MEMBRANE BIOPHYSICS TUTORIAL

AA SIMULATION OF ARTIFICIAL LIPID BILAYER MEMBRANES

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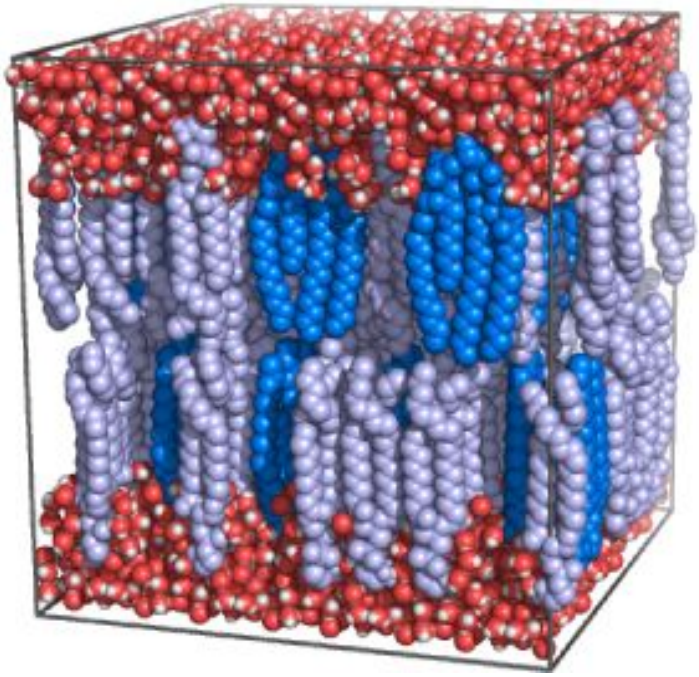

This tutorial will guide a new user through the process of setting up a simulation system containing a symmetric multi-component lipid bilayer membrane in a box of water.

This tutorial assumes you are using a GROMACS version in the 2018.x series.

Please note that the purpose of this tutorial is instructional and is not an endorsement or suggestion that you use these specific parameters/procedure for your simulation.

Generate Topology	Build Bilayer	Solvation	Energy Minimization	Native Structure	Equilibration	Production

Preparing the Topology Files of the Artificial Lipid:

1. Find the Structure of the Lipid closed to the structure of your desired lipid. (Avanti Lipids or some other Database) Open the structure file in Avogadro, and extract it in mol2 file format. (Optional)
   [Open the Structure File → Save As (mol2 file format)]

2. Open the mol2 file/Draw the Entire Structure in Marvin JS Window in Ligand Modeller Module of CHARMM-GUI
   [Caution: Recheck the Charges on Each Atom and Bond Connectivities after making necessary changes to the structure for they would change the partial charges assigned to each atom in the ligand]
   [Link: http://www.charmm-gui.org/?doc=input/ligandrm]

3. Follow the steps and download the final file from their website
   [Use this Video Tutorial to build the Lipid Structure using CHARMM-GUI Ligand Modeller: https://youtu.be/5PYTQiKf0D4]

4. Open the compressed file and extract from it
   - PDB File of the Single Lipid
   - Topology & Restraint Files of the Lipid

Prepare the topology of all the artificial lipids to be used in your system using CHARMM-GUI Ligand Modeller and keep the PDB files of single lipid and their topology files ready in hand before beginning to build the bilayer.

References:

1. S. Jo, T. Kim, V.G. Iyer, and W. Im (2008) CHARMM-GUI: A Web-based Graphical User Interface for CHARMM. (Link: J. Comput. Chem. 29:1859-1865)

2. S. Kim, J. Lee, S. Jo, C.L. Brooks III, H.S. Lee, and W. Im (2017) CHARMM-GUI Ligand Reader and Modeler for CHARMM Force Field Generation of Small Molecules. (Link: J. Comput. Chem. 38:1879-1886)

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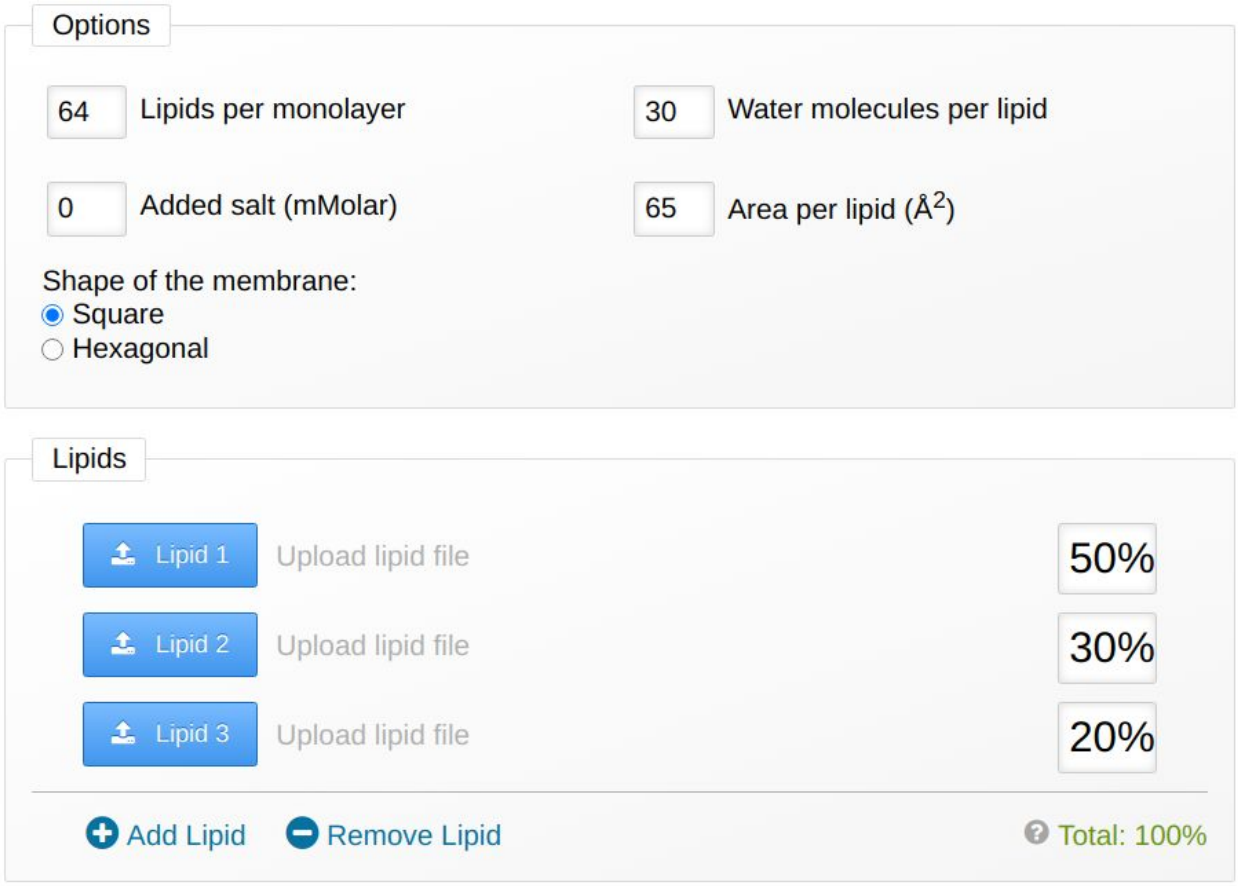
To build the bilayer, we will use the MemGen which generates multi-component lipid membranes given the percentage composition of the required bilayer and the number of lipids/ leaflet in your system. [Make sure you know the quantities required in the below tab: No of Water molecules/lipid (Prescribed: 30), Area/lipid (Prescribed: 65)]



[CAUTION: If you a large system, prepare a smaller patch of the system(~1/4th size of required size) using MemGen Tool and then replicate it along X and Y axis* to form the final patch, for many artificial voids, are seen to form in the bilayer with a higher number of lipids as input. If still voids are seen in the bilayer, the leaflets need to be moved close to each other, until overlap (it can be done in VMD)]

NOTE: To make asymmetric bilayers you may make two bilayers using this tool each of composition of each leaflet and then fuse together a leaflet of each.

Reference:

Christopher J. Knight and Jochen S. HubMemGen: A general web server for the setup of lipid membrane simulation systems (Link: Bioinformatics, 31:2897-2899 (2015)

*`gmx genconf -nbox 2 2 1 -f input.gro -o output.gro`

The final membrane bilayer structure formed after patching usually shows bad contacts among the waters in the system, which prevents us from using it further.

If you face this problem, follow the procedure given below:

1. Remove the water added in the bilayer structure by the MemGen tool. Solvate the bilayer again with water using **gmx_solvate**

2. We need to remove the water that penetrates below the headgroups and create a new structure. [Removing the waters* from the re-solvated structure present within a certain Z_cuttoff (Highest and Lowest coordinate of the P atom** present in the headgroup of the lipids)]

[CAUTION: Make sure you have been updating your topol.top file after this or else you will get lots of nasty error messages ("number of coordinates in the coordinate file does not match topology," etc)]

Reference:

1. R. J. Gowers, M. Linke, J. Barnoud, T. J. E. Reddy, M. N. Melo, S. L. Seyler, D. L. Dotson, J. Domanski, S. Buchoux, I. M. Kenney, and O. Beckstein. MDAnalysis: A Python package for the rapid analysis of molecular dynamics simulations.
2. N. Michaud-Agrawal, E. J. Denning, T. B. Woolf, and O. Beckstein. MDAnalysis: A Toolkit for the Analysis of Molecular Dynamics Simulations. J. Comput. Chem. 32 (2011), 2319-2327

*, ** Has been implemented using Python codes (using MDAnalysis Package)

Generate Topology	Build Bilayer	Solvation	Energy Minimization	Native Structure	Equilibration	Production

Before we can begin dynamics, we ensure that the solvated bilayer system has no steric clashes or inappropriate geometry, by relaxing the structure through energy minimization.

```
gmx grompp -f step6.0_minimization.mdp -o step6.0_minimization.tpr -c
step5_charmm2gmx.pdb -r step5_charmm2gmx.pdb -p topol.top

gmx_d mdrun -v -deffnm step6.0_minimization
```

Generate Topology	Build Bilayer	Solvation	Energy Minimization	Native Structure	Equilibration	Production

The structure of the lipids in the energy minimized is not in their native state. So, running an NVT Equilibration on this system usually makes the system blow up, separating the leaflets on either side of the simulation box.

To avoid this, we run a short NPT equilibration run on the energy-minimized structure. While the native structure is formed.
(**CHECK** the system after this, you might need to bring the leaflets close,(suppose there's a point of unsaturation in the artificial lipid, it will curl up after this creating a void between the leaflets)

$cnt: 3-6

```
gmx grompp -f step6.{$cnt}_equilibration.mdp -o
step6.{$cnt}_equilibration.tpr -c step6.{$pcnt}_equilibration.gro -r
minim.pdb -n index.ndx -p topol.top

gmx mdrun -v -deffnm step6.{$cnt}_equilibration
```

Generate Topology	Build Bilayer	Solvation	Energy Minimization	Native Structure	Equilibration	Production

To begin real dynamics, we must equilibrate the solvent around the membrane. If we were to attempt unrestrained dynamics at this point, the system may collapse. The reason is that the solvent is mostly optimized within itself, and not necessarily with the solute. It needs to be brought to the temperature we wish to simulate and establish the proper orientation about the solute (the membrane). After we arrive at the correct temperature (based on kinetic energies), we will apply pressure to the system until it reaches the proper density.

Equilibration is often conducted in two phases:

1. Temperature Stabilization

The first phase is conducted under an *NVT* ensemble (constant number of particles, Volume, and Temperature). This ensemble is also referred to as "isothermal-isochoric" or "canonical." The timeframe for such a procedure is dependent upon the contents of the system, but in *NVT*, the temperature of the system should reach a plateau at the desired value. If the temperature has not yet stabilized, additional time will be required.

$cnt: 1-2

```
gmx grompp -f step6.{$cnt}_equilibration.mdp -o
step6.{$cnt}_equilibration.tpr -c step6.{$precnt}_equilibration.gro
-r native.pdb -n index.ndx -p topol.top

gmx mdrun -v -deffnm step6.{$cnt}_equilibration
```

2. Pressure Stabilization

We must also stabilize the pressure of the system. Equilibration of pressure is conducted under an *NPT* ensemble, wherein the Number of particles, Pressure, and Temperature are all constant. The ensemble is also called the "isothermal-isobaric" ensemble, and most closely resembles experimental conditions.

$cnt: 3-6

```
gmx grompp -f step6.{$cnt}_equilibration.mdp -o
step6.{$cnt}_equilibration.tpr -c step6.{$precnt}_equilibration.gro
-r native.pdb -n index.ndx -p topol.top

gmx mdrun -v -deffnm step6.{$cnt}_equilibration
```

Generate Topology	Build Bilayer	Solvation	Energy Minimization	Native Structure	Equilibration	Production

Upon completion of the two equilibration phases, the system is now well-equilibrated at the desired temperature and pressure. We are now ready to release the position restraints and run production MD for data collection

```
gmx grompp -f step7_production.mdp -o step7_${cnt}.tpr -c
step6.6_equilibration.gro -n index.ndx -p topol.top

gmx mdrun -v -deffnm step7_${cnt}
```

You should also review the literature and the GROMACS manual for making adjustments to the .mdp files provided here as per your requirement.

If you have suggestions for improving this tutorial, if you notice a mistake, or if anything is otherwise unclear, please feel free to **email me*.**

Happy simulating!

Courtesy: Image in the front page in from MemGen Web Server Tool
The tutorial is designed based on Justin Lemkul's GROMACS Tutorial
(Link: http://www.mdtutorials.com/gmx/)

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SUPPLEMENTARY: Codes for Step 3: Solvation
(Make Necessary Changes)

```
#    Returns the Highest & Lowest Z_Coordinate of the P Atom of
Lipid Headgroup
#   Aniruddha Seal,
#    Theoretical Biophysics Laboratory, Molecular Biophysics Unit,
#    Indian Institute of Science, Bangalore - 560012
#
#    Last Modified: June 30, 2020
#    For any queries regarding usage of the script please feel free
to contact me on
#   aniruddhaseal2011@gmail.com, aniruddha.seal@niser.ac.in

#  Load the required packages
import MDAnalysis as md
import numpy as np
f=md.Universe("/home/aniruddha/Desktop/DPPC-D23-Chol/SIMU-D23/D23_exs
olv_arr.pdb")
p_T=f.select_atoms("name P and prop z > 45")
p_B=f.select_atoms("name P and prop z < 45")
bil=f.select_atoms("resname DPP or resname CHL or resname LIG")
p_T_coo=p_T.positions
p_B_coo=p_B.positions
m=p_T_coo[0][2]
# 112= p_T_coo.size/3
for i in range(112):
    if (p_T_coo[i][2]>m):
        m=p_T_coo[i][2]
    i=i+1
print(m)
n=p_B_coo[0][2]
for i in range(112):
    if(p_B_coo[i][2]<n):
        n=p_B_coo[i][2]
    i=i+1
print(n)
```

```
#    Remove water molecules from within the lipid bilayer between a
defined Z coordinate per leaflet headgroup region
#   Aniruddha Seal,
#    Theoretical Biophysics Laboratory, Molecular Biophysics Unit,
#    Indian Institute of Science, Bangalore - 560012
#
#    Last Modified: June 30, 2020
#    For any queries regarding usage of the script please feel free
to contact me on
#   aniruddhaseal2011@gmail.com, aniruddha.seal@niser.ac.in

#  Load the required packages
import MDAnalysis as md
import numpy as np
u=md.Universe("/home/aniruddha/Desktop/DPPC-D23-Chol/SIMU-D23/step5_c
harmm2gmx.pdb")
lip=u.select_atoms("not (resname SOL)")
P_min=13.39
P_max=78.25
ow=u.select_atoms("name OW")
sol_cut=u.select_atoms("resname SO")
k=len(ow)
for i in range(k):
    ow_resid=ow[i].resid
    sol_resid=u.select_atoms("resid %d"% ow_resid)
    sol_coo=sol_resid.center_of_geometry()[2]
    if ((sol_coo<P_min)|(sol_coo>P_max)):
        sol_cut=sol_cut+sol_resid
new_pdb=lip+sol_cut
new_pdb.write("kcut_step5_charmm2gmx.pdb")
```