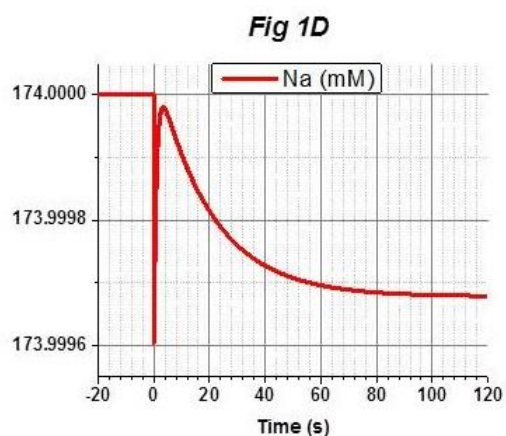
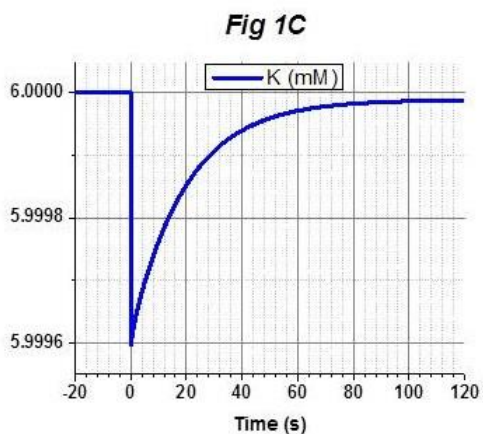
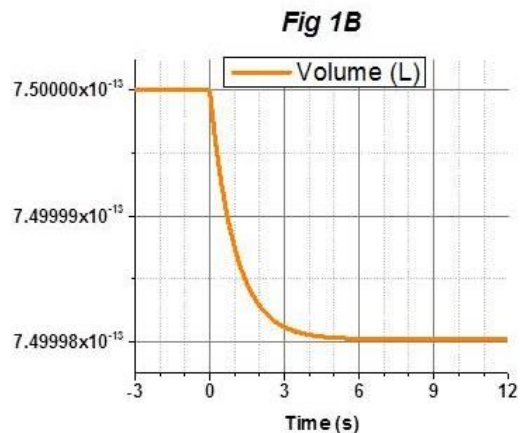
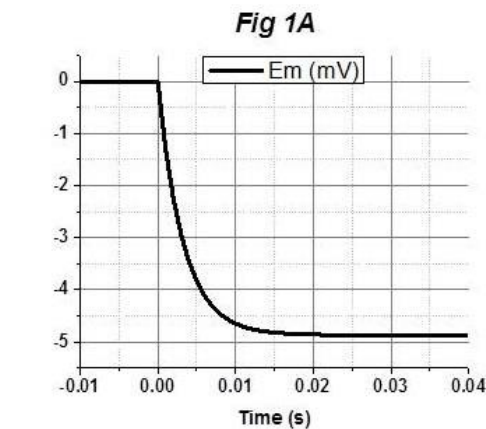


## Cell GUI V3.09 Tutorial, Part 2: Examples

Part 2 of the Tutorial presents practical references on ways to run the Cell GUI program. Reading “Cell GUI V3.09 Tutorial, Part 1: Fields and buttons” before this part is not required, but recommended. The following examples are mostly simulations that were performed during data collection for our paper “The logic of ionic homeostasis: cations are for voltage, but not for volume.” **Example 1** is about simple calculations that can be done with a common calculator, but it serves an important introductory function by presenting basic concepts, demonstrating how to control the flow of calculations, and explaining the structure of output files. We will continue to investigate the case of a sudden change in osmolarity in **example 2** focusing on varying water permeability (the calculations of dynamics that only our program is capable of). **Example 3** explains the importance of proper use of time increments in respect to our, not so popular, method of calculation of membrane potential ( $E_m$ ). **Example 4** will demonstrate how the interface can be used to gather information related to the  $\text{Na}^+/\text{K}^+$  pump (such as expenditure of ATP and contribution of the electrogenic pump to  $E_m$ ) even before starting calculations. **Example 5** will show how to use the program productively to create a graph of relationships between parameters. **Example 6** will give recommendations on simulating “real time” changes of concentrations, voltage, and volume induced by temporal changes in cell parameters.

**Example 1: Finding an equilibrium state in an asymmetrical osmolarity-charge setting.** There are two aims here: 1) to find equilibrium intracellular ionic concentrations and  $E_m$  under certain conditions, which we call osmolarity-charge asymmetry, and 2) to determine dynamics of changes associated with equilibration of osmolarity. Uneven transmembrane distribution of electrically neutral osmolytes and deviation of the mean valence of internal charged osmolyte from -1 (which are both common in real cells) create the osmolarity-charge asymmetry, where the quantity of elementary electrical charges is not equal to the quantity of osmotically active molecules. Assume for this example that the external neutral osmolyte concentration = 6 mM and the mean valence of the internal charged osmolyte = -1.5. Start the Cell GUI program and enter the above mentioned values of external “Neutral Osmolyte” and “Mean Valence” into their respective fields (Note: the program interprets mean valence as an average number of negative charges per molecule; so a positive value should be entered in the field, 1.5 in this case). Change the concentration of internal “Charged Osmolyte” to 90 mM in order to keep  $[\text{Cl}^-]_i = 15$  mM, i.e. to preserve the contribution of  $\text{Cl}^-$  to the total intracellular negative charge (10%). Press the “Save as Defaults” button; that allows us to return to this setting when needed. Press the “Adjust Conc” button, which activates the function that adjusts intracellular concentrations to external osmolarity, and then “OK” in the “Adjustment applied” dialog box that appears. Press the “Adjust Conc” button again and “OK” in the self-explanatory “No adjustment necessary” dialog box that appears. Note the changes in all intracellular concentrations; the ratio between concentrations remains the same as before the adjustment, but internal osmolarity now is equal to external (306 mOsm). The system is osmotically balanced, but not in equilibrium, since there are gradients of concentrations of both cations while  $E_m = 0$ . Make sure that “Detailed Log” radio button is selected and with the default setting of conductances (where the membrane is permeable only to cations) as

well as other default parameters press the “Calculate” button. After a few seconds that are necessary for the program to perform the calculation, press “OK” in the “Calculation complete” dialog box that appears. The values in interface fields were updated:  $E_m$  is -4.8681 mV and tiny changes are shown for concentrations and cell volume. These are the current values of the parameters after one set of calculations, which includes 180,000 iterations of 0.001 sec each and accordingly covers 180 sec, as indicated in the “Number of Iterations”, “Time Step”, and “Duration of Calculation” fields, respectively. But do these concentrations and voltage represent the final equilibrium we are searching for? This can be determined from the text file that was generated during calculation. The file (default name is “exp001.txt”, saved in the same directory where the Cell GUI program resides) contains intracellular concentrations,  $E_m$ , and the cell volume as functions of time. It is a fairly large file since the results of every time step are stored in it (180,000 lines plus 1 for initial conditions). The file can be loaded into a spreadsheet program, such as Microsoft Excel or Origin by OriginLab, for quick visualization of the dynamics. As we can see in **Fig 1A**,  $E_m$  decreased exponentially with a time constant of about 3.75 ms (as expected, since  $R_{input} = 312.5 \text{ M}\Omega$  and capacitance =  $1.2 \cdot 10^{-11} \text{ F}$ ; see respective interface fields), and apparently stabilized after about 25 ms. Very small changes of the cell volume appear to be completed after 7-8 sec (**Fig 1B**), but  $[K^+]_i$  and  $[Na^+]_i$  seem to have needed at least 100 sec to stabilize (**Figs 1C and D**).



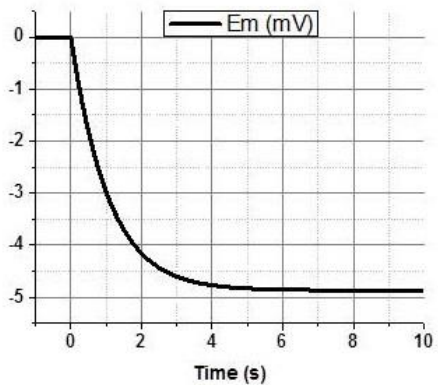
More accurate information can be obtained from the data itself, although it depends on the level of displayed accuracy. For instance, with the standard precision that Excel displays (7 digits),  $[K^+]_i$  reached its maximum value (5.999989 mM) after 134 seconds. All other parameters stabilized earlier. However, if you open the original exp001.txt file with Notepad, which shows the data as generated (up to 17 digits),  $[K^+]_i$ ,  $[Na^+]_i$ , and  $E_m$  are still experiencing some slight changes even at the end of 180 sec. The calculations can be continued for another set of 180 seconds and then for more sets by pressing the "Calculate" button again and again, with each new calculation using the results of the previous one as its initial conditions. (Each set will overwrite exp001.txt with new results unless the file name is updated before each calculation.) But it is recommended instead to switch to "General Log" by choosing the appropriate radio button. Note that "Duration of Calculation" is now 300 minutes because in "General Log" 100 consecutive sets of calculations (each of which in this case lasts 3 minutes) will be performed. A much more compact file will be generated since in "General Log" only the final results of each set of calculation are stored (100 lines plus 1 for initial conditions). Note also that "s" was replaced by "min" in the "Duration of calculation" informational field, and time in the output file will be in minutes too. "Duration of calculation" can be reduced to 20 minutes by decreasing "Number of Iterations" to 12,000 (with the duration of each set of calculations = 12 s, which can be checked by a temporary return to "Detailed Log"). Now with "General Log" selected press the "Calculate" button. The generated file (note that the new file name is still "exp001.txt" since it was not changed and the program overwrites files if you don't change the name) shows that all intracellular values are the same starting from 5 minutes, even with precision of 17 digits. Thus, the concentrations and  $E_m$  obtained after the last calculations are final and represent equilibrium.

Now return to the beginning of this example by pressing the "Restore to Default" button. We have used the "Adjust Conc" function to equilibrate osmolarity before calculations to avoid large initial transmembrane fluxes that could potentially disrupt the flow of calculations. But in fact, the imbalance in the initial state of this sample is not too large, and the program can handle it without "Adjust Conc". Now repeat calculations starting in osmotically uneven conditions. The focus here is still on the values of the parameters in the stable resting state, so it is preferable to use "General Log" because it covers a longer period of time without generating large files. So, choose "General Log", keeping "Number of Iterations" and "Time Step" the same, change the name of the file in the bottom of the interface to "exp002.txt" and press the "Calculate" button. The new values in the interface are practically the same as after our previous simulation, with the noticeable exception for "Volume", which is  $\sim 6.25 \cdot 10^{-13}$  L instead of  $\sim 7.5 \cdot 10^{-13}$  L. This is understandable because in this case we let virtual water flow out of the cell to equilibrate osmolarity. It is also quantitatively predictable because initially internal osmolarity was 1.2 times lower than external. But equilibrium concentrations and  $E_m$  must be independent from the volume. Indeed, the resting state data (except for volume) in "exp001.txt" and "exp002.txt" files are the same with precision of at least 6 digits. Yet the data are not exactly the same and this is normal for these types of calculations. Return to the beginning by pressing the "Restore to Default" button, name the file "exp003.txt" and change "Volume  $\tau$ " to 10. Then calculate in "General Log", keeping timing unchanged. Repeat the same with file name "exp004.txt" and "Volume  $\tau$ " = 0. Compare the final

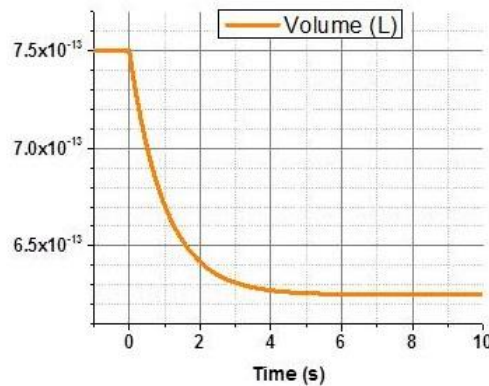
concentrations and  $E_m$  in all four files – they are always *practically* the same, but not necessarily *exactly* the same.

**Example 2: Role of water permeability.** One useful feature of the Cell GIU program is its capability to perform calculations with different water permeability. It is convenient to express water permeability in terms of the time constant of cell volume changes in response to a sudden osmotic change, just like in the current example. In the previous two calculations we have redefined its value in the “Volume  $\tau$ ” field of the interface. Detailed explanations of relations between water permeability and the time constant of cell volume changes are in the Methods section of our paper “The logic of ionic homeostasis: cations are for voltage, but not for volume.” In short, “Volume  $\tau$ ” is inversely proportional to water permeability; the shorter the former, the higher the later. As we have seen (comparing exp002.txt, exp003.txt, and exp004.txt) water permeability is irrelevant to the final concentrations and  $E_m$ , but it does influence the dynamics of changes. Return to the beginning by pressing the “Restore to Default” button and switch to “Detailed Log” which is better suited for analyzing dynamics, because in this mode the program generates files with high time resolution. Keep the same “Number of Iterations” (12,000); in the two cases of high water permeability (with “Volume  $\tau$ ” = 1 s and = 0) that

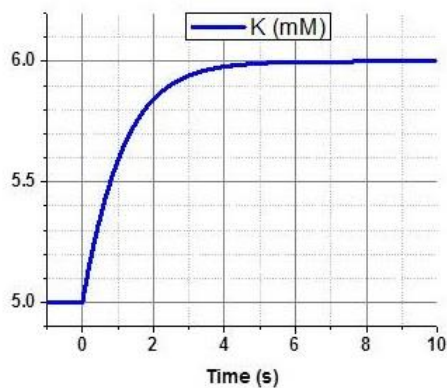
**Fig 2A**



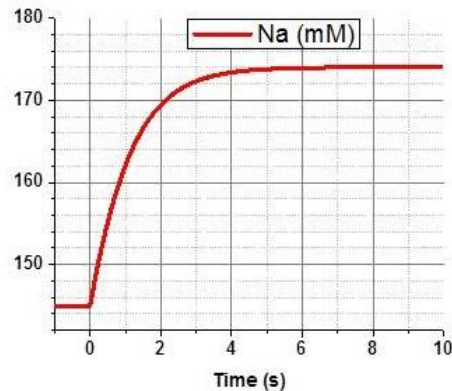
**Fig 2B**



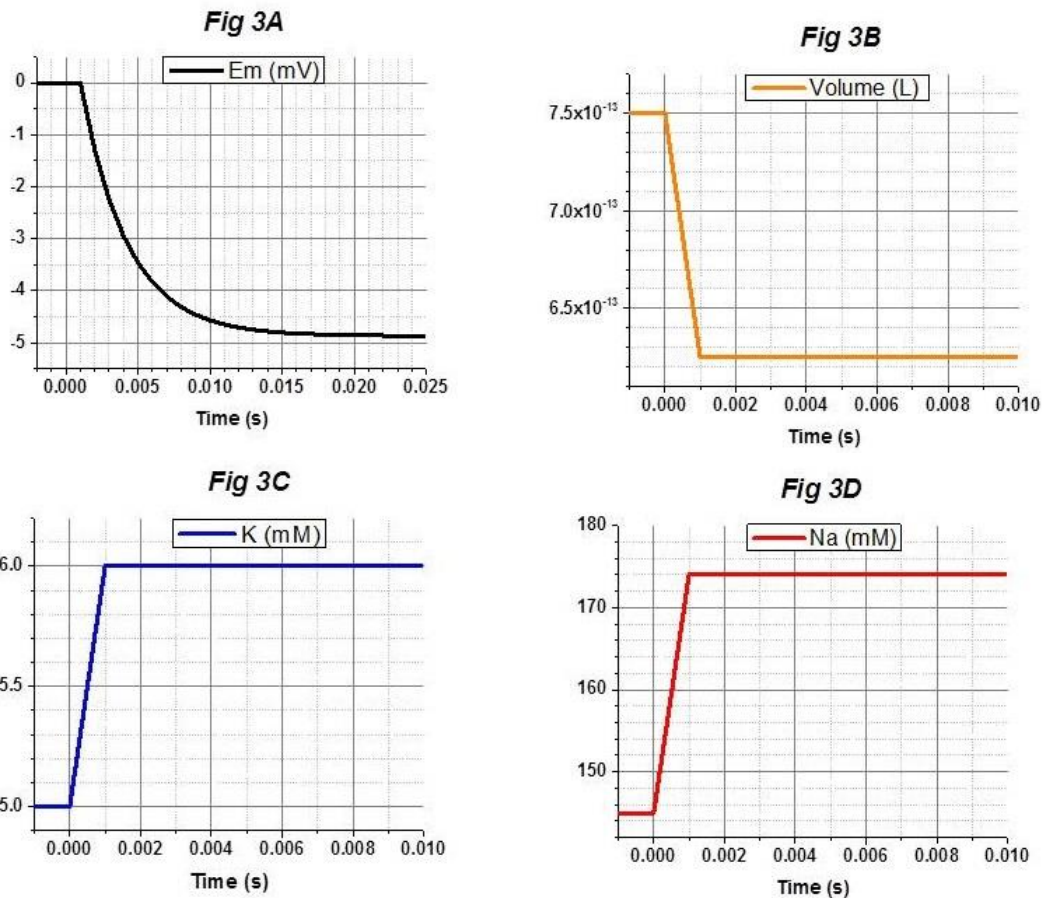
**Fig 2C**



**Fig 2D**

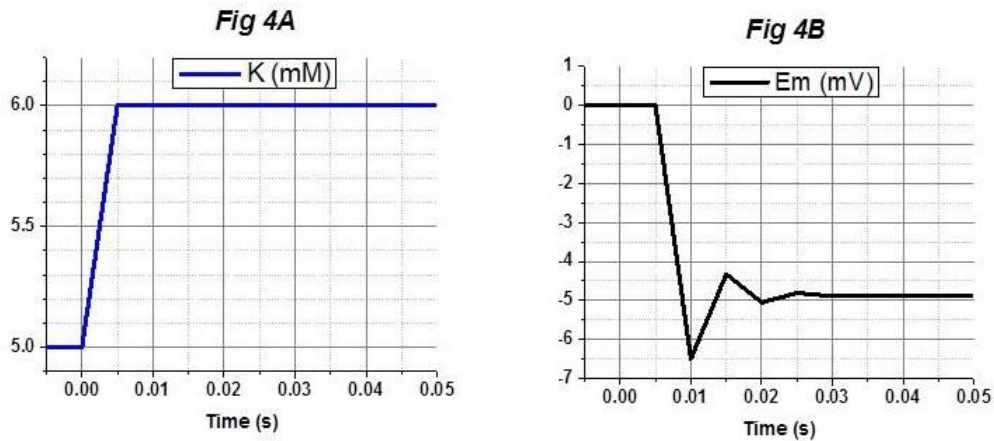


we are going to test, more than 99.9% of changes will be done by 12 sec (= 0.2 min) of the calculation, as “exp002.txt” and “exp004.txt” files show. Change “Volume  $\tau$ ” to 1 s, rename output file to “exp005.txt”, and press the “Calculate” button. Figures above show calculated changes of  $E_m$  (**Fig 2A**), cell volume (**Fig 2B**),  $[K^+]_i$  (**Fig 2C**), and  $[Na^+]_i$  (**Fig 2D**). The volume changed exponentially with a time constant of 1 sec as was defined by setting of “Volume  $\tau$ ”, the concentrations followed the volume, and the voltage followed the concentrations. If we had chosen “Volume  $\tau$ ” = 10 s, changes of the cell volume, concentrations and voltage would be 10 times slower. Now “Restore to Default”, set “Volume  $\tau$ ” = 0, file name to “exp006.txt”, and press the “Calculate” button. In this simulation the time constant of volume changes = 0, i.e. water permeability is infinitely high, as is usually accepted in models concerning volume changes. In this case, only the duration of the time step determines the time necessary for volume and concentrations to equilibrate osmolarity. Accordingly, after 1 ms all “osmotically extra” water has been removed from the cell, the cell volume decreased, and all intracellular concentrations increased (see **Figs 3B, C, and D**). Changed  $[K^+]_i$  and  $[Na^+]_i$  initiate changes of the voltage, but recharging the membrane capacitance required some time determined by the membrane RC. Thus, the changes of  $E_m$  in **Fig 3A** are the same as in **Fig 1A**; they only started 1 ms later. As we can see, limiting water permeability (like we did in previous calculations) makes



simulations more realistic, and although it does not change the values of the final concentrations and the voltage in equilibrium state, it is clearly preferable in simulations concerning dynamics.

**Example 3: Discretization and  $E_m$  calculation.** It is critically important for a smooth flow of calculations to select an appropriate level of discretization, which is defined in the “Time Step” field of the interface. Press “Restore to Default”, set “Time Step” = 0.005 s, “Number of Iterations” = 2,400, file name “exp007.txt”, and press “Calculate”. The newly generated file has 5 times fewer lines than “exp006.txt” but covers the same time. The calculated changes of  $[K^+]_i$  are presented in **Fig 4A**, which looks like **Fig 3C** (the similar calculations with “Time Step” = 0.001 s), but on an expanded time scale. However, the changes of  $E_m$  (**Fig 4B**) are very different from  $E_m$  in **Fig 3A**. In both cases the first time step was spent on volume and concentration changes with  $E_m$  remaining the same. At the beginning of the second time step, uneven cation distribution evoked effluxes of  $K^+$  and  $Na^+$  that hyperpolarized the membrane by 1.3 mV when “Time Step” = 0.001 s (**Fig 3A**), and by 6.5 mV when “Time Step” was 5 times longer (**Fig 4B**).



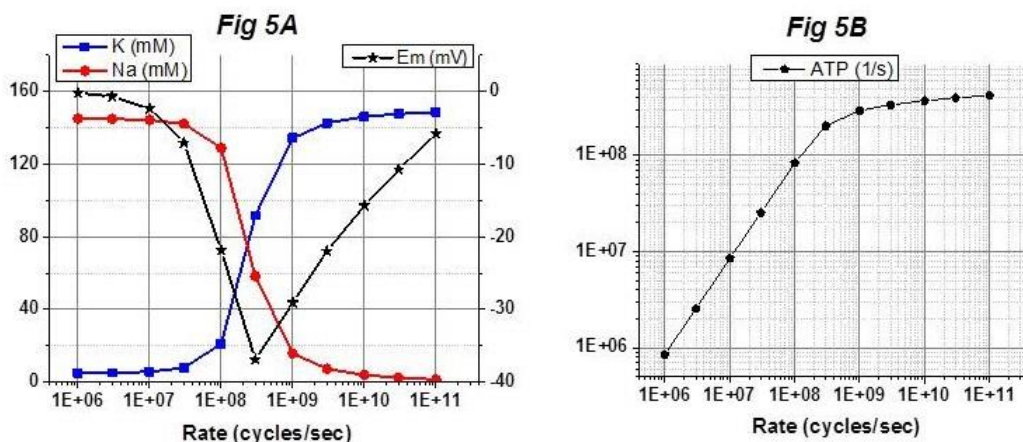
The distinct feature of our program is that  $E_m$  is calculated not from the concentrations of ions and their conductances, but from the transferred electrical charge and the membrane capacitance (the so called “charge-difference” method; for details see our paper “The logic of ionic homeostasis: cations are for voltage, but not for volume”). So, the concentrations and voltage are not linked directly. That creates the possibility that a large transmembrane transfer of charge resulting from too long of a time step could produce an “overshooting” change of  $E_m$ , as we see in **Fig 4B** ( $E_m = -6.5$  mV at 0.01 sec, which is more negative than the  $K^+$  equilibrium potential (-4.87 mV with  $[K^+]_i = 6$  mM and  $[K^+]_o = 5$  mM at 36.7 C°). Accordingly,  $K^+$  (as well as  $Na^+$ ) will enter the cell during next time step, and then the direction of cation fluxes will change that way in each time step until reaching the equilibrium. This case gives us a good demonstration of how the program works, but it is clear that shorter time steps should be used for realistic dynamics. Actually, a limited water permeability (even with a time constant as short as 1 sec) can smooth the flow of calculations, slowing down the volume and consequently concentration and fluxes changes. If the previous calculation was repeated with “Volume  $\tau$ ” = 1 s, the results would be the same as in Fig 2, only in a file that is five times smaller. But increasing “Time Step”

to 0.01 s disrupts the calculations. The large charges of  $E_m$  grow larger with each successive time step, inducing increasingly larger fluctuations in concentration until  $[K^+]_i$  drops to an impossible negative value. As a result, all fields for intracellular concentrations, voltage and volume show “NaN” as an indication of the error. Slowing down volume changes by increasing the time constant does not help until “Volume  $\tau$ ” =  $1e8$  (i.e.  $10^8$  seconds or more than 3 years). Such a long time constant is declared in this program as no water permeability at all. In this case osmotic imbalance does not initiate volume changes and concentrations and voltage remain unchanged.

**Example 4: Using the interface to extract the  $Na^+/K^+$  pump related information.** Start the Cell GUI program, set the “Rate of Na/K-ATPase” =  $1e8$  (i.e.  $10^8$ ) and examine changes in some informational fields. First notice, that the “Spent ATP” field shows a number which is a bit smaller than “Rate of Na/K-ATPase” (about 85% of it). The “Spent ATP” field (besides its obvious energy meaning) shows how many cycles all  $Na^+/K^+$ -ATPase molecules of the modeled cell make each second, replacing internal  $Na^+$  with  $K^+$ , and this is what we call “activity” of the pump. It is directly proportional to “Rate of Na/K-ATPase” (change “Rate ...” to  $1e9$  and notice that “Spent ATP” also increased 10 times) and depends on  $[Na^+]_i$  in a more complex way. Detailed explanations of the  $Na^+$ -dependent relationship between the “Rate ...” and the “Spent ATP” are in the Methods section of our paper “The logic of ionic homeostasis: cations are for voltage, but not for volume”. In short, “Spent ATP” approaches the “Rate ...” when  $[Na^+]_i$  is very high (for illustration enter some impossibly huge number in the internal  $Na^+$  concentration field, such as  $1e15$ ), is a smaller fraction of the “Rate ...” when  $[Na^+]_i$  is decreasing, and is equal to 1/8 of the “Rate...” when  $[Na^+]_i = 8$  mM (enter 8 in the internal  $Na^+$  concentration field to check it).

Return to default internal  $Na^+$  concentration (145 mM) and notice that with “Rate ...” =  $1e9$  the pump generates about -42.6 mV of voltage (the “Voltage by the Pump” field). This is a result of the unequal cation transfer (2  $K^+$  in for 3  $Na^+$  out; see appropriate “Number per Cycle” fields) by the pump, which produces hyperpolarizing current. “Voltage by the Pump” depends on the stoichiometry of the pump (enter 4 in “Number per Cycle /  $K^+$ ” field and the voltage will be positive), the pump activity (change “Rate ...” to  $1e8$  and the voltage also decreases 10 times), and the cell input resistance (increase R input two times, i.e. to 625 M $\Omega$ , by redefining  $Na^+$  conductance = 0 and the voltage doubles). To be correct, only resistance to cations, not the total “R Input”, is relevant in determination of “Voltage by the Pump”. Change “Conductances /  $Cl^-$ ” to  $1e10$  and see that the pump voltage remains the same in spite of a decrease of “R Input”. Change “Conductances /  $Na^+$ ” to  $1e10$ , and “Voltage by the Pump” is halved, while “R input” is reduced to 2/3 of its previous value. Notice also that during all these manipulations  $E_m$  remains at 0. The “Voltage by the Pump” field only shows how much the pump can contribute to the membrane potential under current conditions. Do not be surprised by the excessively large pump voltage in some unrealistic settings. For instance, the voltage generated by a  $3Na^+/2K^+$  pump is expected to be -4256 mV with “Rate of Na/K-ATPase” =  $1e11$  cycles/s and  $[Na^+]_i = 145$  mM, but, as we will see, once calculations start, such a powerful cation transfer quickly decreases  $[Na^+]_i$ , which in turn suppresses the pump activity and reduces the pump voltage to a modest -21 mV.

**Example 5: Creating a graph illustrating dependence of  $[Na^+]_i$ ,  $[K^+]_i$  and  $E_m$  on activity of the  $Na^+/K^+$ -ATPase.** The aim here is to find resting values (i.e. those that remain unchanged with time) of cation concentrations and voltage at various activities of the  $Na^+/K^+$ -pump. Similar to the previously analyzed Example 1, the cell membrane is assumed to be permeable only to cations, but the setting will be osmolarity-charge symmetrical (i.e. the quantity of elementary electrical charges will be equal to the quantity of osmotically active molecules). Re-start the Cell GUI program to clean up all changes that have been done while becoming familiarized with the informational fields. Now we will enter increasing values of “Rate of Na/K-ATPase” and collect resting values of ionic concentrations, voltage and volume. Switch to “General Log” and set “Number of Iterations” to 36,000; one hour should be enough to find the resting state. Enter a small (relative to conductances) “Rate of Na/K-ATPase” of  $1e6$  and press “Calculate”. As the interface fields indicate, the changes were subtle with this pump activity:  $[K^+]_i$  increased a bit,  $[Na^+]_i$  decreased a bit, and the cell hyperpolarized by about  $-0.24$  mV. Open the just created file “exp001.txt” with Notepad, make sure that a stable set of parameters was attained (in this calculation all parameters were stabilized after 6 minutes), copy the last line and paste it into an open worksheet of Excel, Origin or other similar program. In the case of Excel copy it in the 2<sup>nd</sup> row, as the 1<sup>st</sup> row should be reserved for the title line copied from “exp001.txt”. Replace “T (min)” in cell A1 of the worksheet with “Rate”, and “60” in A2 with  $1e6$ ; column “A” will store the values of “Rate of Na/K-ATPase” associated with obtained results. Now enter  $3e6$  in the “Rate ...” field of the Cell GUI program and press “Calculate”. Again open “exp001.txt” file (it must be closed in notepad before calculating, otherwise it will display results of previous calculations), copy the last line and paste it in the next line in the worksheet, replacing A3 with  $3e6$ . Repeat the same for “Rate ...”  $1e7$ ,  $3e7$ ,  $1e8$ ,  $3e8$ ,  $1e9$ ,  $3e9$ ,  $1e10$ ,  $3e10$  and  $1e11$ . The obtained results concerning  $[K^+]_i$ ,  $[Na^+]_i$ , and  $E_m$  are presented in **Fig 5A** (scale on the left for the concentrations, scale on the right for the voltage; the rate scale is logarithmic). Each point is the value of one of the parameters at the end of the calculation for one pump Rate, when the cell has reached a steady state.

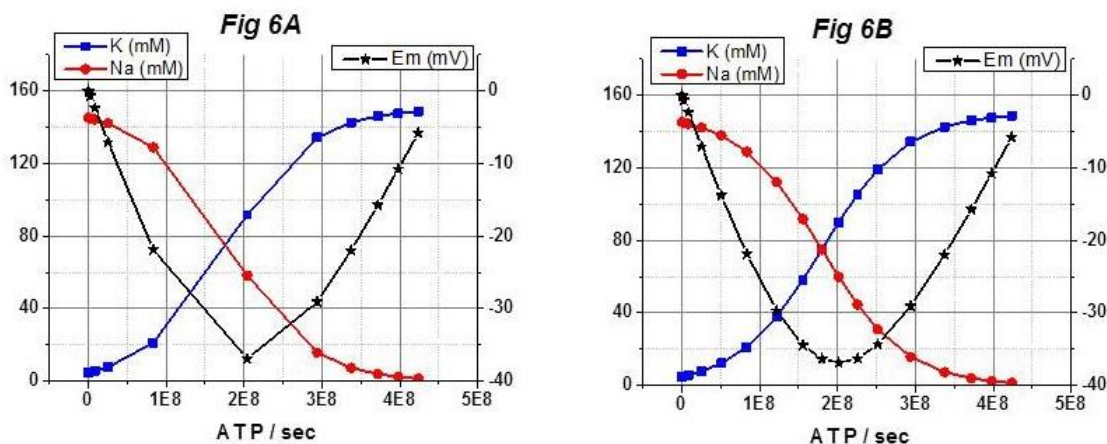


A few remarks about the output file. The Cell GUI program allows the user to choose the name of the output file. For instance, the output files in this sample could be named “PumpRate1e6”, “PumpRate3e6” and so on. The name also can hold information about other parameters associated



with certain calculations. There is a space for about 18 characters in the “Output File Name” field of the interface, but longer names are also permitted, as well as any extension, regardless whether it meaningful for a computer (.txt, .dat, .doc) or meaningless (.abc, .x123, .nnnnn). However, it should be remembered that the output file generated by the program is a simple ASCII text file and extension “.xlsx” will only confuse Excel. The output file name may even have no extension, but it is still recommended to use standard “.txt” which allows opening the file in Notepad simply by double-clicking on it. In fact, we would suggest not to change the output file name at all (and the name always returns to “exp001.txt” when “Restore to Defaults” is pressed); just transfer the obtained data into an appropriate worksheet. In the worksheet project the user can store all relevant information including that which the output file does not contain, like conductances of ions, volume time constant and concentration of external neutral osmolyte.

The **Fig 5A** illustrates the dependence of  $[Na^+]_i$ ,  $[K^+]_i$  and  $E_m$  on rate of the  $Na^+/K^+$ -ATPase, but our aim is to find dependence of the concentrations and voltage on activity of the pump. The rate is a theoretical abstraction that is convenient as an input to the program, since it stays constant during calculation. But it is *activity* which is important, because it is directly related to the quantity of transferred ions and shows the energy cost of the resting state that is reached. The activity does change when  $[Na^+]_i$  is changing and the relation between the rate and the activity (expressed in ATP/s) is presented in **Fig 5B**. The data for this graph were taken from the just created worksheet where the last column contains the values of ATP spent per second. With a relatively low pump rate (from  $1e6$  to  $1e8$ )  $[Na^+]_i$  remains high, and the activity of the pump is mostly proportional to the rate (85% to 83.5 % of rate). But when  $[Na^+]_i$  is significantly decreased by stronger active transport, activity is a much smaller fraction of the rate (less than 0.5% at rate  $1e11$ ). Now copy all the data from the current worksheet into another worksheet and replace the first column (Rate) with the last (ATP (1/s)). Plotting cation concentrations (scale on the left) and voltage (scale on the right) against the pump activity



(ATP/s) produces the graph presented on **Fig 6A**. Obviously the middle part of the graph is lacking data points, so add calculations for the “Rate of Na/K-ATPase” of  $6e7$ ,  $1.5e8$ ,  $2e8$ ,  $3.7e8$ , and  $5e8$  (the pump activity will be about  $5.07e7$ ,  $1.22e8$ ,  $1.56e8$ ,  $2.26e8$ , and  $2.51e8$ , respectively) and insert the data in the worksheet.

These additional pump rates were selected without much consideration, but two more points need more attention. One is the activity at which  $[K^+]_i = [Na^+]_i$ , i.e. the point where the graph for  $K^+$  crosses the graph for  $Na^+$ . This point should be somewhere between an activity of  $1.5e8$  and  $2e8$  ATP/s and, correspondingly, between a rate of  $2e8$  and  $3e8$  cycles/s. Probing different rates in this region and checking results in the interface fields for cation concentrations, it is found that with the rate of  $2.43719E+08$  cycles/s (and activity of  $\sim 1.80e8$ )  $[K^+]_i$  is practically equal to  $[Na^+]_i$  ( $74.9985$  and  $74.9884$  mM, as indicated in the respective interface fields). Insert the data corresponding to this rate into a row in the worksheet.

Another important point is the activity of the pump that produces the most negative  $E_m$ . To find this point another series of calculations is required. Currently the most negative  $E_m$  ( $-36.8734$  mV) is associated with the pump rate  $3e8$  cycles/s (activity  $\sim 2.04e8$ ), but a slightly smaller rate of  $2.9e8$  (activity  $\sim 2.00e8$ ) produces some more hyperpolarization ( $E_m = -36.8787$  mV, as is in the corresponding interface field). Decreasing the rate to  $2.8e8$  (activity  $\sim 1.96e8$ ) diminishes the hyperpolarization ( $E_m = -36.8493$  mV) making it less than at the pump rate of  $3e8$ ; thus the maximum hyperpolarization should be associated with a rate between  $2.9e8$  and  $3e8$  cycles/s. After some more probing by trial and error to identify the exact value, the maximum hyperpolarization ( $E_m = -36.8805$  mV) is found at pump rates from  $2.928e8$  to  $2.938e8$ . The rate of  $2.933e8$ , which is in the middle of the range, (activity  $\sim 2.01e8$ ) can be determined as a point of maximum hyperpolarization. Use the data associated with this rate/activity to replace the worksheet data for rate  $3e8$  (activity  $\sim 2.04e8$ ) to complete the graph presented in **Fig 6B**.

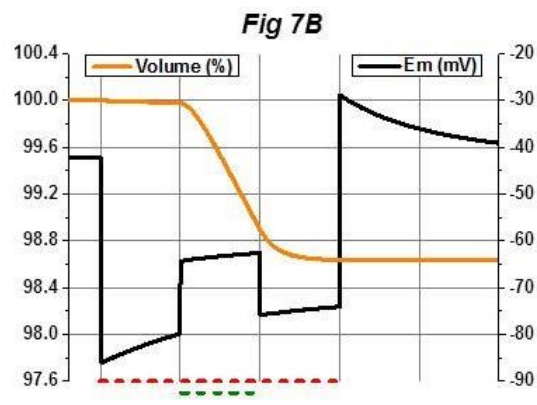
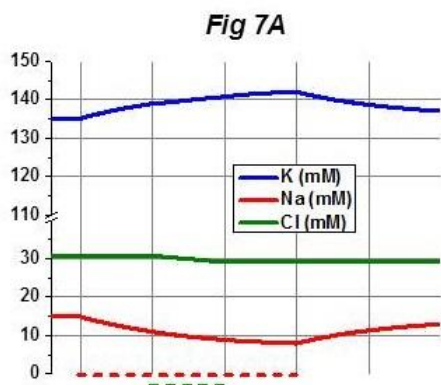
In this example we discuss only cations and voltage because  $Na^+/K^+$ -ATPase directly impacts only cations and voltage. As obtained data show (see "Vol(L)" column of the worksheet), hyperpolarization leads to a tiny decrease of the cell volume due to a microscopic deficiency of cations, which in turn results in tiny increases in concentrations of anions (see "Cl (mM)" and "A (mM)" columns). But the changes in volume and anion concentrations are so small (about 0.002 % of initial values) that it is only worth noting because it demonstrates the ability of the Cell GUI program to generate high resolution of the data.

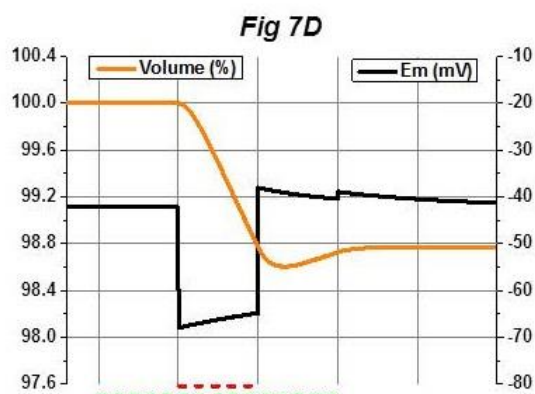
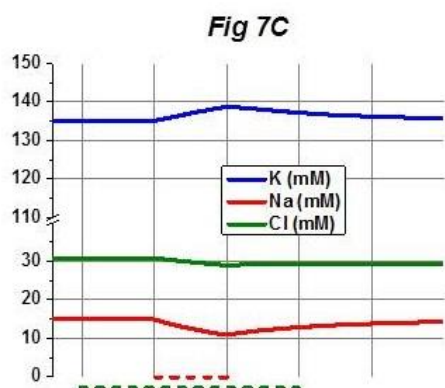
**Example 6: Changes of  $[Na^+]_i$ ,  $[K^+]_i$ ,  $[Cl^-]_i$ ,  $E_m$ , and cell volume evoked by changes in ionic conductances.** In this example a resting state supported by  $Na/K$ -ATPase will be disturbed by sudden and continuous changes of conductances of  $Na^+$  and  $Cl^-$  ( $g_{Na}$  and  $g_{Cl}$ , respectively). Two protocols of conductance changes will be used: 1) a decrease of  $g_{Na}$  for 15 sec and in the middle of this period an increase of  $g_{Cl}$  for 5 sec, and 2) an increase of  $g_{Cl}$  for 15 sec and in the middle of this period a decrease of  $g_{Na}$  for 5 sec. The modelled cell will bear a resemblance to the vertebrate photoreceptor, which experiences a decrease of  $g_{Na}$  under illumination; it will also (like a photoreceptor) have a relatively low membrane potential due to low  $g_K/g_{Na}$ .

Start the Cell GUI V3.09 program and set  $K^+$  conductance to  $1.2e10$  and  $Na^+$  conductance  $8e9$ , so that  $g_K/g_{Na} = 3/2$  and the input resistance remains  $312.5$  M $\Omega$ . Enter  $1e9$  into the "Rate of  $Na/K$ -ATPase" field and select "General Log" to find the well established resting state. Keep all other settings unchanged and press "Calculate". Now activate  $g_{Cl}$  and set it to a high value ( $1e11$ ) to accelerate

reaching the new resting state. Press “Calculate” again and inspect the interface.  $E_m$  did not change and cation concentrations practically did not change, but  $Cl^-$  concentration increased to equilibrate the  $Cl^-$  Nernstian potential with the membrane potential. Accordingly, the concentration of internal impermeant anion (“Charged Osmolyte” in the interface and “A (mM)” column in the output file), with which  $Cl^-$  shares osmotic “room” reserved for anions, decreased due to an increase of the cell volume (“Volume” field in the interface). Reset  $g_{Cl}$  to a very low value ( $1e8$ ) to be able to increase it significantly in coming simulations and press “Calculate” one more time. Nothing changed on the interface, showing that the cations are in the resting state (that costs ~275 million ATP per second) and  $Cl^-$  is in equilibrium. Press “Save as Defaults” and “Reset Time” buttons to complete the preparation to move to further simulations.

Now change “Conductances /  $Na^+$ ” to  $2e9$  and switch to “Detailed Log” since we will be focusing on dynamics of changes. Set “Number of Iterations” to 5,000, which results in 5 sec of “Duration of Calculation”;  $g_{Na}$  will stay low for 15 sec, but after 5 sec we are going to change  $g_{Cl}$ . Press “Calculate”, open the just generated “exp001.txt” file and copy all of its data into a worksheet of Excel, Origin or other similar program. Copy the first row of the data (time = 0), and paste it in a new row inserted just above time=0, changing time to -10; this will show the state of the system before time = 0. Return to the Cell GUI program, change  $g_{Cl}$  to  $1e10$  ions/(V\*s) and press “Calculate”. Open a new “exp001.txt” file, copy all data and paste them to the end of the existing set of data in the worksheet. Note that the first row of the new file starts from 5 sec, the same time that the previous file ended, and that the values of all parameters in this first row are identical to the last row of the previous file, so the files overlap at 5 sec. Make  $g_{Cl}$  low again ( $1e8$ ), perform another calculation for the next 5 sec and add data to the worksheet starting at 10 sec. Finally, return  $g_{Na}$  to its initial value ( $8e9$ ) to simulate recovery. Increase “Number of Iterations” to 10,000 in order to extend the “Duration of Calculation” to 10 sec or to some larger number, if you want to see a longer period of the recovery. Calculate and add the data to the worksheet starting at 15 minutes. The obtained results are graphically presented in **Fig 7A** (concentrations) and **Fig 7B** (volume and voltage). Dashed red lines indicate the time when  $g_{Na}$  was low, dashed green lines indicate the time when  $g_{Cl}$  was high.





Press “Restore to Default” (which also resets time to 0) and repeat the calculations starting this time with an increase of  $g_{Cl}$ , followed by a decrease of  $g_{Na}$ , a return to original  $g_{Na}$ , and a return to original  $g_{Cl}$  for recovery. Do not forget to reset “Number of Iterations” to 5,000, since “Restore to Default” concerns mostly values in the upper part of the interface – concentrations, voltage, volume. The data obtained in this series of calculations are presented in **Fig 7C** (concentrations) and **Fig 7D** (volume and voltage). In general the results demonstrate that changes of cation conductances affected cation concentrations and the membrane potential, but the presence of significant  $Cl^-$  conductance is necessary to change the cell volume. Detailed explanations on interactions between ionic conductances, concentrations, voltage and volume can be found in our paper “The logic of ionic homeostasis: cations are for voltage, but not for volume.”