Molecular Dynamics Simulation of Lysozyme in Water

Using GROMACS

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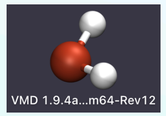
Workflow of MD Simulations

1. Prepare the structure
2. Execute pdb2gmx
3. Define the unit cell & add solvent
4. Add ions
5. Energy minimization
6. Equilibration
7. Production run
8. Analysis

Step 1 - Preparing the Lysozyme Structure

Download PDB file: Obtain the lysozyme structure from the Protein Data Bank (PDB). *Today, we're using alreday cleaned lysozyme pdb file* 1AKI_clean.pdb

Visualize the structure: Use tools like VMD to inspect the downloaded PDB file.

- Open VMD 

To load .pdb flile on VMD:

- Go to File ⟶ New Molecule ⟶ Browse ⟶choose molecule file ⟶ 1AKI_clean.pdb ⟶Load

Step 2 -Execute pdb2gmx

- **Open terminal activate gromax environment using command gmx**

Use command: **gmx pdb2gmx -f 1AKI_clean.pdb -o 1AKI_processed.gro -water spce**

The structure will be processed by pdb2gmx, and you will be prompted to choose a force field:

```
Select the Force Field:
From '/usr/local/gromacs/share/gromacs/top':
 1: AMBER03 protein, nucleic AMBER94 (Duan et al., J. Comp. Chem. 24, 1999-2012, 2003)
 2: AMBER94 force field (Cornell et al., JACS 117, 5179-5197, 1995)
 3: AMBER96 protein, nucleic AMBER94 (Kollman et al., Acc. Chem. Res. 29, 461-469, 1996)
 4: AMBER99 protein, nucleic AMBER94 (Wang et al., J. Comp. Chem. 21, 1049-1074, 2000)
 5: AMBER99SB protein, nucleic AMBER94 (Hornak et al., Proteins 65, 712-725, 2006)
 6: AMBER99SB-ILDN protein, nucleic AMBER94 (Lindorff-Larsen et al., Proteins 78, 1950-58, 2010)
 7: AMBERGS force field (Garcia & Sanbonmatsu, PNAS 99, 2782-2787, 2002)
 8: CHARMM27 all-atom force field (CHARM22 plus CMAP for proteins)
 9: GROMOS96 43a1 force field
10: GROMOS96 43a2 force field (improved alkane dihedrals)
11: GROMOS96 45a3 force field (Schuler JCC 2001 22 1205)
12: GROMOS96 53a5 force field (JCC 2004 vol 25 pag 1656)
13: GROMOS96 53a6 force field (JCC 2004 vol 25 pag 1656)
14: GROMOS96 54a7 force field (Eur. Biophys. J. (2011), 40,, 843-856, DOI: 10.1007/s00249-011-0700-9)
15: OPLS-AA/L all-atom force field (2001 aminoacid dihedrals)
```

For this tutorial, we will use the ***all-atom OPLS force field***, so ***type 15*** at the command prompt, ***followed by 'Enter'***.

Now, we have now generated three new files: **1AKI_processed.gro, topol.top,** and **posre.itp**

Step 3 -Define box & add solvent

Define the box using editconf: **gmx editconf -f 1AKI_processed.gro -o 1AKI_newbox.gro -c -d 1.0 -bt cubic**

- The above command centers the protein in the box (-c), and places it at least 1.0 nm from the box edge (-d 1.0). The box type is defined as a cube (-bt cubic).

Now that we have defined a box, we can fill it with solvent (water):-

Solvation is accomplished using solvate : **gmx solvate -cp 1AKI_newbox.gro -cs spc216.gro -o 1AKI_solv.gro -p topol.top**

- -cp 1AKI_newbox.gro specifies the coordinate file of the system to be solvated, and the configuration of the solvent (-cs) is part of the standard GROMACS installation. We are using spc216.gro, which is a generic equilibrated 3-point solvent model.

Step 4 - Add ions

The output of pdb2gmx (*last line of our `[ atoms ]` directive in topol.top*) told us that the protein has a net charge of +8*e* (based on its amino acid composition).

- The tool for adding ions within GROMACS is called genion
- The .tpr file contains all the parameters for all of the atoms in the system.
- To produce a .tpr file with grompp, we will need an additional input file, with the extension .mdp (molecular dynamics parameter file)
- Grompp will assemble the parameters specified in the .mdp file with the coordinates and topology information to generate a .tpr file.
- .mdp file (the one we will use) is ***ions.mdp***
- Assemble your .tpr file with the following: **gmx grompp -f ions.mdp -c 1AKI_solv.gro -p topol.top. -o ions.tpr**
- Now we have an atomic-level description of our system in the binary file ions.tpr.
- We will pass this file to genion: **gmx genion -s ions.tpr -o 1AKI_solv_ions.gro -p topol.top -pname NA -nname CL -neutral**
- **When prompted, choose group 13 "SOL" for embedding ions.**

Step 5 -Energy minimization

Let's ensure that the system has no steric clashes or inappropriate geometry. The structure is relaxed through a process called energy minimization (EM).

- The process for EM is much like the addition of ions.
- This time, instead of passing the .tpr to genion, we will run the energy minimization through the GROMACS MD engine, mdrun.
- Here, we will use minim.mdp file
- Use command : **gmx grompp -f minim.mdp -c 1AKI_solv_ions.gro -p topol.top -o em.tpr**
- We are now ready to invoke mdrun to carry out the EM: **gmx mdrun -v -deffnm em**

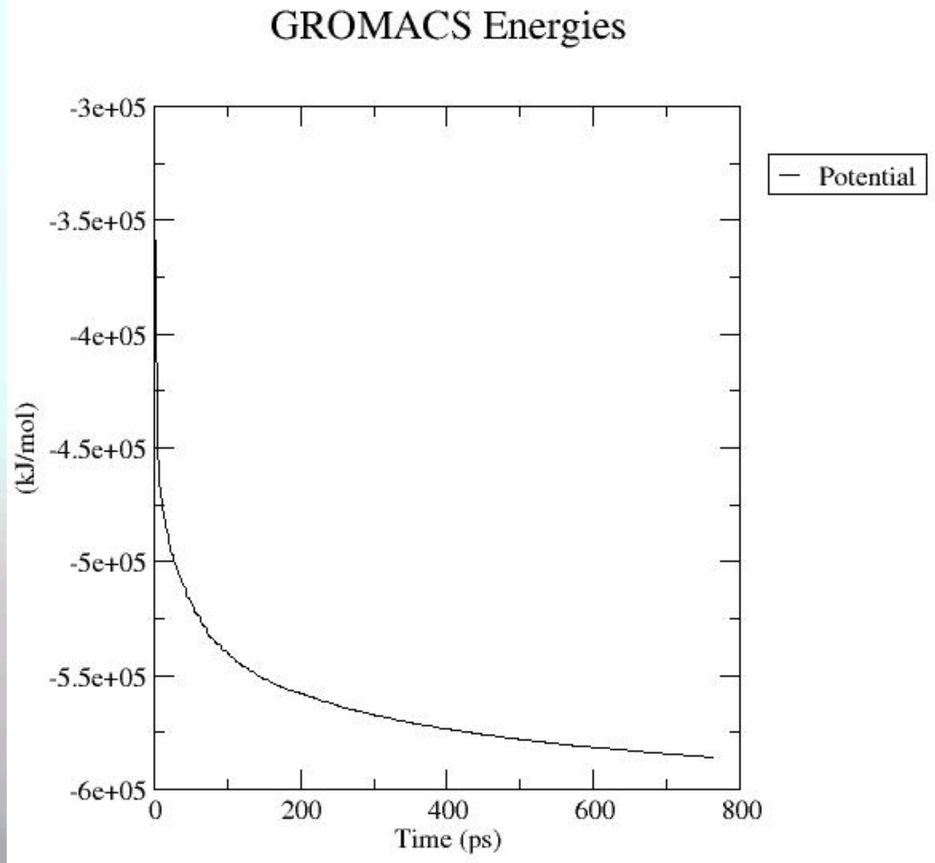
(The -v flag is for the impatient: it makes mdrun verbose, such that it prints its progress to the screen at every step. The -deffnm flag will define the file names of the input and output.)

Let's do a bit of analysis.

- The em.edr file contains all of the energy terms that GROMACS collects during EM. You can analyze any .edr file using the GROMACS energy module: **gmx energy -f em.edr -o potential.xvg**

- At the prompt, type "10 0" to select Potential (10). File called "potential.xvg" will be written. To plot this data, you will need the **Xmgrace** plotting tool.

- The resulting plot should look something like this, demonstrating the nice, steady convergence of E_{pot}:



Step 6 a. - NVT Equilibration

- EM ensured that we have a reasonable starting structure, in terms of geometry and solvent orientation.
- **To begin real dynamics**, we **must equilibrate** the **solvent and ions around the protein**

- **Why are we doing this?**

📌 *The solvent is mostly optimized within itself, and not necessarily with the solute. It needs to be brought to the temperature we wish to simulate and establish the proper orientation about the solute (the protein).*

- **Remember that posre.itp file that pdb2gmx generated a long time ago?**

📌 We're going to use it now. The purpose of posre.itp is to apply a position restraining force on the heavy atoms of the protein (anything that is not a hydrogen).

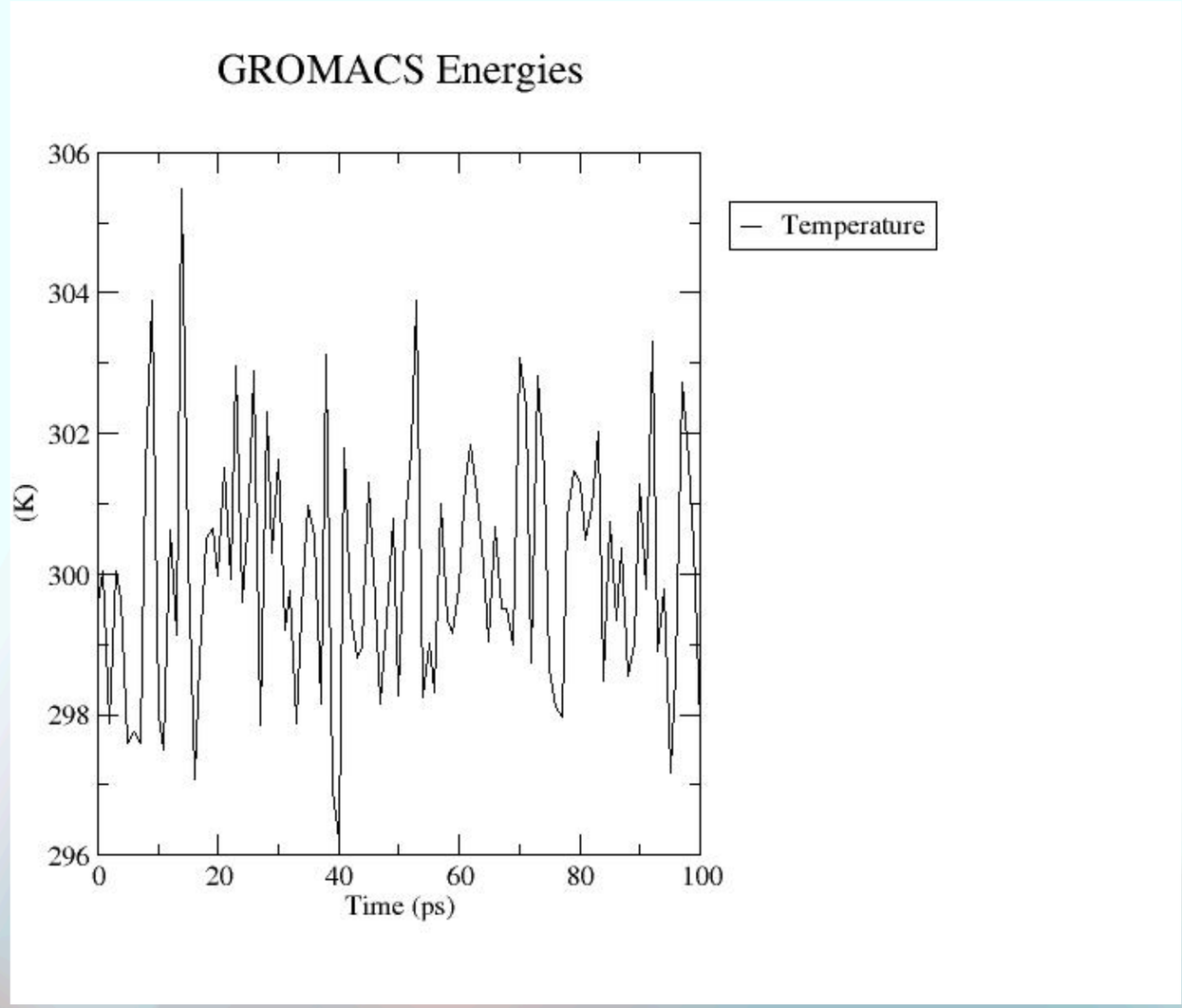
- We're conducting this equlibriation in NVT ensemble or "isothermal-isochoric" or "canonical."
- We will call grompp and mdrun just as we did at the EM step:

gmx grompp -f nvt.mdp -c em.gro -r em.gro -p topol.top -o nvt.tpr
gmx mdrun -deffnm nvt

- Let's analyze the temperature progression, again using energy:

gmx energy -f nvt.edr -o temperature.xvg

- **Type "16 0" at the prompt to select the temperature of the system and exit.**



From the plot, it is clear that the temperature of the system quickly reaches around the target value (300 K), and remains stable over the remainder of the equilibration.

Step 6 b. - NPT Equilibration

- We will call grompp and mdrun just as we did for *NVT* equilibration.
- The -t flag to include the checkpoint file from the *NVT* equilibration; this file contains all the necessary state variables to continue our simulation.

gmx grompp -f npt.mdp -c nvt.gro -r nvt.gro -t nvt.cpt -p topol.top -o npt.tpr

gmx mdrun -deffnm npt

Step 7 -Production MD

- Upon completion of the two equilibration phases, the system is now well-equilibrated at the desired temperature and pressure.

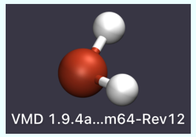
- The script for MD simulation will be in **md.mdp** file.

- Now, use code:

```
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
```

Visualising using VMD

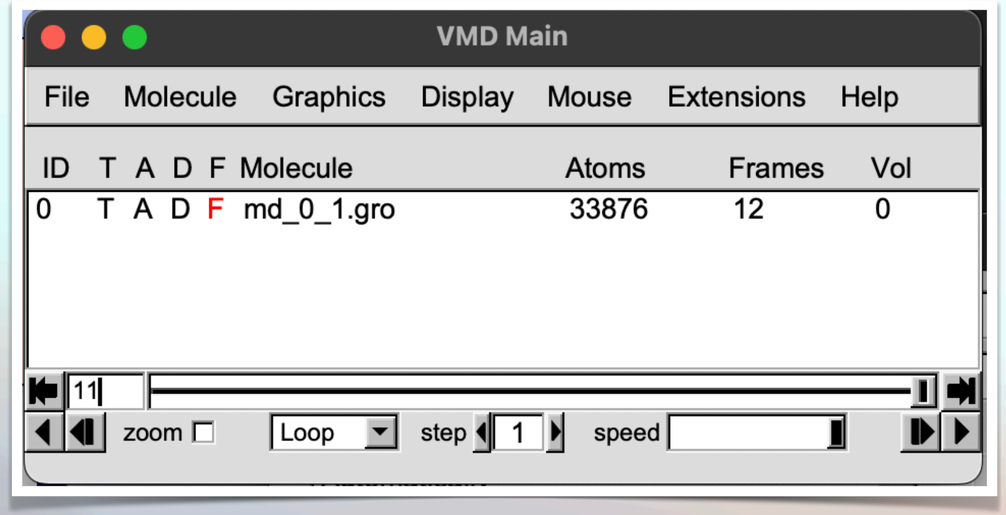
- Now, production is done. It's time to visualise our lysozyme in water.
- Open VMD 

To load .gro flile on VMD:

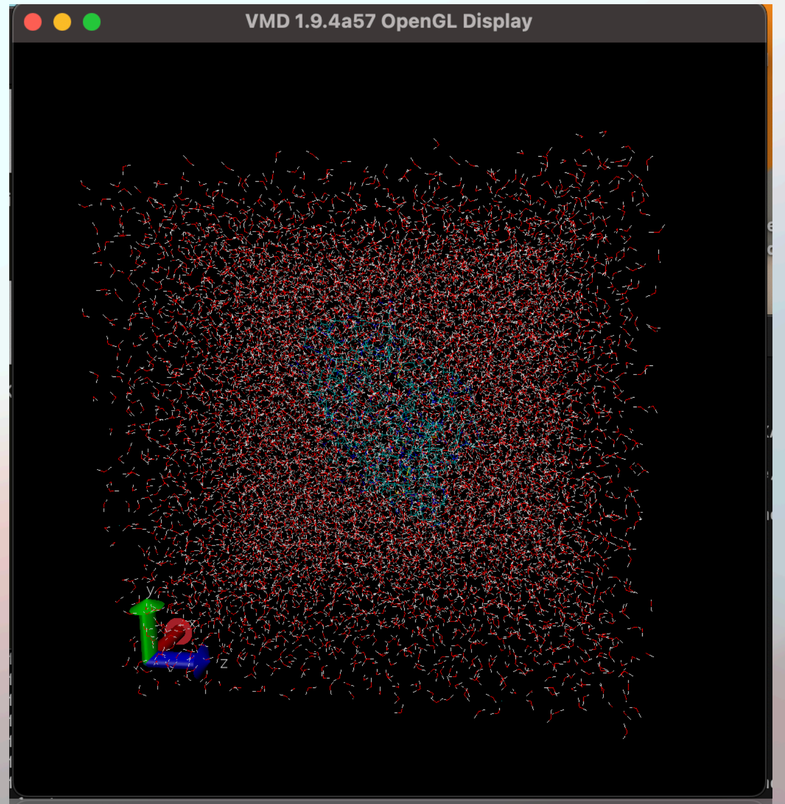
- Go to File ⟶ New Molecule ⟶ Browse ⟶choose molecule file ⟶ md_0_1.gro ⟶Load

To load .xtc file:

- On Molecule File Browser ⟶ Browse ⟶ md_0_1.xtc ⟶ Load



This is what we will get.



Our lysozyme in water

Can you visualise any thing, clearly?

Let's visualise better

- Graphics ⟶ Representation ⟶ Selected Atoms ⟶ Type "protein" ⟶ Press ENTER

⟶ Drawing Method ⟶ New Cartoon

For water

- Click "Create Rep" ⟶ Selected Atoms ⟶ Type "waters" ⟶ Drawing Method ⟶ Quick Surf

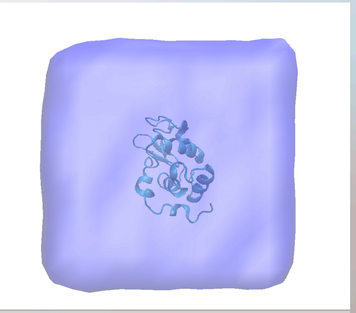
⟶ Coloring Method ⟶ ColorID ⟶ 0(blue) ⟶ Material ⟶ Transparent

- Still we can't see it properly, **let's increase it's resolution** to 4.39 or according to your requirement.
- Let's change background from black to white for more clarity : Graphics ⟶ Colors ⟶ Categories ⟶ Click "Display"

⟶ Background ⟶ Colors ⟶ 8 white.

Finally, on VMD Main window hit the play butoon to see lysozyme in water.

✱Decrease speed if it's too fast.



Lysozyme in water

Thank you!!!

Happy simulating :)