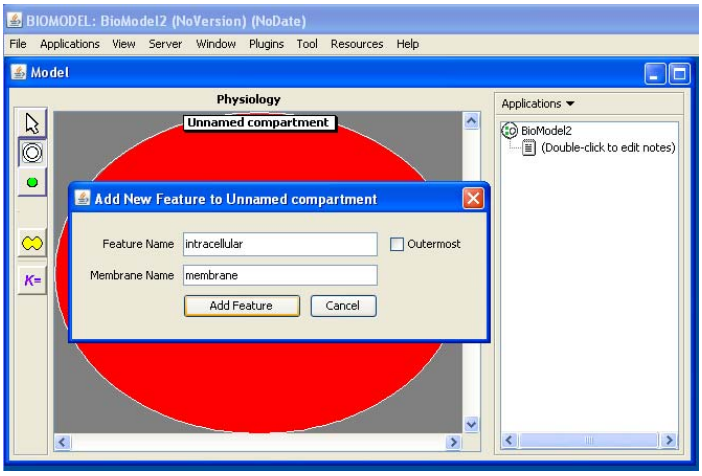
Virtual Cell Version 4.8
Tutorial IV Membrane Potential

Introduction

The following description details the implementation of the Hodgkin-Huxley model of membrane electrophysiology into the Virtual Cell Modeling and Simulation Framework. The Hodgkin-Huxley model serves as a useful demonstration of the functionality of the Electrical Mapping option in the Virtual Cell.. The model includes the sodium and potassium channels and their gating processes; these processes are treated independently of each other however both depend on membrane potential. The total ionic current is described as the sum of the currents for sodium, potassium and a nonspecific ion (leak current).

Following the Tutorial

You can create your own BioModel and Application as you read through the tutorial or you may choose to load the public version of this model. To open the public version, go to File>Open>BioModel>Shared Models>tutorial>Hodgkin_Huxley and press the Open button. You cannot overwrite a public file, so you must save a copy under your own folder in order to make any changes to the BioModel.The BioModel has four different Applications associated with it: voltage only, combined_comp, combined spatial, and voltage_clamp. Each application has its own simulation results.



Creating the BioModel

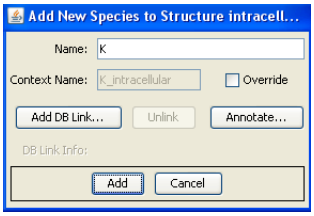
Creating and Defining Compartments

The biological model is defined as a collection of biochemical reactions acting on a set of molecular species localized in specific cellular structures. Cellular structures are defined as mutually exclusive compartments within the cells, as well as the membranes that separate them. The compartments represent three-dimensional volumetric regions while the membranes represent two-dimensional surfaces separating the compartments. All structures can contain molecular species and reactions that describe the biochemical behavior of those species within that structure. Keep in mind when developing your model that it must be mapped to a specific cellular geometry before any quantitative simulation or analysis can be performed.

When the software initiates, you are presented with a new model containing a single compartment. Select the compartment once with the left mouse button, the region will turn red, use the right mouse button to access the Properties menu. Enter the name "extracellular" in the Feature Name text field and press OK.

Select the feature tool  once and click in the extracellular compartment, alternatively you may use the right mouse button to access the Add Feature menu option. A New Feature dialog will open. Enter "intracellular in the Feature Name text field and enter "membrane" in the Membrane Name text field; press Add Feature.

Creating Species



Extracellular Species

Select the species tool  and click in the extracellular compartment. The Add New Species dialog will appear. Enter "K", for potassium, in the Name text field; press Add. Follow the same procedure for adding sodium, Na, and a nonspecific ion, ion. This nonspecific ion is the unknown ion active in the leak current; a small voltage-independent conductance.

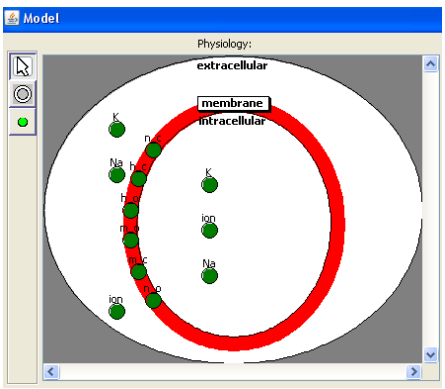
Membrane Species

Use the species tool as you did for creating the extracellular species to create species within the membrane. These are needed to represent the different states of the sodium and potassium channel that regulate the activity of these channels.

Sodium channel opening is controlled by two “gates”. These are typically named the activation gate (m), which is closed at rest and is open when the membrane is depolarized, and the inactivation gate (h), which is open at rest and is closed when the membrane is depolarized. Therefore, at steady state, the sodium channel is closed, both at resting membrane potential, and when the membrane is depolarized (because either one or the other of the gates will be closed). However, opening of the channel is still possible during changes in membrane potential, because the m gate reacts faster to such changes in membrane potential than the h gate. For example, during a depolarization, the m gate will open before the h gate closes. This will open (activate) of the sodium channel. While open, the sodium channel will further contribute to the depolarization, due to the influx of sodium through the open channel. This opening of the channel is transient, since eventually, the slower h gate will close, closing the channel. In contrast, during a repolarization, the m gate will close faster than the h gate will open. Therefore, the sodium channel never gets activated during repolarization.

Potassium channel opening is controlled by a single “gate”. This gate is called an activation gate (n), which is closed at rest and open when the membrane is depolarized. Therefore, at steady state, the potassium channel is closed at resting membrane potential, and open when the membrane is depolarized. It is important to note that the n gate is slowly reacting to changes in membrane potential, slower than either of the two gates that control the sodium channel. This means that during a depolarization, first the sodium channel will activate, transiently (as described above), and only afterwards, the potassium channel will also activate (open). The potassium channel opening is persistent, until the membrane is repolarized. This opening of the potassium channel is actually responsible for the repolarization, due to the outwards flux of potassium through the open channel. Such a combined dynamics of the two channel activities after a depolarization stimulus thus creates an action potential.

You will create separate Species to represent both the open and closed states for each of the three “gates” described above that control the opening of the sodium and potassium channels. These will then be used as “catalysts” controlling the rate of the ionic fluxes passing through the channels (see Membrane Reactions below)..For this model you thus need to create the following membrane Species:

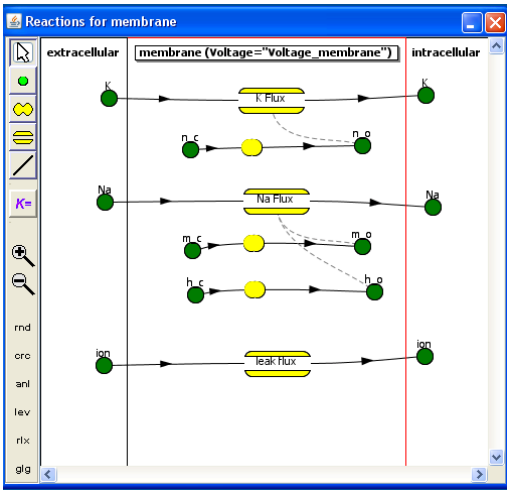


- Potassium Channel Activation Gate-closed "n_c"
- Potassium Channel Activation Gate-open "n_o"
- Sodium Channel Inactivation Gate-closed "h_c"
- Sodium Channel Inactivation Gate-open "h_o"
- Sodium Channel Activation Gate-open "m_o"
- Sodium Channel Activation Gate-closed "m_c"

Intracellular Species

Use the species tool for creating the intracellular species Potassium, Sodium, and a nonspecific ion. Since these species were already created in the extracellular compartment, you can select them and use the right mouse button to access the Edit>Copy command. Click in the intracellular compartment and use the Edit>Paste command, via the right mouse button, to add the newly created Species.

Adding Reactions



Membrane Reactions

Once you have added all the species in your model you can create your reactions. Select the membrane with your left mouse button and then use the right mouse button and select Reactions.

In the Reactions dialog you will set up a total of six reactions; three will be the fluxes across the membrane for the three species defined in the extracellular and intracellular compartments and three will be for defining the sodium and potassium gates. Organize the species according to the image.

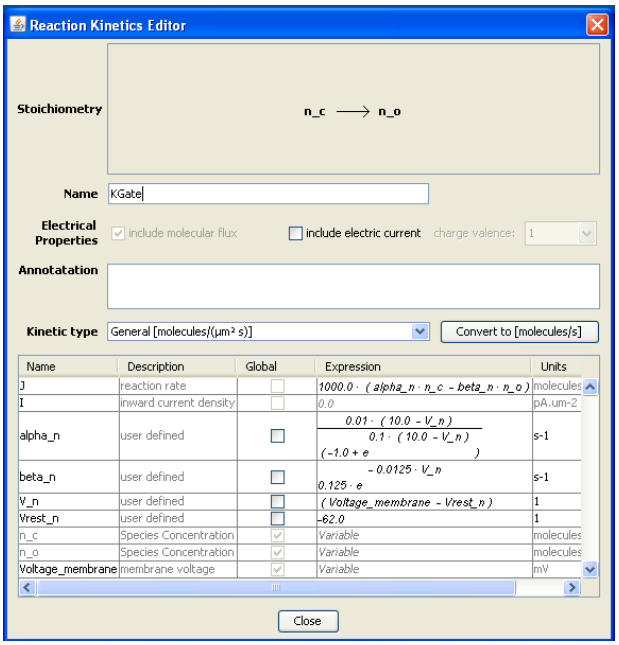
Click on the Reaction tool and click again in the membrane compartment.

We will create the potassium gate first. Use the line tool to connect n_c and n_o to the reaction icon. You always connect from the species to the reaction icon.

Once you have made the connections, select the reaction icon and use the right mouse button to access the Properties menu that will open the Reaction Kinetics dialog for those species.

Change the default reaction name in the Name field to KGate, short for potassium gate, for this reaction. Do not include the electric current and select General for the kinetic type.

When entering the rate equations, please note the units are molecules/(sec-μm^2) and the inward current is pA/μm^2. Please also note the numerical signs. In the Virtual Cell, the software is designed such that the voltage is measured from the outside of the cell going into the cell. Often such measurements are made in the reverse manner.



Double click the Expression field for the reaction rate J and enter the formula to define the gating properties of the potassium channel:

(1000.0 * ((alpha_n * n_c_membrane) - (beta_n * n_o_membrane)))

You now have to define the newly introduced parameters (alpha_n and beta_n), the voltage dependent rate constants, and any additional parameters introduced within those expressions. The software will automatically recognize new parameters and label them as User Defined in the Description field.

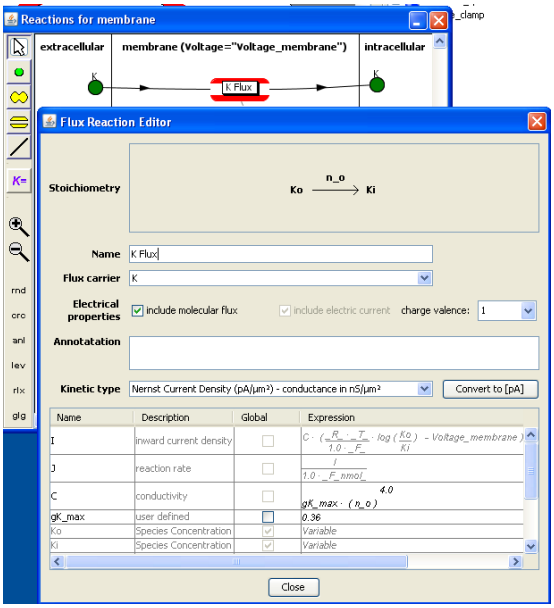
Double click the Expression field and enter the following equation for alpha_n:

(0.01 * (10.0 - V_n) / (exp((0.1 * (10.0 - V_n))) - 1.0))

Once you enter this you will have to define V_n, the total membrane voltage, as (Voltage_membrane - Vrest_n) and then define Vrest_n as -62.0. Define beta_n from the initial rate equation as:

(0.125 * exp((-V_n/80.0)))

Close the Reactions Kinetics Editor



Next we will create the potassium flux. Select the Flux tool and click in the membrane compartment. The Reaction Kinetics dialog will automatically open up. Rename the default flux name in the Name field to K Flux.

Choose the flux carrier by accessing the drop menu with the arrow button. Select K from the list in the Flux Carrier Species dialog. This will automatically connect extracellular and intracellular K to the flux icon. Close the Reaction Kinetics dialogue and connect n_o to the flux icon. Note that the words "catalyst" will appear when you make this connection properly.

Select the flux icon and use the right mouse button to access and select the Properties menu or double-click the icon. Both actions open the Flux Reaction Editor . For Electrical properties be sure that “include molecular flux” is selected. Select Nernst conductance for the Kinetic Type, and note the units.

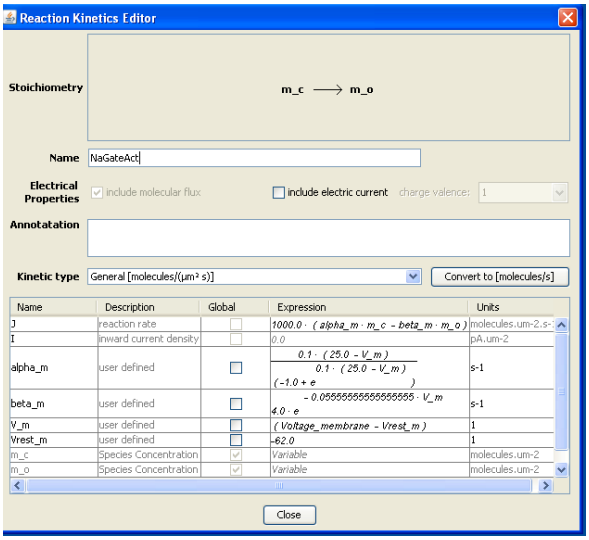
The Charge Valence will automatically become active with this selection; leave the charge at the default of 1. Define the Conductivity, C, in the Expression field as

(gK_max * pow(n_o_membrane, 4.0))

This defines the total potassium current as proportional to n^4: Enter 0.36 in the expression field for gK_max. Note that the equation for inward current density, I, and flux, J reaction rate, is automatically updated based on your conductivity equation. Close the Reaction Kinetics Editor.

Next we will create the activation sodium gate. Select the Reaction tool and place it between m_c and m_o. Once again use the line tool to connect the species to the icon .

Rename the reaction as NaGateAct and select the kinetic type to be General. Double click the following Expression text fields and enter the following information:



Reaction Rate, J:

1000.0 * (alpha_m * m_c_membrane - beta_m * m_o_membrane)

Alpha_m, the rate constant for a particle not activating a gate:

(0.1 * (25.0 - V_m) / (exp((0.1 * (25.0 - V_m))) - 1.0))

Beta_m, the rate constant for a particle activating a gate:

(4.0 * exp((- V_m / 18.0)))

V_m, the total membrane voltage:

(Voltage_membrane - Vrest_m)

Vrest_m as **- 62.0**

Next create the Na inactivation gate. Again, place a Reaction icon between h_c and h_o, where h is the probability that a Na channel is not activated. Open the Reaction Kinetics Editor and enter the following information:

Reaction Name: NaGateInact

Kinetic type: General

Reaction Rate: **(1000.0 * ((alpha_h * h_c_membrane) - (beta_h * h_o_membrane)))**

alpha_h: **(0.07 * exp(-(0.05 * V_h)))**

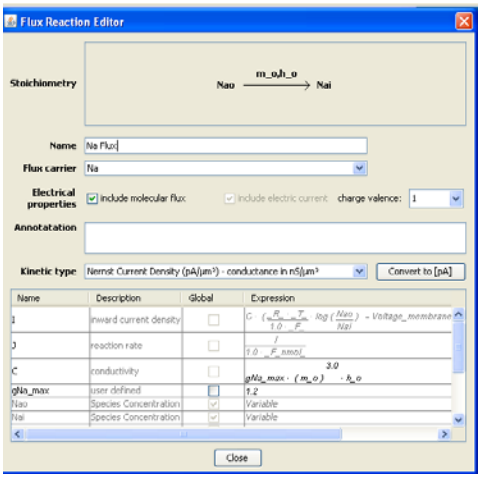
V_h: **(Voltage_membrane - Vrest_h)**

beta_h: **(1.0 / (exp((0.1 * (30.0 - V_h))) + 1.0))**

Vrest_h: **62.0**

The inward current density, I, is predefined and cannot be edited. Close the editor.

To create the Na flux, select the Flux tool and click in the membrane compartment. You can use the line tool to connect the extracellular and intracellular Na to the icon. Click and drag from the species to the flux icon. Be certain that the word “flux” appears as you connect the species. In addition, you will need to connect as catalysts m_o and h_o to the flux icon. Make these connections in the center of the icon and not at the end. Note that the words "catalyst" will appear as you make the connections. Select the icon and open the flux reaction editor.



Name the reaction Na Flux, confirm that the flux carrier is Na. Choose Nernst conductance for the kinetic type and select “include molecular flux”. Leave the charge valence at the default 1.

Maximum sodium conductivity, C:

(gNa_max * pow(m_o_membrane, 3.0) * h_o_membrane)

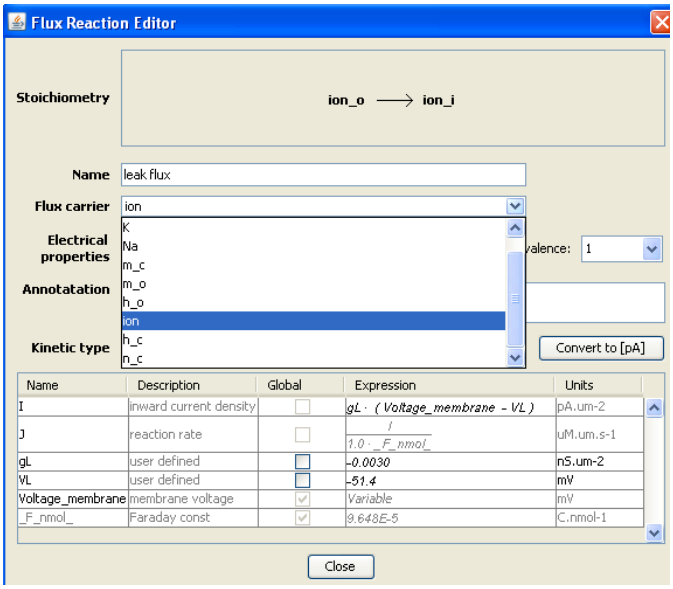
gNa_max: 1.2.

gNa_max assumes the probability that all 3 activation particles (m-activation coefficient) have produced an open channel. H represents the inactivation coefficient. Once again note the units used in this equation.

The inward current density, I, and the reaction rate, J, are predefined and cannot be edited.

Next we create the leak flux. Select the flux tool for the last time and place it in the membrane to create the leak flux between the intracellular and extracellular nonSpecific Ion. This leak is a voltage-independent conductance from an ion other than sodium and potassium.

Rename the flux as leak flux and select ion for the flux carrier. Select include molecular flux, select General Current Density for the kinetic type and leave the charge valence at its default of 1. Define the following:



inward current density, I:
(gL * (Voltage_membrane - VL))

gL, the maximum possible leakage conductance: **-0.0030**

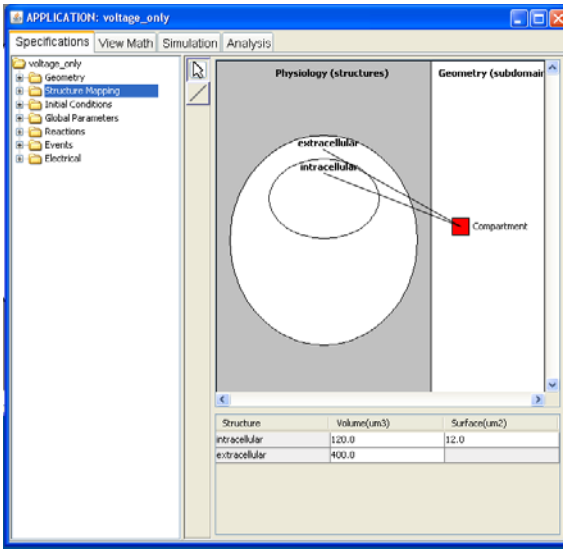
VL, the leakage membrane potential: **- 51.4**.

The reaction rate, J, is predefined and cannot be edited.

Close the dialog and save your model before proceeding further. Go to File>Save As… and enter a model name. Next you will create the Applications that implement different virtual experimental conditions. You will be using both compartmental and spatial models to look at voltage, current and ionic conditions.

Creating the Application

Application I Voltage Only



If you are looking at the database model of the tutorial, you can view the Applications already created. To create Applications yourself (if you have saved the model to your own user account). In the Application panel of the Model document, go to Application >New >Deterministic Application and enter voltage_only or if you choose to load the created Application, double click on voltage_only.

Structure Mapping

The first application, a compartmental model, looks only at voltage changes; sodium and potassium are clamped concentrations. A compartmental model loads by default, so no links need to be drawn to the Geometry panel. We enter the Volume and Surface sizes for the intracellular feature. Select the cell in the Volume (μm^3) column for intracellular, enter 120. Next select the Surface (μm^2) and enter 12. Then enter the Volume sizes for the extracellular feature. Select the cell in the Volume (μm^3) column for extracellular, enter 480.

Initial Conditions

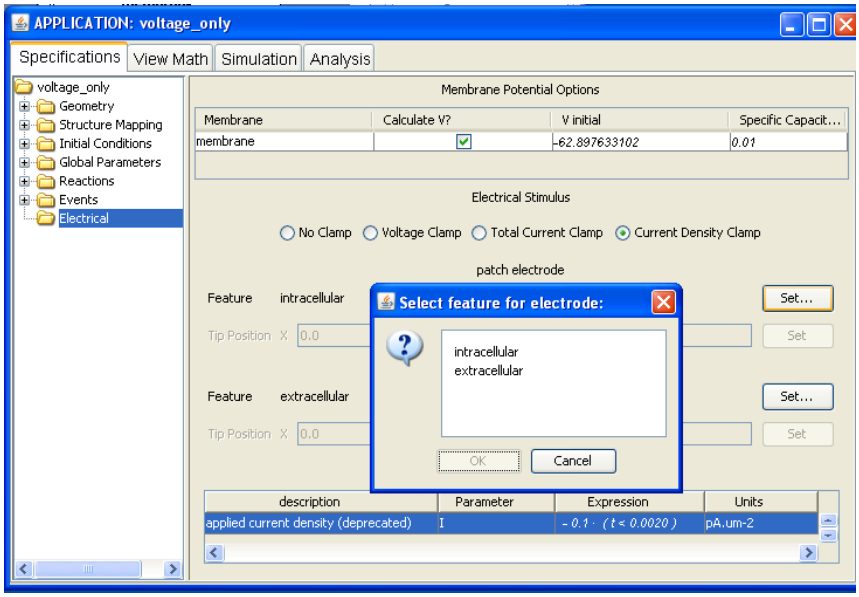
Select the Initial Conditions folder and enter the initial values for the species. Be sure to set the concentrations for potassium, sodium and the non specific ion to Clamped. The values were obtained from steady state values.

Biological Description	Model name	Clamped State	Value
Potassium - extracellular	K	Clamped	20000.0
Sodium - extracellular	Na	Clamped	437000.0
non specific ion - extracellular	ion	Clamped	100.0
Potassium - intracellular	K	Clamped	397000.0
non Specific Ion - intracellular	Ion	Clamped	100.0
Sodium - intracellular	Na	Clamped	50000.0
Potassium Channel Inactive Gate – open	(n_o)		0.304015731
Sodium Channel Activation Gate – closed	(m_c)		0.95240929
Sodium Channel Activation Gate – open	(m_o)		0.04759071
Sodium Channel Inactivation Gate	(h_o)		0.627122591
Sodium Channel Inactivation Gate	(h_c)		0.372877409
Potassium Channel Inactivation Gate	(n_c)		0.695984269

Reaction Mapping

Select the Reactions folder, and make sure all the reactions are enabled. You do not have to select Fast reactions for this Application.

Electrical Mapping



Select the Electrical Mapping folder next. Check calculate V, and enter an initial value of - 62.897633102. Change the specific capacitance to 0.01 pF/μm^2. Note the units here are pF/μm^2 not $\mu F/cm^2$. Select Current Density Clamp in the Electrical Stimulus panel.

To set the patch electrode, press the Set…. button under patch electrode and select intracellular as the physiological feature. The ground electrode is set to extracellular. Next, double click the expression text field for the Applied Current Density and enter (- 0.1 * (t < 0.0020)). Press Enter to accept the value. Note the negative sign accounts for the direction of the current.

Save the Application if you have created your own, and proceed to the Simulation tab.

Running the Simulation

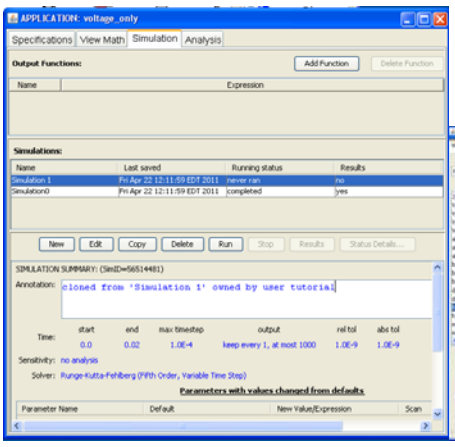
You may load the simulation results, which have already been generated, by selecting the simulation you are interested in and pressing the Results button. Alternatively you may create your own simulation. Select New to create a simulation. Double click in the name field and enter a new name or keep the default name.

Press the Edit button to access the Parameters, Mesh, Task and Advanced tabs. For this particular application you will not change the Parameters or Mesh. The Parameters will remain as defined in the Initial Conditions and the Mesh is disabled for compartmental models. Select the Task tab and enter the run parameters for the simulation. Start Time: 0, End Time: 0.02 and Keep every 1 time sample.

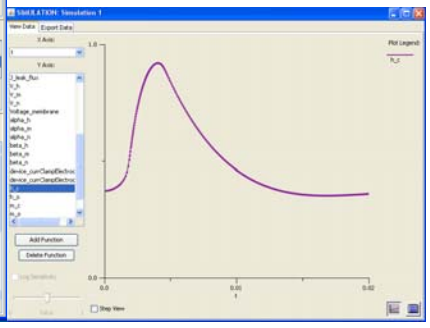
Select the Advanced tab and choose the Runge-Kutta-Fehlberg solver. Set the Starting and Ending Time Bounds to 0.0 and 0.02. Enter 1.0E-8 for the Minimum Time Step, and 1.0E-4 for the Maximum Time Step. The Absolute and Relative Error Tolerances should both be 1.0E-9.

Starting Time Bounds	0.0	Maximum Time Step	1.0E-4
Ending	0.02	Absolute Error Tolerances	1.0E-9
Minimum Time Step	1.0E-8	Relative Error Tolerances	1.0E-9

Close the Edit dialog and make sure the simulation is selected. Press the Run button to initiate the simulation. Once the simulation has generated results, the Results button will become active and you can review them.



Review Chapter 8 of the User Guide for information on additional details on manipulating result views.



Application II - Voltage, sodium, and potassium changes

Structure Mapping

Once again you can either create your own deterministic application based on the following description or you can view the Application "combined_comp". This Application as with the previous one is a compartmental model. The Structure Mapping is in the default setting. However, we will be looking at different volume to surface ratios. Enter the Volume (μm^3) for intracellular as 120 and the Surface (μm^2) as 24. Then enter the Volume (μm^3) for extracellular as 51.43.

Reaction Mapping and Initial Conditions

The Reaction Mapping tab should have all the reactions Enabled. You will be using the same initial condition values as before however this time we will **only Clamp the non Specific ion concentrations**, intracellular and extracellular. You can copy the values from the first application . Under the Initial Conditions tab of the first application right-click a text field and select Copy All. Then open up the current application and right-click a text field and select Paste All. A window will open prompting you to select the specific species that you want values copied for. Click Select All to select all species and then click OK. Then be sure to unselect the Clamp setting for all variables except ion intracellular and extracellular.

Electrical Mapping

Select the Electrical Mapping tab and select “Calculate V” under the Membrane Potential Options panel. Enter the same initial voltage value of - 62.897633102. The specific capacitance is set to 0.01 pF/mm2. Under Electrical Stimulus, select Current Density Clamp. Set the patch electrode Feature to intracellular. The Applied Current Density is different and set to (- 0.1 * (t < 0.05)); press Enter to accept the value.

Running the Simulation

Proceed to the Simulation panel and create a new simulation or review the simulation and stored results. If you are creating a new simulation, click on the Edit button, select the Advanced tab and use the same conditions for the Runge-Kutta-Fehlberg solver:

Starting Time Bounds	0.0	Maximum Time Step	1.0E-4
Ending	0.02	Absolute Error Tolerances	1.0E-9
Minimum Time Step	1.0E-8	Relative Error Tolerances	1.0E-9

Close the Edit dialog and run the simulation. Once again be sure that the simulation is selected before you press Run.

Application III – Voltage, sodium, and potassium changes in a spatial model

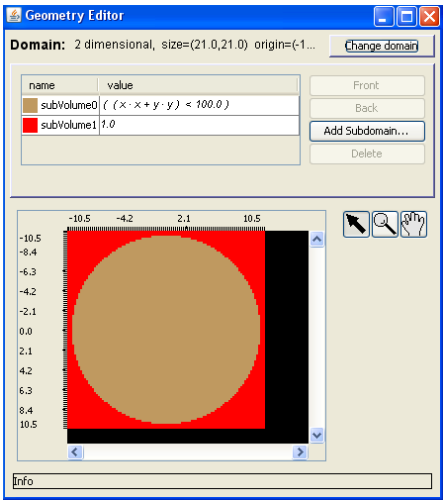
This is a Spatial Application in which you can use the Application and Geometry that are already created or you can create your own deterministic one. If you use the model and Application from the database, the Geometry and the Structure mapping will be complete. If you choose to create your own Application you can either load the Geometry that was already created or once again, create your own.

Creating or Selecting the Geometry

Create a new Application as you did for the previous examples. In the Application window , select the folder on the left side named Geometry. Select the folder.

To load the existing Geometry, open the Application and go to Select Different Geometry>Geometry Names. The database of pre-existing Geometries is displayed. Enter the search term combined_spatial1, select tutorial containing the named geometry and click open.

To create a new geometry, click Create New Geometry. When the Geometry Type window appears, select Analytic Equations (2D) and click OK. The Geometry document will open in the Geometry Editor with a default subdomain automatically created. We will use this as the outermost spatial region and it will be mapped to the extracellular (EC) space represented in our physiology. Double click the name text field for the subdomain. Type in "EC" and press Enter. Leave the value at 1.0.



To represent the spatial region for the cellular compartment, we will add a circle. Press Add Subdomain to create an additional subdomain. From the drop down menu, choose Circle for Subdomain Shape, (0,0) for Center and 10.0 for Radius.

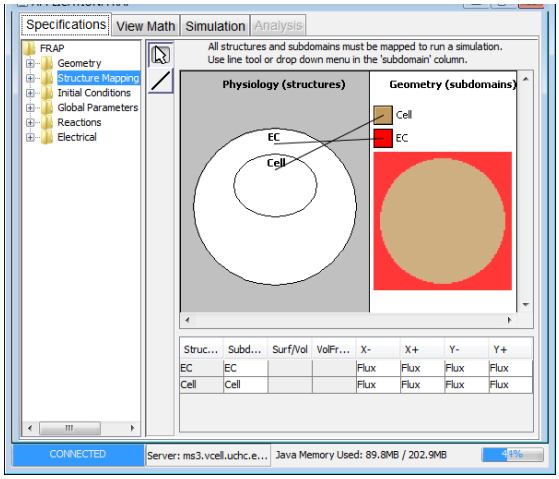
The equation will automatically appear in Analytic Expression. Click Add New Subdomain to confirm it. Double click the name text field for the new subdomain; type in "Cell" and press Enter.

To define the unit size of the domain of the geometry, we need to specify the Geometry Size. Press Change domain to access the Geometry size dialog. Enter "21" in the X and Y size text fields, and enter "-10.5" in the X and Y origin text fields. Press OK to accept the values and to close the window.

Structure Mapping

In the Application dialog, load the geometry you created. Go to View/Change Geometry and navigate to your directory and select your Geometry.

Select the line tool and map the compartments; map extracellular to subdomains1 and intracellular to subdomains0. Leave X-, X+, Y– and Y+ as the default flux.



Reaction Mapping

There is nothing to change on this tab, leave all the reactions Enabled.

Initial Conditions

Copy and paste the initial conditions values for the variables from the previous Application. Open the Initial Conditions folder of the Voltage Only Application, right click to access the menu. Select Copy All. Return to the Initial Conditions folder of the Spatial Application, right click and select Paste All.

Return the initial setting for Clamped variables, i.e. set the concentrations for the intracellular and extracellular **non Specific ion to zero and Clamped**. Now that we have a spatial simulation, **set the diffusion rate constant to 1000000.00** for intracellular potassium. Do the same for extracellular potassium, and extracellular and intracellular sodium.

Electrical Mapping

Select calculate V, and enter an initial value of - 62.897633102. Leave the specific capacitance at 0.01 pF/mm2. Select Current Clamp under Electrical Stimulus. Set the Feature to the intracellular compartment for the patch electrode and enter the Applied Current Density as:

-(0.1 * (t < 0.05))

Save the Application if you have created your own and proceed to running the simulation.

Simulation

Click on the Simulation tab to access the Simulation panel. You can either create your own simulation or review

'Simulation 3' and the results.

If you are creating a new simulation, click on the Edit button and select the Mesh tab. Enter 43 for the X and Y mesh size elements. Select the Task tab and enter the run parameters for the simulation.

Keep the defaults for the Parameters and Advanced tabs. Close the Edit dialog and run the simulation. Once again be sure that the simulation is selected before you press Run to initiate the simulation. Once the simulation has generated results, the Results button will become active, you can review them or wait until the simulation has completed. The results will be displayed automatically. Review Chapter 8 of the User Guide for information on how to review results.

Application IV - Voltage Clamp

This is another Compartmental Application however in this instance you will use a voltage clamp to measure the voltage dependence of the activation and inactivation of ion conductances. Once again, you can use the Application that is already created, voltage_clamp, or create your own. If you decide to create your own, use the Application menu to create another deterministic application.

Structure Mapping

In the Structure Mapping panel, select the cell in the Volume (μm^3) column for intracellular, enter 120. Next select the Surface (μm^2) and enter 12. Then select the Volume (μm^3) column for extracellular and enter 480.

Initial Conditions

Select the Initial Conditions tab. For each species present, double click on the Initial conditions field and enter the following values:

Potassium - extracellular	K	Clamped	20000.0
Sodium - extracellular	Na	Clamped	437000.0
nonSpecific Ion - extracellular	ion	Clamped	100.0
Potassium- intracellular	K	Clamped	397000.0
nonSpecific Ion - intracellular	Ion	Clamped	100.0
Sodium - intracellular	Na	Clamped	50000.0
Potassium Chan Inactivation Gate-O	n_o		0.304015731
Sodium Channel Activation Gate-C	m_c		0.95240929
Sodium Channel Activation Gate-O	m_o		0.04759071
Sodium Channel Inactivation Gate-O	h_o		0.627122159
Sodium Channel Inactivation Gate-C	h_c		0.372877409
Potassium Chan Inactivation Gate-C	n_c		0.695984269

Please note the same 6 species have clamped concentrations.

Reaction Mapping

There is nothing to modify on this tab, leave all the reactions Enabled.

Electrical Mapping

Select calculate V, and enter an initial V value of - 62.897633102. Leave the specific capacitance at 0.01 pF/μm^2. Select Voltage Clamp under Electrical Stimulus and then select the intracellular compartment by pressing the Set... button. The Tip position is not an option since this is a compartmental model. Double click the Applied Voltage text field and enter (-63.0*cos((100.0*t))); press Enter to accept the equation.

Save the Application if you have created your own, and proceed to the Simulation panel. Once again you can either create your own simulation or review the stored simulation, 'Simulation 4', and results.

Simulation

Click on the Edit button, keep the defaults for the Parameters tab.

Starting Time Bounds	0.0
Ending Time Bounds	1.0
Minimum Time Step	1.0E-8
Maximum Time Step	1.0
Absolute Error Tolerances	1.0E-9
Relative Error Tolerances	1.0E-9
Keep every sample, and at most 1000 samples.	1

Select the Advanced tab; choose the Runge-Kutta-Fehlberg solver and set the following values:

Close the Edit dialog and run the simulation. Once again be sure that the simulation is selected before you press Run to initiate the simulation. Once the simulation has generated results, the Results button will become active. You can review them or wait until the simulation has completed; the results will be displayed automatically. Review Chapter 9 of the User Guide for information on how to review results.