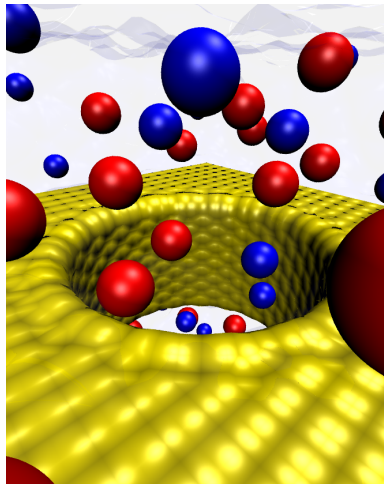


University of Illinois at Urbana-Champaign  
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Computational Biophysics Workshop

# Electrostatic Maps and Ion Conduction

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October 2006

A current version of this tutorial is available at  
<http://www.ks.uiuc.edu/Training/Tutorials/>  
Join the `tutorial-1@ks.uiuc.edu` mailing list for additional help.

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## Introduction

This tutorial is designed to guide users of VMD and NAMD in all the steps required to determine permeation and electrostatic properties of membrane channels. The tutorial assumes that you already have a working knowledge of VMD and NAMD. For the accompanying VMD and NAMD tutorials go to:

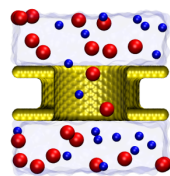
<http://www.ks.uiuc.edu/Training/Tutorials/>

*This tutorial has been designed specifically for VMD 1.8.5 and NAMD 2.6, and should take about X hours to complete in its entirety.*

The tutorial is subdivided into two separate units. The first unit covers the analysis of equilibrium membrane-channel simulations. The second unit describes the steps needed to induce a voltage across a pore-containing membrane and the techniques used to analyze such non-equilibrium simulations.

The examples in the tutorial will focus on the study of a membrane channel model made of an artificial membrane featuring a carbon nanotube pore. Throughout the text, some material will be presented in separate “boxes”. These boxes include complementary information to the tutorial, such as information about the biological relevance of ion transport across membranes, and tips or technical details that can be further explored by the advanced user.

If you have any questions or comments on this tutorial, please email the TCB Tutorial mailing list at [tutorial-l@ks.uiuc.edu](mailto:tutorial-l@ks.uiuc.edu). The mailing list is archived at [http://www.ks.uiuc.edu/Training/Tutorials/mailling\\_list/tutorial-l/](http://www.ks.uiuc.edu/Training/Tutorials/mailling_list/tutorial-l/).



**Membrane Channels.** Selective and controlled transport of solutes (ions and others) across the cellular membrane is essential for the survival of cells and multicellular organisms. Transport of ions through membrane channels play fundamental roles in biological processes such as cell volume regulation and neuronal signaling. In this tutorial we will study permeation properties of membrane channels by using a simplified artificial membrane featuring a pore made of a carbon nanotube. This system is simple and easy to simulate, while the same principles and analysis techniques can be applied to simulations of more complex protein channels.

## Required programs

The following programs are required for this tutorial:

- **VMD:** Available at <http://www.ks.uiuc.edu/Research/vmd/> (for all platforms)
- **NAMD:** Available at <http://www.ks.uiuc.edu/Research/namd/> (for all platforms)
- **MATLAB**

## Getting Started

You can find the files for this tutorial in the `ion-tutorial-files` directory. Below you can see in Fig. 1 the files and directories of `ion-tutorial-files`.

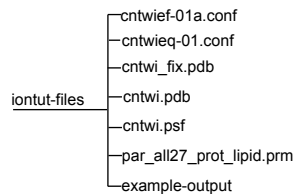


Figure 1: Directory structure of memtut-files

To start VMD type `vmd` in a Unix terminal window, double-click on the VMD application icon in the Applications folder in Mac OS X, or click on the Start → Programs → VMD menu item in Windows.

# 1 Analysis of Equilibrium Simulations

*In this unit you will perform and analyze a simulation of an artificial membrane channel system, learning how to compute densities, osmotic permeabilities, and electrostatic maps.*

## 1.1 The Artificial Membrane Channel System

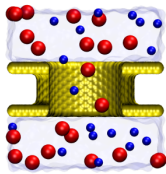
Our first step is to get familiarized with the system under study.

- 1 Navigate to the directory containing the tutorial files ("<your-home-directory>/iontut/ion-tutorial-files/"). Open VMD and load the files `cntwi.psf` and `cntwi.pdb`.
- 2 Choose the Graphics → Representations... menu item and create the following two representations: You should be able to identify two layers of

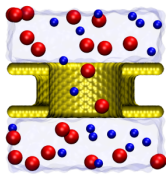
Selection	Drawing Method	Coloring Method
not (water or ions)	VDW	Name
ions	VDW	Name

atoms forming an artificial membrane, a wide pore in the middle of the membrane formed by a carbon nanotube, and ions.

- 3 Turn off the ions representation and select the Mouse → Label → Bond menu item (or use the shortcut key 2). Measure the diameter of the pore by clicking on two atoms located at opposite edges of the pore-forming nanotube. The diameter should be about 26 Å



**Wide and Narrow Membrane Channels.** Membrane proteins form channels with diverse and complex properties. For instance, potassium ion channels feature a pore formed by a very narrow selectivity filter (just wide enough to fit a desolvated ion) and a slightly wider intracellular vestibule (pdb code 1K4C). On the other hand,  $\alpha$ -hemolysin (pdb code 7AHL) exhibits a wide (13 Å in diameter), non-selective pore. Electrostatic and transport properties for both types of channels can be studied with molecular dynamics simulations.



**Carbon Nanotubes.** Nanotubes are cylinders (a few nanometers in diameter) formed by hexagonal graphite layers which exhibit remarkable mechanical, optical, and electronic properties. In the present tutorial, we use a so called (20,12) carbon nanotube to mimick a membrane channel. The nanotube is used as a wide pore suitable for the study of water and ion transport across membranes.

Now we will determine some specific properties of the system such as the number of water molecules and ions in the system.

- 4 Open the Tk Console window by selecting the Extensions → Tk Console menu item. Create selections for all atoms, water, chloride, and sodium ions by typing:

```
set all [atomselect top "all"]
set chloride [atomselect top "name CLA"]
set sodium [atomselect top "name SOD"]
set water [atomselect top "name OH2"]
```

Note that we have selected only oxygen atoms for water.

- 5 Determine the number of atoms in each selection by typing:

```
$chloride num
$sodium num
$water num
```

Can you estimate the ionic concentration used in this system? (Hint: the molar concentration of water is 55 M and  $N_A = 6.022 \times 10^{23}$ )

- 6 Now proceed to determine the radii of all atoms, and of chloride and sodium ions in particular, by typing in the Tk Console:

```
lsort -unique [$all get radius]
lsort -unique [$chloride get radius]
lsort -unique [$sodium get radius]
```

We have used the `get` command to obtain the radius of atoms contained in a selection. The Tcl command `lsort -unique` allowed us to sort the resulting list of radii and delete all redundant elements.



**VMD Radii.** VMD uses a periodic table lookup to determine atomic mass and VDW radii when information about the atom element is available, otherwise it will guess radii using an atom name recognizer heuristic. In the present case, VMD assigned radii of 1.75 Å and 2.27 Å to chloride and sodium ions, respectively. The Pauling radii are actually 0.95 Å and 1.81 Å for  $\text{Na}^+$  and  $\text{Cl}^-$ , while the radii used in simulations is determined from the VDW parameters of the CHARMM force field.

For purely visualization purposes, we will set the radii of  $\text{Cl}^-$  and  $\text{Na}^+$  to the values determined by Pauling.

- 7 Type in the Tk Console:

```
$chloride set radius 1.81
$sodium set radius 0.95
```

8 Finally, determine the charge of your system by typing in the Tk Console:

```
measure sumweights $all weight charge
```

The net charge of your system should be zero. Keep VMD open for the next section.

## 1.2 Membrane Channel in Electrolyte at 0 Volts

Now that you are familiar with the system, we will perform and analyze a molecular dynamics simulation of the membrane channel in electrolyte at 0 Volts.

- 1 Navigate to the tutorial directory named (`ion-tutorial-files`) and open with a text editor the file `cntwieq-01.conf`.
- 2 Look at the NAMD configuration file and note that we are using a 1 fs timestep with a multiple time-stepping algorithm that permits computation of short-range non-bonded interactions every 2 fs, and of long-range non-bonded interactions every 4 fs.
- 3 Locate the section labeled `# Fixed Atoms Constraint`. NAMD will fix atoms which have an Occupancy value different from 0 in the file `cntwi_fix.pdb`.
- 4 Locate the section labeled `# Constant Pressure Control`. The simulation will be performed in the NpT ensemble (constant number of particles N; constant pressure p; constant temperature T). However, note that we will keep the area of the system constant (`useConstantArea yes`), since the artificial-membrane atoms will be kept fixed in this particular example.
- 5 Close the text editor and proceed to run your simulation using NAMD as indicated below.



**Running.** Use NAMD to run your simulation. The usual command to run your job is `namd2 cntwieq-01.conf > cntwieq-01.log`, but it may be different depending on your local system configuration. The simulation should take about 24 hours in a single processor machine. You may want to skip this step and use the trajectory files already available in the `example-output` directory.

We will now proceed to visualize and analyze the outcome of the simulation.

- 6 In the current VMD session, which should be displaying the `cntwi.psf` molecule, load the trajectory `cntwieq-01.dcd` by selecting the File → Load Data Into Molecule... menu item. Be sure you load the file into the existing molecule! As the file loads, you should be able to see ions moving around the simulation box.

Selection	Drawing Method	Coloring Method
water	Lines	Name

- 7 Select the Graphics → Representations... menu item and create the following new representation:

You should be able to see water molecules. Do they permeate the pore?

### 1.3 Computing Water Density

Lets determine the average density of water in all regions of our system.

- 1 If the Tk Console is not open already, select the Extensions → Tk Console menu item. Then type the following commands in the Tk Console:

```
set wat [atomselect top "name OH2"]
volmap density $wat -allframes -combine avg -res 1 -o eqwatdens.dx
```

The execution of the latter command may take a few minutes and will generate a volumetric data set, stored in `eqwatdens.dx`, which contains the average density of water molecules computed over a matrix of cubic voxels (1 Å<sup>3</sup> each).

- 2 Load the generated data set by typing `mol load dx eqwatdens.dx`. Be sure that you are located in the appropriate directory first. The volumetric dataset will be loaded as a new molecule, but in the VMD OpenGL Display window you will only see a cube that delimits the region used to compute the density
- 3 In order to visualize the density, go to the Graphical Representations window. Make sure that under **Selected Molecule** it reads `1:eqwatdens.dx`. The default drawing method for a volumetric data set is **Isosurface**. Change it to **VolumeSlice** and select the **Volume** coloring method.
- 4 In the Graphical Representations window, set the **Render Quality** to **Medium**, and move the slice offset to 0.50. Rotate your molecule, you should be able to see something similar to what is shown in Fig. 2. Turn off the `cntwi.psf` molecule (double click on the D close to the name of the molecule in the VMD Main window) for better visualization.
- 5 Set the **Slice Axis** to **Y** or **Z** and explore the density dataset along the pore.
- 6 Select the Extensions → Visualization → Color Scale Bar menu item; the corresponding window should appear in your screen. Turn **Autoscale On** and click on the **Draw Color Scale Bar** button.





Figure 2: Water density as determined by the VMD volmap plugin.

A color scale bar should appear in your VMD OpenGL Display window. Density values for water fluctuate between  $0.028$  and  $0.037 \times \text{atoms} \times \text{\AA}^{-3}$ . Do these values make sense? Compute the average water density in mol/L using  $1 \text{ L} = 0.001 \text{ m}^3$  and  $N_A = 6.0221 \times 10^{23} \text{ mol}^{-1}$ . Compare it to the standard water concentration value (55 mol/L).

- 7 Press the Delete Color Scale Bar and leave open your VMD session for the next section.

#### 1.4 Computing Ionic Density

We will now follow a similar procedure to compute the density of ions in the simulation cell.

- 1 In the Tk Console type the following commands:

```
mol delete top
set c1 [atomselect top "name CLA"]
volmap density $c1 -allframes -combine avg -res 1 -o eqcldens.dx
```

Computation of chloride density may take a few seconds.

- 2 Once the computation is done, load in VMD the resulting file (`eqcldens.dx`) as a new molecule. In the Graphical Representations window change the default `Isosurface` representation to `VolumeSlice` and then select the Volume Coloring Method. Set the Slice Axis to Z and visually inspect the  $\text{Cl}^-$  density.

The range of values for  $\text{Cl}^-$  densities is more heterogeneous than that observed for water. Use the Color Scale Bar to determine approximate boundaries for the

ionic concentration of the system. Follow the same procedure outlined above to make an estimate of the ionic concentration. Does the number compare well with your earlier estimate of ionic concentration based on the number of ions and water molecules?

3 Once you are done, press the Delete Color Scale Bar button.

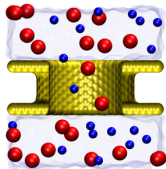
4 Lets follow the same procedure to compute densities for sodium ions:

```
mol delete top
set na [atomselect top "name SOD"]
volmap density $na -allframes -combine avg -res 1 -o eqnadens.dx
```

5 Again, load the resulting file (eqnadens .dx) as a new molecule and explore the Na<sup>+</sup> density. Once you are done exploring, delete the top molecule (corresponding to the Na<sup>+</sup> density file).

## 1.5 Computing Electrostatic Maps

In addition to densities of ions and water, VMD permits one to compute the average electrostatic potential of a molecular dynamics trajectory by using the PMEpot plugin.



**Long Range Electrostatics.** The particle mesh Ewald method (PME) permits the efficient computation of long range electrostatic interactions. The VMD plugin PMEpot evaluates the reciprocal sum of the smooth particle-mesh Ewald method resulting in a smoothed electrostatic potential. In order to use it, you must have a periodic and neutral system. The algorithm approximates point charges using Gaussians with sharpness controlled by the Ewald factor, more details can be found in Aleksij Aksimentiev and Klaus Schulten, *Biophysical Journal*, **88**:3745–3761, 2005

1 Be sure that the top molecule corresponds to cntwi.psf containing all frames of our equilibrium molecular dynamics trajectory. Type the following commands in the Tk Console:

```
package require pmepot
set all [atomselect top "all"]
pmepot -sel $all -frames all -xscfile cntwieq-01.restart.xsc \
-grid {48 48 48} -dxfile eqpot.dx -ewaldfactor 0.25
```

The PMEpot plugin will compute the electrostatic potential for each frame and then will average it over the entire trajectory (VMD will take a few minutes to finish with the computations).

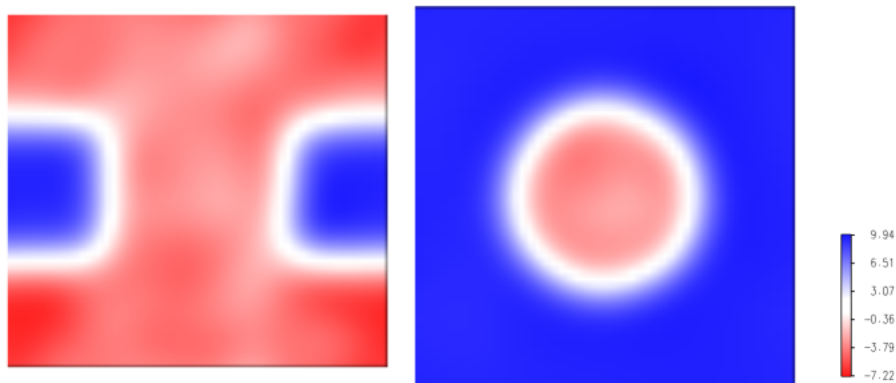



Figure 3: Electrostatic potential as determined by the VMD PMEpot plugin.

The electrostatic potential is calculated using all the atoms of the system, a three dimensional grid of  $48 \times 48 \times 48$  points (which ensures at least one grid point per Å in each direction), and an Ewald factor of 0.25.



**Warning.** Note that the periodic cell size has been read from `cntwieq-01.restart.xsc`, which contains the fixed cell size captured at the end of the equilibrations. Using this file is NOT quite correct, since the simulation was performed at constant pressure and, therefore, the size of the simulation cell changed for each frame. However, for illustration purposes we will use it anyway.

Finally, note that the electrostatic potential will be stored as a three-dimensional data set in the file `eqpot.dx` and will be automatically loaded as a separate molecule in VMD.

- 2 Lets inspect the resulting electrostatic potential map. Open the Graphical Representations window and change the default `Isosurface` representation to `VolumeSlice`. Then select the `Volume Coloring Method`. Set the `Slice Axis` to `Z` and move the slice offset to 0.50.
- 3 Rotate your molecule, you should be able to see something similar to what is shown in Fig. 3.
- 4 Select the `Extensions` → `Visualization` → `Color Scale Bar` menu item; the corresponding window should appear in your screen. Turn `Autoscale On` and click on the `Draw Color Scale Bar` button.

A color scale bar should appear in your VMD OpenGL Display window. The electrostatic potential generated by PMEpot is in units of  $kT/e$ . Using  $T = 300$  K, one PMEpot unit of electrostatic potential is equivalent to 0.0258 Volts.

The computed electrostatic potential ranges from -7.22 to 9.94  $kT/e$ , which is equivalent to a total variation of about 0.5 Volts. The largest variation of electrostatic potential occurs at the interface between water molecules and the membrane/channel atoms. Moreover, the electrostatic potential in the interior of the membrane (vacuum in our artificial example) is homogeneous and positive, while the potential throughout the zones occupied by water is rather inhomogeneous and negative (with variations of at most 0.2 V). Such potential is consistent with the membrane dipole potential caused by interfacial dipoles. The inhomogeneity observed in the region occupied by water is caused by poor sampling.

We now have completed the analysis of a membrane-channel molecular dynamics simulation at 0 Volts. In the next section, we will learn how to induce a voltage difference across our artificial membrane and will analyze the outcome of the corresponding simulations.

## 2 Analysis of Non-Equilibrium Simulations

*In this second unit, you will learn how to induce a voltage difference across a pore-containing membrane and perform analysis of the resulting non-equilibrium molecular dynamics simulation.*

### 2.1 Membrane Channel in Electrolyte at 1 Volt

We will use the same system described in the previous unit, but will now perform a simulation in which an external electric field induces a voltage difference across the pore-containing membrane.

- 1 Navigate to the tutorial directory `ion-tutorial-files` and locate the NAMD configuration file `cntwief-01a.conf`. Open the file with a text editor.
- 2 Locate the section labeled `# Continuing a job from the restart files` and check that we are using the outcome of the simulation performed in unit 1 as a starting point. As usual, its always desirable to start a simulation using an equilibrated system.
- 3 Since we are restarting from a previous simulation, it is not necessary to define the NAMD temperature or the size of the simulation cell, which are commented out in the NAMD configuration file as you can check yourself (Tcl comments always start with the symbol `#`).
- 4 We will perform the simulation in the NVT ensemble (constant number of particles N; constant volume V; constant temperature T). Locate the section labeled `# Constant Pressure Control` and check that is not active (`if{0} {` will always be false and the content inside the brackets will not be taken into account when reading the parameter file).
- 5 Finally, locate the section labeled `## EXTRA PARAMETERS` in the NAMD configuration file. You should find the following NAMD commands:

```
eFieldOn yes
eField 0 0 0.54
```

The first command turns on an electric field that will be applied to all atoms in the system. The second command sets the electric field to be used. The electric field is specified as a vector which, in this particular case, has only a  $z$  component. The electric field unit used by NAMD is the  $\text{kcal/mol}/\text{\AA}/e$ ; one unit is equivalent to  $0.0434 \text{ Volts}/\text{\AA}$ .

- 6 Locate the file named `cntwief-01.restart.xsc`. This file contains information about the periodic-simulation cell size. Open the file with a text editor, you should see text similar to:

```
# NAMD extended system configuration restart file
#$LABELS step a_x a_y a_z b_x b_y b_z c_x c_y c_z o_x o_y o_z s_x s_y s_z s_u s_v s_w
910000 46 0 0 0 46 0 0 0 42.651 0 0 0 -0.000440762 -0.00058589 -9.2304e-05 0 0 0
```

- 7 Note that the size of the system in the  $z$  direction decreased from its original value ( $c_x$  changed from 46 Å to 42.651 Å in the present example). Record the value of  $c_x$  found in your file and close the text editor containing the file `cntwieq-01.restart.xsc`. Then go back to the text editor containing the file `cntwief-01a.conf`.

The constant electric field  $\vec{E}$  applied to the system, as set in the NAMD configuration file (`eField 0 0 0.54`), will induce an electrostatic potential drop across the simulation cell that can be computed from:

$$\nabla\phi = -\vec{E} \quad (1)$$

$$\Rightarrow \phi = E L_z \quad (2)$$

The potential drop across the simulation cell is then about 1 V in our particular example ( $0.54 \times 0.0434 \times 42.651$  V). Since water molecules and ions are free to move around the simulation cell, they will rearrange in order to restore a flat electrostatic potential in bulk electrolyte. Thus, ions and water molecules will generate a reaction potential that, when added to the applied potential, will result in the total electrostatic potential of the system illustrated in Fig. 4.

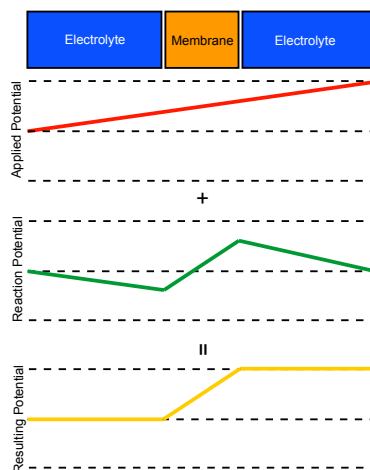
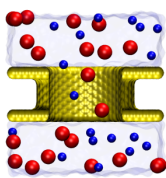


Figure 4: Applied, reaction, and resulting electrostatic potentials

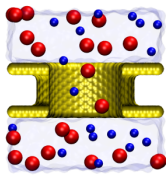
- 8 If you are doing this tutorial with a group of people, set the value of the electrostatic field in the NAMD configuration file `cntwief-01a.conf` so as to apply an electrostatic potential drop equivalent to the month of your birthday multiplied by 0.5 V. For instance, if your birthday is in March, set it to 1.5 V. Note that you have to use the cell size you recorded earlier to determine the value of the electric field in units of  $\text{kcal/mol}/\text{\AA}/e$ . Following our example, we would replace `eField 0 0 0.54` by `eField 0 0 0.162`.

- 9 If you are doing this tutorial by yourself, set the value of the electrostatic field so as to apply an electrostatic potential drop of 1 V. Note that you have to use the cell size you recorded earlier to determine the value of the electric field in units of kcal/mol/Å/e. In our example, the electric field is already set at the correct value.



**Electrostatic Potentials.** Osmotic imbalances between the cell's interior and its surroundings cause electrostatic potentials of a few hundred millivolts to arise across cell membranes. Such potentials influence conformation and permeation properties of membrane proteins. Patch clamp experiments, whereby electrodes placed at opposite sides of a membrane produce an electric field, are used to study the behavior of membrane proteins under the action of a controlled electrostatic potential. The principles behind patch-clamp experiments are the same of those behind molecular dynamics simulations in which an electric field is applied to the whole system. Note that we are using unusually large electrostatic potentials in order to obtain results during the short time-scales achievable by simulations.

- 10 Save and close the modified NAMD configuration file. Proceed then to run your simulation as indicated below.



**Running.** Use NAMD to run your simulation. The usual command to run your job is `namd2 cntwief-01a.conf > cntwief-01a.log`, but it may be different depending on your local system configuration. The simulation should take about 24 hours in a single processor machine. You may want to skip this step and use the trajectory files already available in the `example-output` directory.

- 11 Once the simulation is done, proceed to open VMD, launch the Tk Console window, and load the resulting trajectory by typing:

```
mol load psf cntwi.psf dcd cntwief-01a.dcd
```

- 12 Select the Graphics → Representations... menu item and create the following two representations:

Selection	Drawing Method	Coloring Method
ions	VDW	Name

Rotate the image so as to have the  $z$ -axis pointing up in your screen.

- 13 Use the VMD main window controls to play the trajectory back and forth. You should be able to see positive sodium ions moving across the center of the channel in the positive  $z$  direction, and negative chloride ions moving in the negative  $z$  direction.

## 2.2 Computing density of water

Lets determine whether the applied field changed the average density of water in some regions of the system.

- 1 Type the following commands in the Tk Console window:

```
set wat [atomselect top "name OH2"]
volmap density $wat -allframes -combine avg -res 1 -o efwatdens.dx
```

The execution of the latter command may take a few minutes and will generate a volumetric data set, stored in `efwatdens.dx`, which contains the average density of water molecules computed over a matrix of cubic voxels ( $1 \text{ \AA}^3$  each).

- 2 Following the same approach used in the previous unit, load the generated data set by typing `mol load dx efwatdens.dx`. The volumetric dataset will be loaded as a new molecule, but in the VMD OpenGL Display window you will only see a cube that delimits the region used to compute the density
- 3 In order to visualize the density, go to the Graphical Representations window. Make sure that under **Selected Molecule** it reads `1:efwatdens.dx`. The default drawing method for a volumetric data set is **Isosurface**. Change it to **VolumeSlice** and select the **Volume** coloring method.
- 4 In the Graphical Representations window, set the **Render Quality** to **Medium**, and move the **slice offset** to 0.50. Rotate your molecule, you should be able to see something similar to what is shown in Fig. 5. Turn off the `cntwi.psf` molecule (double click on the D close to the name of the molecule in the VMD Main window) for better visualization. Do you notice a difference between the water density computed for this simulation in which an electric field has been applied, and the water density obtained for our previous simulation in equilibrium?
- 5 Set the **Slice Axis** to **Y** or **Z** and explore the density dataset along the pore.
- 6 Select the **Extensions** → **Visualization** → **Color Scale Bar** menu item; the corresponding window should appear in your screen. Turn **Autoscale On** and click on the **Draw Color Scale Bar** button.

A color scale bar should appear in your VMD OpenGL Display window. Density values for bulk water fluctuate between 0.025 and  $0.038 \times \text{atoms} \times \text{\AA}^{-3}$ . Do these values make sense? Compute the average water density in mol/L using  $1 \text{ L} = 0.001 \text{ m}^3$  and  $N_A = 6.0221 \times 10^{23} \text{ mol}^{-1}$ . Compare it to the standard water concentration value (55 mol/L) and the value you computed in the previous unit.



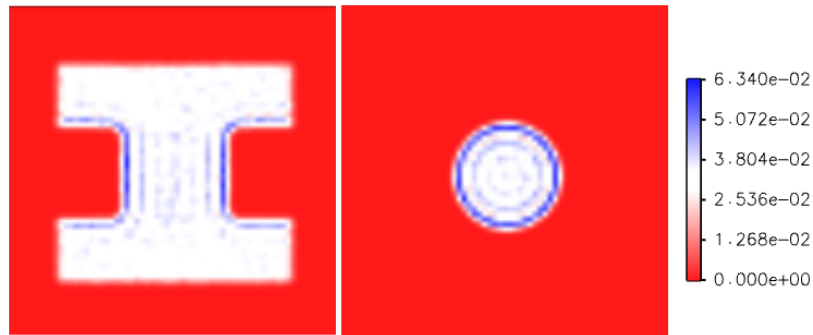


Figure 5: Water density as determined by the VMD volmap plugin.

- 7 Press the Delete Color Scale Bar and leave open your VMD session for the next section.

### 2.3 Computing Density of Ions

We will now follow a similar procedure to compute the density of ions in the simulation cell.

- 1 In the Tk Console type the following commands:

```
mol delete top
set cl [atomselect top "name CLA"]
volmap density $cl -allframes -combine avg -res 1 -o efcldens.dx
```

Computation of chloride density may take a few seconds.

- 2 Once the computation is done, load in VMD the resulting file (`efcldens.dx`) as a new molecule. We will use in this case the default **Isosurface representation** In the Graphical Representations window change the **Isovalue** to 0.001 and then change the **Draw** option from **Points** to **Solid Surface**. Finally, change the **Material** from **Opaque** to **Transparent**. You should be able to see the distribution of Cl ions around the cell as shown in Fig. 6.

The range of values for  $\text{Cl}^-$  densities is more heterogeneous than that observed for water. Use the Color Scale Bar to determine approximate boundaries for the ionic concentration of the system. Follow the same procedure outlined above to make an estimate of ionic concentration. Does the number compare well with your earlier estimate of ionic concentration based on the number of ions and water molecules? Do you observe a difference between the distribution of ions around the simulation cell observed in simulations with and without applied

electric field?

- 3 Once you are done, press the Delete Color Scale Bar button.
- 4 Lets follow the same procedure to compute densities for sodium ions:
 

```
mol delete top
set na [atomselect top "name SOD"]
volmap density $na -allframes -combine avg -res 1 -o efnadens.dx
```
- 5 Again, load the resulting file (`efnadens.dx`) as a new molecule and explore the  $\text{Na}^+$  density using the Isosurface representation. Compare your results with the ionic density computed in equilibrium (without field). Once you are done exploring, delete the top molecule (corresponding to the  $\text{Na}^+$  density file).

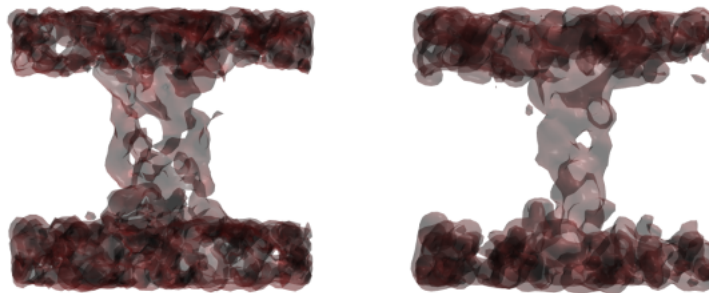


Figure 6: Chloride and Sodium density as determined by the VMD volmap plugin.

## 2.4 Computing Electrostatic Maps

Lets use VMD to compute the average electrostatic potential of the molecular dynamics trajectory by using the PMEPot plugin. Note that the computed electrostatic potential will be the reaction potential described in Fig. ??

- 1 Be sure that the top molecule corresponds to `cntwi.psf` containing all frames of our molecular dynamics trajectory with an applied field. Type the following commands in the Tk Console:

```

package require pmepot
set all [atomselect top "all"]
pmepot -sel $all -frames all -xscfile cntwief-01a.restart.xsc \
-grid {48 48 48} -dxfile efpot.dx -ewaldfactor 0.25

```

The PMEPot plugin will compute the electrostatic potential for each frame and then will average it over the entire trajectory (VMD will take a few minutes to finish with the computations).

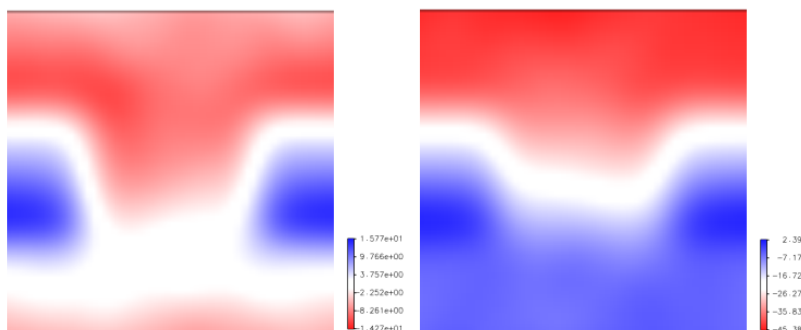


Figure 7: Electrostatic potential as determined by the VMD PMEPot plugin.

The electrostatic potential is calculated using all the atoms of the system, a three dimensional grid of  $48 \times 48 \times 48$  points (which ensures at least one grid point per Å in each direction), and an Ewald factor of 0.25.

Finally, note that the electrostatic potential will be stored as a three-dimensional data set in the file `efpot.dx` and will be automatically loaded as a separate molecule in VMD.

- 2 Lets inspect the resulting electrostatic potential map. Open the Graphical Representations window and change the default `Isosurface` representation to `VolumSlice`. Then select the `Volume Coloring Method`. Set the `Slice Axis` to `Z` and move the slice offset to 0.50.
- 3 Rotate your molecule, you should be able to see something similar to what is shown in Fig. 7. Is the potential similar to the reaction potential shown in Fig. reffig:diagram2?
- 4 Select the `Extensions` → `Visualization` → `Color Scale Bar` menu item; the corresponding window should appear in your screen. Turn `Autoscale On` and click on the `Draw Color Scale Bar` button.

A color scale bar should appear in your VMD OpenGL Display window. As we mentioned before, the electrostatic potential generated by PMEPot is in units

of  $kT/e$ . Using  $T = 300$  K, one PMEpot unit of electrostatic potential is equivalent to 0.0258 Volts.

The computed electrostatic potential ranges from -14.270 to 15.775  $kT/e$ , which is equivalent to a total variation of about 0.77 Volts, close to the expected 1 Volt. Note that since the system studied is small, and the simulation lasted a short time, the potential is unlikely to be completely stabilized. It may take several nanoseconds to achieve an steady state.

The total electrostatic potential of the system can be obtained by adding the linear applied potential to the measured reaction potential. We show in the right panel of Fig. 7 the outcome of the addition, resulting in flat negative and positive potentials at each side of the membrane, as expected for the total electrostatic potential. Future versions of the PMEpot plugin will allow you to add the corresponding applied bias automatically. Future versions of VMD will allow you to extract potential and density one- or two- dimensional profiles.

## 2.5 Computing Ionic Currents

When exploring the trajectory of the simulation in which we applied an external electric field, you noticed that ions translocated across the pore of our artificial membrane. Ionic currents can be computed by counting the number of ions that cross the channel, i.e.:

$$I = \frac{\mathcal{N}q}{\tau} \quad (3)$$

where  $\mathcal{N}$  is the number of crossing events over a time interval  $\tau$ . The ionic current can also be estimated by using:

$$I(t) = \frac{1}{\Delta t L_z} \sum_{i=1}^N q_i [z_i(t + \Delta t) - z_i(t)] \quad (4)$$

as outlined in Aleksij Aksimentiev and Klaus Schulten, *Biophysical Journal*, **88**:3745–3761, 2005.



**Warning.** Be extremely careful when reporting ionic currents, as the values you obtain may depend on several simulation parameters, such as the use of Langevin Dynamics or PME electrostatics. For instance, it is well known that the TIP3P model of water exhibits a diffusion coefficient that is larger than the experimentally observed value. It is also known that the use of Langevin dynamics adds an additional artificial viscosity playing against the ion movement. Thus, we strongly recommend you perform a calibration of ionic currents in bulk electrolyte. Moreover, note that you can estimate the statistical error of your sample by using the root square of the number of permeation events, which for a few events can be rather large ( $I = I_m/\sqrt{N}$ ).



**Homework.** Write a script that permits you to compute the ionic current across a channel given a molecular dynamics trajectory. The script should compute the ionic current by either counting the number of ions crossing the transmembrane pore, or by measuring the displacement current defined in Eq. 4

This ends the Ion Conduction and Electrostatic Maps tutorial. We hope that you learned a lot with it, and that you will be able to apply similar analysis tools to your own molecular dynamics simulations.