

Multiple Choice:

1. A protein that binds two ligands in a non-cooperative manner will:
  - a) **show a hyperbolic binding curve.**
  - b) show a curved Scatchard Plot
  - c) show a curved Hill Plot.
  - d) show a sigmoidal binding curve
2. Once a ligand dissociation constant ( $K_D$ ) has been determined it is possible to calculate:
  - a) the ligand binding constant ( $K_a$ ).
  - b) the  $\Delta G$  for the binding interaction.
  - c) the concentration of ligand required for half-maximal occupancy
  - d) **All of the above are correct**
3. In both hemoglobin and myoglobin the oxygen is bound to:
  - a) the nitrogen atoms on the heme.
  - b) polar pocket in the protein.
  - c) histidine residues in the protein.
  - d) **The iron atom in the heme group.**

Scatchard Plot Fill-In

The Scatchard equation is:

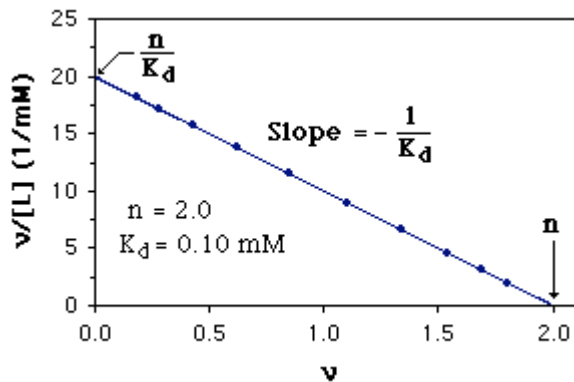
$$v/L = n/K_d - v/K_d, \text{ where}$$

$$v = [L]_{\text{bound}}/[M]_{\text{total}};$$

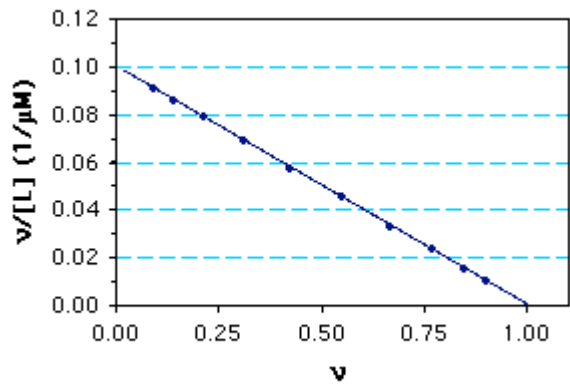
$$L = [L]_{\text{free}};$$

$n$  = # ligands/macromolecule, *i.e.* the stoichiometry;

$K_d$  = the dissociation constant.



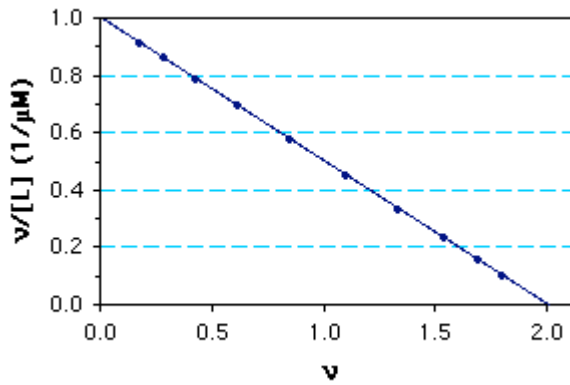
Ligand Binding Curve Problems:



What Type of plot? Scatchard

$N=1$

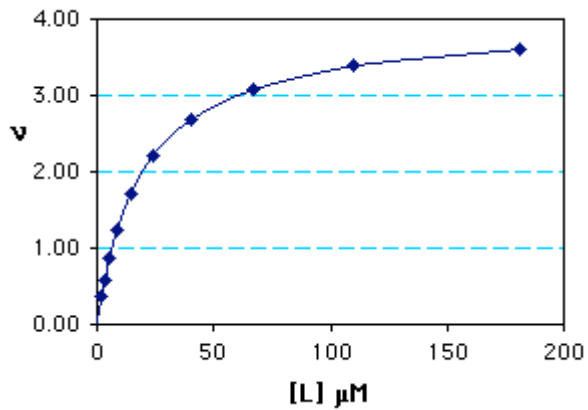
$K_d = 10$



Type of Plot: Scatchard

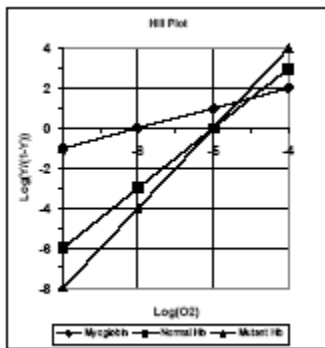
$N=2$

$K_d = 2$



Type of Plot? Saturation binding  
 $n = 4$   
 $K_d = .05$

Hill Plot problem:



**Determine the Hill coefficient and  $K_D$  for the mutant hemoglobin. Please describe your approach**

The hill coefficient is the slope of the line when it crosses the x-axis. So in this case, the hill coefficient is  $4/1 = 4$

The  $K_D$  is the ligand concentration when  $Y = 1/2$ , which is the concentration where the line crosses the X axis.  $\text{Log}(K_D) = -5$  on the graph, so  $K_D = 10^{-5} \text{M}$