
Project Parameters

Periodic Boundary Conditions: Truncated octahedral box, min distance 12Å (solvateoct mol TIP3PBOX 12.0)
 Force Field: Amber f99 + bsc0 + XOL3 (source leaprc.RNA.OL3)
 Water Model: TIP3P (source leaprc.water.tip3p)
 Neutralize RNA (addions mol Na+ 0)
 Add 1 M NaCl (addionsrand mol Na+ X Cl- X)

Thermostat: Langevin 310K, timestep 2 ps, collision freq 1 ps⁻¹ (temp=310; ntt=3 gamma_ln=1.0)
 Barostat: Monte Carlo, 100 step attempt frequency (ntp=1; barostat=2)
 Hydrogen Bond Constraints: SHAKE (ntc=2; ntf=2)
 Restraints: Keep as standard release through equilibration (restraintmask=' :1-12')
 Simulation time: Stepwise minimization and equilibration

Download or create structure

- Folded – AA sequence to AlphaFold server for hairpin structure, randomly choose
- Unfolded – Build AA sequence in PyMol, drag to linear form

Add Hydrogens

- Chimera → Structure editing → Add H
- tleap pdb4amber -i pdbin.pdb -o pdbout.pdb --reduce -dry

tleap system setup

- Calculate required ions with <https://www.phys.ksu.edu/personal/schmit/SLTCAP/SLTCAP.html>

```
# Template RNA Hairpin FOLDED PREP
# MUST REMOVE TERMINAL PHOSPHATES
# Run with tleap -s -f leap.in on CL

source leaprc.RNA.OL3
source leaprc.water.tip3p
mol = loadpdb in.pdb
addions mol Na+ 0
**solvateoct mol TIP3PBOX 12.0
addionsrand mol Na+ 000 Cl- 000**
savepdb mol out.pdb
saveamberparm mol out.prmtop out.inpcrd
saveamberparm mol out.prmtop out.rst7
check mol
quit
```

Initial minimization (min1)

- Let solvent relax around restrained solute
- 1000 steps (500 steepest descent/500 conjugate gradient)
- 500 kcal/mol-Å² restraints (solute)

```
min1 minimization - solvent relaxation, solute restraints
&cntrl
  imin=1,                ! Energy minimization
  irest=0,               ! New simulation - velocities ignored, timestep set to 0
  ntx=1,                 ! Coordinates read from inpcrd file
  maxcyc=1000,           ! 1000 minimization cycles/steps (500 steepest descent, 500 conjugate gradient)
  ncyc=500,              ! Switch from steepest descent to conjugate gradient after 500 cycles
  ntr=1,                 ! Cartesian space restraints with harmonic potential
  restraint_wt=500.0,     ! Positional restraint weight (kcal mol-1-Å2)
  restraintmask=':1-12', ! Restraint mask
  cut=10.0,              ! Non-bonded interaction cutoff
  ntp=100,               ! Energy information printed to mdout and minfo every 100 steps
  ntwx=0,                ! No coordinates written to trajectory file
/
```

Second minimization (min2)

- Let everything relax
- 2500 steps (1000 steepest descents/1500 conjugate gradient)

```
min2 minimization - relaxation
&cntrl
  imin=1,                ! Energy minimization
  irest=0,               ! New simulation
  ntx=1,                 ! Coordinates read from inpcrd file
  maxcyc=2500,           ! 2500 minimization cycles/steps (1000 steepest descent, 1500 conjugate gradient)
  ncyc=1000,             ! Switch from steepest descent to conjugate gradient after 1000 cycles
  cut=10.0,              ! Non-bonded interaction cutoff
  ntp=100,               ! Energy information printed to mdout and mdinfo every 100 steps
  ntwx=0,                ! No coordinates written to trajectory file
/
```

Defrost (md1)

- Begin constant volume to warm to proper temperature with restrained solute
- 100 ps NVT
- Langevin temperature control 0 → 310K
- 25 kcal/mol-Å² restraints (solute)

```
md1 defrost 0 to 310K with restraints on RNA 100ps NVT
&cntrl
  imin=0,                ! No minimization
  irest=0, ntx=1,        ! No restart, only coordinates
  ntb=1,                 ! Periodic boundaries constant volume (NVT)
  ntr=1, restraint_wt=25.0, restraintmask=':1-12', ! Solute restraints
  cut=10.0,              ! Non-bonded interaction cutoff
  ntc=2, ntf=2,          ! ntc=2 Hydrogen bond constraints SHAKE for TIP3P, omitted
  ntt=3, gamma_ln=1.0,   ! Langevin thermostat, collision freq. 1.0-1ps
  tempi=0, temp0=310,     ! Initial temp 0K, reference temp 310K
  nstlim=50000, dt=0.002, ! Timestep 0.002 ps, 50000 steps (100ps)
  ntp=100,               ! Energy printed to mdout/mdinfo every 100 steps
  ntwx=100,              ! Coordinates written to trajectory every 100 steps
  ntwr=1000,             ! Restart written every 1000 steps
/
```

Equilibration (md2)

- Constant pressure to get proper density, gradually release restraints on solute.
- 5-stage release with strong restraints for first 50ps while density is most rapidly changing
- 250 ps NPT
- Langevin temperature control 310K
- “Weak-coupling” pressure control 1.0bar (~1 atm)
- 25 → 5 kcal/mol-Å² restraints(solute)
 1. md2a 25 kcal 50ps
 2. md2b 20 kcal 50ps
 3. md2c 15 kcal 50ps
 4. md2d 10 kcal 50ps
 5. md2e 5 kcal 50ps

```
md2a-e minimization restraint 25kcal 50ps 310K NPT
&cntrl
  imin=0,                ! No minimization
  irest=1, ntx=5,        ! Restart with coord and velocities
  ntb=2,                 ! Periodic boundaries constant pressure (NPT)
  ntp=1, barostat=2,     ! Isotropic position scaling, Monte Carlo barostat
  taup=1.0, pres0=1.0,   ! Pressure relaxation time 1.0, reference pressure 1 bar
  ntc=2, ntf=2,          ! ntc=2 Hydrogen bond constraints SHAKE for TIP3P, omitted
  cut=10.0,              ! Non-bonded interaction cutoff
  ntr=1, restraint_wt=25.0, restraintmask=':1-12', ! Solute restraints
  ntt=3, gamma_ln=1.0,   ! Langevin thermostat, collision freq. 1.0-1ps
  tempi=310.0, temp0=310.0, ! Initial temp 310K, reference temp 310K
  nstlim=25000, dt=0.002, ! Timestep 0.002 ps, 25000 steps (50ps)
  ntp=100, ntwx=1000, ntwr=1000, ! Energy printed/100 steps, coord/100 steps, restart/1000 steps
/
```

Equilibration (md3)

- Release solute restraints and isotropically scale box size to reflect average volume
- 200 ps NPT
- Langevin temperature control 310K
- “Weak-coupling” pressure control 1.0bar (~1.0atm)

```
md3 equilibration 200ps 310K NPT
&cntrl
  imin=0,                ! No minimization
  irest=1, ntx=5,        ! Restart with coord and velocities
  ntb=2,                ! Periodic boundaries constant pressure (NPT)
  ntp=1, barostat=2,     ! Isotropic position scaling, Monte Carlo barostat
  taup=1.0, pres0=1.0,   ! Pressure relaxation time 1.0, reference pressure 1 bar
  cut=10.0,             ! Non-bonded interaction cutoff
  ntc=2, ntf=2,         ! ntc=2 Hydrogen bond constraints SHAKE for TIP3P, omitted
  ntt=3, gamma_ln=1.0,  ! Langevin thermostat, collision freq. 1.0-1ps
  tempi=310.0, temp0=310.0, ! Initial temp 310K, reference temp 310K
  nttx=1,               ! Formatted restart file
  nstlim=100000, dt=0.002, ! Timestep 0.002 ps, 100000 steps (200ps)
  ntp=100, ntwx=1000, ntwr=1000, ! Energy printed/100 steps, coord/100 steps, restart/1000 steps
/
```

Calculate new volume and replace x,y,z in restart file

- New flag: nttx=1

```
postmd3_calcbboxlength.py
1. Box length from first three columns of last line in md3.rst
2. Average volume from md3.mdout
3. Copy md3.rst to md3_NewVolume.rst, replace first three columns of last line with new box length
```

Equilibration (md4)

- Switch to constant volume and equilibrate with scaled box size
- 1 ns NVT
- Remove nttx=1 flag

```
md4 equilibration 1ns 310K NVT
&cntrl
  imin=0,                ! No minimization
  irest=1, ntx=5,        ! Restart with coord and velocities
  ntb=1,                ! Periodic boundaries constant volume (NVT)
  cut=10.0,             ! Non-bonded interaction cutoff
  ntc=2, ntf=2,         ! ntc=2 Hydrogen bond constraints SHAKE for TIP3P, omitted
  ntt=3, gamma_ln=1.0,  ! Langevin thermostat, collision freq. 1.0-1ps
  tempi=310.0, temp0=310.0, ! Initial temp 310K, reference temp 310K
  nstlim=500000, dt=0.002, ! Timestep 0.002 ps, 500000 steps (1000ps)
  ntp=500, ntwx=1000, ntwr=100000, ! Energy printed/500 steps, coord/1000 steps, restart/100000 steps
/
```

Production run: keep the same conditions as the equilibration run

- 1 microsecond
- Copy over md4.rst to production folder, rename as md.rst
- Run production until system fully equilibrates

```
Production run NVT
&cntrl
  imin=0,                ! No minimization
  irest=1, ntx=5,        ! Restart MD with coordinates
  ntb=1,                ! Periodic boundaries, constant volume
  ig=-1,                ! Random seed
  cut=10.0,             ! Non-bonded interactions cutoff 10Å
  ntc=2, ntf=2,         ! ntc=2 Hydrogen bond constraints SHAKE for TIP3P
  ntt=3, gamma_ln=1.0,  ! Langevin thermostat, collision freq. 1.0-1ps
  tempi=310.0, temp0=310.0, ! Initial temp 310K, reference temp 310K
  nstlim=50000000, dt=0.002, ! 50,000,000 steps, 0.002 ps (2 fs) timestep, total time = 100 ns
  ntp=1000 ntwx=500, ntwr=10000, ! Energy printed to mdinfo mdout/1000 steps, coord written/500 steps, rst written/10000 steps
/
```
