**1. Electrical capacitance and energy of the mitochondrial inner membrane**

A capacitor is an electrical circuit element that stores electrical energy in an electric field (Wikipedia REF). The mitochondrial inner membrane acts as an electrical capacitor to store energy in an electrostatic potential different between the milieux on the two sides the membrane.

Electrical capacitance is measured in units of charge per unit voltage, or in standard units, Coulombs per Volt (C/V), equivalently, a Farad. (1 F = 1 C/V)

**1.1. What is the capacitance of mitochondrial inner membrane? Make a reasonable assumption about the size of a mitochondrion and assume that the inner membrane has 5 or 10 times more area than the outer membrane.**

A typical range for the capacity density of a biological membrane is 0.5-1.0 F cm-2 [REF]. Let’s take a mitochondrion as a sphere with radius 1 m. In this case the outer membrane would have a surface area of 4r2, or approximately 12.5 m2. If the inner membrane has 10 times more area than the outer, then the area of the inner membrane is 125 m2. So, we can approximate the capacitance of this mitochondrion’s inner membrane as

F.

**1.2. Express inner mitochondrial membrane capacitance in units of: (a.) farads per liter of mitochondria; (b.) moles per millivolt per unit liter of mitochondria.**

Note that from the previous question, we assume that the mitochondrion has a spherical shape of radius 1 m. Then, the volume of the mitochondrion is m3. Then, in L,

Dividing the capacitance by the volume gives

Converting units gives

**1.3. If the electrostatic potential across the inner mitochondrial membrane is 180 mV, how much electrical energy per unit volume is stored in mitochondria? How much electrical energy is stored in mitochondria per unit volume of myocardium? Express your answer in joules per liter.**

The potential stored on a capacitor is given by

where is the capacitance and is the voltage gradient across the membrane. Then, the energy per unit volume of mitochondria is

Since approximately 1/3 of the volume of myocardium is mitochondria, the energy stored in the myocardium is 5/3 J/L.

**1.4. Approximately much electrical energy is stored in mitochondria in the whole human heart? How does that compare to the amount of energy supplied by a AA battery? How does it compare to the amount of mechanical work the heart does per unit time?**

An average human heart is approximately 250 g. In L, that is

Then, 5/12 J of energy is stored in the inner membrane of the mitochondria.

A typical AA battery contains 0.0039 kWh = 14 kJ.

The LV typically does 1 W of work at baseline: 1 W = 1 J/s.

**2. Converting electrical potential to the ATP hydrolysis chemical potential**

The electrostatic energy potential across the mitochondrial inner membrane is used to drive the synthesis of ATP in the final step of oxidative ATP synthesis. The mammalian mitochondrial F1F0-ATPase synthesizes ATP in the mitochondrial matrix from ADP and inorganic phosphate, coupled to the translocation of protons (H+ ions) from outside to inside of the matrix. The chemical reaction stoichiometry can be expressed:

MgADP1- + HPO42- + (H+)inside = MgATP2- + H2O, (2.1)

where the term (H+)inside indicates that a hydrogen ion from inside the matrix is covalently incorporated into the synthesized ATP. The species MgADP1- and MgATP2- are the magnesium-bound species of ADP and ATP. This chemical reaction is coupled to the transport of nA = 8/3 protons across the inner membrane:

nA(H+)outside = nA (H+)inside. (2.2)

**2.1. Given a free magnesium concentration [Mg2+] and hydrogen ion activity [H+] = 10-pH, how can you compute the concentrations of MgADP1-, MgATP2-, and HPO32- in terms of the total concentrations of the reactants [ADP], [ATP], and [Pi]? (You will need to account for binding of biochemical species to [Mg2+] and [H+].)**

The following solution is for and the others follow similarly. Considering all of the possible intermediates, we have the following reactions and dissociation constants :

|  |  |
| --- | --- |
|  | (1) |
|  | (2) |
|  | (3) |

Note that the last equality of equation (3) substitutes equation (2) for . The, the total measurable ATP concentration can be determined as

|  |  |
| --- | --- |
|  | (4) |

where the last equality substitutes in equations (1)-(3). Then, rearranging equation (4) gives

|  |  |
| --- | --- |
|  | (5) |

Dissociation constants can be found in Table 2 of [1]. Note that the stability constants in [1] are the inverse of those reported here. Similarly, is

|  |  |
| --- | --- |
|  | (6) |

For, , we have

|  |  |
| --- | --- |
|  | (7) |
|  | (8) |

where the last equality substitutes in equation (7) for . Then,

|  |  |
| --- | --- |
|  | (9) |

Rearranging equation (9) gives

|  |  |
| --- | --- |
|  | (10) |

Hence, equations (5), (6), and (10) give the desired result.

[1] Saks, V.A., *et al.* Mitochondrial isoenzyme of creatine phosphokinase: kinetic properties and regulatory action of Mg2+ ions. *Eur J Biochem.* 57, p. 273 – 290. 1975.

**2.2. Derive an expression for the Gibbs free energy change associated with reaction (2.1) in terms of the reference *G*o, the concentrations of biochemical reactants, and the cation concentrations [Mg2+] and [H+]. What is the free energy of ATP hydrolysis in the mitochondrial matrix? Assume that pH = 7.2 and [ADP] = 5 mM, [ATP] = 5 mM, and [Pi] = 1 mM.**

The Gibbs free energy () for ATP hydrolysis (the reverse direction of equation (2.2)) is given by

|  |  |
| --- | --- |
|  | (11) |

**2.3. What is the free energy change of Equation (2.2) at  = 180 mV? How does the free energy change of Equation (2.1) compare to that of Equation (2.2)? How efficient is the transduction of electrical to chemical free energy in this step in ATP synthesis? (What is the ratio of energy stored in ATP to the total energy consumed?)**

**2.4. Given the concentrations assumed in 2.2, what is the minimum value of  at which ATP can be synthesized in the mitochondrial matrix?**

**2.5. Assume that reaction (2.1) proceeds by simple mass-action kinetics, with a constant reverse rate *kr*. How does the forward rate constant necessarily depend on  for the reaction kinetics to be properly thermodynamically balanced?**

**2.6. Write a simple program that simulates the kinetics of [ADP], [ATP] , and [Pi] in the matrix given a fixed membrane potential, pH, and magnesium concentration, and given arbitrary initial conditions. How do the predicted steady-state concentrations depend on membrane potential, pH, and magnesium concentration?**

**Don’t worry about these below**

**What is the free energy of ATP hydrolysis in the myocardium? Express Gibbs free energy in units of joules per mole. In mitochondrial ATP synthesis the chemical synthesis of ATP from ADP and inorganic phosphate is coupled to the movement of positive charges down the electrical gradient from the outside to the inside of the inner membrane. What is the minimum number of charges translocate to synthesize 1 ATP molecule? Assume that there is a 180 mV potential difference across the inner mitochondrial membrane.**

**How efficient is the transduction of electrical to chemical free energy in this step in ATP synthesis? (What is the ratio of energy stored in ATP to the total energy consumed?)**