Steps in MD Simulations

1. Preparation of topology files for target and ligand
2. Solvation
3. Edit Topology
4. Saving MD parameter files
5. Adding ions
6. Energy minimization
7. Restraining the ligand
8. Thermostats
9. Constant Number of atoms, Volume, and temperature (NVT) equilibrium.
10. Constant Number of atoms, pressure, and temperature (NPT) equilibrium.
11. Production MD
12. MD extension
13. RMSD computation
14. RMSF computation
15. RDF computation
16. Radius of Gyration
17. Hydrogen bonds
18. Energy, enthalpy, entropy, etc. computations
19. Charts

- **Force Fields:** CHARMM, AMBER, GROMOS, OPLS, and COMPASS, UFF, MM2, MM3 and MM4, CFF (consistent force field) and MMFF (Merck molecular force field)
- **Energy Minimization Techniques**: Simplex Method, Sequential univariate search method, steepest descents method, Conjugate gradients minimization, Newton-Raphson method, Quasi-Newton method

Steps:

1. System topology
   - **i.** Protein Topology
   - **ii.** The Ligand Topology

2. Define Box & Solvation
3. Add Ions
4. Energy Minimization
5. Equilibration Phase1
6. Equilibration Phase2
7. Production MD
8. Analysis:
   - **i.** Recentering and Rewrapping Coordinates
   - **ii.** Analyzing Protein-Ligand Interactions and Ligand Dynamics
   - **iii.** Protein-Ligand Interaction Energy
   - **iv.** RMSD RMSF
   - **v.** Radius of Gyration
   - **vi.** H bonds

#LINUX and GROMACS UNIVERSAL TUTORIAL

----INSTALL UBUNTU-----

UPGRADE AND UPDATE LIBRARIES OF UBUNTU BY FOLLOWING COMMANDS:

sudo apt update

sudo apt upgrade

sudo apt install gcc

sudo apt install cmake

sudo apt install build-essential

sudo apt install libfftw3-dev OR

sudo apt-get install -y libfftw3-dev

ONCE THE ABOVE COMMANDS ARE INTERED AND LINUX SYSTEM IS UPDATED, PROCEED TO INSTALL GROMACS

----------GROMACS DIRTY INSTALLATION COMMAND----------

sudo apt install gromacs

#sudo apt remove gromacs

----------Install Pymol----------

sudo apt-get install -y pymol

----------Install google chrome----------

wget https://dl.google.com/linux/direct/google-chrome-stable_current_amd64.deb

sudo apt install ./google-chrome-stable_current_amd64.deb

----------Install Chimera----------

Download the set-up file from "https://www.cgl.ucsf.edu/chimera/download.html"

Move the file to desired folder.

In that folder open terminal

Command:

ls (to check the name of setup file)

chmod +x CHIMERA-INSTALLER.bin

./CHIMERA-INSTALLER.bin

----------Autodoc-Vinna----------

Go to download page "http://vina.scripps.edu/download.html"

Move the file to desired folder.

In that folder open terminal

Command:

ls (to check the name of setup file)

tar -xzvf autodock_vina_1_1_2_linux_x86.tgz

----------Install-GRACE----------

sudo apt-get install grace

----------VMD----------

https://www.ks.uiuc.edu/Development/Download/download.cgi?PackageName=VMD

1. Save the .tar.gz file in working folder

2. open the extracted folder and run command './configure' by opening the termianl

3. Open the folder src in terminal and run the command 'sudo make install'

4. type 'vmd' in termial and program should run.

##########MANNUAL-GROMACS-COMPILATION##########

Download Gromacs from :

https://manual.gromacs.org/current/download.html#

# #

##########CUDA TOOLKIT:##########

https://developer.nvidia.com/cuda-downloads

#Follow the steps and copy the commands

# #

##########Gromacs Compilation Process##########

tar xfz gromacs-2020.2.tar.gz

cd gromacs-2020.2

mkdir build

cd build

cmake .. -DGMX_GPU=CUDA -DCUDA_TOOLKIT_ROOT_DIR=/usr/local/cuda

make

make check

sudo make install

source /usr/local/gromacs/bin/GMXRC

##########

#########STEPS FOR MD AS FOLLOWS#########

---PREPARE LIGAND AND RECEPTOR IN CHIMERA----

1. open the best pose ligand with the receptor Protein.pdb file
2. Delete the chain of protein, in the residual ligand, add hydrogens and save it as LIG.mol2 as 'LIG.mol2'
   #Open the LIG.mol2 file and in the second line
   #Correction to be made in LIG.mol2
   #Open LIG.mol2 by using gedit command or simplyopening file in any text editor.

2.1. "@<TRIPOS>MOLECULE" make sure this is the first line in file

2.2. delete the header and empty space if you have to

2.3. "@<TRIPOS>MOLECULE" there will be name after this line maybe xxx.pdb or ****** or anything else

2.4. change it to LIG

2.5. bond orders "@<TRIPOS>BOND" will be arranged differently in each file

3. arrange them in specific order to avoid errors use
4. perl sort_mol2_bonds.pl LIG.mol2 LIG.mol2 script
5. Go to SwissParam "http://www.swissparam.ch/" and upload the 'Lig.mol2 file'
6. Download the .zip folder

7. Again open the best pose ligand with the receptor .pdb file, delete ligand, Perform DockPrep of protein as save it as .pdb file as 'REC.pdb'
8. Make a working Folder for Gromacs, copy contents of the downloaded zip file into this folder, copy the DockPrep 'rec.pdb' in to working folder
9. Copy all the .mdp files into this working folder
10. Open the terminal in this working folder and proceed with Gromacs.

---------GROMACS UBUNTU TUTORIAL-----------

source /usr/local/gromacs/bin/GMXRC (If Gromacs is manually compiled / not for dirty install)

gmx pdb2gmx -f REC.pdb -ignh

8 (CHARMM27)

1 (TIP3P)

gmx editconf -f LIG.pdb -o LIG.gro

gedit conf.gro LIG.gro

*(Copy content from 3rd line of lig.gro to the conf.gro file up to the 2nd last line)

*(Check the column number from where the lig.gro data ends (x) in conf.gro and replace the value in 2nd line by x-3)

*(Open file in chimera to check ligand and receptor)

-----EDIT THE FOLLOWING in topol.top -----

gedit topol.top

(add

; Include ligand topology

#include "LIG.itp"

below- Include forcefield parameters

#include "amberGS.ff/forcefield.itp")

AT THE BOTTOM OF THE SAME FILE PERFORM FOLLOWING CHANGES

(add LIG 1

align exactly below-

Protein_chain_E 1)

-----EDIT THE FOLLOWING in lig.itp -----

gedit lig.itp

[moleculetype]

; Name nrexcl

lig_gmx2 3

TO

[moleculetype]

; Name nrexcl

LIG 3

(in certain cases this will already be LIG 3 so for such case no change is needed)

---

gmx editconf -f conf.gro -d 1.0 -bt triclinic -o box.gro

gmx solvate -cp box.gro -cs spc216.gro -p topol.top -o box_sol.gro

gmx grompp -f ions.mdp -c box_sol.gro -p topol.top -o ION.tpr

(OR)

gmx grompp -f ions.mdp -c box_sol.gro -maxwarn 2 -p topol.top -o ION.tpr

gmx genion -s ION.tpr -p topol.top -conc 0.1 -neutral -o box_sol_ion.gro

15

gmx grompp -f EM.mdp -c box_sol_ion.gro -p topol.top -o EM.tpr (OR)

gmx grompp -f EM.mdp -c box_sol_ion.gro -maxwarn 2 -p topol.top -o EM.tpr

gmx mdrun -v -deffnm EM

gedit nvt.mdp (This file is already modified)

Now make index files

gmx make_ndx -f LIG.gro -o index_LIG.ndx

> 0 & ! a H*

> q

gmx genrestr -f LIG.gro -n index_LIG.ndx -o posre_LIG.itp -fc 1000 1000 1000

> select group "3"

Now, open topol.top file

at the end of the document

after

"; Include Position restraint file

#ifdef POSRES

#include "posre.itp"

#endif

"Here"

add this

; Ligand position restraints

#ifdef POSRES

#include "posre_LIG.itp"

#endif

Again, Make other Index file for System

gmx make_ndx -f EM.gro -o index.ndx

> 1 | 13

> q

-----[NVT]-----

gedit NVT.mdp (This file is already modified)

gmx grompp -f NVT.mdp -c EM.gro -r EM.gro -p topol.top -n index.ndx -maxwarn 2 -o NVT.tpr

gmx mdrun -deffnm NVT

-----[NPT]-----

gedit NPT.mdp (This file is already modified)

gmx grompp -f NPT.mdp -c NVT.gro -r NVT.gro -p topol.top -n index.ndx -maxwarn 2 -o NPT.tpr

gmx mdrun -deffnm NPT

-----[FINAL MD RUN/PRODUCTION]-----

gedit NPT.mdp (Change MD RUN TIME as per your need)

gmx grompp -f MD.mdp -c NPT.gro -t NPT.cpt -p topol.top -n index.ndx -maxwarn 2 -o MD.tpr

gmx mdrun -deffnm MD

----[Recentering and Rewrapping Coordinates]----

gmx trjconv -s MD.tpr -f MD.xtc -o MD_center.xtc -center -pbc mol -ur compact

#Choose "Protein" for centering and "System" for output.

#To extract the first frame (t = 0 ns) of the trajectory, use trjconv -dump with the recentered trajectory:

gmx trjconv -s MD.tpr -f MD_center.xtc -o start.pdb -dump 0

------RMSD Calculations-----

gmx rms -s MD.tpr -f MD_center.xtc -o rmsd.xvg

gmx rms -s MD.tpr -f MD_center.xtc -o rmsd.xvg -tu ns

4

13

#(Select appropritate 2 options one by one and then open the output files in Grace) Select Backbone and then LIG

xmgrace rmsd.xvg

------RMSF Calculations-----

gmx rmsf -s MD.tpr -f MD_center.xtc -o rmsf.xvg

4

(Select appropritate Backbone open the output files in Grace)

xmgrace output.xvg

----------h-bonds----------

gmx hbond -s MD.tpr -f MD_center.xtc -num hb.xvg

gmx hbond -s MD.tpr -f MD_center.xtc -num hb.xvg -tu ns

1

13

xmgrace hb.xvg

----------Gyration Radius----------

gmx gyrate -s MD.tpr -f MD_center.xtc -o gyrate1.xvg

#Choose the group of your choice

xmgrace gyrate1.xvg

----------ENERGY Calculations----------

gmx energy -f MD.edr -o energy1.xvg

#Choose the option of your choice

xmgrace -nxy energy1.xvg

GROMACS

1. **System topology:**
    1.1.Protein Topology::pdb2gmx:

grep JZ4 3HTB_clean.pdb > jz4.pdb

#tar -zxvf charmm36-jul2022.ff.tgz

gmx pdb2gmx -f 3HTB_clean.pdb -o 3HTB_processed.gro -ter

1.2.The Ligand Topology:

perl sort_mol2_bonds.pl jz4.mol2 jz4_fix.mol2

python cgenff_charmm2gmx.py JZ4 jz4_fix.mol2 jz4.str charmm36-jul2022.ff

gmx editconf -f jz4_ini.pdb -o jz4.gro

2. **Define Box & Solvation:**

gmx editconf -f complex.gro -o newbox.gro -bt dodecahedron -d 1.0

gmx solvate -cp newbox.gro -cs spc216.gro -p topol.top -o solv.gro

3. **Add Ions:**

gmx grompp -f ions.mdp -c solv.gro -p topol.top -o ions.tpr

gmx genion -s ions.tpr -o solv_ions.gro -p topol.top -pname NA -nname CL -neutral

4. **Energy Minimization:**

gmx grompp -f em.mdp -c solv_ions.gro -p topol.top -o em.tpr

gmx mdrun -v -deffnm em

5. **Equilibration Phase1:**

gmx make_ndx -f jz4.gro -o index_jz4.ndx

...

> 0 & ! a H*

> q

gmx genrestr -f jz4.gro -n index_jz4.ndx -o posre_jz4.itp -fc 1000 1000 1000

#tc-grps = Protein JZ4 SOL CL

gmx make_ndx -f em.gro -o index.ndx

> 1 | 13

> q

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

gmx mdrun -deffnm nvt

6. Equilibration Phase2:

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

gmx mdrun -deffnm npt

7. Production MD:

gmx grompp -f md.mdp -c npt.gro -t npt.cpt -p topol.top -n index.ndx -o md_0_10.tpr

gmx mdrun -deffnm md_0_10

8. Analysis:

8.1.Recentering and Rewrapping Coordinates:

gmx trjconv -s md_0_10.tpr -f md_0_10.xtc -o md_0_10_center.xtc -center -pbc mol -ur compact

gmx trjconv -s md_0_10.tpr -f md_0_10_center.xtc -o start.pdb -dump 0

gmx trjconv -s md_0_10.tpr -f md_0_10_center.xtc -o md_0_10_fit.xtc -fit rot+trans

8.2.Analyzing Protein-Ligand Interactions and Ligand Dynamics:

#gmx distance -s md_0_10.tpr -f md_0_10_center.xtc -select 'resname "JZ4" and name OAB plus resid 102 and name OE1' -oall

#gmx make_ndx -f em.gro -o index.ndx

...

> 13 & a OAB | a H12

(creates group 23)

> 1 & r 102 & a OE1

(creates group 24)

> 23 | 24

> q

gmx angle -f md_0_10_center.xtc -n index.ndx -ov angle.xvggmx make_ndx -f em.gro -n index.ndx

...

> 13 & ! a H*

> name 26 JZ4_Heavy

> q

gmx rms -s em.tpr -f md_0_10_center.xtc -n index.ndx -tu ns -o rmsd_jz4.xvg

8.3.Protein-Ligand Interaction Energy:

gmx grompp -f ie.mdp -c npt.gro -t npt.cpt -p topol.top -n index.ndx -o ie.tpr

gmx mdrun -deffnm ie -rerun md_0_10.xtc -nb cpu

gmx energy -f ie.edr -o interaction_energy.xvg

xmgrace rmsd.xvg

Example GROMACS commands

#To convert pdb to gro format and topology generation

gmx pdb2gmx -f <protein_filename.pdb> -o <output_filename.gro> -water spce

#To create a box

gmx editconf -f <input_file.gro> -o <output_file.gro> -c -d 1.0 -bt cubic

#To solvate box (fille with water)

gmx solvate -cp <input_file.gro> -cs spc216.gro -o solv.gro -p topol.top

#Add ions to neutralize the system

gmx grompp -f ions.mdp -c solv.gro -p topol.top -o ions.tpr

gmx genion -s ions.tpr -o solv_ions.gro -p topol.top -pname NA -nname CL -neutral

#Energy minimization

gmx grompp -f minim.mdp -c solv_ions.gro -p topol.top -o em.tpr

gmx mdrun -v -deffnm em

#NVT and NPT ensembling

#NVT

gmx grompp -f nvt.mdp -c em.gro -r em.gro -p topol.top -o nvt.tpr

gmx mdrun -deffnm nvt

#NPT

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

gmx mdrun -deffnm npt

#Production run

gmx grompp -f md.mdp -c npt.gro -t npt.cpt -p topol.top -o md_0_1.tpr

gmx mdrun -deffnm md_0_1