Membrane Transport (BIOL 4151) Assignment One (2 October 2014)

Due 10 October 2014 by 5:00 PM at FS229

QUESTION ONE:

Part One. Propose a mechanism and/or structures that modify the properties of lipids to resist the effect of high pressure. Recall that organisms can survive the remarkable pressures of the oceanic depths (and even survive the 'nil' pressure of outer space).

Part Two. Compare your mechanism to known pressure resistant organisms. These can be from any biological clade (it is not just Archaea, but also plants and animals that survive high pressures). I am especially interested in the physical/chemical mechanisms.

Hint: Make sure you approach the question from a physico-chemical perspective. You should be able to find many papers on the effect of hydrostatic pressure on membranes, but probably very little on the lipid structures that are most resistant to the effects of pressure. Focus on mechanisms and provide explanations that can be understood by your professor and fellow students.

QUESTION TWO:

How long will it take for you to die of dehydration if increasing extracellular [K^+] is the causal agent?

Assumptions.

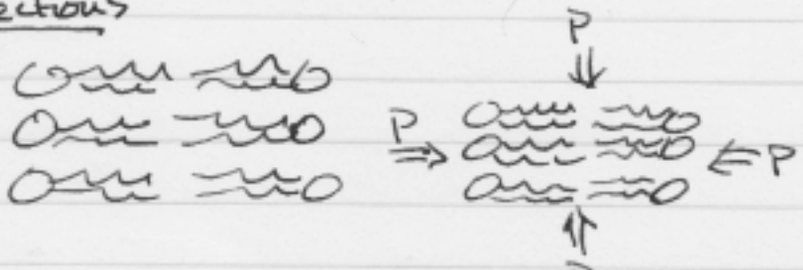
1) Your excitable cells have a potential of –80 mV, and the AP trains that would result in fibrillation are triggered at a threshold potential of –30 mV.
2) Cellular volume is unchanged (and that there is no osmotic water flow)
3) Do not considered kidneys or other regulatory mechanisms that would allow you to survive for at least a bit longer….

Hint: Show your units (points off if you don't)! Any additional assumptions you need to make (for example, your volume, your extracellular water volume and the rate at which you lose water) should be reasonable assumptions (Wikipedia may provide useful values).

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). I strongly prefer handwritten assignments. Excessive length is not encouraged.

KEY (high pressure - membrane effects and adaptations)

At high pressure, the "squeezing" is in <u>both directions</u>



10 (5 for one direction)

The lipid is compressible: 5.6×10^{-7} kPa^{-1}
slightly more so than water 4.5×10^{-7} kPa^{-1}

Physico-chemical aspects: Either from the chemical potential term $\bar{V}_P$ where $\bar{V}$ is the partial molal volume, or Clausius-Clapeyron

$\frac{dT_M}{dP} = \frac{T_M \Delta V}{\Delta H}$ (~~Berkeley~~ UC-Davis PhysWiki)
(pressure raises T_M, exponentially)

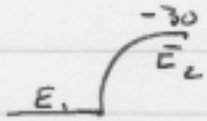
20

Counter strategies?
- more unsaturated acyl chains?
- longer acyl chains?
- bulkier head groups?
- less sterol?

70

Most common amongst barophiles is greater unsaturation & longer length
For example, C22:6 has lots (6!) of unsaturated bonds
Possibly, even fatty acids (single acyl chain)

KEY (could elevated $[K^+]_o$ be a cause of death due to dehydration?)



$E_1 - E_2 = -50$ mV

$$\underbrace{\frac{RT}{zF}\ln\frac{C_o}{C_i}}_{-80\text{ mV}} - \underbrace{\frac{RT}{zF}\ln\frac{C_o'}{C_i}}_{25\text{ mV}} = -50$$

for C_o = 5 mM, C_i = 123 mM, and C_o' is 37mM

solve for... solve for...

$$\frac{5\text{ mMoles}}{x\,l} = \frac{37\text{ mMoles}}{l}$$

→ 0.135 l Must lose 86.5% of extracellular fluid (1 − 0.135 = 0.865)

If ECF is 15 l, must lose 13 l
At 2 liters/day → 6.5 days

Student Answers (stem & leaf)

3	23
2	
2	
1	6
1	13
0	5666666667777789999 ← 6.5 69 days
0	0344

(multiply stem by 10 for days)

Name:_____**KEY**_____
Student ID:_____
Be sure to write your name and student ID above. Read the two questions carefully, think, then write your answers in the lined space (front and back of this page). When finished, please hand your answer in.

Question One.

Design two bilayer membranes using the structures provided. One membrane should be resistant to high pressure. The other membrane should be resistant to cold temperatures. Use no more than 3–5 lipid species per membrane. Explain from a physico-chemical perspective why the pressure-resistant and cold-resistant membranes are different.

General Considerations:
• As is common in most biological membranes, a major requirement is for a zwitterionic headgroup (with both –ve and +ve groups and net charge of zero). This will maximize hydrophilicity of the head group and encourage spontaneous bilayer formation.
• Diacyl lipids will optimize the molecular spacing in the hydrophobic interior of the membrane (monoacyl lipids disrupt membrane integrity). At high pressure, monoacyl lipids may be useful, but it is unlikely.

*The only diacyl lipid with a net charge is **ceramide-1-phosphate (18:1 & 8:0, 1 –ve charge)**, so this could be a major component of both pressure-resistant and cold-resistant membranes. Other diacyl lipids have 3 hydroxyl (OH) groups that provide dipole to interact with water: the **N-16:0 and N-24:0 phytosphingosines** have the best acyl chain lengths.*

At high pressure lateral and transverse compression will occur. The lateral compression will cause membrane rigidity (and a higher Tm), which can be offset by unsaturated kinks in the acyl groups. The transverse compression would cause the membrane to be thinner, and can be offset by the use of long chain acyl groups.

*In addition to **ceramide-1-phosphate (18:1 & 8:0)** and **N-24:0 phytosphingosine**, shorter acyl chains with unsaturated kinks, are **ceramide (18:1 & 12:0)** and **ceramide (chicken) (16:1 & 16:0)**.*

At cold temperatures, the fluidity of the membrane will be seriously impaired. Unsaturated acyl groups can offset this. The acyl chain lengths should be similar to biological membranes at normal temperatures (about 18 carbons).

*In addition to **ceramide-1-phosphate (18:1 & 8:0)** and **N-16:0 phytosphingosine**, **ceramide (18:1 & 12:0)** and **ceramide (chicken) (16:1 & 16:0)** provide the best mix of unsaturation and acyl chain length.*

Scoring (___/100):
effort (minimal effort may get less) (50)
zwitterion headgroup (10)
use of diacyl lipids (10)
longer acyl groups for high pressure (10)
unsaturated acyls for both high pressure and cold temperatures (10)
physico-chemical difference between pressure and cold explained clearly (10)

Question Two.

Two molecular species (a and b) have similar molecular weights. Species a has a permeability coefficient of 10^{-4} cm s^{-1} and no net charge. Species b has a permeability coefficient of 10^{-9} cm s^{-1} and a net charge of +ve 1. If the concentration outside of a 10 μm square cell is 10 mM and 1 mM inside, what is the flux of species a? At what electrical potential will the flux of species b equal that of species a?
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.

Here are the constants and other values we require

> $R := 1.987 \cdot 10^{-3} \frac{[\![kcal]\!]}{[\![mol]\!][\![K]\!]} : T := 293[\![K]\!] : F := 23.06 \frac{[\![kcal]\!]}{[\![mol]\!][\![V]\!]} : \psi := -0.12\,[\![V]\!] :$

Now we need to consider the magnitude of the fluxes, using species concentrations in units of mols per cubic meter. First, for the uncharged species a

> $P_a := 10^{-6} \frac{[\![m]\!]}{[\![s]\!]} : P_b := 10^{-11} \frac{[\![m]\!]}{[\![s]\!]} : C_o := 0.01 \cdot 10^3 \frac{[\![mol]\!]}{[\![m^3]\!]} : C_i := 0.001 \cdot 10^3 \frac{[\![mol]\!]}{[\![m^3]\!]} :$

> $solve\left(J = -P_a \cdot \left(C_o - C_i\right), J\right)$

$$-\frac{9.00 \times 10^{-6}\,[\![m]\!]\,[\![mol]\!]}{[\![s]\!]\,[\![m^3]\!]} \qquad (1)$$

This is an inward flux of 9 umol m^{-2} s^{-1}, or 90 nmol cm^{-2} s^{-1}.

For species b, with one net positive charge, we can guess by using different values of the potential. It soon becomes clear that a very large potential must be used, given the low permeability coefficient of the charged species.

> $solve\left(J = P_b \cdot \frac{F \cdot (-2275[\![V]\!])}{R \cdot T} \cdot \frac{C_o - C_i \cdot \exp\left(\frac{F \cdot (-2275[\![V]\!])}{R \cdot T}\right)}{1 - \exp\left(\frac{F \cdot (-2275[\![V]\!])}{R \cdot T}\right)}, J\right)$

$$-\frac{9.01 \times 10^{-6}\,[\![m]\!]\,[\![mol]\!]}{[\![s]\!]\,[\![m^3]\!]} \qquad (2)$$

About -2.4 kiloVolt. The is extremely negative (and would cause breakdown of the membrane)
scoring (/10): set-up (6/10); correct answer (4/10)

If we evaluate the flux at a more normal potential (-100 mV), the flux is very small compared to the neutral species.

> $P_b \cdot \frac{F \cdot \psi}{R \cdot T} \cdot \frac{C_o - C_i \cdot \exp\left(\frac{F \cdot \psi}{R \cdot T}\right)}{1 - \exp\left(\frac{F \cdot \psi}{R \cdot T}\right)}$

The flux is 10^4 lower than the uncharged molecule. So, a very large negative potential is necessary to 'pull' the cation into the cell. A large negative potential means that exp(FΨ/RT)~0. Setting the expenential terms to zero simplifies the equation, allowing an accurate estimate of the potential

$$-\frac{4.79 \times 10^{-10}\,[\![m]\!]\,[\![mol]\!]}{[\![s]\!]\,[\![m^3]\!]} \qquad (3)$$

Scoring (___/100):
neutral flux equation setup and value (50)
equation for cation flux (constant field equation) (20)
setup (20)
answer (10)

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 : $D = \frac{1}{2} \bullet \frac{\Delta^2}{\tau}$, $\langle x^2 \rangle = 2 \bullet D \bullet t$, and $\langle r^2 \rangle = 6 \bullet D \bullet t$

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:

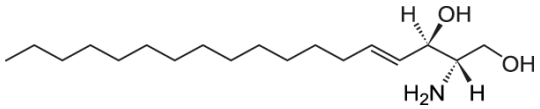
$C = 4 \cdot \pi \cdot r^2 \cdot C'$ C' is the capacitance per unit area

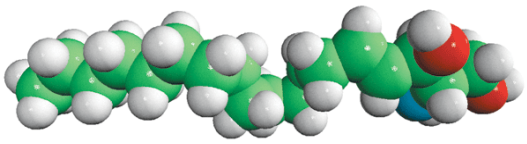
(about 1 microFarad per square centimeter for cells).

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)
	$5.822 \cdot 10^2$	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) used for $\log_{10}$
	1.342	kcal mol^{-1}	At 20 ºC (293 ºK) used for $\log_{10}$
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)

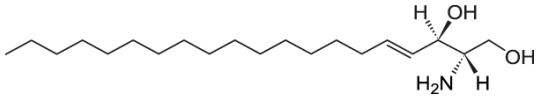
Source: Nobel, Park S (1991) Physicochemical and Environmental Physiology

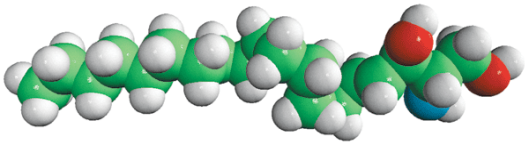
Sphingosine (d18:1) (D-*erythro*-Sphingosine



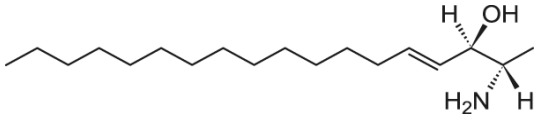


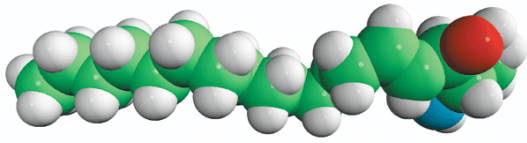
Sphingosine (d20:1) (D-*erythro*-Sphingosine)



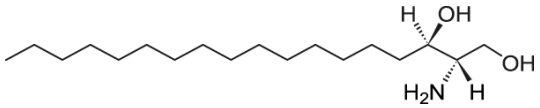


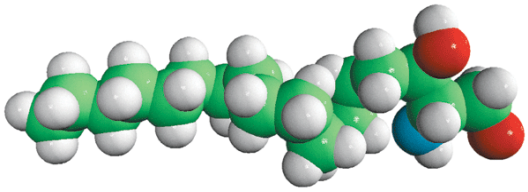
1-deoxysphingosine (m18:1)



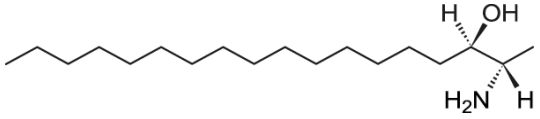


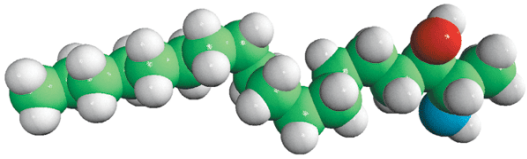
Sphinganine (d18:0) (D-*erythro*-sphinganine)



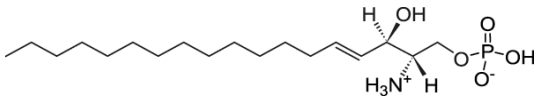


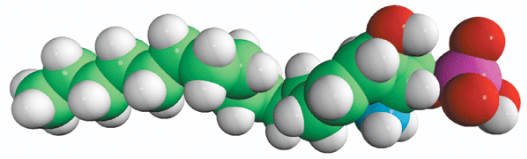
1-deoxysphinganine (m18:0)



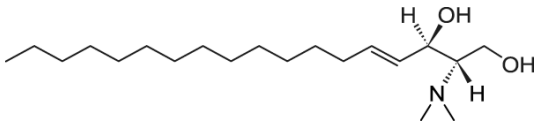


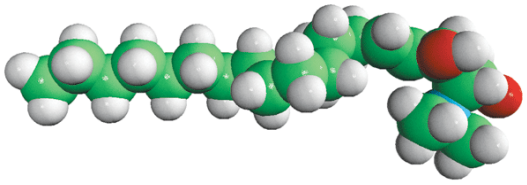
Sphingosine-1-Phosphate (d18:1) (D-*erythro*-sphingosine-1-phosphate)



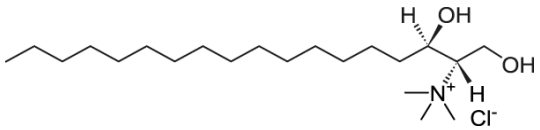


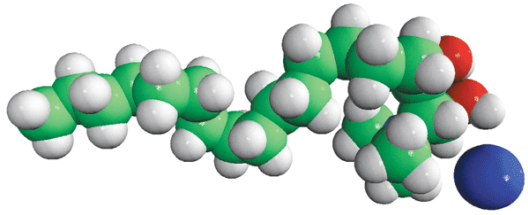
Dimethyl Sphingosine (d18:1) (N,N-dimethyl-D-*erythro*-sphingosine)



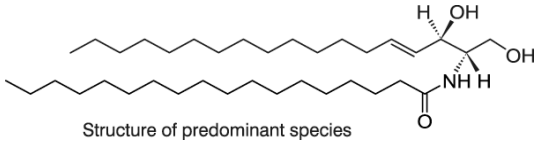


Trimethyl Sphinganine (d18:0) (N,N,N-trimethyl-D-*erythro*-dihydrosphingosine) (chloride salt)

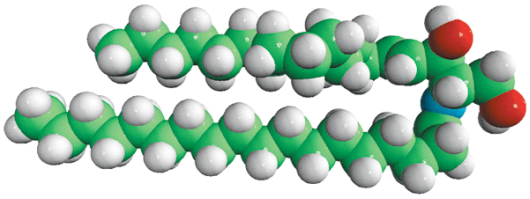




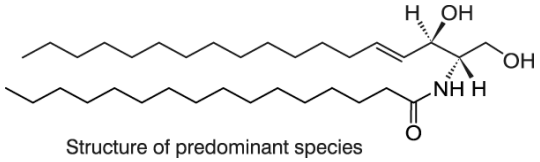
Ceramide (Brain, Porcine)



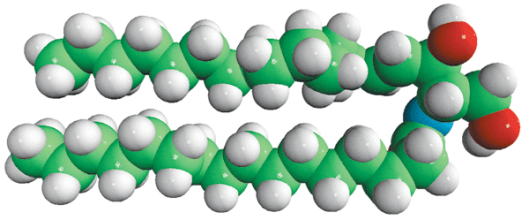
Structure of predominant species



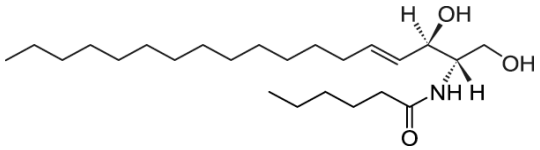
Ceramide (Egg, Chicken)

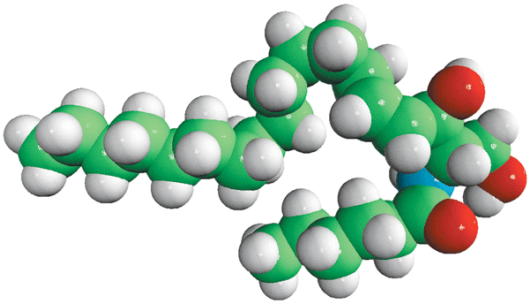


Structure of predominant species

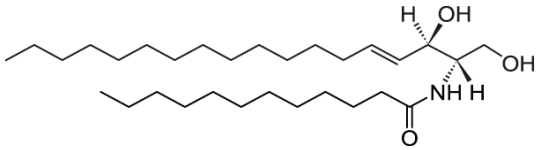


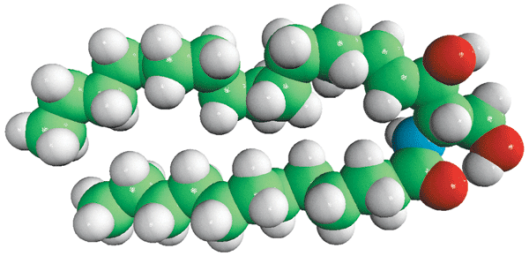
Ceramide (d18:1/6:0) (N-hexanoyl-D-*erythro*-sphingosine)



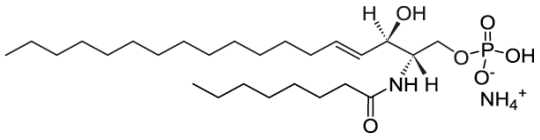


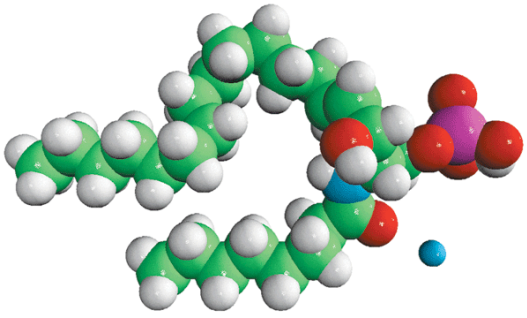
Ceramide (d18:1/12:0) (N-lauroyl-D-*erythro*-sphingosine)



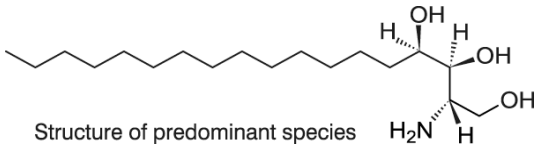


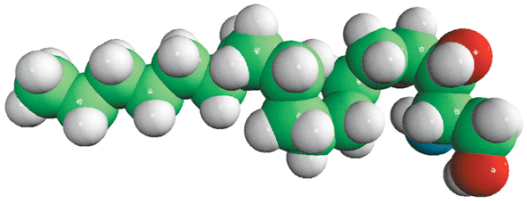
Ceramide-1-Phosphate (d18:1/8:0) (N-octanoyl-ceramide-1-phosphate (ammonium salt)



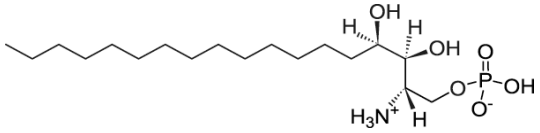


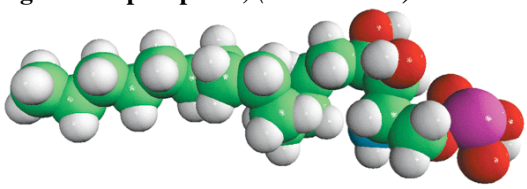
D-ribo-Phytosphingosine (4-hydroxysphinganine) ***(Saccharomyces cerevisiae)***



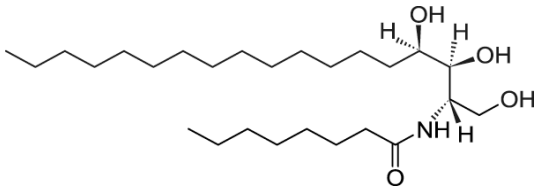


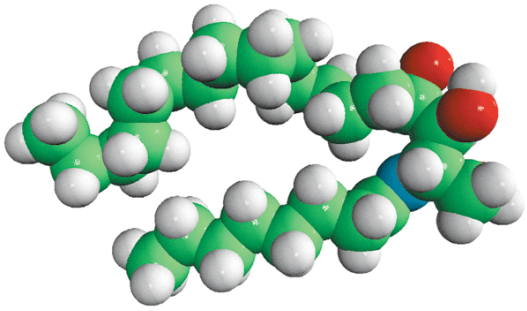
D-ribo-Phytosphingosine-1-Phosphate (4-hydroxysphinganine-1-phosphate) ***(S. cerevisiae)***



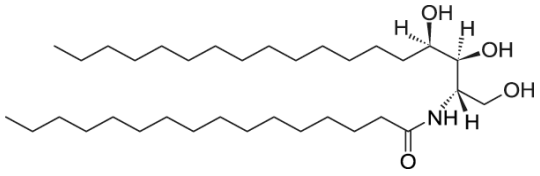


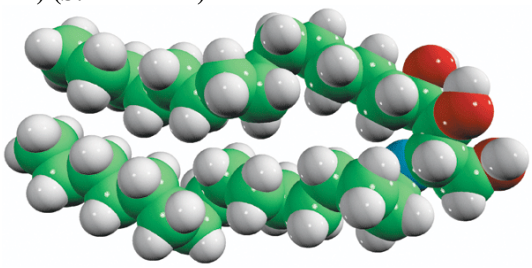
N-08:0 Phytosphingosine (N-octanoyl 4-hydroxysphinganine) ***(S. cerevisiae)***



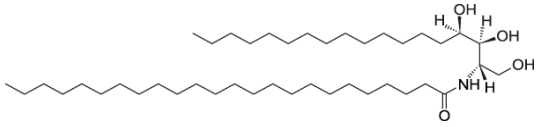


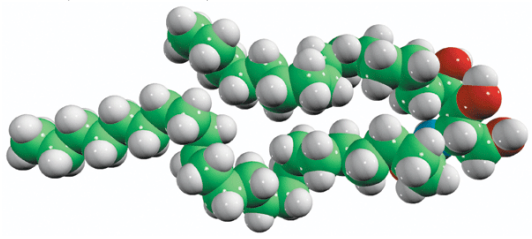
N-16:0 Phytosphingosine (N-palmitoyl-phytosphingosine) (*S. cerevisiae*)



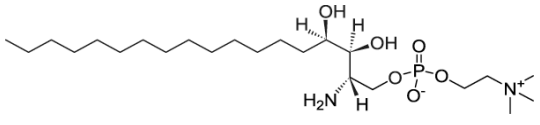


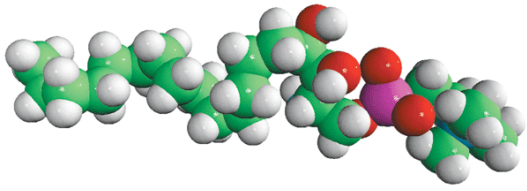
N-24:0 Phytosphingosine)N-lignoceroyl-phytosphingosine (*S. cerevisiae*)





Phytosphingosine Phosphocholine (4-hydroxysphinganine-1-phosphocholine) ***(S. cerevisiae)***





Assignment Two (28 October 2014) **Due 6 November 2014 by 4:30 PM at FS229**

QUESTION ONE: Arsenic is not the only element that can be poisonous, and for which multiple transport mechanisms serve to extrude it from cells. The transition metals zinc, cadmium and mercury can be either essential (zinc at low concentrations), or toxic. Cadmium and mercury are transition metals of especial concern to human health because they are accumulated in the food chain.

Identify the
1) chemical properties of cadmium and mercury relevant to transport.
2) biochemical mechanisms of transport, and
3) energetics of transport.

> Hints: There is a large literature on the genes that play a role in alleviating –or increasing—accumulation within cells. Research on biochemical mechanisms of transport is not so well developed. It is the latter that you should focus on to address the three aspects of the assignment. The best work on this has been done on bacteria, plants and yeast. Chemical properties relevant to the forms that may exist in the cytoplasm are most interesting to a transport physiologist. Biochemical mechanisms should include the type of experiment that was done (radioisotope? cellular? isolated membranes? etcetera). If you find $K_{1/2}$'s and other biochemical measures of enzymatic activity, try to reference their relevance to normal elemental concentrations in the environment. Energetics should include calculations of how effectively the biochemical mechanism can exclude the element.

Ground Rules: Students may (or may not) wish to work with each other on the assignment (especially on bioenergetics calculations). Please ensure that the work you hand in is in your own words (it's your voice I want to hear). I prefer handwritten: it's easier to include diagrams (which I like) and discourages copy/paste. I don't expect to see more than 2–4 substantive references (that is, to the scientific literature); 4–5 pages should suffice.

Name:_____KEY_____

Question One.

In a bacterial plasma membrane, suppose the electrochemical proton gradient is the driving force for a sodium extrusion system (antiport). For two H^+ coming into the cell, one Na^+ ion is pumped out. In turn, the Na^+ gradient is used to drive the efflux of Ca^{2+} out of the cell. For each Na^+ ion coming into the cell, one Ca^{2+} ion is pumped out. At steady state, if the $[Ca^{2+}]_{(outside)}$ is 5 mM and $[Ca^{2+}]_{(inside)}$ is 0.005 mM, what is the required proton electrochemical gradient, assuming the potential is -120 mV? What if the H^+/Na^+ antiporter stoichiometry is 1 H^+ per 1 Na^+?

Question One
Here are the constants and other values we require

```
> R := 1.987·10^-3 [[kcal]]/([[mol]][[K]]) : T := 293[[K]] : F := 23.06 [[kcal]]/([[mol]][[V]]) : ψ := -0.12 [[V]] : n := 0.5 :
```

Here are the concentrations we require. H_{inside} is assumed to be neutral pH, Na_{inside} is set to 1 mM

```
> H_inside := 10^-7 [[mol]]/[[liter]] : Ca_inside := 5·10^-6 [[mol]]/[[liter]] : Ca_outside := 5·10^-3 [[mol]]/[[liter]] : Na_inside := 1
      ·10^-6 [[mol]]/[[liter]] :
```

Now we calulate. First, for $Na_{outside}$. Note that z is equal to 2 for Ca^{2+}:

```
> solve(R·T·ln(Ca_inside/Ca_outside) + 2· F·ψ = R·T·ln(Na_inside/Na_outside) + F·ψ, Na_outside)
```

$$\frac{1.16 \times 10^{-1}\,[[mol]]}{[[L]]} \qquad (1)$$

Then, for $H_{outside}$ (2:1 stoichiometry):

```
> solve(R·T·ln(Na_inside/(1.16 × 10^-1 [[mol]]/[[L]])) + F·ψ = 2· R·T·ln(H_inside/H_outside) + 2· F·ψ, H_outside)
```

$$\frac{3.16 \times 10^{-6}\,[[mol]]}{[[L]]} \qquad (2)$$

An acid external concentration (pH of 5.5) is required to drive Ca^{2+} extrusion.

For $H_{outside}$ with a 1:1 stoichiometry:

```
> solve(R·T·ln(Na_inside/(1.16 × 10^-1 [[mol]]/[[L]])) + F·ψ = R·T·ln(H_inside/H_outside) + F·ψ, H_outside)
```

$$\frac{1.16 \times 10^{-2}\,[[mol]]}{[[L]]} \qquad (3)$$

A very acid external concentration (pH of 1.9) is required to drive Ca^{2+} extrusion

Clarity and logic were important adjuncts to the very general grading scheme to the right.	equating delta-mu's correctly	(50/100)
	Electrochemical gradient (either ratio or $[H^+]$ or pH	(25/100)
	Correct answer	(25/100)

Question Two.

A bacteria (for the sake of simplicity, assume it is a square, with 2 μm sides that are very rigid) has an internal pressure of 1 MPa. It is subjected to a hypo-osmotic shock that dilutes the external medium from an osmotically active concentration of 500 mM to 1 mM. Without mechanosensitive channels, the bacteria would lyse and die in 60 seconds. How many mechanosensitive channels would be required if the current per channel is 10 pA? For the sake of simplicity, assume that the movement of ions through the channel does not affect the membrane potential of the bacterium.

Question Two

Here are the constants and other values we require. Concentrations are done on a cubic meter basis.

> $R := 2.437 \frac{[\![m]\!]^3 [\![Pa]\!]}{[\![mol]\!][\![K]\!]}$: $T := 293[\![K]\!]$: $F := 9.649 \cdot 10^4 \frac{[\![C]\!]}{[\![mol]\!]}$: $c_{initial} := 0.5 \frac{[\![mol]\!]}{1 \cdot 10^{-3}[\![m]\!]^3}$: $c_{final} := 0.001 \frac{[\![mol]\!]}{1 \cdot 10^{-3}[\![m]\!]^3}$: $Avogadro := 6.023 \cdot 10^{23} \frac{1}{[\![mol]\!]}$:

Here are the dimensions we require.

> $h := 2 \cdot 10^{-6}[\![m]\!]$:

Now we calculate volume and area of the bacteria:

> volume = h^3

$$volume = 8.00 \times 10^{-18}\ [\![m]\!]^3 \quad (1)$$

> $area = 6 \cdot h^2$

$$area = 24.00 \times 10^{-12}\ [\![m]\!]^2 \quad (2)$$

Then, the change in pressure inside the cell:

> $solve\left(P_{change} = R \cdot T \cdot \left(c_{initial} - c_{final}\right), P_{change}\right)$

$$3.56 \times 10^5\ [\![Pa]\!] \quad (3)$$

The concentration change inside the cell to allieviate the pressure change:

> $solve\left(3.56 \times 10^5\ [\![Pa]\!] = R \cdot T \cdot \left(c_{cell}\right), c_{cell}\right)$

$$\frac{4.99 \times 10^2\ [\![mol]\!]}{[\![m]\!]^3} \quad (4)$$

This is equivalent to molecules in the cell: $molecules = c_{cell} \cdot volume \cdot Avogadro's\ number$

> $molecules = \frac{4.99 \times 10^2\ [\![mol]\!]}{[\![m]\!]^{3.00 \times 10^0}} \cdot 8.00 \times 10^{-18}\ [\![m]\!]^{3.00} \cdot Avogadro$

$$molecules = 2.40 \times 10^9 \quad (5)$$

If each channel has a current of 10 pA, then in 60 seconds, the number of molecules that pass through each channel are:

> $channel = 10 \cdot 10^{-12} \frac{[\![C]\!]}{[\![s]\!]} \cdot \frac{1}{F} \cdot 60[\![s]\!] \cdot Avogadro$

$$channel = 3.74 \times 10^9 \quad (6)$$

Dividing molecules/channel estimates the number of channels required:

> $number\_of\_channels = \frac{2.40 \times 10^9}{3.74 \times 10^9}$

$$number\_of\_channels = 6.42 \times 10^{-1} \quad (7)$$

Less than one is sufficient to 'save' the cell from hyposmotic shock

>

Clarity and logic were important adjuncts to the general grading scheme to the right.	$P=RT(C_i-C_o)$	(20/100)
	C_i change	(20/100)
	Cell volume	(10/100)
	Number of molecules	(20/100)
	Channel molecules in molecules per sec (*60 sec)	(20/100)
	Number of Channels	(10/100)

Name:_____
Student ID:_____
Be sure to write your name and student ID above. Read the questions carefully, think, then write your answers in the lined spaces. When finished, please hand your answer in.

Question One.

Arachaeal lipids contain isopentenyl subunits that create a bulkier hydrophobic component due to methyl (and cyclic) side-groups on the carbon backbone. Students differed on whether the presence of the methyl (and cyclic) side-groups would increase or decrease fluidity of the hydrophobic core of the Archaeal membrane. Propose an experiment that would directly measure the fluidity of the Archaeal membrane.
Hints:
• You should find the equation for fluorescence polarization helpful:

$$P = \frac{I_{parallel} - I_{perpendicular}}{I_{parallel} + 2 \cdot I_{perpendicular}}$$

• A diagram explaining how it works is required.

• Suggest two different control experiments that validate your proposed experiment.

As described in lectures on the physical properties of membranes, perylene is one example of a probe of fluidity. By exciting the planar molecule with polarized light ($I_{parallel}$), and measuring the fluorescent light (both $I_{parallel}$ and $I_{perpendicular}$), it is possible to measure the relative fluidity: More $I_{perpendicular}$ means higher fluidity.
To be able to assess the effect of side groups on the isopentenyl subunits, it would be necessary to use artificial membranes, with either methyl or cyclic side-groups and all other parts of the structure identical.

By measuring fluidity as a function of both temperature and pressure, one could attempt to remove the added complexity of transverse packing effects of the two side-groups --which have significantly different molecular 'bulk'. These would be two different and helpful controls.

Experimentally fluorescence anisotropy is the difference between fluorescence intensities emitted parallel to and perpendicular to the polarity of the exciting light, divided by the total emitted fluorescence[1]:

$$A = (I_{\parallel} - I_{\perp}) / (I_{\parallel} + 2 \cdot I_{\perp})$$

where $2 \cdot I_{\perp}$ refers to the two perpendicular directions of emission (perpendicular to the y-axis, as shown below, and perpendicular to the x-axis (parallel to the y-axis).

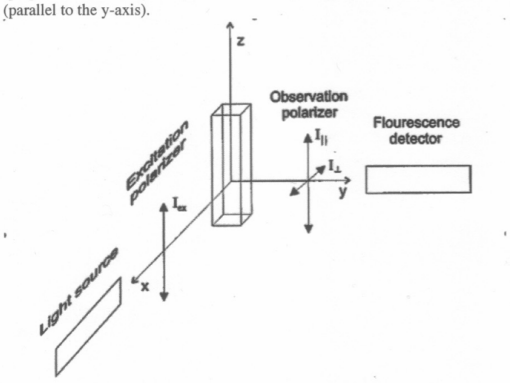


FIG. 1. Schematic diagram for measurement of fluorescence anisotropy of a cylindrically symmetrical emission field.

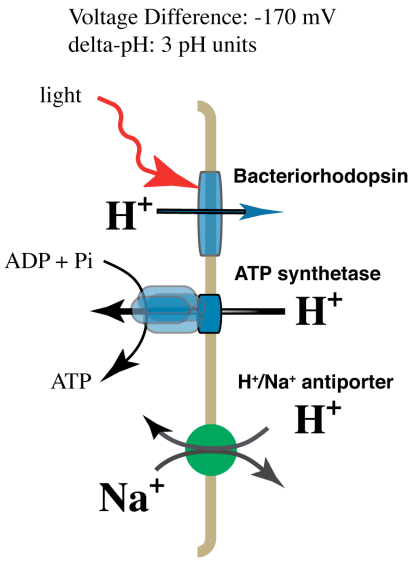
In the older literature, polarization was commonly used:

$$P = (I_{\parallel} - I_{\perp}) / (I_{\parallel} + I_{\perp}).$$

[1] Source: Bloomfield, VA (2000) Survey of biomolecular hydrodynamics. On-Line Biophysics Textbook Volume: Separations and Hydrodynamics (Todd M. Schuster, editor) Chapter 1

Question Two.

Although Halobacteria halobium is a halophile, it still needs to exclude Na^+ from the cytoplasm. Suppose that 40 mM Na^+ is the threshold for cytoplasmic toxicity, and the extracellular NaCl is the same as the Dead Sea (12-18% w/v, a concentration of 2.57 M). Are a voltage difference of –170 mV and a delta-pH of 3 pH units sufficient to exclude Na^+?



• Show explicitly for an antiporter with stoichiometries of $2H^+$ per Na^+ and $1\ H^+$ per $2Na^+$.

Is there sufficient energy in light to create the proton motive force?

• Show explicitly. You will need to know that the energy per photon (at 550 nm) is $3.61 \cdot 10^{-19}$ joules.

Please be sure that you show units. This is an important internal check, both for you and for me.

Question Two
Here are the constants and other values we require

```
> R := 1.987·10^-3 [[kcal]]/([[mol]][[K]]) : T := 293[[K]] : F := 23.06 [[kcal]]/([[mol]][[V]]) : ψ := -0.17 [[V]] :
```

Here are the concentrations we require. H_{inside} is assumed to be neutral pH, $Na_{outside}$ is set to 2.57 M

```
> H_inside := 10^-7 [[mol]]/[[liter]] : H_outside := 10^-4 [[mol]]/[[liter]] : Na_outside := 2.57 [[mol]]/[[liter]] :
```

Now we calculate. First, for Na_{inside} with a 2 proton to 1 sodium stochiometry

```
> solve(2·R·T·ln(H_inside/H_outside) + 2·F·ψ = R·T·ln(Na_inside/Na_outside) + F·ψ, Na_inside)
```

$$\frac{3.06 \times 10^{-9}\ [[mol]]}{[[L]]} \quad (1)$$

Internal sodium will be 3 nM, well below the toxic limit.
For a stoichiometry of 1 proton and 2 sodiums:

```
> solve(R·T·ln(H_inside/H_outside) + F·ψ = 2·R·T·ln(Na_inside/Na_outside) + 2·F·ψ, Na_inside)
```

$$\frac{2.36 \times 10^{0}\ [[mol]]}{[[L]]} \quad (2)$$

Internal sodium is well above the toxic threshold.

To assess the 'power of light'. First, the PMF in joules/mole

```
> solve(PMF = 8.314 [[joule]]/([[mol]][[K]])·T·ln(H_inside/H_outside) + 9.649·10^4 [[joule]]/([[mol]][[V]])·ψ, PMF)
```

$$-\frac{3.32 \times 10^{4}\ [[J]]}{[[mol]]} \quad (3)$$

Then per molecule

```
> -(3.32 × 10^4 [[J]]/[[mol]]) / (6.023·10^23 [[mole]]/[[mol]])
```

$$-\frac{5.51 \times 10^{-20}\ [[J]]}{[[mol]]} \quad (4)$$

Less than the power of a blue light photon, so there is sufficient energy.

```
>
```

Question Three.

Molecular biologists often focus on the gene expression as a proxy for function ("If it's up-regulated, it must be necessary"). But bioengineers know that varying levels of gene expression can have minimal to nil effect on physiological functions; it depends upon the gene and the function. In the context of transport, provide biophysical evidence that changes in gene expression for an ion channel may have no effect on cell function. A mathematical explanation is required. Do the same thing for an ATP-dependent pump, but explain why changes in gene expression are more likely to affect function.

For ion channels, the membrane capacitance is key. It is reasonably trivial to calculate that very little flux is required for electrical signaling --the common function of ion channels. Adding channels won't have an effect. Nor will more channels affect the kinetics of signaling, since gating is central to kinetics. So, up-regulation of ion channel genes is probably without function or biological meaning.

$Q = C \cdot \Delta E$ (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:

$C = 4 \cdot \pi \cdot r^2 \cdot C'$ C' is the capacitance per unit area (about 1 microFarad per square centimeter for cells).

Active pumps are a completely different subject! There, fluxes are much lower than for an ion channel: 100 ions per second compared to 100,000 ions per second. Additive flux will have a very significant impact, so gene regulation of ion pumps is likely to have a biological 'meaning'.

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: $6 \bullet h^2$ 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: $D = \frac{1}{2} \bullet \frac{\Delta^2}{\tau}$, $\langle x^2 \rangle = 2 \bullet D \bullet t$, and $\langle r^2 \rangle = 6 \bullet D \bullet t$

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:

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

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).

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 Transport: Water Fluxes

$$\text{Volume Flow: } J_V \propto \frac{\partial P}{\partial x}$$

$$\text{Flow through a Pipe: } J_V = -\frac{r^2}{8 \bullet \eta} \bullet \frac{\partial P}{\partial x}$$

Flow into / out of a cell:

$$J_V = -\frac{1}{A} \bullet \frac{\partial V}{\partial t}$$

$$J_V = L_p \bullet [P - RT(c_i - c_o)]$$

$$J_V = L_p \bullet \Delta\Psi$$

where $RT(c_i - c_o) = \pi_i - \pi_o$

when $J_V = 0$: $P = RT(c_i - c_o)$

Cell volume, pressure and osmotic relations

$$\frac{\partial P}{\partial V} = \frac{\varepsilon}{V} \approx \frac{\Delta P}{\Delta V} = \frac{P - P_0}{V - V_0}$$

$$\frac{\partial \pi_i}{\partial V} \approx \frac{\Delta \pi_i}{\Delta V} = \frac{\pi_i - \pi_{i,0}}{V - V_0}$$

$$P(t) = (P - P_e) \bullet e^{\left(-L_p \bullet A \bullet \frac{\varepsilon + \pi_i}{V} \bullet t\right)}$$

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)
	$5.822 \cdot 10^2$	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) used for $\log_{10}$
	1.342	kcal mol^{-1}	At 20 ºC (293 ºK) used for $\log_{10}$
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