NAME:_____ BIOL/CHEM 300
Fall 2014

This exam and the data presented in the context of questions is based on:

Thoden, J. B., Holden, H. M., and Grant, G. A. (2014 Dec 9). Structure of L-Serine Dehydratase from *Legionella pneumophilia*: Novel Use of the C-terminal Cysteine as an Intrinsic Competitive Inhibitor. **Biochemistry 53**: 7615-24.

Thoden *et al.* use the Hill analysis to model their cooperative kinetic enzyme data. The first set of exercises will have you reconsider their data in the context of the MWC model for cooperative systems. While the MWC model has an additional parameter compared to the Hill model, we will gain some additional molecular insight into the function of the enzyme with this reanalysis. To begin to understand the application of the MWC model to kinetic data, complete the following columns. The left column is for ligand binding as in classwork; the right column is for a MM reaction process.

$$\theta = \frac{[L]_{free}}{K_D + [L]_{free}}$$

1. Write a simplified expression for (1-θ). Note that "1" is the maximum value of θ.

2. Write a simplified expression for $\left(\frac{\theta}{1-\theta}\right)$.

3. Write a simplified expression for $\log\left(\frac{\theta}{1-\theta}\right)$ in the form of a linear equation.

4. On a Hill plot $\left(\log\left(\frac{\theta}{1-\theta}\right) \text{ vs. } \log[L]_{free}\right)$ for a noncooperative ligand binding process:
   a. The slope =
   b. The y_{int} =

$$v_o = \frac{V_{\max}[S]_o}{K_M + [S]_o}$$

1. Write a simplified expression for $\left(V_{\max} - v_o\right)$.

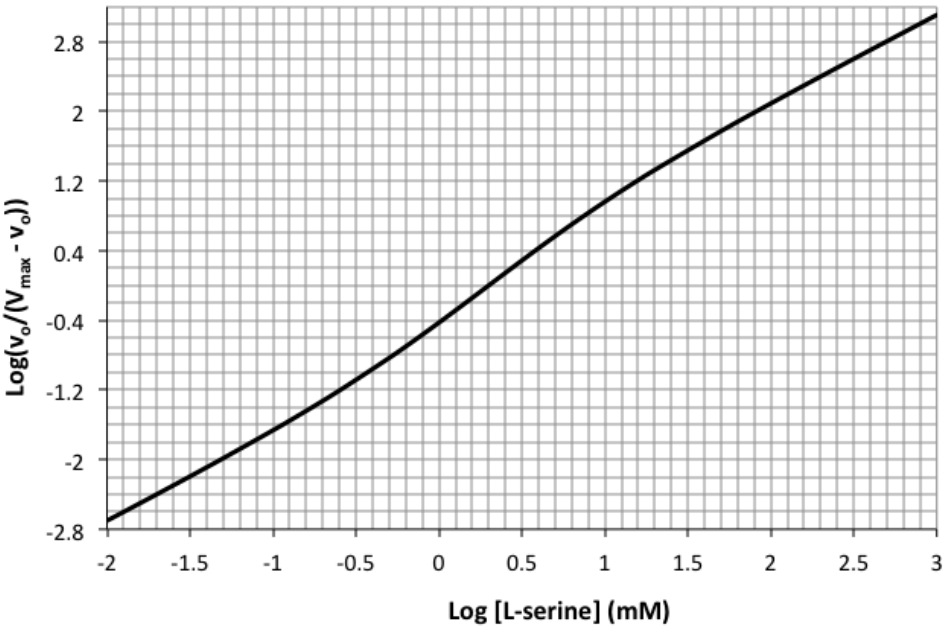
2. Write a simplified expression for $\left(\frac{v_o}{V_{\max} - v_o}\right)$.

3. Write a simplified expression for $\log\left(\frac{v_o}{V_{\max} - v_o}\right)$ in the form of a linear equation.

4. On a Hill plot $\left(\log\left(\frac{v_o}{V_{\max} - v_o}\right) \text{ vs. } \log[S]_o\right)$ for a noncooperative enzyme kinetic process:
   a. The slope =
   b. The y_{int} =

Using the kinetic parameters reported by Thoden *et al.*, initial rate values could be computed. The following table and graph present the Hill analysis for the WT enzyme with L-serine as the substrate.

Log $[L\text{-Serine}]_o$	**Log $(v_o/(V_{max}-v_o))$**
-2.00	**-2.70**
-1.70	-2.40
-1.52	-2.22
-1.40	-2.09
-1.30	-1.99
-1.00	**-1.66**
-0.82	-1.47
-0.60	-1.21
-0.30	-0.83
-0.12	-0.60
0.00	**-0.43**
0.10	-0.29
0.35	0.07
0.40	0.14
0.48	0.25
0.54	0.35
0.57	0.39
0.65	0.50
0.70	0.56
0.72	0.59
0.85	0.76
0.90	0.84
0.95	0.90
1.00	**0.96**
1.04	1.01
1.08	1.06
1.11	1.10
1.30	1.33
1.70	1.77
2.00	**2.09**
2.30	2.40
2.48	2.58
2.60	2.70
2.70	2.80
2.78	2.88
2.90	3.01
3.00	**3.10**



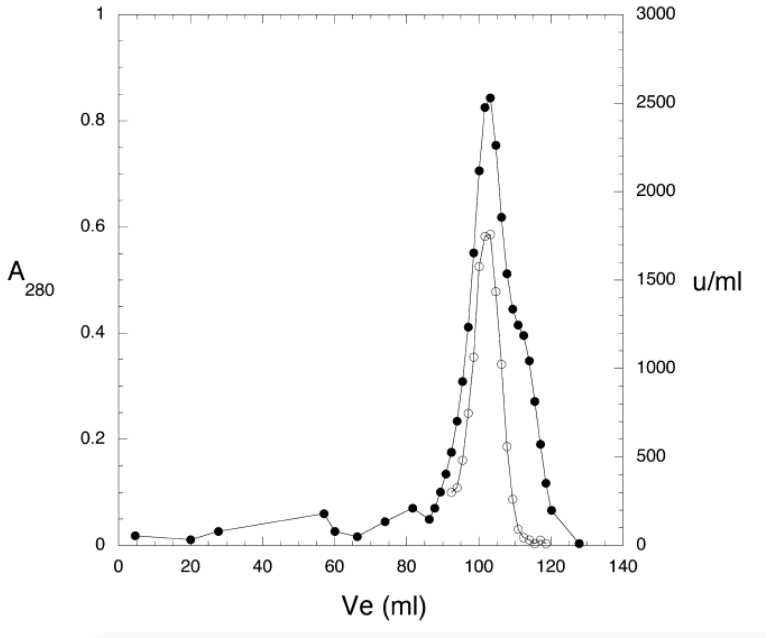
1. What quantitative observation allows you to conclude that the enzyme functions in a cooperative mechanism? Show your work.

2. At low and high [L-serine], what quantitative observation allows you to conclude that the enzyme functions in non-cooperative mechanism (identified as the *T-state* in low levels of L-serine and the *R-state* in high levels of L-serine)? Show your work.

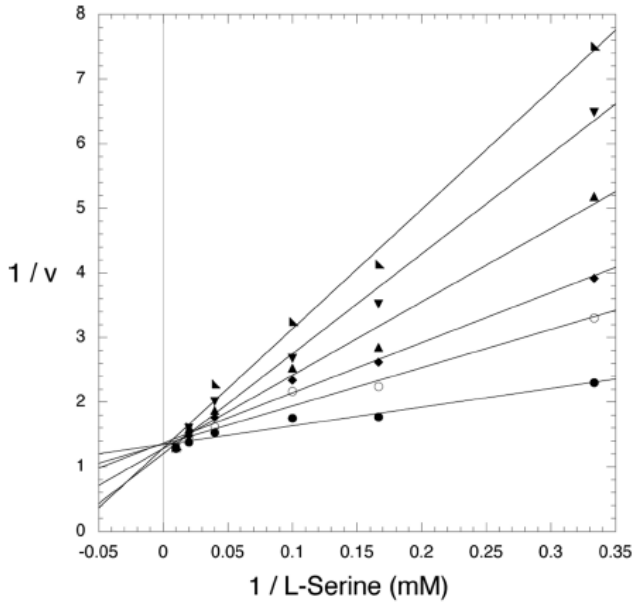
3. Determine the values of $K_M^{T-state}$ and $K_M^{R-state}$. Show your work.

Thoden *et al.* expressed and purified *L. pneumophilia* L-Serine Dehydratase from *E. coli*. Annotate Figure 1 from the paper (e.g., add text and diagrams, do not write a paragraph) to indicate how the authors were able to conclude:

(*i*) that the enzyme exists in both monomeric and dimeric states and
(*ii*) that the dimeric form is active while the monomeric form is inactive.



Thoden *et al.* conclude that L-cysteine is a competitive inhibitor of *L. pneumophilia* L-Serine Dehydratase Annotate Figure 4 from the paper (e.g., add text and diagrams, do not write a paragraph) to indicate how the authors were able to draw this conclusion:



Thoden *et al.* stored their final purified *L. pneumophilia* L-Serine Dehydratase in 10 mM Tris buffer with 200 mM NaCl at pH 8.0. Explain how to make 1 L of this solution using the minimum amount of each of the following materials from Sigma Aldrich (e.g. no concentrated stocks are needed):

- Trizma® Base
- Trizma® HCl
- NaCl
- Water

Use PyMol and the paper to identify [amino acid and/or primary structure position number (1 – 485)] for:

(*i*) each Cys that is coordinating the Fe-S cluster
(*ii*) the proposed identity of "*E*"

Scheme 1