-the simulation will be performed at the previously docked receptor and ligand(Remedisivir) as it had a higher docking score than Azithromycin.

-copy pose1(complex of protein and the receptor) file and paste it on the MD folder, open it with chimera to see it. File-> open -> desktop -> pose1.pdb. change the color of the ligand: Select-> Chain-> A-> Pose1.pdb(#0.2) , Actions-> Color-> Red.

-Download the zipped file from Github (DweipayanG/GROMACS-Protein-Ligand). Code -> Download ZIP. Save it to MD folder. In the extracted folder there are 8 files.

- In the gromacs code file, there are the steps for MD simulation. Move pose1 file to the downloaded folder.

-First we need to make the topology for each protein(receptor) and ligand separately. So we have to split pose1 file of the complex into receptor file and ligand file.

-Open chimera. File -> open pose1.pdb -> open.

Select-> chain-> A-> pose1(protein). Actions-> Atoms/Bonds -> Delete. Now we have only the ligand. Add hydrogens to it. Tools-> Structure Editing-> AddH-> Ok. Then save it: File-> Save Mol2. In the same working directory, File name: LIG, make sure that Add mole2 box is checked. Save. Close session. In our directory there is a LIG.mol2 file.

- We have to make the same steps for protein and delete the ligand. In chimera, File-> open -> pose1 -> Select-> Chain -> A -> Pose1(#0.2), now the ligand is selected. Actions-> Atoms/Bonds -> delete. Tools-> Surface/Binding Analysis-> Dock Prep. Uncheck the Write Mol2 file-> ok-> Add hydrogens -> ok. Assign charges for Dock Prep -> Gasteiger -> ok -> Specify Net Charges -> Gasteiger -> ok. In the status bar: Dock prep finished. Save PDB to the working directory. File name: REC(for receptor) -> Save. Close chimera. Now we have REC.pdb file.

Files splitted are: Pose1.pdb – LIG.mol2 - REC.pdb

- In the working directory: Open in Terminal. To open LIG.mol2 file type: gedit LIG.mol2, the file is now opened. The first line should be: @<TRIPOS>MOLECULE.

-the second line has to be the same name of the file itself. So change it from pose1.pdb to LIG only, save and close.

-copy the command: perl sort\_mol2\_bonds.pl LIG.mol2 LIG.mol2, to arrange bonds in the same order to terminal and enter.

- now we have to create topology file for LIG.mol2 file to gromacs. So, go to [www.swissparam.ch](http://www.swissparam.ch/) : choose file and upload LIG.mol2 file and press submit. Wait for about 10 secs and press the link. Refresh. Press LIG.zip link. A zipped file is downloaded. Move it to your directory and unzip it to our directory and choose replace nothing.

- to run gromacs, in the working directory terminal type: gmx, it will not be found, so first type:

source /usr/local/gromacs/bin/GMXRC

* This is needed for the compiled installation of gromacs not for dirty installation.

now if we type gmx or gmx -- version -> it will appear.

-now copy and paste commands from gromacs codes file:

1) gmx pdb2gmx -f REC.pdb -ignh

-choose the forcefield that are required: 8: CHARMM27 all-atom force field (for proteins)

-select the water molecule: 1: recommended.

- a file named as topol.top is created in our directory which is the topology file of the protein(receptor). you can delete the #topol.top.1# file.

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

LIG.pdb is obtained from swissparam. Now he have a file named as LIG.gro

-the topology files of protein (conf.gro) and ligand (LIG.gro) are created separately, now we have to merge the topology of protein and ligand together (manually).

- to open the two files type: gedit conf.gro LIG.gro

- in LIG.gro, copy everything from the third line till down except the last line, go to conf.gro file, at the second last line press enter and paste in the empty space. The last line is at 6931. At the second line overwrite the number by 6928 (6931-3) as the first two lines and the last line doesn’t contain any information about the topology. Then save.

-the file of conf.gro is now containing the topology of both protein and ligand, to check that the copy and paste has been done properly, open conf.gro file using chimera. You will see that the file has opened properly. If there is any error, the file will not opened by chimera.

-from select -> Residue you will find LIG, choose it, actions-> Color-> Red.

- open topol.top file: gedit topol.top , add:

; Include ligand topology

#include "LIG.itp"

below:

Include forcefield parameters (line 20)

#include "charmm27.ff/forcefield.itp"

-then:

At the very end of the file you will find:

[ molecules ]

; compound #mols

Protein\_chain\_A 1

Now add and align exactly below:

LIG 1

The final lines are now:

[ molecules ]

; compound #mols

Protein\_chain\_A 1

LIG 1

Save and close topol.top file.

- open LIG.itp file: gedit LIG.itp

--EDIT THE FOLLOWING in LIG.itp:

[ moleculetype ] (line 58)

; 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).

Save and close.

-create the virtual box around the complex:

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

-fill the box with the solvent:

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

-to see how the box looks like: vmd box\_sol.gro

gmx grompp -f ions.mdp -c box\_sol.gro -p topol.top -o ION.tpr

gmx genion -s ION.tpr -p topol.top -conc 0.1 -neutral -o box\_sol\_ion.gro

Select a group: 15

gmx grompp -f EM.mdp -c box\_sol\_ion.gro -p topol.top -o EM.tpr

gmx mdrun -v -deffnm EM

- Now make index files

gmx make\_ndx -f LIG.gro -o index\_LIG.ndx

> 0 & ! a H\* copy and paste them

0: System, &: and, !: not, a: atom, H: hydrogen atoms , \*: all hydrogen atoms

> q save and quit

gmx genrestr -f LIG.gro -n index\_LIG.ndx -o posre\_LIG.itp -fc 1000 1000 1000

> select group "3" 3: System\_&\_!H\*

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

Align them properly. Save and close.

Again, Make other Index file for System:

gmx make\_ndx -f EM.gro -o index.ndx

> 1 | 13 ,1: Protein, 13: LIG

> q

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

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

gmx mdrun -v -deffnm NVT

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

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

gmx mdrun -v -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 -v -deffnm MD

-using the trajectory files we will do the visualization of the results:

Open chimera: Tools-> MD/Ensemble Analysis -> MD Movie:

Trajectory format: GROMACS

Run Input (.tpr): MD.tpr (set Input Location)

Trajectory (.tpr or .xtc): MD.xtc (set Input Location)

Ok.

MD Movie: play

-For better visualization, keep protein and ligand and delete rest of the things: Select-> Residue-> CL , Actions-> Atoms/Bonds-> Delete.

Do the same for SOL and NA.

Change the color of ligand.

-Open MD movies and slow the -> Playback speed -> play.

Stop the movie at any position (Frame) save it: File-> Save PDB.

To save a movie: File-> Record movie.

\_Post simulation assessment:

-open the terminal in your directory:

source /usr/local/gromacs/bin/GMXRC

----[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.

Select a group for centering: 1 (Protein)

Select a group for output: 0 (System)

-#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

Select group for least squares fit: 1 (protein)

Select group for RMSD calculation: 13 (LIG)

-open the file and see how its looks:

xmgrace rmsd.xvg

-the time is appeared in picosecond, to convert it to nanosecond:

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

Enter: 1, 13

xmgrace rmsd.xvg

now the time is in nanosecond.

------RMS Fluctuation (RMSF) Calculations-----

gmx rmsf -s MD.tpr -f MD\_center.xtc -o rmsf.xvg

4 (backbone) , 13 (LIG)

open the output files in Grace:

xmgrace rmsf.xvg

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

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

1 (protein)

13 (LIG)

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

xmgrace hb.xvg

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

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

#Choose the group of your choice : 1

xmgrace gyrate1.xvg

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

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

#Choose the option of your choice

xmgrace -nxy energy1.xvg