Simulation report of Lysosome_in_water (1aki.pdb)

Firstly, download the .pdb file from protein data bank (ID: 1AKI). After downloading a file we can visualize the protein structure using VMD, Chimera, PyMOL, etc.
Create one folder (Desktop/Lysosome_in_water)
Open terminal
cd Desktop
cd Lysosome_in_water
cd give direction to the folder or we can go to Lysosome_in_water folder and right click to open terminal.

```
user@gcf51:~$ cd Desktop
user@gcf51:~/Desktop$ cd Lysosome_in_water
user@gcf51:~/Desktop/Lysosome_in_water$
```

grep –v HOH 1aki.pdb > 1aki_clean.pdb
The "grep" command will remove HOH molecules from the downloaded protein file and save it in a new file i.e, 1AKI_clean.pdb.

gmx pdb2gmx -f 1aki _clean.pdb -o 1aki _processed.gro -water spc
The gmx pdb2gmx allow us to set a topology. -f specify the input file i.e, 1AKI_clean.pdb and -o specify the output generated file and process it in .gro format and -water spc specify the water model to be use. After running this command, we have to select a force field. In this tutorial we select 15 (OPLS-AA/L all-atom force field) as the force field. Three files were created:

1. .gro: Format structure file which contain all atoms defined within the force field.
2. .itp: Contain information used to restrain the positions of heavy atoms.
3. .top: contain the system topology

Structure of the .top file

```
; Include forcefield parameters
#include "oplsaa.ff/forcefield.itp"

[ moleculetype ]
; Name            nrexcl
Protein_chain_A     3

[ atoms ]
;   nr       type  resnr residue  atom   cgnr     charge       mass  typeB    chargeB      massB
; residue   1 LYS rtp LYSH q +2.0
     1   opls_287      1    LYS      N      1       -0.3    14.0027
     2   opls_290      1    LYS     H1      1       0.33      1.008
     3   opls_290      1    LYS     H2      1       0.33      1.008
```

Figure 1: structure of the topological file

#include "oplsaa.ff/forcefield.itp" calls parameter within the OPLSAA force field. Protein_chain_A defined molecule name. [atoms] defined atom in the protein.
nr: Atom number
type: Atom type
resnr: Amino acid residue number
residue: Amino acid residue name
atom: Atom name
chgnr: charge group number

We defined a box dimension and then filled it with solvent.
gmx editconf -f 1aki _processed.gro -o 1aki _newbox.gro -c -d 1.0 -bt cubic

This command defined a box using editconf. The function of –f and –o was already mentioned in the above command. –c will keep the protein in the centre of the box. –d 1.0 is the distance of the protein from the edge box. –bt is use to define the box shape (cube). Next, we are ready to fill the solvent.

gmx solvate -cp 1aki _processed.gro -cs spc216.gro -o 1aki _solv.gro -p topol.top
solvate is a tool use to add solvent molecule. Here –cp specifies the input coordinate file for solvent. –cs specify the configuration of the solvent. –p function is to update the file.

gmx grompp -f ions.mdp -c 1aki _solv.gro -p topol.top -o ions.tpr
The grompp module can process the coordinate file and topology file to generate an atomic level input i.e, .tpr format which is a binary file. This file contains all the parameters for all atoms in the system.

gmx genion -s ions.tpr -o 1aki _solv_ions.gro -p topol.top -pname NA -nname CL –neutral
genion is a tool to add ions. It replaced the water molecules with the ions that the user specifies and updates the topological file. –s structure the file as an input. Now –p will process the topology file; remove the water molecules and ad ions.

Energy minimization
This process helps is stabilizing the structure and resolve steric clashes. Download the file em.mdp. This file is use as an input into grompp to generate a .tpr file.

gmx grompp -f em.mdp -c 1aki _solv_ions.gro -p topol.top -o em.tpr
Here we use the grompp module to process the coordinate file and topology file to generate an atomic level input i.e, .tpr format.

gmx mdrun -v -deffnm em
The flag -v will print the progress to the screen at every step. The –deffnm define file name of the input. After executing this line, we get the following files:
1. em.log: Which is ASCII-text log file of energy minimization process
2. em.edr: This is a binary energy file. It contains all the energy terms that the gromac collect during the energy minimization.
3. em.trr: This is the binary full-precision trajectory file
4. em.gro: Energy minimized structure

gmx energy -f em.edr -o potential.xvg
We can analyse the .edr file using energy module. For analysing the potential, we have to select from the prompt “10 0”. The data can be plot using xmgrace plotting tools.

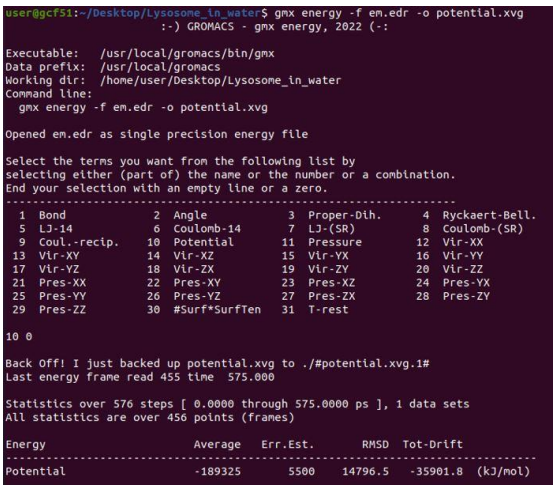

Figure 1: Finding the potential energy

xmgrace potential.xvg

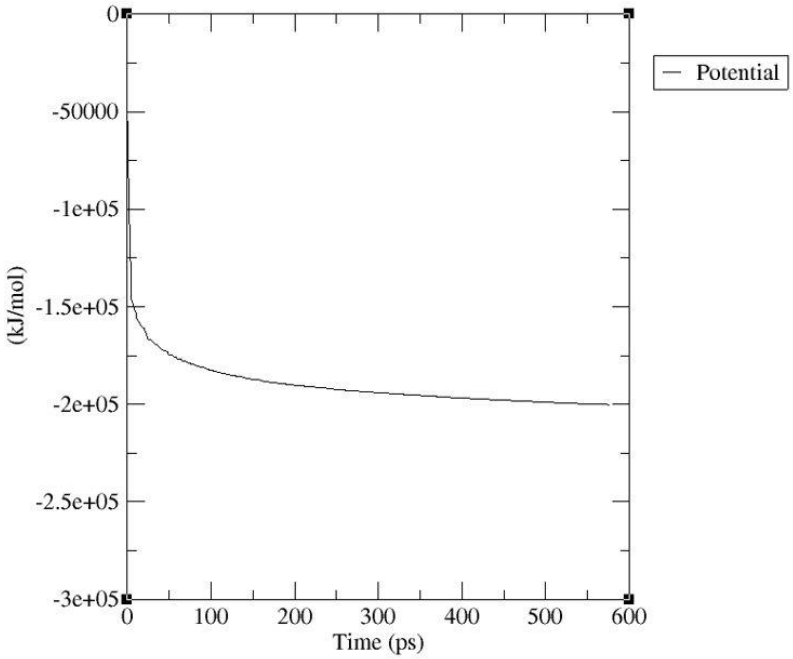

Figure 2: Potential energy vs. time

Equilibration is conducted in two phases:
1. NVT (constant Number of particles, Volume, and Temperature) which sometimes referred to as "isothermal-isochoric" or "canonical". This phase helps in stabilizing the temperature of the system. Download the nvt.mdp file

gmx grompp -f nvt.mdp -c em.gro -r em.gro -p topol.top -o nvt.tpr
The grompp module again is used to process the coordinate file and topology file to generate an atomic level input i.e, .tpr format.
gmx mdrun -deffnm nvt
We can analyse the temperature progression using the energy module
gmx energy -f nvt.edr -o temperature.xvg

```
user@gcf51:~/Desktop/Lysosome_in_water$ xmgrace temperature.xvg
user@gcf51:~/Desktop/Lysosome_in_water$ gmx energy -f nvt.edr -o temperature.xvg
                      :-) GROMACS - gmx energy, 2022 (-:

Executable:   /usr/local/gromacs/bin/gmx
Data prefix:  /usr/local/gromacs
Working dir:  /home/user/Desktop/Lysosome_in_water
Command line:
  gmx energy -f nvt.edr -o temperature.xvg

Opened nvt.edr as single precision energy file

Select the terms you want from the following list by
selecting either (part of) the name or the number or a combination.
End your selection with an empty line or a zero.
-------------------------------------------------------------------
  1  Bond             2  Angle            3  Proper-Dih.      4  Ryckaert-Bell.
  5  LJ-14            6  Coulomb-14       7  LJ-(SR)          8  Disper.-corr.
  9  Coulomb-(SR)    10  Coul.-recip.     11  Position-Rest.  12  Potential
 13  Kinetic-En.     14  Total-Energy     15  Conserved-En.   16  Temperature
 17  Pres.-DC        18  Pressure         19  Constr.-rmsd    20  Vir-XX
 21  Vir-XY          22  Vir-XZ           23  Vir-YX          24  Vir-YY
 25  Vir-YZ          26  Vir-ZX           27  Vir-ZY          28  Vir-ZZ
 29  Pres-XX         30  Pres-XY          31  Pres-XZ         32  Pres-YX
 33  Pres-YY         34  Pres-YZ          35  Pres-ZX         36  Pres-ZY
 37  Pres-ZZ         38  #Surf*SurfTen    39  T-Protein       40  T-non-Protein
 41  Lamb-Protein                         42  Lamb-non-Protein

16 0

Back Off! I just backed up temperature.xvg to ./#temperature.xvg.1#
Last energy frame read 100 time  100.000

Statistics over 50001 steps [ 0.0000 through 100.0000 ps ], 1 data sets
All statistics are over 501 points

Energy                      Average   Err.Est.       RMSD  Tot-Drift
-------------------------------------------------------------------------------
Temperature                 299.81      0.099    3.52137   0.594327  (K)

GROMACS reminds you: "Engage" (J.L. Picard)
```

Figure 3: Finding temperature

xmgrace temperature.xvg

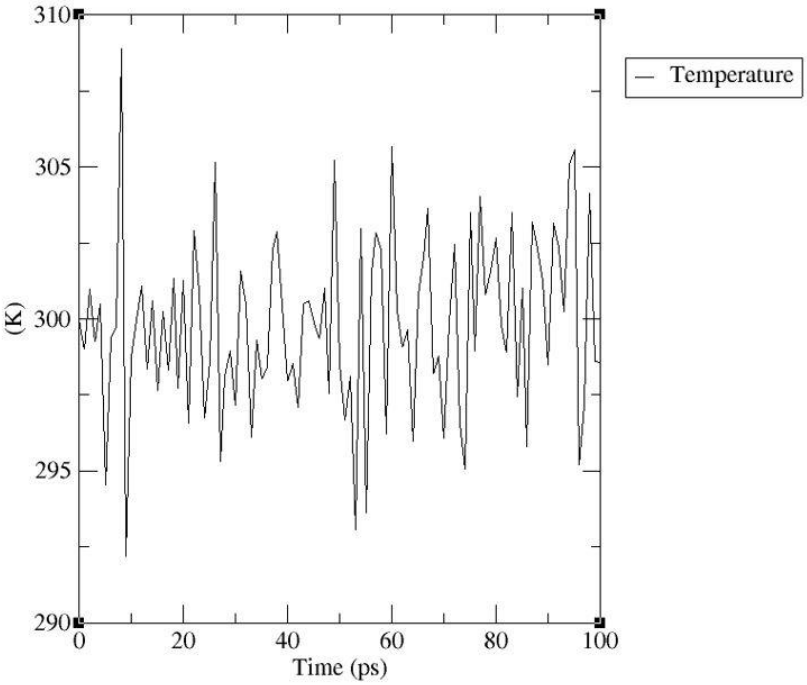

Figure 4: Temperature vs. time

2. NPT (constant Number of particles, Pressure, and Temperature)
#npt (Number of particles, Pressure, and Temperature)
gmx grompp -f npt.mdp -c nvt.gro -r nvt.gro -t nvt.cpt -p topol.top -o npt.tpr
gmx mdrun -v -deffnm npt
gmx energy -f npt.edr -o pressure.xvg

```
user@gcf51:~/Desktop/Lysosome_in_water$ gmx energy -f npt.edr -o pressure.xvg
                    :-) GROMACS - gmx energy, 2022 (-:

Executable:   /usr/local/gromacs/bin/gmx
Data prefix:  /usr/local/gromacs
Working dir:  /home/user/Desktop/Lysosome_in_water
Command line:
  gmx energy -f npt.edr -o pressure.xvg

Opened npt.edr as single precision energy file

Select the terms you want from the following list by
selecting either (part of) the name or the number or a combination.
End your selection with an empty line or a zero.
-------------------------------------------------------------------
  1  Bond             2  Angle            3  Proper-Dih.      4  Ryckaert-Bell.
  5  LJ-14            6  Coulomb-14       7  LJ-(SR)          8  Disper.-corr.
  9  Coulomb-(SR)    10  Coul.-recip.     11  Position-Rest.  12  Potential
 13  Kinetic-En.     14  Total-Energy     15  Conserved-En.   16  Temperature
 17  Pres.-DC        18  Pressure         19  Constr.-rmsd    20  Box-X
 21  Box-Y           22  Box-Z            23  Volume          24  Density
 25  pV              26  Enthalpy         27  Vir-XX          28  Vir-XY
 29  Vir-XZ          30  Vir-YX           31  Vir-YY          32  Vir-YZ
 33  Vir-ZX          34  Vir-ZY           35  Vir-ZZ          36  Pres-XX
 37  Pres-XY         38  Pres-XZ          39  Pres-YX         40  Pres-YY
 41  Pres-YZ         42  Pres-ZX          43  Pres-ZY         44  Pres-ZZ
 45  #Surf*SurfTen   46  Box-Vel-XX       47  Box-Vel-YY      48  Box-Vel-ZZ
 49  T-Protein                            50  T-non-Protein
 51  Lamb-Protein                         52  Lamb-non-Protein

18 0

Back Off! I just backed up pressure.xvg to ./#pressure.xvg.1#
Last energy frame read 100 time  100.000

Statistics over 50001 steps [ 0.0000 through 100.0000 ps ], 1 data sets
All statistics are over 501 points

Energy                    Average   Err.Est.       RMSD  Tot-Drift
-------------------------------------------------------------------------------
Pressure                 -132.957         14    340.442    71.8269  (bar)
```

Figure 5: Finding pressure

xmgrace pressure.xvg

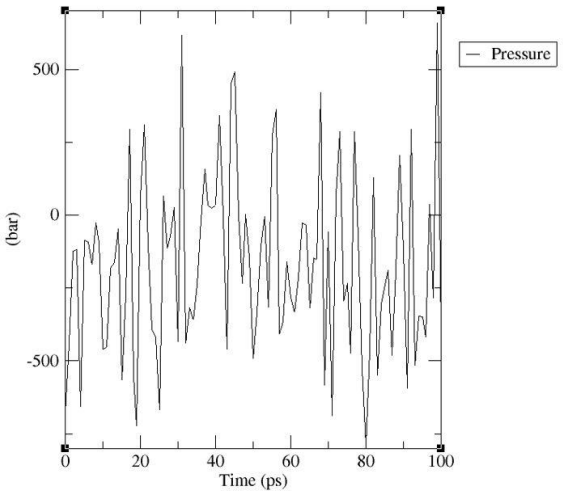

Figure 6: Pressure vs. time

gmx energy -f npt.edr -o density.xvg

```
user@gcf51:~/Desktop/Lysosome_in_water$ gmx energy -f npt.edr -o density.xvg
                      :-) GROMACS - gmx energy, 2022 (-:

Executable:   /usr/local/gromacs/bin/gmx
Data prefix:  /usr/local/gromacs
Working dir:  /home/user/Desktop/Lysosome_in_water
Command line:
  gmx energy -f npt.edr -o density.xvg

Opened npt.edr as single precision energy file

Select the terms you want from the following list by
selecting either (part of) the name or the number or a combination.
End your selection with an empty line or a zero.
-------------------------------------------------------------------
  1  Bond             2  Angle            3  Proper-Dih.      4  Ryckaert-Bell.
  5  LJ-14            6  Coulomb-14       7  LJ-(SR)          8  Disper.-corr.
  9  Coulomb-(SR)    10  Coul.-recip.    11  Position-Rest.  12  Potential
 13  Kinetic-En.     14  Total-Energy    15  Conserved-En.   16  Temperature
 17  Pres.-DC        18  Pressure        19  Constr.-rmsd    20  Box-X
 21  Box-Y           22  Box-Z           23  Volume          24  Density
 25  pV              26  Enthalpy        27  Vir-XX          28  Vir-XY
 29  Vir-XZ          30  Vir-YX          31  Vir-YY          32  Vir-YZ
 33  Vir-ZX          34  Vir-ZY          35  Vir-ZZ          36  Pres-XX
 37  Pres-XY         38  Pres-XZ         39  Pres-YX         40  Pres-YY
 41  Pres-YZ         42  Pres-ZX         43  Pres-ZY         44  Pres-ZZ
 45  #Surf*SurfTen   46  Box-Vel-XX      47  Box-Vel-YY      48  Box-Vel-ZZ
 49  T-Protein                           50  T-non-Protein
 51  Lamb-Protein                        52  Lamb-non-Protein

24 0

Back Off! I just backed up density.xvg to ./#density.xvg.1#
Last energy frame read 100 time  100.000

Statistics over 50001 steps [ 0.0000 through 100.0000 ps ], 1 data sets
All statistics are over 501 points

Energy                      Average   Err.Est.       RMSD  Tot-Drift
-------------------------------------------------------------------------------
Density                     1045.28       0.67    4.47883    1.88447  (kg/m^3)
```

Figure 7: Finding the density

xmgrace density.xvg

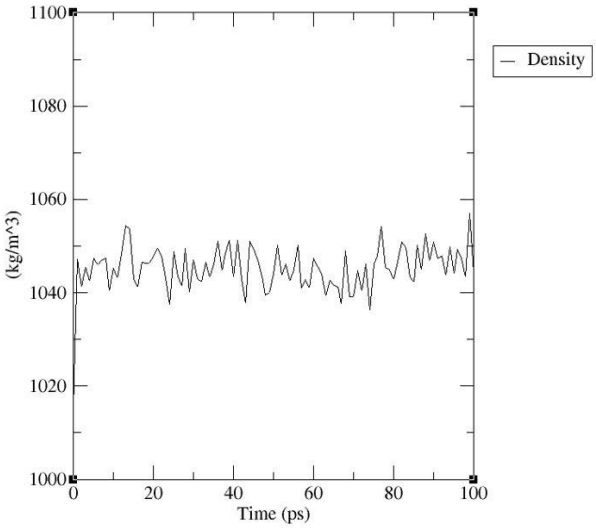

Figure 8: Density vs. time

```
gmx grompp -f md.mdp -c npt.gro -t npt.cpt -p topol.top -o md_0_1.tpr
gmx mdrun -v -deffnm md_0_1
gmx trjconv -s md_0_1.tpr -f md_0_1.xtc -o md_0_1_noPBC.xtc -pbc mol –center
gmx rms -s md_0_1.tpr -f md_0_1_noPBC.xtc -o rmsd.xvg -tu ns
xmgrace rmsd.xvg
```

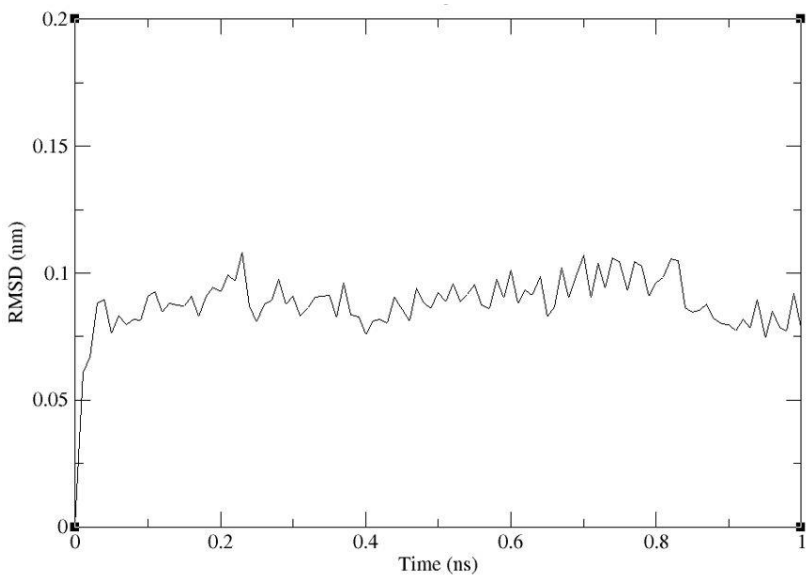

Figure 9: Root mean square

```
gmx gyrate -s md_0_1.tpr -f md_0_1_noPBC.xtc -o gyrate.xvg
xmgrace gyrate.xvg
```

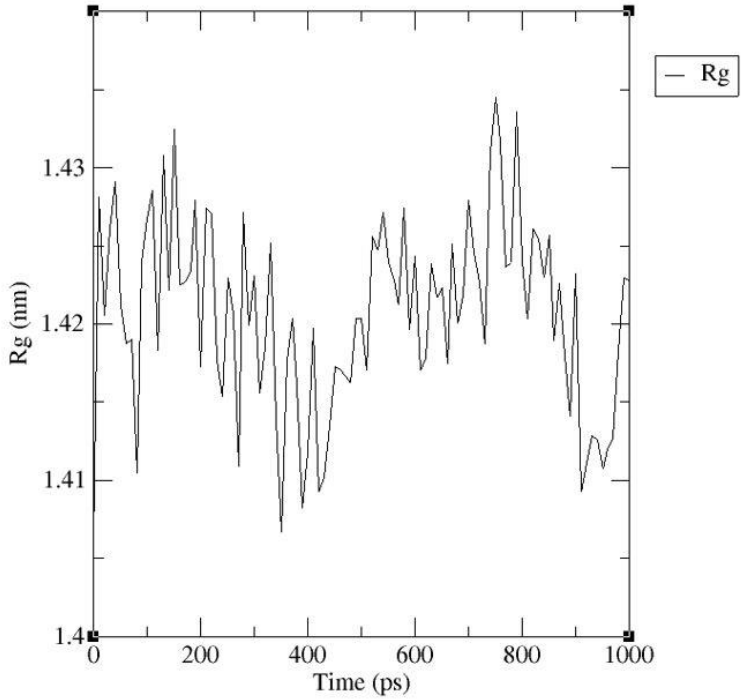

Figure 10: Radius of Gyration