

REGULATION OF ORGANELLE ACIDITY

Introduction

Intracellular compartments are largely defined by their luminal pH. Regulation of organelle acidity is a vital aspect of cellular homeostasis.

This lesson will show how to:

- Import predefined functions
- Import and fit experimental data

Additionally, we will explore in greater detail the mechanisms of ion transport.

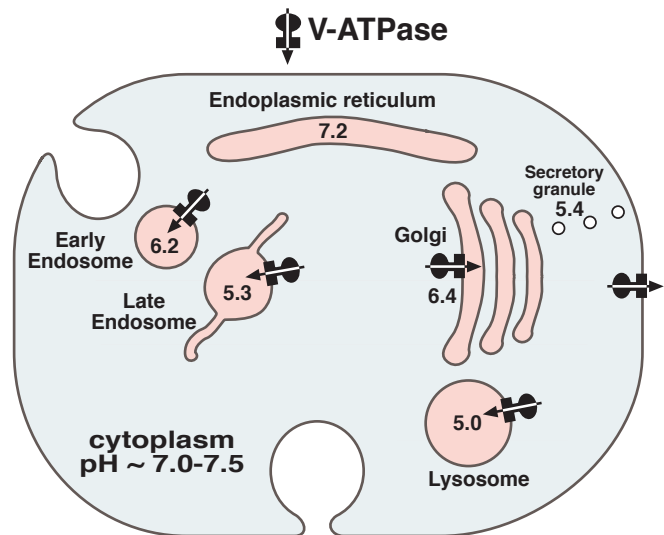


Figure 1. Typical organelle pH values.

A Two Compartment Model

Biological Background

Acidity must be tightly regulated to sustain life. Blood pH is typically 7.4 pH units; when this value falls to just 7.2 severe acidosis ensues and massive system failure follows and, if not treated, will result in death. Treatment involves the addition of buffer solutions to the blood. All cellular compartments maintain a distinctive pH that is essential to their function (see **Figure 1**); For example, lysosomes have pH ~ 5.0, in order to degrade harmful substances. The acidification of endosomes as they mature from early to late stages is required for the dissociation of receptors and ligands so that receptors can be recycled to the cell surface. A comprehensive understanding of how cellular organelles maintain their pH does not exist. As we will see, modeling can be a useful tool for interpreting experimental data.

The principal components of organelle pH regulation are listed in **Figure 2**. It is believed that the competition between the proton pumping V-ATPase (item #1) and channel mediated proton leaking (item #2), determines the steady state pH of many organelles. In the crudest terms, this is like trying to fill up a swimming pool with a bunch of holes in it. The pool will fill until the input hose is overpowered by the leaks. The case of ion transport is complicated by other factors. The lumen of the organelle buffers many of the protons that are pumped from the cytoplasm (item #4). Additionally, as positive protons move across the organelle membrane an electric membrane potential builds up. This membrane potential is offset by the counter-movement of ions like potassium (item #3). All of these effects can be combined into a coherent model.

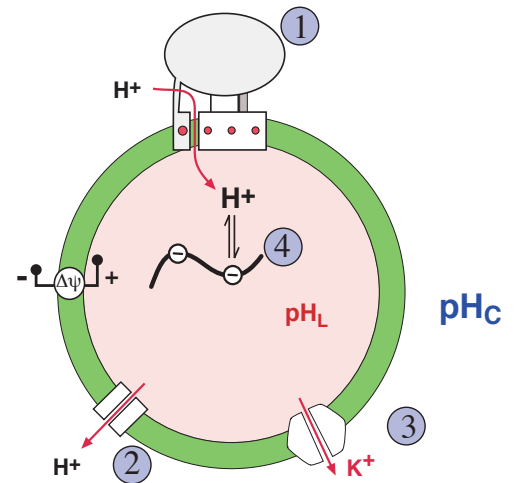


Figure 2. Key pH regulatory elements. 1) V-ATPases, 2) proton leaks, 3) K^+ leaks, and 4) luminal buffering.

Mathematical Description

THE V-ATPASE PROTON PUMP ACIDIFIES ORGANELLES

The hydrolysis of ATP provides the energy for pumping protons against their concentration gradient. For our purposes, we require the average pumping rate of a single V-ATPase as a function of membrane potential and pH gradient, $J(\Delta\Psi, \Delta pH)$. This function is defined numerically in the file **VATPASE**; it has been computed from another more complicated model.

THE PASSIVE LEAK OF IONS DEPENDS UPON CONCENTRATION AND MEMBRANE POTENTIAL

Intact bilayers are somewhat permeable to protons, but impermeable to other ions. Ion-specific channels allow an organelle to equilibrate specific ions between the lumen and cytoplasm. Movement of these ions through the channel is driven by the transmembrane concentration difference and the membrane potential. The simplest model for the diffusion flux of ions in the presence of a membrane potential can be described by

$$\begin{aligned}
 J_{Hleak} &= P_H \cdot S \cdot \frac{U \cdot ([H^+]_L - [H^+]_C \cdot e^{-U})}{1 - e^{-U}} \\
 J_{Kleak} &= P_K \cdot S \cdot \frac{U \cdot ([K^+]_L - [K^+]_C \cdot e^{-U})}{1 - e^{-U}}
 \end{aligned}
 \tag{1}$$

where P is the permeability of the membrane to each ion, S is the surface area of the compartment, C refers to cytoplasmic concentrations, L refers to luminal concentrations, and U is the reduced membrane potential, $U = \Psi F / (RT)$. Note that U has no units; it is dimensionless. F is Faraday's constant, R is the gas constant, and T is absolute temperature. The value of $F / (RT)$ in milliVolts at room temperature is given in Table 2.

PROTONS BECOME BUFFERED AFTER THEY CROSS THE MEMBRANE

Cellular spaces are sponges for protons and other ions. Proteins and molecules are constantly binding and releasing ions from solution. The buffering capacity, β (units: [M/pH]), measures the ability of the luminal matrix to bind protons. When protons cross the lipid bilayer a certain fraction of them are immediately bound and do not contribute to the pH. The change in proton concentration of the lumen and the change in pH are given by:

$$(\text{Change in proton concentration}) = \Delta[H^+]_L = -\beta \cdot \Delta\text{pH}
 \tag{2}$$

In general, spaces have different buffering capacities at different pH values, but we will assume that the buffering power is a fixed constant. For measured values see Table 1.

ACCUMULATED CHARGE AS A MODEL FOR MEMBRANE POTENTIAL

The membrane potential affects the flow of ions across lipid membranes and biases the distributions of those ions at steady state. Electroneutrality requires every small volume to be electrically neutral. The membrane potential arises from the microscopic deviation from electroneutrality at a lipid boundary. We use an explicit form for the membrane potential *across the bilayer* in terms of the excess charge inside the organelle. This treatment is very similar to the treatment of the membrane potential in the axon models. We assume that the net charge localizes to the luminal leaflet, so that we can treat the membrane as a parallel plate capacitor. The potential drop across the bilayer is then written as:

$$\Delta\psi = \frac{F}{C_0} \cdot \frac{V}{A} \cdot \left(\underbrace{[K^+]_L}_1 + \underbrace{\beta \cdot (pH_C - pH_L)}_2 - \underbrace{B}_3 \right) \quad (3)$$

where A is the surface area of the membrane (called S everywhere else in the text), C_0 is the capacitance per unit area of the membrane ($C_0 \cdot A$ is the total capacitance of the membrane), V is the volume of the organelle, and the numbered terms giving the concentrations of charged particles are:

1. Total concentration of potassium ions.
2. Total amount of buffered and free protons in the lumen. β is the buffering capacity. We assume that protons do not contribute to the membrane potential when the luminal pH is equal to the cytoplasmic pH.
3. Molar concentration of all impermeant charges. This term primarily represents fixed negative protein charges trapped in the lumen and we call these Donnan particles.

Despite the complexity of this system, it is only a two-tank model. In the next section we will construct this model in Berkeley Madonna™.

Assembling the Model

Before we begin constructing the model, we must think carefully about the time-dependent variables and their relationship to other variables in the model. As with other exercises, we will keep track of the *number* of protons, NH, and the *number* of potassium ions, NK, that enter the organelle. So make these the tanks. The cytoplasmic values of these ions change very little during acidification; therefore, we will take these values as constants.

Pure Proton and Potassium Leak

Using the Flowchart, start by constructing tanks and flows corresponding to the number of protons, NH, and potassium ions, NK, in the lumen. Have only one flow *into* each tank and call the flows **H Flow** and **K Flow**. How can we use only 1 flow if the ions can move in and out of the organelle? Now fill in the passive flows as described by Equation 1. Fill in a few of the variables needed for the passive flow by using Function Balls. This will begin to assemble the corresponding differential equations in the Equations Window. Once you have the basic tanks and flows set up, even though

you do not have anything filled in, go to **Flowchart/Discard Flowchart** and get rid of the Flowchart. At this point, only include the passive leak for each time dependent variable (see Equation (1)). In general, reservoirs should represent numbers of things. Here we have chosen number of particles, but previously we have used moles. We will need to compute the proton and potassium ion concentrations, which are closely related to NH and NK. For now, we will let:

$$[K^+]_L = NK/Na/V$$

and

$$[H^+]_L = NH/Na/V; \text{pH}_L = -\text{LOG}_{10}([H^+]_L) = -\text{LOG}_{10}(NH/Na/V),$$

but these last two equations are only true in the absence of proton buffering. Na is Avogadro's Number.

The initial values for NK and NH must be set before the model can be solved. We will assume that the initial total number of protons in the lumen is given by the initial pH value of the lumen. This boils down to assuming that there are no buffered protons at the beginning of the simulation:

$$\text{INIT NH} = 10^{(-\text{pH}_L_INIT)} * Na * V$$

where pH_L_INIT is the variable for the initial luminal pH, which we call pH_{initial}. Since cellular spaces are largely electroneutral, we will set the initial number of potassium ions such that the total membrane potential starts at zero according to Eq. 3:

$$\text{INIT NK} = (B - \text{Beta} * (\text{pH}_C - \text{pH}_L)) * Na * V$$

B, Beta, Na, V, and pH_C (the pH of the cytoplasm) are all constants that you can define in the equations. To start, set B to 0.1 M and Beta equal to 0.01 M/pH.

HANDLING THE FLOWS. Next we will construct the leak flows for the protons and potassium. These equations require special care, as we now discuss. Often we encounter equations such as $y = x/x$ that we want to evaluate when the denominator is equal to zero. In this case, the answer is 1, but if we ask the computer to evaluate this it will return a divide by zero error. Computers do not take limits easily. Equation (1) exhibits this problem when the membrane potential is equal to zero. The easiest way to handle this problem is to rewrite the equation in an equivalent form at the troubled areas. For Equation 1, we use the form:

$$J_{H \text{ leak}} = \begin{cases} P_H \cdot S \cdot \frac{U \cdot ([H^+]_L - [H^+]_C \cdot e^{-U})}{1 - e^{-U}} & \text{for } -0.01 > U > 0.01 \\ P_H \cdot S \cdot \frac{([H^+]_L - [H^+]_C \cdot e^{-U})}{1 - \frac{U}{2} + \frac{U^2}{6}} & \text{for } -0.01 < U < 0.01 \end{cases} \quad (4)$$

Use the **IF** statement in Berkeley MadonnaTM to implement this form of the leak term for the protons and the potassium ions. The Madonna Code appears as follows:

```
J_H_Leak = IF abs(U) > 0.01
THEN
Permh*S*U*(H_L - H_C*exp(-U))/(1-EXP(-U))*Na/1000
ELSE
Permh*S*(H_L - H_C*exp(-U))/(1-U/2+U*U/6)*Na/1000,
```

where we have used `Permh` for the proton permeability for $J_{H \text{ leak}}$, `Na` for Avogadro's Number, and `H_L` and `H_C` for the luminal and cytoplasmic proton concentrations. Additionally, the model will keep track of the total *number* of particles, so the flows must be in ions per second. Analysis of the parameter values in Equations 1 or 5 reveals that the equation has dimensions of

$(\text{cm}^3/\text{sec}) \cdot (\text{moles/liter})$. Remembering that 1000 cm^3 is equal to 1 liter, this reduces to:

$\text{moles/sec}/1000$. Finally, since 1 mole is equal to `Na` (Avogadro's Number), *we realize that we need to multiply Eq. 1 by `Na/1000`* to be consistent with our Tanks representing the number of ions.

Hence, in the Madonna code above you will see this factor at the end of the equation for the flows.

Answer the following questions by using the numbers for the Golgi from Table 1. You will have to set Ψ , PSI , equal zero, and we will assume that the proton permeability is $P_H = 1.3 \times 10^{-8} \text{ cm/s}$. Next, you should implement a corresponding **IF/THEN** statement for the K^+ flow. Once you have added the flows to your Equations Window, make sure that the differential equations for NH and NK are as follows:

$$\frac{d(\text{NH})}{dt} = -J_{H \text{ Leak}} \quad \text{AND} \quad \frac{d(\text{NK})}{dt} = -J_{K \text{ Flow}}$$

Based on Equation 4, why do we need the negative sign in each equation?

Exercise 1. Start off with the luminal pH = 8.4, 5.4, and 4.4 and watch the pH change until it is the same as the cytoplasmic value. What is the time constant for the pH changes? How does this change if you look at proton concentration?

Exercise 2. Track the corresponding changes to the luminal K^+ concentration. What is the time constant for this movement? The ratio of time constants is related to the ratio of permeabilities. When the model reaches steady state, if the luminal and cytoplasmic quantities are not equal then the channels are not being treated properly.

Accounting for Proton Buffering

Use $\Delta pH = -1/\beta \cdot \Delta[H^+]$ to rewrite the equation for the luminal pH, pH_L . We rewrite Eq. 2 as follows:

$$pH_L = pH_{initial} - 1/\beta \cdot (NH/Na/V - 10^{(-pH_{initial})}), \quad (5)$$

where $pH_{initial}$ is the initial pH of the lumen at the beginning of the simulation. Notice that when there is buffering, $NH/Na/V$ is no longer the luminal proton concentration but rather the total number of protons in the lumen, bound plus free. We need to rewrite the luminal proton concentration as:

$$[H^+]_L = 10^{(-pH_L)}.$$

Once you have added buffering, carry out the following exercise.

Exercise 3. Compare the time constants for the luminal proton concentration when the buffering is 0.01, 0.02, and 0.04 M. Notice that the proton movement is extremely slow now. Additionally, the change in pH_L is proportional to the proton concentration not the pH. Therefore, the solution is not an exponential and the time constant depends upon the initial values. You should set the stop time to 10^8 s, and DTMAX to 10^6 .

Couple in the Membrane Potential

So far the leak terms should be well behaved. When we add in the membrane potential, which couples the ion flows, we might find that our equations are not quite right. Expect problems here.

Represent the membrane potential as in Equation (3). Let the Donnan particle concentration, $B = 0.1$ M. Explore the model and once you are convinced that it is giving reasonable results continue.

Add in Proton Pumping

Select Data Sets and import the function VATPASE as a 2-D matrix. We can set the pump rate equal to this function by defining it like this:

$$J_{pump} = N_pump * \#VATPASE(psi, pH_L) \quad (6)$$

This function returns the number of protons per second per pump. What factors must this function be multiplied by so that it can be used in the differential equation for NH? Anything? You must define a new parameter, N_pump = number of pumps on the organelle membrane. Once the entire model is complete move on to the case studies.

NOTE: Predefined numerical functions are only defined over a certain domain. If during a simulation your variables extend beyond this domain the predefined function will not give the correct results.

VATPASE is defined for $4.0 < pH_L < 7.6$ and $-80 \text{ mV} < \Psi < 260 \text{ mV}$.

Case 1. Endosomal Acidification

Endosomes have been extracted from cells and are bathing in a 7.4 pH solution with 100 mM K^+ .

The V-ATPase proton pumps are not working because the solution lacks ATP. The pH of the endosome is measured using pH sensitive dyes. At 0 seconds, ATP is reintroduced to the bathing solution and the endosomes begins to acidify from pH 7.47. Load the file **MVB_74** into your model (this is the acidification data).

Exercise 4. Using the endosome (MVB) parameters from Table 1 determine the number of V-ATPase pumps and the proton permeability by fitting the model pH to the experimental data. Start with the $pH_{initial}$ set to 7.47. Using the fact that the endosomal pH = 7.4 in the absence of any proton pumping, determine the concentration of Donnan particles (B).

Case 2. Membrane Leakiness

We want to determine the proton permeability of the Golgi and secretory granule. We have provided you with two data sets each containing five experiments:

Data Set #1 - [SG_1; SG_2; SG_3; SG_4; SG_5]

Data Set #2 - [Golgi_1; Golgi_2; Golgi_3; Golgi_4; Golgi_5]

Load this data into your program. In each of these experiments, intact cells have been loaded with pH fluorescent dyes that localize to specific organelles in the cell. The pH of the organelle can then be measured by recording the light emitted from the cell. At time zero, the cell was washed with a drug, bafilomycin, that inhibits the proton pump so that the organelles can no longer maintain their acidity, and they begin to alkalinize. This can be seen in the data sets.

Exercise 5. For each experimental curve, begin with the initial pH near the pH of the first data point. Fit the model to the data using the curve fit procedure. Allow the program to adjust the proton permeability and the Donnan particle concentration, B . Record the best-fit proton permeability. Remember that the proton pumps have been “turned off” experimentally. This means that $N_{\text{pump}} = 0$ in your model. Repeat this for all the data sets and compute the mean and standard deviation for each organelle. Do these experiments show a noticeable difference in the bilayer leakiness between the Golgi and secretory granule?

NOTE: Remember to use the correct parameters from Table 1 when analyzing different organelles.

References

Grabe, M. and G. Oster (2001). Regulation of organelle acidity. *J. Gen. Physiol.* **117**:329-344.

Rybak S, F. Lanni, and R. Murphy (1997). Theoretical Considerations on the Role of Membrane Potential in the Regulation of Endosomal pH. *Biophys. J.* **73**:674-687.

Tables

Parameter	Golgi	Secretory Granule	Endocytic vesicle
Surface Area [cm ²]	5.14×10^{-6}	1.26×10^{-9}	8.35×10^{-10}
Volume [L]	2.6×10^{-14}	4.2×10^{-18}	2.27×10^{-18}
Potassium permeability [cm/s]	1×10^{-5}	1×10^{-5}	1×10^{-5}
Buffering capacity [M/pH]	0.01	0.02	0.04

Table 1. Typical values for Golgi, secretory granules, and endosomes.

Parameter	Value
Cytoplasmic pH	7.4
Cytoplasmic potassium [M]	0.100
Membrane capacitance [kF/cm ²]	1×10^{-9}
Faraday's Constant [moles/Coulomb]	96,480
Avogadro's Number [molecules/mole]	6.02×10^{23}
RT/F [mV]	25.69

Table 2. Constants and typical variables.