Problem 1: An Exotic Hemoglobin

In remote lake Baikal, in eastern Siberia, biologists recently found a hitherto unknown aquatic creature, with a most unusual Hemoglobin: It consists of two amino-acid chains only, with one heme group each, so as to have a total of two binding sites of Oxygen per macromolecule.

You are to establish the possible shapes of the oxygen saturation curve: Saturation $Y$ versus $p_{O_2}$.

**Notation:** There are four states of oxygen binding, which will be called

- $M_0 =$ bare macromolecule
- $M_{1a}, M_{1b} =$ one $O_2$ bound, on site a or b, respectively
- $M_2 =$ two $O_2$ bound

The associated chemical potentials will be called

- $\mu_{M0} = \mu_{M0}^0 + kT \ln X_0$
- $\mu_{M1a} = \mu_{M1a}^0 + kT \ln X_{1a} = \mu_{M0} + \Delta\mu_{1}^0 + kT \ln R_{1a}$
- $\mu_{M1b} = \mu_{M1b}^0 + kT \ln X_{1b} = \mu_{M0} + \Delta\mu_{1}^0 + kT \ln R_{1b}$
- $\mu_{M2} = \mu_{M2}^0 + kT \ln X_2 = \mu_{M0} + \Delta\mu_{2}^0 + kT \ln R_2$

Notice that the R’s are the mole fractions relative to the unliganded macromolecule. Also, sites a and b are assumed to have the same free energy $\Delta\mu_{1}^0$ of binding. Also observe that $R = R_{1a} + R_{1b}$, giving a degeneracy factor of 2.

Finally, let $\mu_{O_2} = \mu_{O_2}^0 + kT \ln \left( \frac{P_{O_2}}{P_f} \right)$ stand for the oxygen chemical potential.

**Question 1:** Write down the equilibrium conditions for the reactions

- $M_0 + O_2 \leftrightarrow M_{1a}$ (or $M_{1b}$)
- $M_0 + 2O_2 \leftrightarrow M_2$
Question 2: Find the expressions for \( R_1 \) and \( R_2 \).

Question 3: Introducing the fugacity \( z \) for Oxygen: \( z = e^{\mu_0 / kT} \), show that the relative abundances \( R \) can be written as

\[
R_0 = 1, \quad R_1 = B_1 z, \quad R_2 = B_2 z^2
\]

and give expressions for the coefficients \( B_1 \) and \( B_2 \).

Question 4: Show that the degree of \( Y \) of oxygen saturation \((0 \leq Y \leq 1)\) is given by

\[
Y = \frac{1 + \frac{R_1 + 2R_2}{2(1 + R_1 + R_2)}}{\frac{1}{2} \frac{\partial}{\partial z} \ln P(z)} \quad \text{where} \quad P(z) = 1 + B_1 z + B_2 z^2.
\]

N.B. \( P(z) \) is called the binding polynomial.

Question 5: Show that in the case where the free energy of binding of \( O_2 \) to one site does not depend on whether the other site is occupied or not, one has the relation \( \Delta \mu_0^2 = 2 \Delta \mu_0^1 \). Also show that, as a consequence, one has the relation between coefficients \( B_1 \) and \( B_2 \): \( B_2 = B_1^2 / 4 \). Finally show that in this case, the expression for the saturation \( Y \) reduces to

\[
Y = \frac{B_1 z / 2}{1 + B_1 z / 2}
\]

Question 6: Shape of the saturation curve: \( Y = Y(P_{O2}) \).

First it is clear from the definition of the fugacity \( z \) that \( z \) is proportional to the oxygen pressure \( p_{O2} \). This means that the binding polynomial \( P(z) \) can be written in the form

\[
P(p_{O2}) = 1 + 2 \frac{p_{O2}}{p_1} + \left( \frac{p_{O2}}{p_2} \right)^2
\]

Now show that the saturation \( Y(p) \) may be written as

\[
Y(p) = \frac{1}{2} p \frac{\partial}{\partial p} \ln P(p).
\]

Then show that the pressures \( p_1 \) and \( p_2 \) have a simple interpretation:

\[
\left( \frac{dY}{dp_{O2}} \right)_{p_{O2}=0} = \frac{1}{p_1} \quad \text{and that} \quad Y(p_2) = \frac{1}{2}, \quad \text{so that} \quad p_1 / 2 = p_2
\]

Question 7: Now use this information to show that the saturation curve will have the characteristic sigmoid shape provided one has \( p_2 < \frac{1}{2} p_1 \). (Hint: Make a sketch extending the tangent to the saturation curve at \( p = 0 : T(p) + \frac{p}{p_1} \) to and beyond the half saturation point \( p = p_2 \), and draw in the hypothetical sigmoid saturation curve.)
**Question 8:** What relation between the chemical potentials $\Delta \mu_1^0$ and $\Delta \mu_2^0$ is implied by the condition for a sigmoid shape?

**Problem 2: Linkage Relations.**

Consider a macromolecule $M$ with one binding site each for two distinct ligand species “a” and “b”. There will be four states: $M$, $M_a$, $M_b$, $M_{ab}$, with the following chemical potentials:

- $\mu_M = \mu_M^0 + kT \ln X_M$
- $\mu_{Ma} = \mu_M + \Delta \mu_{Ma}^0 + kT \ln R_a$ (where $R_a = X_{Ma}/X_M$, etc.)
- $\mu_{Mb} = \mu_M + \Delta \mu_{Mb}^0 + kT \ln R_b$
- $\mu_{Mab} = \mu_M + \Delta \mu_{Mab}^0 + kT \ln R_{ab}$

In addition, one has the chemical potentials $\mu_a$ and $\mu_b$ of the unliganded species “a” and “b”.

**Question 1:** State the equilibrium conditions for the reactions

- $M + a \leftrightarrow M_a$, $M + b \leftrightarrow M_b$, $M_a + b \leftrightarrow M_{ab} \leftrightarrow M + a$

**Question 2:** Obtain expressions for the relative probabilities $R_a$, $R_b$, and $R_{ab}$ for $M_a$, $M_b$, and $M_{ab}$, in terms of the quantities $\Delta \mu_{Ma}^0$, $\Delta \mu_{Mb}^0$, $\Delta \mu_{Mab}^0$, and the fugacities $z_a = \exp(\mu_a/kT)$ and $z_b = \exp(\mu_b/kT)$.

**Question 3:** Now let species “a” be an oxygen molecule, and species “b” a proton (that is, and H$^+$ ion). One has then

- $\mu_a/kT = \ln p_{O2} + \text{const}$, and $\mu_b/kT = -2.3 \text{ pH} + \text{const'}$

It is then convenient to express the binding polynomial

$$P = 1 + R_a + R_b + R_{ab} = 1 + B_a z_a + B_b z_b + B_{ab} z_a z_b = P(\mu_a, \mu_b)$$

in terms of a “Free Energy” $F$: $F(\mu_a, \mu_b) = -kT \ln P$

Show that the mean oxygenation $Y$ and the mean electric charge $Q$, given by

$$Y(p_{O2}) = <v_a> = \frac{R_a + R_{ab}}{1 + R_a + R_b + R_{ab}}$$

and

$$Q = <v_b> = \frac{R_b + R_{ab}}{1 + R_a + R_b + R_{ab}}$$

may be expressed as

$$<v_a> = -\frac{\partial F}{\partial \mu_a}$$

and

$$<v_b> = -\frac{\partial F}{\partial \mu_b}$$

**Question 4:** Show now how the “Maxwell relation” $\partial <v_a>/\partial \mu_b = \partial <v_b>/\partial \mu_a$ leads to the linkage relation

$$\left( \frac{\partial Y}{\partial \ln p_{O2}} \right)_{p_{O2}} = -2.3 \left( \frac{\partial Q}{\partial \ln p_{O2}} \right)_{pH}$$

**Question 5:** Legendre transformed Linkage Relations
Given that the differential \( dF \) has the structure:

\[
dF = - <v_a> d\mu_a - <v_b> d\mu_b
\]

show that the functions \( G_a = F + <v_a>\mu_a \) and \( G_b = F + <v_b>\mu_b \) may be viewed as functions of the sets \( \{<v_a>,\mu_b\} \) and \( \{\mu_a,<v_b>\} \) respectively.

This gives two new linkage relations

\[
\frac{\partial}{\partial \mu_b} \left( \frac{\partial G_a}{\partial <v_a>} \right) = \frac{\partial}{\partial <v_a>} \left( \frac{\partial G_b}{\partial \mu_b} \right)
\]

and one with \((a,b)\) interchanged.

State the explicit form of these relations, in terms of the quantities \( Y, Q, p_{O_2}, \) and \( \text{pH} \).

**Question 6**: One of the relations obtained in question 5 should be of the form

\[
\left( \frac{\partial Y}{\partial Q} \right)_{p_{O_2}} = 2.3 \left( \frac{\partial \text{pH}}{\partial \ln p_{O_2}} \right)_{Q}
\]

This relation can be integrated to give

\[
\Delta Y = \{Y(Q = 1) - Y(Q = 0)\}_{p_{O_2} = \text{const}} = \frac{2.3}{\partial \ln p_{O_2}} \int_0^1 \text{pH}(p_{O_2}, Q) dQ
\]

To do this integral, express \( \text{pH} \) in terms of \( Q \), by showing that \( Q + <v_b> \) may be written as

\[
Q = \frac{z_0}{z_0 + \frac{1}{1 + B_a z_a}} = \frac{[H^+]}{[H^+] + [H^+]^{1/2}}
\]

with \([H^+]\) being the hydrogen ion concentration, and \([H^+]^{1/2}\) that at \( Q = \frac{1}{2} \).

Show finally that \( \Delta Y \) is given, in terms of \( \text{pH}_{1/2} \) by

\[
\Delta Y = +2.3 \frac{\partial \text{pH}_{1/2}}{\partial \ln p_{O_2}}
\]

How could one read this number off a curve which plots \( Y \) versus \( p_{O_2} \) for various values of \( \text{pH} \)?

**Problem 3: Kinetic Equations and Equilibrium.**

In Chemistry, it is customary to look at a reaction process in kinetic terms. So, for the binding of a ligand \( a \) to a macromolecule \( M \): \( M + a \leftrightarrow M_a \), one writes the rate equation (in terms of concentrations \( C \) of the species involved)

\[
\frac{dC_{Ma}}{dt} = k^+ C_M C_a - k^- C_{Ma}
\]

**Question 1**: You clearly get from this the relation valid at equilibrium:

\[
R_a = \frac{C_{Ma}}{C_M} = \frac{C_a}{K_{eq}} = \frac{X_a}{K'_{eq}}
\]
Xₐ being the mole fraction of “a” in the “soup”. You previously dealt with an expression for Rₐ. What is the expression for the constant K’_{eq} in terms of the relevant chemical potential differences?

**Question 2:** If the concentration C_{Ma} depends on time t, so will Cₐ, since we have the conservation law

\[ C_{M} + C_{Ma} = C_{M,\text{tot}} = \text{const} \]

What is the rate equation for C_{Ma}: \[ \frac{dC_{Ma}}{dt} = ??? \]

Consider now the case of two distinct ligands “a” and “b”, capable of binding to the macromolecule M. In this case, one has the four species M, Mₐ, Mₐ, and Mₐₖ. The kinetic equation for Mₐ is then

\[ \frac{dC_{M_a}}{dt} = +k^+_a C_{Ma} C_a - k^-_a C_{Ma} + k^+_b C_{Mb} C_a - k^-_b C_{M_a} C_b. \]

**Question 3:** What is the interpretation of the four terms on the rhs?

**Question 4:** An equation similar to the above holds for \( \frac{dC_{Mb}}{dt} \). Write it out! What are the two additional equations, for \( \frac{dC_{M_a}}{dt} \) and \( \frac{dC_{M_{ab}}}{dt} \), respectively? Coefficients will appear in them, which by the conservation law

\[ C_{M} + C_{Ma} + C_{Mb} + C_{M_{ab}} = \text{const} \]

can be expressed in terms of those appearing in the eqs. for \( \frac{dC_{Ma}}{dt} \) and \( \frac{dC_{Mb}}{dt} \).

**Question 5:**
A) Calculate the mean square fluctuation \((\Delta v)^2\) in the number of oxygen molecules bound to the hemoglobin tetramer using the model of equal binding free energy for each subunit. Express your result in terms of the mean number \( \bar{v} \) bound.
B) Compare your result with the fluctuations expected using Poisson statistics.
C) Give a qualitative explanation for the departure from the result expected from Poisson statistics.
D) Answer parts A, B, and C also using the Pauling tetrahedral model which includes homotropic interactions between bound oxygen molecules.
E) Hint: For Poisson statistics \( P(v, \bar{v}) = \frac{\left(\bar{v}\right)^v e^{-\bar{v}}}{v!} \)