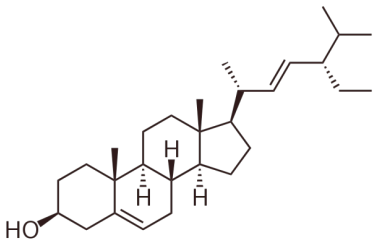
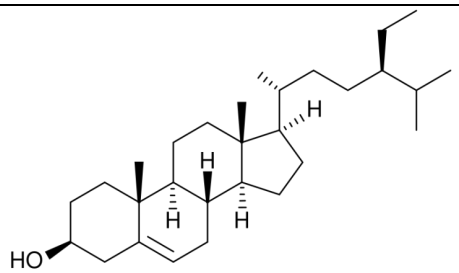
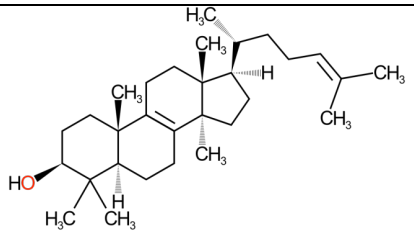
Assignment One (28 September 2010) **ANSWER KEY**



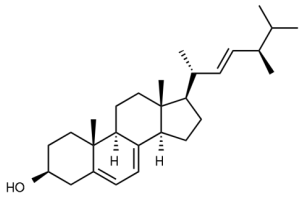
stigmasterol



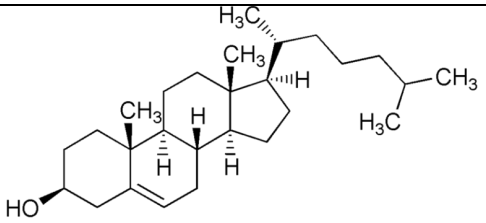
sitosterol



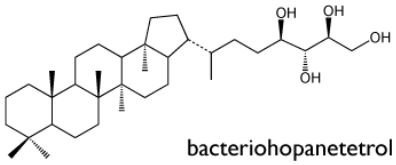
lanosterol



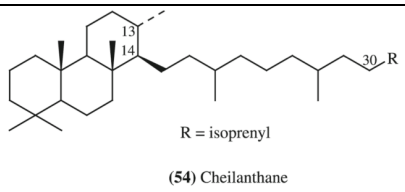
ergosterol



cholesterol



bacteriohopanetetrol



cheilanthane

QUESTION ONE: Some sterols of eukaryotic origin are shown, along with a bacterial 'homolog' (a hopanetetrol). The final structure (cheilanthane —left panel) is commonly found in rock and is believed to be of biogenic origin (Brocks et al., 2003. Geochimica et Cosmochimica Acta 67:4321–4335). Could the bacteriohopanetetrol and cheilanthane play the same functional role in biological membranes as the other compounds do? Explain.

QUESTION TWO: What is/are the structural and functional difference(s) between a sterol and a steroid. Pick an example of each and draw the structure showing the salient difference(s). Will both intercalate into membranes? Explain.

QUESTION THREE: Design a novel ion channel. If it is an amino acid sequence, it should be no more than 8 amino acids long, give the sequence and hydropathies. If some other chemical structure(s) (similar in length to a peptide 8 amino acids long), provide hydropathies as best you can. Give an explanation why your chemical structure would work as an ion channel.

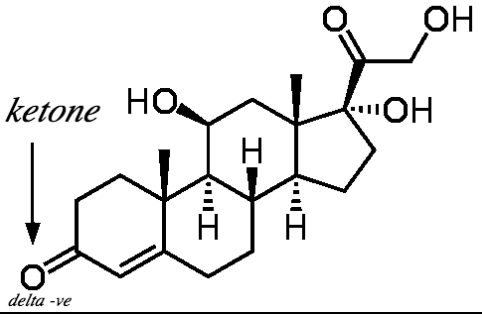
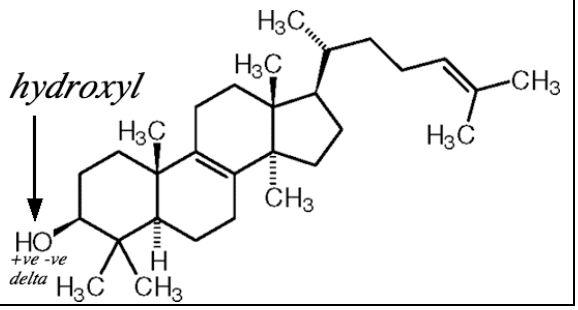
Ground Rules: I expect that students may (or may not) wish to work with each other on the assignment (depending on personal preference), and may certainly come to me for help. But, please ensure that the work you hand in is your own.

ANSWER ONE: If the amphiphilic properties of the compounds are a key aspect of their ability to intercalate into the membrane in an orderly fashion, then one would expect the hopanetetrol to operate as a 'functional' sterol, even though its orientation would be 'backwards' compared to the sterols, because the hydrophilic hydroxyls are on the opposite end of the molecule compared to the sterols. Indeed, this has been proposed by many scientists, but with conflicting experimental evidence. The argument against is that hopanetetrols are not ubiquitous in prokaryotes. And, in some instances, they are synthesized only under special conditions; for example, during spore formation, or in aerial structures, implying a role as a 'waxy' barrier rather than a membrane constituent. Thus definitive evidence for or against a role as functional sterols is unclear. What is intriguing from the standpoint of biology is their long presence in the evolutionary record. The geobiologist Tanja Bosak referred to them as fossil fats[1]. The cheilanthanes

[1] Bosak, T. (2007) Fossil fats. *In* Small Things Considered —The Microbe Blog— American Society of Microbiology. http://schaechter.asmblog.org/schaechter/2007/11/fossil-fats.html (accessed 02oct2010)

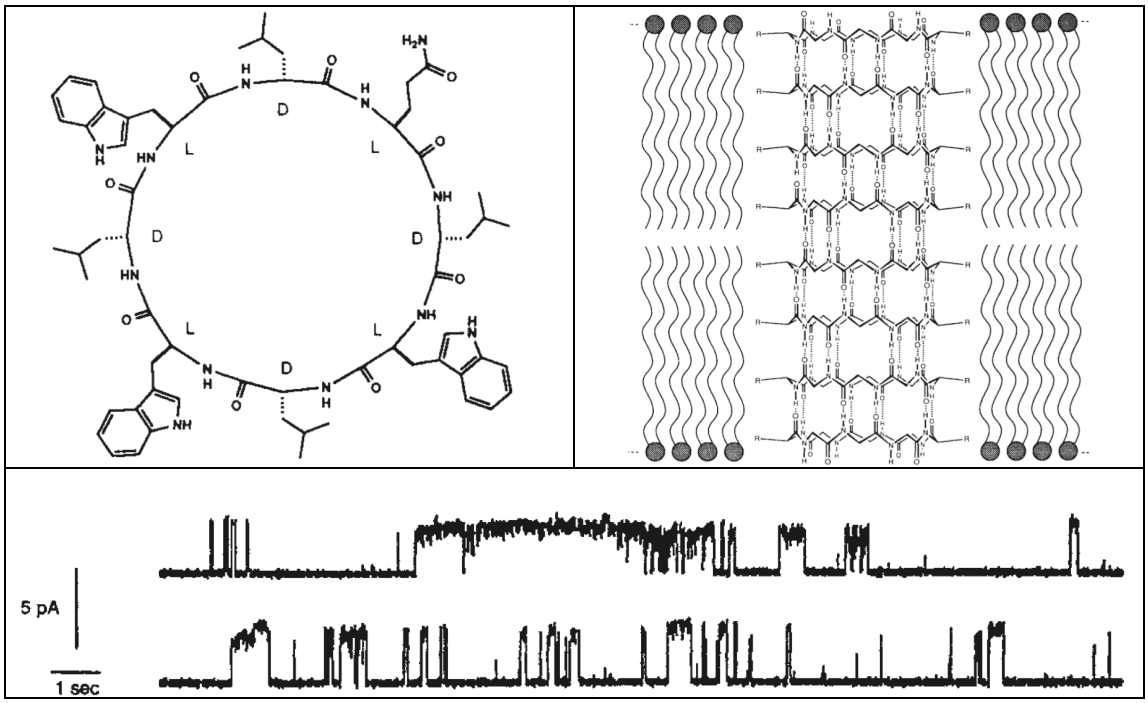
are abundant in sediments of all ages. In the absence of an amphiphilic structure, they may not have a role as membrane stabilizers like the sterols, but will certainly intercalate into membranes. (scoring: 75% base, –5 to –10% for failing to identify hydrophilic variants, +5 to +10% for clarity of presentation)

ANSWER TWO: Chemically, there is not much difference between sterols and steroids. As the names imply, sterols contain an —OH group that is usually absent in steroids (replaced by a carbonyl ==O). Both are dipoles due to the electronegativity of oxygen, but the polarity is reversed. Two examples of steroids (*left*) and sterol (*right*):

The steroid cortisol. Note the carbonyl (ketone) on carbon three, although some steroid compounds (e.g., estrogens) have an —OH group on carbon three.	The sterol lanosterol has an hydroxyl (alcohol group, —OH) on carbon three. Note the acyl 'tail' with terminal methyl groups.
	

Both steroids and sterols are mostly hydrophobic, thus lipophiles and can easily intercalate into the membrane. However, their physiological roles are quite different: sterols as membrane stabilizers and steroids as hormones, especially sexual development (estrogens and testosterones) but other things as well. (scoring: 80% base, –5 to –10% for failing to identify structural variants, +5 to +10% for clarity of presentation)

ANSWER THREE: The problem is difficult: a peptide 8 amino acids long won't traverse the membrane, no way, no how. Some students opted to extend the length by disulfide bonds, which seemed a bit unfair to me. Many left the complete charge of the carboxyl and/or amino terminuses dangling in the middle of the membrane, an energetically daunting prospect. The experimentally proven answer is deceptively simple[2]: a cyclic peptide 8 amino acids long with the sequence *cyclo*-Trp-**D**Leu-Trp-**D**Leu-Trp-**D**Leu-Gln-**D**Leu. Essentially, alternating **L**-Tryptophan and **D**-Leucine. The structure of the cyclic peptide monomer is shown below (*left* panel), along with a model of how the rings stack —stabilized by hydrogen bonding (*right* panel). The *lower* panel shows the ion channel behavior of the cyclic peptides in a membrane: typical step-wise changes in current, where gating is due to alignment of the cyclic peptide rings. The channel is cation-selective. (scoring: 75% base, –5 to –15% for failing to identify hydropathy values, 'stretching' 8 a.a. to traverse the membrane etc., +5 to +10% for clarity of presentation or creative solutions, +15% for the 'right' answer)



[2] Ghadiri, MR, Granja JR, & Buehler LK (1994) Artificial transmembrane ion channels from self-assembling peptide nanotubes. Nature 369:301–304.

Assignment Two (09 November 2010) **Due Friday 12 November 2010 at 4:30 PM**

QUESTION ONE: In class, we explored the nature of the membrane potential and its dependence on various ion species. Notably, in the resting state, animal cells are usually K^+-permeant. An example of data is presented in Table I[1]:

Table I: Cytoplasmic and extracellular K^+, Na^+ and Cl^- concentrations, calculated Nernst Potentials and Permeability Coefficients. Note the data are 'hypothetical' (but realistic).

Ion	[Cytoplasm], mM	[Extracellular], mM	Nernst Potential (mV)	P_{ion}, cm/sec
K^+	135	4	–92	$1 \cdot 10^{-7}$
Na^+	12	140	+64	$1 \cdot 10^{-9}$
Cl^-	4	116	–88	$1 \cdot 10^{-8}$

If a cell is excitable (that is, capable of generating an action potential), the action potential can be triggered by a depolarizing the potential by approximately +15 mV from its normal value of about –90 mV. How much 3 M KCl would you have to add to the bloodstream to cause a massive induction of action potentials in excitable cells?

Hints: Wikipedia says that the average blood volume of a human adult is in the range of 5 liters. For the sake of simplicity, use this volume in your calculations. Assume that extracellular [Na^+] is unaffected, leaving you to consider *only* extracellular [K^+] and [Cl^-].

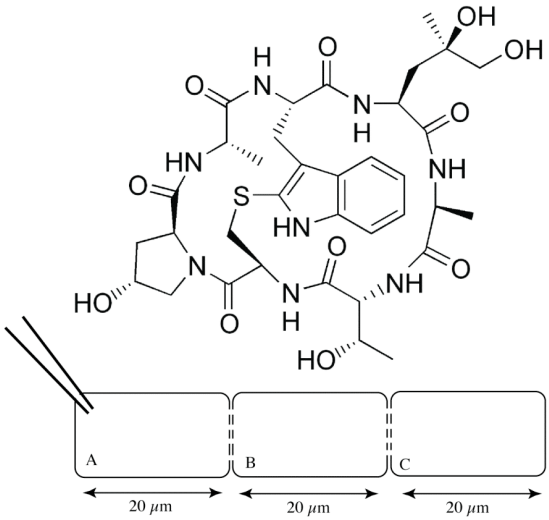
QUESTION TWO: Choose examples of a cation and an anion with similar atomic radii. What are their hydrated radii? How many water molecules are in the hydrated shell(s)? How does the hydrated radii compare to known channels (gramicidin and potassium come to mind, but there are others)? If the ion traverses the channel in a hydrated state, predict the cation *versus* anion selectivity.

Hints: Data on atomic radii and mobilities are provided in the course notes mounted on the website; lecture notes provide additional information.

Ground Rules: I expect that students may 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 your own. And, if you cite articles, a complete citation helps me greatly. As always, excessive length is not encouraged.

[1] Wright, SH (2004) Generation of resting membrane potential. Adv. Physiol. Educ. 28:139–142.

Cell-to-cell coupling is very common in multicellular organisms. In plants, the connections between cells are called plasmodesmata; in animals, they are known as gap junctions. In either case, diffusion of substances can follow an intercellular pathway, so that the substance never has to leave the system of inter-connected cells, instead passing through the intercellular plasmodesmata or gap junctions. This is true for normal cell constituents, as well as toxins. One such toxin is phalloidin. Produced by some fungi (*Amanita*, the death cap, is the best known), phalloidin is a deadly toxin of molecular formula $C_{35}H_{48}N_8O_{11}S$ and molecular weight 788.87 g/mol. It is known that the toxin is capable of traveling from cell-to-cell: Microinjecting phalloidin into one cell causes the death of nearby cells[1].



One. Is the phalloidin permeable through the membrane? Give a realistic estimate of its permeability coefficient. Explain.

Two. Estimate the effect of a transmembrane electrical potential on permeation of phalloidin into the cell.

Three. Predict the diffusion coefficient (be sure your prediction is realistic and how you estimated it).

Four. Calculate the mean time required for the toxin to diffuse through three cells (assume the intercellular pores do not limit diffusion).

Guidelines: Equations and constants (and data on diffusion and permeability coefficients) are provided. Should you require some other equation, ask. Please be sure that you show units. This is an important internal check, both for you and for me.

[1] Muller H, von Eichel-Streiber C, Habermann E (1992) Morphological changes of cultured endothelial cells after microinjection of toxins that act on the cytoskeleton. Infection and Immunity 60:3007–3010.

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 $I_d = 4 \bullet \pi \bullet a \bullet D \bullet C_\infty$

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	$liter\ MPa\ mol^{-1}$	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	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	$1.004 \bullet 10^{-3}$	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

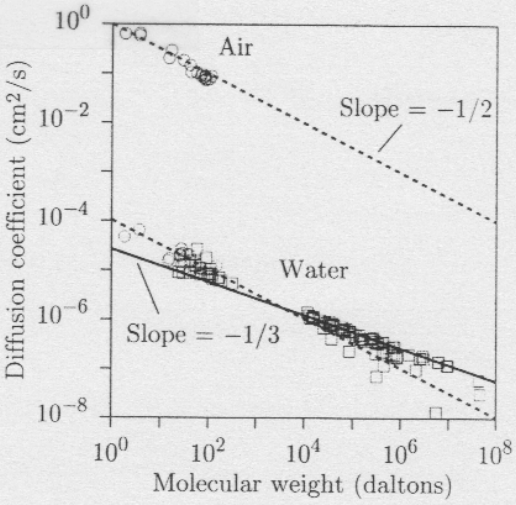


Figure 3.11 The measured diffusion coefficient D is plotted in logarithmic coordinates as a function of the molecular weight M for 19 gases diffusing in air and for 123 solutes diffusing in water. The data are from several sources (Tanford, 1961; Cohn and Edsall, 1965; Cussler, 1984; Lide, 1990). The measurements of solutes in water were made at temperatures in the range of 20–25°C and are extrapolated to infinite dilution. Those in air were made at atmospheric pressure and at temperatures in the range of 0–26.1°C. Circles represent gases diffusing either in air or in water; squares represent other solutes.

Table 3.3 Diffusion coefficients of selected molecules in air (above line) and water (below line). All these are included in Figure 3.11, which also gives citations. M is the molecular weight; D is the diffusion coefficient.

Molecule	Medium	Temp. (°C)	M (g/mol)	D (cm²/s)
Hydrogen	Air	0	2	6.11×10^{-1}
Helium	Air	3	4	6.24×10^{-1}
Oxygen	Air	0	32	1.78×10^{-1}
Benzene	Air	25	78	9.60×10^{-2}
Hydrogen	Water	25	2	4.50×10^{-5}
Helium	Water	25	4	6.28×10^{-5}
Oxygen	Water	25	32	2.10×10^{-5}
Urea	Water	25	60	1.38×10^{-5}
Benzene	Water	25	78	1.02×10^{-5}
Sucrose	Water	25	342	5.23×10^{-6}
Ribonuclease	Water	20	13,683	1.19×10^{-6}
Hemoglobin	Water	20	68,000	6.90×10^{-7}
Catalase	Water	20	250,000	4.10×10^{-7}
Myosin	Water	20	493,000	1.16×10^{-7}
DNA	Water	20	6,000,000	1.30×10^{-8}
Tobacco mosaic virus	Water	20	50,000,000	3.00×10^{-8}

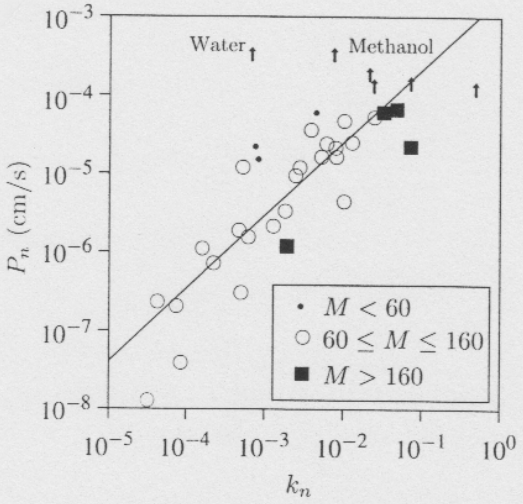


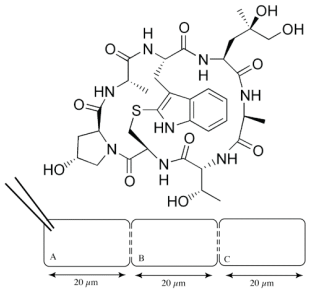
Figure 3.37 The permeability, P_n, of the membrane of the cell of the plant *Chara ceratophylla* versus the olive oil : water partition coefficient, k_n, for a number of solutes plotted on double logarithmic coordinates (adapted from Collander and Bärlund, 1933 Tables 6 and 10). The symbols are used to indicate the molecular weights of the solutes. The points with arrows indicate that the permeability of that solute was too large to be measured, but is above the value at the base of the arrow. The regression line has the equation $\log P_n = 0.930 \log k_n - 2.74$ with a correlation coefficient of 0.86.

Table 3.6 Membrane permeabilities of selected solutes in *Chara, Nitella,* human erythrocyte, and artificial lipid membranes (Collander, 1954; Stein, 1990). M is the molecular weight, and k is the olive oil : water partition coefficient. .

Solute characteristics			*Membrane permeability (cm/s)*			
Name	M	k	*Chara ceratophylla*	*Nitella mucronata*	Human erythrocytes	Artificial lipid
Water	18	1.3×10^{-3}	6.6×10^{-4}	2.5×10^{-3}	1.2×10^{-3}	2.2×10^{-3}
Formamide	45	1.1×10^{-6}	2.2×10^{-5}	7.6×10^{-6}	1.1×10^{-6}	1.0×10^{-4}
Ethanol	46	3.6×10^{-2}	1.6×10^{-4}	5.5×10^{-4}	2.1×10^{-3}	
Ethanediol	58	4.9×10^{-4}	1.1×10^{-5}		2.9×10^{-5}	8.8×10^{-5}
Butyramide	87	1.1×10^{-6}	5.0×10^{-5}	1.4×10^{-5}	1.1×10^{-6}	
Glycerol	92	7.0×10^{-5}	2.0×10^{-7}	3.2×10^{-9}	1.6×10^{-7}	5.4×10^{-6}
Erythritol	122	3.0×10^{-5}			6.7×10^{-9}	

KEY

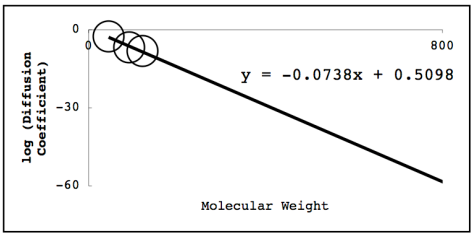
Cell-to-cell coupling is very common in multicellular organisms. In plants, the connections between cells are called plasmodesmata; in animals, they are known as gap junctions. In either case, diffusion of substances can follow an intercellular pathway, so that the substance never has to leave the system of inter-connected cells, instead passing through the intercellular plasmodesmata or gap junctions. This is true for normal cell constituents, as well as toxins. One such toxin is phalloidin. Produced by some fungi (*Amanita*, the death cap, is the best known), phalloidin is a deadly toxin of molecular formula $C_{35}H_{48}N_8O_{11}S$ and molecular weight 788.87 g/mol. It is known that the toxin is capable of traveling from cell-to-cell: Microinjecting phalloidin into one cell causes the death of nearby cells.



One. Is the phalloidin permeable through the membrane? Give a realistic estimate of its permeability coefficient. Explain.

We are searching for an estimate of permeability that can be obtained by referring to the data provided at the end of the test. We have no information about partitioning, but can extrapolate from data for increasing molecular weight and increasing polar uncharged groups (carbonyls, hydroxyls and imides):

Ethanol	MW 46	$2.1 \cdot 10^{-3}$
Glycerol	MW 92	$1.6 \cdot 10^{-7}$
Erythritol	MW 122	$6.7 \cdot 10^{-9}$
Phalloidin	MW 798	$1.9 \cdot 10^{-58}$ cm sec^{-1}



The value is very low, unrealistically so. Nevertheless, it indicates very clearly that phalloidin will not be permeable through the membrane of the cell[2].

[Identification of polar groups (5/10); comparison with P-values (2/10); power function $x \cdot \log P = P^x$) (2/10); partial points for effort]

Two. Estimate the effect of a transmembrane electrical potential on permeation of phalloidin into the cell.

First, the structure reveals no ionizable groups at normative pH (neutral in cellular cytoplasm), thus its permeation though the membrane is unaffected by the normal –ve inside potential of a cell. If it did have ionizable groups, the impact would be small because of the low permeability. If the net charge is negative, efflux would be increased. If the net charge is positive, efflux would be decreased in accordance with the GHK equation: For ±100 mV, *ca* $\exp(\pm 100/25)$ or $10^{\pm 2}$.

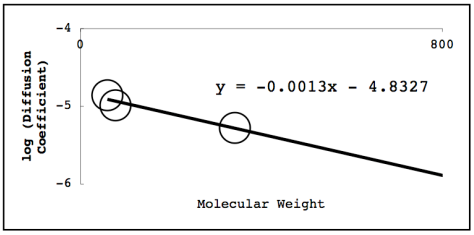
[neutral, Ψ-independent (8/10) or GHK (5/10); partial points for effort]

[2] Fluorescent conjugates of phalloidin are used to visualize actin,but it is a very common observation that the cells must be fixed, since the heptapeptide does not penetrate cells (PNAS 76:4498–4502, 1979). Nothnagel et al. treated Chara cells to 1 mM phalloidin for up to 72 hours without effect; with injection, inhibition occurred within 60 minutes (J. Cell Biol 88:364–372, 1981). Phalloidin's toxic effects in animals are due to specific binding to liver cells, in which they are taken up by a transporter of bile salts (PNAS 81:5232–5236, 1984)

KEY (continued)

Three. Predict the diffusion coefficient (be sure your prediction is realistic and how you estimated it).

The estimate of a diffusion coefficient should be more accurate, because the extrapolation is not so large.



Urea	MW 60	$1.38 \cdot 10^{-5}$
Benzene	MW 78	$1.02 \cdot 10^{-5}$
Sucrose	MW 342	$5.23 \cdot 10^{-6}$
Phalloidin	MW 798	$1.39–4.8 \cdot 10^{-6}$ cm^2 sec^{-1}
Ribonuclease	MW 13683	$1.19 \cdot 10^{-6}$

The value is about 0.92 to 0.26–fold lower than sucrose, depending on extrapolation technique.

[realistic estimate from data provided (8/10); partial points for effort].

Four. Calculate the mean time required for the toxin to diffuse through three cells (assume the intercellular pores do not limit diffusion).

This is a straightforward ‘plug-in the numbers’. Three dimensional diffusion, while not completely accurate is a better metric than two-dimensional, thus $t = (0.006\ cm)^2/(6 \cdot 1.39 \cdot 10^{-6}\ cm^2\ sec^{-1})$: 4.3 seconds.

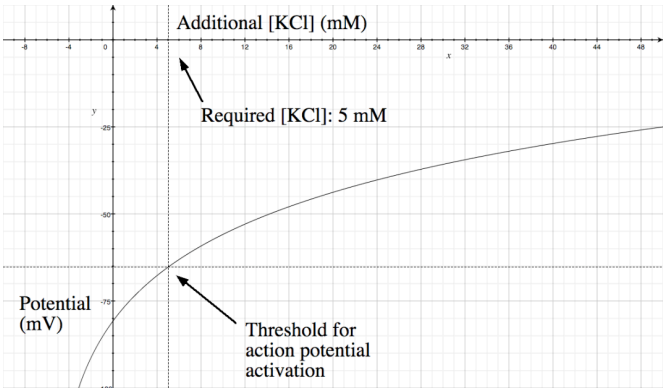
[correct use of equation (10/10); partial points for effort
]

Assignment Two (09 November 2010) **Due Friday 12 November 2010 at 4:30 PM**

Question One: A simple approach is to graph the membrane potential versus added extracellular [KCl] using the GHK equation (and the data supplied in the question) where x is the added KCl:

$$\Psi = \frac{(10^{-9} \bullet 140) + (10^{-7} \bullet (4 + x)) + (10^{-8} \bullet 4)}{(10^{-9} \bullet 12) + (10^{-7} \bullet 135) + (10^{-8} \bullet (116 + x))}$$

A depolarization to the threshold causing action potentials will occur when additional [KCl] is about 5 mM.



For a blood volume of 5 liters, the required amount of 3 M KCl would be:

[(0.005 M)•(5 liters)] / [(3 M)] = 0.008 liters

Most students chose to solve the GHK equation for the depolarized threshold (*ca* –75 mV) to obtain the required added KCl. Problems arose if students elected to use the Nernst Potential alone, since this does not account for the countervailing effect of the Cl^- in the added KCl.

Question Two: This is surprisingly difficult to assess, as students discovered. Calculations of the hydrated radius from Stokes relation:

$$u = \frac{z \bullet e}{8 \bullet \pi \bullet \eta \bullet r}, \text{ or } r = \frac{z \bullet 1.6 \bullet 10^{-19}}{8 \bullet \pi \bullet 1 \bullet 10^{-3} \bullet u}$$

give values smaller than expected. For example, K^+ (u = 7.62•10^{-8} (m/s)/(V/m)) yields a radius of:

$$r = \frac{1.6 \bullet 10^{-19}}{8 \bullet \pi \bullet 10^{-3} \bullet 7.62 \bullet 10^{-8}} = 0.84 \text{ Angstroms}$$

even though its ionic radii is reported to be about 1.33 Angstroms. A similar conundrum occurs when considering the anion. The literature is replete with reported hydrated radii, many are based on calculations and or model fitting such that practically all ions of interest to biologists have about 5–10 H_2O molecules in the first 'hydration sphere'. What is clear, however, is that size/hydration alone cannot explain the 'cation-bias' of the gramicidin channel. So, either the pore carbonyl dipoles repulse the anion, or, more likely repulse the hydrated anion because of the outward facing negative dipole of the coordinated water molecule. A lower bound when considering a *non-selective* ion channel (of which there are many) would be a pore size of 8–10 Angstroms (to shield pore carbonyls and amides from the net charge of the H_2O dipoles in the hydrated ion). Some students opted solely to cite reported values of hydrated ions, which was acceptable. Not providing a citation was unacceptable.

Question One. Transport coupling is very common. Conventionally, transport of some solute is coupled to either a H^+ gradient or a Na^+ gradient, where 'downhill' flow of the H^+ or Na^+ is coupled to the 'uphill' flow of the solute. In the case of arsenate/arsenite transport, efflux of the arsenical out of the cell can be coupled to ATP hydrolysis (ars AB), to the membrane potential (ars B) and even to the electrochemical proton gradient (one H^+ in causing efflux of one arsenate/arsenite molecule out of the cell). For these three mechanisms, calculate the effectiveness (how well they remove arsenicals from the cell). For all sub-questions, use an extracellular arsenate/arsenite concentration of 10 mM and determine the cytoplasmic concentration at equilibrium.

One. Membrane potential driven efflux (Ars B). Assume the cytoplasm membrane potentials is –160 mV (–ve inside) and the charge on the arsenate/arsenite is –ve 2.

Two. ATP-driven arsenate/arsenite efflux (Ars AB). Assume the charge on the arsenate/arsenite is zero (that is, no net negative charge).

Three. H^+ gradient coupled efflux. Assume the cytoplasmic pH is 7, the extracellular pH is 5, the membrane potential is -180 mV, that arsenate/arsenite has two negative charges, and that the stoichiometry is 1 H^+ per arsenate/arsenite.

Question Two. From a consideration of electro-neutrality and capacitance, what net concentration of arsenate (–ve 2 charge) would be required to create a potential of -180 mV in a rod-shaped (cylindrical) bacterial cell of dimensions 1 micron by 5 micron.

Guidelines: Equations and constants (and data on diffusion and permeability coefficients) are provided. Should you require some other equation, ask. Please be sure that you show units. This is an important internal check, both for you and for me.

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 $I_d = 4 \bullet \pi \bullet a \bullet D \bullet C_\infty$

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	$liter\ MPa\ mol^{-1}$	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 (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	$1.004 \bullet 10^{-3}$	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

1.1) $\psi = \frac{RT}{zF} \cdot \ln \frac{C_o}{C_i}$, so, $\underbrace{z\frac{F}{RT}\psi}_{-2\frac{1}{25.3}-160 = 12.648} = \ln \frac{C_o}{C_i}$ 4/5

$e^{12.648} = \frac{C_o}{C_i}$, so, $C_i = \frac{C_o}{311209} = 3.213 \times 10^{-5}$ mM 1/5

1.2) $\Delta G_{TOT} = \Delta\mu_{ion} + \Delta G_{ATP} = 0$ (at equilibrium)

$\Delta\mu_{ion} \to 0$, $\Delta G_{ATP} \to \Delta G^\circ_{ATP} + RT \ln \frac{[ADP][P_i]}{[ATP]}$

$RT \ln \frac{C_o}{C_i} + \cancel{zF\psi} = \Delta G^\circ_{ATP} + RT \ln \frac{[ADP][P_i]}{[ATP]}$

(ΔG°_{ATP} = 10 kcal/mol)

$0.583 \frac{kcal}{mol} \ln \frac{[0.001][0.001]}{[0.005]} = -4.96$ kcal/mol 4/5

$RT \ln \frac{C_o}{C_i} = 10 - 4.96 = 5.04$ 1/5

$C_i = \frac{10}{e^{5.04}} = 6.47 \times 10^{-2}$ mM

1.3) $\Delta G_{TOT} = \Delta\mu_{ATP} + \Delta\mu_H = 0$

$RT \ln \frac{C_o}{C_i} + \underbrace{zF\psi}_{8.302} = \underbrace{RT \ln \frac{10^{-5}}{10^{-7}}}_{2.684} + \underbrace{zF\psi}_{4.1508}$

(-ve 2, +ve 1)

note that all terms will "force" arsenate exclusion.

$\ln \frac{C_o}{C_i} = \frac{15.136}{0.583}$ 4/5

$C_i = \frac{10}{e^{25.963}} = 5.302 \times 10^{-11}$ mM 1/5

2) $Q = C \cdot \Delta E$ ($\Delta E = 0.18\ V$)

$Q = \pi r^2 h \frac{c}{2} \cdot F$ (c = 2 e⁻/molecule) $\qquad C = 4\pi r h\ (1\times10^{-6}\ F/cm^2)$

$$C = \frac{4\pi r h\ (1\times10^{-6}\ F/cm^2)}{\pi r^2 h \frac{1}{2} F} \cdot 0.18\ V$$ 4/5

$$C = \frac{4\ (1\times10^{-6}\ F/cm^2)}{(0.5\times10^{-4}\ cm)\ \frac{1}{2}\ (9.649\times10^{4}\ \frac{coulomb}{mole})} \cdot 0.18\ Volt$$ (coulomb/Volt)

$= 2.98\times10^{-7}\ mole/cm^3 = 2.98\times10^{-4}\ M$

1/5

Assignment Three (30 November 2010) **Due Friday 10 December 2010 at 4:30 PM**

QUESTION ONE: Please explain the role of mechanosensitive channels in hearing (assume a mammal (e.g., human) sensory system, rather than insect or somesuch). Please focus on the mechanosensitive component of the hearing mechanism (that is, the auditory sensory cells). The explanation should be at a level that is understandable to a second year science student.

Ground Rules: I expect students to work independently on this assignment. So, please ensure that the work you hand in is your own. It's okay to be a bit creative, but don't go *crazy*. If you cite articles, a complete citation helps me greatly. **Excessive length is not encouraged**. Handwritten is preferred to typewritten, because hand-drawn diagrams will be helpful. Finally, clarity of explanation will be very important in grading of the assignments.

KEY

As an explanatory assignment, much of the score was devoted to clarity of explanation and suitable diagrams. Of all the sensory transduction systems, the auditory-sensory one is the least understood, even though the anatomy of the system has been well described. Hearing is a power function, using a logarithmic scale of decibels[1]. Thus, like vision, the dynamic range is quite remarkable. Within the cochlea, hair cells are deflected by pressure waves, resulting in activation of channels that in turn cause neurotransmitter release to activate action potentials in afferent nerves. Activation of the channels in the hair cells is caused by mechanical linkages between the bundled stereocilia. Calcium influx mediates neurotransmitter release. Of especial note is the unusual ionic composition of the cochlear (endolymph) fluid —high in potassium— and the high potential of this extracellular fluid relative to the perilymph. That high potential (+80 mV) is crucial for hearing function and is generated through a highly complex and coordinated array of transport systems[2].

[1] Decibels (dB) = 20 $\log_{10}[P/P_{reference}]$.

[2] Nin F, Hibino H, Doi K, Suzuki T, Hisa Y, Kurachi Y (2008) The endocochlear potential depends on two K^+ diffusion potentials and an electrical barrier in the stria vascularis of the inner ear. PNAS 105:1751–1756.

CHOOSE THREE OF THE FOLLOWING FOUR QUESTIONS

QUESTION ONE

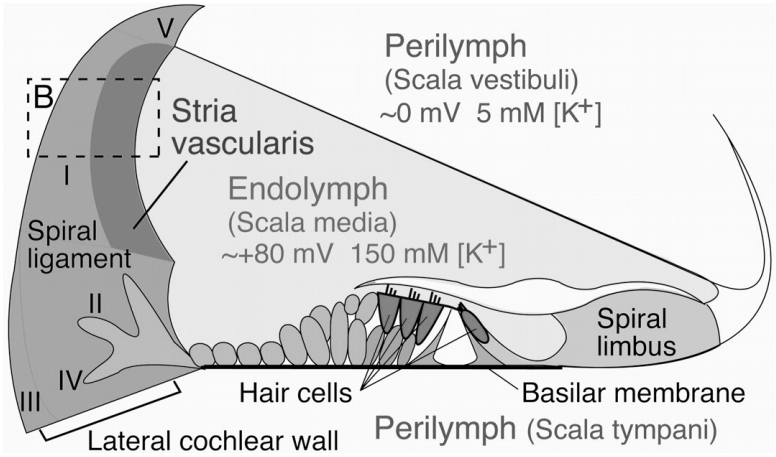
Data on the ionic composition of cochlear endolymph fluid (bathing the surface of the auditory-sensory cells in the mammalian hearing apparatus) is provided for Guinea pig in the table below[1]. For comparative purposes, ionic composition of cerebrospinal fluid (bathing neuronal cells and similar to ion composition of the perilymph fluid) is also shown, as is an estimate of intracellular ion levels.

Ionic species	Endolymph fluid	Cerebro-spinal fluid	Intracellular
	(milliEquivalents per liter)		
Na^+	26	150	12
K^+	142.0	4.0	135
Cl^-	110	122	4
Ca^{2+}	3.0 ± 0.2	3.0 ± 0.2	0.0002
Mg^{2+}	0.9 ± 0.2	2.0 ± 0.2	2.5
Nota bene For the divalent ions, divide by two to obtain milliMolar concentration			

• For the four major ions (Na^+, K^+ Ca^{2+} and Cl^-), what are the Nernst Potentials for the auditory-sensory cells protruding into the endolymph? If hair cells have an intracellular potential of –50 mV, calculate the relative permeability ratios for the four major ions.

• Mechanosensation is supposedly mediated by influx of Ca^{2+}. Test this by calculating the Gibbs free energy for Ca^{2+} flux: inward or outward and the magnitude (kcal/mole preferred)?

• The endolymph has an extracellular potential of +100 mV relative to the perilymph[2]. How would this impact electrical signaling?



[1] Citron L, Exley D (1957) Recent work on the biochemistry of the labyrinthine fluids. Proceedings of the Royal Society of Medicine 50:697–701.

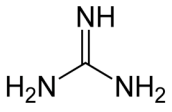
[2] Nin F, Hibino H, Doi K, Suzuki T, Hisa Y, Kurachi Y (2008) The endocochlear potential depends on two K^+ diffusion potentials and an electrical barrier in the stria vascularis of the inner ear. PNAS 105:1751–1756.

QUESTION TWO

From the data tabulated 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.

• Predict the enthalpy of hydration for the cation Guanidinium$^+$ 

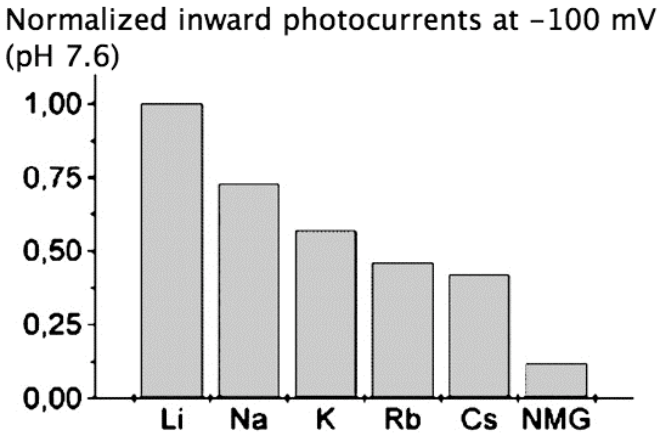
• Show how you extrapolated the hydration value, and give supporting rationale for your extrapolation.

QUESTION THREE

The data below show permeability ratios for Channelrhodopsin–2[3] (*nota bene* the channel is impermeant to anions).

Ion	P_X/P_{Na}
H^+	$\sim 10^6$
Guanidinium$^+$ 	13 ± 5
Methylammonium ($CH_3NH_3^+$)	6 ± 2
diMethylammonium ($(CH_3)_2NH_2^+$)	4 ± 2
Li^+	2 ± 0.5
Na^+	1
K^+	0.5 ± 0.3
tetraMethylammonium ($(CH_3)_4N^+$)	<0.06

The relative conductances can be inferred from the following data showing inward photocurrents for various cations (at an extracellular concentration of 115 mM) (*nota bene* the NMG^+ (N-methyl-d-glucamine$^+$) is a measure of H^+ conductance, but at a very low [H^+] of 25 nM).



• Explain how the permeability ratios were determined.

• Estimate the relative conductance (g_X/g_{Na})

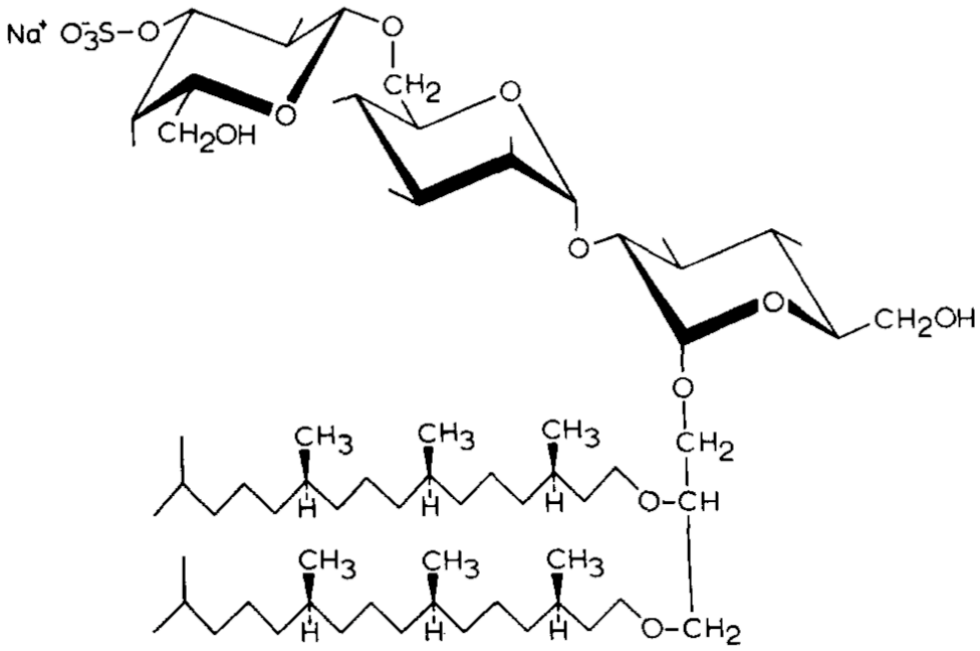
• Propose a gate and/or pore structure that would explain the unusual permeability ratios.

[3] Nagel G, Szellas T, Huhn W, Kateriya S, Adeishvili N, Berthold P, Ollig D, Hegemann P, Bamberg E (2003) Channelrhodopsin-2, a directly light-gated cation-selective membrane channel. PNAS 100:13940–13945.

QUESTION FOUR

In the archaeal extremophile *Halobium cutirubrum* (whose purple membranes are identical to those of *H. halobium* (*salinarum*)[4]), about 25% of the polar lipid is a sulfur-containing glycolipid[5] (shown below).

$Na^+O_3^-SO$-3-Gal*p*-β-(1→6)-Man*p*-α-(1→2)-Glc*p*-α-(1→1)-2,3-diphytanyl-*sn*-glycerol



• Compare and contrast the structural elements of this sulfur-containing glycolipid with 'normal' Archaeal ether lipids. Predict the adaptive (if any) role(s) that these difference(s) would have in the context of the extreme environment and physiological function(s) of *Halobium*.

[4] Kushwaha SC, Kates M, Stoeckenius W (1976) Comparison of purple membrane from *Halobacterium cutirubrum* and *Halobacterium halobium*. Biochimica et Biophysica Acta 426:703-710.

[5] Kates M, Deroo PW (1973) Structure determination of the glycolipid sulfate from the extreme halophile *Halobacterium cutirubrum*. Journal of Lipid Research 14:438–445.

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

Goldman - Hodgkin - Katz (GHK) equation

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

Equations relevant to Membrane Transport: Water Fluxes

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

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

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

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

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

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:

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

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).

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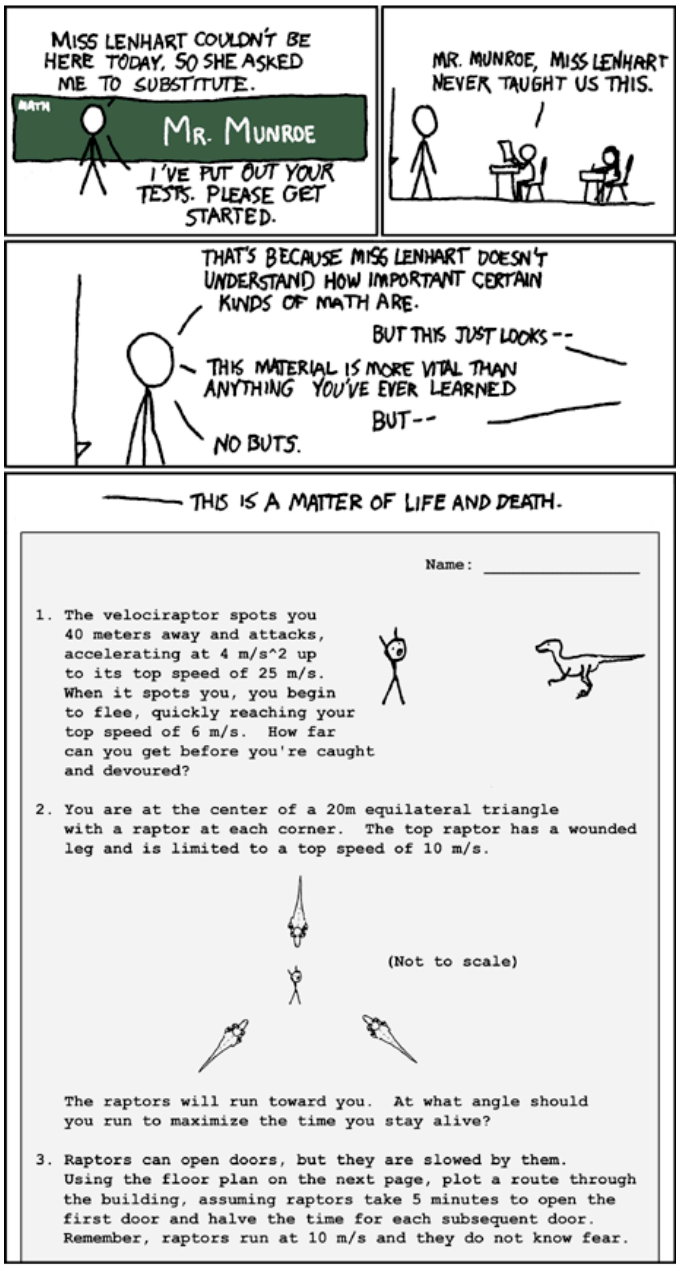
ν_w	$1.004 \bullet 10^{-6}$	$m^2\ sec^{-1}$	kinematic viscosity of water at 20 ºC (viscosity/density)

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

THIS IS NOT A QUESTION. IT IS PROVIDED AS A RESPITE FROM THE FINAL EXAM. SOLELY FOR YOUR ENJOYMENT AND AS A REMINDER THAT MATH IS A MATTER OF LIFE AND DEATH (AT LEAST ACCORDING TO RANDALL MUNROE)



from Randall Munroe (http://xkcd.com)

Finis

QUESTION ONE KEY

Nernst Potential

$E = (RT/zF) \bullet ln(c_o/c_i) = 25.3/z \bullet ln(c_o/c_i)$

$E_{Na} = 25.3/z \bullet ln(c_o/c_i) = 25.3/1 \bullet ln(26/12) = +19.7$ mV
$E_K = 25.3/z \bullet ln(c_o/c_i) = 25.3/1 \bullet ln(142/135) = +1.3$ mV
$E_{Ca} = 25.3/z \bullet ln(c_o/c_i) = 25.3/2 \bullet ln(0.0002/3) = +121.6$ mV
$E_{Cl} = 25.3/z \bullet ln(c_o/c_i) = 25.3/-1 \bullet ln(110/4) = -83.8$ mV
(/10)

Permeability ratios can be derived from:

Goldman - Hodgkin - Katz (GHK) equation

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

but it has to be simplified to only one unknown. For example, normalizing by P_{Na}, the solution for Na^+ relative to K^+ is:

$$\frac{P_K}{P_{Na}} = \frac{e^{-2}c^i_{Na} - c^o_{Na}}{c^o_K - e^{-2}c^i_K}$$

By inspection of the Nernst potentials, we know that Cl^- must be the most permeant ion, and Ca^{2+} the least permeant. So first, $P_{Cl}/P_{Na} = 2.247$. Now substituting this value for the next most permeant, K^+:

$$e^{-2} = \frac{c^o_{Na} + 2.247c^i_{Cl} + \frac{P_K}{P_{Na}}c^o_K}{c^i_{Na} + 2.247c^o_{Cl} + \frac{P_K}{P_{Na}}c^i_K} = \frac{.035 + \frac{P_K}{P_{Na}}c^o_K}{.259 + \frac{P_K}{P_{Na}}c^o_K}$$

$P_K/P_{Na} = 3 \bullet 10^{-4}$

If we were to first consider P_{Cl}/P_K first, we would use:

$$\frac{P_{Cl}}{P_K} = \frac{e^{-2}c^i_K - c^o_K}{c^i_{Cl} - e^{-2}c^o_{Cl}} = 11$$

That is P_{Cl} is greater than *either* P_{Na} or P_K.

The P_{Ca}/P_{Na} must be extremely small to account for the –ve inside potential given the +ve Nernst potential for Ca. Checking for *only* three ions (using our P_X/P_{Na} values):

$$E = 25 \bullet \ln\left[\frac{[0.026] + 2.247[0.004] + 3\times10^{-4}[0.142]}{[0.012] + 2.247[0.110] + 3\times10^{-4}[0.135]}\right] = -50mV$$

(/10)

For Ca^{2+} Gibbs free energy difference, we only need to be concerned with the chemical potential difference, so:

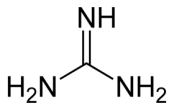
$$\Delta\mu_{Ca} = RT \bullet \ln\left[\frac{c^o_{Ca}}{c^i_{Ca}}\right] + zF\Delta\Psi = 5.833\times10^{-1} \bullet \ln\left[\frac{1.5}{0.0002}\right] + 2\times23.06\times0.05 = 7.51 \text{ kcal/mole}$$

The chemical potential of both terms is directed *inwards*
(/5) .

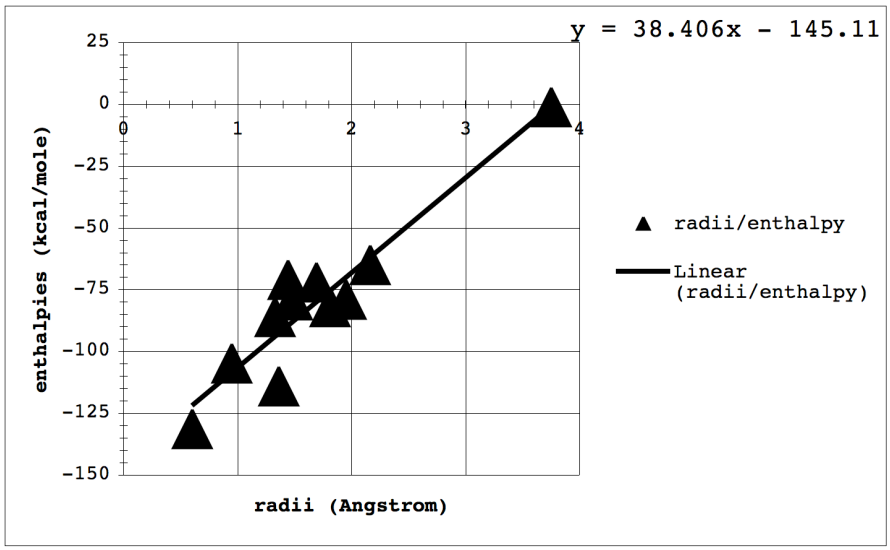
Finally, the hair cell is exposed to two very different extracellular media, one similar to a 'normal' extracellular media, the other quite different. The +ve endolymph potential may impact the driving force for Ca^{2+} inward flux, dramatically so, as well as K^+ influx
(/5).

QUESTION TWO KEY

Plotting the hydration enthalpies *versus* atomic radii will allow us to predict an enthalpy for guanidinium$^+$. However, we need to estimate what the radii of this relatively complex molecule.



Noting that two 'similar' molecules are provided amongst the tabulated data gives us a foundation for am estimate: NH_4^+ has an atomic radius of 1.48 Angstroms, NO_3^- has a radius of 2.9. Hydrogen does not contribute that much to the overall radius of a molecule (for example, the water molecule is dominated by the oxygen). This is supported by the much larger size of NO_3^- compared to NH_4^+. So, using NH_4^+ as the base measure, we can estimate that Guanidinium$^+$ is ×2 to ×3, or *ca* 3 to 4.5 (say, 3.75) Angstroms.

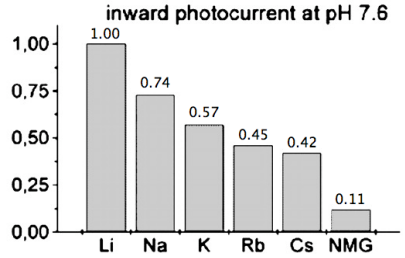


This results in an estimate of near zero enthalpy of hydration for guanidinium$^+$.

(/10)

QUESTION THREE KEY

A bilayer chamber can be used to determine permeability ratios. Ions are added to one side of the membrane and the change in the potential when ion flux is zero (the reversal potential is measured). For example, if a 10-fold increase in KCl on one side of the membrane (*cis*) causes the reversal potential to become –55 mV relative to the *trans* side, we can infer that the membrane is permeant to K^+ but not to Cl^-. If a 10-fold increase in KCl (*cis*) and a 10-fold increase in NaCl (*trans*) cause the potential to become –27.5 mV, we can infer that $P_{Na}/P_K = 1$.



Ion	(g_X/g_{Na})	
Li	1.35	(that is, 1.00/0.74=1.35)
Na	1.00	
K	0.77	
Rb	0.61	
Cs	0.57	

First, the channel is remarkably non-selective with the exception of H^+ (which is highly permeant). And, the channel passes all measured ions with relative ease (conductances are not very different amongst the alkali metals). Thus the concept of a gate or pore structure conferring selectivity is difficult to envisage. However, the channel is anion-impermeant. To exclude anions, there must be a repelling –ve charge associated with the entrance into the channel. This could be formed of carbonyls. The pore diameter will not be narrow, given its non-selectivity and ability to pass bulky ions like the methyl ammonium(s). In fact, for these ions to pass through the channel, the pore must be larger than 4 to 5 Angstroms based on comparison with ammonium data from Question 2.

(/10)

QUESTION FOUR KEY

The major elements that are unique are explicit in the name sulfur-containing glycolipid. The presence of a sulfate group is very unusual. Multi-chain sugar groups are found in chloroplasts (the digalactosyldiglyceride) but are otherwise fairly rare.

Other elements, the ether linkage, the phytanal tail, are shared with other archaeal ether lipids.

So, what function could the negatively charged sulfate and tri-glyco chain have? For the negatively charged sulfur, we could infer a role similar to that of cardiolipin in mitochondria. At 25% of polar lipid, the –ve charges would create a strongly negative surface potential on the faces of the bacterial membranes. These could act to 'proton tunnel' H^+, such that they stayed near the membrane surface and were quickly re-cycled during light-driven proton pumping and ATP synthesis.

As to the sugars, they might act to protect the bacterial membrane from oxidative damage, similar to a potential role of galactosyl lipids in chloroplasts.

Could they also play a role in halotolerance? It's hard to envision how binding sodium at the membrane surface would have a protective role.

(/10)