

Conformation of curcumin in water: A tutorial

Daniel M Shadrack and Ali A Hassanali

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1 Introduction

Aim

This tutorial aims to introduce learners to molecular dynamics simulation using Gromacs engine. In this tutorial you will perform a simulation of a single curcumin molecule in bulk solvent, the molecule is chosen as a model natural product with versatile biological activities whose clinical application is hampered by poor water solubility among others. At the end of this tutorial you will be able to understand the behavior of curcumin in bulk solvent. The same model can be used to study the behavior of other natural products with similar properties.

Objectives

Once this tutorial is completed participants/students will be able:

- Write and prepare an input file for MD simulation using gromacs code
- Perform a molecular dynamics simulation of the system of interest
- Perform some analysis from the trajectory generated

Resources

The resources for this tutorial are available at:

`scp dshadrac@ssh.ictp.it:/afs/ictp.it/home/d/dshadrac/curcumin_tutorial.tar.`

2 Some theory

From the theory of classical MD simulation you have learned this week, we are now going to make the practical of the same using a simple system i.e curcumin in water. For the purpose of this tutorial we will use OPLS-AA force field and TIP4P water model.

3 Simulation details

Solvation

We are now going to define our simulation box with curcumin at the center and then fill box with water. In this tutorial we will use the cubic box as our unit cell. We will do this in two ways.

- Define the box dimension using the editconf module by executing the following command:

```
gmx editconf -f CURC.gro -o box.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). The distance to the edge of the box is an important parameter.

To see some help on making the box or increasing/reducing the box size to the dimension you wish, type the following command:

```
gmx editconf -h
```

For example: if you want to make a box of 2x2x2 in the above command include the following flag, -box 2 2 2.

To view your box and curcumin inside the box, we will use VMD tool. To do that, just execute the following command:

```
vmd box.gro. Then, type in the vmd terminal pbz box
```

You will get something like below.

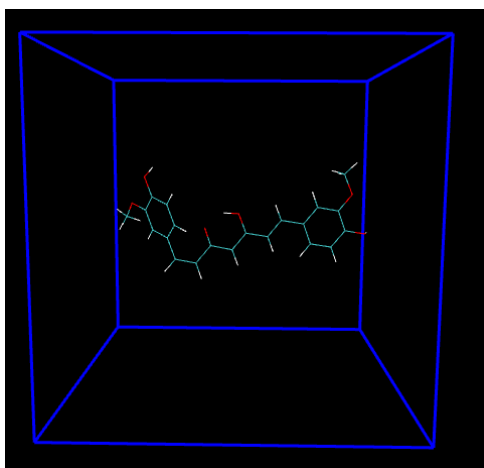


Figure 1: Our simulation box

- Ok, did you achieve!!, Congratulations. Now, lets go to solvate our system with TIP4P water model. This is the second step we call it **Solvation**. Solvation is done by solvate module in gromacs, now execute the following command.

```
gmx solvate -cp box.gro -cs tip4p.gro -o solv.gro -p CURC.top.
```

When solvating, dont forget to update your topology, otherwise you will get error related to *number in the coord.. file does not match with the topol. file* in the next stage.

Now, you can view your solvated system using vmd command as we did in the previous step. you will see something like this.

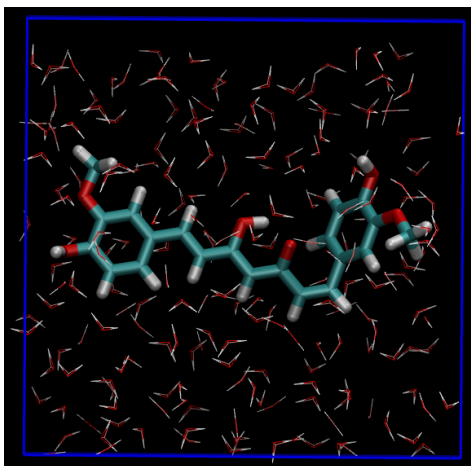


Figure 2: Curcumin solvated with TIP4P water

Now, check your .top file, you will see how many waters was added to solvate your curcumin in a given box. to open your .top file use *gedit name of your file*, or just type in your terminal *tail name of your .top file*

Energy minimization

Before we start our MD simulation, we need to minimize to avoid any unrealistic interactions or clashes (**remember you have learned from the theory**). Now let us go on with the practical. We will use steepest decent algorithm to slightly moves the atomic positions optimizing the interatomic interactions and the potential energy of the system. You will use the following input file called *min.mdp*. You can open the file to see the information it contains.

****NOTE: before you minimize your system of interest, make sure it is electroneutral, by adding positive or negative charges. and make**

sure you update the .top file, otherwise you will get errors related to number in coordinate and topology not matching

Now, to minimize our system we will use the grompp in gmx. execute the following command:

```
gmx grompp -f min.mdp -c solv-ions.gro -p CUR.top -o em.tpr
```

Now you have generated the em.tpr, we will use it to minimize our system using the mdrun module. execute the following command:

```
gmx mdrun -deffnm em -v
```

In the above command: the -deffnm flag, specifies or defines the file names for input and output. the -v makes the mdrun process verbose, in a such a way you can print and see the progress.

After minimization process you will get the following files:

- em.gro: minimized structure,
- em.trr: binary full-precision trajectory
- em.edr: binary energy file
- em.log: log file for EM process

Let us now do some analysis of our system: We will check the energy of our system. The file *em.edr* contains information about energy of the your system. **Two factors can help to decide if our system is energy minimized, (1), the potential energy printed at the end of the minimization process, the potential energy should be negative but depends on the system and the amount of water added. (2) the maximum force (Fmax) is another important factor, we have set a emtol =1000 kJ/mol /nm indicating that a target force should not be greater than that value. If your system is reaching the Epot with Fmax greater than 1000, You can also change some parameters in your min.mdp file e.g emsteps, integrator** To check our energy profile execute the following command and when prompted choose 10 for potential and 0 to close e.g (10 0) then press enter.

```
gmx energy -f em.edr -o potential.xvg
```

Then, use xmgrace to view your energy profile: write *xmgrace potential.xvg*

- What is the nature of your profile? did you expect?
- From your energy profile, what can you comment? Is the system minimize?

Equilibration

Our EM system, has now a good starting structures (geometry and solvent orientation). Now, to start a real dynamics we need to equilibrate our system. **Why do you think is necessary to equilibrate?.**

Equilibration is normally done in two steps (1) using the NVT ensemble (constant number of particles, volume and temperature), this ensemble is also known as canonical. The second equilibration is done under NPT ensemble (constant Pressure) also known as isothermal-isobaric ensemble.

For the purpose of this tutorial as previously reported for the same system see the work by T.Patsahan et al., in Condensed Matter Physics,2017,Vol.20,No2,23003:1–20; DOI:10.5488/CMP.20.23003; and <https://doi.org/10.1016/j.molliq.2016.08.098> for further references. We are going to perform our MD simulation in NPT ensemble.

In our NPT equilibration we will use the Parrinello-Rahman barostat with pressure at 1 bar and temperature at 300 K. we are going to use the input file named *npt.mdp* with the following command:

```
gmx grompp -f npt.mdp -c em.gro -r e m.gro -p CUR.top -o npt.tpr
```

```
gmx mdrun -deffnm npt -v
```

Again, let us do some analysis on pressure as we did before using the energy.

```
gmx energy -f npt.edr -o pressure.xvg
```

When prompted type 15 0 to select the pressure of the system and exit.

What did you observe from your plot and why? did you get a plot like this?

The pressure value fluctuates widely over the course of the 200-ps equilibration phase, do you expect this behaviour?. The running average of these data are plotted as the red line in the same plot with the average pressure of 8.5 ± 435 bar see Fig. 3. Pressure is the quantity which fluctuate more during the MD simulation as it is observed from the RMSD of 435.

Lets check the density using the energy, when prompted enter 21 0

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

As for the pressure the average value for density is $998 \pm 8 \text{ kg m}^{-3}$ as indicated in Fig. 3.

Some discussion:

1. Is your density close to experimental value i.e 1000 kg m^{-3} ?
2. Is your density stable over the simulation time?

GROMACS Energies

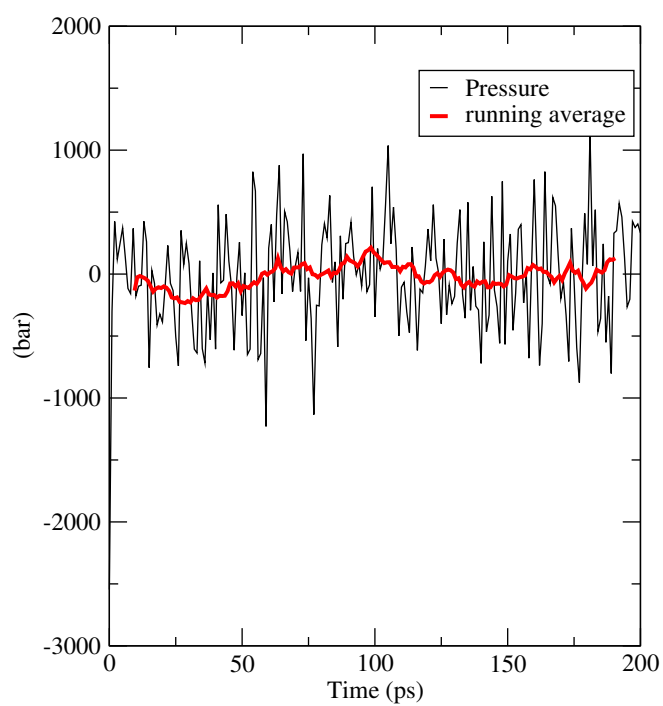


Figure 3: Pressure of the system over 200 ps

GROMACS Energies

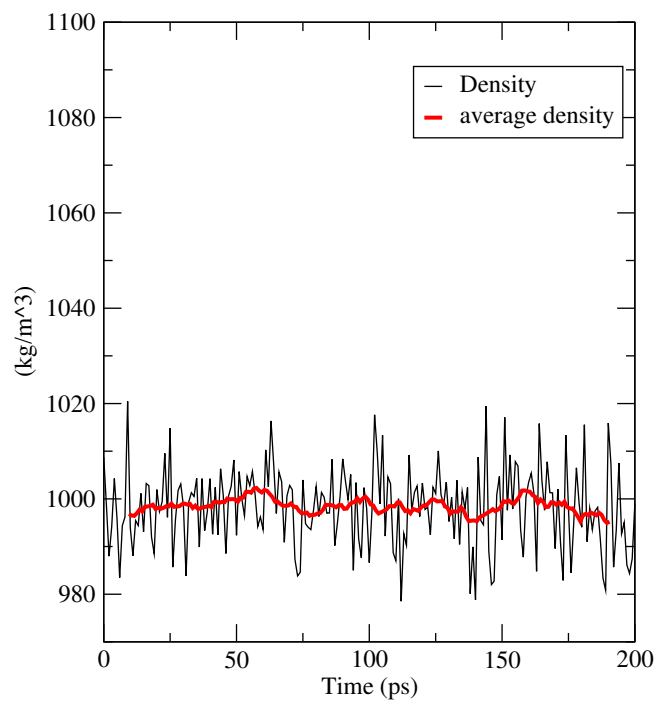


Figure 4: Density of the system over 200 ps

Production simulation

We have now equilibrated and the system is prepared to run the simulation. The process is similar to equilibration, except simulation parameters file and initial coordinates file. Now we will use the *md.mdp* file to run our production.

Now execute the following two steps command to perform the MD production:

```
gmx grompp -f md.mdp -c npt.gro -t npt.cpt -p CUR.top -o md.tpr
```

```
gmx mdrun -deffnm md -v
```

Analysis

We will perform the following analysis and make a discussion

- Pair correlation function/Radial distribution function ($g(r)$) between water oxygen and different groups of curcumin e.g O and H.
- Keto-enol distance
- End to end distance
- Hydrogen bond
- RMSD & Rg
- dihedral angles