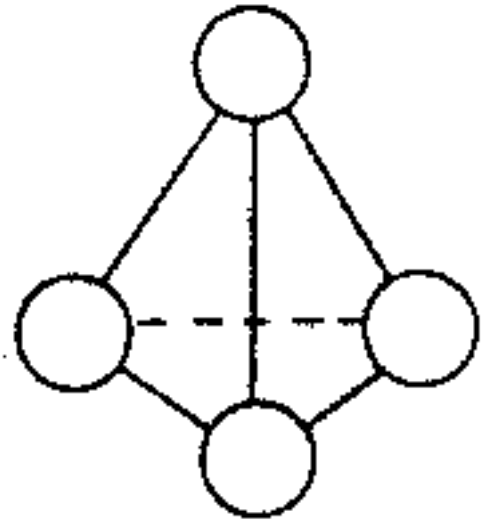
Bio5312 Feb. 8, 2016
Problem Set-4 ***due Feb. 15***

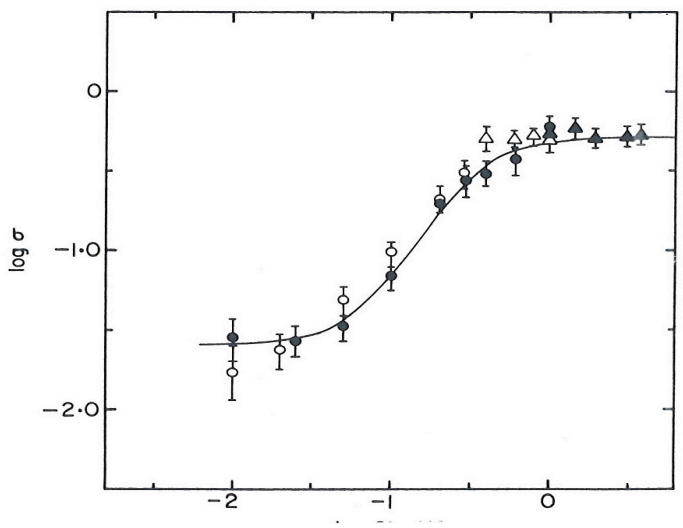
1. A homo-tetrameric protein (identical subunits) has four binding sites for a ligand X, one site per subunit. X binds to each subunit with a site binding constant, κ. Subsequent ligands bind with cooperativity. The subunits of the tetramer can be viewed as forming a tetrahedron such that cooperative interactions can occur between any pair of sites, with cooperative interaction constant, σ.



a.) Write the binding polynomial for this system and obtain an expressions for <X>.

b.) Obtain expressions for the step-wise microscopic constants k_i in terms of κ and σ.

c.) The Figure below shows a plot of log σ vs. log Y, where Y is a second ligand that can also bind to the tetramer. Is there linkage between the binding of X and Y? If so, what type?



2. Protein, P, has one site to which three ligands, A, B, and C, can bind competitively with equilibrium constants k_A, k_B and k_C, respectively.

a.) Write an expression for the Binding Polynomial for this system.

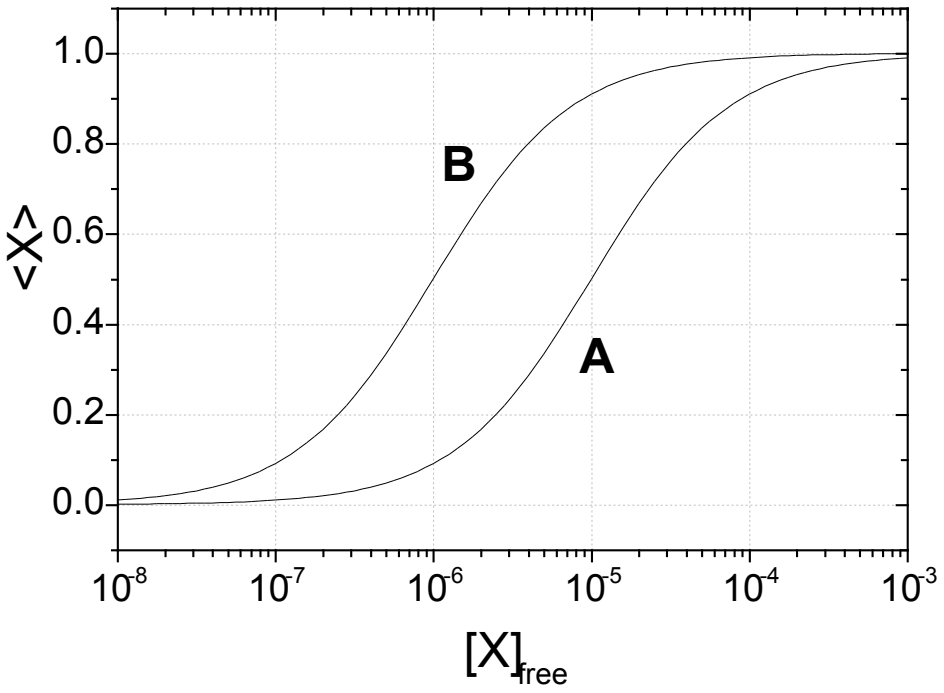
b.) What type of linkage describes the binding of ligands A and C?

c.) Obtain an expression for the average moles of B bound per protein molecule.

3. A protein exists in equilibrium between two conformations, A and B, where, $K_{obs} = [B]/[A]$.

$$A \overset{K_{obs}}{\leftrightarrow} B$$

A ligand, X, can bind to both forms of the protein and the binding isotherms (<X> vs. free ligand concentration, x) for X binding to each form of the protein are shown below.



Construct a qualitative plot of ($d\log K_{obs}/d\log[x]$) vs. [x]. Explain your answer including the equations that you used to construct this plot.

4. A ligand, L, can bind to three sites on a protein with site binding constants, κ_1, κ_2, and κ_3. Cooperativity can exist when sites 2 and 3 are occupied, with cooperativity parameter, c.

   Given $\kappa_1 = 1 \times 10^5\ M^{-1}$ and the values of the equilibrium constants shown below (at 20˚C), determine the standard state free energy change, ΔG_3° for binding of X to site 3.