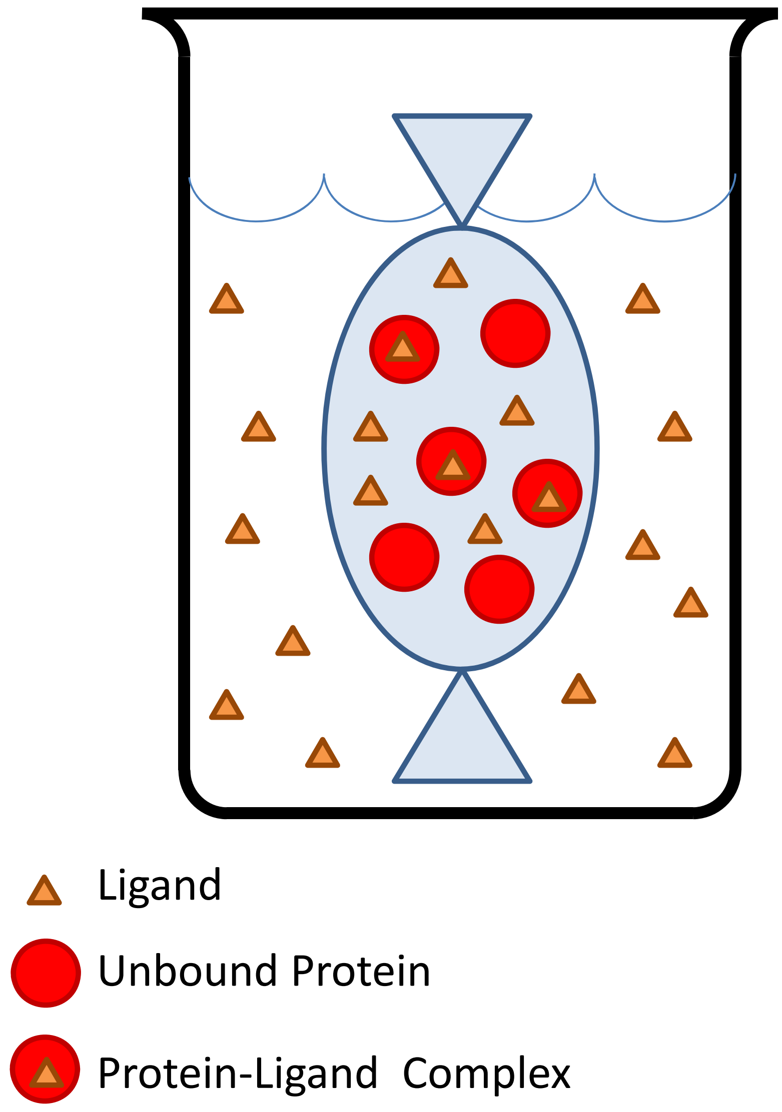
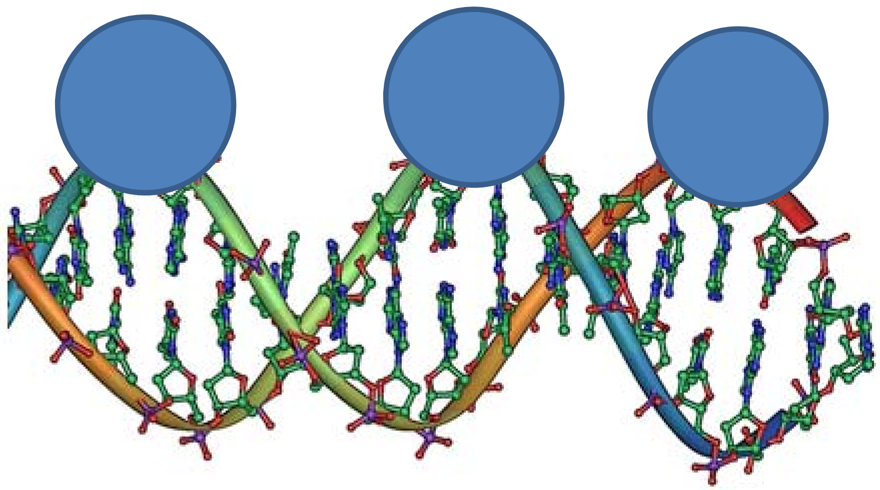
Equilibrium Dialysis

- Proteins can't pass through membrane
- Small molecules (drug/ligand) can
- μ of free (unbound) ligand must be equal inside *and* outside of the bag



Multiple-Site Binding



- Many proteins bind to DNA: control of transcription & replication
  - Sites are relatively independent
- Can we develop a mathematical model?

Source: Wikipedia

Why Do We Multiply in the Numerator?

- Terms are:

$$\frac{\#\ \text{moles P bound}}{\text{L of solution}}$$

- Think of it this way:

$$\frac{\#\ \text{moles P bound}}{\#\ \text{moles } \mathrm{P_xD}} \times \frac{\#\ \text{moles } \mathrm{P_xD}}{\text{L of solution}}$$

- Example:

$$\frac{2\ \text{mol P in } \mathrm{P_2D}}{1\ \text{mol } \mathrm{P_2D}} \times [P_2D] = 2[P_2D]\ \frac{\text{mol P bound}}{\text{L solution}}$$

Thinking Question:

Discuss At Your Tables

- Do we expect the concentration of the following species to differ:

[100] vs. [010] vs. [001]

- Why or why not?

Observations: The Structure of the Equation

- Degree of binding for 3 independent site system:

$$\bar{\nu} = \frac{0[000] + 1([100] + [010] + [001]) + 2([110] + [101] + [011]) + 3[111]}{[000] + ([100] + [010] + [001]) + ([110] + [101] + [011]) + [111]}$$

$$\bar{\nu} = \frac{0(1)[P_0D] \ + 1(3)[P_1D] \ + 2(3)[P_2D] \ + 3(1)[P_3D]}{(1)[P_0D] + (3)[P_1D] + (3)[P_2D] + (1)[P_3D]}$$

- Two observations:
  - Combinatorics seems to play a role
  - Numerator seems related to the denominator

Combinatorics Review

- # of ways to arrange *n* things taken *m* at a time

$$ {}_{n}C_{m} \text{ or } \binom{n}{m} = \frac{m!}{m!\,(n-m)!} $$

- **Example:** The number of ways to fill 3 binding sites with two sites occupied at a time.

Three Site Binding: The Final Form

$$\bar{\nu} = \frac{3K[P][P_0D] + 6K^2[P]^2[P_0D] + 3K^3[P]^3[P_0D]}{[P_0D] + 3K\,[P][P_0D] + 3K^2[P]^2[P_0D] + K^3[P]^3[P_0D]}$$

$$\bar{\nu} = \frac{3K[P](1 + 2K[P] + K^2[P]^2)}{1 + 3K\,[P] + 3K^2[P]^2 + K^3[P]^3}$$

$$\bar{\nu} = \frac{3K[P](1 + K[P])^2}{(1 + K\,[P])^3}$$

$\bar{\nu} = \frac{3K[P]}{1+K\,[P]} = \frac{3[P]}{K_D+[P]}$ where $K_D = K^{-1}$

- Look familiar?

Summary

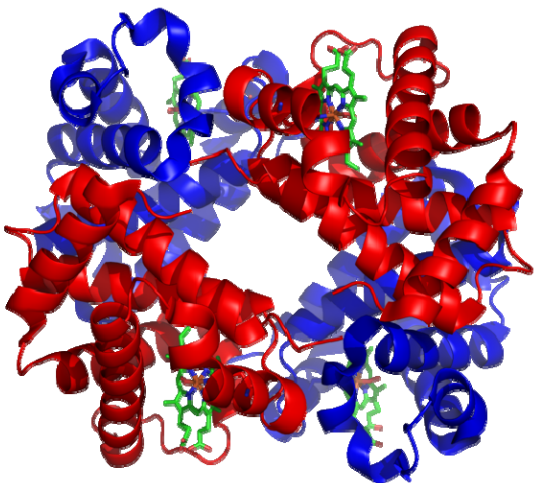
- N identical, independent binding sites:

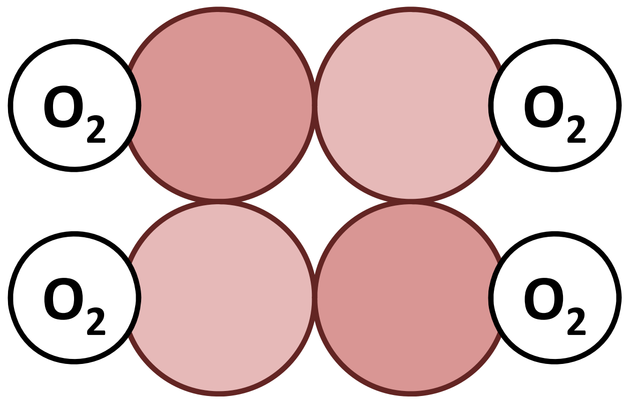
$$\bar{\nu} = \frac{N[L]}{K_D + [L]} = \frac{NK[L]}{1 + K[L]}$$

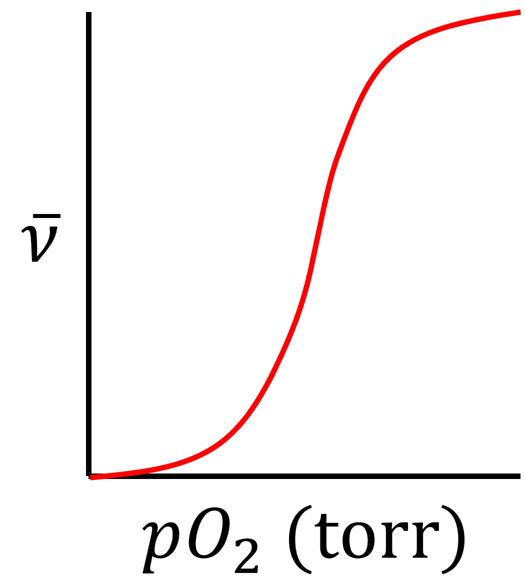
- $\bar{\nu}$ ranges from 0 to N
  - Asymptote at N may be hard to identify from a binding curve

- **Remember:** We should *always* be at equilibrium in a binding experiment! ($\Delta \bar{G} = 0$)

What About Hemoglobin?







- Oxygen binding is *cooperative*: binding one site makes adjacent binding more favorable

Hemoglobin. http://en.wikipedia.org/wiki/hemoglobin (PDB 1GZX)

Allostery and Cooperativity

- **Allostery:** Binding at one site affects the binding at another site
  - It can become more or less favorable
- **Cooperativity:** Binding at one site increases affinity at another site (sometimes positive cooperativity)
- This behavior shows up in *many* biological systems:
  - Can we model it?
  - What is the molecular (physical) basis of allostery?