Quick Start Guide: MD Simulation of a Protein-Ligand complex in Water (GROMACS - Amber Forcefield - Acpype)

Input Files:

protein.pdb (without water and any ligands)

Ligand.pdb (obtained after docking)

ions.mdp

minim.mdp

nvt.mdp

npt.mdp

md.mdp

Step 1: Prepare the protein Topology

gmx pdb2gmx -f protein.pdb -o protein_processed.gro

When prompted, select the desired Amber force field and compatible water model (e.g., TIP3P)

Step 2: Prepare the Ligand Topology

Setting ACPYPE Alias

alias acpype='/usr/local/acpype/acpype_lib/acpype.py'

Note: Replace /usr/local/... with the actual installation path on your system

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Source Amber Environment Variables

source /usr/local/amber18/amber.sh

Note: Replace /usr/local/... with the actual installation path on your system

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Convert pdb to mol2

obabel -ipdb Ligand.pdb -O Ligand.mol2

Run acpype

acpype -di Ligand.mol2 -c gas

Note: The -c gas option uses the partial charges already present in the MOL2 file and does not perform quantum mechanical charge calculation.

For more accurate molecular dynamics simulations, it is recommended to compute ligand charges using quantum-based methods (e.g., AM1-BCC or RESP) prior to topology generation

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From the Ligand.acpype directory, copy the following files into your working directory:

Ligand_GMX.itp

Ligand_NEW.pdb

Rename them as follows:

Ligand_NEW.pdb → lig.pdb

Ligand_GMX.itp → lig.itp

Notes: Ensure the molecule name (lig) matches what is defined in lig.itp and lig.pdb.

Make ligand gro file

gmx editconf -f lig.pdb -o lig.gro

Step 3: Update the Topology and Coordinate files for protein-ligand complex

Combine Protein and Ligand Coordinates

Merge the ligand coordinates (lig.gro) with the protein coordinates (protein_processed.gro) to create a single .gro file.

Ensure that atom numbers are updated correctly and that the structure is consistent.

Save the combined structure as: complex.gro

Include Ligand Topology in topol.top

Add the following line at the top of the topology file, after the force field inclusion but before the protein topology:

; Include ligand topology

#include "lig.itp"

Define Molecules in topol.top

At the end of topol.top, in the [molecules] section, specify the number of protein and ligand molecules:

[molecules]
; Compound #mols
Protein 1
lig 1

Step 4: Defining the Unit Cell & Adding Solvent

Defining the Unit Cell

gmx editconf -f complex.gro -o complex_newbox.gro -bt cubic -d 1.0

Adding Solvent

gmx solvate -cp complex_newbox.gro -cs spc216.gro -o complex_solv.gro -p topol.top

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Step 5: Adding Ions

Prepare the system for ion addition

gmx grompp -f ions.mdp -c complex_solv.gro -p topol.top -o ions.tpr

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Add ions to neutralize the system and reach physiological salt concentration

gmx genion -s ions.tpr -o complex_solv_ions.gro -p topol.top -pname NA -nname CL -neutral -conc 0.15

When prompted, select the SOL group.

Step 6: Energy Minimization

Prepare the system for Energy Minimization

gmx grompp -f minim.mdp -c complex_solv_ions.gro -p topol.top -o em.tpr

Run Energy Minimization

gmx mdrun -v -deffnm em

Step 7: Restraining the lig

Generate position restraint file

gmx genrestr -f lig.gro -o posre_lig.itp -fc 1000 1000 1000

When prompted, select the ligand

Include the position restraints of ligand in topology

Place it after including the ligand topology

; lig position restraints
#ifdef POSRES
#include "posre_lig.itp"
#endif

Step 8: Temperature coupling

Create Index File

gmx make_ndx -f em.gro -o index.ndx

Combine protein and ligand groups (Protein|lig)

Quit with q

Step 9: NVT Equilibration

Prepare the system for NVT Equilibration

gmx grompp -f nvt.mdp -c em.gro -r em.gro -p topol.top -n index.ndx -o nvt.tpr

Run NVT Equilibration

gmx mdrun -v -deffnm nvt

Step 10: NPT Equilibration

Prepare the system for NPT Equilibration

gmx grompp -f npt.mdp -c nvt.gro -r nvt.gro -p topol.top -n index.ndx -o npt.tpr

Run NPT Equilibration

gmx mdrun -v -deffnm npt

Step 11: Production MD

Prepare the system for Production MD

gmx grompp -f md.mdp -c npt.gro -p topol.top -n index.ndx -o md.tpr

Run Production MD

gmx mdrun -v -deffnm md