- SIMULATION (Trp cage protein as example)

Preparation

1. Clean the pdb file manually by deleting unnecessary ions/water etc and rename as trp
2. gmx pdb2gmx -f trp_clean.pdb -o trp_processed.gro -water spce -ignh

   (choose 9: gromacs96)

Solvation

3. gmx editconf –f trp_processed.gro -o trp_newbox.gro -c -d 1.0 -bt cubic
4. gmx solvate -cp trp_newbox.gro -cs spc216.gro -o trp_solv.gro -p topol.top

   Ions addition

5. gmx grompp -f ions.mdp -c trp_solv.gro -p topol.top -o ions.tpr -maxwarn 3
6. gmx genion -s ions.tpr -o trp_solv_ions.gro -p topol.top -pname NA -nname CL -neutral -conc 0.15

   Energy minimisation

7. gmx grompp -f minim.mdp -c trp_solv_ions.gro -p topol.top -o em.tpr -maxwarn 3
8. gmx mdrun -v -deffnm em

   Check: a. gmx energy -f em.edr -o potential.xvg (select 10: potential and 2 times enter)

   b. xmgrace potential.xvg

   Nvt equilibration

9. gmx grompp -f nvt.mdp -c em.gro -r em.gro -p topol.top -o nvt.tpr -maxwarn 3
10. gmx mdrun -v -deffnm nvt

    Note: the temperature of the system van be changed at this step, by changing one line in the nvt.mdp file i.e., **ref_t = 300 300** to **ref_t = 363 363**

    Check: a.gmx energy -f nvt.edr -o temperature.xvg (select temperature)

    b. Xmgrace temperature.xvg (to check the temperature of system)

    Npt equilibration

11. **gmx grompp -f npt.mdp -c nvt.gro -r nvt.gro -t nvt.cpt -p topol.top -o npt.tpr -maxwarn 3**
12. gmx mdrun -deffnm npt

Note: the temperature of the system has to changed at this step also, by changing one line in the npt.mdp file i.e., **ref_t = 300 300** to **ref_t = 363 363**

Check: **a.** gmx energy -f npt.edr -o pressure.xvg (select presure and 2 times enter)

b. xmgrace pressure.xvg

c. gmx energy -f npt.edr -o density.xvg (select density & 2 times enter)

d. xmgrace density.xvg

Production

13. **gmx grompp -f md.mdp -c npt.gro -t npt.cpt -p topol.top -o md_1.tpr -maxwarn 3**
14. gmx mdrun –v -deffnm md_1

- ANALYSIS

1. Deviation in Ramachandran basin of 4th and 8th residue w.r.t. to starting structure

   Step 1: need to calculate the phi psi of the starting structure

   gmx rama -f 1pgb_processed.gro -s em.tpr -o rama.xvg

   (it will generate the phi psi of the 1 frame only which is the starting structure generated at the prepaption step)

   A. After EM

   gmx rama -f em.gro -s em.tpr -o rama.xvg

   (it will generate the phi/psi of the 1 frame only of the EM state from the gro file)

   B. After 10 ns simulation (here there are 10000 frames only)

   gmx rama -f md_0_1.xtc -s md_0_1.tpr -b 9999 -e 10000 -o rama.xvg

   Or gmx rama -f md_0_1.xtc -s md_0_1.tpr -b 10000 -o rama.xvg

   Or gmx rama –f md.grp -s md.tpr -o rama.xvg

2. Energy with time

A. gmx energy -f md_0_1.edr -o total_energy.xvg

(put 13 & 0, it will calculate the total energy of the system with time)

B. gmx energy -f md_0_1.edr -o coul_sr.xvg
(select 9 & 0, it will calulate the coulomb SR energy of s)

C. gmx energy -f md_0_1.edr -o lj-sr.xvg

(select 7 & 0)

2. **Radius of gyration calculate**

gmx gyrate -f md_1.xtc -s md_1.tpr -o gyrate.xvg

(select protein, it will give the rog evolution with whole time)

3. Cluster Analysis

gmx cluster -f md_1.xtc -s md_1.tpr -method gromos -cutoff 0.15 -g –cl

(select 3 and 1; gromos method is used for calculation with 0.15 cutoff, it will generate the log file, rmsd file and the clustered pdb file)

gmx cluster -f md_1.xtc -s md_1.tpr -method gromos -cutoff 0.1 -g -cl

gmx cluster -f md_1.xtc -s md_1.tpr -method gromos -cutoff 0.30 -g -cl