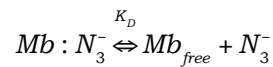


## NONCOOPERATIVE LIGAND BINDING: HOW STRONGLY DOES AZIDE BIND TO MYOGLOBIN?

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<sub>2</sub> in tissue by allowing O<sub>2</sub> to coordinate to an Fe<sup>2+</sup> ion within the myoglobin heme group. O<sub>2</sub> 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<sub>3</sub><sup>-</sup>) 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<sup>3+</sup> in the heme group instead of Fe<sup>2+</sup>. O<sub>2</sub> does not bind to the coordinated Fe<sup>3+</sup> and will not compete for azide binding in these assays.



1. Define the dissociation equilibrium constant K<sub>D</sub> 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]<sub>free</sub>
  - b. Plug this expression for [Mb]<sub>free</sub> into your expression for K<sub>D</sub> 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