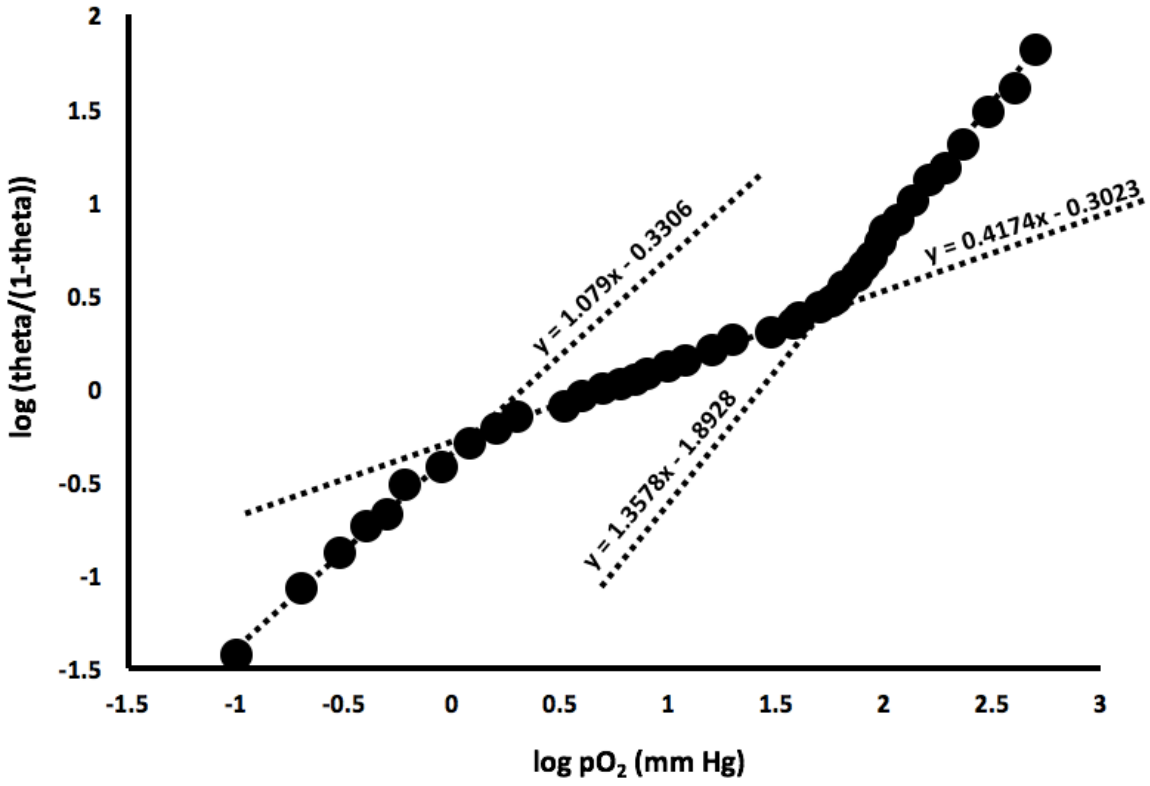
Name: _____ Exam 3

All references to data in this exam are from:
Shigenori Nagatomo, Yukifumi Nagai, Yayoi Aki, Hiroshi Sakurai, Kiyohiro Imai, Naoki Mizusawa, Takashi Ogura, Teizo Kitagawa, and Masako Nagai. (2015) An Origin of Cooperative Oxygen Binding of Human Adult Hemoglobin: Different Roles of the α and β Subunits in the $\alpha_2\beta_2$ Tetramer. **PLoS ONE 10**(8): e0135080.

In the assigned paper, the authors discuss several different models used to describe the binding of O_2 to hemoglobin. One of these widely-used, discussed models is the MWC model. This model is poplar because it provides a context to understand the binding process with biologically-relevant parameters (i.e., the binding affinity of oxygen to hemoglobin in the lungs as compared to the tissues) and the effect of allosteric effector molecules (i.e., 2,3-bisphosphoglycerate or inositol hexaphosphoate) on the conformational state of hemoglobin. When the authors studied the α87His$\rightarrow$Gly mutant hemoglobin, they found it presented with negative cooperativity. A significant limitation to the MWC model is that it cannot explain negative cooperativity. The first series of questions on this exam will walk you through exploring the origin of this limitation in the MWC model. The following graph presents the data from the Hill plot of oxygen binding by rHB(αH87G) in the paper. I have re-normalized the data to be on a traditional Hill plot as we studied in class (e.g., the axes are back to normal).



1. (5 pts) Looking at the authors' data (as presented in the above graph), what aspect provides the direct clue that the rHB(αH87G) acts with negative cooperativity?

____/5 pts

The MWC model proposes that hemoglobin exists in two conformations, one that binds O_2 **weakly (T-state)** and one that binds O_2 **tightly (R-state)**. The four, individual oxygen-binding sites act independently (non-cooperativity) when hemoglobin is in the T-state.

2. (10 pts) Determine the <u>association</u> binding constant for the <u>weakly-binding T-state</u> (call $K_{site,\,T}$).

3. (5 pts) What direct observation from the Hill plot supports that this hemoglobin acts non-cooperatively in the T-state?

Since there are multiple sites and ways for each oxygen molecule to bind, the T-state binding constant receives a statistical factor for the process of binding at all four sites.

4. (5 pts) Determine the value of the equilibrium constant at each point during the binding scheme below:

$$T_0 + 4O_2 \overset{4\,K_{site,T}}{\Longleftrightarrow} T_1 + 3O_2 \overset{\frac{3}{2}K_{site,T}}{\Longleftrightarrow} T_2 + 2O_2 \overset{\frac{2}{3}K_{site,T}}{\Longleftrightarrow} T_3 + O_2 \overset{\frac{1}{4}K_{site,T}}{\Longleftrightarrow} T_4$$

The four, individual oxygen-binding sites act independently (non-cooperativity) when hemoglobin is in the R-state.

5. (10 pts) Determine the <u>association</u> binding constant for the <u>tightly-binding R-state</u> (call $K_{site,\,R}$).

6. (5 pts) What direct observation from the Hill plot supports that this hemoglobin acts non-cooperatively in the R-state?

Since there are multiple sites and ways for each oxygen molecule to bind, the R-state binding constant receives a statistical factor for the process of binding at all four sites.

7. (5 pts) Determine the value of the equilibrium constant at each point during the binding scheme below:

$$R_0 + 4O_2 \overset{4\,K_{site,R}}{\Longleftrightarrow} R_1 + 3O_2 \overset{\frac{3}{2}K_{site,R}}{\Longleftrightarrow} R_2 + 2O_2 \overset{\frac{2}{3}K_{site,R}}{\Longleftrightarrow} R_3 + O_2 \overset{\frac{1}{4}K_{site,R}}{\Longleftrightarrow} R_4$$

____/40 pts

8. (10 pts) At low oxygen concentrations without oxygen bound, is this mutant hemoglobin overwhelmingly in the T or the R state?

9. (5 pts) Consider that there is a 1000 to 1 ratio for the more abundant conformation to the less abundantconformation. What is the value for $K_{To \rightarrow Ro} = \frac{[Ro]}{[To]}$?

10. (10 pts) Determine the value of the equilibrium constant describing the conversion of the T-state to the R-state at each point along the binding pathway:

$K_{T1 \rightarrow R1} =$

$K_{T2 \rightarrow R2} =$

$K_{T3 \rightarrow R3} =$

$K_{T4 \rightarrow R4} =$

11. (10 pts) Explain why your answer to question #10 using the MWC model is incompatible with the observed data for the mutant hemoglobin from the above graph?

The sequential model, also known as the KNF model, was developed by Linus Pauling and can mathematically model negatively cooperative processes. It benefits from having only two fit-parameters as opposed to the three of the MWC model (e.g., less degrees of freedom and better determined values); however, its parameters are not directly relatable to biologically-relevant processes as the MWC model. There are always tradeoffs when picking models. The sequential mechanism only has four (sequential) steps:

$$Hb_0 + 4O_2 \overset{K_{0\rightarrow1}}{\Longleftrightarrow} Hb_1 + 3O_2 \overset{fK_{0\rightarrow1}}{\Longleftrightarrow} Hb_2 + 2O_2 \overset{f^2K_{0\rightarrow1}}{\Longleftrightarrow} Hb_3 + O_2 \overset{f^3K_{0\rightarrow1}}{\Longleftrightarrow} Hb_4$$

One can derive an equation that mathematically describes the sequential mechanism for hemoglobin:

$$\theta = \frac{K_{0\rightarrow1}(pO_2) + 3f\left(K_{0\rightarrow1}(pO_2)\right)^2 + 3\left(fK_{0\rightarrow1}(pO_2)\right)^3 + f^6\left(K_{0\rightarrow1}(pO_2)\right)^4}{1 + 4K_{0\rightarrow1}(pO_2) + 6f\left(K_{0\rightarrow1}(pO_2)\right)^2 + 4\left(fK_{0\rightarrow1}(pO_2)\right)^3 + f^6\left(K_{0\rightarrow1}(pO_2)\right)^4}$$

_____/35 pts

(exam continues on back)

12. (20 pts) The authors collected ^{1}H NMR data in 50 mM phosphate buffer at pH 7.0. Determine the mass of each phosphate species required to prepare 250 mL of this buffer. The pKa of phosphate is 7.21.

NaH_2PO_4 (119.98 g $mole^{-1}$) Na_2HPO_4 (141.96 g $mole^{-1}$)

_____/20 pts