Molecular dynamics workshop

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- https://ub.cbm.uam.es/downloads/MD-workshop-gr-2026.pdf
- https://tinyurl.com/MDGRWS26)

Create Linux VM (if missing)

- Download image: https://tinyurl.com/LU22BIO
- Uncompress it
- Launch Virtual Box
  - Click on “Nueva”
    - Nombre: “Bioinfo-Lubuntu22”
    - Tipo: “Linux”
    - Version: “Lubuntu (64-bit)”
  - Click on “Siguiente”
    - Memoria base: 3072
    - Procesadores: 4
  - Click on “Siguiente”
    - “Usar un archivo de disco virtual existente”
    - Path to uncompressed file
  - Click on “Siguiente”
  - Click on “Terminar”
  - Select the new virtual machine and click on “Configuración”
  - Click on “Pantalla” and increase “Memoria de vídeo” up to 128MB

Gromacs install with mamba (skip)

Open terminal 

Install micromamba:

- **"${SHELL}" <(curl -L micro.mamba.pm/install.sh)**

- Exit terminal and open a new one

micromamba config append channels bioconda --env

micromamba update -n base -c defaults conda

micromamba activate base

micromamba install -c cdat xmgrace

micromamba install pocl gromacs vmd

Prepare environment

Open console:

Activate environment:

micromamba activate base

Set variables:

exampdir=/home/$USER/examp-md-cdk2

workdir=/home/$USER/md-cdk2

pdb=cdk2_mod.BL00010001.pdb

pdbr=cdk2_mod.BL00010001

export DSSP=~/bin/dssp

Prepare environment II

Make example and working directories:

mkdir $workdir

mkdir $exampdir

Go to example files directory:

cd $exampdir

Get files and uncompress files:

wget https://saco.csic.es/s/yeLKeqZmWijWLQS

tar -xzf cdk2-md.tgz

Molecular dynamics simulations

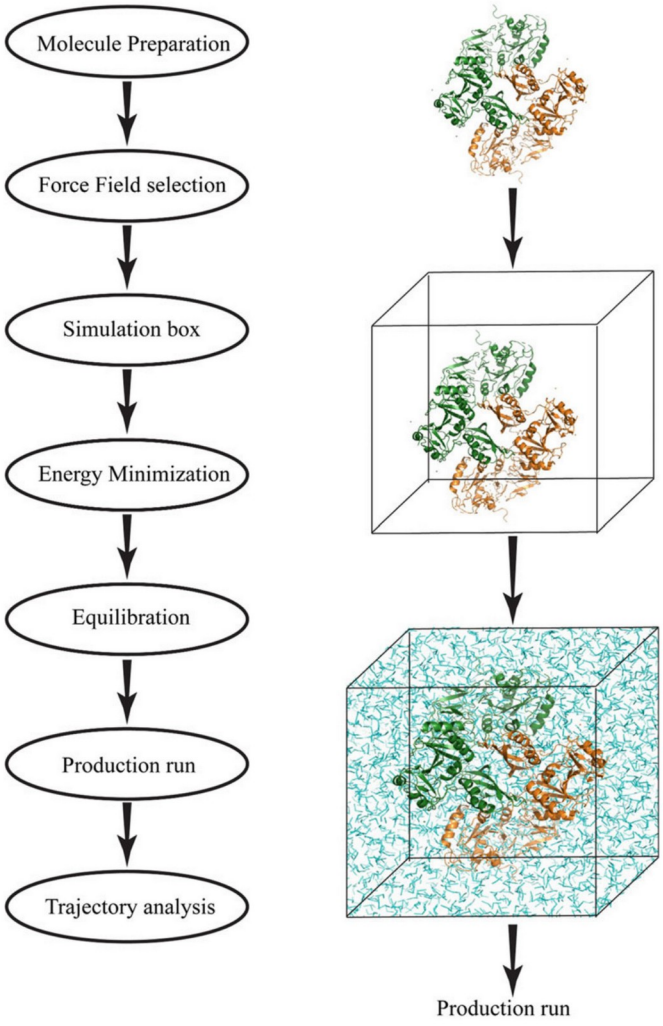


Fig. 1 The flow diagram for the MD simulation run

Redkar, A.S., Ramakrishnan, V. (2023). Molecular Dynamics Simulations. In: Ramakrishnan, V. (eds) Biophysical Characterization of Functional Peptides. Springer Protocols Handbooks. Humana, New York, NY. https://doi.org/10.1007/978-1-0716-3405-9_3

System preparation

- Go to working directory:

  cd $workdir

- copy model structure:

  cp $exampdir/cdk2_mod.BL00010001.pdb .

- copy gromacs run definitions:

  cp $exampdir/*.mdp .

- Add hydrogens and generate topology file:

  gmx pdb2gmx -f $pdb -water tip3p -ff amber03 -ignh

- Generated files:

  Topology: topol.top

  Coordinates: conf.gro

  List of restrained atoms: posre.itp

Water box and ions

- Define water box size and shape:

  gmx editconf -d 1.0 -bt triclinic -o ${pdbr}_newbox.gro

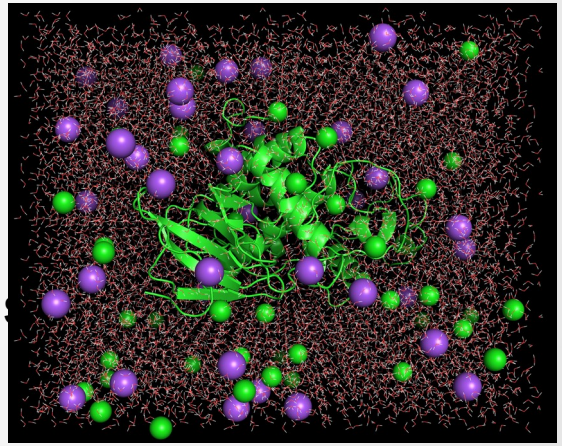
- Add waters to the system:

  gmx solvate -cp ${pdbr}_newbox.gro -cs spc216.gro -o ${pdbr}_solv.gro -p topol.top

- This generates new topology and coordinates files:

  cdk2_mod.BL00010001_solv.gro

  topol.top



- Prepare de system for adding ions

  gmx grompp -f ions.mdp -c ${pdbr}_solv.gro -p topol.top -o

  - This generates gromacs input:
    - cdk2_mod.BL00010001_ions.tpr

- Add ions to neutralize protein net charge and to a final concentration of 0'15M

  echo 13|gmx genion -s ${pdbr}_ions.tpr -o ${pdbr}_ions.gro -p topol.top -neutral -conc 0.15

  - This generates new topology and coordinates files:

    cdk2_mod.BL00010001_ions.gro

    topol.top

Minimization

- Prepare the system

  gmx grompp -f minim.mdp -c ${pdbr}_ions.gro -p topol.top -o ${pdbr}_em.tpr

- Run the minimization (~ 2min)

  gmx mdrun -v -deffnm cdk2_mod.BL00010001_em

Minimization

- Minimization input file:

```
; minim.mdp - used as input into grompp to generate em.tpr
; Parameters describing what to do, when to stop and what to save
integrator      = steep  ;Algorithm (steep = steepest descent minimization)
emtol           = 1.0    ; Stop minimization when the maximum force < 1.0 kJ/mol/nm
emstep          = 0.01   ; Energy step size
nsteps          = 1000   ; Maximum number of (minimization) steps to perform
; Parameters describing how to find the neighbors of each atom and how to calculate the interactions
nstlist         = 10     ; Frequency to update the neighbor list and long range forces
ns_type         = grid   ; Method to determine neighbor list (simple, grid)
rlist           = 1.0    ; Cut-off for making neighbor list (short range forces)
coulombtype = PME    ; Treatment of long range electrostatic interactions
rcoulomb        = 1.0    ; Short-range electrostatic cut-off
rvdw            = 1.0    ; Short-range Van der Waals cut-off
pbc             = xyz    ; Periodic Boundary Conditions (yes/no)
```

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Minimization analysis

- Generated files

  cdk2_mod.BL00010001_em.edr #Energies

  cdk2_mod.BL00010001_em.trr #Trajectory

  cdk2_mod.BL00010001_em.log #Log

  cdk2_mod.BL00010001_em.gro #Final coordinates

- Analyze potential energy evolution

  echo '10 0' | gmx energy -f ${pdbr}_em.edr -o ${pdbr}_potential.xvg

- View graph

  xmgrace ${pdbr}_potential.xvg

Equilibration NVT with restrains

- NVT: Canonical ensemble (amount, volume and temperature constant)
- Prepare the system

  gmx grompp -f nvt.mdp -c ${pdbr}_em.gro -r ${pdbr}_em.gro -p topol.top -o ${pdbr}_nvt.tpr

- Run the equilibration (5ps, ~ 1min)

  gmx mdrun -deffnm ${pdbr}_nvt

Equilibration NVT Input file

- title = Amber03 NVT equilibration
- **define = -DPOSRES** ; position restrain the protein
- ; Run parameters
- integrator = md ; leap-frog integrator
- **nsteps = 2500** ; 2fs * 2500steps = 5 ps
- dt = 0.002 ; 2 fs
- ; Output control
- ;nstxout = 100 ; save coordinates every 0.2 ps
- ;nstvout = 100 ; save velocities every 0.2 ps
- nstxout-compressed = 100 ; xtc compressed trajectory output every 0.2ps
- nstenergy = 100 ; save energies every 0.2 ps
- nstlog = 100 ; update log file every 0.2 ps
- nstcalcenergy = -1
- ; Bond parameters
- continuation = **no** ; first dynamics run
- constraint_algorithm = lincs ; holonomic constraints
- constraints = h-bonds ; bonds involving H are constrained
- lincs_iter = 1 ; accuracy of LINCS
- lincs_order = 4 ; also related to accuracy
- ; Neighborsearching
- cutoff-scheme = Verlet ; Buffered neighbor searching
- ns_type = grid ; search neighboring grid cells
- nstlist = 10 ; 20 fs, largely irrelevant with Verlet scheme
- rlist = 1.0 ; short-range neighborlist cutoff (in nm)
- rcoulomb = 1.0 ; short-range electrostatic cutoff (in nm)
- rvdw = 1.0 ; short-range van der Waals cutoff (in nm)
- ; Electrostatics
- coulombtype = PME ; Particle Mesh Ewald for long-range electrostatics
- pme_order = 4 ; cubic interpolation
- fourierspacing = 0.12 ; grid spacing for FFT
- ; Temperature coupling is on
- tcoupl = V-rescale ; modified Berendsen thermostat
- tc-grps = Protein Non-Protein ; two coupling groups - more accurate
- tau_t = 0.1 0.1 ; time constant, in ps
- ref_t = 300 300 ; reference temperature, one for each group, in K
- ; Pressure coupling is off
- **pcoupl = no** ; no pressure coupling in NVT
- ; Periodic boundary conditions
- pbc = xyz ; 3-D PBC
- ; Dispersion correction
- DispCorr = EnerPres ; account for cut-off vdW scheme
- ; Velocity generation
- **gen_vel = yes** ; assign velocities from Maxwell distribution
- **gen_temp = 300** ; temperature for Maxwell distribution
- gen_seed = -1 ; generate a random seed

Equilibration NVT analysis

- Generated files

  cdk2_mod.BL00010001_nvt.cpt #Checkpoint

  cdk2_mod.BL00010001_nvt.edr #Energies

  cdk2_mod.BL00010001_nvt.xtc #Trajectory

  cdk2_mod.BL00010001_nvt.log #Log

  cdk2_mod.BL00010001_nvt.gro #Final coordinates

- Check temperature evolution

  echo '16 0' | gmx energy -f ${pdbr}_nvt.edr -o ${pdbr}_temperature.xvg

- View graph

  xmgrace ${pdbr}_temperature.xvg

Equilibration NPT with restrains

- Prepare the system

  gmx grompp -f npt.mdp -c ${pdbr}_nvt.gro -r ${pdbr}_nvt.gro -t ${pdbr}_nvt.cpt -p topol.top -o ${pdbr}_npt.tpr

- Run the equilibration (15 ps, ~ 3min)

  gmx mdrun -deffnm ${pdbr}_npt

Equilibration NPT Input file

```
title                    = Amber03 NPT equilibration
define                   = -DPOSRES  ; position restrain the protein
; Run parameters
Integrator               = md        ; leap-frog integrator
Nsteps                   = 7500      ; 2fs * 7500steps = 15ps
Dt                       = 0.002     ; 2 fs
; Output control
;nstxout                 = 100       ; save coordinates every 0.2 ps
;nstvout                 = 100       ; save velocities every 0.2 ps
Nstxout-compressed       = 100       ; xtc compressed trajectory output every 0.2 ps
nstenergy                = 500
; save energies every 1 ps
nstlog                   = 100
; update log file every 0.2 ps
; Bond parameters
Continuation             = yes
; Restarting after NVT
constraint_algorithm     = lincs     ; holonomic constraints
Constraints              = h-bonds   ; bonds involving H are constrained
lincs_iter               = 1         ; accuracy of LINCS
lincs_order              = 4         ; also related to accuracy
; Neighborsearching
ns_type                  = grid
; search neighboring grid cells
Nstlist                  = 10        ;20 fs, largely irrelevant with Verlet scheme
cutoff-scheme            = Verlet
Rlist                    = 1.0       ; short-range neighborlist cutoff (in nm)
Rcoulomb                 = 1.0       ; short-range electrostatic cutoff (in nm)
Rvdw                     = 1.0       ; short-range van der Waals cutoff (in nm)
; Electrostatics
coulombtype              = PME       ; Particle Mesh Ewald for long-range electrostatics
pme_order                = 4         ; cubic interpolation
Fourierspacing           = 0.12      ; grid spacing for FFT
; Temperature coupling is on
tcoupl                   = V-rescale ; modified Berendsen thermostat
tc-grps                  = Protein Non-Protein ; two coupling groups - more accurate
tau_t                    = 0.1 0.1   ; time constant, in ps
ref_t                    = 300 300   ; reference temperature, one for each group, in K
; Pressure coupling is on
pcoupl                   = Berendsen  ; Pressure coupling on in NPT
Pcoupltype               = isotropic  ; uniform scaling of box vectors
tau_p                    = 2.0        ; time constant, in ps
ref_p                    = 1.0        ; reference pressure, in bar
compressibility = 4.5e-5              ; isothermal compressibility of water, bar^-1
refcoord_scaling = com
; Periodic boundary conditions
pbc                      = xyz        ; 3-D PBC
; Dispersion correction
DispCorr                 = EnerPres   ; account for cut-off vdW scheme
; Velocity generation
gen_vel                  = no         ; Velocity generation is off
```

Equilibration NPT analysis

- Check pressure evolution

  echo '18 0' |gmx energy -f ${pdbr}_npt.edr -o ${pdbr}_pressure.xvg
- View graph

  xmgrace cdk2_mod.BL00010001_pressure.xvg
- Check density evolution

  echo '24 0' |gmx energy -f ${pdbr}_npt.edr -o ${pdbr}_density.xvg
- View graph

  xmgrace cdk2_mod.BL00010001_density.xvg
- Analyze rmsd evolution

  echo '4 4' |gmx rms -s ${pdbr}_npt.tpr -f ${pdbr}_npt.xtc -o ${pdbr}-bkbone-rmsd-npt.xvg
- View graph

  xmgrace cdk2_mod.BL00010001-bkbone-rmsd-npt.xvg

MD production

- Prepare the system

  gmx grompp -f md.mdp -c ${pdbr}_npt.gro -t ${pdbr}_npt.cpt -p topol.top -o ${pdbr}_md.tpr

- Run the production (40ps, 5min)

  gmx mdrun -deffnm ${pdbr}_md -cpt 5

MD production Input file

```
title                    = Amber03 MD
;Don't use restrains
; Run parameters
Integrator               = md        ; leap-frog integrator
Nsteps                   = 20000     ; 2 * 20000 = 40 ps
Dt                       = 0.002     ; 2 fs
; Output control
;nstxout                 = 100       ; save coordinates every 0.2
ps
;nstvout                 = 100       ; save velocities every 0.2 ps
Nstxout-compressed = 100         ; xtc compressed trajectory
output every 2 ps
nstenergy                = 500                                ;
save energies every 1 ps
nstlog                   = 500                                ;
update log file every 1 ps
; Bond parameters
continuation             = yes                    ; Restarting
after NPT
constraint_algorithm   = lincs      ; holonomic constraints
constraints              = h-bonds ; bonds involving H are
constrained
lincs_iter               = 1         ; accuracy of LINCS
lincs_order              = 4         ; also related to accuracy
; Neighborsearching
cutoff-scheme            = Verlet    ; Buffered neighbor
searching
ns_type                  = grid      ; search neighboring grid
cells
Nstlist                  = 10        ;20 fs, largely irrelevant with
Verlet scheme
Rcoulomb                 = 1.0       ; short-range electrostatic
cutoff (in nm)
Rvdw                     = 1.0       ; short-range van der Waals
cutoff (in nm)
; Electrostatics
Coulombtype    = PME                 ; Particle Mesh
Ewald for long-range electrostatics
pme_order        = 4
      ; cubic interpolation
Fourierspacing = 0.12                ; grid spacing for
FFT
; Temperature coupling is on
tcoupl                   = V-rescale             ; modified
Berendsen thermostat
tc-grps                  = Protein Non-Protein ; two coupling
groups - more accurate
tau_t                    = 0.1 0.1               ; time constant,
in ps
ref_t                    = 300 300               ; reference
temperature, one for each group, in K
; Pressure coupling is on
pcoupl                   = Parrinello-Rahman  ; Pressure
coupling on in NPT
Pcoupltype         = isotropic             ; uniform scaling
of box vectors
tau_p                    = 2.0                   ; time constant,
in ps
ref_p                    = 1.0                   ; reference
pressure, in bar
compressibility    = 4.5e-5                ; isothermal
compressibility of water, bar^-1
; Periodic boundary conditions
pbc                      = xyz                   ; 3-D PBC
; Dispersion correction
DispCorr                 = EnerPres              ; account for cut-
off vdW scheme
; Velocity generation
gen_vel                  = no                    ; Velocity
generation is off
```

MD production analysis I

- Check energy evolution

echo '13 0' | gmx energy -f ${pdbr}_md.edr -o ${pdbr}_ener_md.xvg

View graph

xmgrace ${pdbr}_ener_md.xvg

- Check temperature evolution

echo '15 0' | gmx energy -f ${pdbr}_md.edr -o ${pdbr}_temp_md.xvg

- View graph

xmgrace ${pdbr}_temp_md.xvg

- Check pressure evolution

echo '17 0' | gmx energy -f ${pdbr}_md.edr -o ${pdbr}_press_md.xvg

- View graph

xmgrace ${pdbr}_press_md.xvg

- Remove waters and ions from trajectory

echo '1 1'| gmx trjconv -pbc mol -center -s ${pdbr}_md.tpr -f ${pdbr}_md.xtc -o ${pdbr}_md_nw.xtc

- Generate parameters file without water and ions

echo '1' | gmx convert-tpr -s ${pdbr}_md.tpr -o ${pdbr}_md_nw.tpr

- Extract first frame from production to .gro

echo '0'|gmx trjconv -f ${pdbr}_md_nw.xtc -s ${pdbr}_md_nw.tpr -o ${pdbr}_md_nw.gro -dump 0

- View trajectory

vmd ${pdbr}_md_nw.gro -xtc ${pdbr}_md_nw.xtc

MD production analysis II

- Analyze fluctuations by residue
  echo 1| gmx rmsf -s ${pdbr}_md_nw.tpr -f ${pdbr}_md_nw.xtc -o ${pdbr}-rmsf-md.xvg -ox ${pdbr}-md-avg.pdb -res -oq ${pdbr}-md-bfact.pdb
- View graph
  xmgrace ${pdbr}-rmsf-md.xvg
- View PDB
  pymol ${pdbr}-md-bfact.pdb
  Action → Preset → b factor putty
- Analyze RMSD
  echo '4 4' |gmx rms -s ${pdbr}_md_nw.tpr -f ${pdbr}_md_nw.xtc -o ${pdbr}-bkbone-rmsd-md.xvg
- View graph
  xmgrace ${pdbr}-bkbone-rmsd-md.xvg

Compare with real cdk2 structure

- Set variables:

  realdir=/home/usuario/3qqg-md

  pdbr=3qqg
- Make example directory:

  cd

  mkdir $realdir
- Go to example files directory:

  cd $realdir
- Get files:

  wget ub.cbm.uam.es/members/dabia/3qqg-md.tgz
- Uncompress files:

  tar -xzf 3qqg-md.tgz