We will be using the **NAMD** program.

I will use the protein Ubiquitin, while you should do this exercise on the `pdb` file of your structure (Experimental structure or Alphafold2 model)

Before you begin.

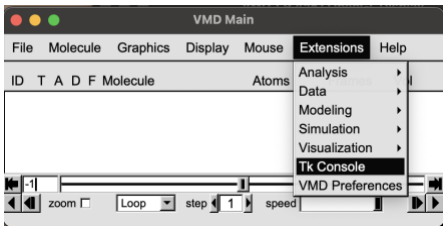
Create a new directory to work in, e.g., named `myrun`. Copy your `pdb` file into this directory.

Also copy all the files provided to you on SUCourse for the purposes of this tutorial.

Launch VMD from your directory, `myrun`.

Part 1. Constructing `psf` files – we will do this first without any water, then for the same protein soaked in water and finally with added ions.

1A. Open your `pdb` file in VMD. Activate 'Tk Console' as shown.



In the command line type

```
source makepsf.tcl
```

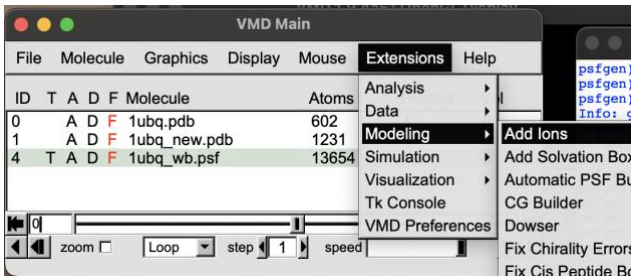
Load your original pdb file into VMD as a new molecule; also load your new pdb file into VMD as another new molecule. Take note of how many atoms there were in your original protein and how many there are after the matching with the topology file. Comment on the source of the differences.

1B. Now go back to your Tk Console and type

```
source makepsfwithwater.tcl
```

How many water molecules are added in your system?

1C. Finally we will add ions to your system. Open the `Add Ions` option as follows:



Your `psf` and `pdb` files should contain the latest one you made – with water. Select a prefix for the files to be created in this step (`ionized` is fine). The choose your salt (this will depend on the environment of your protein – KCl for cytoplasmic ones and NaCl for extracellular ones is usually fine. Neutralize the system at a concentration of 150mM. Set minimum distance from protein and between ions to 3 Å. Hit `Autoionize`.

How many of each type of ion was added into your system? How did you find out?

How many atoms does your system have before and after adding the ions? Rationalize this count.

Part 2. Getting ready to run your MD – configuring the conf file to get your MD on the go.

2A. First start by going back to your Tk Console and typing

```
source measurebox.tcl
```

This computes for you the size (in Å) of the box you created as well as the position of the center of mass coordinates of your system.

2B. Now open the file ionized.conf. **We will edit this together** for your needs and link the given information to what we discussed in class in the last three lectures.

2C. Once the editing is done, run your job; the sample below is for my own computer's settings:

```
charmrun namd2 ionized.conf +p4 > out.txt
```

`charmrun` and `namd2` should point to where these executable files are; +p4 points to the number of processors to use – my computer has four of them. You should find out what this is for your own computer.

Wait for the run to finish. This should not take more than a few minutes.

Part 3. Analyzing output.

3A. We will predict how long an actual MD run will take on your computer. Open the out.txt file with an editor, find the lines which have the word 'Benchmark' in them; read the numbers next to days/ns. This should give you an idea about how much it will take you to get to where you want.

For example, in my case these numbers are around 0.145 – 0.155 days/ns; so, I would get about 6.5 ns/day on my computer if I did nothing else on it. If I am interested in a 1 μs run, this means it would take 153 days on my computer – about half a year! That's why you will do your runs on the toSUn cluster and nevertheless limit your time horizon to 100 ns for the purposes of this course.

3B. Now load your trajectory into VMD. To do so, read `ionized.psf` as a new molecule and then using `Load data into molecule` (or `Load files for`), read in the newly created file with the `dcd` extension. This file has accumulated the coordinates of all the atoms in your generated trajectory. It is good practice to select `Load all at once` to save time during loading.

How many frames does it load? Howcome?

3C. Now let's look at the RMSD profile of your system in this short trajectory. To do this, we will turn to `Extensions` → `Analysis` → `RMSD Trajectory Tool`. First write,

```
protein and name CA
```

in the box on the upper left-hand corner, then select `Align`. This aligns the trajectory to your first snapshot, i.e., your PDB file coordinates, but the fit is done to the C_α coordinates only. Note that, you align all your trajectory to your protein structure, but the distances between C_α atoms are optimized. This is standard procedure, getting rid of all diffusive motions your protein makes during the MD run. Next select RMSD. Make sure the `Plot` box is ticked to get a plot of the data; ticking `save` writes the same data in a file for you to plot using other programs (e.g., excel) afterwards.

What do your data tell you? Which part of the recorded data is for minimization and which part is for the actual MD run?

3D. Some other analyses you can do on your own:

Inspect your own periodic boundary conditions – Under `Graphics` → `Representations`, select the line which displays your protein, click the `Periodic` tab and add the periodic images of your protein in the different directions of your choice.

Calculate contact map – Under `Extensions` → `Analysis` → `Contact Map`, select the `Calculate` → `Calc. res-res dists` option. You will see the of the active snapshot (probably the last one loaded); if you wish to see the differences in the contact map with time, go to another snapshot (set the initial one) and repeat the process.

Find salt bridges – Under `Extensions` → `Analysis` → `Salt Bridges`, keep the defaults to write outputs to files as directed in the boxes. Then hit `Find salt bridges`. You can inspect the output files written in your default folder to see the positions, the exact distances between pairs, etc.

Calculate hydrogen bonds – Under `Extensions` → `Analysis` → `Hydrogen Bonds`, keep the defaults to write outputs to files as directed in the boxes. Then hit `Find hydrogen bonds`. You should provide a file name to write the outputs; otherwise, the graphic you get will not make too much sense. You can inspect the output files written in your default folder to see the positions, the exact distances between pairs, etc. If you know more about the character of your hydrogen bonds, you can knowledgably play with the default settings.