

Instructions for the Use of the Computer Simulations

'Preamble'

Even if you find very detailed instructions for these simulation environments, I strongly encourage you to play with the programs. There is nothing to break and a lot to learn. The best use in the end is to develop your own hypotheses and then test them with the simulator.

If you have questions ask them during the lecture or email me:
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Introduction

I cannot get these files to work on a Mac - they run in a Windows environment. Some of the files are outdated and your computer might consider them harmful - but I tested them and they are not - you can safely override a potential security warning.

The zip file contains three different simulation systems

- A) A flash application modeling the Goldman equation (folder Goldman).
- B) A browser-based collection of Java modules to simulate diffusion and channel gating (folder UChigaco).
- C) A Hodgkin-Huxley equation based simulator for voltage activated channels and action potential generation (folder CESE).

A. The Nernst and Goldman Equation Simulator

<http://www.nernstgoldman.physiology.arizona.edu/>

Locate the folder 'Goldmann', once you unzipped the file. Open it and doubleclick on the 'nsgwin.exe' application. Click anywhere on the first screen to get started. If you do not want the application to fill the entire screen hit the 'Esc' button.

Activate the 'Goldmann @ 37°C' tab by clicking on it (1)

Adobe Flash Player 9

File View Control Help

⊕ Potassium [K⁺] (mM)

PK⁺ 100 2

[K⁺]_o 10

[K⁺]_i 100 3

⊕ Sodium [Na⁺] (mM)

PNa⁺ 1

[Na⁺]_o 100

[Na⁺]_i 10

⊖ Chloride [Cl⁻] (mM)

PCl⁻ 10

[Cl⁻]_o 100

[Cl⁻]_i 10

Temperature 37

HELP ION/PERMEABILITY PRESETS: DEFAULT

Nernst Nernst @37°C Goldman Goldman @37°C 1

$E_m = -61.5 \log \frac{P_K [K^+]_i + P_{Na} [Na^+]_i + P_{Cl} [Cl^-]_o}{P_K [K^+]_o + P_{Na} [Na^+]_o + P_{Cl} [Cl^-]_i} = -59.2$ (mV)

Na⁺

K⁺ Cl⁻

E_K -61.5 (mV)

E_{Na} 61.5 (mV)

E_{Cl} -61.5 (mV)

The parameters in the model are adjusted by clicking and dragging on the different sliders. Three parameters each can be adjusted for three different ion species. You can control the conductance or permeability P (2) and the concentration $[\]$ (3) inside and outside of the cell.

Tasks:

1. Alter the outside potassium concentration. Observe how the potassium reversal potential is altered (right hand graph red line). How does the membrane potential behave (black line)? Why? Do the same thing with the sodium concentration inside the cell. How does the sodium reversal potential react? How does the membrane potential react? Why are the reactions so different for the two ion species? Put the values for $[K]_o$ and the value for $[Na]_i$ back to 10.
2. Alter the chloride ion conductance (PCl). What is happening? How is the behavior of the cell changed? Put PCl back to 10.
3. Try to simulate (to the extent possible) an action potential in the cell – accurate timing is not important. Which parameters of which channels do you have to change? Reset the simulator to its ground state, as seen in the screenshot above.
4. Set the PCl slider to 9999. Repeat your action potential simulation. What has changed? How do we call this phenomenon? When does it occur in neurons?
5. Formulate your own hypotheses and test them.

B. The Simulators of the University of Chicago

<http://nerve.bsd.uchicago.edu/>

Locate and open the folder 'UChicago' wherever you copied zip file contents to. Find the file called 'start.htm' and open it by double clicking. This should open a browser window (Chrome, Explorer or Firefox).

Not all the software works without auxiliary application, we will therefore only work with the ones marked (1,2 and 3) in the figure below.

ELECTROPHYSIOLOGY
and The Molecular Basis of Excitability

The Nerve Impulse This is a brief Textbook that covers the basics of the initiation and propagation of the nerve impulse using equivalent circuits with reference to ionic conductances. Voltage dependent conductances are explained on the basis of single channel properties. The text contains links to the simulation programs [Download pdf file](#)

Nerve Impulse Laboratory Manual Experiments using the NERVE program

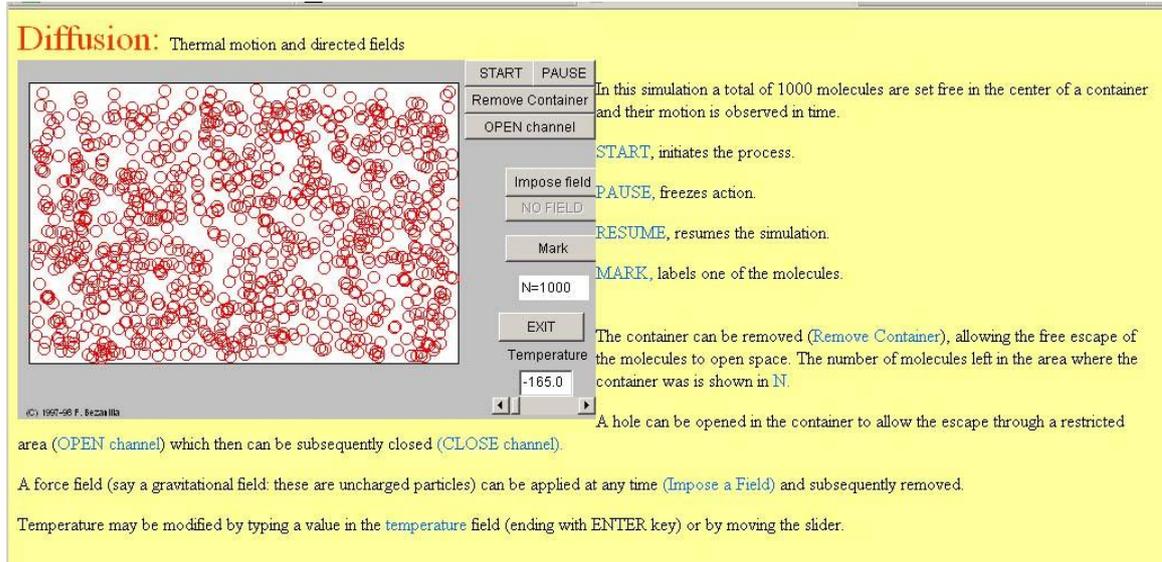
Simulation Programs

1 <u>Diffusion</u>	2 <u>Membrane</u>
NERVE (Includes Cable simulation of propagated AP)	Cable Properties of the Axon
3 <u>Voltage dependent K Channel</u>	Sodium Channel Simulation
Equivalent Circuit of The Axon	NEW!! Model of Shaker K channel gating

Contact me for comments or questions: peezani@ucla.edu

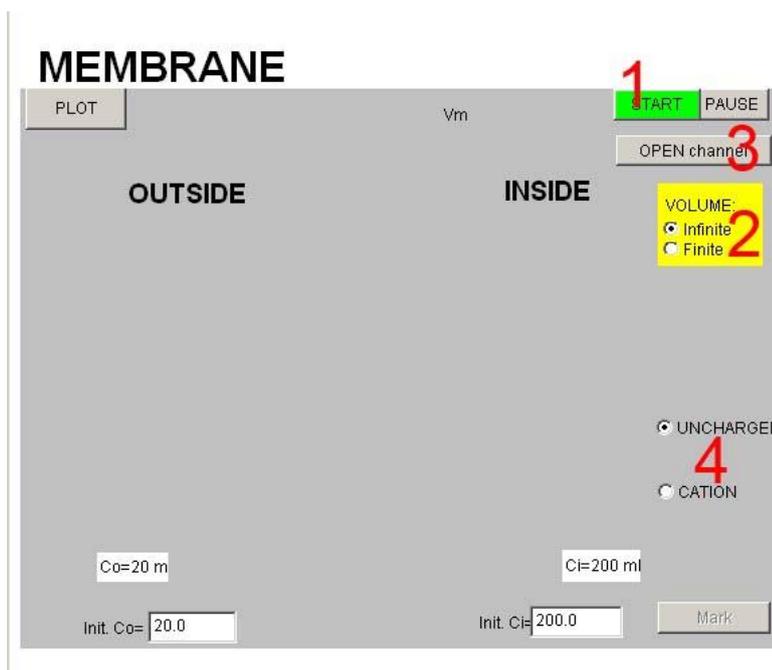
In simulation 1 you will look at a few fundamental principles of diffusion, in simulation 2 you can elaborate on these and study diffusion through a pore. In simulation 3 you will look at single channel gating and single channel events and their summation to a macroscopic current.

Click the hyperlink 'Diffusion'. The user interface is self-explanatory.



1. Start the simulation. Why do the molecules move? Can you use this movement to do work? What are the boundary conditions? What is the effect of temperature in all of this?

2. Return to the main page and click on the hyperlink 'Membrane'. On the page that opens click on 'Membrane' again.



Set the volume of the containers to 'Finite' (2) and start the simulator (1). Click on the button 'OPEN channel' and observe the diffusion. When do you reach equilibrium? How do you define equilibrium in this case? Now stop the simulation and set the volumes to 'Infinite' (2). Start the simulation and open the channel. When do you reach the equilibrium? Stop the simulation again and click on the 'CATION' radio button. What the model does not tell you is that the pore is selective for cations. Repeat the simulations again with finite and infinite containers. How is the situation different for charged and uncharged particles? What can you say about the different equilibrium states?

3 Return to the main page and click on the 'Voltage dependent K channel' link.

K Channel

This simulation shows a simplified view of the channel from the outside of the cell membrane. The S0 segments of the pore (blue) represent the conduction pathways and the voltage sensors are represented by the S4 segments (brown). The link between the S0 and the pore is simply indicated as a bar (yellow) connecting the inner end of the S4 with the inner end of the S0 segment.

START initiates pulsing.
 STOP stops pulsing and resets the average I_o.
 PLOT restores the plot window.
 click here for FAST simulation

To modify the pulse, type a new value in the Vpulse window, followed by ENTER.

(C) F. Bezanilla, 1998

On the page that opens click on 'CHANNELOPERATION' and on the following page on 'FAST simulation' (see red mark on figure left).

You should have the following two windows open. One is the webpage with the controls and two is the java applet window.

PLOT

Parameters of Channel Operation

Pulse V: 10.0 **1**

Post V: -100.0

Pulse Dur: 30.0

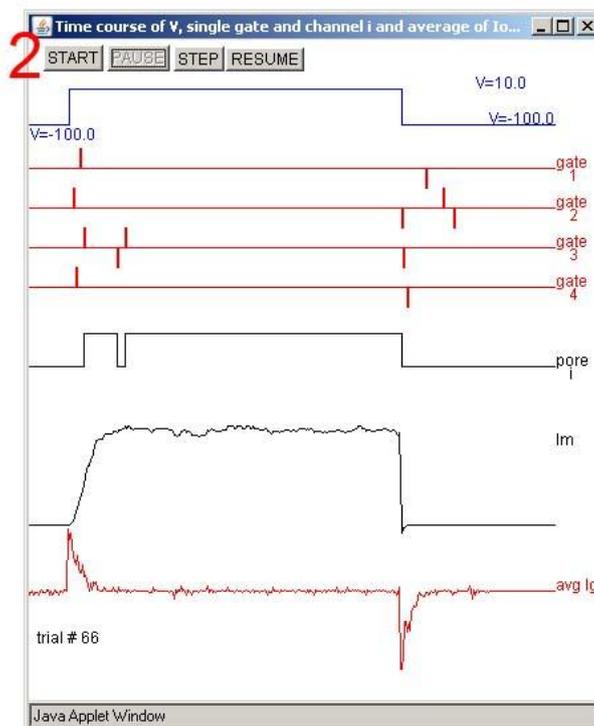
Hold V: -100.0

SIMULATION OF K CHANNEL OPERATION (fast option)

- START - reset averages and initiates pulsing
- PAUSE- stops pulsing
- RESUME- continues pulsing
- STEP- one pulse at a time
- PLOT -restores the plot window.

To change parameters, type a new value in the appropriate window and press ENTER.

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Window two shows a simulation in progress – you get this view by pressing the ‘START’ button.

The top trace is the voltage pulse applied to the channel – we are simulating a voltage-clamp experiment. In the four red rows below you can see the state transitions of the four gates (upwards open, downwards close). The black trace below shows the actual open state of the channel pore. Note that the pore is only open if all four gates open. The trace below shows the sum of the currents through the channel. The lowermost red trace shows the gating current – you can ignore it if you want.

The simulation runs one trial after the other in rapid succession. The same stimulus is applied over and over and the results summed up. That is the reason for the gradual smoothing of the curve. One important experimental observation has been that the summed current looks the same if 100 trials for one channel are made in succession or if 100 channels are looked at in one trial. What does that indicate for the interaction of channels? In order to observe things more clearly you can pause the simulation (‘PAUSE’) and then look at a single even in ‘STEP’ mode.

Vary the step in window (1) to -40 mV - the voltage command will take the membrane voltage from -100 mV to -40 mV for 30 ms and then back to -100 mV again. Click the ‘PLOT’ button and start the simulation again. How does the behavior of the channel change? How can we best describe this dependence on the voltage? Close the browser at the end.

C. CESE

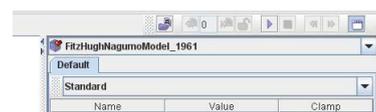
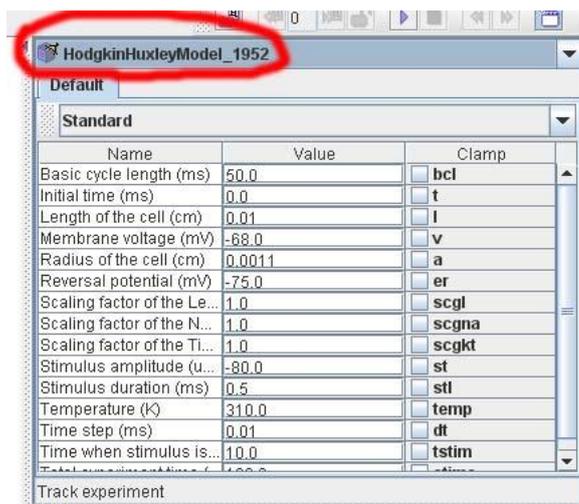
<http://cese.sourceforge.net/>

Open the folder ‘CESE’ double click ‘cese.exe’.

You may get a splash screen asking for feedback – fill out whatever you want and go on - it is an annoying quirk of the program to ask for feedback the first time it is activated.....

CESE stands for ‘cell electrophysiology simulation environment’. It is designed to let you simulate the behavior of electrically excitable cells. Several of the models deal with cardiac cells (you may explore them and find out just how differently they behave). The model we will be using is based on the Hodgkin-Huxley equations. Developed after the end of WWII they were meant to mathematically describe the behavior of a squid giant axon action potential. The quality of the experimental data it was based on allowed a remarkably precise modeling, which is used to this day. Many of the parameters were shown to have clear molecular correlates – remarkable for a model which was developed before ion channels were described.

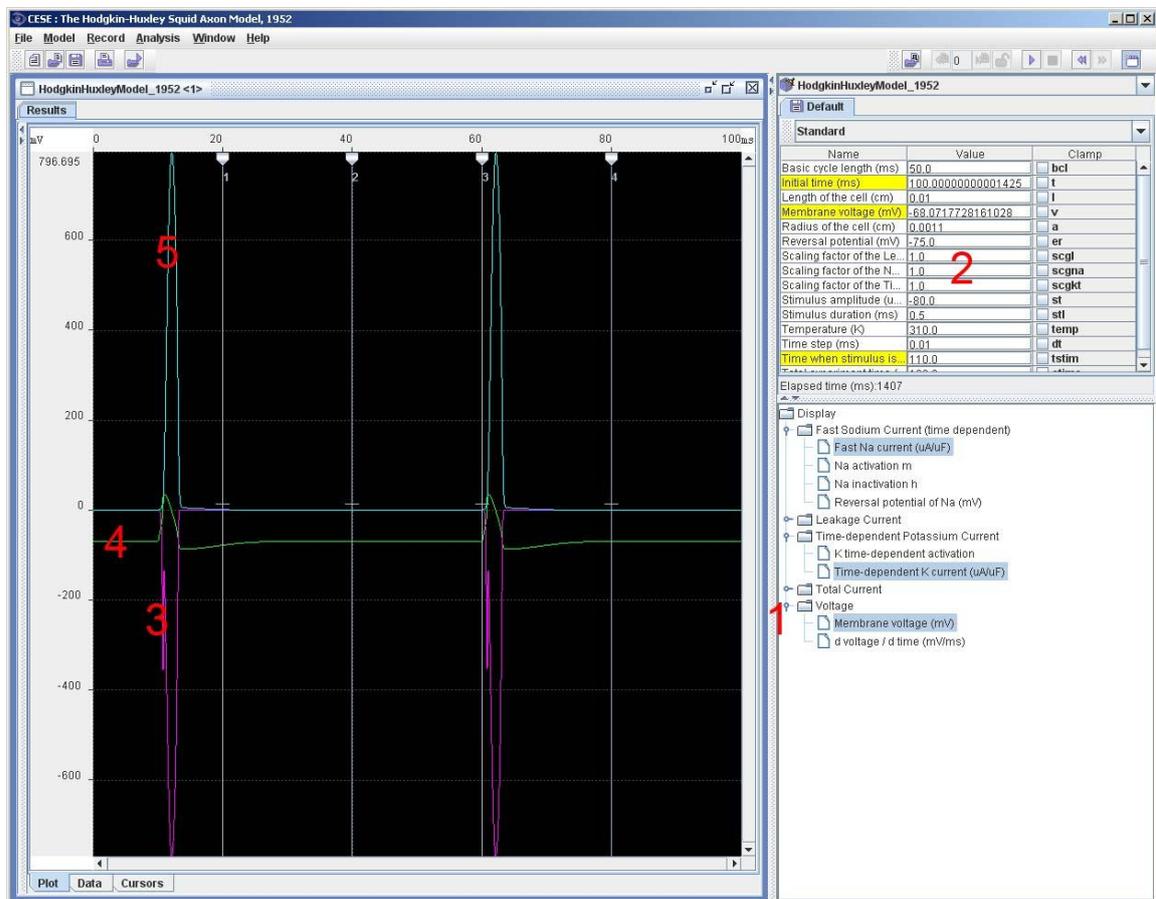
Please select the Hodgkin-Huxley model.



There are a great number of parameters to change. At the beginning concentrate on the number of available voltage-gated sodium and potassium conductances (2). The parameter is the fraction of available channels (from 0-1).

First you have to choose the parameters you want displayed. Choose the membrane voltage by clicking on the small circle left of the 'Voltage' folder (1) and click on 'Membrane voltage'. Expand in the same way the 'Fast sodium current' and the 'Time dependent potassium current'. Choose the parameters shown below - by holding the 'Ctrl' or 'Strg' key you can select multiple parameters.

Press the 'F5' key to start a simulation (you should now see the picture below). Sodium current (3), membrane voltage (4) and potassium current (5) are traced. The modes simulates two brief injections of current (in current-clamp mode) resulting in two action potentials.



Now reduce in several steps the (scgkt)(2) potassium conductance down to 0.01 and redo a simulation every time. Observe the effect on the action potential. How would you experimentally produce the same effect in a neuron? Set the potassium conductance back to 1. Now do the same with the sodium conductance (scgna) (2). Why can you still observe a depolarization even though the sodium current is at 0? If you observe closely the sodium current has two peaks. Why?

Close and reopen the simulator. Choose the Hodgkin-Huxley model again. Change the parameters that control the timing and duration of the injected current (confirm the entries individually with the 'Enter' key): $bcl=150$ ms, $t=0$ ms, $stl=50$ ms, $tstim=50$ ms und $etime=150$ ms. This is a much longer injection than before. Choose Membrane Voltage to be displayed as before.



You should end up with a graph similar to the one seen here. How do you explain this outcome? Alter the potassium conductance again and observe the effect.

Play with the model and try to formulate and test your own hypotheses.