

## Lab III: MD II & Analysis - Windows

In this lab you will:

- 1) Add water, ions, and set up and minimize a solvated protein system
- 2) Learn how to create fixed atom files and systematically minimize a new structure.
- 3) Set up NVT and NPT molecular dynamics simulations including PBC/PME.
- 4) Analyze output files from MD runs, print and plot thermodynamic and structural variables.

**Working directory: C:\Users\yourname\Desktop\aps\_2016\exercises\lab\_III**

NOTE: in this lab, the “>” symbol means that you are in an active terminal window and what follows this symbol is what you type. Make sure you know what directory you are in (> pwd) and what files are in your current directory (> ls). The symbol “vmd>” means you type what follows inside the VMD console (not a Linux terminal). One final note, in problem 5 (d&e) you will run a plotting program called Gnuplot. Once started, it presents the user with a prompt “gnuplot>”. Therefore, you type what is past this prompt in the active Gnuplot program.

### 1. Add water and ions to solvate the protein hydrolase.

In Lab II yesterday you built several water, protein, and complex systems. You minimized one (or more structures) in vacuum. In this problem, you will build a more applicable model system for the protein hydrolase (“protein\_II”) from Lab II. You will use VMD GUI tools to add a solvation box, then you will use another VMD GUI to add ions, and you will type some TCL commands in the VMD console to measure the resulting periodic box size. You will need these numbers for subsequent problems.

Directories:

solvate\_and\_add\_ions\

Files: you will generate these!

Procedure:

- a) Change to the solvate\_and\_add\_ions directory and copy the complete directory for “protein\_II” that you generated yesterday to this location.
  - i. > **robocopy**  
**C:\Users\yourname\Desktop\aps\_2016\exercises\lab\_II\psfgen\protein\_II protein\_II /S**
  - ii. NOTE: The /S flag to robocopy copies subdirectories. If your version of robocopy does not correctly copy the expected files replace the /S flag with /s /i.
- b) *Open VMD* and change to the working directory (C:\Users\username\Desktop\aps\_2016\exercises\lab\_III\solvate\_and\_add\_ions\)
- c) Load the new\_hydrolase.psf and new\_hydrolase.pdb files.
- d) Using the pull-down menu, choose the Extensions -> Modeling -> Add Solvation Box item.
- e) Uncheck the ‘Waterbox Only’ box.
- f) Check the Input PSF: and Input PDB: fields to make sure that they are pointing at the full path and filenames for the “new\_hydrolase” system.
- g) In the Output field, name the output file “solvated\_proteinII”.
- h) Make sure that the ‘Use Molecular Dimensions’ box is selected (checked).
- i) Enter the value “12.0” for each of the Box Padding for all min and max values of x, y, and z.
- j) Hit the ‘Solvate’ button at the bottom.
- k) The resulting files (solvated\_proteinII.psf and solvated\_proteinII.pdb) will be created in the VMD working directory. If you placed them somewhere other than

Desktop\aps\_2016\exercises\lab\_III\solvate\_and\_add\_ions\ then move them to your current location:

- i. **> move <location>\solvated\_proteinII\* .**
  - ii. <location> should be replaced with the full path to the location in which the files were saved (e.g. C:\Users\yourname\Desktop\aps\_2016\exercises\stuff).
  - iii. NOTE: the trailing period at the end of the command above.
  - iv. NOTE2: The "\*" symbol merely tells the "move" program to move all files that start with solvated\_proteinII and have any additional characters following (including NO additional characters). The "\*" is called a wild-card. The trailing period means "put it HERE".
- l) Now, using the pull-down menu, choose the Extensions -> Modeling -> Add Ions box item.
  - m) Input the newly created solvated\_proteinII (.psf & .pdb) files (Browse if needed).
  - n) Enter "ionized\_solvated\_proteinII" in the Output prefix: field.
  - o) Enter "ION" in the Segment name of placed ions: field.
  - p) Make sure that the 'Only neutralize with NaCl' box is checked.
  - q) Look at Figure 1 below to check your input values. Then, click the Autoionize button at the bottom.
  - r) The resulting files (ionized\_solvated\_proteinII.psf and ions\_solvated\_proteinII.pdb) will again be created in the VMD working directory. If necessary move them to the Desktop\aps\_2016\exercises\lab\_III\solvate\_and\_add\_ions\ directory with a similar command to before (replacing solvated\_proteinII with ionized\_solvated\_proteinII).
  - s) Using the "2" button (or the Mouse -> Label -> Bonds from the VMD GUI), measure the x, y, z dimensions of the system. Write down your values.
  - t) Enter the commands listed below into the TkConsole to use TCL commands to measure the dimensions of your system. Write down your values. Type the text AFTER the "vmd >" prompt below.  

```
vmd> set everyone [atomselect top all]
vmd> measure minmax $everyone
```
  - u) Compare your measured values in step (q) above to those obtained in step (r).
  - v) Visualize your final system using VMD. Use VMD "Representations" to depict the protein as "New Cartoon", the water molecules by "Points" and the ions by VDW spheres.

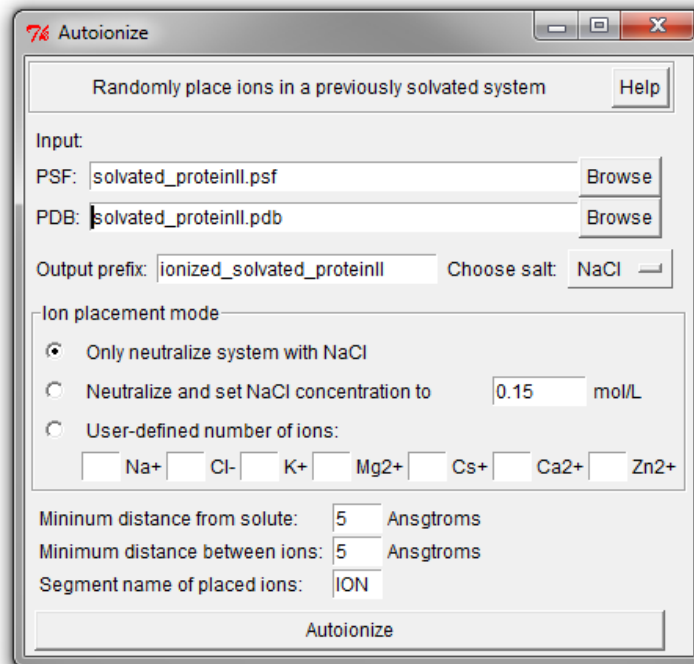


Figure 1. Autoionize input GUI.

## 2. Create fixed atom files to be used to systematically minimize the solvated hydrolase system.

In the lecture, we went through the process of generating fixed atom files so that we could keep parts of our system rigid while we relax everything else. In this problem you will generate ONE of the same fixed atom files. The others will be provided to you due to time limitations. Of course, it would be beneficial if you can create all three fixed atom files yourself. If you wish to do this, refer to the slides in the talk for the correct TCL commands to do this. You will use these fixed atom PDB files in the remainder of this lab.

Directories:

```
namd\  
  minimization_II\  
    output\  
      
```

Files: there are files in both the minimization\_II and minimization\_II\output directories.

```
minimization_II\  
  smin0
```

```
minimization_II\output\  
  fixed_backbone_atoms.pdb  
  fixed_secondary_structure.pdb
```

Procedure:

- a) Change to the namd\minimization\_II directory.
- b) Copy the final files you created in (1) to the namd\minimization\_II\output directory.
  - i. **> copy ../../solvate\_and\_add\_ions/ionized\* output\**
  - ii. NOTE the “../../” at the beginning of the statement above means go up to directory levels (of course one set of “..” would tell the copy command to go up a single level.
  - iii. NOTE2: the “\*” is the wild-card and the final “output” tells to put the files into the local directory named “output”.
- c) If VMD is still open, close the program and *restart VMD* by clicking the icon on the desktop.
- d) Load the ionized\_solvated\_proteinII.psf file and then load the coordinates into the same molecule by loading the ionized\_solvated\_proteinII.pdb file.
- e) Create a new fixed atom file named: fixed\_protein\_atoms.pdb using the commands listed in the figure below in the TkConsole. Examine the resulting output fixed\_protein\_atoms.pdb file. Confirm that the correct atoms have either 1.00 or 0.00 in the beta column.

```
vmd> set everyone [atomselect top all]  
vmd> $everyone set beta 0.00  
vmd> set sell [atomselect top "protein"]  
vmd> $sell set beta 1.00  
vmd> $everyone writepdb fixed_protein_atoms.pdb
```
- f) Make sure that the newly created fixed\_protein\_atoms.pdb file is in the namd\minimization\_II\output directory (move it there if it is created somewhere else, such as “namd\minimization”).
- g) List the minimization\_II\output directory and inspect the other fixed\_atom files that are there (fixed\_backbone\_atoms.pdb and fixed\_secondary\_structure.pdb). Make sure that you understand what these files are doing (if needed, open them up as new molecules and color using the BETA value).
- h) If you have time, you can try to create your own versions of fixed\_backbone\_atoms.pdb and fixed\_secondary\_structure.pdb files. The slides in the MD-II lecture cover have the exact commands to execute.

3. **Set up and run a series of restrained minimization runs of the ionized\_solvated\_proteinII system using the fixed atom files created in step (2).**

In the lecture we showed how you sequentially relax a newly built system in order to retain the original biological structure. In this problem, you will use the fixed atom files (one or more of which you generated in (2) above) in a series of minimization runs to relax your newly built system. You will write (and edit) a set of input files for the minimization runs and run the jobs using NAMD. The final file from this sequence of minimization runs (smin3.coor) will be used in problem 4 below.

Directories:

```
namd\  
  minimization_II\  
    output\  
      
```

Files: there are files in both the minimization\_II and minimization\_II\output directories.

```
minimization_II\  
  smin0
```

```
minimization_II\output\  
  fixed_backbone_atoms.pdb  
  fixed_secondary_structure.pdb  
  fixed_protein_atoms.pdb (created in step (2e) above)  
  ionized_solvated_proteinII.psf (copied here in step (2b) above)  
  ionized_solvated_proteinII.pdb (copied here in step (2b) above)
```

Procedure:

- a) Change to the namd\minimization\_II\ directory.
- b) Inspect the NAMD input file named smin0.
  - i. Inspect the cellbasisvector lines, how do they compare to the dimensions of the system as measured in step 2?
- c) Start the minimization by typing:
  - a. **> START ""**

To open a new window then

- b. **>namd2 smin0 > smin0.out**
- c. ALTERNATIVELY, since we have dual-core CPUs, you could run this job in parallel using the command:
  - i. **> charmrun +p 2 \$(PATH\_TO\_NAMD)/namd2 smin0 > smin0.out**  
or  
**> namd2 +p 2 smin0 > smin0.out**  
See lab2 for an explanation of the two commands.
  - ii. **DON'T DO IT BOTH WAYS AT THE SAME TIME.** Choose one. In either case the output is saved to a file called "smin0.out".
- d) Observe the progress of the minimization by typing :  
**> tail -90 smin0.out**
- e) repeat the command as the run progresses
- f) Copy the smin0 file and to a new file called smin1  
**> copy smin0 smin1**
- g) Edit the smin1 file to use the output coordinates from the last run and change the fixed atom file to use the fixed\_backbone\_atoms.pdb file. Also change the name of the output file (see lecture slides).
- h) Minimize the system using similar commands as used above (c-e).
- i) Copy the smin1 file and to a new file called smin2

- ```
> copy smin1 smin2
```
- j) Edit the smin2 file to use the output coordinates from the last run and change the fixed atom file to use the fixed\_secondary\_structure.pdb file. Also change the name of the output file (see lecture slides).
  - k) Minimize the system using similar commands as used above (c-e). You need to change the actual names of the input and output files accordingly!!!
  - l) Copy the smin2 file and to a new file called smin3
 

```
> copy smin2 smin3
```
  - m) Edit the smin3 file to use the output coordinates from the last run and remove the fixed atom lines. This final minimization run will let all atoms move. Also change the name of the output file (see lecture slides). Make sure that the output file for this run is smin3 (you will need smin3.coor for the problems described below).
  - n) Minimize the system using similar commands as used above (c-e).
  - o) Examine the final structure(s) with VMD. NOTE: the final coordinate file you should visualize is in a file called smin3.coor in the output/ directory. Since we save the coordinates as text files (i.e. not using the binary output option) NAMD writes these files with the “.coor” suffix, but it is really a PDB file. When you open this file with VMD it will think that the input file type is NAMDBIN. You have to use the pull down menu to tell VMD that it is a PDB file. Alternatively, you can merely make a copy of the smin3.coor file so that it has the “.pdb” suffix that VMD will associate with a PDB formatted file.

#### 4. NAMD NVT and NPT molecular dynamics.

In this problem you will take your minimized hydrolase system and start a molecular dynamics trajectory in the NVT ensemble and then continue the same run in the NPT ensemble. NVT merely means that the number of particles (atoms), volume, and temperature will be held fixed, while NPT means that the number of particles (atoms), pressure and temperature will be held fixed. We will perform these simulations at 1 atmosphere at 300 K.

The NPT MD run is set up to run for 1 million time-steps (i.e.  $10^6$  femtoseconds = 1 nanoseconds). Your laptop will not be able to finish this run in time for our analysis lab tomorrow, so while you can let this job continue for the duration of the course, we'll use completed NVT & NPT runs in the next problem (i.e. we'll give you the finished files). You should probably cancel this run before you start other assignments in the days ahead as they are computationally demanding as well.

Directories:

```
namd\
  dynamics\
    output\
```

Files: there are files in the dynamics directory. You will move files created in steps (2) and (3) above to the dynamics\output directory.

```
dynamics\
  dyn0
  dyn1
```

```
dynamics\output\
```

- a) Change to the namd\dynamics directory.
- b) Copy the files ionized\_solvated\_proteinII.psf and smin3.coor into the output\directory. You created these files in part 1o and 3n above.

- i. > **copy**  
`..\minimization_II\output\ionized_solvated_proteinII.psf`  
`output\`
- ii. > **copy** `..\minimization_II\output\smin3.coor` `output\`
- c) Inspect the NAMD NVT dynamics input file (dyn0). Write down the name of the output files that will be created.
- d) Start the dynamics run using your input file.
  - i. > **START** ""  
to open a new window
  - ii. > **namd2** `dyn0` > `dyn0.out`
  - iii. ALTERNATIVELY, you could run this job in parallel using the command:  
> **charmrun** `+p 2 $(PATH_TO_NAMD)/namd2 dyn0 > dyn0.out`  
or  
> **namd2** `+p 2 dyn0 > dyn0.out`  
See lab2 for an explanation of the two commands.  
DON'T DO IT BOTH WAYS AT THE SAME TIME. Choose one. In either case the output is saved to a file called "dyn0.out".
  - iv. THIS RUN WILL TAKE SOME TIME, so proceed to section 5 below before completing the rest of this section.
- e) Examine the output file and resulting final structure using VMD.
- f) Inspect the NAMD NPT dynamics input file (dyn1). Write down the name of the output files that will be created.
- g) The dyn1 script will take a really long time to run (at least a day). You can start the run to convince yourself that it works using the similar commands to those you used for dyn0 (pressing Ctrl and C at the same time will quit the run). However, a better way than tying up your laptop for a day is to run it using SASSIE-web.
  - i. Login to your SASSIE-web account.
  - ii. From the main menu select 'Simulate'
  - iii. Click the button to enter the 'Energy Minimization' module (note: this doesn't only perform energy minimization but allows you to specify a NAMD input file).
  - iv. Select 'supply input file' from the 'run type' listbox and check the 'checkbox to enter restart files' checkbox.
  - v. Enter the appropriate files from the current and minimization\_II directories as input such that your screen looks like the following Figure.

**Energy Minimization**

run name:

reference pdb:  or  Local: ionized\_solvated\_proteinII.pdb

input filename (dcd or pdb):  or  Local: dyn0.coor

PSF file name:  or  Local: ionized\_solvated\_proteinII.psf

output file name (dcd):

number of processors:

keep run output files:

run type:

namd input file:  or  Local: dyn1

check box to enter restart files:

velocity restart file:  or  Local: dyn0.rest.pdb.vel

extended system restart file:  or  Local: dyn0.rest.pdb.xsc

Figure 2: Input for the SASSIE-web NPT molecular dynamics run

- vi. Click 'Submit'

- vii. This run will take a long time but your laptop remains free. In the section 5 we will analyse data from a similar run that has been run previously. You can reconnect to this job at any point from the Job Management screen.
- h) Examine the output file (dyn0.out) and resulting final structure from the NVT run (dyn0.coor) using VMD.

## 5. Analysis of MD runs.

In this problem, you will analyze the output from a completed 1 nanosecond trajectory of the hydrolase system you set up in this lab. This lab will introduce you to the way you can “source” TCL scripts (code, commands, etc.) in the VMD console to perform simple analyses.

Directories:

```
namd\  
  finished_dynamics\  
    output\  
      
```

Files: there are files in both the finished\_dynamics\ and finished\_dynamics\output\ directories.

```
finished_dynamics\  
  dyn0  
  dyn1  
  dyn0.out  
  dyn1.out  
  dyn0_no_wrap  
  dyn0_no_wrap.out  
  dyn1_no_wrap  
  dyn1_no_wrap.out  
  rmsd.tcl  
  rmsd_no_correction.tcl  
  residue_rmsd.tcl  
  namdstats.tcl
```

```
finished_dynamics\output\  
  dyn0.coor  
  dyn0.dcd  
  dyn0.rest.pdb.coor  
  dyn0.rest.pdb.vel  
  dyn0.rest.pdb.xsc  
  dyn0.vel  
  dyn0.xsc  
  dyn0_nw.coor  
  dyn0_nw.dcd  
  dyn0_nw.rest.pdb.coor  
  dyn0_nw.rest.pdb.vel  
  dyn0_nw.rest.pdb.xsc  
  dyn0_nw.vel  
  dyn0_nw.xsc  
  dyn1.coor  
  dyn1.dcd  
  dyn1.rest.pdb.coor  
  dyn1.rest.pdb.vel  
  dyn1.rest.pdb.xsc  
  dyn1.vel  
  dyn1.xsc  
  dyn1.xst
```

```
dyn1_nw.coor
dyn1_nw.dcd
dyn1_nw.rest.pdb.coor
dyn1_nw.rest.pdb.vel
dyn1.rest.pdb.xsc
dyn1_nw.vel
dyn1_nw.xsc
dyn1_nw.xst
ionized_solvated_proteinII.psf
smin3.coor
```

Some notes: Essentially there are four sets of files in the finished\_dynamics and finished\_dynamics/output directories.

NVT, 10 ps with wrapped coordinates → dyn0  
NVT, 10 ps without wrapped coordinates → dyn0\_nw

NPT, 1 ns with wrapped coordinates → dyn1  
NPT, 1 ns without wrapped coordinates → dyn1\_nw

- a) Change to the namd\finished\_dynamics directory.
- b) First, let's look at the averages by analyzing the output file from the 1 ns NPT run (with wrapped coordinates).
  - *Open VMD* and change the working directory to the namd\finished\_dynamics directory by typing something like (depending on the location from which you start):

```
vmd> cd
```

```
C:\Users\username\Desktop\aps_2016\exercises\lab_III\namd\finished_dynamics
```

- At the vmd prompt type: **vmd> source namdstats.tcl**
- At the vmd prompt type: **vmd> data\_avg dyn1.out**
- c) Now, let's create files that contain only some of the thermodynamics variables. Your laptop is probably too slow to run more than one of these ... pick one of the following. You will have to alter your plot command in section (d) below if you chose something other than TEMP.
  - **vmd> data\_time TEMP dyn1.out**
  - **vmd> data\_time VDW dyn1.out**
  - **vmd> data\_time ELECT dyn1.out**
- d) View these files by using gnuplot. In a Linux terminal that is in the namd\finished\_dynamics\ directory, type
  - **> gnuplot**
  - Then within the gnuplot command window, you can type, for example,
  - **gnuplot> plot "TEMP.dat" w l**
  - NOTE that the gnuplot program has a "gnuplot>" prompt!
- e) Now, let's take a look at the root-mean square deviation of the protein structure over the 1 ns trajectory.
  - *Open a new VMD session* and change the working directory to the namd\finished\_dynamics directory by typing something like (depending on the location from which you start):

```
vmd> cd
```

```
C:\Users\username\Desktop\aps_2016\exercises\lab_III\namd\finished_dynamics
```

- Load the output\ionized\_solvated\_proteinII.psf and output\dyn1.dcd into a new molecule
- At the vmd prompt type:



- i. `vmd> source rmsd_no_correction.tcl`
  - ii. `vmd> source rmsd.tcl`
    - This will create two new files with rmsd data: `rmsd_no_correction.dat` and `rmsd.dat`.
    - View the results using Gnuplot in a similar manner as was done in section (d) above.
- f) Close VMD and reopen, then load `output/ionized_solvated_proteinII.psf` and `output/dyn1_nw.dcd` into a new molecule
- g) To color the protein with RMSD values for each individual amino acid follow averaged over the entire 1 ns trajectory we are going to use a script supplied by VMD to calculate the RMSD for each alpha carbon atom. The TCL script keeps the results in a special place (called "User") so that the calculated values can be shown on the molecule in question. This is similar to what we have done with the BETA field, but by using the "User" capability we do not have to create a special PDB file with BETA values that correspond to the RMSD of each particular amino acid. Run the `residue_rmsd.tcl` script within the TkConsole as follows:
- ```
vmd> source residue_rmsd.tcl
vmd> set sel_resid [[atomselect top "protein and alpha"
get resid]
vmd> rmsd_residue_over_time top $sel_resid
```
- h) Now, you will use the VMD GUI to color the molecule through the "User" selection. Access the "User" data by merely selecting the "User" option in the "Coloring Method" pull down menu of the "Representation" pop-up menu in VMD. Higher versions of VMD may list "User" option under "Trajectory" in the "Coloring Method" pull down menu.
- i) Repeat the analysis for (b) through (f) using the 1 ns NPT run (without wrapped coordinates). If time allows, repeat (b) through (f) for the 10 ps NVT run(s).
- j) VMD has an odd default coloring scheme (counter intuitive). If you want to color the molecule so that "blue" corresponds to "cold" or "rigid" portions of the molecule and RED corresponds to "hot" or "flexible" portions, then you can reconfigure the color scale settings
- In the VMD GUI, use the pull-down menu to get the color scale settings by clicking on Graphics → Colors, then choose the "Color Scale" tab. Then change the default setting from RWB to BWR.
- k) Since the RMSD calculation is for the protein part only, you may want to exclude the solvent in VMD representation. In "Graphical Representations", type "protein" in the "Selected Atoms" box.