**Lipid Bilayer Simulation (Protein+Ligand – Bilayer)**

🡪Make the ***Forcefield*** (<http://www.bevanlab.biochem.vt.edu/Pages/Personal/justin/gmx-tutorials/membrane_protein/02_topology.html>)

🡪Convert ‘protein.pdb’ to ‘protein.gro’ and obtain ‘topol.top’ of the protein in the process

gmx pdb2gmx -f protein.pdb -o protein.gro -ignh -ter -water spc

🡪download these files:

* dppc128.pdb🡪dppc128.gro
* dppc.itp
* dppc\_topol.top
* lipid.itp
* minim.mdp
* nvt.mdp
* npt.mdp
* md.mdp

🡪gmx grompp -f minim.mdp -c dppc128.gro -p topol\_dppc.top -o em.tpr

🡪gmx trjconv -s em.tpr -f dppc128.gro -o dppc128\_whole.gro -pbc mol -ur compact

*For Transmembrane proteins that span across both the leaflets of bilayer, use this command*

🡪gmx editconf -f protein.gro -o protein\_newbox.gro -princ -box <X coordinate of lipid> <Y coordinate of lipid> < Z coordinate of protein> -rotate <x> <y> <z> -center <X/2> <Y/2> <Z/2>

*For Proteins that is completely immersed in Bilayer or just skims out of the surface of the leaflets of the bilayer*

🡪gmx editconf -f protein.gro -o protein\_newbox.gro -princ -box <lipid coordinates of X> <lipid coordinates of Y> < Z coordinate of protein> -rotate <x> <y> <z> -center <X/2> <Y/2> <Z/2>

🡪cat protein\_newbox.gro dppc\_whole.gro >system.gro

**🡪**The system needs to be inflated and shrink using perl script and alternative energy minimization process

genrestr -f protein\_newbox.gro -o strong\_posre.itp -fc 100000 100000 100000

Select protein

*If ligand is bound to protein then add resrtrain to ligand as well, mentioned below:*

genrestr -f protein\_newbox.gro -o strong\_posre\_ligand.itp -fc 100000 100000 100000

Select ligand

Make changes in ‘strong\_posre\_ligand.itp’

Change the first column numbering, start the numbering of the first column from “1”, following “2” in first column second row and so on (“3” in first column third row…….).

🡪Update topology for calling the strongposition restrain.itp files (Refer: <http://www.bevanlab.biochem.vt.edu/Pages/Personal/justin/gmx-tutorials/membrane_protein/03_solvate.html>)

Add define = -DSTRONG\_POSRES -DSTRONG\_POSRES\_LIGAND in ‘.mdp file’( Add “DSTRONG\_POSRES\_LIGAND”, if ligand bound to protein is present).

perl inflategro.pl system.gro 4 DPPC 14 system\_inflated.gro 5 area.dat

Troubleshoot: If you are not able to visualize the system\_inflated.gro, Try deleting alternate lines (if any, present in ‘system\_inflated.gro’) and save as system\_inflated.gro

Update the ‘topol.top’ depending upon the number of lipid atom deleted

**ENERGY MINIMIZATION**

gmx grompp -f minim.mdp -c system\_inflated.gro -p topol.top -o new.tpr

gmx mdrun -v -deffnm new

perl inflategro.pl new.gro 0.95 DPPC 0 shrink.gro 5 area\_shrink.dat

gmx grompp -f minim.mdp -c shrink.gro -p topol.top -o new1.tpr

mdrun -v -deffnm new1

perl inflategro.pl new1.gro 0.95 DPPC 0 shrink1.gro 5 area\_shrink1.dat

Carry it on till you get new30.gro

If you not open new30.gro then use previous .gro file ie-: new29.gro, new28.gro for further commands

(Don’t stick to the protocol and start visualizing the structure after ‘new20.gro’, it is not necessary that your protein will be completely shrinked at ‘new30.gro’, it may happen early as well. Take the ‘Energy minimized’ structure and not the ‘shrink’ structure.)

**SOLVATION**

gmx editconf -f new30.gro -o new30\_newbox.gro -d <0.01-1.0> -bt triclinic

0.01-1.0 is the range of box size that is desirable

The quickest way I have found to solvate membrane systems is to make a local copy of vdwradii.dat and change the value of C from 0.15 to 0.375.

After solvating, check your structure to be sure no water molecules are present in the hydrophobic core of the bilayer. If there are a few stray molecules, you can either delete them manually, continue to adjust the van der Waals. Make sure you update the topology file if you delete any molecule of water manually.

gmx genbox -cp new30\_newbox.gro -cs spc216.gro -o new30\_solv.gro -p topol.top

Visualize the structure to make sure no water molecules are present in the bilayer and if everything works cool delete the local copy of vdwradii.dat before continuing.

**IONIZATION**

gmx grompp -f ions.mdp -c new30\_solv.gro -p topol.top -o ions.tpr

See the total charge on the system and add NA or CL accordingly

gmx genion -s ions.tpr -o new30\_solv\_ions.gro -p topol.top -pname NA -nname CL -nn <10>

<10>, if the whole system charge is +10

Do it as per your system charge

**MINIMIZATION**

Remove thhe Restrains from minim.mdp file KEEP NO STRONG RESTRAINS DURING EM

gmx grompp -f minim.mdp -c new30\_solv\_ions.gro -p topol.top -o em.tpr

gmx mdrun -v -deffnm em

Troubleshoot: If the minimization is not successful then do it once again. First time with the strong restrains and second time without restrains.

energy -f em.edr -o potential.xvg

See the potential graph and carry forward.

make\_ndx -f em.gro -o index.ndx

Update the nvt.mdp for the groups we have made(we can have different groups, for example Protein\_JZ4\_DPPC –one group ,SOL\_CL –second group [for this you would need to made only first and last group as the dppc would already exist)this will increase precision ]

(Refer: <http://www.bevanlab.biochem.vt.edu/Pages/Personal/justin/gmx-tutorials/membrane_protein/06_equil.html>)

**NVT RUN**

This run has to be just of 100 ps you might have to leave pc on for the night as it will take 8-12 hours.

Update the file for you groups of interest.

grompp -f nvt.mdp -c em.gro -p topol.top -n index.ndx -o nvt.tpr

mdrun -deffnm nvt

energy -f nvt.edr -o temperature.xvg

**NPT RUN**

I don’t think you would need to update the ‘npt,mdp’ file.

keep it 50ns or 5,000,000 steps

gmx grompp -f npt.mdp -c nvt.gro -t nvt.cpt -p topol.top -o npt.tpr

gmx mdrun -deffnm npt

energy -f npt.edr -o pressure.xvg

**MD RUN**

Make sure you change the number of steps to 200ns or 20,00,00,000 steps and update the ‘md.mdp’ file for our group of interest.

gmx grompp -f md.mdp -c npt.gro -t npt.cpt -p topol.top -n index.ndx -o md\_0\_1.tpr

gmx mdrun -nt X -deffnm md\_0\_1

gmx trjconv -s md\_0\_1.tpr -f md\_0\_1.xtc -o md\_0\_1\_noPBC.xtc -pbc mol -ur compact

gmx rms -s md\_0\_1.tpr -f md\_0\_1\_noPBC.xtc -o rmsd.xvg -tu ns