Assignment One (KEY) **Due 12 October 2012 by 4:30 PM at FS229**

QUESTION ONE:

> Part One. Propose a mechanism that uses the properties of lipids to sense temperature. Be explicit about the way that you would 'tune' the lipids for various temperature ranges, showing structures as required.
>
> Part Two. Compare your mechanism to known temperature-sensing transporters. These can be from any biological clade (although the animal world should provide you with the most fascinating examples). I am especially interested in the mechanism. In other words, you may find that heat activates channels, but how does it activate them?

Hint: I am not interested in a literature review, or essay-style approach. You will find many papers on heat sensing channels, but probably very little on the heat-sensing mechanism. It's the latter I am interested in. For example, are lipids involved? Is there an infrared absorbing pigment? Do scientists actually know? And if not, what do you propose?

For Part One, lipid acyl chain length, unsaturation, and head group (3/5 points) are the major determinants of gel to fluid transition and in various combinations can be used to 'tune' the temperature-sensing mechanism to specific temperatures (2/5 points).

For Part Two, partial credit was given for 'effort' (2/5). This was mostly for descriptions of trp or similar ion channels, for which downstream effects of T-sensing are well documented, but the T-sensing mechanism itself is unknown. Many students identified a well-understood bacterial T-sensing mechanism, in which a small sequence at the lipid aqueous interface is perturbed because chilling increases the membrane thickness, resulting in a cascade of downstream effects that result in changes in unsaturation or saturation of the lipid acyl chains. Other students identified a short a.a. sequence in an ion channel for which a.a. substitutions provide good evidence that it functions in T-sensing, though the actual mechanisms remains unclear (5/5).

I learned a lot from assignments, and thank you! I had no idea how central T-sensing is and how little is known! Students commonly found papers published in the past two years or so, evidence of a very active area of research!

Parts one (/5) and two (/5) added to 10 points total (out of 20 for the entire assignment).

QUESTION TWO: Assuming that action potentials would be triggered if the resting potential of excitable cells is depolarized by 30 mV from the normal resting potential of –80 mV, calculate the LD_{50} (lethal dose, at which 50% of the population would die) for bananas. Assume that potassium clearing is not occurring and for the sake of simplicity, assume you need only consider blood volume.

For this question, it was crucial to show calculations of the Nernst potential and the need to calculate it for the initial potential (–80 mV) *minus* the final potential (–50 mV): $\psi_{initial} - \psi_{final} = 30$ mV (10/10). Some students used toxicity data *only* (of which INCHEM is one of the most comprehensive) and were awarded partial credit (2/10). Student calculations for the number of 'lethal' bananas ranged from 1 banana to 55. I suspect that both you and I will never look at a banana the way we used to....

Question Two (/10) was 10 points total (out of 20 for the entire assignment).

ANSWER ONLY ONE OF THE FOLLOWING TWO QUESTIONS

Question One.

Equlibrium concentrations of intracellular and extracellular ions are shown for a 'normal' animal cell in the table below.

Part One. Calculate the inward flux of Ca^{2+} if the membrane potential is –100 mV (negative-inside). Assume the permeability coefficient for Ca^{2+} is 10^{-10} cm sec^{-1}.

Ion	Extracellular (mM)	Intracellular (mM)
Na^+	145	12
K^+	4	155
Ca^{2+}	1.5	$<10^{-4}$
Cl^-	123	4.2

Part Two. If the cell is a typical size (about 10 micron in diameter), calculate how high the concentration of Ca^{2+} would be after 60 minutes.

Part Three. If the Ca^{2+} influx occurred at a single location on the cell, how long would it take to diffuse to the other side of the cell?

Question Two.

Equlibrium concentrations of intracellular and extracellular ions are shown for a 'normal' marine algal cell (*Valonia ventricosa*) in the table below. The reported membrane potential is –70 mV (negative-inside)[1]

Part One. For the four ions below, predict the relative permeability coefficients based on known mathematical relations of ions and fluxes (that is, quantitatively). Show your calculations and assumptions, if any, that you must make.

Ion	Extracellular (mM)	Intracellular (mM)
Na^+	490	40
K^+	10	435
Ca^{2+}	1.0	$<10^{-4}$
Cl^-	560	140

Part Two. For the most permeable ion (in Part One), calculate the net concentration of imbalanced charge that would cause a potential of –100 mV. *Valonia* is a spherical cell with a diameter of 1 centimeter.

Guidelines: Equations and constants are provided. Please be sure that you show units. This is an important internal check, both for you and for me.

[1] Gutknecht J (1966) Sodium, potassium, and chloride transport and membrane potentials in *Valonia ventricosa*. The Biological Bulletin 130:331–344.

Equations relevant to Membrane Transport: Geometry, Diffusion and Flux

Sphere Area : $4 \bullet \pi \bullet r^2$ Sphere Volume : $\frac{4}{3} \bullet \pi \bullet r^3$

Cylinder Area : $4 \bullet \pi \bullet r \bullet h$ Cylinder Volume : $\pi \bullet r^2 \bullet h$

Cube Area : $6 \bullet \mathrm{h}^2$ Cube Volume : h^3

Fick's Diffusion : $J = D \bullet \frac{dc}{dx}$ Fick's Diffusion : $\frac{dc}{dt} = D \bullet \frac{d^2c}{dx^2}$

Einstein's Random Walks : $D = \frac{1}{2} \bullet \frac{\Delta^2}{\tau}$, $\left\langle x^2 \right\rangle = 2 \bullet D \bullet t$, and $\left\langle r^2 \right\rangle = 6 \bullet D \bullet t$

Membrane Diffusion : $J = P \bullet \left(c_{outside} - c_{inside} \right)$

Membrane Diffusion : $J = -(uRT) \bullet \frac{dc}{dx} - (zFuc) \bullet \frac{d\Psi}{dx}$

Membrane Diffusion : $J = -P \bullet \left(\frac{zF\Psi}{RT} \right) \bullet \left(\frac{c_o - c_i \bullet e^{zF\Psi / RT}}{1 - e^{zF\Psi / RT}} \right)$

Nernst Equation : $\Psi = \left(\frac{RT}{zF} \right) \bullet \ln \left(\frac{c_o}{c_i} \right)$

Ohm's Law : $V = I \bullet R$, $I = g \bullet V$, $R = \rho \bullet \left(l / A \right)$, and $J = I/(zF)$

Radial Diffusion : $C(r) = C_\infty \bullet \left(1 - \frac{a}{r} \right)$, and $J(r) = -D \bullet C_\infty \bullet \left(\frac{a}{r^2} \right)$

Radial Currents : $I_m = 4 \bullet \pi \bullet a^2 \bullet \beta$, and $I_d = 4 \bullet \pi \bullet a \bullet D \bullet C_\infty$

Dimensionless relations $P_e = \frac{2 \bullet a \bullet v}{D}$ and $R_e = \frac{\rho \bullet v \bullet l}{\eta}$

Goldman - Hodgkin - Katz (GHK) equation

$$\Psi = \frac{RT}{F} \ln \left(\frac{P_H c_H^o + P_{Na} c_{Na}^o + P_K c_K^o + P_{Cl} c_{Cl}^i}{P_H c_H^i + P_{Na} c_{Na}^i + P_K c_K^i + P_{Cl} c_{Cl}^o} \right)$$

Equations relevant to membrane capacitance

$Q = C \cdot \Delta E$ (coulombs) = (coulombs/volt) (volt)

Charge (Q) for a spherical cell of radius r:

$Q = 4/3 \cdot \pi \cdot r^3 \cdot c \cdot F$

c is the concentration of net charge.

Capacitance of a spherical cell of radius r:

$C = 4 \cdot \pi \cdot r^2 \cdot C'$ C' is the capacitance per unit area

(about 1 microFarad per square centimeter for cells).

Symbol	Value	Units	Comments
GAS CONSTANT			
R	8.314	J mol^{-1} K^{-1}	R is the Boltzmann constant times Avogadro's Number ($6.023 \cdot 10^{23}$)
	1.987	cal mol^{-1} K^{-1}	
	8.314	m^{-3} Pa mol^{-1} K^{-1}	
RT	$2.437 \cdot 10^3$	J mol^{-1}	At 20 ºC (293 ºK)
	$5.833 \cdot 10^2$	cal mol^{-1}	At 20 ºC (293 ºK)
	2.437	liter MPa mol^{-1}	At 20 ºC (293 ºK)
RT/F	25.3	mV	At 20 ºC (293 ºK)
2.303 • RT	5.612	kJ mol^{-1}	At 20 ºC (293 ºK)
	1.342	kcal mol^{-1}	At 20 ºC (293 ºK)
FARADAY CONSTANT			
F	$9.649 \cdot 10^4$	coulombs mol^{-1}	F is the electric charge times Avogadro's Number
	$9.649 \cdot 10^4$	J mol^{-1} V^{-1}	
	23.06	kcal mol^{-1} V^{-1}	
CONVERSIONS			
kcal	4.187	J (joules)	Joules is an energy unit (equal to 1 Newton•meter)
Watt	1	J sec^{-1}	
Volt	1	J $coulomb^{-1}$	
Amperes	1	coulomb sec^{-1}	
Pascal (Pa)	1	Newton $meter^{-2}$	Pascal is a pressure unit (equal to 10^{-5} bars)
Siemens	1	Ohm^{-1}	Siemens (S) is conductance, the inverse of resistance (Ohm)
PHYSICAL PROPERTIES			
η_w	$1.004 \cdot 10^{-3}$	Pa sec	viscosity of water at 20 ºC
ν_w	$1.004 \cdot 10^{-6}$	m^2 sec^{-1}	kinematic viscosity of water at 20 ºC (viscosity/density)

Source: Nobel, Park S (1991) Physicochemical and Environmental Physiology

1.1 Ca^{2+} influx - driven by both Ψ & concentration

$$J = -P \underbrace{\frac{zF\Psi}{RT}} \frac{c_o - c_i \exp\left(\frac{zF\Psi}{RT}\right)}{1 - \exp\left(\frac{zF\Psi}{RT}\right)}$$ 5/10

note! $z=2$ & $F/RT = \frac{1}{25\,mV}$ 2/10

$$\frac{(2)(-100\,mV)}{25\,mV} = -8$$

$$J = (10^{-10}\,\tfrac{cm}{sec})(-8)\,\frac{\overbrace{0.0015 - 10^{-7} e^{-8}}^{1.4991 \times 10^{-6}}}{\underbrace{1 - e^{-8}}_{0.9997}}\ \frac{mole}{1000\,cm^3}$$

cm/sec

$$\frac{mole}{cm^2\,sec} = J = 1.2 \times 10^{-18}\ mole/cm^2\,sec$$ 3/10

1.2 cell area $4\pi (0.5 \times 10^{-3}\,cm)^2 = 3.14 \times 10^{-6}\,cm^2$ 3/10

cell volume $4/3\pi (0.5 \times 10^{-3}\,cm)^3 = 5.24 \times 10^{-10}\,cm^3$ 3/10

J area time

$$\frac{(1.2 \times 10^{-18}\,\frac{mole}{cm^2\,sec})(3.14 \times 10^{-6}\,cm^2)(60\,\frac{sec}{min})(60\,min)}{5.24 \times 10^{-10}\,cm^3}$$

volume

$$= 2.59 \times 10^{-11}\,\frac{mole}{cm^3}$$

or $0.026\,\mu M$ (plus $0.1\,\mu M$) 4/10

1.3 From $r^2 = 6 \cdot D \cdot t$, $t = \frac{r^2}{6D}$ 5/10

but what is D? Most ions will have diffusion coefficients in the range of $10^{-10}\,cm^2/sec$ (measured value is $7.9 \times 10^{-10}\,\frac{cm^2}{sec}$ *)

$$t = \frac{(1 \times 10^{-3}\,cm)^2}{6\ 10^{-10}\,cm^2/sec} = 1670\,sec$$ 5/10

marks out of 30
(10 per question point)

* next page.

1.3 (continued)

It may be possible to estimate D from the given P-value (10^{-10} cm/sec).

$$P = D \frac{K_p}{d} \quad \text{or} \quad D = P \frac{d}{K_p}$$

d is the thickness of the membrane (2-3 nm)
K_p is the partitioning $\frac{C_{membrane}}{C_{aqueous}}$

This will be very low because of the charge on the Ca^{2+}, say 1 in a million? (10^{-6})

$$D = 10^{-10} \frac{cm}{sec} \frac{2-3 \times 10^{-7} cm}{10^{-6}}$$

$$= 2-3 \times 10^{-11} cm^2/sec.$$

7.1 Relative permeabilities

We can take an indirect approach by calculating Nernst potentials for the given ions and comparing to the resting potential.

	ext	int	E_N*	
Na^+	490	40	+63 mV	(+ve inside)
K^+	10	435	−94	
Ca^{2+}	1	10^{-4}	+115 mV	(divide by $z=2$)
Cl^-	560	140	−35	(−ve inside)

* $E = \frac{RT}{F} \ln \frac{C_o}{C_i}$

Relative permeabilities:

K^+	−94 − (−70)	+24	most permeable
Cl^-	−35 − (−70)	+35	
Na^+	+63 − (−70)	+133	
Ca^{2+}	+115 − (−70)	+185	least permeable

Now, we can solve for two of the ions to account for concentration

For example, $\psi = \frac{RT}{F} \ln \frac{P_K[K^+]_o + P_{Cl}[Cl]_i}{P_K[K^+]_i + P_{Cl}[Cl]_o}$ $\times \frac{1}{P_K}$ $\times \frac{1}{P_K}$

chloride

$$\ln \frac{[K^+]_o + \frac{P_{Cl}}{P_K}[Cl]_i}{[K^+]_i + \frac{P_{Cl}}{P_K}[Cl]_o}$$

$$\exp \frac{-70}{25} = \frac{10 + 140x}{435 + 560x}$$

$$x \left(\frac{P_{Cl}}{P_K}\right) = 0.16$$

For calcium

divalent

$$\psi = \frac{RT}{F} \ln \frac{P_K [0.01] + P_{Ca} [0.001]^2}{P_K [0.435] + P_{Ca} [10^{-7}]^2} \quad \begin{matrix} 1/P_{Ka} \\ 1/P_K \end{matrix}$$

$$e^{-70/25} \approx \frac{[0.01] + x [0.001]^2}{[0.435] + x [10^{-7}]^2}$$

$e^{-70/25} \rightsquigarrow 0.0608$

$$0.0608 [0.435] + 0.0608 \cdot 10^{-14} \cdot x = [0.01] + 10^{-6} x$$

$$0.0608 [0.435] - [0.01] = 10^{-6} x - 6.0608 \cdot 10^{-14} x$$

$$\sim 10^{-6} x$$

$$1.6 \times 10^4 = P_{Ca}/P_K$$

That is, P_{Ca} is more permeable

chk

$$25 \ln \frac{[0.01] + 1.6 \times 10^4 [0.001]^2}{[0.435] + 1.6 \times 10^4 [10^{-7}]^2} \quad \frac{0.026}{0.435}$$

$= -70$ mV

Assignment One (31 October 2012) **Due 9 November 2012 by 4:30 PM at FS229**

QUESTION ONE: For arsenate transport —utilizing both arsA and arsB, or arsB alone— calculate the relative efficiencies of arsenate exclusion from the bacterium. What are the effects of stoichiometry and the net charge of the arsenate oxyanion on exclusion?

(Hints: You will need to consider the Gibbs free energy difference. For ATP hydrolysis, use $\Delta G^{o} = 10$ kcal/mole. For comparisons of the effects of stoichiometry and net oxyanion charge, use more than 2 values, to identify trends, if any. Bacterial membrane potentials are difficult to measure, most measured values cluster around –100 mV, negative inside. Finally, it is probably easiest to keep the extracellular concentration constant —10 mM might work well.)

QUESTION TWO: Based on the biochemical properties of the various oxidative states and chemical forms of arsenic, is there any advantage to modifying the oxidative state or chemical form of the arsenic to minimize the energetic requirements for transport out of the cell? Propose a mechanism, with explanation.

(Hint: Bear in mind that some chemical modifications may require energy input.)

Ground Rules: I expect that students may (or may not) wish to work with each other on the assignment (depending on personal preference), and may certainly come to me for help. But, please ensure that the work you hand in is in your own words (it's your voice I want to hear).

Assignment Two (9 November 2012) **KEY**

Question One

For arsenate transport —utilizing both arsA and arsB, or arsB alone—calculate the relative efficiencies of arsenate exclusion from the bacterium. What are the effects of stoichiometry and the net charge of the arsenate oxyanion on exclusion?

(Hints: You will need to consider the Gibbs free energy difference. For ATP hydrolysis, use ΔG^{o} = 10 kcal/mole. For comparisons of the effects of stoichiometry and net oxyanion charge, use more than 2 values, to identify trends, if any. Bacterial membrane potentials are difficult to measure, most measured values cluster around –100 mV, negative inside. Finally, it is probably easiest to keep the extracellular concentration constant —10 mM might work well.)

Key

Calculations (using Maple for the sake of simplicity) are shown separately. For ArsB alone —the oxyanion channel— expelling a more negatively charged oxyanion is more efficacious —resulting in a much lower internal [As]. Coupling efflux to ATP hydrolysis (Ars A and B) is even more efficacious. However, if the stoichiometry is increased from 1 to 2 or 3 arsenic molecules per ATP, the efficacy declines. This is due to the higher energy cost of moving more than one molecule per cycle (the Gibbs free energy for ATP hydrolysis remains constant).

Scoring was 'generous'. However, calculations that led to very unrealistic values of internal arsenic resulted in lower scores.

(10/10)

Question Two

Based on the biochemical properties of the various oxidative states and chemical forms of arsenic, is there any advantage to modifying the oxidative state or chemical form of the arsenic to minimize the energetic requirements for transport out of the cell? Propose a mechanism, with explanation.
(Hint: Bear in mind that some chemical modifications may require energy input.)

Key

Students provided a number of fairly distinct options with respect to potential mechanisms. For the first, they cleaved closely to the mechanism used by *E. coli*, reduction to arsenite followed by transport out of the cell. Other students provided evidence in other organisms for energy production mechanisms relying on electron donation from arsenic (arsenite) to produce ATP as a means to offset energetic costs. Another approach was to invoke methylation of arsenic, as it occurs in humans and other mammals followed by transport out of the cell by an ABC cassette transporter —this approach is problematic because of the metabolic costs of synthesizing methyl groups. Finally, some students provided evidence of the conversion of arsenic to volatile forms that could easily diffuse out of the cell into the atmosphere.

Scoring depended upon the clarity of presentation, novelty, and consideration of energetic costs.

(10/10)

The calculations were performed using Maple, for the ease of presentation.

Here are the constants and other values we require

> $R := 1.987 \cdot 10^{-3} \frac{[\![kcal]\!]}{[\![mol]\!][\![K]\!]} : T := 293[\![K]\!] : F := 23.06 \frac{[\![kcal]\!]}{[\![mol]\!][\![V]\!]} : G := -10 \frac{[\![kcal]\!]}{[\![mol]\!]} : \psi := -0.1\,[\![V]\!] : As_o := 0.010 \frac{[\![mol]\!]}{[\![liter]\!]} :$

First, we solve for the oxyanion channel alone (ArsB), with the potential as the only driving force

> $solve\left(0 = R \cdot T \cdot \ln\left(\frac{As_i}{As_o}\right) + -1 \cdot F \cdot \psi, As_i\right)$

$$\frac{1.90 \times 10^{-4}\,[\![mol]\!]}{[\![L]\!]}$$

> $solve\left(0 = R \cdot T \cdot \ln\left(\frac{As_i}{As_o}\right) + -2 \cdot F \cdot \psi, As_i\right)$

$$\frac{3.63 \times 10^{-6}\,[\![mol]\!]}{[\![L]\!]}$$

> $solve\left(0 = R \cdot T \cdot \ln\left(\frac{As_i}{As_o}\right) + -3 \cdot F \cdot \psi, As_i\right)$

$$\frac{6.91 \times 10^{-8}\,[\![mol]\!]}{[\![L]\!]}$$

For Ars B alone, we need to consider only the chemical potential for arsenate, and solve for the internal [As]. Note that an increase in charge (to more negative values, arrows creates a very strong driving force for arsenic exclusion from the cell, because of the negative inside potential.

Now we need to consider the contribution of ATP hydrolysis to arsenate exclusion (Ars AB), calculating delta-G

> $ATP := 0.0025 : ADP := 0.0005 : P := 0.0005 :$

> $solve\left(Gibbs = G + R \cdot T \cdot \ln\left(\frac{ADP \cdot P}{ATP}\right), Gibbs\right)$

$$-\frac{15.36\,[\![kcal]\!]}{[\![mol]\!]}$$

We can calculate the Gibbs free energy for ATP hydrolysis, and perform the same calculation —this time with changing stoichiometry (arrows).

We now solve for stoichiometries of 1, 2 and 3

> $solve\left(-\frac{15.36\,[\![kcal]\!]}{[\![mol]\!]} = 1 \cdot R \cdot T \cdot \ln\left(\frac{As_i}{As_o}\right) + -1 \cdot F \cdot \psi, As_i\right)$

$$\frac{6.63 \times 10^{-16}\,[\![mol]\!]}{[\![L]\!]}$$

> $solve\left(-\frac{15.36\,[\![kcal]\!]}{[\![mol]\!]} = 2 \cdot R \cdot T \cdot \ln\left(\frac{As_i}{As_o}\right) + -2 \cdot F \cdot \psi, As_i\right)$

$$\frac{3.55 \times 10^{-10}\,[\![mol]\!]}{[\![L]\!]}$$

> $solve\left(-\frac{15.36\,[\![kcal]\!]}{[\![mol]\!]} = 3 \cdot R \cdot T \cdot \ln\left(\frac{As_i}{As_o}\right) + -3 \cdot F \cdot \psi, As_i\right)$

$$\frac{2.89 \times 10^{-8}\,[\![mol]\!]}{[\![L]\!]}$$

Note the increasing the number of arsenic molecules per pump cycle decreases the efficacy. This is because pumping more molecules requires more energy, while the energy provided by ATP hydrolysis remains constant.

If the charge on the arsenate was -3

> $solve\left(-\frac{15.36\,[\![kcal]\!]}{[\![mol]\!]} = 3 \cdot R \cdot T \cdot \ln\left(\frac{As_i}{As_o}\right) + -9 \cdot F \cdot \psi, As_i\right)$

$$\frac{1.05 \times 10^{-11}\,[\![mol]\!]}{[\![L]\!]}$$

Increasing the charge on the molecule increases efficacy.

ANSWER ONE OF THE FOLLOWING TWO QUESTIONS

Kuroda et al. (1997)[1] examined the effects of pH on $^{73}AsO_2^-$ transport through the ArsB protein alone using vesicles containing ArsB (Figure 1).

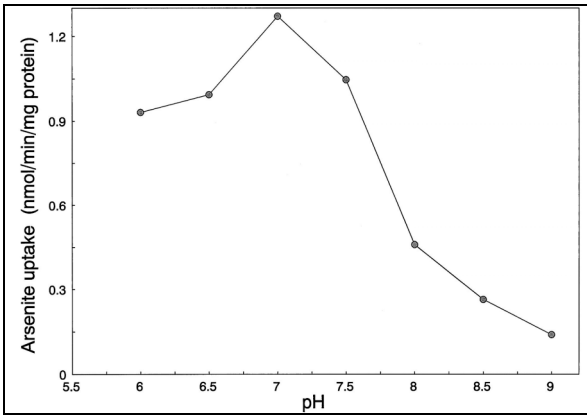


Figure 1. Effect of pH on NADH-driven arsenite transport in membrane vesicles. The initial rate of arsenite uptake was measured in a buffer consisting of 50 mM PIPES, 50 mM Bicine, containing 0.15 M K_2SO_4, 0.25 M sucrose, and 2.5 mM $MgSO_4$. The pH was adjusted to the indicated values with either H_2SO_4 or KOH. Assume the x-axis pH values correspond to external pH.

QUESTION ONE

Could the pH data be explained by an alternative mechanism for the Ars A & B pump, that involves ATP hydrolysis and arsenite efflux (out of the bacteria) coupled to proton (H^+) influx (into the cell)? To answer this question, use the following reaction scheme:

$$ATP + HAsO_3^{-2}{}_{inside} + H^+{}_{outside} \rightleftarrows ADP + P_i + HAsO_3^{-2}{}_{outside} + H^+{}_{inside}$$

• Show the complete accounting for the Gibbs free energy difference, including ATP hydrolysis, efflux of the arsenite ion with a net charge of negative 2, and influx of the H^+ ion with a net charge of positive 1.

You may need to know that the external concentration of $[HAsO_3^{-2}]_{outside} = 10^{-2}$ M, $[H^+]_{outside} = 10^{-5.5}$ M, and $[H^+]_{inside} = 10^{-7.5}$ M; [ATP] = 0.0005 M, [ADP] = 0.0004 M, and $[P_i] = 0.0025$ M.

• Calculate $[HAsO_3^{-2}]_{inside}$ when the membrane potential is 0 mV and –100 mV (negative inside).

[1] Kuroda M, Dey S, Sanders OI and Rosen BP (1997) Alternate energy coupling of ArsB, the membrane subunit of the Ars anion-translocating ATPase. The Journal of Biological Chemistry 272:326–331.

Here are the constants and other values we require

> $R := 1.987 \cdot 10^{-3} \frac{[\![kcal]\!]}{[\![mol]\!][\![K]\!]} : T := 293[\![K]\!] : F := 23.06 \frac{[\![kcal]\!]}{[\![mol]\!][\![V]\!]} : G := -10 \frac{[\![kcal]\!]}{[\![mol]\!]} : \psi := -0.1\,[\![V]\!] : As_o := 0.010 \frac{[\![mol]\!]}{[\![liter]\!]} : H_o := 10^{-5.5} : H_i := 10^{-7.5} :$

The $\Delta\mu_H$ can be calulated for 0 and −0.1 Volts :

> $R \cdot T \cdot \ln\left(\frac{H_o}{H_i}\right)$

$$\frac{2.68 \times 10^{0}\ [\![kcal]\!]}{[\![mol]\!]} \quad \textbf{(1)}$$

> $R \cdot T \cdot \ln\left(\frac{H_o}{H_i}\right) - 1 \cdot F \cdot \psi$

$$\frac{4.99 \times 10^{0}\ [\![kcal]\!]}{[\![mol]\!]} \quad \textbf{(2)}$$

Both will increase the net Gibbs free energy difference in the *negative* direction (towards more arsenite exclusion). Now we need to consider the contribution of ATP hydrolysis to arsenite exclusion, calculating delta-G (Gibbs)

> $ATP := 0.0005 : ADP := 0.0004 : P := 0.0025 :$

> $solve\left(\text{Gibbs} = G + R \cdot T \cdot \ln\left(\frac{ADP \cdot P}{ATP}\right), Gibbs\right)$

$$-\frac{13.62\ [\![kcal]\!]}{[\![mol]\!]} \quad \textbf{(3)}$$

We now solve for the internal arsenite at equilibrium with the membrane potential 'driving force' of -0.1 Volt. Note that -ve inside drives arsenite *out* and H+ *in*.

> $solve\left(-\frac{13.62\ [\![kcal]\!]}{[\![mol]\!]} = R \cdot T \cdot \ln\left(\frac{As_i}{As_o}\right) + -2 \cdot F \cdot \psi + R \cdot T \cdot \ln\left(\frac{H_o}{H_i}\right) - 1 \cdot F \cdot \psi, As_i\right)$

$$\frac{4.78 \times 10^{-20}\ [\![mol]\!]}{[\![L]\!]} \quad \textbf{(4)}$$

And, when the membrane potential is 0

> $solve\left(-\frac{13.62\ [\![kcal]\!]}{[\![mol]\!]} = 1 \cdot R \cdot T \cdot \ln\left(\frac{As_i}{As_o}\right) + R \cdot T \cdot \ln\left(\frac{H_o}{H_i}\right), As_i\right)$

$$\frac{6.92 \times 10^{-15}\ [\![mol]\!]}{[\![L]\!]} \quad \textbf{(5)}$$

Without the inward driving force of more H+ outside, less arsenite is excluded:

> $solve\left(-\frac{13.62\ [\![kcal]\!]}{[\![mol]\!]} = 1 \cdot R \cdot T \cdot \ln\left(\frac{As_i}{As_o}\right), As_i\right)$

$$\frac{6.92 \times 10^{-13}\ [\![mol]\!]}{[\![L]\!]} \quad \textbf{(6)}$$

Rubric: Equation set-up (10/20); Proton gradient increases efficacy (lower internal arsenite) (4/20); graph analysis (expect increased flux at acid pH, not observed) (4/20); correct answer (2/20)

Kuroda et al. (1997)[2] also tested the competitive effect of a number of other oxyanions — AsO_4^{3-}, PO_4^{3-}, NO_3^-, NO_2^-, SO_3^{2-}, and SeO_3^{2-} — which had no effect on $^{73}HAsO_2^{-2}$ transport via ArsB (Table I). The results indicate that ArsB does not catalyze nonspecific anion movement.

Table I. Effect of oxyanions on NADH-dependent arsenite transport via ArsB. The initial rate of arsenite were measured in the presence of the oxyanions shown to determine if they were transported through ArsB.

Addition	Initial velocity of arsenite uptake nmol/mg membrane protein/min	Percent
None	0.82	100
10 mM Na_2HAsO_4	0.79	96
10 mM K_2HPO_4	0.73	89
10 mM KNO_2	0.74	90
10 mM KNO_3	0.74	90
10 mM Na_2SO_3	0.81	99
10 mM K_2SeO_3	0.75	91

QUESTION TWO

• Calculate the expected channel pore diameter for the Ars B oxyanion channel. Explain your calculation.

• Propose a selectivity mechanism that ensures closely related oxyanions like phosphate, nitrate, etcetera are excluded from the channel.

Key: Data provides us with the sole relevant estimator of arsenite radius, the radius of nitrate: 2.90 angstroms. The problem arises from the complete lack of competition by other oxyanions — AsO_4^{3-}, PO_4^{3-}, NO_3^-, NO_2^-, SO_3^{2-}, and SeO_3^{2-} —, including those with similar oxidation states (NO_2^-). There is no doubt that the ArsB, while considered a channel, is unlikely to have a typical channel pore structure. Instead, like chloride channels, an occlusion in the pore must exist, that passes arsenite by a well-defined steric binding that excludes other oxyanions.

[2] Kuroda M, Dey S, Sanders OI and Rosen BP (1997) Alternate energy coupling of ArsB, the membrane subunit of the Ars anion-translocating ATPase. The Journal of Biological Chemistry 272:326–331.

Equations relevant to Membrane Transport: Geometry, Diffusion and Flux

Sphere Area : $4 \cdot \pi \cdot r^2$ Sphere Volume : $\frac{4}{3} \cdot \pi \cdot r^3$

Cylinder Area : $4 \cdot \pi \cdot r \cdot h$ Cylinder Volume : $\pi \cdot r^2 \cdot h$

Cube Area : $6 \cdot \mathrm{h}^2$ Cube Volume : h^3

Fick – 1st Diffusion Law : $J = D \cdot \frac{dc}{dx}$ Fick – 2d Diffusion Law : $\frac{dc}{dt} = D \cdot \frac{d^2c}{dx^2}$

Einstein – Random Walks : $D = \frac{1}{2} \cdot \frac{\Delta^2}{\tau}$, $\langle x^2 \rangle = 2 \cdot D \cdot t$, and $\langle r^2 \rangle = 6 \cdot D \cdot t$

Membrane Diffusion : $J = P \cdot (c_{outside} - c_{inside})$, $\mathrm{P} = \mathrm{D}\frac{\mathrm{k_p}}{\mathrm{d}}$

Membrane Diffusion : $J = -(uRT) \cdot \frac{dc}{dx} - (zFuc) \cdot \frac{d\Psi}{dx}$

Membrane Diffusion : $J = -P \cdot \left(\frac{zF\Psi}{RT}\right) \cdot \left(\frac{c_o - c_i \cdot e^{zF\Psi/RT}}{1 - e^{zF\Psi/RT}}\right)$

Nernst Equation : $\Psi = \left(\frac{RT}{zF}\right) \cdot \ln\left(\frac{c_o}{c_i}\right)$

Ohms Law : $V = I \cdot R$, $I = g \cdot V$, $R = \rho \cdot \left(l/A\right)$, and $J = I/(zF)$

Radial Diffusion : $C(r) = C_\infty \cdot \left(1 - \frac{a}{r}\right)$, and $J(r) = -D \cdot C_\infty \cdot \left(\frac{a}{r^2}\right)$

Radial Currents : $I_m = 4 \cdot \pi \cdot a^2 \cdot \beta$, and $I_d = 4 \cdot \pi \cdot a \cdot D \cdot C_\infty$

Dimensionless relations $P_e = \frac{2 \bullet a \bullet \nu}{D}$ and $R_e = \frac{\rho \bullet \nu \bullet l}{\eta}$

Goldman - Hodgkin - Katz (GHK) equation

$$\Psi = \frac{RT}{F} \ln\left(\frac{P_H c^o_H + P_{Na} c^o_{Na} + P_K c^o_K + P_{Cl} c^i_{Cl}}{P_H c^i_H + P_{Na} c^i_{Na} + P_K c^i_K + P_{Cl} c^o_{Cl}}\right)$$

Equations relevant to Bioenergetics

For the vectorial chemical reaction:

$\mathrm{ATP} + \mathrm{n} \bullet \mathrm{ion}^{\mathrm{i}} \leftrightarrow ADP + P_i + \mathrm{n} \bullet \mathrm{ion}^{\mathrm{o}}$

(n is the stoichiometry)

At equilibrium: $\Delta \mathrm{G}_{\mathrm{total}} = n \sum \Delta \mu_{ion} + \Delta G_{ATP}$

$$\Delta G_{ATP} = \Delta G^{o}_{ATP} + RT \ln \frac{[ADP][P_i]}{[ATP]}$$

$$\Delta \mu_{ion} = RT \ln \frac{c^{o}_{ion}}{c^{i}_{ion}} + zF\Delta\Psi$$

Note that ΔG^{o}_{ATP} varies with pH and $[Mg^{2+}]$. For our purposes, specifying –10 kcal mole^{-1} is a reasonable estimate.

Equations relevant to membrane capacitance

$Q = C \cdot \Delta E$ (coulombs) = (coulombs/volt) (volt)

Charge (Q) for a spherical cell of radius r:

$Q = \frac{4}{3} \cdot \pi \cdot r^3 \cdot c \cdot F$

c is the concentration of net charge.

Capacitance of a spherical cell of radius r:

$C = 4 \cdot \pi \cdot r^2 \cdot \mathrm{C}'$ C' is the capacitance per unit area

(about 1 microFarad per square centimeter for cells).

Symbol	Value	Units	Comments
GAS CONSTANT			
R	8.314	J mol^{-1} K^{-1}	R is the Boltzmann constant times Avogadro's Number ($6.023 \bullet 10^{23}$)
	1.987	cal mol^{-1} K^{-1}	
	8.314	m^{-3} Pa mol^{-1} K^{-1}	
RT	$2.437 \bullet 10^{3}$	J mol^{-1}	At 20 ºC (293 ºK)
	$5.833 \bullet 10^{2}$	cal mol^{-1}	At 20 ºC (293 ºK)
	$2.437 \bullet 10^{-3}$	liter MPa mol^{-1}	At 20 ºC (293 ºK)
RT/F	25.3	mV	At 20 ºC (293 ºK)
2.303 • RT	5.612	kJ mol^{-1}	At 20 ºC (293 ºK)
	1.342	kcal mol^{-1}	At 20 ºC (293 ºK)
FARADAY CONSTANT			
F	$9.649 \bullet 10^{4}$	coulombs mol^{-1}	F is the electric charge times Avogadro's Number
	$9.649 \bullet 10^{4}$	J mol^{-1} V^{-1}	
	23.06	kcal mol^{-1} V^{-1}	
CONVERSIONS			
kcal	4.187	kJ (kiloJoules)	Joules is an energy unit (equal to 1 Newton•meter)
Watt	1	J sec^{-1}	
Volt	1	J $coulomb^{-1}$	
Amperes	1	coulomb sec^{-1}	
Pascal (Pa)	1	Newton $meter^{-2}$	Pascal is a pressure unit (equal to 10^{-5} bars)
Siemens	1	Ohm^{-1}	Siemens (S) is conductance, the inverse of resistance (Ohm)
PHYSICAL PROPERTIES			
η_w	$1.004 \bullet 10^{-3}$	Pa sec	viscosity of water at 20 ºC
ν_w	$1.004 \bullet 10^{-6}$	m^2 sec^{-1}	kinematic viscosity of water at 20 ºC (viscosity/density)

Source: Nobel, Park S (1991) Physicochemical and Environmental Physiology

Table 9. System of the van der Waals radii (Å) of elements

Li 2.2 2.63	Be 1.9 2.23											B 1.8 2.05	C 1.7 1.96	N 1.6 1.79	O 1.55 1.71	F 1.5 1.65
Na 2.4 2.77	Mg 2.2 2.42											Al 2.1 2.40	Si 2.1 2.26	P 1.95 2.14	S 1.8 2.06	Cl 1.8 2.05
K 2.8 3.02	Ca 2.4 2.78	Sc 2.3 2.62	Ti 2.15 2.44	V 2.05 2.27	Cr 2.05 2.23	Mn 2.05 2.25	Fe 2.05 2.27	Co 2.0 2.25	Ni 2.0 2.23	Cu 2.0 2.27	Zn 2.1 2.24	Ga 2.1 2.41	Ge 2.1 2.32	As 2.05 2.25	Se 1.9 2.18	Br 1.9 2.10
Rb 2.9 3.15	Sr 2.55 2.94	Y 2.4 2.71	Zr 2.3 2.57	Nb 2.15 2.46	Mo 2.1 2.39	Tc 2.05 2.37	Ru 2.05 2.37	Rh 2.0 2.32	Pd 2.05 2.35	Ag 2.1 2.37	Cd 2.2 2.37	In 2.2 2.53	Sn 2.25 2.46	Sb 2.2 2.41	Te 2.1 2.36	I 2.1 2.22
Cs 3.0 3.30	Ba 2.7 3.05	La 2.5 2.81	Hf 2.25 2.52	Ta 2.2 2.42	W 2.1 2.36	Re 2.05 2.35	Os 2.0 2.33	Ir 2.0 2.34	Pt 2.05 2.37	Au 2.1 2.41	Hg 2.05 2.25	Tl 2.2 2.53	Pb 2.3 2.53	Bi 2.3 3.52	Po	At
		Th 2.4 2.75	U 2.3 2.65													

Table 9 presents the set of the recommended crystallographic (upper numbers) and equilibrium (lower numbers) van der Waals radii. The equilibrium radii are where there is a minimum in the potential of van der Waals interaction between two isolated atoms.
Source: Batsanov SS (2001) Van der Waals radii of elements. *Inorganic Materials*. 37(9):871–885.

Tabulated data for atomic radii, enthalpies of hydration and mobilities:

ATOMIC RADII, HYDRATION ENTHALPIES AND MOBILITIES			
Ion	Atomic Radii (Å)	Enthalpies of Hydration (kcal/mole)	Mobility (10^{-4}) (cm/sec)/(V/cm)
Tl^+	1.44	71	7.74
H^+	.	-269	36.3
NH_4^+	1.48	.	7.52
Cs^+	1.69	-72	8.01
Rb^+	1.48	-79.2	8.06
K^+	1.33	-85.8	7.62
Na^+	0.95	-104.6	5.19
Li^+	0.60	-131.2	4.01
Cl^-	1.81	-82	7.92
F^-	1.36	-114	5.74
Br^-	1.95	-79	8.09
I^-	2.16	-65	7.96
NO_3^-	2.90	.	7.41
Mg^{2+}	0.65	-476	2.75
Ca^{2+}	0.99	-397	3.08
Sr^{2+}	1.13	-362	3.08
Mn^{2+}	0.80	-458	.
Ba^{2+}	1.35	-328	3.30
Co^{2+}	0.74	-502	.
Ni^{2+}	0.72	-517	.
Zn^{2+}	0.74	-505	.

Source: Hille, B (1991) Ionic Channels of Excitable Membranes. Sinauer Associates. pp. 157 & 166.

Assignment Three (22 November 2012) **Due Monday 3 December 2012 at 5:30 PM**

QUESTION ONE: Please explain *Diffusion to Capture* and the role of advective flow so that even your professor will understand it. That is, the explanation should be at a level that is understandable to a second year science student. Your explanation should be *grounded* in the seminal paper by Berg and Purcell (1977) (Physics of chemoreception. Biophysical Journal 20:193–219) and Chapters 2 and 3 in Berg (1993) Random Walks in Biology (pp. 25–47).

Ground Rules: I expect students to work independently on this assignment. So, please ensure that the work you hand in is your own. You will need to select just a few ideas that you think are important (explaining why you think so). **Excessive length is not encouraged**. I think that 4 to 6 pages are sufficient. Handwritten is preferred to typewritten, because hand-drawn diagrams will be helpful. I am especially interested in your ability to make the ideas understandable. Stating 'the fluxes are described by this equation' is not helpful, unless you explain what the equation means or what it implies. Finally, clarity of explanation will be very important in grading of the assignments.

Rubric

By the nature of the assignment, the rubric is fairly general. Described in order of descending importance

• Mastery of the topic. How well you explained the underlying physics to explain the biological physics of diffusion to capture and the effect of advective flow. _____(/10)

• Your ability to explain the subject effectively. That is, logical clarity and flow. _____(/15)

• Writing is a craft. Your craftsmanship improves with experience, so grammatical skill and writing style was also considered. _____(/15)

Overall Score _____(/40)

CHOOSE TWO OF THE FOLLOWING THREE QUESTIONS

QUESTION ONE

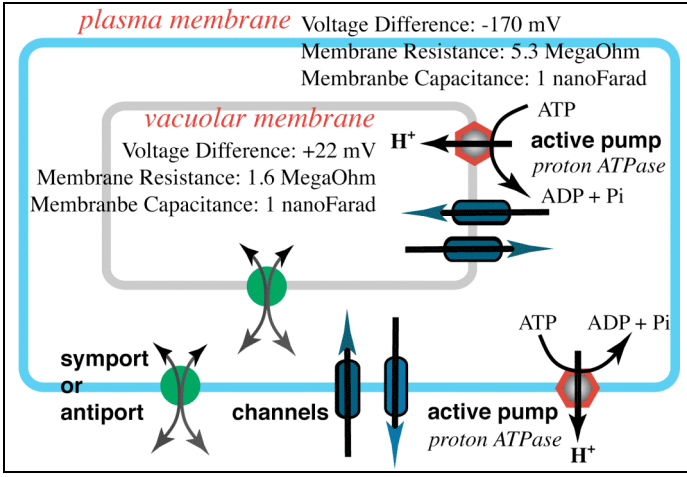
Data on the ionic composition of a plant cell[1] (and the extracellular media) is provided in the table below.

Ionic species	extracellular	cytoplasmic	vacuolar
	(milliEquivalents per liter)		
Na^+	1.0	15	65
K^+	0.1	120	75
Cl^-	1.3	80	160
Ca^{2+}	0.1	0.0005	0.1
Mg^{2+}	0.1	5	0.5
H^+	10^{-6} M	$10^{-7.2}$ M	10^{-5} M
Nota bene For the divalent ions, divide by two to obtain milliMolar concentration			

• For the five major ions (Na^+, K^+, Ca^{2+}, H^+ and Cl^-), what are the Nernst Potentials across the vacuolar membrane and plasma membrane?

• Note that the cytoplasm is –170 mV relative to the extracellular medium and the vacuole is +22 mV relative to the cytoplasm. Which ion is likely to be most permeant at the plasma membrane? At the vacuolar membrane? Explain why.

1



General architecture of a plant cell. The plasma membrane is polarized, negative-inside by 170 mV. The vacuole is polarized, positive-inside (compared to the cytoplasm) by 22 mV. Other electrical properties are shown the diagram, as are some (but not all) possible transporters at the two membranes. A typical plant cell is rectangular (20×20×80 microns). The vacuole occupies 80% of the cell volume.

Question One (30 points)

Here are the constants and other values we require

> $R := 1.987 \cdot 10^{-3} \frac{[\![kcal]\!]}{[\![mol]\!][\![K]\!]} : T := 293[\![K]\!] : F := 23.06 \frac{[\![kcal]\!]}{[\![mol]\!][\![V]\!]} : \psi := -0.17 [\![V]\!] : Na_o := 0.001 \frac{[\![mol]\!]}{[\![liter]\!]} : Na_i := 0.015 \frac{[\![mol]\!]}{[\![liter]\!]} : K_o := 0.0001 \frac{[\![mol]\!]}{[\![liter]\!]} : K_i := 0.12 \frac{[\![mol]\!]}{[\![liter]\!]} : Cl_o := 0.0013 \frac{[\![mol]\!]}{[\![liter]\!]} : Cl_i := 0.08 \frac{[\![mol]\!]}{[\![liter]\!]} : Ca_o := 0.0005 \frac{[\![mol]\!]}{[\![liter]\!]} : Ca_i := 2.5 \cdot 10^{-7} \frac{[\![mol]\!]}{[\![liter]\!]} : Mg_i := 0.0025 \frac{[\![mol]\!]}{[\![liter]\!]} : Mg_o := 0.00005 \frac{[\![mol]\!]}{[\![liter]\!]} : H_i := 10^{-7.2} \frac{[\![mol]\!]}{[\![liter]\!]} : H_o := 10^{-6} \frac{[\![mol]\!]}{[\![liter]\!]} :$

First, we solve for the Nernse potentials at the plasma membrane

> $solve\left(0 = R \cdot T \cdot \ln\left(\frac{Na_i}{Na_o}\right) + 1 \cdot F \cdot E, E\right)$

$$-68.37 \times 10^{-3} [\![V]\!] \quad \textbf{(1)}$$

> $solve\left(0 = R \cdot T \cdot \ln\left(\frac{K_i}{K_o}\right) + 1 \cdot F \cdot E, E\right)$

$$-179.00 \times 10^{-3} [\![V]\!] \quad \textbf{(2)}$$

> $solve\left(0 = R \cdot T \cdot \ln\left(\frac{Ca_i}{Ca_o}\right) + 2 \cdot F \cdot E, E\right)$

$$95.95 \times 10^{-3} [\![V]\!] \quad \textbf{(3)}$$

> $solve\left(0 = R \cdot T \cdot \ln\left(\frac{Cl_i}{Cl_o}\right) + -1 \cdot F \cdot E, E\right)$

$$104.01 \times 10^{-3} [\![V]\!] \quad \textbf{(4)}$$

> $solve\left(0 = R \cdot T \cdot \ln\left(\frac{Mg_i}{Mg_o}\right) + 2 \cdot F \cdot E, E\right)$

$$-49.38 \times 10^{-3} [\![V]\!] \quad \textbf{(5)}$$

> $solve\left(0 = R \cdot T \cdot \ln\left(\frac{H_i}{H_o}\right) + 1 \cdot F \cdot E, E\right)$

$$69.76 \times 10^{-3} [\![V]\!] \quad \textbf{(6)}$$

Of all the ions, the closes match to the -170 mV potential is potassium. Therefore, to a first approximation, the potassium is likely to be the most permeant ion at the plasma membrane.

scoring (/15): correct Nernst values (2 pts each). Identification of potassium (5 pts)

Now, we need to consider the Nernst potentials at the vacuole

> $Na_{vac} := 0.065 \frac{[\![mol]\!]}{[\![liter]\!]} : K_{vac} := 0.075 \frac{[\![mol]\!]}{[\![liter]\!]} : Cl_{vac} := 0.16 \frac{[\![mol]\!]}{[\![liter]\!]} : Ca_{vac} := 0.0005 \frac{[\![mol]\!]}{[\![liter]\!]} : Mg_{vac} := 0.00025 \frac{[\![mol]\!]}{[\![liter]\!]} : H_{vac} := 10^{-5} \frac{[\![mol]\!]}{[\![liter]\!]} :$

> $solve\left(0 = R \cdot T \cdot \ln\left(\frac{Na_{vac}}{Na_i}\right) + 1 \cdot F \cdot E, E\right)$

$$-37.02 \times 10^{-3}\ [\![V]\!] \quad \textbf{(7)}$$

> $solve\left(0 = R \cdot T \cdot \ln\left(\frac{K_{vac}}{K_i}\right) + 1 \cdot F \cdot E, E\right)$

$$11.87 \times 10^{-3}\ [\![V]\!] \quad \textbf{(8)}$$

> $solve\left(0 = R \cdot T \cdot \ln\left(\frac{Ca_{vac}}{Ca_i}\right) + 2 \cdot F \cdot E, E\right)$

$$-95.95 \times 10^{-3}\ [\![V]\!] \quad \textbf{(9)}$$

> $solve\left(0 = R \cdot T \cdot \ln\left(\frac{Cl_{vac}}{Cl_i}\right) + -1 \cdot F \cdot E, E\right)$

$$17.50 \times 10^{-3}\ [\![V]\!] \quad \textbf{(10)}$$

> $solve\left(0 = R \cdot T \cdot \ln\left(\frac{Mg_{vac}}{Mg_i}\right) + 2 \cdot F \cdot E, E\right)$

$$29.07 \times 10^{-3}\ [\![V]\!] \quad \textbf{(11)}$$

> $solve\left(0 = R \cdot T \cdot \ln\left(\frac{H_{vac}}{H_i}\right) + 1 \cdot F \cdot E, E\right)$

$$-127.89 \times 10^{-3}\ [\![V]\!] \quad \textbf{(12)}$$

Excepting Mg2+ (which was not listed in the question), the Nernst potentials closest to the measured vacuole potentials are chloride and potassium. Thus, chloride is likely to be the most permeant ion, followed by potassium.

scoring (/15): correct Nernst values (2 pts each). Identification of chloride and potassium (2.5 pts each)

QUESTION TWO (Use the data provided in Question One)

Mechanosensation is supposedly mediated by elevation of Ca^{2+} in the cytoplasm, which may be due to Ca^{2+} influx from the extracellular medium. Some scientists believe that the source of the Ca^{2+} is the vacuole.

• Test this by calculating the Gibbs free energy for Ca^{2+} flux into the cytoplasm from 1) the extracellular media and 2) the vacuole. Units of kcal/mole are preferred.

• Calculate the fluxes using a realistic permeability coefficient (10^{-8} cm s).

• How much time would be required to elevate the cytoplasmic Ca^{2+} to a concentration sufficient to trigger signaling cascades (about 1 μM)?

Question Two (30 points)

Gibbs free energy for calcium. First for the extracellular/cytoplasm:

> $solve\left(\text{Gibbs} = R\cdot T\cdot \ln\left(\frac{Ca_i}{Ca_o}\right) + 2\cdot F\cdot \psi, \text{Gibbs}\right)$

$$-\frac{12.27\ [\![kcal]\!]}{[\![mol]\!]} \tag{13}$$

Then for the vacuole/cytoplasm

> $solve\left(\text{Gibbs} = R\cdot T\cdot \ln\left(\frac{Ca_i}{Ca_{vac}}\right) + 2\cdot F\cdot 0.022[\![V]\!], \text{Gibbs}\right)$

$$-\frac{3.41\ [\![kcal]\!]}{[\![mol]\!]} \tag{14}$$

Both are negative, indicating a net energy directed to flux into the cytoplasm.

scoring (/10): set-up (6/10); correct answer (4/10)

Now we need to consider the magnitude of the calcium fluxes into the cytoplasm, using calcium concentrations in units of mols per cubic meter.

> $P_{Ca} := 10^{-10}\frac{[\![m]\!]}{[\![s]\!]} : C_o := 0.0005\cdot 10^3\frac{[\![mol]\!]}{[\![m^3]\!]} : C_i := 2.5\cdot 10^{-4}\frac{[\![mol]\!]}{[\![m^3]\!]} : C_v := 0.0005\cdot 10^3\frac{[\![mol]\!]}{[\![m^3]\!]} :$

> $solve\left(J = -P_{Ca}\cdot\frac{2\cdot F\cdot\psi}{R\cdot T}\cdot\frac{C_o - C_i\cdot\exp\left(\frac{2\cdot F\cdot\psi}{R\cdot T}\right)}{1-\exp\left(\frac{2\cdot F\cdot\psi}{R\cdot T}\right)}, J\right)$

$$\frac{6.73\times 10^{-10}\ [\![m]\!]\ [\![mol]\!]}{[\![s]\!]\ [\![m^3]\!]} \tag{15}$$

So the flux is $6.73\times 10^{-10}\left(\text{mol m}^{-2}\text{ s}^{-1}\right)$ for flux into the cytoplasm from the extracellular medium, or $6.73\times 10^{-14}\left(\text{mol cm}^{-2}\text{ s}^{-1}\right)$.

>

> $solve\left(J = -P_{Ca}\cdot\frac{2\cdot F\cdot 0.022[\![V]\!]}{R\cdot T}\cdot\frac{C_v - C_i\cdot\exp\left(\frac{2\cdot F\cdot 0.022[\![V]\!]}{R\cdot T}\right)}{1-\exp\left(\frac{2\cdot F\cdot 0.022[\![V]\!]}{R\cdot T}\right)}, J\right)$

$$\frac{1.84\times 10^{-11}\ [\![m]\!]\ [\![mol]\!]}{[\![s]\!]\ [\![m^3]\!]} \tag{16}$$

And $1.84\times 10^{-11}\left(\text{mol m}^{-2}\text{ s}^{-1}\right)$ for flux into the cytoplasm from the vacuole, or $1.84\times 10^{-15}\left(\text{mol cm}^{-2}\text{ s}^{-1}\right)$.

scoring (/10): set-up (6/10); correct answer (4/10)

>

The volumes of the cytoplasm and vacuole can be estimated. Using the values provided.

> $solve\left(area_{cell} = \left(\left(20\cdot 10^{-6}\right)^2\cdot 2 + 20\cdot 10^{-6}\cdot 80\cdot 10^{-6}\cdot 4\right), area_{cell}\right)[\![m^2]\!]$

$$7.20\times 10^{-9}\ [\![m^2]\!] \tag{17}$$

> $solve\left(volume_{cytoplasm} = 0.2\cdot\left(20\cdot 10^{-6}\cdot 20\cdot 10^{-6}\cdot 80\cdot 10^{-6}\right), volume_{cytoplasm}\right)[\![m^3]\!]$

$$6.40\times 10^{-15}\ [\![m^3]\!] \tag{18}$$

vacuole area can be estimated by scaling the dimensions by 0.8

> $solve\left(area_{vacuole} = \left(\left((0.8\cdot 20)\cdot 10^{-6}\right)^2\cdot 2 + (0.8\cdot 20)\cdot 10^{-6}\cdot(0.8\cdot 80)\cdot 10^{-6}\cdot 4\right), area_{vacuole}\right)[\![m^2]\!]$

$$4.61\times 10^{-9}\ [\![m^2]\!] \tag{19}$$

We are starting with 250

$\times 10^{-9}$ moles per liter, and need to determine the time required to elevate it to 10^{-6} moles per liter. Given the cytoplasmic volume of the cell, we can caluate the required mols.

> $solve\left(\text{mol} = \left(10^{-6}\frac{[\![mol]\!]}{[\![liter]\!]} - 2.5\cdot 10^{-7}\frac{[\![mol]\!]}{[\![liter]\!]}\right)\cdot 6.40\times 10^{-15}\,[\![m^3]\!]\cdot 10^3\frac{[\![liter]\!]}{[\![m^3]\!]}, mol\right)$

$$4.80 \times 10^{-18}\ [\![mol]\!] \quad \textbf{(20)}$$

So, we require 4.80×10^{-18} mol. How much time?

For the plasma membrane, about 1 second.

> $solve\left(t = \frac{4.80\times 10^{-18}\,[\![mol]\!]}{\frac{6.73\times 10^{-10}\,[\![m]\!]\,[\![mol]\!]}{[\![s]\!]\,[\![m^3]\!]}}\cdot\frac{1}{7.20\times 10^{-9}\,[\![m^2]\!]}, t\right)$

$$\frac{9.91 \times 10^{-1}\ [\![s]\!]\ [\![m^3]\!]}{[\![m]\!]\ [\![m^2]\!]} \quad \textbf{(21)}$$

For the vacuolar source, about 60 seconds.

> $solve\left(t = \frac{4.80\times 10^{-18}\,[\![mol]\!]}{\frac{1.84\times 10^{-11}\,[\![m]\!]\,[\![mol]\!]}{[\![s]\!]\,[\![m^3]\!]}}\cdot\frac{1}{4.61\times 10^{-9}\,[\![m^2]\!]}, t\right)$

$$\frac{5.66 \times 10^{1}\ [\![s]\!]\ [\![m^3]\!]}{[\![m]\!]\ [\![m^2]\!]} \quad \textbf{(22)}$$

scoring (/10): set-up (6/10); correct answer (4/10)

>

QUESTION THREE

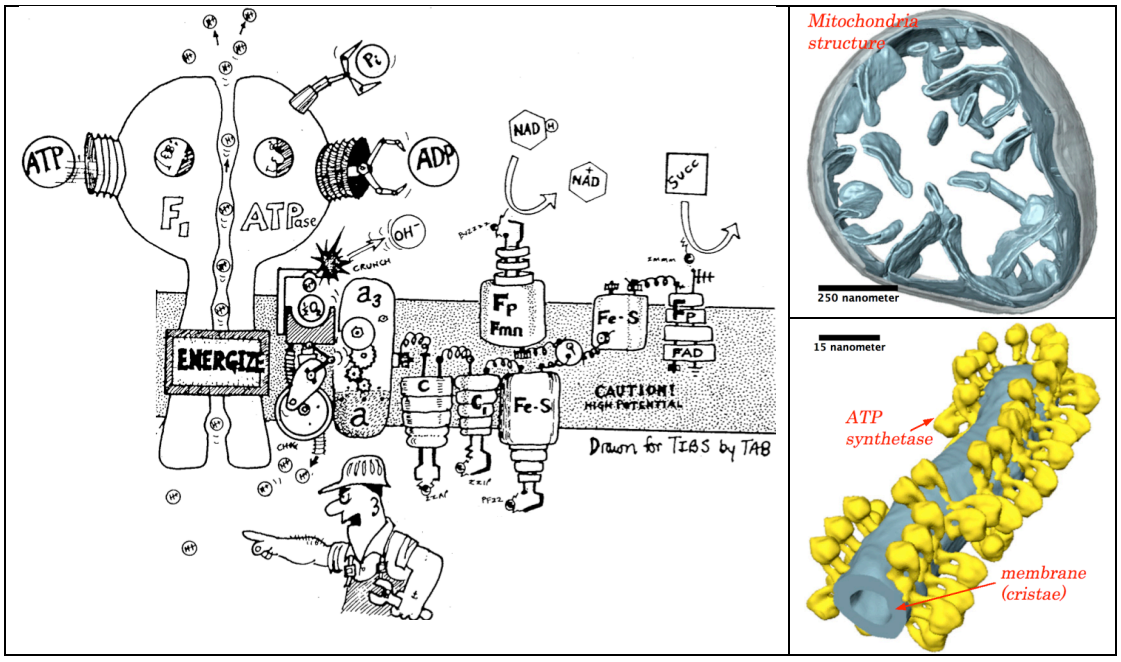
Berg and Purcell focus on aspects of diffusion and advective flow in the context of chemoreception. Some of the ideas they discuss are relevant to ion fluxes within the cell, specifically at the mitochondria, where H^+ pumped across the membrane by the electron transport chain re-enter through the F_1F_o ATP synthetase (see diagrams). Some have argued that H^+ tunneling along the membrane surface plays a crucial role, minimizing losses that would occur if the proton enters the bulk medium. The two-stage capture model of Adam and Delbruck (as described in Berg and Purcell) proposes a mean time to capture (t_s):

$$\bar{t}_s = \frac{1.1 \cdot a^2}{N \cdot D_s} \ln\left[\frac{1.2 \cdot a^2}{N \cdot s^2}\right]$$

where a is the radius of the cell, N is the number of receptors (F_1F_o ATP synthetase in this case), D_s is the coefficient of surface diffusion, and s is the radius of the binding site on the F_1F_o ATP synthetase. The diffusion coefficient for H^+ in water is about 7×10^{-5} cm^2/sec.

• Propose a model that incorporates the two-stage capture mechanism of Adam and Delbruck, and accounts for the novel lipid composition of the mitochondrial membrane. Pay special attention to the effect on diffusion times in two dimensions (D_s) and three dimensions (D), and adsorption.

• Compare the units of D_s and D. Explain why they are the same, or different.



Question Three (30 points)

The question actually provides a 'Fermi Question' approach to testing the two stage mechanism of diffusion to capture proposed by Adam and Delbruck, because we now have a reasonable idea of the density of ATP synthetase on the cristal membrane, and its turnover rate (about 100 per sec). From the question and diagram, we can obtain values and dimensions:

> $Diffusion := 7 \cdot 10^{-9} \frac{[\![m]\!]^2}{[\![s]\!]} : a := 15 \cdot 10^{-9} [\![m]\!] : s := 0.5 \cdot 10^{-9} [\![m]\!] : N := 20 :$

> $solve\left(t = \frac{1.1 \cdot a^2}{N \cdot Diffusion} \cdot \ln\left(\frac{1.2 \cdot a^2}{N \cdot s^2} \right), t \right)$

$$7.05 \times 10^{-9}\ [\![s]\!] \qquad \textbf{(1)}$$

So, to a first approximation, the time to capture is fast (7 nanoseconds), much faster than the cycle time of the ATP synthetase (1 millisecond). The actual value will be different. We are simplifying the geometry to a sphere and our estimates of the binding site (5 angstroms) and number of binding sites 'per sphere' are only reasonable guesses. But the mean time to capture is not strongly affected by any one of these factors, so the estimate is reasonably robust --within a few orders of magnitude.

>

A number of other factors serve to maximize time to capture.

The first is that the protons are enclosed within the cyclindrical membrane of the critae. That is, they can't 'escape', instead returning to the surface over and over again. Thus the very low probabilities for multiple returns to the surface are circumvented.

The second is the strong impact on adsorption provided by the phospholipids. There are two phospholipids with net negative charges --cardiolipin and phosphatidylserine-- that comprise 45% of the total phospholipid. This is an astonishingly high negative charge that would certainly encourage strong adsorption of the protons, whether due to direct protonation of the cardiolipin phosphates and phosphatidylserine carboxyl, or due to smeared negative charge located at the membrane surface.

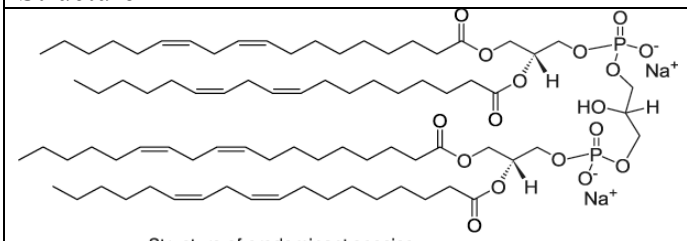
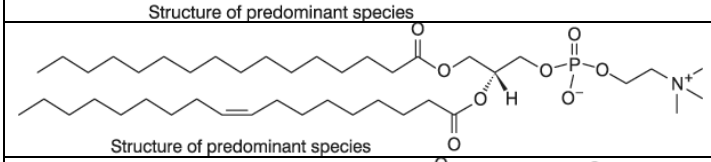
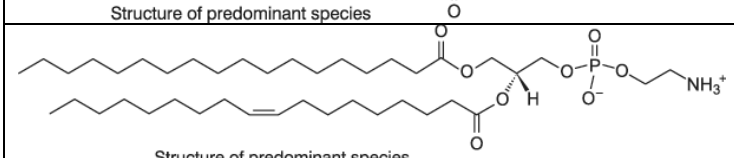
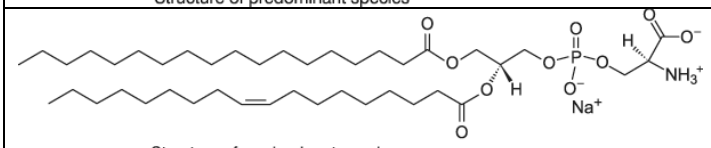
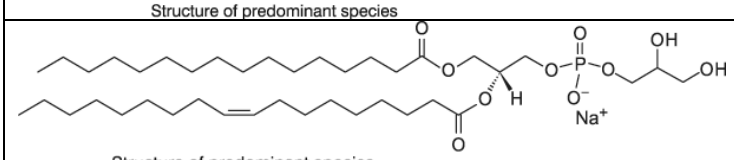
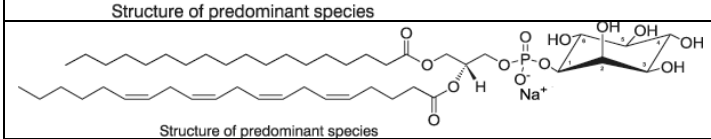
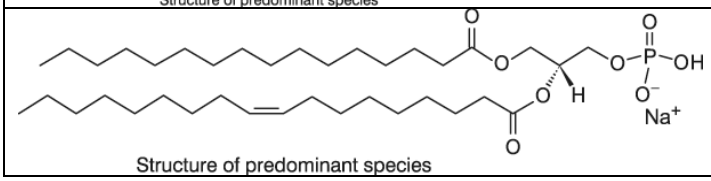
It is reasonable to speculate that 'Biology knows what it is doing'. That is, selective pressure to maximize the architectural efficiency of the electron transport chain and ATP synthetase in mitochondria has likely resulted in an optimal design that is difficult to improve on through bioengineering.

scoring (/30): set-up and calculation of time to capture(15/30); understanding of the roles of geometry and charged phospholipids (15/30)

ATOMIC RADII, HYDRATION ENTHALPIES AND MOBILITIES			
Ion	Atomic Radii (Å)	Enthalpies of Hydration (kcal/mole)	Mobility (10^{-4}) (cm/sec)/(V/cm)
Tl^+	1.44	71	7.74
H^+	.	-269	36.3
NH_4^+	1.48	.	7.52
Cs^+	1.69	-72	8.01
Rb^+	1.48	-79.2	8.06
K^+	1.33	-85.8	7.62
Na^+	0.95	-104.6	5.19
Li^+	0.60	-131.2	4.01
Cl^-	1.81	-82	7.92
F^-	1.36	-114	5.74
Br^-	1.95	-79	8.09
I^-	2.16	-65	7.96
NO_3^-	2.90	.	7.41
Mg^{2+}	0.65	-476	2.75
Ca^{2+}	0.99	-397	3.08
Sr^{2+}	1.13	-362	3.08
Mn^{2+}	0.80	-458	.
Ba^{2+}	1.35	-328	3.30
Co^{2+}	0.74	-502	.
Ni^{2+}	0.72	-517	.
Zn^{2+}	0.74	-505	.

Source: Hille, B (1991) Ionic Channels of Excitable Membranes. Sinauer Associates. pp. 157 & 166.

Examples of Naturally Occurring Phospholipids

Structure	Name
 Structure of predominant species	Cardiolipin (mitochondrial membranes normally contain 20%)
 Structure of predominant species	Phosphatidylcholine (mitochondrial membranes normally contain 27%)
 Structure of predominant species	Phosphatidylethanolamine (mitochondrial membranes normally contain 29%)
 Structure of predominant species	Phosphatidylserine (mitochondrial membranes normally contain 25%)
 Structure of predominant species	Phosphatidylglycerol (mitochondrial membranes normally contain 0%)
 Structure of predominant species	Phosphatidylinositol (mitochondrial membranes normally contain 0%)
 Structure of predominant species	Phosphatidic acid (mitochondrial membranes normally contain 0%)

Equations relevant to Membrane Transport: Geometry, Diffusion and Flux

Sphere Area: $4 \cdot \pi \cdot r^2$ Sphere Volume: $\frac{4}{3} \cdot \pi \cdot r^3$

Cylinder Area: $4 \cdot \pi \cdot r \cdot h$ Cylinder Volume: $\pi \cdot r^2 \cdot h$

Cube Area: $6 \cdot \mathrm{h}^2$ Cube Volume: h^3

Fick – 1st Diffusion Law: $J = D \cdot \frac{dc}{dx}$ Fick – 2d Diffusion Law: $\frac{dc}{dt} = D \cdot \frac{d^2c}{dx^2}$

Einstein – Random Walks: $D = \frac{1}{2} \cdot \frac{\Delta^2}{\tau}$, $\langle x^2 \rangle = 2 \cdot D \cdot t$, and $\langle r^2 \rangle = 6 \cdot D \cdot t$

Membrane Diffusion: $J = P \cdot (c_{outside} - c_{inside})$, $\mathrm{P} = \mathrm{D}\frac{\mathrm{k_p}}{\mathrm{d}}$

Membrane Diffusion: $J = -(uRT) \cdot \frac{dc}{dx} - (zFuc) \cdot \frac{d\Psi}{dx}$

Membrane Diffusion: $J = -P \cdot \left(\frac{zF\Psi}{RT}\right) \cdot \left(\frac{c_o - c_i \cdot e^{zF\Psi/RT}}{1 - e^{zF\Psi/RT}}\right)$

Nernst Equation: $\Psi = \left(\frac{RT}{zF}\right) \cdot \ln\left(\frac{c_o}{c_i}\right)$

Ohms Law: $V = I \cdot R$, $I = g \cdot V$, $R = \rho \cdot \left(l/A\right)$, and $J = I/(zF)$

Radial Diffusion: $C(r) = C_\infty \cdot \left(1 - \frac{a}{r}\right)$, and $J(r) = -D \cdot C_\infty \cdot \left(\frac{a}{r^2}\right)$

Radial Currents: $I_m = 4 \cdot \pi \cdot a^2 \cdot \beta$, and $I_d = 4 \cdot \pi \cdot a \cdot D \cdot C_\infty$

Cell Intake by Diffusion: $J = 4 \cdot \pi \cdot D \cdot C_\infty \cdot N \cdot s \cdot a/(N \cdot s + \pi \cdot a)$

Capture Probability: $P_s = a/(a + s)$

Dimensionless relations $P_e = \frac{2 \bullet a \bullet v}{D}$ and $R_e = \frac{\rho \bullet v \bullet l}{\eta}$

Goldman - Hodgkin - Katz (GHK) equation

$$\Psi = \frac{RT}{F} \ln\left(\frac{P_H c_H^o + P_{Na} c_{Na}^o + P_K c_K^o + P_{Cl} c_{Cl}^i}{P_H c_H^i + P_{Na} c_{Na}^i + P_K c_K^i + P_{Cl} c_{Cl}^o}\right)$$

Equations relevant to Bioenergetics

For the vectorial chemical reaction:

$$\mathrm{ATP} + \mathrm{n} \bullet \mathrm{ion}^{\mathrm{i}} \leftrightarrow ADP + P_i + \mathrm{n} \bullet \mathrm{ion}^{\mathrm{o}}$$

(n is the stoichiometry)

$$\text{At equilibrium: } \Delta \mathrm{G}_{\mathrm{total}} = n \sum \Delta \mu_{ion} + \Delta G_{ATP}$$

$$\Delta G_{ATP} = \Delta G^{o}_{ATP} + RT \ln \frac{[ADP][P_i]}{[ATP]}$$

$$\Delta \mu_{ion} = RT \ln \frac{c^{o}_{ion}}{c^{i}_{ion}} + zF\Delta\Psi$$

Note that ΔG^{o}_{ATP} varies with pH and [Mg^{2+}]. For our purposes, specifying 10 kcal $mole^{-1}$ is a reasonable estimate.

Equations relevant to membrane capacitance

$$Q = C \cdot \Delta E \text{ (coulombs)} = \text{ (coulombs/volt) (volt)}$$

Charge (Q) for a spherical cell of radius r:

$$Q = \tfrac{4}{3} \cdot \pi \cdot r^3 \cdot c \cdot F$$

c is the concentration of net charge.

Capacitance of a spherical cell of radius r:

$C = 4 \cdot \pi \cdot r^2 \cdot \mathrm{C}'$ C' is the capacitance per unit area

(about 1 microFarad per square centimeter for cells).

Symbol	Value	Units	Comments
GAS CONSTANT			
R	8.314	J mol^{-1} K^{-1}	R is the Boltzmann constant times Avogadro's Number ($6.023 \bullet 10^{23}$)
	1.987	cal mol^{-1} K^{-1}	
	8.314	m^{-3} Pa mol^{-1} K^{-1}	
RT	$2.437 \bullet 10^{3}$	J mol^{-1}	At 20 ºC (293 ºK)
	$5.833 \bullet 10^{2}$	cal mol^{-1}	At 20 ºC (293 ºK)
	$2.437 \bullet 10^{-3}$	liter MPa mol^{-1}	At 20 ºC (293 ºK)
RT/F	25.3	mV	At 20 ºC (293 ºK)
2.303 • RT	5.612	kJ mol^{-1}	At 20 ºC (293 ºK)
	1.342	kcal mol^{-1}	At 20 ºC (293 ºK)
FARADAY CONSTANT			
F	$9.649 \bullet 10^{4}$	coulombs mol^{-1}	F is the electric charge times Avogadro's Number
	$9.649 \bullet 10^{4}$	J mol^{-1} V^{-1}	
	23.06	kcal mol^{-1} V^{-1}	
CONVERSIONS			
kcal	4.187	kJ (kiloJoules)	Joules is an energy unit (equal to 1 Newton•meter)
Watt	1	J sec^{-1}	
Volt	1	J $coulomb^{-1}$	
Amperes	1	coulomb sec^{-1}	
Pascal (Pa)	1	Newton $meter^{-2}$	Pascal is a pressure unit (equal to 10^{-5} bars)
Siemens	1	Ohm^{-1}	Siemens (S) is conductance, the inverse of resistance (Ohm)
PHYSICAL PROPERTIES			
η_w	$1.004 \bullet 10^{-3}$	Pa sec	viscosity of water at 20 ºC
ν_w	$1.004 \bullet 10^{-6}$	m^2 sec^{-1}	kinematic viscosity of water at 20 ºC (viscosity/density)

Source: Nobel, Park S (1991) Physicochemical and Environmental Physiology