NOTE: The star “\*” stands for the name of any file.

NOTE: The expansion of the pdb file and other more basic commands can be found in the NbyN tutorial.

In order to incorporate a protein in a monolayer/bilayer, we first have to have the respective pdb files of the protein and the monolayer/bilayer. In order to generate the proteins pdb file, you can use programs such as CHARMM GUI. The pdb file of the protein has to be opened through a text editor ( either with the vim command or on the midnight commander). The easiest way to make a pdb file containing both the protein and the monolayer is to display both on the program UCSF CHIMERA, and then save them into one pdb using the options in UCSF CHIMERA.   
  
Before saving the pdb, there are 2 sets of modifications that have to made in order for the pdb file to work properly.

Firstly, if the protein is charged, you have to add ions which contain the exact amount of opposite charge to have a net neutral system ( this can be achieved through input generating machines such as CHARMM GUI).

Secondly, the height of the monolayer might need to be modified so that the protein is in the right place ( it is important to understand that the “right place” differs based on the hydrophobicity of the protein).

This can be achieved through the following command:

Thirdly, the collisions between the protein and the monolayer have to be taken care of. For this, under “tools” in the surface/binding analysis, the “Find collisions/contacts” option has to be utilized. Through this option, all of the collisions can be selected and then deleted/hidden from the screen so that the final saved pdb file does not contain collisions.

After the generation of the new pdb file, the rest of the steps are similar to the NbyN simulations. The pdb file needs to be expanded in the x and y direction through the following command:

To expand the size of a pdb file ( in this case the monolayer pdb file) the following command has to be used:

Module load gromacs

Gmx editconf -f \*.pdb -o \*.pdb -translate X Y Z

In this command, the variables X Y and Z are the numbers indicating the factor to which the pdb file is expanded in the x y and z direction respectively. For instance, the command -translate 4 4 1 would expand our monolayer to a square 16 fold are ( with vertices multiplied by 4) but would keep the height of the monolayer constant.

The topology file has to contain the ITP file of the protein, which can be found in the folder produced by the input generator machine.

The rest of the process is similar to previous cases as we have to make a new index file, modify the topology file to make sure the numbers of atoms/molecules correspond to the newly modified pdb file and run the two step energy minimization and equilibration.

To make a new index file we use the following command:

Module load gromacs

Gmx make\_ndx -f \*.pdb -o index.ndx

Finally the Topology file has to be edited for so that the number of molecules correspond to the new number of molecules.

The rest of the process is similar to the normal bilayer:

there are two separate steps that have to be taken, energy minimization and the mdrun. The necessary files (pdb, top, itp) used in these steps have to be generated through an engine such as CHARMGUI or should be designated from the server (in the case of the itp files). These commands of these steps should be incorporated in a “sh” file ( which is a file containing the commands and the designated computing core )and submitted to the central computing system using the following command:

qsub \*.sh

You can track the progress of the simulation either through the following command

Qstat

which gives you information on the recent runs on the central system, or through the error file which is a file named after the “sh” file in the folder of operation and contains information about the current status of the simulation. (NOTE:The first error in the error file is usually the source of the problems of the simulation and is therefore the first thing that has to be addressed).

In the case that there are unnecessary warnings, you can add a “ -maxwarn N” to the end of the command lines, N being a number.

The index file which will be used in the two steps can be made through the following command from the pdb file:

gmx make-ndx -f \*.pdb -o \*.ndx

The two steps include the following commands:

Module load gromacs

Step #1: energy minimization

Gmx grompp -f \*.mdp -c \*.pdb -n \*.ndx -o \*.tpr

Gmx mdrun -c \*.pdb -s \*.tpr deffnm emin -tabel \*.xvg

Step #2: mdrun

Gmx grompp -f \*.mdp -c \*.pdb -n \*.ndx -o \*.tpr

Gmx mdrun -c \*.pdb -s \*.tpr deffnm mdrun -tabel \*.xvg

The xvg and tpr files generated from the second step can be used to observe the trajectory through programs such as UCSF CHIMERA.