NONCOOPERATIVE LIGAND BINDING: HOW STRONGLY DOES AZIDE BIND TO MYOGLOBIN?

Adapted by T.J. Gries from Marcoline, A.T. and Elgren, T.E. (1998) A Thermodynamic Study of Azide Binding to Myoglobin. JCE 75: 1622-1623.

Pre-Lab

Myoglobin's major purpose is to increase the solubility of O_2 in tissue by allowing O_2 to coordinate to an Fe^{2+} ion within the myoglobin heme group. O_2 is a difficult ligand to study in the biochemistry lab since it is also a component of air. We are going to study the binding of azide (N_3^-) to myoglobin (Mb). Since azide does not have a detectable vapor pressure and is not a component of the atmosphere, controlling its concentration is much easier. You will be studying oxidized myoglobin (metmyoglobin), which contains Fe^{3+} in the heme group instead of Fe^{2+}. O_2 does not bind to the coordinated Fe^{3+} and will not compete for azide binding in these assays.

$$Mb:N_3^- \overset{K_D}{\Leftrightarrow} Mb_{free} + N_3^-$$

1. Define the dissociation equilibrium constant K_D in terms of molar concentrations of products and reactants.

2. You need to rearrange your definition in Question #1 to look like the value of θ:

$$\theta = \frac{[Mb:N_3^-]}{[Mb]_T}$$

$$[Mb]_T = [Mb]_{free} + [Mb:N_3^-]$$

   a. Solve for $[Mb]_{free}$
   b. Plug this expression for $[Mb]_{free}$ into your expression for K_D from Question #1.
   c. Use algebra to solve for θ (i.e., $\frac{[Mb:N_3^-]}{[Mb]_T}$)

3. Read Pollard, T.D. (2010). A Guide to Simple and Informative Binding Assays. **Molecular Biology of the Cell. 21:**4061-4067.

4. Calculate the amount of each stock required to prepare the eleven solutions described in Table 1: Myoglobin Binding Set.

Stock solutions:

2 mM metmyoglobin (Mb)
20 mM Sodium Phosphate buffer pH 7.4
0.500 mM Sodium azide
0.500 mM Sodium Chloride

Table 1: Myoglobin Binding Set

Tube	[Mb] (mM)	[azide] (mM)	[NaCl] (mM)	Total Vol. (mL)
1	0.050	0.00000	0.16667	3.00
2	0.050	0.00083	0.16583	3.00
3	0.050	0.00250	0.16417	3.00
4	0.050	0.00500	0.16167	3.00
5	0.050	0.01000	0.15667	3.00
6	0.050	0.01500	0.15167	3.00
7	0.050	0.02000	0.14667	3.00
8	0.050	0.04000	0.12667	3.00
9	0.050	0.06000	0.10667	3.00
10	0.050	0.10000	0.06667	3.00
11	0.050	0.16667	0.00000	3.00