Worksheet: MD simulations on protein-ligand complex using GROMOS forcefield.

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This worksheet provides a step-by-step guide for performing molecular dynamics (MD) simulations on protein-ligand complexes using the GROMOS force field. The GROMOS force field is particularly well-suited for simulations of complex systems containing ligands due to its accurate treatment of non-bonded interactions between ligands and proteins.

Compared to other commonly used force fields such as CHARMM and AMBER, GROMOS has been shown to perform particularly well in simulations of protein-ligand complexes, especially in terms of reproducing experimental binding affinities and capturing the thermodynamics of binding. This is due in part to the force field's optimized treatment of ligand-protein interactions, which includes explicit treatment of the ligand partial charges and accurate modeling of the solvation environment.

For this study, we will use the protein-ligand complex 6ddi as a model system to demonstrate the efficacy of GROMOS in simulating protein-ligand interactions. The 6ddi complex comprises human bromodomain-containing protein 2 (BRD2), a member of the bromodomain and extraterminal domain (BET) family of proteins that play crucial roles in regulating gene expression by binding to acetylated histones and transcription factors. BRD2 has been implicated in various cellular processes, including cell cycle regulation, differentiation, and apoptosis. Aberrant expression or activity of BRD2 has been associated with the development and progression of several types of cancer, such as breast, prostate, and lung cancer. Studies have shown that BRD2 overexpression can enhance cell proliferation, inhibit apoptosis, and induce drug resistance, thereby promoting tumor growth and survival. Tetrahydroquinoline is a class of compounds that has demonstrated potential for inhibiting cancer through various mechanisms, including the inhibition of histone acetyltransferases (HATs) such as BRD2. Therefore, this study aims to investigate the conformational changes of BRD2 induced by Tetrahydroquinoline.

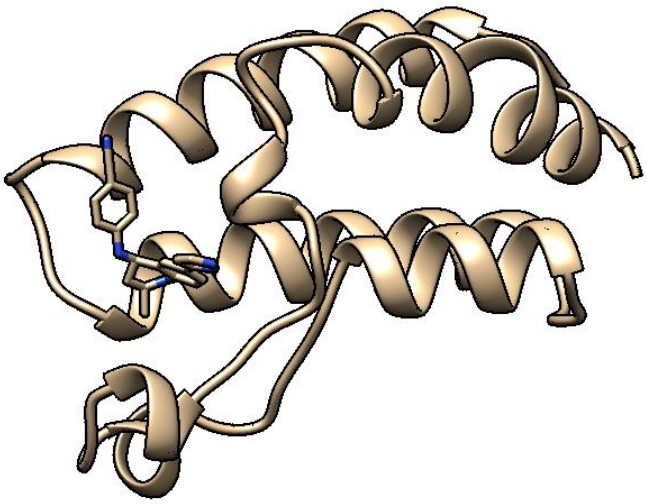


Figure1: Crystal Structure of the human BRD2 BD1 bromodomain in complex with a Tetrahydroquinoline analogue.

1. **Preparation of complex structure.**

Download the complex structure from RCSB: https://www.rcsb.org/structure/6DDI

Then, we need to make a new pdb file out of it by using VMD. Because the initial pdb file is consists of unrecognized DNA base residue. Thus, we need to correct DNA residue again.

$ vmd 6ddi.pdb

Click: Graphics > Representations

After that, Graphical Representations pop-up window will show up. On the "**Selected Atoms**" section type: **chain C and not waters**. Then click apply or press "**enter**".

Then, save the complex coordinate by clicking on 4da3.pdb and: **File > Save coordinates..**

On "**Selected atoms:**" section select for "**chain C and not waters**" then click save. Save the complex as "**start.pdb**".

2. **Preparation of ligand topology.**

Next, we need to separate ligand and protein because we can't directly apply forcefield into complex structure. Due to the forcefield is not able to recognize ligand molecule. Thus, we need to apply each of them separately.

$ grep LIG start1.pdb > lig.pdb

$ grep -v LIG start1.pdb > receptor.pdb

After we have a ligand and protein pdb files. We need to create a topology and forcefield for ligand. To do this, we need to use "Automated Topology Builder (ATB) and Repository" webserver. This webserver is a topology builder tool, which based on GROMOS forcefield.

Before we upload the structure. We need to prepare the ligand by using Biovia discovery studio to open **LIG.pdb** file. Then use right click and select "**Apply Forcefield**". After that, save and we're ready to upload the ligand into ATB webserver (always check that you save the ligand as pdb file).

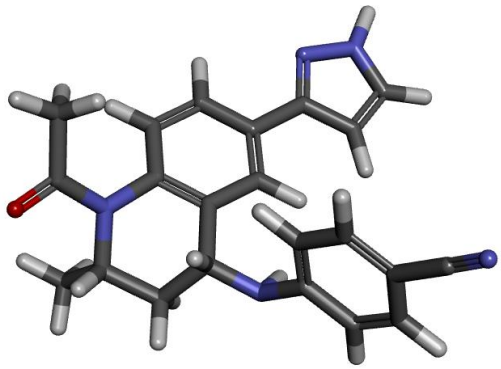
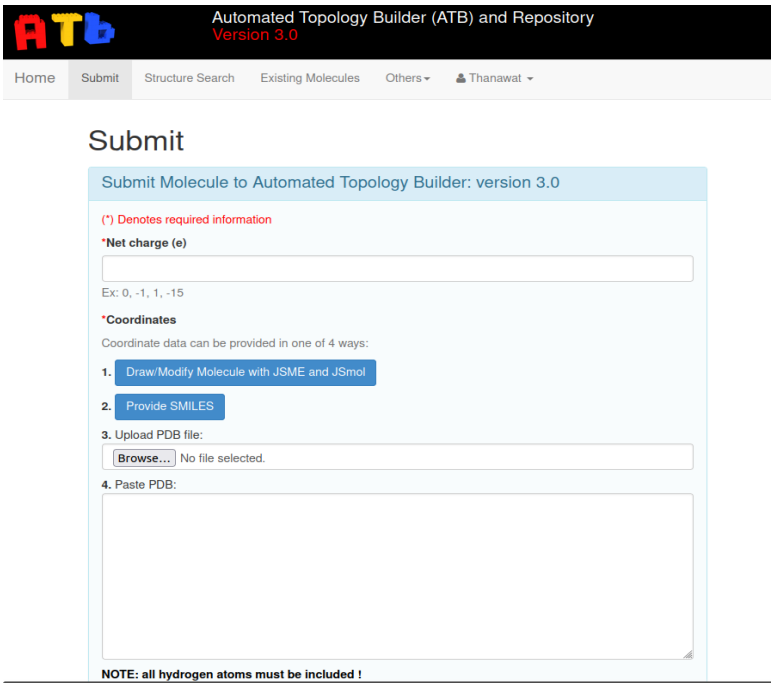


Figure 2: Tetrahydroquinoline analogue use as ligand.

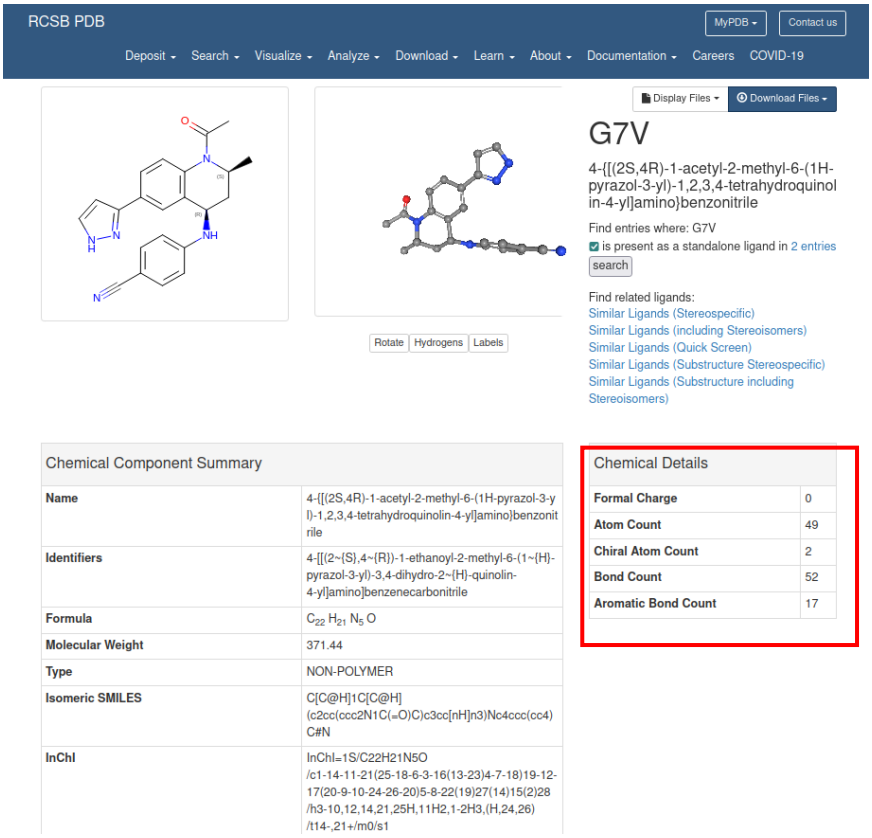
To do this, we need to access with following link: http://atb.uq.edu.au/

You need to register for the account and password only for the first time.

Once you got an email from ATB. You need to click on "**Submit**" tab.



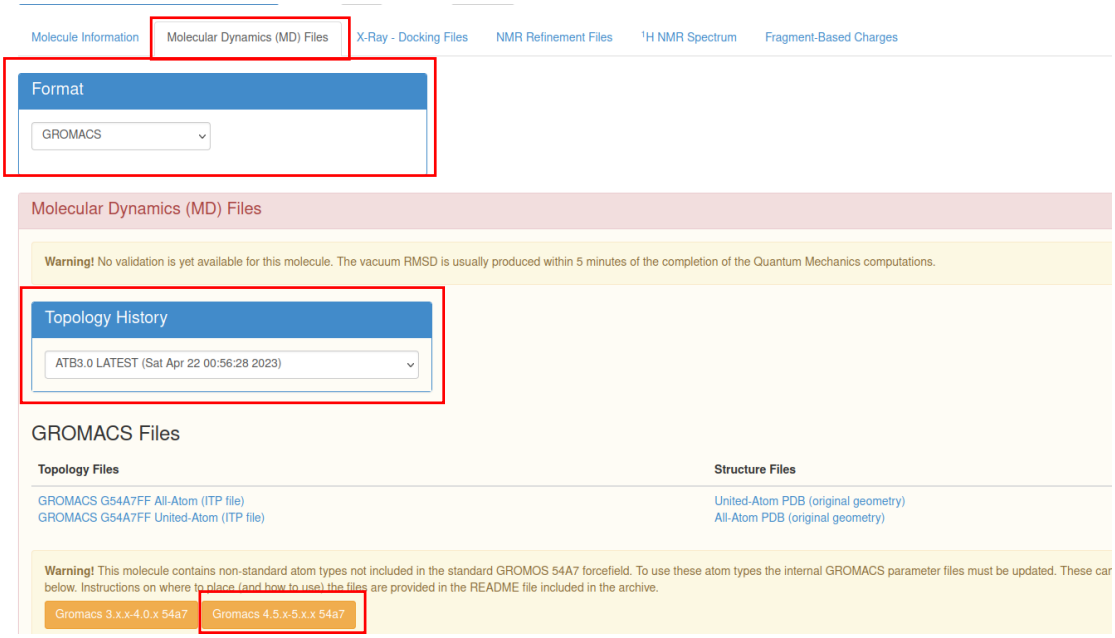
Then select "**0**" for "**Net charge (e)**". To get the value of the **Net charge**, you can check it from ligand section in RCSB:



After that, you can select either **upload with PDB** or **Provide SMILES**. In this tutorial, we going to **upload with PDB**. Upload your PDB file then click **“Next”** and **“Submit this Molecule”**.

(optional) If you want to proceed with **SMILES**. Then you can click on **Provide SMILES** and use **Isomeric SMILES** of your ligand instead of pdb file. After that you can continue the same step as upload with PDB.

Normally, this step could take a several minute. Once the server is finish, go to "**Molecular Dynamics (MD) Files**" tab.



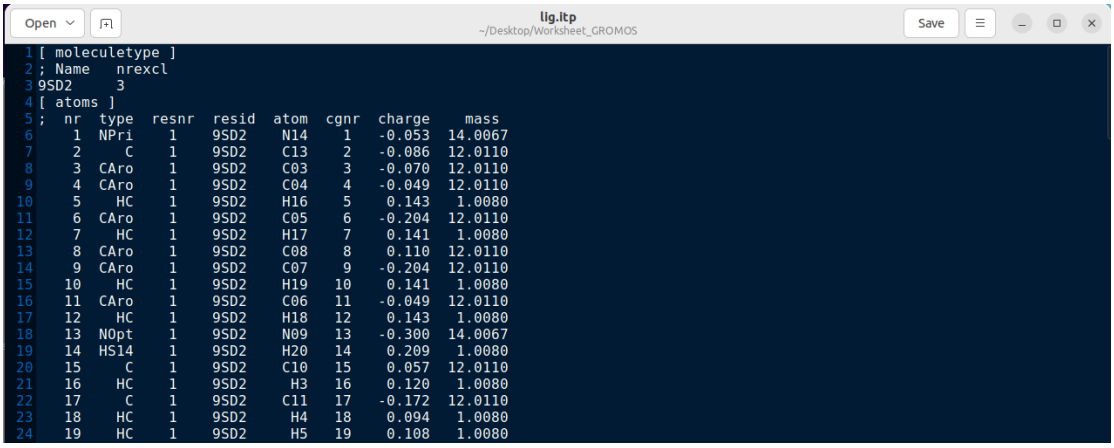
Choose "**GROMACS**" for Format. For Topology History choose "**ATB3.0 LATEST**". And then download as "**Gromacs 4.5.x-5.x.x 54a7**".

Then move the **downloaded file** to your working directory with **“mv”** and untar the file with **“tar -xvf”**.

After that, you will have new directory name **“gromos54a7_atb.ff”**. Move this directory into your gromacs topology directory. Normally, the pathway must be: **/usr/local/gromacs/share/gromacs/top**. To move the directory please use the following command:

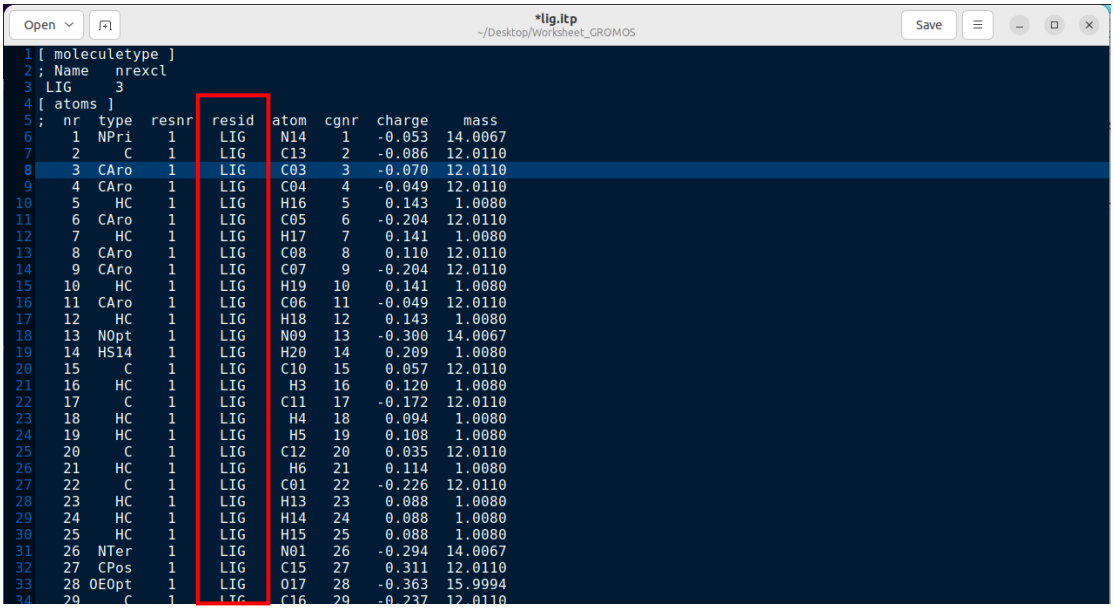
$ sudo mv gromos54a7_atb.ff /usr/local/gromacs/share/gromacs/top

Then, head back to the **ATB website**. In order to archive the more accurate Protein-Ligand complex MD, we need to perform an all-atom MD simulation. Thus, we need the all atom topology for ligand. Click the "**GROMACS G54A7FF All-Atom (ITP file)**". Copy all the line from “[moleculetype]” and save it in your work directory as "lig.itp".




```
[ moleculetype ]
; Name   nrexcl
9SD2     3
[ atoms ]
;  nr  type  resnr  resid  atom  cgnr  charge    mass
    1  NPri    1    9SD2    N14    1   -0.053  14.0067
    2     C    1    9SD2    C13    2   -0.086  12.0110
    3  CAro    1    9SD2    C03    3   -0.070  12.0110
    4  CAro    1    9SD2    C04    4   -0.049  12.0110
    5    HC    1    9SD2    H16    5    0.143   1.0080
    6  CAro    1    9SD2    C05    6   -0.204  12.0110
    7    HC    1    9SD2    H17    7    0.141   1.0080
    8  CAro    1    9SD2    C08    8    0.110  12.0110
    9  CAro    1    9SD2    C07    9   -0.204  12.0110
   10    HC    1    9SD2    H19   10    0.141   1.0080
   11  CAro    1    9SD2    C06   11   -0.049  12.0110
   12    HC    1    9SD2    H18   12    0.143   1.0080
   13  NOpt    1    9SD2    N09   13   -0.300  14.0067
   14  HS14    1    9SD2    H20   14    0.209   1.0080
   15     C    1    9SD2    C10   15    0.057  12.0110
   16    HC    1    9SD2     H3   16    0.120   1.0080
   17     C    1    9SD2    C11   17   -0.172  12.0110
   18    HC    1    9SD2     H4   18    0.094   1.0080
   19    HC    1    9SD2     H5   19    0.108   1.0080
```

Change all “**9SD2**” into “**LIG**” as following figure:




```
[ moleculetype ]
; Name   nrexcl
 LIG     3
[ atoms ]
;  nr  type  resnr  resid  atom  cgnr  charge    mass
    1  NPri    1     LIG    N14    1   -0.053  14.0067
    2     C    1     LIG    C13    2   -0.086  12.0110
    3  CAro    1     LIG    C03    3   -0.070  12.0110
    4  CAro    1     LIG    C04    4   -0.049  12.0110
    5    HC    1     LIG    H16    5    0.143   1.0080
    6  CAro    1     LIG    C05    6   -0.204  12.0110
    7    HC    1     LIG    H17    7    0.141   1.0080
    8  CAro    1     LIG    C08    8    0.110  12.0110
    9  CAro    1     LIG    C07    9   -0.204  12.0110
   10    HC    1     LIG    H19   10    0.141   1.0080
   11  CAro    1     LIG    C06   11   -0.049  12.0110
   12    HC    1     LIG    H18   12    0.143   1.0080
   13  NOpt    1     LIG    N09   13   -0.300  14.0067
   14  HS14    1     LIG    H20   14    0.209   1.0080
   15     C    1     LIG    C10   15    0.057  12.0110
   16    HC    1     LIG     H3   16    0.120   1.0080
   17     C    1     LIG    C11   17   -0.172  12.0110
   18    HC    1     LIG     H4   18    0.094   1.0080
   19    HC    1     LIG     H5   19    0.108   1.0080
   20     C    1     LIG    C12   20    0.035  12.0110
   21    HC    1     LIG     H6   21    0.114   1.0080
   22     C    1     LIG    C01   22   -0.226  12.0110
   23    HC    1     LIG    H13   23    0.088   1.0080
   24    HC    1     LIG    H14   24    0.088   1.0080
   25    HC    1     LIG    H15   25    0.088   1.0080
   26  NTer    1     LIG    N01   26   -0.294  14.0067
   27  CPos    1     LIG    C15   27    0.311  12.0110
   28 OEOpt    1     LIG    O17   28   -0.363  15.9994
   29     C    1     LIG    C16   29   -0.237  12.0110
```

3. Preparation of complex topology

Now, we already have prepared forcefield and topology for ligand. Next, we need to apply forcefield into receptor.pdb by using following command:

$ gmx pdb2gmx -f receptor.pdb -o receptor_processed.gro -ignh -missing

Select for GROMOS forcefield and SPC as water molecule itp.

15: GROMOS96 54a7 force field

1: SPC

Then, we need to introduce ligand topology “**lig.itp**” into “**topol.top**”. To do this we need to edit “**topol.top**” file. (You can use either $ nano or Text editor)

$ nano topol.top

Then paste: **#include "lig.itp"**. After the line: **#include "gromos54a7_atb.ff/forcefield.itp"**. As show in following figure:

```
;
;       File 'topol.top' was generated
;       By user: thanawat (1000)
;       On host: thanawat-virtual-machine
;       At date: Sat Apr 22 22:15:21 2023
;
;       This is a standalone topology file
;
;       Created by:
;                           :-) GROMACS - gmx pdb2gmx, 2023 (-:
;
;       Executable:   /usr/local/gromacs/bin/gmx
;       Data prefix:  /usr/local/gromacs
;       Working dir:  /home/thanawat/Desktop/Worksheet_GROMOS
;       Command line:
;         gmx pdb2gmx -f receptor.pdb -o receptor_processed.gro -ignh -missing
;       Force field was read from the standard GROMACS share directory.
;

; Include forcefield parameters
#include "gromos54a7_atb.ff/forcefield.itp"
#include "lig.itp"

[ moleculetype ]
; Name            nrexcl
Protein_chain_C     3

[ atoms ]
;   nr       type  resnr residue  atom   cgnr     charge       mass  typeB    chargeB      massB
; residue  76 THR rtp THR  q +1.0
     1         NL     76    THR      N      1      0.129    14.0067
     2          H     76    THR     H1      1      0.248      1.008
     3          H     76    THR     H2      1      0.248      1.008
     4          H     76    THR     H3      1      0.248      1.008
     5        CH1     76    THR     CA      2      0.127     13.019
```

And also add "**LIG 1**" at **[molecules]** section (add LIG before protein). You should have something like this:

```
;  i funct       fcx        fcy        fcz
   1    1       1000       1000       1000
#endif

; Include topology for ions
#include "gromos54a7_atb.ff/ions.itp"

[ system ]
; Name
Protein

[ molecules ]
; Compound        #mols
LIG                 1
Protein_chain_C     1
```

And change [system] section into: **LIG and Protein**.

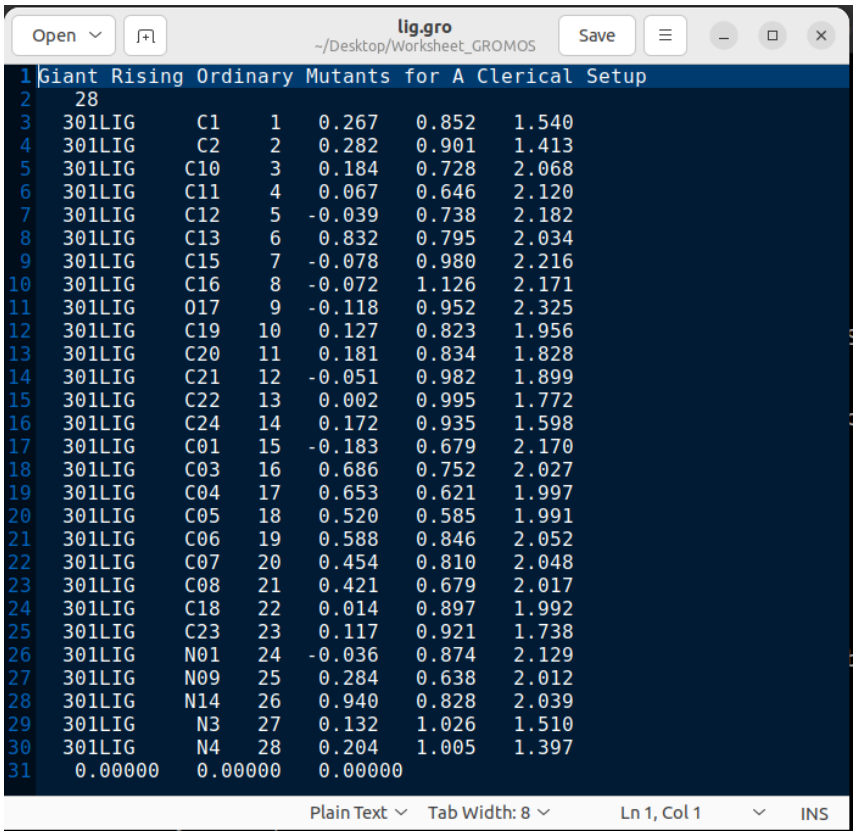
```
[ system ]
; Name
LIG and Protein

[ molecules ]
; Compound        #mols
LIG                 1
Protein_chain_C     1
```

Next, creat **"lig.gro"** file by using following command:

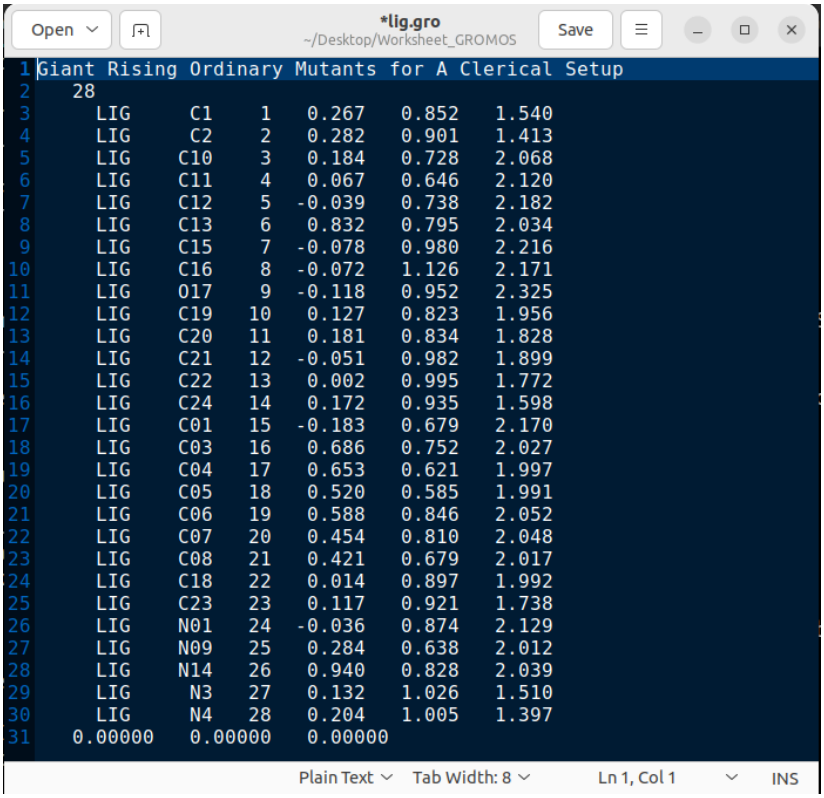
$ gmx editconf -f lig.pdb -o lig.gro

Do not forget, you need to use the lig.pdb file the one that you upload in ATB webserver.

After that, open "**lig.gro**" and change the first column from **301LIG** following figure:

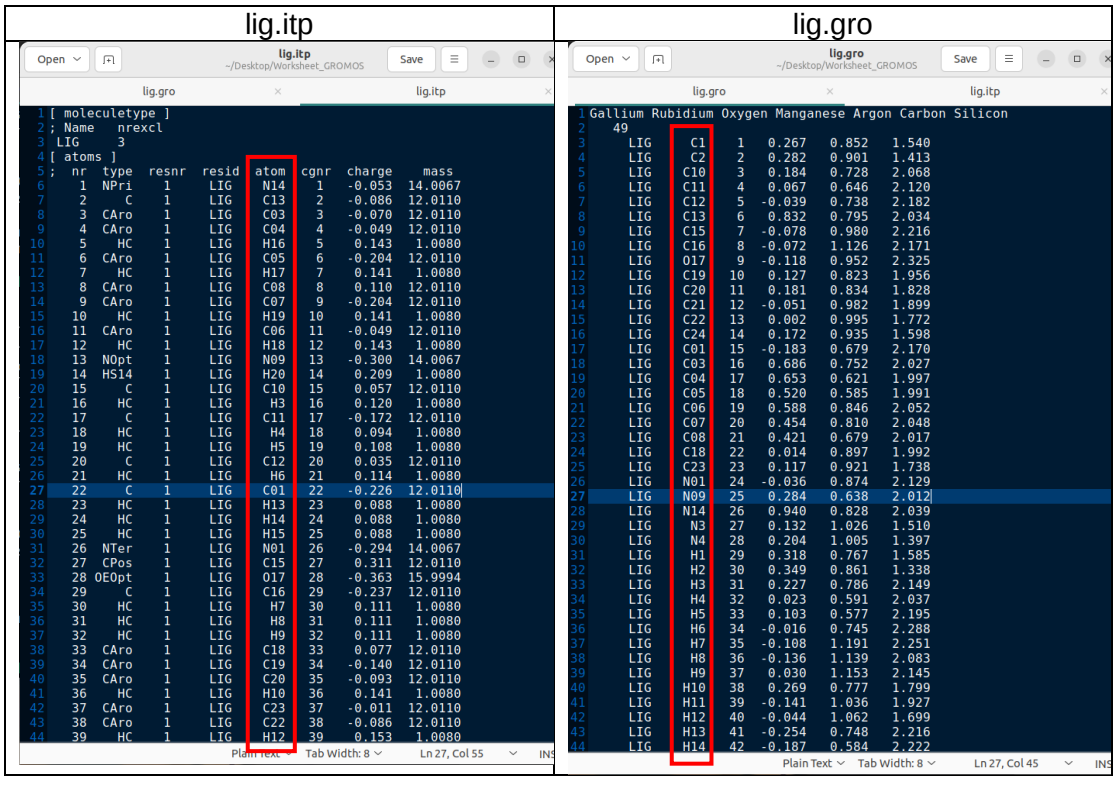


Into **LIG** as following figure:



Next, to do Protein-Ligand MD simulation we need to make sure that the atom order between GROMACS ligand file is relate to the atom order in ligand topology file that we already created.

The atom order in “**lig.gro**” needs to be order in the same order as written in “**lig.itp**”.



As you see, the atom order between **lig.itp** and **lig.gro** is still different ordered.

To order the atom, there are 2 ways to order either do it by hand or use python script.

To use python script, you need to install git by using:

$ sudo apt install git

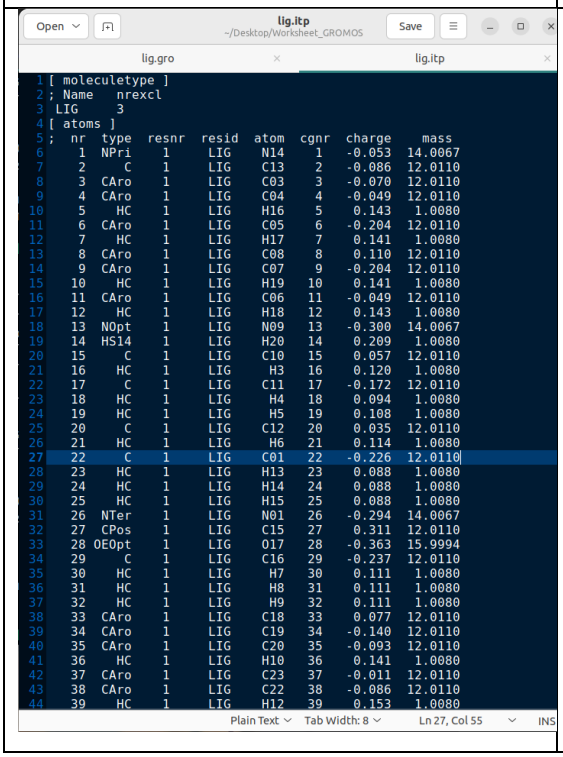
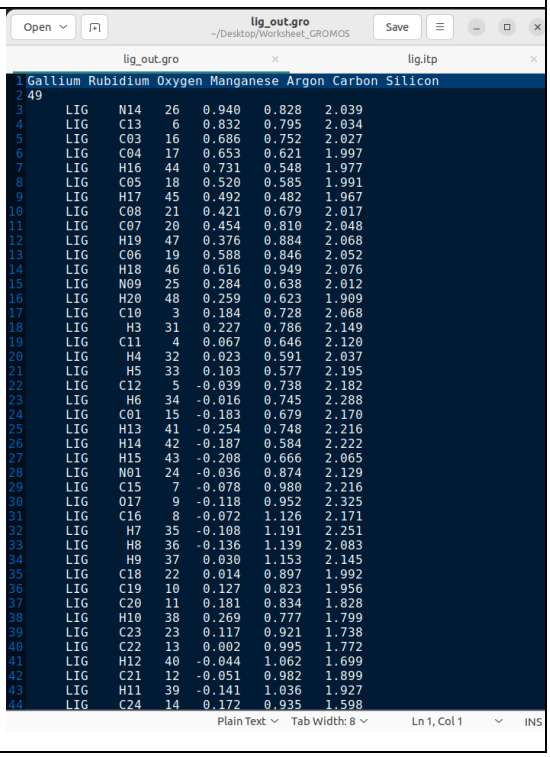
Then, clone the python script from github. The python script was written and developed by Thanawat Thaingtamtanha, under supervised by PD. Dr. Stephan Baeurle. If the script has any problem, please send email to "**thaingtamtanhat@gmail.com**"

$ git clone https://github.com/thaingtamtanha/ATOM_ReORDER_GROMOS_Forcefield.git

After git clone, you'll have new directory name "**ATOM_ReORDER_GROMOS_Forcefield**" go into this directory and copy python script name "**reorder_lig_gro.py**" into your working directory.

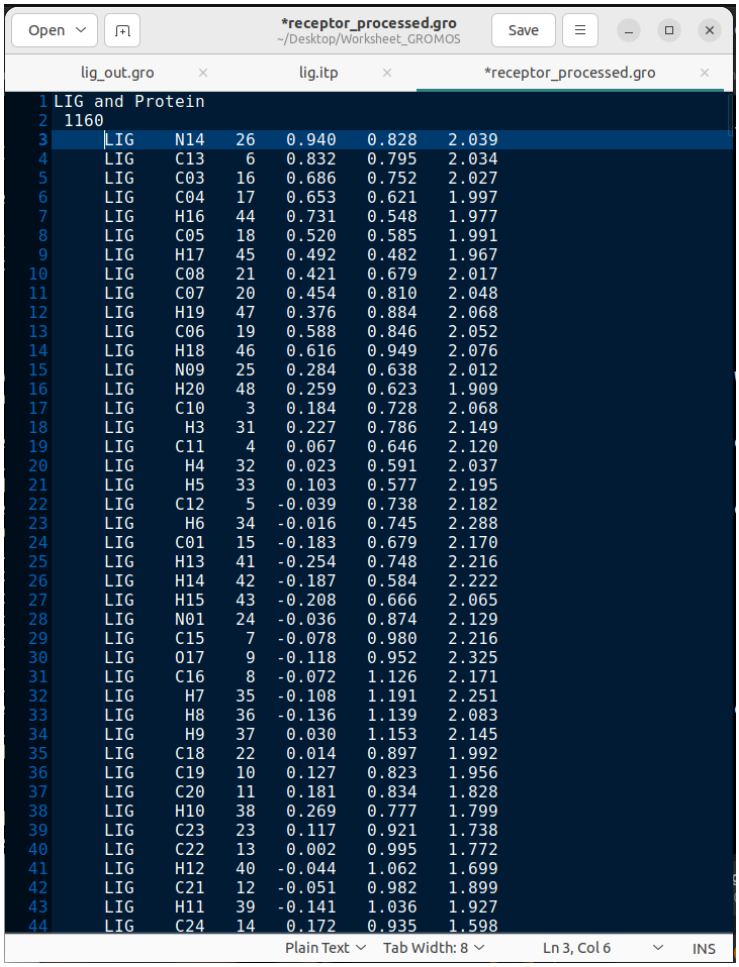
After that, use the following command to reorder the atom:

$ python reorder_lig_gro.py lig.itp lig.gro lig_out.gro

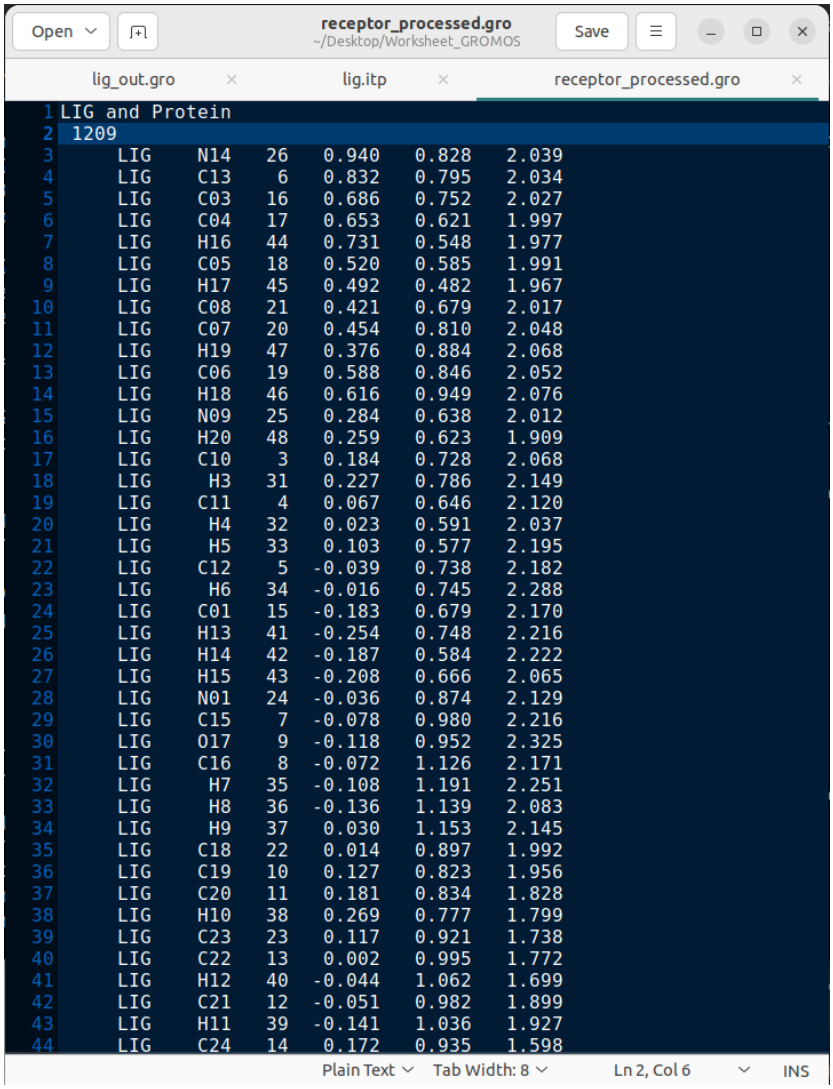
lig.itp	lig_out.gro
	

Now you'll have "**lig_out.gro**", which the atom of ligand already re-arranges.

Next, we need to build the **complex.gro**. To do this we need to copy all the "**LIG**" lines from "**lig_out.gro**" and paste these lines into "**receptor_processed.gro**", **paste these lines before protein residue**. And also change the header into "**LIG and Protein**" as following figure:



Then because we introduce ligand atom into this gro file. Thus, we need to change the amount of atom from **1160** into **1209**. Finally, the **receptor_processed.gro** must look like:



Next, we need to create the boundary box for simulation and place the complex into the center of the box. To do this, we need to make an index for LIG and protein:

$ gmx make_ndx -f receptor_processed.gro -o group.ndx

2 | 3

q

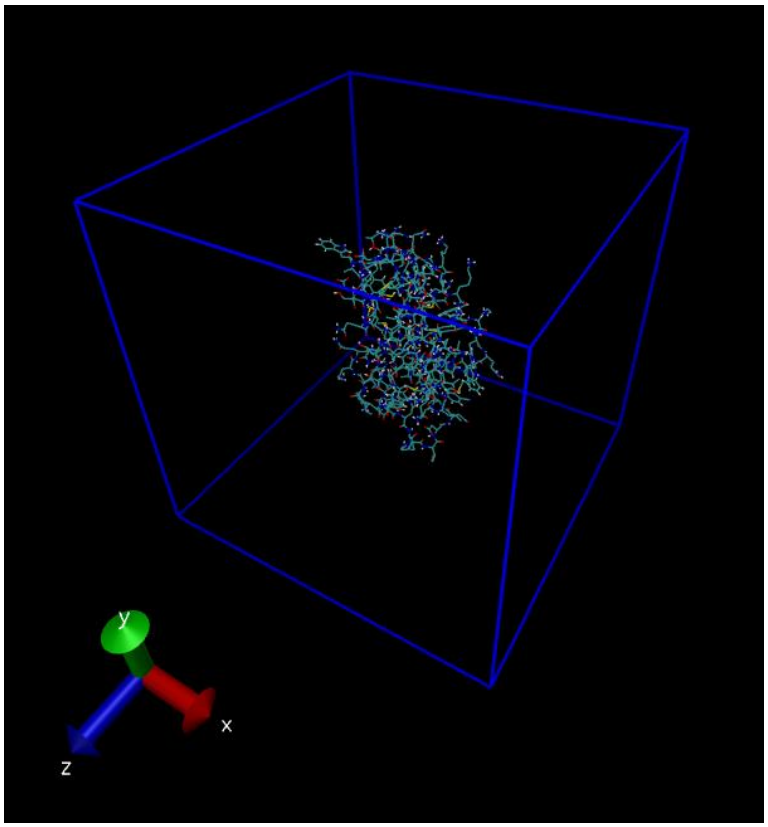
$ gmx editconf -f receptor_processed.gro -o newbox.gro -c -d 1.0 -bt cubic -n group.ndx

14

0

To make sure that the complex is really place at the center of the box. We can check by using **VMD**. Open **newbox.gro** with **VMD,** then click **Extensions > Tk console**.

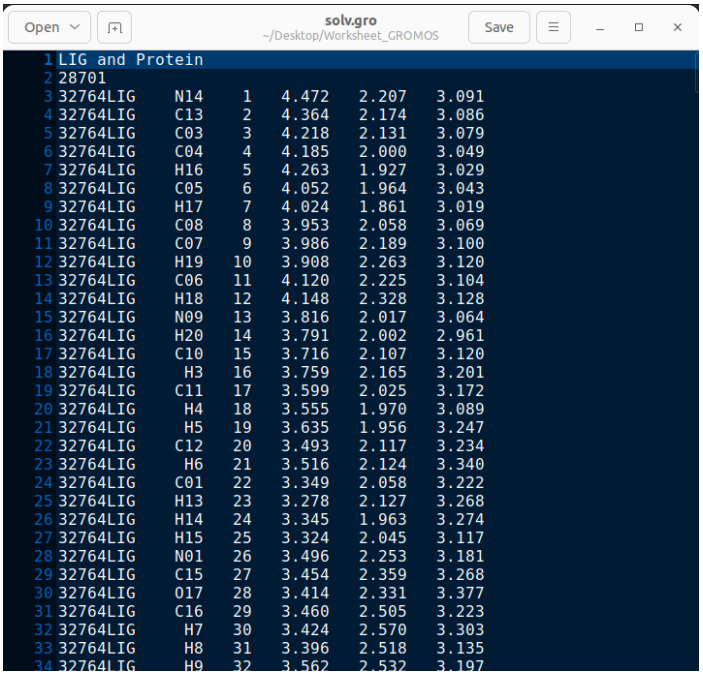
In the Tk console type "**pbc box**" and press enter. Then you should have the same as following figure:



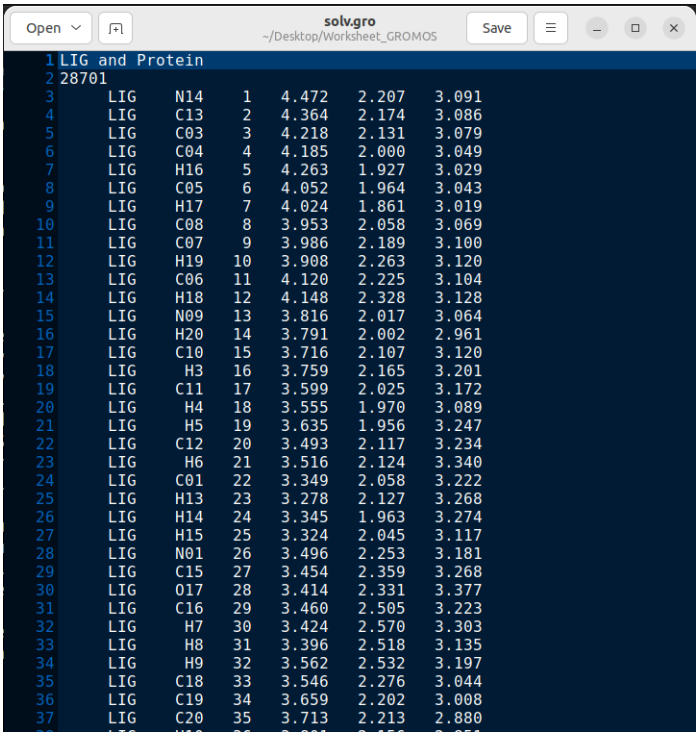
Then, solvated the system by using following command:

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

After this part, the software will automatically change the residue name. But we need to keep the residue name as "**LIG**". Therefore, use "nano" or "Text editor" to read "**solv.gro**". And change the residue name from **32764LIG** into **LIG**:



After change:



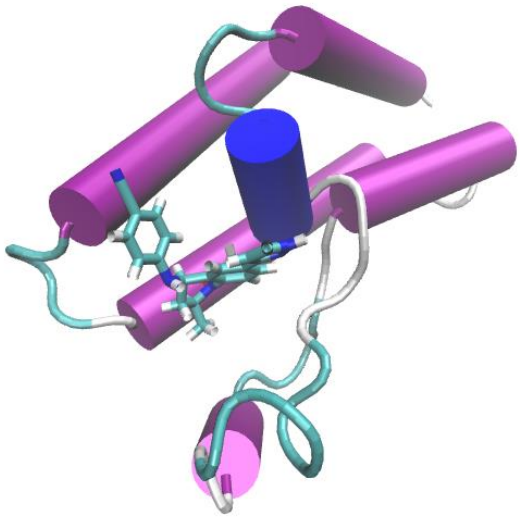
Then save.

After that, download again “**ions.mdp**” file from previous worksheet and apply ions into system by using:

$ gmx grompp -f ions.mdp -c solv.gro -p topol.top -o ions.tpr -maxwarn 5

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

In this step, if you did everything correct and following the step. Your complex could be complete as following figure:



Otherwise, your ligand molecule will be destroyed due to GROMACS will replace some of ligand molecule into ion molecule.

Next, we need to constrain ligand by using constant uniform force. First, create index for ligand:

$ 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

3

Then copy and paste the following line in "topol.top" after the line: #include "lig.itp":

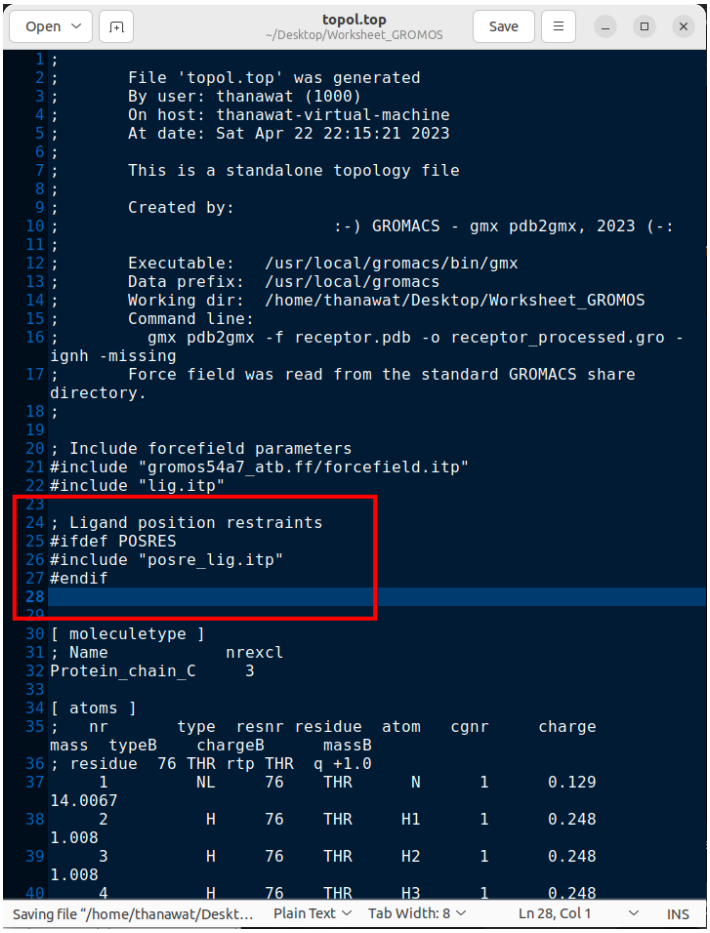
; Ligand position restraints

#ifdef POSRES

#include "posre_lig.itp"

#endif

You should have topol.top like this:



Save and quit.

4. Energy minimization, Equilibration and MD production on Protein-Ligand complex

Then continue with Energy minimization > Equilibration > MD production.

$ gmx grompp -f em.mdp -c solv_ions.gro -p topol.top -o em.tpr -maxwarn 1

$ gmx mdrun -v -deffnm em

Then, we need to control temperature coupling for ligand and protein (2 | 4).

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

2 | 4

q

Put "**LIG_Protein**" and "**Water_and_ions**" into tc-grps every mdp files.

For NPT:

title	= Protein-ligand complex NPT equilibration
define	= -DPOSRES ; position restrain the protein and ligand

```
; Run parameters
integrator              = md        ; leap-frog integrator
nsteps                  = 50000     ; 2 * 50000 = 100 ps
dt                      = 0.002     ; 2 fs
; Output control
nstenergy               = 500       ; save energies every 1.0 ps
nstlog                  = 500       ; update log file every 1.0 ps
nstxout-compressed      = 500       ; save coordinates every 1.0 ps
; Bond parameters
continuation            = yes       ; continuing from NVT
constraint_algorithm    = lincs     ; holonomic constraints
constraints             = h-bonds   ; bonds to H are constrained
lincs_iter              = 1         ; accuracy of LINCS
lincs_order             = 4         ; also related to accuracy
; Neighbor searching and vdW
cutoff-scheme           = Verlet
ns_type                 = grid      ; search neighboring grid cells
nstlist                 = 20        ; largely irrelevant with Verlet
rlist                   = 1.2
vdwtype                 = cutoff
vdw-modifier            = force-switch
rvdw-switch             = 1.0
rvdw                    = 1.2       ; short-range van der Waals cutoff (in nm)
; Electrostatics
coulombtype             = PME       ; Particle Mesh Ewald for long-range electrostatics
rcoulomb                = 1.2
pme_order               = 4         ; cubic interpolation
fourierspacing          = 0.16      ; grid spacing for FFT
; Temperature coupling
tcoupl                  = V-rescale                     ; modified Berendsen thermostat
tc-grps                 = LIG_Protein Water_and_ions    ; two coupling groups - more accurate
tau_t                   = 0.1   0.1                     ; time constant, in ps
ref_t                   = 300   300                     ; reference temperature, one for each group, in K
; Pressure coupling
pcoupl                  = Berendsen                     ; pressure coupling is on for NPT
pcoupltype              = isotropic                     ; uniform scaling of box vectors
tau_p                   = 2.0                           ; time constant, in ps
ref_p                   = 1.0                           ; reference pressure, in bar
compressibility         = 4.5e-5                        ; isothermal compressibility of water, bar^-1
refcoord_scaling        = com
; Periodic boundary conditions
pbc                     = xyz       ; 3-D PBC
; Dispersion correction is not used for proteins with the C36 additive FF
DispCorr                = no
```

```
; Velocity generation
gen_vel                 = no       ; velocity generation off after NVT
```

For NVT:

```
title                   = Protein-ligand complex NVT equilibration
define                  = -DPOSRES  ; position restrain the protein and ligand
; Run parameters
integrator              = md       ; leap-frog integrator
nsteps                  = 50000    ; 2 * 50000 = 100 ps
dt                      = 0.002    ; 2 fs
; Output control
nstenergy               = 500   ; save energies every 1.0 ps
nstlog                  = 500   ; update log file every 1.0 ps
nstxout-compressed      = 500   ; save coordinates every 1.0 ps
; Bond parameters
continuation            = no       ; first dynamics run
constraint_algorithm    = lincs    ; holonomic constraints
constraints             = h-bonds   ; bonds to H are constrained
lincs_iter              = 1        ; accuracy of LINCS
lincs_order             = 4        ; also related to accuracy
; Neighbor searching and vdW
cutoff-scheme           = Verlet
ns_type                 = grid     ; search neighboring grid cells
nstlist                 = 20       ; largely irrelevant with Verlet
rlist                   = 1.2
vdwtype                 = cutoff
vdw-modifier            = force-switch
rvdw-switch             = 1.0
rvdw                    = 1.2      ; short-range van der Waals cutoff (in nm)
; Electrostatics
coulombtype             = PME      ; Particle Mesh Ewald for long-range electrostatics
rcoulomb                = 1.2      ; short-range electrostatic cutoff (in nm)
pme_order               = 4        ; cubic interpolation
fourierspacing          = 0.16     ; grid spacing for FFT
; Temperature coupling
tcoupl                  = V-rescale                    ; modified Berendsen thermostat
tc-grps                 = LIG_Protein Water_and_ions    ; two coupling groups - more accurate
tau_t                   = 0.1   0.1                    ; time constant, in ps
ref_t                   = 300   300                    ; reference temperature, one for each group, in K
; Pressure coupling
pcoupl                  = no       ; no pressure coupling in NVT
; Periodic boundary conditions
pbc                     = xyz      ; 3-D PBC
```

```
; Dispersion correction is not used for proteins with the C36 additive FF
DispCorr                = no
; Velocity generation
gen_vel                 = yes       ; assign velocities from Maxwell distribution
gen_temp                = 300       ; temperature for Maxwell distribution
gen_seed                = -1        ; generate a random seed
```

For MD:

```
title                   = Protein-ligand complex MD simulation
; Run parameters
integrator              = md        ; leap-frog integrator
nsteps                  = 5000000   ; 2 * 5000000 = 10000 ps (10 ns)
dt                      = 0.002     ; 2 fs
; Output control
nstenergy               = 500000      ; save energies every 10.0 ps
nstlog                  = 500000      ; update log file every 10.0 ps
nstxout-compressed      = 500000      ; save coordinates every 10.0 ps
; Bond parameters
continuation            = yes       ; continuing from NPT
constraint_algorithm    = lincs     ; holonomic constraints
constraints             = h-bonds   ; bonds to H are constrained
lincs_iter              = 1         ; accuracy of LINCS
lincs_order             = 4         ; also related to accuracy
; Neighbor searching and vdW
cutoff-scheme           = Verlet
ns_type                 = grid      ; search neighboring grid cells
nstlist                 = 20        ; largely irrelevant with Verlet
rlist                   = 1.2
vdwtype                 = cutoff
vdw-modifier            = force-switch
rvdw-switch             = 1.0
rvdw                    = 1.2       ; short-range van der Waals cutoff (in nm)
; Electrostatics
coulombtype             = PME       ; Particle Mesh Ewald for long-range electrostatics
rcoulomb                = 1.2
pme_order               = 4         ; cubic interpolation
fourierspacing          = 0.16      ; grid spacing for FFT
; Temperature coupling
tcoupl                  = V-rescale                     ; modified Berendsen thermostat
tc-grps                 = LIG_Protein Water_and_ions    ; two coupling groups - more accurate
tau_t                   = 0.1   0.1                     ; time constant, in ps
ref_t                   = 300   300                     ; reference temperature, one for each group, in K
; Pressure coupling
```

```
pcoupl                  = Parrinello-Rahman             ; pressure coupling is on for NPT
pcoupltype              = isotropic                     ; uniform scaling of box vectors
tau_p                   = 2.0                           ; time constant, in ps
ref_p                   = 1.0                           ; reference pressure, in bar
compressibility         = 4.5e-5                        ; isothermal compressibility of water, bar^-1
; Periodic boundary conditions
pbc                     = xyz       ; 3-D PBC
; Dispersion correction is not used for proteins with the C36 additive FF
DispCorr                = no
; Velocity generation
gen_vel                 = no        ; continuing from NPT equilibration
```

After that, untar "**gromos54a7_atb.ff.tar.gz**" again in your work directory. Then tar your work directory:

$ tar -cvf GROMOS_worksheet.tar <Your work directory>

Then upload GROMOS_worksheet.tar into OMNI cluster. Untar the file and create a SLURM file by using:

$ nano WK_GROMOS.sh

And using script as following lines:

```
#!/bin/bash
#SBATCH --nodes=4
#SBATCH --ntasks-per-node=18
#SBATCH --time=1-00:00:00
#SBATCH --partition=medium

module load gromacs/2018.6

gmx_mpi grompp -f nvt.mdp -c em.gro -r em.gro -p topol.top -n index.ndx -o nvt.tpr -maxwarn 5
mpirun gmx_mpi mdrun -deffnm nvt
gmx_mpi grompp -f npt.mdp -c nvt.gro -t nvt.cpt -r nvt.gro -p topol.top -n index.ndx -o npt.tpr -
maxwarn 5
mpirun gmx_mpi mdrun -deffnm npt
gmx_mpi grompp -f md.mdp -c npt.gro -t npt.cpt -p topol.top -n index.ndx -o md_0_1.tpr -maxwarn 5
mpirun gmx_mpi mdrun -deffnm md_0_1
```

Submit for the calculation with:

$ sbatch WK_GROMOS.sh

5. **MD simulations analysis**

Once the calculation is finish, we need to perform MD simulations analysis.

$ srun --pty /bin/bash

$ module load gromacs/2018.6

Centering the Protein-Ligand complex by using following command:

$ mpirun gmx_mpi trjconv -s md_0_1.tpr -f md_0_1.xtc -o md_0_1_center.xtc -n index.ndx -pbc mol -center

20

0

Extract for start.pdb and end.pdb:

$ mpirun gmx_mpi trjconv -s md_0_1.tpr -f md_0_1_center.xtc -o start.pdb -n index.ndx -dump 0

20

$ mpirun gmx_mpi trjconv -s md_0_1.tpr -f md_0_1.xtc -o end.pdb -n index.ndx -dump 10000

20

Extract for full-trajectory:

$ gmx_mpi trjconv -s md_0_1.tpr -f md_0_1.xtc -o fulltraj.pdb -b 0 -e 10000 -skip 1 -n index.ndx

20

Protein RMSD:

$ mpirun gmx_mpi rms -s md_0_1.tpr -f md_0_1.xtc -o rmsd.xvg -tu ns

7

7

$ mpirun gmx_mpi gyrate -s md_0_1.tpr -f md_0_1.xtc -o gyrate.xvg

4

6. **Ligand interaction analysis**

Create active binding site on complex:

$ mpirun gmx_mpi make_ndx -f md_0_1.tpr -o activesite.ndx

r 98 | r 101 | r 103 | r 108 | r 110 | r 156 | r 165

We'll get new group number 22 for active binding site:

22 r_98_r_101_r_103_r_108_r_110_r_156_r_165: 65 atoms

Then, we will plot the COM distance between drug and the binding site:

$ mpirun gmx_mpi distance -f md_0_1_center.xtc -s md_0_1.tpr -n activesite.ndx -oav distance.xvg -oall dist.xvg -select 'com of group 22 plus com of group 2' -tu ns

Distance.xvg is for the average COM distance change, dist.xvg is all distance as function of time.

Analyzed for how much the ligand binding pose has changed over the course of the simulation:

$ mpirun gmx_mpi rms -s md_0_1.tpr -f md_0_1_center.xtc -n index_lig.ndx -tu ns -o rmsd_LIG.xvg

3

3

As soon as the analysis is finished, send the calculation results on OMNI back on your laptop and unpack them.