1) The data below are for the binding of oxygen to squid hemacyanin. The percent saturation is the parameter \( \frac{v}{N} \times 100\% = f \times 100\% \).

<table>
<thead>
<tr>
<th>( P_{O2} ) (mmHg)</th>
<th>Percent saturation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.13</td>
<td>0.30</td>
</tr>
<tr>
<td>7.72</td>
<td>1.92</td>
</tr>
<tr>
<td>31.71</td>
<td>8.37</td>
</tr>
<tr>
<td>100.5</td>
<td>32.9</td>
</tr>
<tr>
<td>136.7</td>
<td>55.7</td>
</tr>
<tr>
<td>203.2</td>
<td>73.4</td>
</tr>
<tr>
<td>327.0</td>
<td>83.4</td>
</tr>
<tr>
<td>566.9</td>
<td>89.4</td>
</tr>
<tr>
<td>736.7</td>
<td>91.3</td>
</tr>
</tbody>
</table>

a) Construct a Hill plot of the data shown

\[
\log \frac{v}{1-v} \text{ versus } \log P_{O2}
\]
b) From the plot, determine whether the binding is cooperative or independent. Explain.
The slope = 1.3 so the binding is not independent.

c) Estimate the number of oxygen molecules that can be attached to a single hemacyanin molecule.

The slope of this ‘line’ is 1.3, therefore $N = 2$.

2) Consider a monomeric protein which can exist between two forms T and R. Either T or R can bind a ligand A to form TA or RA.

\[
T \underset{k_r}{\overset{k_T}{\rightleftharpoons}} R \\
\Downarrow k_T \quad \Downarrow k_R \\
TA \iff RA
\]

a) Write an expression for the total fraction of bound proteins $\bar{V}$ in terms of the concentrations $[T]$, $[R]$, $[TA]$, $[RA]$.

\[
\bar{V} = \frac{protein \ molecules \ bound}{total \ protein \ molecules} = \frac{[TA] + [RA]}{[T] + [R] + [TA] + [RA]}
\]

b) Using the definitions of L, $k_T$ and $k_R$, write the expression for $\bar{V}$ in part a in terms of L, $k_T$, $k_R$, $[R]$, $[T]$, and $[A]$.

\[
k_r = \frac{[T][A]}{[TA]}, k_R = \frac{[R][A]}{[RA]}, \text{and } L = \frac{[T]}{[R]}
\]

Define $\bar{V} = \frac{[TA] + [RA]}{[T] + [R] + [TA] + [RA]} = \frac{\frac{[T][A]}{k_r} + \frac{[R][A]}{k_R}}{L \left(\frac{[T]}{[R]}\right) + \frac{[T][A]}{k_r} + \frac{[R][A]}{k_R}}$

\[
= \frac{\frac{[T][A]}{k_r} + \frac{[R][A]}{k_R}}{L + \frac{[T][A]}{k_r} + \frac{[R][A]}{k_R}} = \frac{1}{L + \frac{1}{k_r} + \frac{1}{k_R}}
\]

c) Note in the ligand-free environment the T form dominates so $L = \frac{[T]}{[R]} \gg 1$.

Assume also that T binds A more weakly than R binds A so the equilibrium constant $k_T = [T][A]/[TA]$ is much greater than $k_R = [R][A]/[RA]$, i.e. $k_T >> k_R$. Using these assumptions, simplify your expression for $\bar{V}$ in part b.

\[
\bar{V} = \frac{\frac{[T][A]}{k_r} + \frac{[R][A]}{k_R}}{L + \frac{1}{k_r} + \frac{1}{k_R}} = \frac{\frac{[A]}{k_R}}{L + \frac{1}{k_r} + \frac{1}{k_R}} = \frac{1}{L + \frac{1}{k_r} + \frac{1}{k_R}} [A]
\]
d) From the expression in part c, will the binding between A and the protein will be cooperative or independent? Explain.

Independent binding has the form $\bar{V} = \frac{K[A]}{1 + K[A]}$. We can put the equation for the mechanism above in this form...

$$\bar{V} = \frac{\frac{1}{L_k}[A]}{1 + \frac{1}{L_k}[A]} = \frac{K[A]}{1 + K[A]}$$

*where $K = \frac{1}{L_k}$*

This is the equation for independent site binding…

3) Consider the following data for the binding of a ligand A to a protein:

<table>
<thead>
<tr>
<th>[A] (M)</th>
<th>$\bar{V}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$10^{-6}$</td>
<td>0.101</td>
</tr>
<tr>
<td>$5 \times 10^{-6}$</td>
<td>0.381</td>
</tr>
<tr>
<td>$10^{-5}$</td>
<td>0.591</td>
</tr>
<tr>
<td>$5 \times 10^{-5}$</td>
<td>1.116</td>
</tr>
<tr>
<td>$10^{-4}$</td>
<td>1.409</td>
</tr>
<tr>
<td>$5 \times 10^{-4}$</td>
<td>1.813</td>
</tr>
<tr>
<td>$10^{-3}$</td>
<td>1.899</td>
</tr>
<tr>
<td>$5 \times 10^{-3}$</td>
<td>1.955</td>
</tr>
</tbody>
</table>

a) Make a Scatchard plot of the data. Show your result.
b) Make a Hill plot of the data. Show your result.

From the x intercept of the Scatchard plot $N=2$. So $f = \frac{\sqrt{2}}{2} \Rightarrow \frac{f}{1-f} = \frac{\sqrt{2}}{2-\sqrt{2}}$

![Hill Plot](image)

\[ \text{Hill Plot} \]

$c)$ From these two plots tell as much as you can about the binding. Is it cooperative or independent? What is (are) the binding constant(s)? How many binding sites?

**Solution:**
From the Scatchard plot we get non-linear behavior, which may arise from independent binding to 2 different types of sites. From the x intercept of the plot $N=2$. From lecture we see that a non-linear Scatchard plot has the 2-slope appearance if there is independent binding to two types of sites with $N_1=N_2=1$ but $K_1>K_2$. From the y intercept we get $K_1 N_1$ which is then $K_1=115,000$. $K_2$ is hard to obtain accurately without doing a simulation. But if $K_2$ were much smaller than $K_1$ (i.e. $K_1=1000K_2$) the x intercept would be 1. But the x intercept is 2 so $K_2$ is likely about a tenth of $K_1$.

The Hill plot shows a small non-linearity…which supports the two site independent binding model. The low [A] slope is 1 and the high [A] slope is 1 which rules out highly cooperative binding to two sites…in which case the slope would be 2. The data are consistent with independent binding to two inequivalent sites…but we cannot rule out a partly cooperative mechanism.
4) The following table gives the adsorption of butane onto nickel-magnesium catalyst as a function of butane pressure. V (mL) is the adsorbed gas volume, measured at S.T.P. (T=273K, P=760 mmHg). The saturated vapor pressure of butane at T=273K is P₀=774.4 mmHg. The data in the table are taken at T=273K. The nickel-magnesium catalyst weighs 1.876 grams.

<table>
<thead>
<tr>
<th>Pressure P (mmHg)</th>
<th>Adsorbed Volume V (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>56.39</td>
<td>17.09</td>
</tr>
<tr>
<td>89.47</td>
<td>20.62</td>
</tr>
<tr>
<td>125.22</td>
<td>23.74</td>
</tr>
<tr>
<td>156.61</td>
<td>26.09</td>
</tr>
<tr>
<td>179.30</td>
<td>27.77</td>
</tr>
<tr>
<td>187.46</td>
<td>28.30</td>
</tr>
</tbody>
</table>

a) Using the data in the table, and for \( x = \frac{P}{P_0} \), make a graph of \( \frac{x}{V(1-x)} \) as a function of x. Do the data fit the B.E.T. isotherm equation

\[
\frac{P}{V(P_0 - P)} = \frac{1}{V_m c} + \frac{c-1}{V_m c} \frac{P}{P_0^n} \]

? Explain.

Solution: According to the B.E.T. equation, a plot of \( \frac{x}{V(1-x)} \) as a function of x results in a straight line. The appropriate graph is

And the linear form indicates the B.E.T. equation is appropriate.
b) Using the B.E.T. equation, obtain an expression for \( V_m \), the volume of gas, measured under S.T.P. conditions, that is adsorbed into the first monolayer, in terms of the slope of the graph in part a and the y intercept.

Solution: According to the B.E.T. equation the slope is \( m = \frac{c - 1}{V_m c} = 0.039mL^{-1} \) and the y-intercept is \( b = \frac{1}{V_m c} = 0.0019mL^{-1} \).

c) From the graph in part a, determine the constants, \( V_m \) and \( c \).
Solution: You can eliminate \( c \) from these expressions and obtain

\[
V_m = \frac{1}{m + b} = \frac{1}{0.039 + 0.0019} mL = 24.5mL
\]

Then \( c \) can be obtained from the y-intercept equation…

\[
c = \frac{1}{V_m b} = \frac{1}{(24.5mL)(0.0019mL^{-1})} = 21.48
\]

d) Using your result in part c, calculate the surface area of the nickel-magnesium catalyst, assuming the cross sectional area of a single butane molecule is \( 44.6 \times 10^{-20} \) m\(^2\). Recall that the molar volume of a gas at S.T.P. is 22.415L. Give your answer in units of meters squared. Also, calculate the specific surface area, which is the surface area per gram of catalyst.

Surface Area=

\[
\sigma V_m N_A = \frac{44.6 \times 10^{-20} m^2 / molecule}{(24.5mL/ gm)(6.02 \times 10^{23}molecules / mole)}
\]

\[
= 293.8 m^2 / gm
\]

5) A membrane is passively permeable to water and to Cl\(^-\) ion, but not to H\(^+\). The electrostatic potential \( E_\phi \) and the equilibrium concentrations of Cl\(^-\) and H\(^+\) inside and outside the membrane are given below. Assume the ionic strength inside the membrane is sufficiently small so that the activity coefficients of univalent ions may be taken to be 1 inside the membrane. \( T=298 \).

<table>
<thead>
<tr>
<th>Outside Membrane</th>
<th>Inside Membrane</th>
</tr>
</thead>
<tbody>
<tr>
<td>E(\phi) = 0.150 volts</td>
<td>E(\phi) = 0.000</td>
</tr>
<tr>
<td>([H^+]) = 5x10(^{-6})M</td>
<td>([H^+]) = 10(^{-7}) moles/liter</td>
</tr>
<tr>
<td>([Cl^-]) = 5x10(^{-2})M</td>
<td>([Cl^-]) = ?</td>
</tr>
<tr>
<td>([Na^+]) =0.2 M</td>
<td>([SO_4^{2-}] =0.075 M</td>
</tr>
</tbody>
</table>

a) Calculate the ionic strength outside of the membrane.
Solution:
\[ I = \frac{1}{2} \left( 12[\text{Na}^+] + 12[\text{Cl}^-] + 2^2[\text{SO}_4^{2-}] \right) = \frac{1}{2} (12(0.2) + 12(0.05) + 2^2(0.075)) = 0.275 \]

b) Using the Debye-Huckel expression for the mean activity coefficient (even though in some cases ion concentrations may exceed the limits of that theory) calculate the mean activity coefficient \( \gamma \) and the activity of \( \text{Cl}^- \) outside the membrane.

Solution:
\[ \log \gamma \approx -0.509z^2 \sqrt{I} = (-0.509)(0.275)^{1/2} = -0.267; \gamma = 10^{-0.267} = 0.541 \]

c) What is the equilibrium ratio of the activity of \( \text{Cl}^- \) inside the membrane to that outside the membrane?

\[ \mu^\circ + RT \ln a_{\text{outside}} + zF \phi_{\text{outside}} = \mu^\circ + RT \ln a_{\text{inside}} + zF \phi_{\text{inside}} \]

substituting \( z = -1 \) and cancelling the \( \mu^\circ \)’s

\[ RT \ln a_{\text{outside}} - FE_{\phi_{\text{outside}}} = RT \ln a_{\text{inside}} - FE_{\phi_{\text{inside}}} \]

dividing through by \( RT \) and rearranging

\[ \frac{F(\phi_{\text{inside}} - \phi_{\text{outside}})}{RT} = \ln \left( \frac{a_{\text{inside}}}{a_{\text{outside}}} \right) \]

making appropriate numerical substitutions

\[ \frac{-96500(0.150)}{8.314 \times 298} = -5.842 = \ln \left( \frac{a_{\text{inside}}}{a_{\text{outside}}} \right) \]

\[ \left( \frac{a_{\text{inside}}}{a_{\text{outside}}} \right) = e^{-5.842} = 0.00290 \]

d) What is the activity and the equilibrium concentration of \( \text{Cl}^- \) inside the membrane? Hint: calculate the activity of \( \text{Cl}^- \) outside the membrane.

Solution:
\[ a_{\text{outside}} = \gamma \ [\text{Cl}^-] = (0.541)5 \times 10^{-2} \text{ moles/liter} = 0.0271 \text{M} \]

You have also calculated the ratio of activities, (from the potential) in part d so you can get the activity inside

\[ a_{\text{inside}} = [\text{Cl}^-] = a_{\text{outside}}(0.00290) = (0.0271 \text{M})(0.00290) = 7.86 \times 10^{-5} \text{M} \]

e) Calculate the difference between the chemical potentials of [\( \text{H}^+ \)] inside and outside the membrane.
Solution:

\[ \mu_{\text{inside}} - \mu_{\text{outside}} = \{ \mu^0 + RT \ln \text{a}_{\text{inside}} + zF\phi_{\text{inside}} \} - \{ \mu^0 + RT \ln \text{a}_{\text{outside}} + zF\phi_{\text{outside}} \} \]

substituting \( z = 1 \) and cancelling the \( \mu^0 \)s

\[ \{ \mu_{\text{inside}} - \mu_{\text{outside}} \} = \{ RT \ln \text{a}_{\text{inside}} + F\phi_{\text{inside}} \} - \{ RT \ln \text{a}_{\text{outside}} + F\phi_{\text{outside}} \} \]

rearranging and factoring out \( RT \)

\[ \{ \mu_{\text{inside}} - \mu_{\text{outside}} \} = RT \ln \left( \frac{\text{a}_{\text{inside}}}{\text{a}_{\text{outside}}} \right) + F(\phi_{\text{inside}} - \phi_{\text{outside}}) \]

\[ \{ \mu_{\text{inside}} - \mu_{\text{outside}} \} = (8.31)(298) \ln(10^{-7}/(0.541)(5 \times 10^{-6})) - 96,500(0.150) \]

\[ = -8,166 \text{J/mole} - 14,475 \text{J/mole} = -22,641 \text{J/mole} \]

f) The reaction \( ATP \rightleftharpoons ADP + P_i \) \( \Delta G^\circ = -31 \text{ kJ/mole} \) is coupled to the transport of \( H^+ \) with an efficiency of 50% (i.e., half the free energy from ATP hydrolysis is recovered and available to drive the active transport of \( H^+ \)). Assuming the concentration of inorganic phosphate is 0.01M, what ratio of ATP to ADP is required to drive the transport of \( H^+ \)?

Solution: By driving the transport of \( H^+ \) we mean transporting \( H^+ \) from inside to outside. This occurs against a concentration gradient and the work required is the opposite sign of the work calculated in part e. This means the work is positive...i.e the transport must be driven by hydrolyzing ATP.

\[ \Delta G = 0 \text{ (at equilibrium)} \]

\[ \Delta G = \Delta G(\text{Cl}^-) + \Delta G(H^+) + \Delta G(\text{ATP}/\text{ADP}) = 0 \]

But \( \Delta G(\text{Cl}^-) = 0 \ldots \) because the membrane is permeable to \( \text{Cl}^- \). \( \Delta G(H^+) = \mu_{\text{outside}} - \mu_{\text{inside}} = 22,641 \text{J}, \) from part e.

\[ \Delta G(\text{ATP}/\text{ADP}) = \Delta G^\circ + RT \ln Q = -31,000 \text{J} + RT \ln Q \text{ where Q is the desired ratio} \]

\[ \frac{[ADP][P_i]}{[ATP]} \]

\[ \Delta G = 0 = \Delta G(H^+) + (0.5) \Delta G(\text{ATP}/\text{ADP}) = 22,641 \text{J} - 15,500 \text{J} + (0.5)RT \ln Q \]

\[ 0 = 7141 \text{J} + (0.5)(8.31)(298) \ln Q = 7141 \text{J} + 1238.2 \ln Q \]

Therefore \( \ln Q = -5.77 \) and

\[ Q = 0.0031 = \frac{[ADP][P_i]}{[ATP]} = \frac{[ADP](0.01)}{[ATP]} \]

\[ \therefore \frac{[ADP]}{[ATP]} = \frac{0.0031}{(0.01)} = 0.31 \]
6) The electrostatic potential difference $\Delta \psi$ across a biological membrane can be calculated using the Goldman equation. The following conditions exist in the resting squid axon:

<table>
<thead>
<tr>
<th>Ion</th>
<th>Concentration Inside</th>
<th>Concentration Outside</th>
<th>Relative Permeability</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K^+$</td>
<td>400mM</td>
<td>20mM</td>
<td>1.00</td>
</tr>
<tr>
<td>$Na^+$</td>
<td>50mM</td>
<td>440mM</td>
<td>0.04</td>
</tr>
<tr>
<td>$Cl^-$</td>
<td>50mM</td>
<td>560mM</td>
<td>0.45</td>
</tr>
</tbody>
</table>

a) Use the Goldman equation to calculate the membrane potential at $T=293K$. Note relative permeabilities are just ratios of absolute permeabilities $P$. The relative permeabilities listed above are expressed relative to the membrane permeability of potassium. Hence, for example, the relative permeability of sodium is $\frac{P_{Na^+}}{P_{K^+}}=0.04$.

Solution: The Goldman equation gives the value of the membrane potential $E$ as a function of ion concentrations inside and outside the membrane and ion membrane permeabilities $P$. As explained in the lecture notes the Goldman equation has the form:

$$E_{membrane} = \frac{RT}{3} \ln \left[ \frac{[K^+]_{out}}{[K^+]_{in}} \frac{P_{K^+}}{P_{Na^+}} + \frac{[Na^+]_{out}}{[Na^+]_{in}} \frac{P_{Na^+}}{P_{Cl^-}} + \frac{[Cl^-]_{out}}{[Cl^-]_{in}} \frac{P_{Cl^-}}{P_{Na^+}} \right]$$

$$E_{membrane} = \frac{RT}{3} \ln \left[ \frac{[K^+]_{out}}{[K^+]_{in}} \frac{P_{K^+}}{P_{Na^+}} + \frac{[Na^+]_{out}}{[Na^+]_{in}} \frac{P_{Na^+}}{P_{Cl^-}} + \frac{[Cl^-]_{out}}{[Cl^-]_{in}} \frac{P_{Cl^-}}{P_{Na^+}} \right]$$

$$= \frac{8.31J/K}{96485C} \ln \frac{20}{400} \frac{1}{1} + \frac{440}{50} \frac{0.04}{0.45} + \frac{50}{560} \frac{0.45}{0.45} = 0.0252V \ln \frac{20 + 17.6 + 22.5}{400 + 2 + 252}$$

$$=(0.0252V)\ln(60.1/654)=-60.2mV$$

b) Suppose that upon transition to the excited state, there occurs a 330-fold increase in the $Na^+$ permeability to the squid axon membrane relative to potassium (i.e. the $Na^+$ gates open). What happens to the membrane potential? Support your answer with a calculation using the Goldman equation.

$$E_{membrane} = \frac{8.31J/K}{96485C} \ln \frac{20}{400} \frac{1}{1} + \frac{440}{50} \frac{0.04}{0.45} + \frac{330}{560} \frac{0.45}{0.45}$$

$$= 0.0252V \ln \frac{20 + 5808 + 22.5}{400 + 660 + 252} = 0.0252V \ln \frac{5851}{1312} = 25.2mV \ln 4.46 = 168mV$$