Name:_____ Exam II

NOTE: All quoted texts are from Storz and Moriyama. (2008). Mechanisms of Hemoglobin Adaption to High Altitude Hypoxia. *High Altitude Medicine & Biology 9*: 148-157.

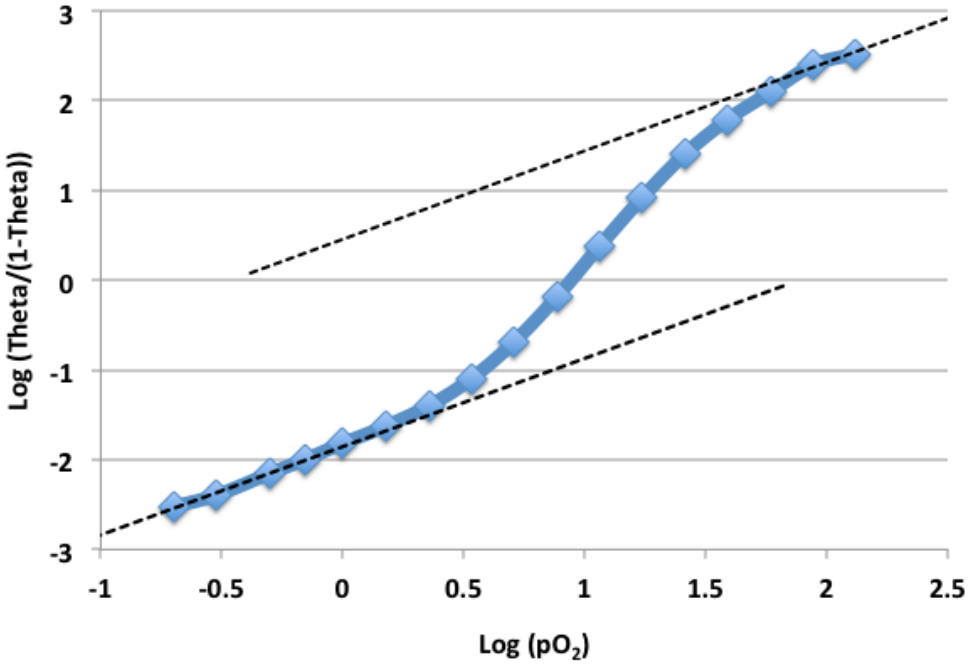
> "One of the most celebrated case studies of high altitude adaptation involves a pair of distantly related waterfowl species, the bar-headed goose (*Anser indicus*) and the Andean goose (*Chloephaga melanoptera*), that have independently evolved exceptionally high Hb–O_2 affinities. The bar-headed goose spends the breeding season on high alpine lakes at 4000 to 6000 m on the Tibetan Plateau and spends the winter months in wetland habitats in different parts of the Indian subcontinent. This requires an annual round-trip migratory flight over the crest of the Himalaya at altitudes of nearly 10,000 m where ambient PO_2 is less than one-third of that at sea level. As might be expected for an animal capable of sustaining powered flight at such altitudes, the bar-headed goose is characterized by an exceptionally high Hb–O_2 affinity relative to its lowland sibling species, the greylag goose (*Anser anser*; P_{50}, the PO_2 at 50% saturation of Hb 29.7 vs. 39.5 torr at 37°C, pH 7.4). The observed difference in P_{50} is attributable to a small difference in the intrinsic Hb–O_2 affinity..."

1. **Draw a single graph of theta vs. PO_2 (torr = mmHg), which contains expected oxygen binding curves to (*i*) bar-headed goose hemoglobin and (*ii*) graylag goose hemoglobin.**

2. **The difference between the oxygen affinities of the hemoglobins of these geese "is attributable to a small difference in the intrinsic Hb-O_2 affinity". If only the intrinsic affinity of oxygen is different between the geese hemoglobins, fill in each blank with "<", ">", or "=" for the parameters of the MWC model. [NOTE: K_{site} parameters are association equilibrium constants.]**

$$K_{site}^{T}(bar-headed) \_\_\_\_ K_{site}^{T}(graylag)$$
$$K_{site}^{R}(bar-headed) \_\_\_\_ K_{site}^{R}(graylag)$$
$$K_{o}^{T\rightarrow R}(bar-headed) \_\_\_\_ K_{o}^{T\rightarrow R}(graylag)$$

Imagine that the curve on the Hill plot below is for the bar-headed goose hemoglobin. Using the relative values of the parameters of the MWC that you indicated above, add a curve for the graylag goose hemoglobin.



"In principle, substitutions at any one of these [DPG] binding sites can alter the sensitivity of Hb to the various allosteric effectors [i.e., DPG], thereby altering the equilibrium between the T- and R-state quaternary structures. Since the binding of allosteric effectors [i.e., DPG] typically stabilizes T-state deoxyHb, amino acid substitutions that inhibit effector binding will typically increase Hb–O_2 affinity by shifting the equilibrium in favor of R-state oxyHb."

"The relatively high Hb–O_2 affinity of Andean camelids (i.e., llama, vicuña, alpaca, and guanaco) is attributable to a His→Asn substitution that eliminates two of the seven DPG-binding sites per tetramer."

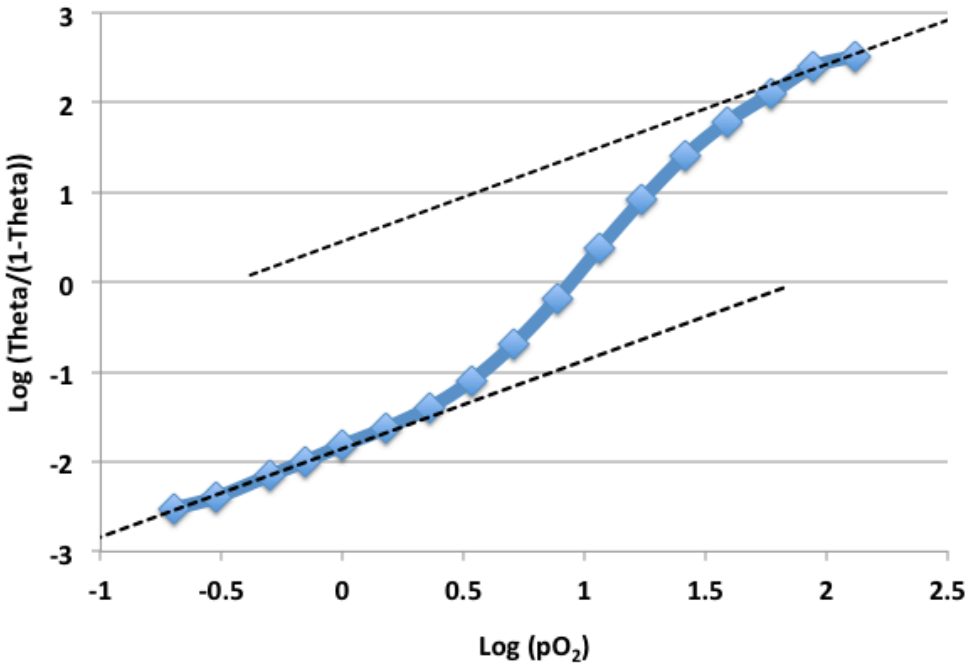
"The suppression of DPG binding that accounts for the high Hb–O_2 affinity of Andean camelids also accounts for the high O_2-binding affinity of human fetal Hb (HbF). The γ-chain subunits of HbF are encoded by the γ-globin gene, which is distinguished from the adult β-globin gene by the substitution His→Ser at key DPG-binding sites. The increased affinity of HbF relative to adult Hb is advantageous, because it facilitates placental O_2 transfer from the maternal circulation to the fetal circulation."

3. **Draw a single graph of theta vs. PO_2, which contains expected oxygen binding curves to (*i*) adult hemoglobin and (*ii*) fetal hemoglobin.**

4. **Fill in each blank with "<", ">", or "=" for the parameters of the MWC model. [NOTE: K_{site} parameters are association equilibrium constants.]**

$$K^{T}_{site}(adult) \_\_\_\_ K^{T}_{site}(fetal)$$
$$K^{R}_{site}(adult) \_\_\_\_ K^{R}_{site}(fetal)$$
$$K^{T\rightarrow R}_{o}(adult) \_\_\_\_ K^{T\rightarrow R}_{o}(fetal)$$

Imagine that the curve on the Hill plot below is for adult hemoglobin. Using the relative values of the parameters of the MWC that you indicated above, add a curve for fetal hemoglobin.

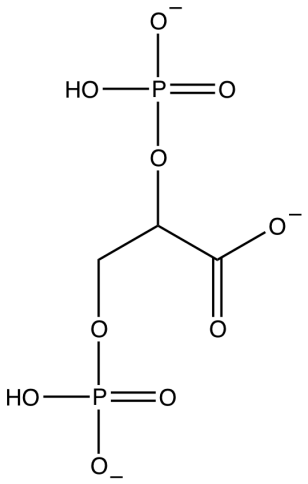


5. Fill in each blank with "<", ">", or "=" to compare the dissociation equilibrium binding constant (K_D) of the hemoglobin effector molecule DPG:

$$K_D^{DPG}(non-Andean\ camelid)\_\_\_\_K_D^{DPG}(Andean\ camelid)$$

$$K_D^{DPG}(adult)\_\_\_\_K_D^{DPG}(fetal)$$

The chemical structure of DPG is below. Explain the molecular basis for the observation that the His→Asn and His→Ser found in Andean camelids and fetal hemoglobins, respectively, suppresses DPG binding.



Activity Assay

SHOW ALL WORK for CALCULATIONS. INCLUDE UNITS.

1. Open the Beer's Law 2013 Excel Template. Accept any messages that may appear.
2. Wash the cuvette with dH_2O.
3. Add 750 uL of 1.0 mM ONPG to a cuvette.
4. Add 200 uL of Disruption Buffer to the cuvette.
5. Mix the solution by gentle pipetting.
6. Click the "time" button on the Beer's Law program.
7. Blank the spectrophotometer.
8. Add 50 uL of enzyme to the cuvette and mix by gentle pipetting.
9. Read the absorbance of the sample at 410 nm every 15 seconds for at least 3 minutes.
10. **List the equation of the best-fit line to linear portion your data for A_{410} vs. time in seconds. Include the correlation coefficient.**

11. Wash the cuvette with dH_2O. The reaction solution can go down the drain with water.
12. Close the Beer's Law 2013 Excel Template. Do not save the data.
13. **Use the mM absorptivity of the product ONP ($\varepsilon = 4.8\ mM^{-1}\ cm^{-1}$) to convert the slope to units of mM of ONP per minute.**

14. **Convert the slope to nmole of ONP per minute. This is defined as milliUnits (or mU) of activity.**

15. **Divide the mU of activity by the volume of enzyme solution added to the activity assay in units of mL to determine the specific activity in mU per mL.**