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

$$> R := 1.987 \cdot 10^{-3} \frac{[\![kcal]\!]}{[\![mol]\!][\![K]\!]} : T := 293[\![K]\!] : F := 23.06 \frac{[\![kcal]\!]}{[\![mol]\!][\![V]\!]} : \psi := -0.12\,[\![V]\!] : n := 0.5 :$$

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

$$> H_{inside} := 10^{-7} \frac{[\![mol]\!]}{[\![liter]\!]} : Ca_{inside} := 5 \cdot 10^{-6} \frac{[\![mol]\!]}{[\![liter]\!]} : Ca_{outside} := 5 \cdot 10^{-3} \frac{[\![mol]\!]}{[\![liter]\!]} : Na_{inside} := 1 \cdot 10^{-6} \frac{[\![mol]\!]}{[\![liter]\!]} :$$

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

$$> solve\left(R \cdot T \cdot \ln\left(\frac{Ca_{inside}}{Ca_{outside}} \right) + 2 \cdot F \cdot \psi = R \cdot T \cdot \ln\left(\frac{Na_{inside}}{Na_{outside}} \right) + F \cdot \psi,\ Na_{outside} \right)$$

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

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

$$> solve\left(R \cdot T \cdot \ln\left(\frac{Na_{inside}}{\frac{1.16 \times 10^{-1}\,[\![mol]\!]}{[\![L]\!]}} \right) + F \cdot \psi = 2 \cdot R \cdot T \cdot \ln\left(\frac{H_{inside}}{H_{outside}} \right) + 2 \cdot F \cdot \psi,\ H_{outside} \right)$$

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

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

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

$$> solve\left(R \cdot T \cdot \ln\left(\frac{Na_{inside}}{\frac{1.16 \times 10^{-1}\,[\![mol]\!]}{[\![L]\!]}} \right) + F \cdot \psi = R \cdot T \cdot \ln\left(\frac{H_{inside}}{H_{outside}} \right) + F \cdot \psi,\ H_{outside} \right)$$

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

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

Clarity and logic were important adjuncts to the <u>very general</u> 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

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Clarity and logic were important adjuncts to the <u>general</u> 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)