Protocol for coarse grained simulation of protein ligand system using GROMACS

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Protocol status: Working
We use this protocol and it's working

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PROTOCOL integer ID: 82789

ABSTRACT

Coarse-grained (CG) simulations are a powerful tool for studying the behavior of biomolecular systems. They are becoming increasingly important tools for drug discovery, as they can be used to study a wide variety of systems over long timescales.
CG simulations are faster than all-atom MD simulations, which allows researchers to study larger systems over longer timescales. They can also be used to study systems that are too large or too complex to be studied with all-atom MD simulations. Additionally, CG simulations can be used to study systems that are difficult or impossible to study experimentally. CG simulations are typically 100-1000 times faster than all-atom MD simulations.

This protocol provides steps along with a video tutorial perform CG simulation for protein ligand system. The advantages and test cases are but not limited to identification of active site, detection of cryptic pockets on protein, competitive binding between two or more ligands and many more

The link the video tutorial is available in https://youtu.be/xjfbA1G3PIM

BEFORE START INSTRUCTIONS

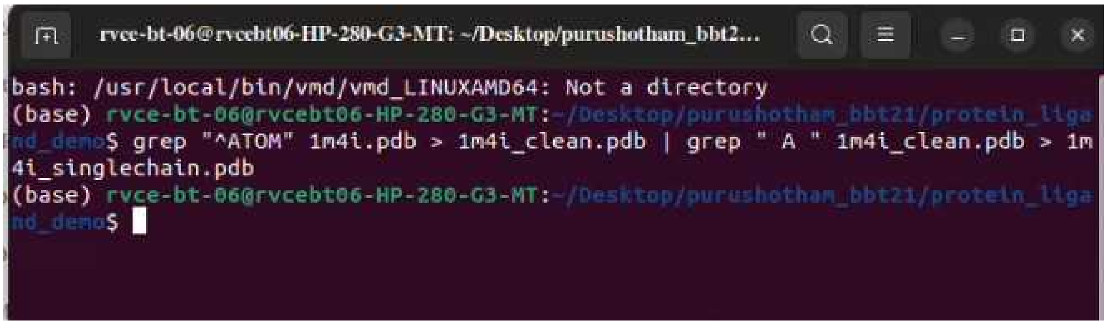
A basic understanding on gromacs and simulations.
For visual assistance refer to https://youtu.be/xjfbA1G3PIM

PART-1
The tutorial on Protocol for the development of coarse-grained structures for macro molecular simulation using GROMACS is available at https://protocols.io/view/protocol-for-the-development-of-coarse-grained-str-cp64vrgw.html and visual assistance for the same at https://youtu.be/QMR4f4eRSbs

1 Preprocessing of protein

Removal of Heteroatoms and if required removing other chains

grep "^ATOM" 1m4i.pdb > 1m4i_clean.pdb | grep " A " 1m4i_clean.pdb > 1m4i_singlechain.pdb


rvce-bt-06@rvcebt06-HP-280-G3-MT: ~/Desktop/purushotham_bbt2...

```
bash: /usr/local/bin/vmd/vmd_LINUXAMD64: Not a directory
(base) rvce-bt-06@rvcebt06-HP-280-G3-MT:~/Desktop/purushotham_bbt21/protein_liga
nd_demo$ grep "^ATOM" 1m4i.pdb > 1m4i_clean.pdb | grep " A " 1m4i_clean.pdb > 1m
4i_singlechain.pdb
(base) rvce-bt-06@rvcebt06-HP-280-G3-MT:~/Desktop/purushotham_bbt21/protein_liga
nd_demo$ 
```

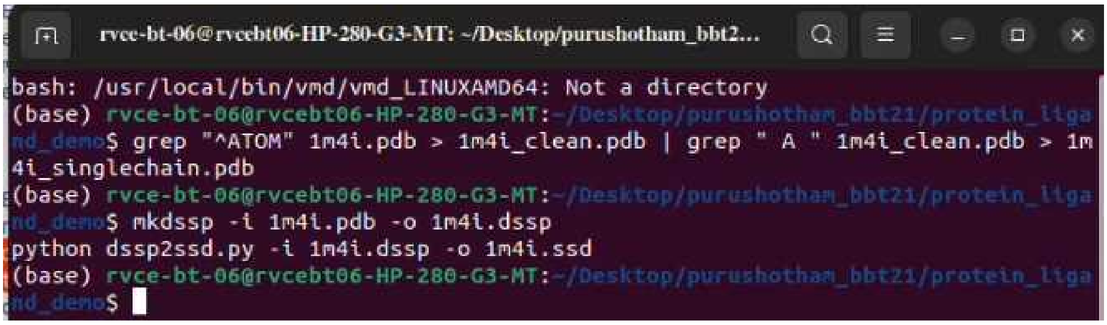
Preprocessing of protein

2 Finding secondary structure of 1m4i (AAC2)

mkdssp -i 1m4i.pdb -o 1m4i.dssp

python dssp2ssd.py -i 1m4i.dssp -o 1m4i.ssd

Second line of ssd file contains required secondary structure of 1m4i chain A

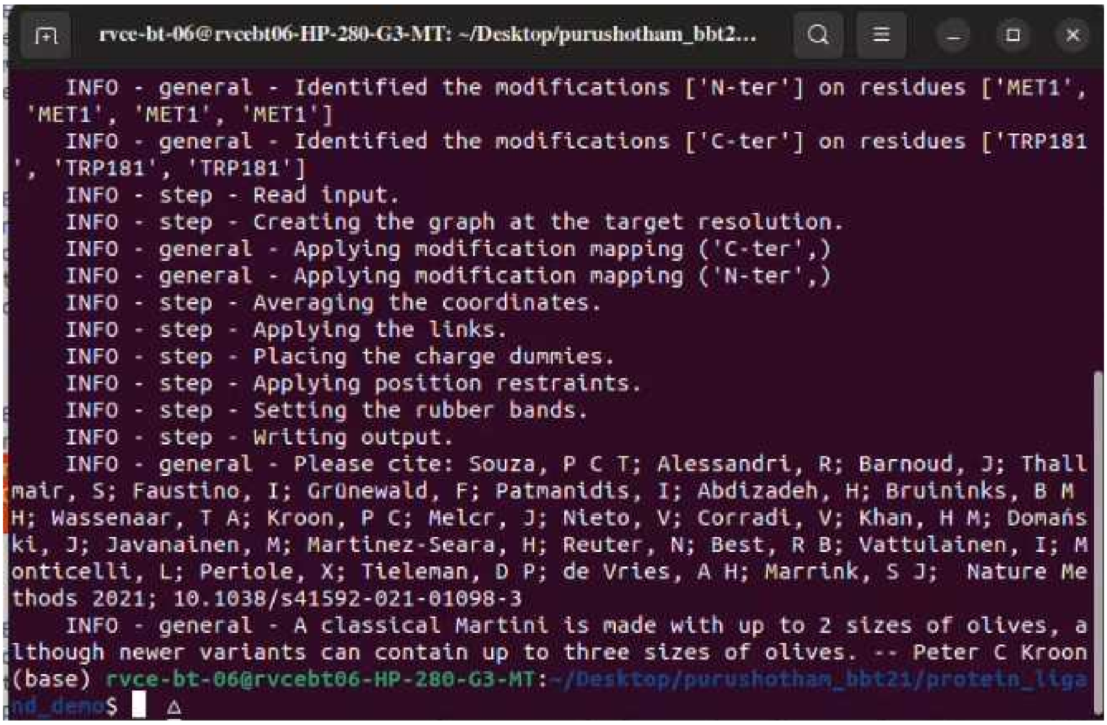

rvce-bt-06@rvcebt06-HP-280-G3-MT: ~/Desktop/purushotham_bbt2...

```
bash: /usr/local/bin/vmd/vmd_LINUXAMD64: Not a directory
(base) rvce-bt-06@rvcebt06-HP-280-G3-MT:~/Desktop/purushotham_bbt21/protein_liga
nd_demo$ grep "^ATOM" 1m4i.pdb > 1m4i_clean.pdb | grep " A " 1m4i_clean.pdb > 1m
4i_singlechain.pdb
(base) rvce-bt-06@rvcebt06-HP-280-G3-MT:~/Desktop/purushotham_bbt21/protein_liga
nd_demo$ mkdssp -i 1m4i.pdb -o 1m4i.dssp
python dssp2ssd.py -i 1m4i.dssp -o 1m4i.ssd
(base) rvce-bt-06@rvcebt06-HP-280-G3-MT:~/Desktop/purushotham_bbt21/protein_liga
nd_demo$ 
```

Secondary structure of 1m4i

3 Martinize: Convert All atomic model of AAC2 to Coarse grain model

martinize2 -f 1m4i_singlechain.pdb -o 1m4i_ONLY.top -x 1m4i_CG.pdb -ss CCCCTTCCEEEEGGGCCHHHHHHHHHHHHHHTTTCCCHHHHHHTCSSEEEEEEETTEEEEEEEEEEE EEEETTEEEEEEEEEEEEECGGGTTSSHHHHHHHHHHHHHHHHCSEEEEECCTTTHHHHHHTTCEEC CSCEEEEETTEEEECGGGTTTEEEEESSCCCCTTSCEEEECCSSCCC -p backbone -ff martini3001 -elastic -ef 500.0 -el 0.5 -eu 0.8 -scfix -cy auto


rvce-bt-06@rvcebt06-HP-280-G3-MT: ~/Desktop/purushotham_bbt2...

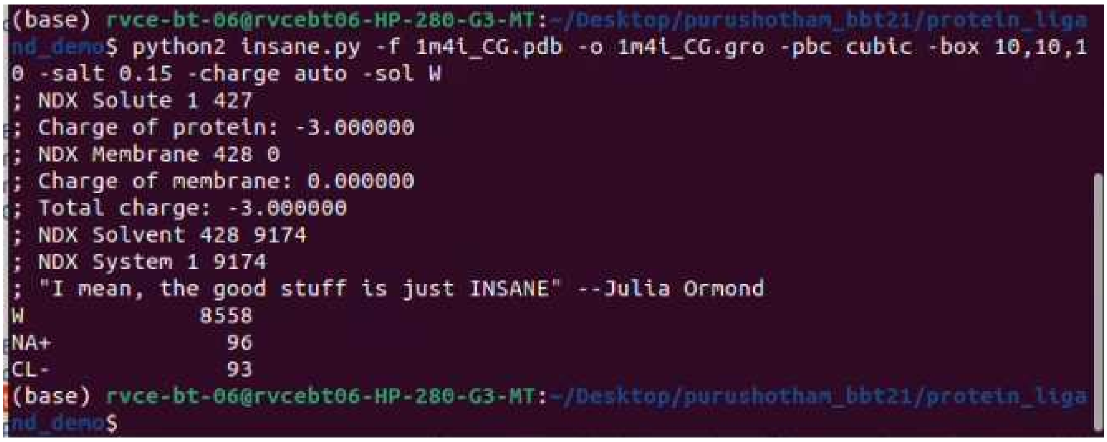
```
    INFO - general - Identified the modifications ['N-ter'] on residues ['MET1',
 'MET1', 'MET1', 'MET1']
    INFO - general - Identified the modifications ['C-ter'] on residues ['TRP181
', 'TRP181', 'TRP181']
    INFO - step - Read input.
    INFO - step - Creating the graph at the target resolution.
    INFO - general - Applying modification mapping ('C-ter',)
    INFO - general - Applying modification mapping ('N-ter',)
    INFO - step - Averaging the coordinates.
    INFO - step - Applying the links.
    INFO - step - Placing the charge dummies.
    INFO - step - Applying position restraints.
    INFO - step - Setting the rubber bands.
    INFO - step - Writing output.
    INFO - general - Please cite: Souza, P C T; Alessandri, R; Barnoud, J; Thall
mair, S; Faustino, I; Grünewald, F; Patmanidis, I; Abdizadeh, H; Bruininks, B M
H; Wassenaar, T A; Kroon, P C; Melcr, J; Nieto, V; Corradi, V; Khan, H M; Domański, J; Javanainen, M; Martinez-Seara, H; Reuter, N; Best, R B; Vattulainen, I; M
onticelli, L; Periole, X; Tieleman, D P; de Vries, A H; Marrink, S J;  Nature Me
thods 2021; 10.1038/s41592-021-01098-3
    INFO - general - A classical Martini is made with up to 2 sizes of olives, a
lthough newer variants can contain up to three sizes of olives. -- Peter C Kroon
(base) rvce-bt-06@rvcebt06-HP-280-G3-MT:~/Desktop/purushotham_bbt21/protein_liga
nd_demo$
```

Martinize: Conversion of All atomic to Coarse grain model

4 Add water and ions using Insane.py script

python2 insane.py -f 1m4i_CG.pdb -o 1m4i_CG.gro -pbc cubic -box 10,10,10 -salt 0.15 -charge auto -sol W

copy the number of water and ions (without signs) to Topology file (1m4i_ONLY.top), also add required itp files and rename it to 1m4i.top



```
(base) rvce-bt-06@rvcebt06-HP-280-G3-MT:~/Desktop/purushotham_bbt21/protein_liga
nd_demo$ python2 insane.py -f 1m4i_CG.pdb -o 1m4i_CG.gro -pbc cubic -box 10,10,1
0 -salt 0.15 -charge auto -sol W
; NDX Solute 1 427
; Charge of protein: -3.000000
; NDX Membrane 428 0
; Charge of membrane: 0.000000
; Total charge: -3.000000
; NDX Solvent 428 9174
; NDX System 1 9174
; "I mean, the good stuff is just INSANE" --Julia Ormond
W              8558
NA+              96
CL-              93
(base) rvce-bt-06@rvcebt06-HP-280-G3-MT:~/Desktop/purushotham_bbt21/protein_liga
nd_demo$
```

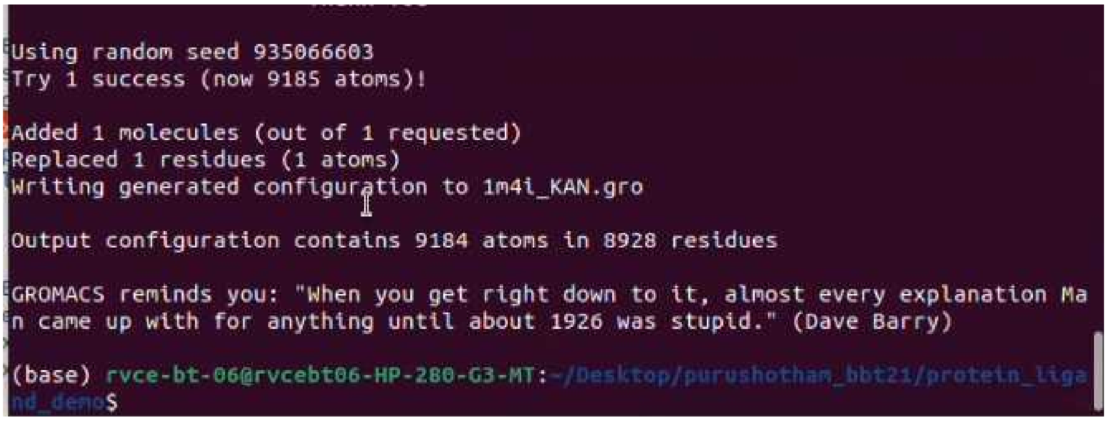
Solvation and Ionisation

5 Insert Ligand molecule

Ligand has been parameterised and included for simulation

gmx insert-molecules -f 1m4i_CG.gro -nmol 1 -ci KAN.gro -o 1m4i_KAN.gro -replace

replace water by pressing the water selection. After successfull execution, change the number of water molecules and add kanamycin in molecules section and itp file of kanamycin in the topology file.



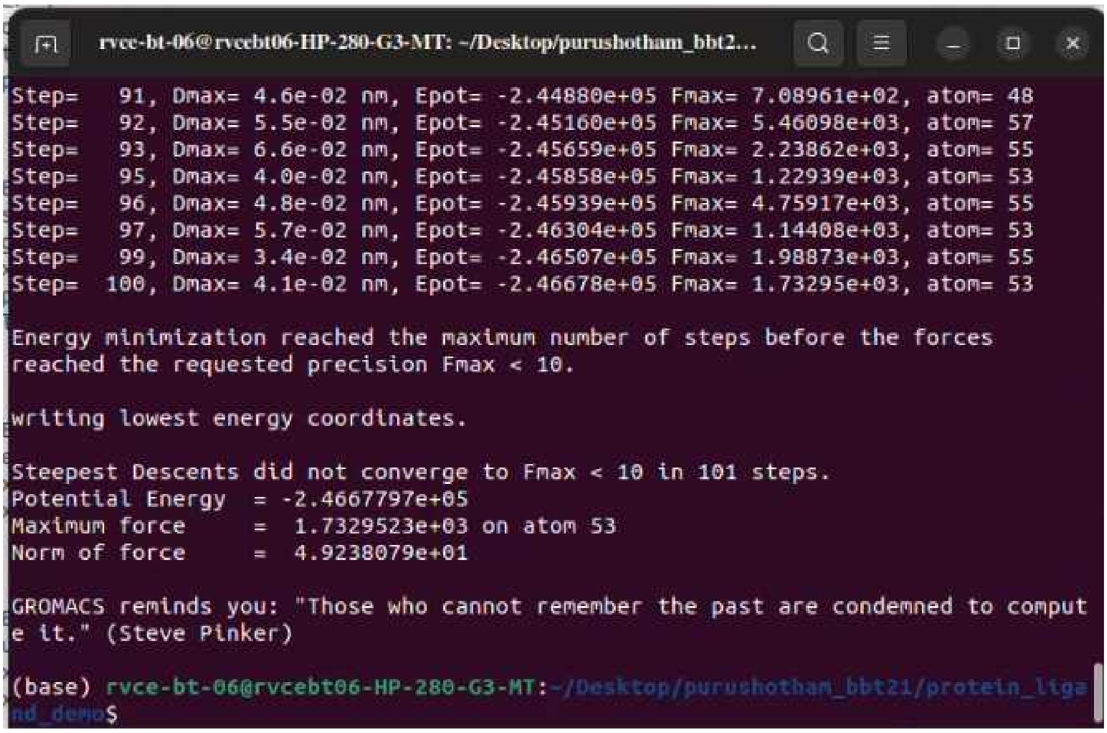
```
Using random seed 935066603
Try 1 success (now 9185 atoms)!

Added 1 molecules (out of 1 requested)
Replaced 1 residues (1 atoms)
Writing generated configuration to 1m4i_KAN.gro

Output configuration contains 9184 atoms in 8928 residues

GROMACS reminds you: "When you get right down to it, almost every explanation Ma
n came up with for anything until about 1926 was stupid." (Dave Barry)

(base) rvce-bt-06@rvcebt06-HP-280-G3-MT:~/Desktop/purushotham_bbt21/protein_liga
nd_demo$
```

Addition of small molecule

6 Energy Minimization

gmx grompp -p 1m4i_KAN.top -f min.mdp -c 1m4i_KAN.gro -r 1m4i_KAN.gro -o em.tpr -maxwarn 1

gmx mdrun -v -deffnm em

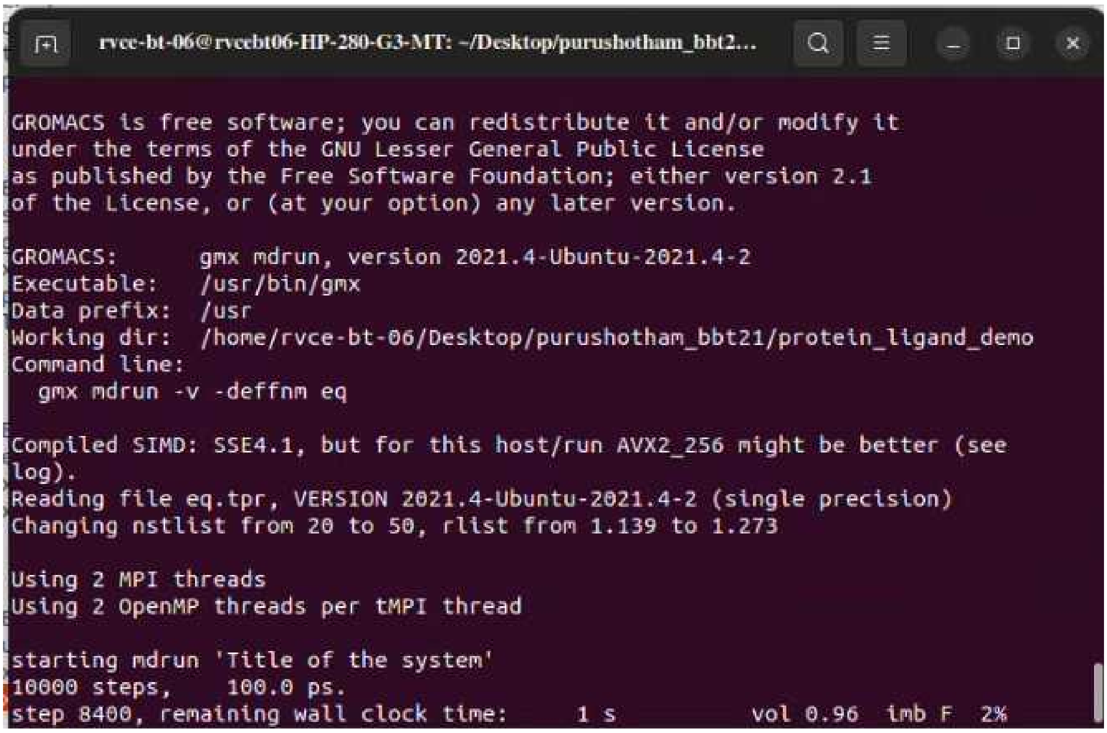

rvce-bt-06@rvcebt06-HP-280-G3-MT: ~/Desktop/purushotham_bbt2...

```
Step=   91, Dmax= 4.6e-02 nm, Epot= -2.44880e+05 Fmax= 7.08961e+02, atom= 48
Step=   92, Dmax= 5.5e-02 nm, Epot= -2.45160e+05 Fmax= 5.46098e+03, atom= 57
Step=   93, Dmax= 6.6e-02 nm, Epot= -2.45659e+05 Fmax= 2.23862e+03, atom= 55
Step=   95, Dmax= 4.0e-02 nm, Epot= -2.45858e+05 Fmax= 1.22939e+03, atom= 53
Step=   96, Dmax= 4.8e-02 nm, Epot= -2.45939e+05 Fmax= 4.75917e+03, atom= 55
Step=   97, Dmax= 5.7e-02 nm, Epot= -2.46304e+05 Fmax= 1.14408e+03, atom= 53
Step=   99, Dmax= 3.4e-02 nm, Epot= -2.46507e+05 Fmax= 1.98873e+03, atom= 55
Step=  100, Dmax= 4.1e-02 nm, Epot= -2.46678e+05 Fmax= 1.73295e+03, atom= 53

Energy minimization reached the maximum number of steps before the forces
reached the requested precision Fmax < 10.

writing lowest energy coordinates.

Steepest Descents did not converge to Fmax < 10 in 101 steps.
Potential Energy  = -2.4667797e+05
Maximum force     =  1.7329523e+03 on atom 53
Norm of force     =  4.9238079e+01

GROMACS reminds you: "Those who cannot remember the past are condemned to comput
e it." (Steve Pinker)

(base) rvce-bt-06@rvcebt06-HP-280-G3-MT:~/Desktop/purushotham_bbt21/protein_liga
nd_demo$
```

Energy Minimization

7 Equilibration

gmx grompp -p 1m4i_KAN.top -f eq.mdp -c em.gro -r 1m4i_KAN.gro -o eq.tpr -maxwarn 1
gmx mdrun -v -deffnm eq


rvce-bt-06@rvcebt06-HP-280-G3-MT: ~/Desktop/purushotham_bbt2...

```
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of the License, or (at your option) any later version.

GROMACS:      gmx mdrun, version 2021.4-Ubuntu-2021.4-2
Executable:   /usr/bin/gmx
Data prefix:  /usr
Working dir:  /home/rvce-bt-06/Desktop/purushotham_bbt21/protein_ligand_demo
Command line:
  gmx mdrun -v -deffnm eq

Compiled SIMD: SSE4.1, but for this host/run AVX2_256 might be better (see
log).
Reading file eq.tpr, VERSION 2021.4-Ubuntu-2021.4-2 (single precision)
Changing nstlist from 20 to 50, rlist from 1.139 to 1.273

Using 2 MPI threads
Using 2 OpenMP threads per tMPI thread

starting mdrun 'Title of the system'
10000 steps,    100.0 ps.
step 8400, remaining wall clock time:     1 s          vol 0.96  imb F  2%
```

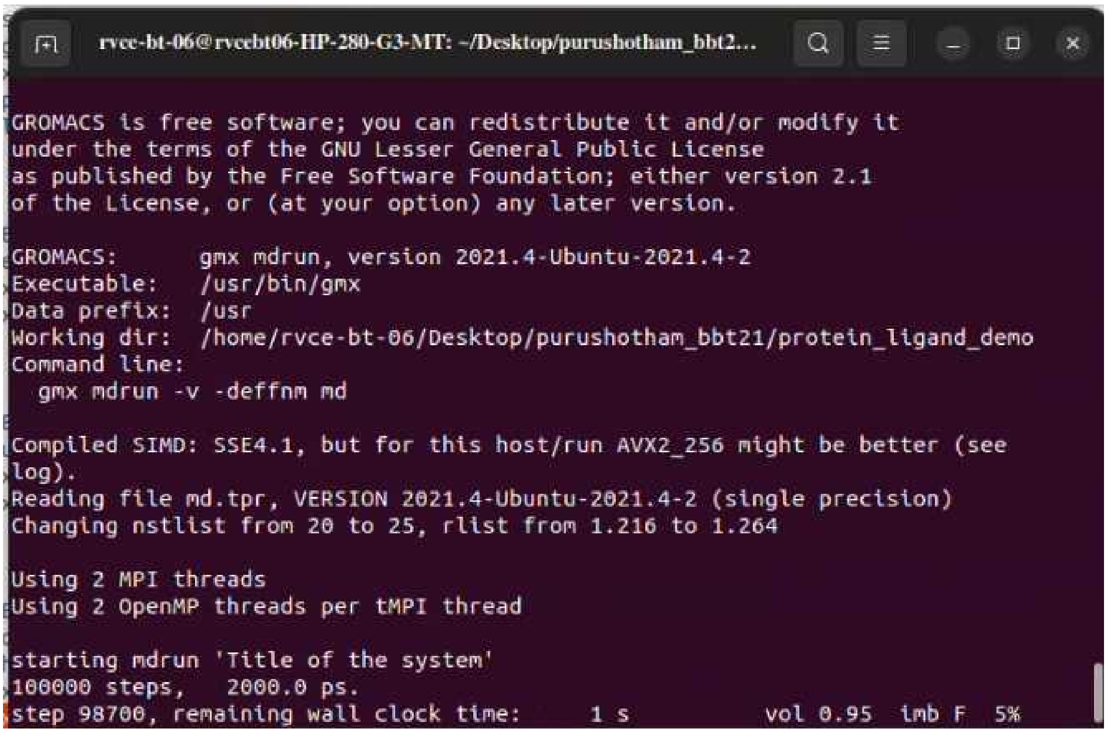
Equilibration

8 MD Production

I have ran for 2ns only to show, but you should increase it to case study.

gmx grompp -p 1m4i_KAN.top -f md.mdp -c eq.gro -o md.tpr

gmx mdrun -v -deffnm md



rvce-bt-06@rvcebt06-HP-280-G3-MT: ~/Desktop/purushotham_bbt2...

```
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as published by the Free Software Foundation; either version 2.1
of the License, or (at your option) any later version.

GROMACS:      gmx mdrun, version 2021.4-Ubuntu-2021.4-2
Executable:   /usr/bin/gmx
Data prefix:  /usr
Working dir:  /home/rvce-bt-06/Desktop/purushotham_bbt21/protein_ligand_demo
Command line:
  gmx mdrun -v -deffnm md

Compiled SIMD: SSE4.1, but for this host/run AVX2_256 might be better (see
log).
Reading file md.tpr, VERSION 2021.4-Ubuntu-2021.4-2 (single precision)
Changing nstlist from 20 to 25, rlist from 1.216 to 1.264

Using 2 MPI threads
Using 2 OpenMP threads per tMPI thread

starting mdrun 'Title of the system'
100000 steps,   2000.0 ps.
step 98700, remaining wall clock time:     1 s          vol 0.95  imb F  5%
```

MD Production