
Project Parameters — AMBER RNA.OL3 + TIP3P

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 0.1 M NaCl (addionsrand mol Na+ X Cl- X)

Thermostat: Langevin 300K, timestep 2 ps, collison freq 1 ps-1 (temp=300; 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

Project Parameters — DE Shaw RNA + TIP4P-D + CHARMM22

Periodic Boundary Conditions: Truncated octahedreal box, min distance 12 A (solvateOct mol TIP4PDBOX 12.0)
Force Field: DE Shaw RNA (source leaprc.RNA.Shaw)
Water Model: TIP4P-D (source leaprc.water.tip4pd)
Neutralize RNA (addions mol Na+ 0)
Add 0.1 M NaCl (addionsrand mol Na+ X Cl- X)

Thermostat: Langevin 300K, timestep 2 ps, collison freq 1 ps-1 (temp=300; 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=' :1-13')
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

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tleap system setup

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

```
# Template RNA Hairpin FOLDED PREP
# pdb4amber -i 1hs3.pdb -o 1hs3_pdb4amber.pdb --reduce –dry
# 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
```

```
source leaprc.RNA.Shaw
source leaprc.water.tip4pd
mol = loadpdb 1hs3_pdb4amber.pdb
addions mol Na+ 0
solvateOct mol TIP4PDBOX 12.0
addionsrand mol Na+ 000 Cl- 000
check mol
savepdb mol 1hs3folded_shawFinal.pdb
saveamberparm mol 1hs3_shaw.prmtop 1hs3_shaw.inpcrd
saveamberparm mol 1hs3_shaw.prmtop 1hs3_shaw.rst7
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=':1-13',           ! Restraint mask
  cut=10.0,                        ! Non-bonded interaction cutoff
  ntpr=100,                        ! Energy information printed to mdout and mdinfo 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
  ntpr=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 → 300K
- 25 kcal/mol^{-1}–Å^{2} restraints (solute)

```
md1 defrost 0 to 300K 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=':1-13', ! 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=300,                              ! Initial temp 0K, reference temp 300K
  nstlim=50000, dt=0.002,                          ! Timestep 0.002 ps, 50000 steps (100ps)
  ntpr=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 reslease restraints on solute.
- 5-stage release with strong restraints for first 50ps while density is most rapidly changing
- 250 ps NPT
- Langevin temperature control 300K
- “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 300K 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=':1-13',     ! Solute restraints
  ntt=3, gamma_ln=1.0,                                 ! Langevin thermostat, collision freq. 1.0-1ps
  tempi=300.0, temp0=300.0,                            ! Initial temp 300K, reference temp 300K
  nstlim=25000, dt=0.002,                              ! Timestep 0.002 ps, 25000 steps (50ps)
  ntpr=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 300K
- “Weak-coupling” pressure control 1.0bar (~1.0atm)

```
md3 equilibration 200ps 300K 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=300.0, temp0=300.0,                    ! Initial temp 300K, reference temp 300K
  ntxo=1,                                      ! Formatted restart file
  nstlim=100000, dt=0.002,                     ! Timestep 0.002 ps, 100000 steps (200ps)
  ntpr=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: ntxo=1

```
postmd3_calcboxlength.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 ntxo=1 flag

```
md4 equilibration 1ns 300K 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=300.0, temp0=300.0,                  ! Initial temp 300K, reference temp 300K
  nstlim=500000, dt=0.002,                   ! Timestep 0.002 ps, 500000 steps (1000ps)
  ntpr=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=300.0, temp0=300.0,        ! Initial temp 300K, reference temp 300K
  nstlim=50000000, dt=0.002,       ! 50,000,000 steps, 0.002 ps (2 fs) timestep, total time = 100 ns
  ntpr=1000 ntwx=500, ntwr=10000,  ! Energy printed to mdinfo mdout/1000 steps, coord written/500 steps, rst written/10000 steps
 /
```
