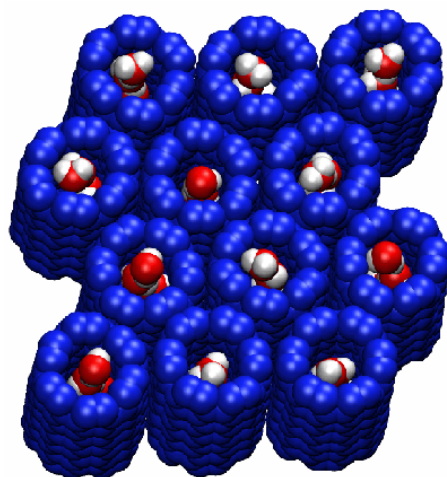University of Illinois at Urbana-Champaign
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Simulation of Water Permeation Through Nanotubes

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Contents

1 Initial Setup

In order to complete this tutorial, you will be required to have up-to-date versions of the following software, properly installed on your computer:

- VMD www.ks.uiuc.edu/Research/vmd/

  - You will need to install VMD 1.8.4 or later.

- NAMD www.ks.uiuc.edu/Research/namd

- a text editor of your choice (we offer a few easy-to-use recommendations):

  - UNIX: nedit (www.nedit.org)
  - Windows XP: NotePad (included with OS)
  - Mac OS X: TextEdit (included with OS)

- a command prompt, such as a terminal in UNIX, Terminal.app in Mac OS X, or the DOS command prompt (found under Accessories) in Windows.

If you downloaded the tutorial from the web, the files that you will be needing can be found in a directory called `files`. If you got the files through a workshop, they can be found in the ~/`Workshop/nanotubes-tutorial` directory. In the rest of this tutorial, we will refer to this directory as the `nanotubes-tutorial` working directory.

In a Terminal window, you can move to this directory by typing:

```
cd <path to nanotubes-tutorial working directory>
```

You can list the content of this directory, by using the command:

`ls`, in a UNIX or Mac OS X terminal, or
`dir`, at the Windows command prompt.

2 Description of the system

In the following exercises, you will be investigating the permeation of water through nanotubes, as a model for transmembrane permeation of substrates through channels. Before starting the simulations and analyzing them, let's have a look at the system that you will be using for your exercises. For the simulations you will be using a set of four nanotubes arranged side by side that separates two layers of water. This is, in fact, your **unit cell**, and in actual simulations will be replicated in three dimensions using **periodic boundary**

conditions (PBC).

1. Start VMD, and load a new molecule with the files `nanotubes.psf` and `nanotubes.pdb`, located in the `nanotubes-tutorial` working directory. You should see four nanotubes arranged in a membrane with water on both sides. This is your **unit cell**. The dimensions of the unit cell are 24.07 by 20.85 by 34.00 Å^3.

We now wish to see what the periodic system will look like (since the NAMD simulation will be using periodic boundary conditions).

2. In VMD, open the TkCon console (from the Extensions menu), and set the periodic cell dimensions by typing:
```
molinfo top set a 24.07
molinfo top set b 20.85
molinfo top set c 34.
```
`a`, `b` and `c` refer to the periodic cell dimensions in x, y and z, respectively. The above commands tell VMD the dimensions of the periodic cell that you are using.

3. Replace the current representation to only show the nanotubes. Open the Representations window and enter `carbon` for the text selection, and VdW for the Drawing Method. Then choose the Display → Reset View menu item in the Main window. You should now see a top view of the nanotubes.

4. Now click on the Periodic tab in the Representation window. Making sure that your "carbon" representation is selected, click on the +X, -X, +Y and -Y checkboxes to display more unit cells. Your molecule should now resemble Fig. 1.
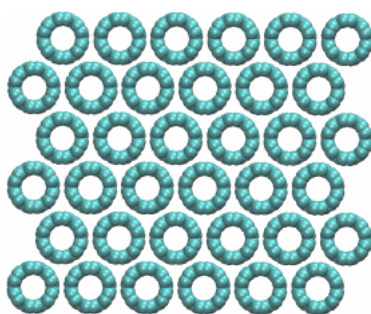
Figure 1: The simulated nanotube array.

5. Now rotate the system sideways, uncheck all the ±X and ±Y checkboxes, and check the ±Z boxes instead.

6. Add a `water` representation and make it periodic in Z as well, just like you previously did for the carbon nanotubes. Your molecule should now resemble Fig. 2.
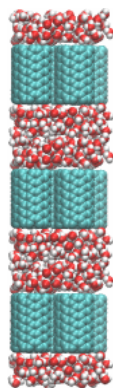
Figure 2: The layers of nanotubes and water in the simulation.

Constructing a nanotube. A tcl script, `make_arm.tcl`, is provided to build psf and pdb files for nanotubes of various sizes. The procedure takes three arguments, the width (m), the length (n), and the name for the output files. After sourcing the file in VMD (`source make_arm.tcl`), it can be used by typing, e.g.,

```
make_arm 6 12 nanotube
```

which will construct a single nanotube of the same size as those in `nanotubes.psf/pdb`.

3 Water diffusion and permeation through nanotubes

In this section, you will observe water movement through an array of nanotubes under two types of simulation conditions, namely, free water diffusion in equilibrium and directional water flow under a hydrostatic pressure difference.

All simulations in this section are performed under periodic boundary conditions, with constant temperature (300 K) and constant volume. To make the simulations faster and analysis simpler, all atoms in the nanotubes are fixed, i.e., only water molecules can move.

For water diffusion, you will look at a 3 ns equilibrium MD simulation that has been provided to you in the form of an already computed NAMD trajectory. Using this trajectory, you will look at water orientation in the nanotubes as well as water diffusion through the nanotubes. Then you will perform another type of simulation, where a hydrostatic pressure difference is induced through the application of forces on some water molecules. In this simulation you will be able to observe a directional water flow through the nanotubes. You will run a short simulation (40 ps) of this type yourself, but we have also provided a longer trajectory (1 ns), which you can use for your analysis. Finally, you will look at a 1 ns MD trajectory of modified nanotubes (with charges assigned to a few atoms), and find out whether the water in the nanotubes behaves differently.

3.1   Submit your simulation

Here, you will submit and start a simulation. The details of the simulation and how you will analyze it will be explained in a later section. Since the simulation will take some time to finish, you need to submit it now so that when you reach analysis of the results, your simulation will be completed.

The following files will be used for your NAMD job
`cnt.psf`
`cnt.pdb`
`par_all27_prot_lipid.prm`
`initial.coor`
`initial.vel`
`sim_short.conf`

1. Make sure you are in the `nanotubes-tutorial` working directory, using `cd` or `dir` in a terminal window.

2. Run the NAMD job. In the same terminal window, type:

       `namd2 sim_short.conf`

Windows users: Instead of typing "namd2", you will need to specify the full path to NAMD, depending on where you installed it. For example, if you installed namd2 in `C:\NAMD\`*, you would type:* `C:\NAMD\namd2 sim_short.conf`

You should now see NAMD's output indicating that the job is running. Now let's move to the next section. You will come back to this job and analyze the results after it has finished.

3.2   Water diffusion in equilibrium

Here, you will look closely at a long (3 ns) trajectory of an MD simulation in equilibrium, which shows free water diffusion through the nanotubes. The respective simulation has been already computed and the results are provided to you in the `nanotubes` working directory.

1. In VMD, delete the molecule that you loaded in the previous section, and create a new molecule by loading the files `cnt.psf` and `eq.dcd`. Loading the trajectory might take a minute or two.

2. Create different representations for nanotubes and water. You can use atom selections `carbon` and `water`, for nanotubes and water, respectively. Feel free to choose your favorite `Drawing Method` and `Coloring Method` for these representations.

3. Observe water orientation inside the nanotubes. Also look at how the water molecules are aligned. You should find that water molecules in the same nanotube are all aligned along the same direction (i.e., either all with their O atoms up and H atoms down, or all with H atoms up and O atoms down). Think about why they prefer such a concerted alignment. Play the trajectory with the VMD animation controls, and see whether the orientation remains stable during the simulation. In particular, did you observe any flipping of their orientation? (If yes, how many times?)

4. Observe single file water diffusion. To identify individual water molecules, you can label a couple of them in a nanotube (choose Mouse → Label → Atoms in VMD). Now play the trajectory. How do water molecules move with respect to each other? Is any water molecule ever found to pass another one in the nanotube? Otherwise do they always move in concert? Also look at the direction of the water movement in nanotubes. Does it move back and forth, just like one-dimensional Brownian motion?

5. Observe permeation events. A permeation event is defined as a water molecule entering from one end of a nanotube and leaving the other end, therefore traversing the entire length of the nanotube. Can you find some of the permeation events in the trajectory? Obviously, it is a tedious job to find all of them in a long trajectory. So we provide a script, `permeation.tcl`, to help you. To use the script in VMD, you should first make sure that the trajectory to be analyzed is the "top" molecule. Then open the TkCon window in VMD and after making sure you are in the `files` directory, type into the window:

```
source permeation.tcl
```

NOTE: While the script is running, VMD may appear to freeze and not respond to other commands. Depending on your machine, this may take 10-30 seconds.

The script will list the *resid* number of all the water molecules permeating through the nanotubes, the frame at which the permeation event completes, and its direction into the TKCon window. You can pick a few water molecules from the list, and check whether they have indeed completed the permeation event as reported by the script. In the last two lines of the output, the script also tells you the total number of permeation events in each direction. Are the two numbers close to each other, as would be expected?

Some additional details: You won't find any permeation events in the very beginning (say, the first 200 ps) of the trajectory. This is because for water molecules initially inside a nanotube, you don't know from which end of the nanotube they had entered. Consequently, when they leave the nanotubes, no permeation event can be counted. Therefore, for quantitative study, the beginning portion of the trajectory is usually excluded from statistics. In your case, the numbers of permeation events reported by the script `permeation.tcl` do not include those in the first 500 frames (500 ps), thus are actually for permeation events within the last 2.5 ns of the simulation.

6. After all of the above steps are done, delete the molecule from VMD, since it takes up a significant amount of memory in your machine.

3.3   Simulation with induced pressure difference

Now you will come back to the simulation that you submitted at the beginning. First, let's learn something about the background and motivation for this type of simulation.

In experiments, a typical method to study water channels is to set up different concentrations of (impermeable) solutes on the two sides of the channel, thus giving rise to an osmotic pressure difference, and to measure the net directional water flux. Obviously, the relationship between the water flux and the osmotic pressure difference cannot be studied by equilibrium simulations.

It is known that an osmotic pressure is equivalent to a hydrostatic pressure. Therefore, if one can generate a hydrostatic pressure difference in MD simulations, one could mimic the experiments mentioned above. This can be done by applying a constant force along the z direction on a layer of bulk water molecules. The force will induce a pressure gradient in the bulk water layer, which results in a pressure difference on the two sides of the nanotubes due to the periodic boundary conditions (refer to Fig. 2). One appealing feature of this method is that the amount of pressure difference can be easily calculated and tuned, thus the results can be compared to experiments quantitatively.

In the submitted simulation, a constant force of 0.4 Kcal/mol/Å along the $+z$ direction was applied to a 5.4 Å-thick water layer. In every time step of the simulation, the force will be applied to the O atoms of water molecules whose coordinates (in the unit cell of the periodic system) satisfy $z > 12.5$Å or $z < -12.5$Å. This is a fairly large force, but it is needed to induce a very fast water flux that can be observed in a very short simulation time (practically you can only afford a 40 ps simulation for the purpose of this tutorial). You may take a few moment to inspect the NAMD configuration file `sim_short.conf` for this simulation.

1. Check whether the simulation has already finished. If not, wait a few minutes for it to finish (after 40,000 steps).

2. In VMD, create a new molecule with the files `cnt.psf` and `sim_short.dcd` (the DCD file generated by your simulation).

3. Play the trajectory and observe the water flow. Since water molecules in the nanotubes move concertedly, you can label any of them in a nanotube, and its movement will indicate the movement of the whole water chain. Did you observe the drift of the water chains toward $+z$ direction in the trajectory? What is the net water flow through the nanotubes during this simulation?

NOTE: The net water flow is in general different from the number of permeation events. For example, when each water molecule moves one step in the single file, effectively a water molecule is transferred, thus resulting in a net water flow of one water molecule, although this move may not cause a permeation event. In the equilibrium simulation you looked at earlier, there was little net water flow, although you observed a lot of permeation events. In contrast, here in the present (short) trajectory, you might not see any individual water molecule crossing all the way through the nanotubes, but you can observe a directional water flow.

The trajectory of your simulation (40 ps) is too short for good statistics. Therefore, here we provide to you another trajectory, which was run at exactly the same conditions as the simulation you ran, except that this one is longer (1 ns), which allows you to observe more water flow.

4. In VMD, delete all the frames of the current molecule (which should be `cnt.psf`), by going to Molecule → Delete Frames → Delete. Then load into the DCD file `sim.dcd`, by first selecting the `cnt.psf` molecule and then calling the File → Load Data Into Molecule... menu item.

5. Observe the water flow in the long trajectory. Since the total water flow is fairly large, it will be too tedious to count it by hand. Therefore, we have provided to you a script, `flow.tcl`, to count the water flow. In VMD, make sure that the 1 ns trajectory is the "top" molecule, and then type in the TKCon

window `source flow.tcl`. The script will report the amount of net water flow and its direction.

NOTE: While the script is running, VMD will freeze and will not respond to other commands. Depending on your machine, this may take 10-20 seconds.

Exercise: Can you calculate the hydrostatic pressure difference in this simulation? The pressure difference is $\Delta P = nf/A$. Here you know that f is 0.4 Kcal/mol/Å, the unit cell of the periodic system has dimensions of 23 Å× 19.9 Å× 30.4 Å, and n, the number of water molecules in the 5.4 Å-thick layer (water molecules on which you are applying force), can be roughly estimated by the volume of the layer and the molar volume of water (55.5 mol/l). What is your calculated pressure difference? How does it compare to one atmosphere (10^5 Pa)? Now you may realize how "hard" it is to induce a significant water flow within 40 ps.

6. You may also look at water orientation in the nanotubes as water is being conducted. Does the fast flow alter the water orientation that you had observed in the equilibrium case?

3.4 Water properties in modified nanotubes

So far, you have been looking at uncharged nanotubes. Now you will look at the effect of modifications of the nanotubes.

Here is your new system: a positive charge (+1e) is assigned to the center of the nanotubes, and two negative charges (-0.5e each) are assigned to the edges of the nanotubes. Other parts of the new system are exactly identical to your previous system. You will see later how you can do such a modification by changing the psf file.

We ran a 1 ns simulation on this new system, with induced pressure difference as described earlier. The simulation conditions were exactly the same as the previous simulation of the unmodified nanotubes. The trajectory is provided to you in the form of the file `sim_mod.dcd` available in the `nanotubes` working directory.

1. In VMD, create a new molecule with the files `cnt_mod.psf` and `sim_mod.dcd`.

2. Visualize the charges of the modified nanotubes: highlight the charges, by adding a *CPK* representation of atom selection `carbon and charge!=0` in VMD, and choose `Charge` in the Coloring Method.

3. Observe water orientation in the modified nanotubes. Does the water orientation in the nanotubes now look very different than what you observed before?

You should see a "bipolar" orientation here, in which the water molecules at the top and bottom of the channels are oriented along opposite directions. Play the trajectory. Is such a bipolar orientation well conserved during the simulation? You may want to pay particular attention to the water molecule in the center of each nanotube and the positively charged atoms there. Do they seem to be interacting strongly with each other?

4. Observe water permeation in modified nanotubes. Play the trajectory and look at the movement of single water files inside the nanotubes. Do they seem to move as fast as what you observed before in the unmodified nanotubes? Now count the net water flow in this 1 ns trajectory (you can do this by hand, or by using the script `flow.tcl` following earlier instructions). How does it compare to the water flow in the simulation of unmodified nanotubes (which were performed under the same conditions)? Now you may get an idea how much the permeation property of the nanotubes can be altered by introduction of charges. Think about the reason for such a difference.

4 "Decorating" the nanotubes

4.1 Running a simulation with AutoIMD

Here, we describe a VMD extension called AutoIMD (where IMD stands for Interactive Molecular Dynamics). The purpose of this tool is to automatically setup a NAMD simulation that can be visualized in real-time in VMD. AutoIMD takes care of generating all the necessary files (PDB/PSF/NAMD config) and of launching and connecting to a NAMD simulation. Once you have connected VMD to a running simulation, you can see your molecule's motion as it is being computed!

1. In VMD and in the `nanotubes` working directory, load the files `imdnanotube.psf` and `imdnanotube.pdb` into a new molecule.

2. Create a new representation for the molecule in VMD. Select only atoms of the nanotube for the new representation by typing `carbon` into the atom selection window. Choose the Charge Coloring Method. This will display different charges using different colors.

To get started with AutoIMD, follow these steps:

3. Open the AutoIMD window through the VMD Extensions →Simulation →AutoIMD menu item.

4. In the AutoIMD window, select the Settings → Simulation Parameters... menu item. A new window should pop up (Fig. 3). In this new window, com-

pletely erase the contents of the text input field named CHARMM Params, and then click on the Add... button. You will now need to locate the CHARMM parameter file called par_nanotubes.inp in the nanotubes working directory and click on Open. When you have specified the right parameter file, you can dismiss the Simulation Parameters window (by clicking on its close box).
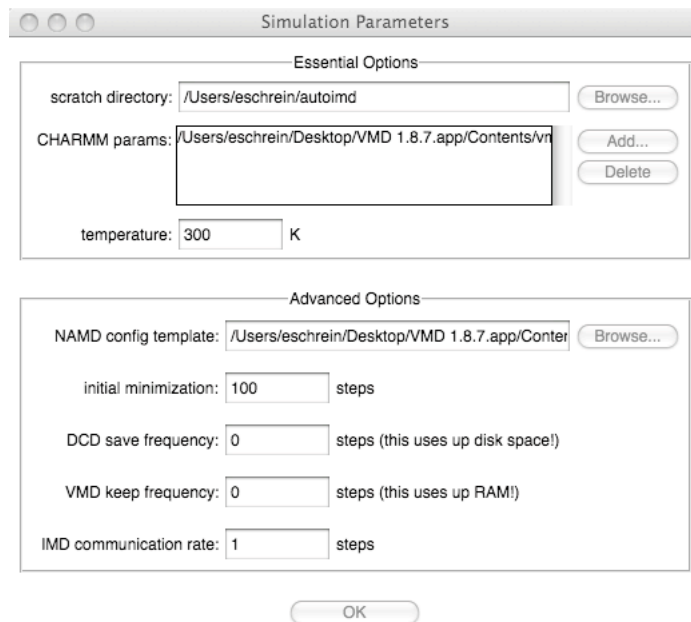

Figure 3: The AutoIMD Simulation Parameters dialog.

AutoIMD allows you to specify a region of your molecule that you wish to simulate. To do this, it separates the molecule into three regions: a *molten* region, a *fixed* region and an *excluded* region. The molten region is the one that will be moving in the simulation. To hold the molten region in place, AutoIMD finds all the atoms that surround it (this is the fixed region) and uses them as unmoving constraints for the molten region. Atoms that are very far from the molten region are not simulated in order to make the simulation faster; they constitute the excluded region.

5. In the AutoIMD window, in the molten selection text input box, enter same residue as water and (x*x + y*y < 16) . This corresponds to the cylinder of water of radius 4 Å that we wish to simulate. The other atoms will become part of the "fixed" region.

6. Then, choose Local from the next menu in the window. This will make the

simulation run on your local machine. After these steps, your AutoIMD window should look like Fig. 4.
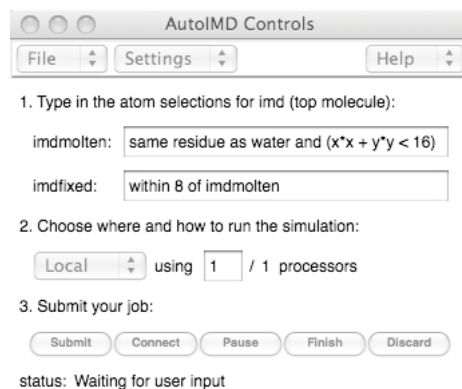

Figure 4: The filled-in AutoIMD window.

7. Now, click on Submit. This should create and launch a NAMD simulation on your machine. You should now see a second molecule called AutoIMD Simulation, which will be used to connect to the running simulation.

NOTE: If AutoIMD asks to create a directory called `autoimd` *in your home directory, answer* Yes.

NOTE: If you get an error message saying: `Unable to open psf file imdnanotube.psf`*, you need to reload your PSF file on top of your current molecule one more time and click* Submit *again.*

Windows users: AutoIMD will ask for the location of the `namd2` *executable. Simply point it to where you installed NAMD.*

8. Finally, click on the Connect button to start displaying your simulation. After 100 steps of minimization, the water molecules in your VMD graphics window will begin to move. There will be a time counter in the AutoIMD window's status line indicating the minimization steps, and later, the simulation time (in ps).

NOTE: It might take a few seconds for the NAMD simulation to get started. During this time, VMD will not be able to connect and you will see some error messages of the type: `Error connecting to localhost on port 2314`*. This is perfectly normal, and these errors can be ignored. VMD will connect as soon as NAMD is ready.*

9. The nanotube will appear by default as bunch of chubby spheres. To see the water through it, select the "imdhetero" representation in the AutoIMD molecule, and change the drawing method from VdW to Bonds.

10. When you are done (after, say, 10 ps or more), click on the Discard button. This will halt the simulation and bring you back to the molecule as it was before you started AutoIMD. If you had clicked on Finish instead, the final coordinates of the simulation would have been saved into your initial molecule (overwriting the old coordinates).

11. If you want to run a new simulation with different initial coordinates or modified parameters (or simply to re-run your old simulation) you would now only need to repeat steps 8 to 10 (*i.e.*, click on Submit, Connect and Discard).

4.2   Modifying the charges

Here, we will learn how to modify the electric charge of individual atoms, as they appear in the NAMD simulation.

Exercise: We have seen that water permeates through the nanotubes in a single file of oriented water molecules. We would like to modify the nanotube such that we get a bipolar configuration of water, such as the one seen in the glyceroaquaporin pore (*i.e.*, the water oxygens at both ends of the pore point toward the center of the pore). One way to achieve this is to assign charges to a few carbon atoms in the nanotube. Think of a way that you can charge 3 or more of the nanotube's carbon atoms to achieve this. You will soon run a simulation using these charges to test your predictions.

The following instructions detail how to change the charge of an atom in a PSF file (which in turn is used by NAMD simulations).

1. Make a copy of the `imdnanotube.psf` file (let's call it `mynanotube.psf`, for example) and open it in your favorite text editor. In the PSF file, you should see a bunch of lines that look like:

```
64 NT22 1    ARM  C063 CA      0.000000       12.0110        0
65 NT22 1    ARM  C064 CA      0.000000       12.0110        0
66 NT22 1    ARM  C065 CA      0.000000       12.0110        0
```

2. You want to change the charges of a few carbons in the nanotube, but first you need to identify these atoms by their unique index. Using the Mouse → Query mode in VMD and then clicking on atoms, get the indices of the carbon atoms whose charges you have decided to change (with the query mouse mode, you can read the atom indices in the console window or the Graphics → Labels window).

Note: VMD counts atoms starting from 0, while the PDB and PSF files start at 1. Thus, you need to add 1 to the index you get from VMD to get the atom index in the PSF file.

3. In the PSF file (in the text editor), find the row corresponding to the atom index that you are looking for (the atomic index is in the first column). Do not forget to add 1. *Example:* if you wish to change the charge of the atom with index 65 in VMD, you need to edit the row starting with `66`.

4. Now, change the column that says `0.000000` to a new number. This number should be a multiple or fraction of the electron charge e. *Important:* Make sure not to change the column alignment, as every space in the PSF file is important! For example, to assign a charge of *-e/2* to the 65th (VMD's index) atom, you would substitute the line

```
  66 NT22 1     ARM  C065 CA      0.000000        12.0110         0
```

by

```
  66 NT22 1     ARM  C065 CA     -0.500000        12.0110         0
```

5. Save your changes to the new PSF file.

6. Back in VMD, load your new PSF file (`mynanotubes.psf`) *into* your previous molecule (by selecting it and choosing the File → Load Data Into Molecule... menu item, *etc.*). This will replace the charges that were previously there. Select your nanotube (`carbon`) representation in the Graphical Representations window and click on the Apply button to redraw the nanotube with the correct charge colors. You should now see different colors for different charges, as in Fig. 5.

7. You can repeat this process as many times as you want, until you get the charge distribution that you like to experiment with. Once you have a decorated nanotube, you are ready to run the simulation on your local machine with AutoIMD. Just follow instructions 8 to 10 from section 4.1 (*i.e.*, click on Submit, Connect and Discard).

4.3   Experimenting with different charge configurations

Now that you know how to change the charges in a simulation, here are a few numerical experiments that you could do:

- Try placing a single large negative charge (*i.e.*, -4e) at the center of the nanotube. Observe the simulation for about 10 ps. What happens to the water file inside the nanotube? Can you explain what causes this behavior?
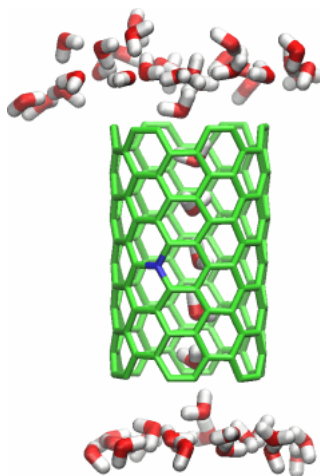
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Figure 5: A decorated nanotube, showing a negatively charged carbon atom (in blue) near its center.

- What happens if you place instead a positive charge at the center?

- You may experiment with a few charge distributions of your choosing. For example, what happens if you reverse the charges that you have applied in the previous section? What happens if all your charges are vertically aligned along the nanotube, is it the same as if they were distributed somewhere else around the tube? And finally, would the answer to this last question remain the same if you inverted all the charges in the tube?

4.4   Modifying the VdW parameters

Here, you will learn how to modify the VdW radius of a certain type of atom in a NAMD simulation. you will significantly reduce the radius of the nanotube's carbon. This will make the pore wider.

Question: Try to predict what will happen if you make the nanotube wider. As you will soon see, the answer to this will be quite surprising.

1. Make a copy of the **par_nanotubes.inp** parameter file (let's call it **par_mynanotubes.inp**; it is very important that it starts with **par_** and ends in **.inp**!) and open it in a text editor. Near the end of the file, you should see the following lines:

```
!atom  ignored    epsilon     Rmin/2
CA     0.000000  -0.070000    1.992400
HT     0.000000  -0.046000    0.224500
OT     0.000000  -0.152100    1.768200
```

These lines specify the van der Waals parameters for each given atom type, which will be used by the NAMD simulation. Fig. 6 shows the meaning of the epsilon and Rmin/2 parameters in the van der Waals potential energy function.
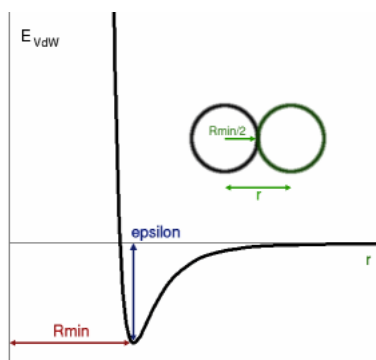

Figure 6: The van der Waals potential energy function.

2. Reduce the VdW radius parameter by a factor of 4 for all the `CA` atoms, by editing the line:

```
CA      0.000000   -0.070000     1.992400
```

so that it now says:

```
CA      0.000000   -0.070000     0.5
```

3. Save your changes to the parameter file.

4. To run an AutoIMD simulation with this new parameter file, you need to open AutoIMD's Settings → Simulation Parameters... window. Completely erase the contents of the CHARMM params line, click on Add... and then select your modified `par_mynanotubes.inp` file. Your next AutoIMD session will use these modified parameters.

5. Make sure that you are using an uncharged nanotube by reloading the original `imdnanotube.psf` PSF file back into your molecule again (to override your previous changes).

6. Finally, start AutoIMD (by clicking on AutoIMD's Submit button, *etc.*) and observe what happens to the water file after 10-20 ps.

If you are surprised, do you think that you could have predicted what just happened? In order to explain why the water departed from the pore, it might be helpful if you used VMD to display both the nanotube and the permeating

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waters by using the VdW Draw Style. In order to display, the carbons' approximate size, you can shrink their VdW Sphere Radii (in the VMD Representation window) by a factor of 4 (*i.e.*, Sphere Radius = 1.0/4 = 0.2-0.3). By inspection, what can you say about the water-nanotube interaction with the big and the small carbons. Remember that at the VdW radius (*i.e.* when the "spheres" touch) the VdW interaction is maximally attractive.

4.5   Experimenting with VdW parameters

Now that you know how to change the VdW parameter, here are a few more experiments that you can do.

- When you made the pore larger by reducing the carbons' radii, you decreased the surface of the favorable VdW interactions between the permeating water molecules and the nanotube. Instead of being surrounded by carbon, the waters are now only attracted to one side, and therefore the water-nanotube interaction is much smaller. Can you change the carbon's VdW maximum binding energy (`epsilon` in the parameter file) so that the water file remains stable inside the pore (as opposed to leaving the pore as before), while still keeping a small carbon VdW radius parameter of `0.5`? Make sure that epsilon is always negative or else the simulation won't run.

- Revert your changes by recopying the original parameter file. What happens if you slightly increase the carbons' VdW radius parameter so as to increase the contact area with water? How can you observe this increased interaction in your water file? Can you find the optimal `epsilon` that will maximize the number of water molecules in the pore?

Acknowledgments