit
1 peptide chains
Monomer
Binds one Heme
Binds one O_2

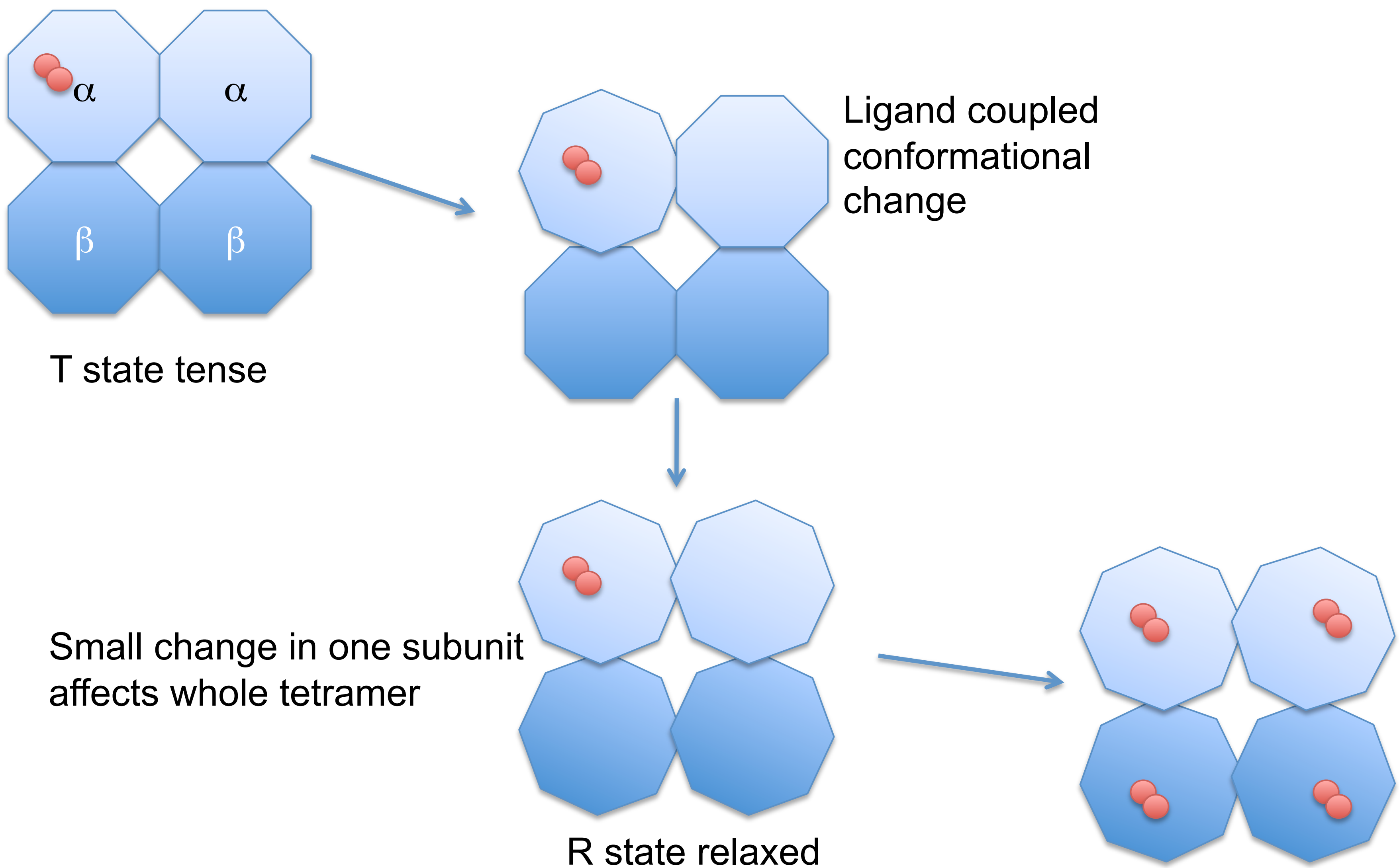
Hemoglobin Quaternary Structure



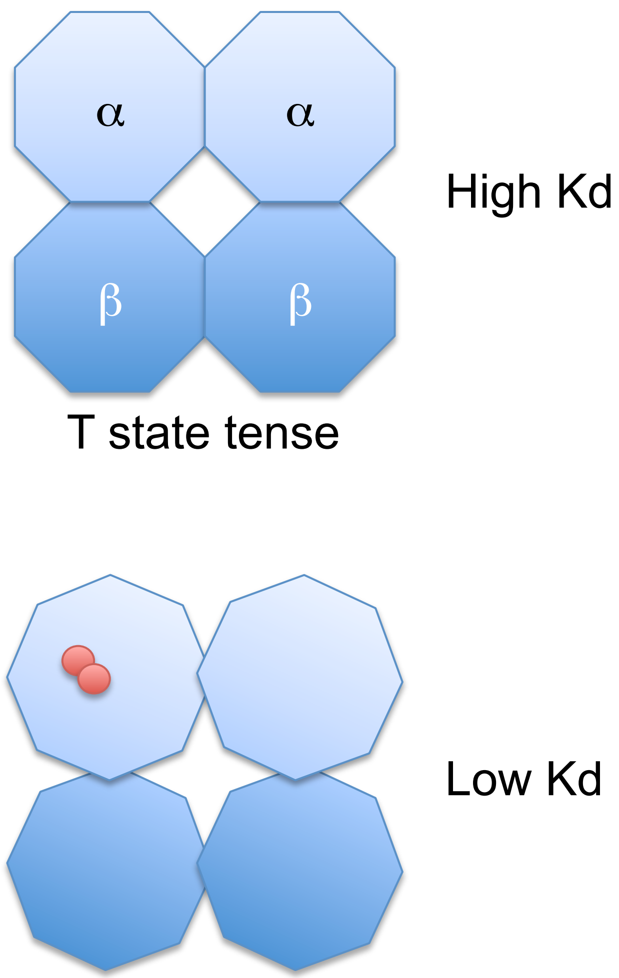
Hemoglobin Quaternary Structure

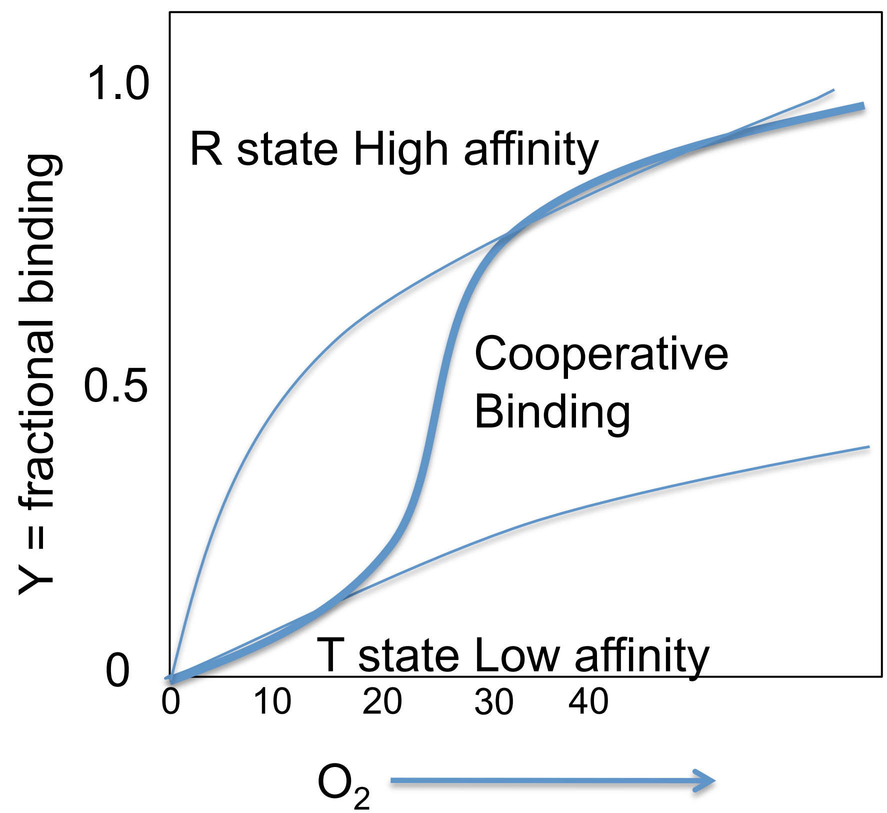


Hemoglobin has allostery



Each state has a different Kd





Modeling cooperative binding

For Myoglobin:

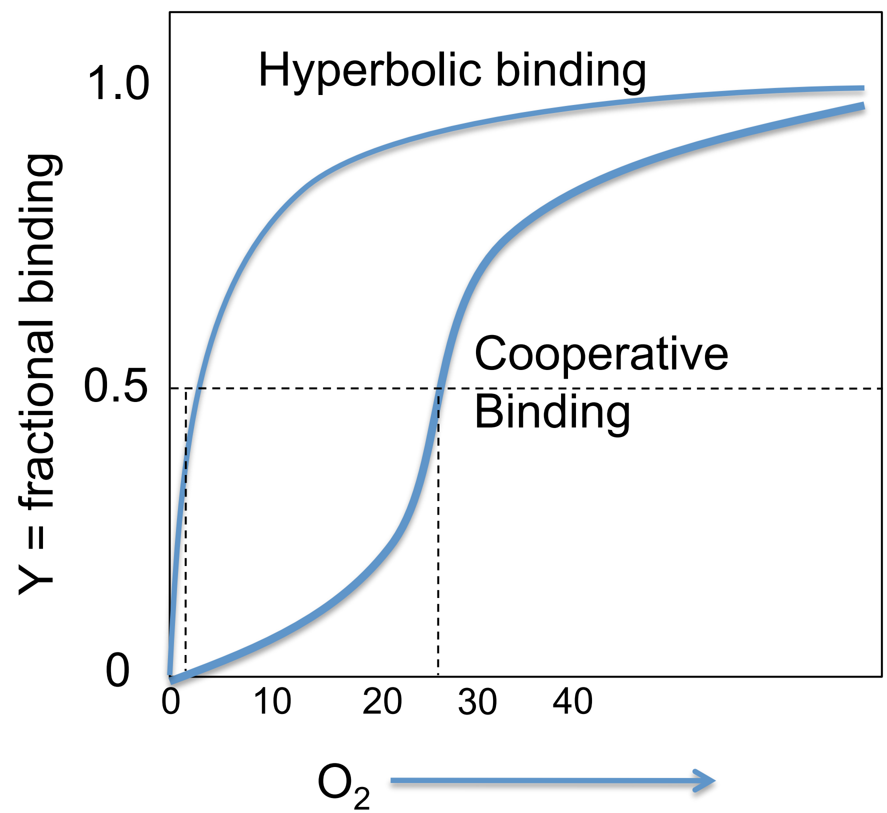
$$Y = \frac{[O_2]}{(K_d + [O_2])}$$

Hyperbolic binding
Simple one phase binding

For Hemoglobin:

$$Y = \frac{[O_2]^n}{(K_d^n + [O_2]^n)}$$

Sigmoidal binding
Where **n** is extent of cooperativity



More binding this afternoon

- In lab we will be looking at two things that bind Hemoglobin and Myoglobin
  - Oxygen
  - Carbon Monoxide