

## 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.

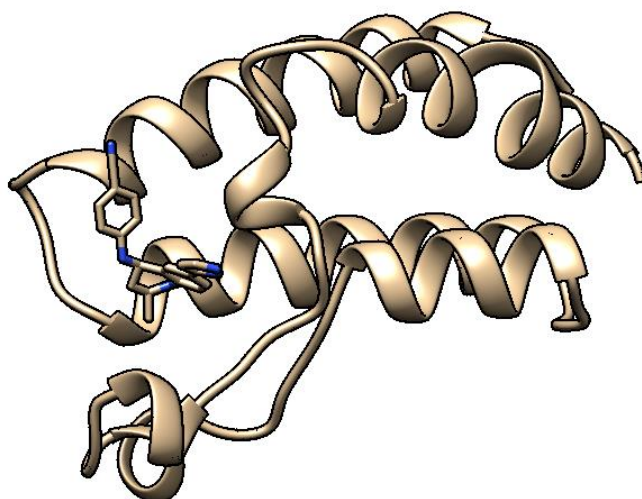


Figure1: Crystal Structure of the human BRD2 BD1 bromodomain in complex with a Tetrahydroquinoline analogue.

## 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 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**).

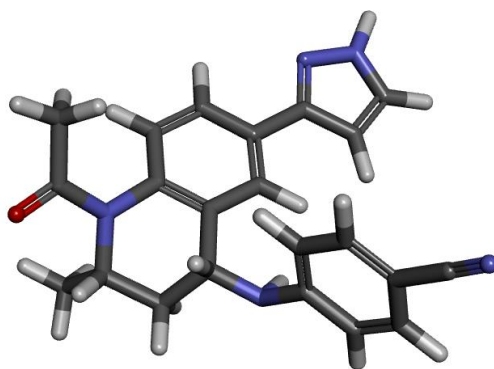


Figure 2: Tetrahydroquinoline analogue use as ligand.

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.

**ATB** Automated Topology Builder (ATB) and Repository  
Version 3.0

Home Submit Structure Search Existing Molecules Others Thanawat

### Submit

Submit Molecule to Automated Topology Builder: version 3.0

(\*) Denotes required information

**\*Net charge (e)**

Ex: 0, -1, 1, -15

**\*Coordinates**

Coordinate data can be provided in one of 4 ways:

1. Draw/Modify Molecule with JSME and JSmol
2. Provide SMILES
3. Upload PDB file:  
 No file selected.
4. Paste PDB:

**NOTE: all hydrogen atoms must be included !**

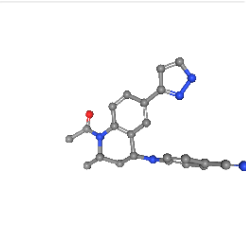
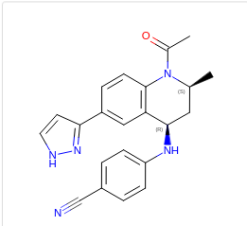
Then select “0” for “Net charge (e)”. To get the value of the **Net charge**, you can check it from ligand section in RCSB:

RCSB PDB

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### G7V

4-[[[(2S,4R)-1-acetyl-2-methyl-6-(1H-pyrazol-3-yl)-1,2,3,4-tetrahydroquinolin-4-yl]amino]benzonitrile

Find entries where: G7V  
☒ is present as a standalone ligand in 2 entries

Find related ligands:  
[Similar Ligands \(Stereospecific\)](#)  
[Similar Ligands \(including Stereoisomers\)](#)  
[Similar Ligands \(Quick Screen\)](#)  
[Similar Ligands \(Substructure Stereospecific\)](#)  
[Similar Ligands \(Substructure including Stereoisomers\)](#)

Chemical Component Summary	
Name	4-[[[(2S,4R)-1-acetyl-2-methyl-6-(1H-pyrazol-3-yl)-1,2,3,4-tetrahydroquinolin-4-yl]amino]benzonitrile
Identifiers	4-[[[(2-[S],4-[R])-1-ethanoyl-2-methyl-6-(1-[H]-pyrazol-3-yl)-3,4-dihydro-2-[H]-quinolin-4-yl]amino]benzenecarbonitrile
Formula	C <sub>22</sub> H <sub>21</sub> N <sub>5</sub> O
Molecular Weight	371.44
Type	NON-POLYMER
Isomeric SMILES	C[C@H]1[C@H](c2cc(ccc2N1C(=O)C)c3cc[nH]n3)Nc4ccc(cc4)C#N
InChI	InChI=1S/C22H21N5O/c1-14-11-21(25-18-6-3-16(13-23)4-7-18)19-12-17(20-9-10-24-26-20)5-8-22(19)27(14)15(2)28/h3-10,12,14,21,25H,11H2,1-2H3,(H,24,26)/t14-,21-/m0/s1

Chemical Details	
Formal Charge	0
Atom Count	49
Chiral Atom Count	2
Bond Count	52
Aromatic Bond Count	17

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.

Molecule Information Molecular Dynamics (MD) Files X-Ray - Docking Files NMR Refinement Files <sup>1</sup>H NMR Spectrum Fragment-Based Charges

Format

GROMACS

Molecular Dynamics (MD) Files

Warning! No validation is yet available for this molecule. The vacuum RMSD is usually produced within 5 minutes of the completion of the Quantum Mechanics computations.

Topology History

ATB3.0 LATEST (Sat Apr 22 00:56:28 2023)

GROMACS Files

Topology Files	Structure Files
<a href="#">GROMACS G54A7FF All-Atom (ITP file)</a>	<a href="#">United-Atom PDB (original geometry)</a>
<a href="#">GROMACS G54A7FF United-Atom (ITP file)</a>	<a href="#">All-Atom PDB (original geometry)</a>

Warning! This molecule contains non-standard atom types not included in the standard GROMOS 54A7 forcefield. To use these atom types the internal GROMACS parameter files must be updated. These can be found below. Instructions on where to place (and how to use) the files are provided in the README file included in the archive.

Gromacs 3.x.x-4.0.x 54a7 Gromacs 4.5.x-5.x.x 54a7

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".

```

1 [ moleculetype ]
2 ; Name      nrexcl
3 9SD2      3
4 [ atoms ]
5 ; nr      type      resnr      resid      atom      cgnr      charge      mass
6 1      NPri      1      9SD2      N14      1      -0.053      14.0067
7 2      C      1      9SD2      C13      2      -0.086      12.0110
8 3      CAro      1      9SD2      C03      3      -0.070      12.0110
9 4      CAro      1      9SD2      C04      4      -0.049      12.0110
10 5      HC      1      9SD2      H16      5      0.143      1.0080
11 6      CAro      1      9SD2      C05      6      -0.204      12.0110
12 7      HC      1      9SD2      H17      7      0.141      1.0080
13 8      CAro      1      9SD2      C08      8      0.110      12.0110
14 9      CAro      1      9SD2      C07      9      -0.204      12.0110
15 10      HC      1      9SD2      H19      10      0.141      1.0080
16 11      CAro      1      9SD2      C06      11      -0.049      12.0110
17 12      HC      1      9SD2      H18      12      0.143      1.0080
18 13      NOpt      1      9SD2      N09      13      -0.300      14.0067
19 14      HS14      1      9SD2      H20      14      0.209      1.0080
20 15      C      1      9SD2      C10      15      0.057      12.0110
21 16      HC      1      9SD2      H3      16      0.120      1.0080
22 17      C      1      9SD2      C11      17      -0.172      12.0110
23 18      HC      1      9SD2      H4      18      0.094      1.0080
24 19      HC      1      9SD2      H5      19      0.108      1.0080

```

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

```

1 [ moleculetype ]
2 ; Name      nrexcl
3 LIG      3
4 [ atoms ]
5 ; nr      type      resnr      resid      atom      cgnr      charge      mass
6 1      NPri      1      LIG      N14      1      -0.053      14.0067
7 2      C      1      LIG      C13      2      -0.086      12.0110
8 3      CAro      1      LIG      C03      3      -0.070      12.0110
9 4      CAro      1      LIG      C04      4      -0.049      12.0110
10 5      HC      1      LIG      H16      5      0.143      1.0080
11 6      CAro      1      LIG      C05      6      -0.204      12.0110
12 7      HC      1      LIG      H17      7      0.141      1.0080
13 8      CAro      1      LIG      C08      8      0.110      12.0110
14 9      CAro      1      LIG      C07      9      -0.204      12.0110
15 10      HC      1      LIG      H19      10      0.141      1.0080
16 11      CAro      1      LIG      C06      11      -0.049      12.0110
17 12      HC      1      LIG      H18      12      0.143      1.0080
18 13      NOpt      1      LIG      N09      13      -0.300      14.0067
19 14      HS14      1      LIG      H20      14      0.209      1.0080
20 15      C      1      LIG      C10      15      0.057      12.0110
21 16      HC      1      LIG      H3      16      0.120      1.0080
22 17      C      1      LIG      C11      17      -0.172      12.0110
23 18      HC      1      LIG      H4      18      0.094      1.0080
24 19      HC      1      LIG      H5      19      0.108      1.0080
25 20      C      1      LIG      C12      20      0.035      12.0110
26 21      HC      1      LIG      H6      21      0.114      1.0080
27 22      C      1      LIG      C01      22      -0.226      12.0110
28 23      HC      1      LIG      H13      23      0.088      1.0080
29 24      HC      1      LIG      H14      24      0.088      1.0080
30 25      HC      1      LIG      H15      25      0.088      1.0080
31 26      NTer      1      LIG      N01      26      -0.294      14.0067
32 27      CPos      1      LIG      C15      27      0.311      12.0110
33 28      OEOpt      1      LIG      O17      28      -0.363      15.9994
34 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:

```

1;
2;      File 'topol.top' was generated
3;      By user: thanawat (1000)
4;      On host: thanawat-virtual-machine
5;      At date: Sat Apr 22 22:15:21 2023
6;
7;      This is a standalone topology file
8;
9;      Created by:
10;                  :-( ) GROMACS - gmx pdb2gmx, 2023 (-(
11;
12;      Executable: /usr/local/gromacs/bin/gmx
13;      Data prefix: /usr/local/gromacs
14;      Working dir: /home/thanawat/Desktop/Worksheet_GROMOS
15;      Command line:
16;          gmx pdb2gmx -f receptor.pdb -o receptor_processed.gro -ignh -missing
17;      Force field was read from the standard GROMACS share directory.
18;
19;
20; Include forcefield parameters
21#include "gromos54a7_atb.ff/forcefield.itp"
22#include "lig.itp"
23;
24[ moleculetype ]
25; Name          nrexcl
26Protein_chain_C 3
27
28[ atoms ]
29; nr      type  resnr residue  atom    cgnr      charge      mass  typeB    chargeB    massB
30; residue 76  THR rtp  THR  q +1.0
31    1      NL    76   THR    N      1      0.129    14.0067
32    2      H     76   THR    H1     1      0.248     1.008
33    3      H     76   THR    H2     1      0.248     1.008
34    4      H     76   THR    H3     1      0.248     1.008
35    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:

```

7505; i funct      fcx      fcy      fcz
7506  1      1      1000      1000      1000
7507#endif
7508
7509; Include topology for ions
7510#include "gromos54a7_atb.ff/ions.itp"
7511
7512[ system ]
7513; Name
7514Protein
7515
7516[ molecules ]
7517; Compound      #mols
7518LIG              1
7519Protein_chain_C 1

```

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

```

7511
7512[ system ]
7513; Name
7514LIG and Protein
7515
7516[ molecules ]
7517; Compound      #mols
7518LIG              1
7519Protein_chain_C 1

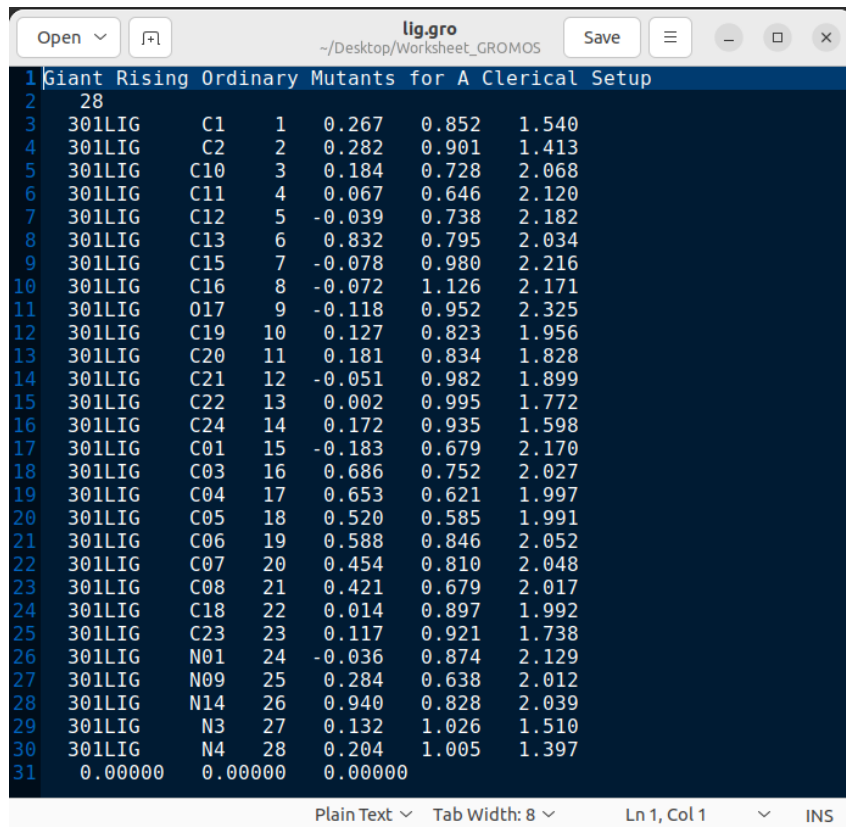
```

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:



Line	Column 1	Column 2	Column 3	Column 4	Column 5	Column 6
1	Giant Rising Ordinary Mutants for A Clerical Setup					
2	28					
3	301LIG	C1	1	0.267	0.852	1.540
4	301LIG	C2	2	0.282	0.901	1.413
5	301LIG	C10	3	0.184	0.728	2.068
6	301LIG	C11	4	0.067	0.646	2.120
7	301LIG	C12	5	-0.039	0.738	2.182
8	301LIG	C13	6	0.832	0.795	2.034
9	301LIG	C15	7	-0.078	0.980	2.216
10	301LIG	C16	8	-0.072	1.126	2.171
11	301LIG	017	9	-0.118	0.952	2.325
12	301LIG	C19	10	0.127	0.823	1.956
13	301LIG	C20	11	0.181	0.834	1.828
14	301LIG	C21	12	-0.051	0.982	1.899
15	301LIG	C22	13	0.002	0.995	1.772
16	301LIG	C24	14	0.172	0.935	1.598
17	301LIG	C01	15	-0.183	0.679	2.170
18	301LIG	C03	16	0.686	0.752	2.027
19	301LIG	C04	17	0.653	0.621	1.997
20	301LIG	C05	18	0.520	0.585	1.991
21	301LIG	C06	19	0.588	0.846	2.052
22	301LIG	C07	20	0.454	0.810	2.048
23	301LIG	C08	21	0.421	0.679	2.017
24	301LIG	C18	22	0.014	0.897	1.992
25	301LIG	C23	23	0.117	0.921	1.738
26	301LIG	N01	24	-0.036	0.874	2.129
27	301LIG	N09	25	0.284	0.638	2.012
28	301LIG	N14	26	0.940	0.828	2.039
29	301LIG	N3	27	0.132	1.026	1.510
30	301LIG	N4	28	0.204	1.005	1.397
31	0.00000	0.00000	0.00000			

Into **LIG** as following figure:

Open [icon] \*lig.gro ~/Desktop/Worksheet\_GROMOS Save [icon] [icon] [icon] [icon]

1 Giant Rising Ordinary Mutants for A Clerical Setup

2 28

3	LIG	C1	1	0.267	0.852	1.540
4	LIG	C2	2	0.282	0.901	1.413
5	LIG	C10	3	0.184	0.728	2.068
6	LIG	C11	4	0.067	0.646	2.120
7	LIG	C12	5	-0.039	0.738	2.182
8	LIG	C13	6	0.832	0.795	2.034
9	LIG	C15	7	-0.078	0.980	2.216
10	LIG	C16	8	-0.072	1.126	2.171
11	LIG	017	9	-0.118	0.952	2.325
12	LIG	C19	10	0.127	0.823	1.956
13	LIG	C20	11	0.181	0.834	1.828
14	LIG	C21	12	-0.051	0.982	1.899
15	LIG	C22	13	0.002	0.995	1.772
16	LIG	C24	14	0.172	0.935	1.598
17	LIG	C01	15	-0.183	0.679	2.170
18	LIG	C03	16	0.686	0.752	2.027
19	LIG	C04	17	0.653	0.621	1.997
20	LIG	C05	18	0.520	0.585	1.991
21	LIG	C06	19	0.588	0.846	2.052
22	LIG	C07	20	0.454	0.810	2.048
23	LIG	C08	21	0.421	0.679	2.017
24	LIG	C18	22	0.014	0.897	1.992
25	LIG	C23	23	0.117	0.921	1.738
26	LIG	N01	24	-0.036	0.874	2.129
27	LIG	N09	25	0.284	0.638	2.012
28	LIG	N14	26	0.940	0.828	2.039
29	LIG	N3	27	0.132	1.026	1.510
30	LIG	N4	28	0.204	1.005	1.397
31	0.00000	0.00000	0.00000			

Plain Text Tab Width: 8 Ln 1, Col 1 INS

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**”.

lig.itp										lig.gro									
Open [icon] [icon] lig.itp ~/Desktop/Worksheet_GROMOS Save [icon] [icon] [icon] [icon]										Open [icon] [icon] lig.gro ~/Desktop/Worksheet_GROMOS Save [icon] [icon] [icon] [icon]									
lig.gro										lig.gro									
1 [ moleculetype ]										1 Gallium Rubidium Oxygen Manganese Argon Carbon Silicon									
2 ; Name nrexcl										2 49									
3 LIG 3										3 LIG C1									
4 [ atoms ]										4 LIG C2									
5 ; nr type resnr resid	atom	cgmr	charge	mass						5 ; nr type resnr resid	atom	cgmr	charge	mass					
6 1 NPri 1	LIG	N14	1	-0.053	14.0067					6 1 NPri 1	LIG	N14	1	-0.053	14.0067				
7 2 C 1	LIG	C13	2	-0.086	12.0110					7 2 C 1	LIG	C13	2	-0.086	12.0110				
8 3 CAro 1	LIG	C03	3	-0.070	12.0110					8 3 CAro 1	LIG	C03	3	-0.070	12.0110				
9 4 CAro 1	LIG	C04	4	-0.049	12.0110					9 4 CAro 1	LIG	C04	4	-0.049	12.0110				
10 5 HC 1	LIG	H16	5	0.143	1.0080					10 5 HC 1	LIG	H16	5	0.143	1.0080				
11 6 CAro 1	LIG	C05	6	-0.204	12.0110					11 6 CAro 1	LIG	C05	6	-0.204	12.0110				
12 7 HC 1	LIG	H17	7	0.141	1.0080					12 7 HC 1	LIG	H17	7	0.141	1.0080				
13 8 CAro 1	LIG	C08	8	0.110	12.0110					13 8 CAro 1	LIG	C08	8	0.110	12.0110				
14 9 CAro 1	LIG	C07	9	-0.204	12.0110					14 9 CAro 1	LIG	C07	9	-0.204	12.0110				
15 10 HC 1	LIG	H19	10	0.141	1.0080					15 10 HC 1	LIG	H19	10	0.141	1.0080				
16 11 CAro 1	LIG	C06	11	-0.049	12.0110					16 11 CAro 1	LIG	C06	11	-0.049	12.0110				
17 12 HC 1	LIG	H18	12	0.143	1.0080					17 12 HC 1	LIG	H18	12	0.143	1.0080				
18 13 NOpt 1	LIG	N09	13	-0.300	14.0067					18 13 NOpt 1	LIG	N09	13	-0.300	14.0067				
19 14 HS14 1	LIG	H20	14	0.209	1.0080					19 14 HS14 1	LIG	H20	14	0.209	1.0080				
20 15 C 1	LIG	C10	15	0.057	12.0110					20 15 C 1	LIG	C10	15	0.057	12.0110				
21 16 HC 1	LIG	H3	16	0.120	1.0080					21 16 HC 1	LIG	H3	16	0.120	1.0080				
22 17 C 1	LIG	C11	17	-0.172	12.0110					22 17 C 1	LIG	C11	17	-0.172	12.0110				
23 18 HC 1	LIG	H4	18	0.094	1.0080					23 18 HC 1	LIG	H4	18	0.094	1.0080				
24 19 HC 1	LIG	H5	19	0.188	1.0080					24 19 HC 1	LIG	H5	19	0.188	1.0080				
25 20 C 1	LIG	C12	20	0.035	12.0110					25 20 C 1	LIG	C12	20	0.035	12.0110				
26 21 HC 1	LIG	H6	21	0.114	1.0080					26 21 HC 1	LIG	H6	21	0.114	1.0080				
27 22 C 1	LIG	C01	22	-0.226	12.0110					27 22 C 1	LIG	C01	22	-0.226	12.0110				
28 23 HC 1	LIG	H13	23	0.088	1.0080					28 23 HC 1	LIG	H13	23	0.088	1.0080				
29 24 HC 1	LIG	H14	24	0.088	1.0080					29 24 HC 1	LIG	H14	24	0.088	1.0080				
30 25 HC 1	LIG	H15	25	0.088	1.0080					30 25 HC 1	LIG	H15	25	0.088	1.0080				
31 26 NTer 1	LIG	N01	26	-0.294	14.0067					31 26 NTer 1	LIG	N01	26	-0.294	14.0067				
32 27 CPos 1	LIG	C15	27	0.311	12.0110					32 27 CPos 1	LIG	C15	27	0.311	12.0110				
33 28 OEOpt 1	LIG	017	28	-0.363	15.9994					33 28 OEOpt 1	LIG	017	28	-0.363	15.9994				
34 29 C 1	LIG	C16	29	-0.237	12.0110					34 29 C 1	LIG	C16	29	-0.237	12.0110				
35 30 HC 1	LIG	H7	30	0.111	1.0080					35 30 HC 1	LIG	H7	30	0.111	1.0080				
36 31 HC 1	LIG	H8	31	0.111	1.0080					36 31 HC 1	LIG	H8	31	0.111	1.0080				
37 32 HC 1	LIG	H9	32	0.111	1.0080					37 32 HC 1	LIG	H9	32	0.111	1.0080				
38 33 CAro 1	LIG	C18	33	0.077	12.0110					38 33 CAro 1	LIG	C18	33	0.077	12.0110				
39 34 CAro 1	LIG	C19	34	-0.140	12.0110					39 34 CAro 1	LIG	C19	34	-0.140	12.0110				
40 35 CAro 1	LIG	C20	35	-0.093	12.0110					40 35 CAro 1	LIG	C20	35	-0.093	12.0110				
41 36 HC 1	LIG	H10	36	0.141	1.0080					41 36 HC 1	LIG	H10	36	0.141	1.0080				
42 37 CAro 1	LIG	C23	37	-0.011	12.0110					42 37 CAro 1	LIG	C23	37	-0.011	12.0110				
43 38 CAro 1	LIG	C22	38	-0.086	12.0110					43 38 CAro 1	LIG	C22	38	-0.086	12.0110				
44 39 HC 1	LIG	H12	39	0.153	1.0080					44 39 HC 1	LIG	H12	39	0.153	1.0080				

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3	LIG	C1	1	0.267	0.852	1.540
4	LIG	C2	2	0.282	0.901	1.413
5	LIG	C10	3	0.184	0.728	2.068
6	LIG	C11	4	0.067	0.646	2.120
7	LIG	C12	5	-0.039	0.738	2.182
8	LIG	C13	6	0.832	0.795	2.034
9	LIG	C15	7	-0.078	0.980	2.216
10	LIG	C16	8	-0.072	1.126	2.171
11	LIG	017	9	-0.118	0.952	2.325
12	LIG	C19	10	0.127	0.823	1.956
13	LIG	C20	11	0.181	0.834	1.828
14	LIG	C21	12	-0.051	0.982	1.899
15	LIG	C22	13	0.002	0.995	1.772
16	LIG	C24	14	0.172	0.935	1.598
17	LIG	C01	15	-0.183	0.679	2.170
18	LIG	C03	16	0.686	0.752	2.027
19	LIG	C04	17	0.653	0.621	1.997
20	LIG	C05	18	0.520	0.585	1.991
21	LIG	C06	19	0.588	0.846	2.052
22	LIG	C07	20	0.454	0.810	2.048
23	LIG	C08	21	0.421	0.679	2.017</



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](mailto: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

Open	lig.itp	Save			
~/Desktop/Worksheet_GROMOS					
lig.gro			lig.itp		
1 [ moleculetype ]					
2 ; Name nrexcl					
3 LIG 3					
4 [ atoms ]					
5 ;	nr	type	resnr	resid	atom cgmr charge mass
6	1	NPri	1	LIG	N14 1 -0.053 14.0067
7	2	C	1	LIG	C13 2 -0.086 12.0110
8	3	CAro	1	LIG	C03 3 -0.070 12.0110
9	4	CAro	1	LIG	C04 4 -0.049 12.0110
10	5	HC	1	LIG	H16 5 0.143 1.0080
11	6	CAro	1	LIG	C05 6 -0.204 12.0110
12	7	HC	1	LIG	H17 7 0.141 1.0080
13	8	CAro	1	LIG	C08 8 0.110 12.0110
14	9	CAro	1	LIG	C07 9 -0.204 12.0110
15	10	HC	1	LIG	H19 10 0.141 1.0080
16	11	CAro	1	LIG	C06 11 -0.049 12.0110
17	12	HC	1	LIG	H18 12 0.143 1.0080
18	13	NOpt	1	LIG	N09 13 -0.380 14.0067
19	14	HS14	1	LIG	H20 14 0.209 1.0080
20	15	C	1	LIG	C10 15 0.057 12.0110
21	16	HC	1	LIG	H3 16 0.120 1.0080
22	17	C	1	LIG	C11 17 -0.172 12.0110
23	18	HC	1	LIG	H4 18 0.094 1.0080
24	19	HC	1	LIG	H5 19 0.108 1.0080
25	20	C	1	LIG	C12 20 0.035 12.0110
26	21	HC	1	LIG	H6 21 0.114 1.0080
27	22	C	1	LIG	C01 22 -0.226 12.0110
28	23	HC	1	LIG	H13 23 0.088 1.0080
29	24	HC	1	LIG	H14 24 0.088 1.0080
30	25	HC	1	LIG	H15 25 0.088 1.0080
31	26	NTer	1	LIG	N01 26 -0.294 14.0067
32	27	CPos	1	LIG	C15 27 0.311 12.0110
33	28	OEOpt	1	LIG	O17 28 -0.363 15.9994
34	29	C	1	LIG	C16 29 -0.237 12.0110
35	30	HC	1	LIG	H7 30 0.111 1.0080
36	31	HC	1	LIG	H8 31 0.111 1.0080
37	32	HC	1	LIG	H9 32 0.111 1.0080
38	33	CAro	1	LIG	C18 33 0.077 12.0110
39	34	CAro	1	LIG	C19 34 -0.140 12.0110
40	35	CAro	1	LIG	C20 35 -0.093 12.0110
41	36	HC	1	LIG	H10 36 0.141 1.0080
42	37	CAro	1	LIG	C23 37 -0.011 12.0110
43	38	CAro	1	LIG	C22 38 -0.086 12.0110
44	39	HC	1	LIG	H12 39 0.153 1.0080

Plain Text ▾ Tab Width: 8 ▾ Ln 27, Col 55 ▾ INS

lig\_out.gro

Open	lig_out.gro	Save			
~/Desktop/Worksheet_GROMOS					
lig_out.gro			lig.itp		
1 Gallium Rubidium Oxygen Manganese Argon Carbon Silicon					
2 49					
3	LIG	N14	26	0.940	0.828 2.039
4	LIG	C13	6	0.832	0.795 2.034
5	LIG	C03	16	0.686	0.752 2.027
6	LIG	C04	17	0.653	0.621 1.997
7	LIG	H16	44	0.731	0.548 1.977
8	LIG	C05	18	0.520	0.585 1.991
9	LIG	H17	45	0.492	0.482 1.967
10	LIG	C08	21	0.421	0.679 2.017
11	LIG	C07	20	0.454	0.810 2.048
12	LIG	H19	47	0.376	0.884 2.068
13	LIG	C06	19	0.588	0.846 2.052
14	LIG	H18	46	0.616	0.949 2.076
15	LIG	N09	25	0.284	0.638 2.012
16	LIG	H20	48	0.259	0.623 1.909
17	LIG	C10	3	0.184	0.728 2.068
18	LIG	H3	31	0.227	0.786 2.149
19	LIG	C11	4	0.067	0.646 2.120
20	LIG	H4	32	0.023	0.591 2.037
21	LIG	H5	33	0.103	0.577 2.195
22	LIG	C12	5	-0.039	0.738 2.182
23	LIG	H6	34	-0.016	0.745 2.288
24	LIG	C01	15	-0.183	0.679 2.170
25	LIG	H13	41	-0.254	0.748 2.216
26	LIG	H14	42	-0.187	0.584 2.222
27	LIG	H15	43	-0.208	0.666 2.065
28	LIG	N01	24	-0.036	0.874 2.129
29	LIG	C15	7	-0.078	0.980 2.216
30	LIG	O17	9	-0.118	0.952 2.325
31	LIG	C16	8	-0.072	1.126 2.171
32	LIG	H7	35	-0.108	1.191 2.251
33	LIG	H8	36	-0.136	1.139 2.083
34	LIG	H9	37	0.030	1.153 2.145
35	LIG	C18	22	0.014	0.897 1.992
36	LIG	C19	10	0.127	0.823 1.956
37	LIG	C20	11	0.181	0.834 1.828
38	LIG	H10	38	0.269	0.777 1.799
39	LIG	C23	23	0.117	0.921 1.738
40	LIG	C22	13	0.002	0.995 1.772
41	LIG	H12	40	-0.044	1.062 1.699
42	LIG	C21	12	-0.051	0.982 1.899
43	LIG	H11	39	-0.141	1.036 1.927
44	LIG	C24	14	0.172	0.935 1.598

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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:

*receptor_processed.gro						
~/Desktop/Worksheet_GROMOS						
Save						
lig_out.gro x lig.itp x *receptor_processed.gro x						
1	LIG and Protein					
2	1160					
3	LIG	N14	26	0.940	0.828	2.039
4	LIG	C13	6	0.832	0.795	2.034
5	LIG	C03	16	0.686	0.752	2.027
6	LIG	C04	17	0.653	0.621	1.997
7	LIG	H16	44	0.731	0.548	1.977
8	LIG	C05	18	0.520	0.585	1.991
9	LIG	H17	45	0.492	0.482	1.967
10	LIG	C08	21	0.421	0.679	2.017
11	LIG	C07	20	0.454	0.810	2.048
12	LIG	H19	47	0.376	0.884	2.068
13	LIG	C06	19	0.588	0.846	2.052
14	LIG	H18	46	0.616	0.949	2.076
15	LIG	N09	25	0.284	0.638	2.012
16	LIG	H20	48	0.259	0.623	1.909
17	LIG	C10	3	0.184	0.728	2.068
18	LIG	H3	31	0.227	0.786	2.149
19	LIG	C11	4	0.067	0.646	2.120
20	LIG	H4	32	0.023	0.591	2.037
21	LIG	H5	33	0.103	0.577	2.195
22	LIG	C12	5	-0.039	0.738	2.182
23	LIG	H6	34	-0.016	0.745	2.288
24	LIG	C01	15	-0.183	0.679	2.170
25	LIG	H13	41	-0.254	0.748	2.216
26	LIG	H14	42	-0.187	0.584	2.222
27	LIG	H15	43	-0.208	0.666	2.065
28	LIG	N01	24	-0.036	0.874	2.129
29	LIG	C15	7	-0.078	0.980	2.216
30	LIG	O17	9	-0.118	0.952	2.325
31	LIG	C16	8	-0.072	1.126	2.171
32	LIG	H7	35	-0.108	1.191	2.251
33	LIG	H8	36	-0.136	1.139	2.083
34	LIG	H9	37	0.030	1.153	2.145
35	LIG	C18	22	0.014	0.897	1.992
36	LIG	C19	10	0.127	0.823	1.956
37	LIG	C20	11	0.181	0.834	1.828
38	LIG	H10	38	0.269	0.777	1.799
39	LIG	C23	23	0.117	0.921	1.738
40	LIG	C22	13	0.002	0.995	1.772
41	LIG	H12	40	-0.044	1.062	1.699
42	LIG	C21	12	-0.051	0.982	1.899
43	LIG	H11	39	-0.141	1.036	1.927
44	LIG	C24	14	0.172	0.935	1.598

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:

receptor_processed.gro						
~/Desktop/Worksheet_GROMOS						
lig_out.gro x lig.itp x receptor_processed.gro x						
1	LIG and Protein					
2	1209					
3	LIG	N14	26	0.940	0.828	2.039
4	LIG	C13	6	0.832	0.795	2.034
5	LIG	C03	16	0.686	0.752	2.027
6	LIG	C04	17	0.653	0.621	1.997
7	LIG	H16	44	0.731	0.548	1.977
8	LIG	C05	18	0.520	0.585	1.991
9	LIG	H17	45	0.492	0.482	1.967
10	LIG	C08	21	0.421	0.679	2.017
11	LIG	C07	20	0.454	0.810	2.048
12	LIG	H19	47	0.376	0.884	2.068
13	LIG	C06	19	0.588	0.846	2.052
14	LIG	H18	46	0.616	0.949	2.076
15	LIG	N09	25	0.284	0.638	2.012
16	LIG	H20	48	0.259	0.623	1.909
17	LIG	C10	3	0.184	0.728	2.068
18	LIG	H3	31	0.227	0.786	2.149
19	LIG	C11	4	0.067	0.646	2.120
20	LIG	H4	32	0.023	0.591	2.037
21	LIG	H5	33	0.103	0.577	2.195
22	LIG	C12	5	-0.039	0.738	2.182
23	LIG	H6	34	-0.016	0.745	2.288
24	LIG	C01	15	-0.183	0.679	2.170
25	LIG	H13	41	-0.254	0.748	2.216
26	LIG	H14	42	-0.187	0.584	2.222
27	LIG	H15	43	-0.208	0.666	2.065
28	LIG	N01	24	-0.036	0.874	2.129
29	LIG	C15	7	-0.078	0.980	2.216
30	LIG	O17	9	-0.118	0.952	2.325
31	LIG	C16	8	-0.072	1.126	2.171
32	LIG	H7	35	-0.108	1.191	2.251
33	LIG	H8	36	-0.136	1.139	2.083
34	LIG	H9	37	0.030	1.153	2.145
35	LIG	C18	22	0.014	0.897	1.992
36	LIG	C19	10	0.127	0.823	1.956
37	LIG	C20	11	0.181	0.834	1.828
38	LIG	H10	38	0.269	0.777	1.799
39	LIG	C23	23	0.117	0.921	1.738
40	LIG	C22	13	0.002	0.995	1.772
41	LIG	H12	40	-0.044	1.062	1.699
42	LIG	C21	12	-0.051	0.982	1.899
43	LIG	H11	39	-0.141	1.036	1.927
44	LIG	C24	14	0.172	0.935	1.598

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
```

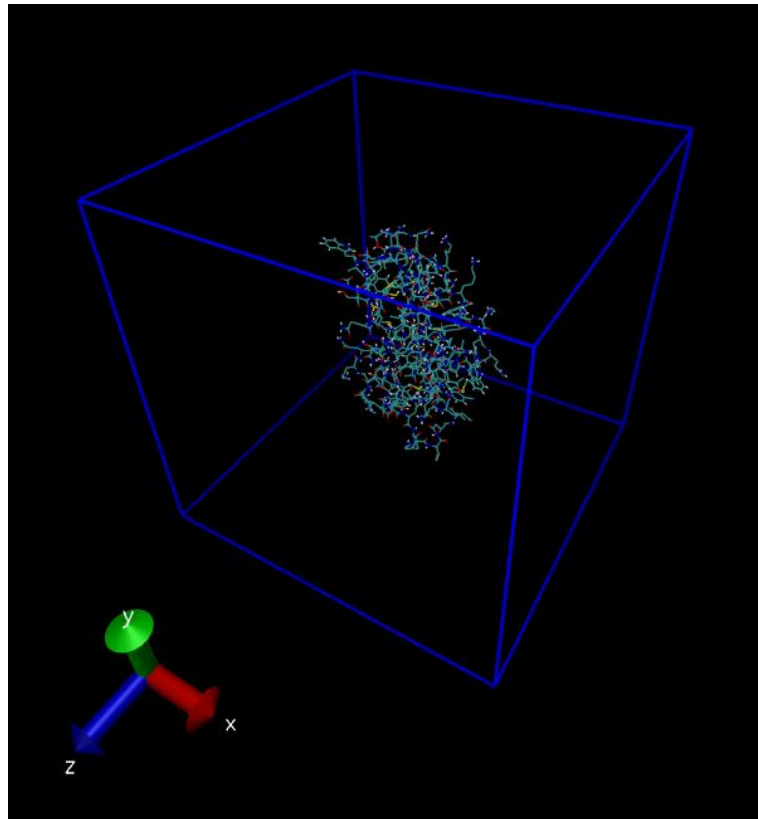
```
$ gmx editconf -f receptor_processed.gro -o newbox.gro -c -d 1.0 -bt cubic -n group.ndx
```

```
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 “**pbx 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**:

1	LIG and Protein					
2	28701					
3	32764LIG	N14	1	4.472	2.207	3.091
4	32764LIG	C13	2	4.364	2.174	3.086
5	32764LIG	C03	3	4.218	2.131	3.079
6	32764LIG	C04	4	4.185	2.000	3.049
7	32764LIG	H16	5	4.263	1.927	3.029
8	32764LIG	C05	6	4.052	1.964	3.043
9	32764LIG	H17	7	4.024	1.861	3.019
10	32764LIG	C08	8	3.953	2.058	3.069
11	32764LIG	C07	9	3.986	2.189	3.100
12	32764LIG	H19	10	3.908	2.263	3.120
13	32764LIG	C06	11	4.120	2.225	3.104
14	32764LIG	H18	12	4.148	2.328	3.128
15	32764LIG	N09	13	3.816	2.017	3.064
16	32764LIG	H20	14	3.791	2.002	2.961
17	32764LIG	C10	15	3.716	2.107	3.120
18	32764LIG	H3	16	3.759	2.165	3.201
19	32764LIG	C11	17	3.599	2.025	3.172
20	32764LIG	H4	18	3.555	1.970	3.089
21	32764LIG	H5	19	3.635	1.956	3.247
22	32764LIG	C12	20	3.493	2.117	3.234
23	32764LIG	H6	21	3.516	2.124	3.340
24	32764LIG	C01	22	3.349	2.058	3.222
25	32764LIG	H13	23	3.278	2.127	3.268
26	32764LIG	H14	24	3.345	1.963	3.274
27	32764LIG	H15	25	3.324	2.045	3.117
28	32764LIG	N01	26	3.496	2.253	3.181
29	32764LIG	C15	27	3.454	2.359	3.268
30	32764LIG	O17	28	3.414	2.331	3.377
31	32764LIG	C16	29	3.460	2.505	3.223
32	32764LIG	H7	30	3.424	2.570	3.303
33	32764LIG	H8	31	3.396	2.518	3.135
34	32764LIG	H9	32	3.562	2.532	3.197

After change:

1	LIG and Protein					
2	28701					
3	LIG	N14	1	4.472	2.207	3.091
4	LIG	C13	2	4.364	2.174	3.086
5	LIG	C03	3	4.218	2.131	3.079
6	LIG	C04	4	4.185	2.000	3.049
7	LIG	H16	5	4.263	1.927	3.029
8	LIG	C05	6	4.052	1.964	3.043
9	LIG	H17	7	4.024	1.861	3.019
10	LIG	C08	8	3.953	2.058	3.069
11	LIG	C07	9	3.986	2.189	3.100
12	LIG	H19	10	3.908	2.263	3.120
13	LIG	C06	11	4.120	2.225	3.104
14	LIG	H18	12	4.148	2.328	3.128
15	LIG	N09	13	3.816	2.017	3.064
16	LIG	H20	14	3.791	2.002	2.961
17	LIG	C10	15	3.716	2.107	3.120
18	LIG	H3	16	3.759	2.165	3.201
19	LIG	C11	17	3.599	2.025	3.172
20	LIG	H4	18	3.555	1.970	3.089
21	LIG	H5	19	3.635	1.956	3.247
22	LIG	C12	20	3.493	2.117	3.234
23	LIG	H6	21	3.516	2.124	3.340
24	LIG	C01	22	3.349	2.058	3.222
25	LIG	H13	23	3.278	2.127	3.268
26	LIG	H14	24	3.345	1.963	3.274
27	LIG	H15	25	3.324	2.045	3.117
28	LIG	N01	26	3.496	2.253	3.181
29	LIG	C15	27	3.454	2.359	3.268
30	LIG	O17	28	3.414	2.331	3.377
31	LIG	C16	29	3.460	2.505	3.223
32	LIG	H7	30	3.424	2.570	3.303
33	LIG	H8	31	3.396	2.518	3.135
34	LIG	H9	32	3.562	2.532	3.197
35	LIG	C18	33	3.546	2.276	3.044
36	LIG	C19	34	3.659	2.202	3.008
37	LIG	C20	35	3.713	2.213	2.880

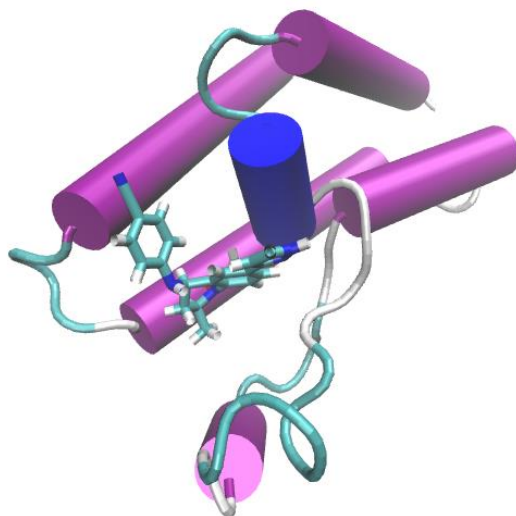
Then save.

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:

```

1;
2;      File 'topol.top' was generated
3;      By user: thanawat (1000)
4;      On host: thanawat-virtual-machine
5;      At date: Sat Apr 22 22:15:21 2023
6;
7;      This is a standalone topology file
8;
9;      Created by:
10;                  :-) GROMACS - gmx pdb2gmx, 2023 (-:
11;
12;      Executable: /usr/local/gromacs/bin/gmx
13;      Data prefix: /usr/local/gromacs
14;      Working dir: /home/thanawat/Desktop/Worksheet_GROMOS
15;      Command line:
16;      gmx pdb2gmx -f receptor.pdb -o receptor_processed.gro -
17;      ighn -missing
18;      Force field was read from the standard GROMACS share
19;      directory.
20;
21; Include forcefield parameters
22#include "gromos54a7_atb.ff/forcefield.itp"
23#include "lig.itp"
24; Ligand position restraints
25#ifdef POSRES
26#include "posre_lig.itp"
27#endif
28
29
30[ moleculetype ]
31; Name          nrexcl
32Protein_chain_C 3
33
34[ atoms ]
35; nr      type  resnr residue  atom  cgnr      charge
36mass  typeB  chargeB  massB
37; residue 76  THR  rtp  THR  q +1.0
381         NL   rtp  76   THR   N       1       0.129
3914.0067
402         H    76   THR   H1    1       0.248
411.008
423         H    76   THR   H2    1       0.248
431.008
444         H    76   THR   H3    1       0.248

```

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 gmh_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 gmh_mpi trjconv -s md_0_1.tpr -f md_0_1_center.xtc -o start.pdb -n index.ndx -dump 0
```

```
20
```

```
$ mpirun gmh_mpi trjconv -s md_0_1.tpr -f md_0_1_center.xtc -o end.pdb -n index.ndx -dump 10000
```

```
20
```

Extract for full-trajectory:

```
$ gmh_mpi trjconv -s md_0_1.tpr -f md_0_1_center.xtc -o fulltraj.pdb -b 0 -e 10000 -skip 1 -n index.ndx
```

```
20
```

Protein RMSD:

```
$ mpirun gmh_mpi rms -s md_0_1.tpr -f md_0_1_center.xtc -o rmsd.svg -tu ns
```

```
7
```

```
7
```

```
$ mpirun gmh_mpi gyrate -s md_0_1.tpr -f md_0_1_center.xtc -o gyrate.svg
```

```
4
```

## 6. Ligand interaction analysis

Create active binding site on complex:

```
$ mpirun gmh_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.