Additional Materials: NVT and NPT equilibration

About parameters in mdp file

- To comment a line out, use semicolon (;)at the beginning.
- Title is optional (But always recommended to write to keep the track)
- define = -DPOSRES is positional restrained if to be used.
  - It is specified in the grompp command with the flag -r file.gro. If there is no constraint on the residues in the system comment out the line.
  - If specified, please check a keyword “#include ‘posres.itp’” is present or not, if not then add it.
- Integrator specifies the algorithm to be used, such as steep (short form of “steepest decent “ for energy minimization), velocity-verlett for md and so on.
- nsteps is the number of steps user can specify for the simulation.
- dt is the step duration. More than 4fs is not recommended.
  - ***Total duration of simulation = dt * nsteps***
- ref_t is set to the desired temperature, here it is 300K.
- Temperature (tcoupl) can be controlled by using different methods, here tcoupl is set to V-scale. For detailed understanding of the method, user may refer to Gromacs manual/documentation.
- pcoupl = “no” means, there is no pressure coupling.
- pcoupl= “Parrinello-Rahman” is the pressure coupling method used here in the NPT step
- ref_p is the reference pressure in bar, and set to 1 here.
- Compressibility parameter allows user to set compressibility of the solvent, here set to = 4.5e-5 (compressibility of water).

About pressure equilibration

- The pressure generally shows very large fluctuation and sometimes does not converge to reference average pressure.
- Beginners may visualize it as follows. To maintain the pressure, the program (Gromacs) attached a mechanical wall to box and adjust the volume such that the pressure should remain constant. But the problem is that, we use a small box and because of that the volume fluctuation is less which result in less stabilization of pressure. if one wish to get accurate pressure, we should use a large box but again it will cost in terms of computational power.
- Bottom rule is that do not worry about the average pressure. The best parameter to see the pressure equilibration is density. If the system density is around water density then everything is fine.
- To calculate the density use same command "**gmx energy -f npt.edr -o density.xvg**" but this time choose density option.

Terminal commands used in the tutorial

Grace is installed in the system. **Windows** users may use **QtGrace**, which is a fork of **Grace**. Windows usets can drag the **.xvg** file icon on the graph window to avoid error messages.

1. Type, **cd Documents/firstmd** and press **Enter**.
2. Type, **gmx grompp -f nvt.mdp -c em.gro -r em.gro -p topol.top -o nvt.tpr** and press **Enter**.
3. Type **ls** and press **Enter** to list the files.
4. Type, **gmx mdrun -v -deffnm nvt** and press **Enter** to start the temperature equilibration. This step generates, several files with **nvt** prefix.
5. Type, **gmx energy -f nvt.edr -o temperature.xvg** and press **Enter**. Choose 16 and press **Enter** to write the file.
    - Use the number for temperature as appropriate, it may be different for you.
1. Type, **xmgrace temperature.xvg** and press **Enter**.
2. Type, **gmx grompp -f npt.mdp -c nvt.gro -t nvt.cpt -p topol.top -o npt.tpr** and press **Enter**.
3. **gmx mdrun -v -deffnm npt** and press **Enter**.
4. Press **Ctrl+C** to abort the process.
5. Type, **gmx energy -f npt.edr -o pressure.xvg** and press **Enter**. Type **18** and press **Enter**.
    - This number may be different for you.

1. Type, **xmgrace pressure.xvg** and press **Enter** to open the pressure- time graph.