MD Study of a tripeptide (asa)

Nicolás Rojo Esteban

1 Introduction

Molecular dynamics (MD) is a computational technique that models the time-dependent behavior of atomic and molecular systems by numerically integrating Newton's equations of motion. Given an initial configuration and a potential energy function (force field), MD generates trajectories that describe how each atom moves under the influence of bonded and nonbonded interactions. By sampling these trajectories, one can compute thermodynamic and kinetic properties.

In this work we apply MD to a small tripeptide, ACE–Ala–Ser–Ala–NME (ASA),which serves as a minimal model to investigate backbone and side-chain dynamics in aqueous solution. The peptide is acetylated at the N-terminus (ACE) and amidated at the C-terminus (NME) to mimic the electronic environment of a longer polypeptide chain.

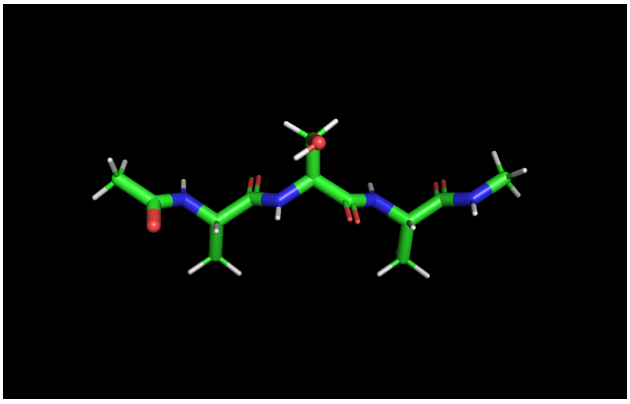

Figure 1: Structure of the tripeptide (asa) shown as licorice

2 Computational Details

All molecular dynamics simulations were carried out using GROMACS 2023 with the CHARMM27 all-atom force field and the TIP3P water model. After topology generation and solvation, two successive NVT runs were performed: a equilibration (4ps) and a production run (2ps). The full contents of the parameter files are given below.

Equilibration parameters (`equiNVT.mdp`)

```
; 4 ps NVT equilibration at 298 K
integrator       = md
dt               = 0.0005      ; 0.5 fs
nsteps           = 8000        ; 4 ps total
define           = -DFLEXIBLE -DCHARMM_TIP3P
constraints      = none
comm-mode        = Linear
nstcomm          = 1000        ; remove COM every 0.5 ps
```

```
nstxout          = 200         ; coordinates every 0.1 ps
nstvout          = 200         ; velocities every 0.1 ps
nstfout          = 0

Tcoupl           = v-rescale
tc-grps          = System
tau_t            = 0.1
ref_t            = 298

gen_vel          = yes
gen_temp         = 298.0
gen_seed         = 12345678
```

Production parameters (runNVT.mdp)

```
; 2 ps NVT production at 298 K
integrator       = md
dt               = 0.0005      ; 0.5 fs
nsteps           = 4000        ; 2 ps total
define           = -DFLEXIBLE -DCHARMM_TIP3P
constraints      = none
comm-mode        = Linear
nstcomm          = 1000        ; remove COM every 0.5 ps

nstxout          = 2           ; coordinates every 1 fs
nstvout          = 2           ; velocities every 1 fs
nstfout          = 0

Tcoupl           = v-rescale
tc-grps          = System
tau_t            = 0.1
ref_t            = 298

gen_vel          = no
```

3 Results

System Preparation

1. **Obtain the ASA PDB and generate the topology.** We start from the ASA structure file asa.pdb downloaded from the Protein Data Bank, which provides the 3D coordinates of the peptide already which already has ACE and CT3 terminals. Using GROMACS we convert this PDB into a structure (asa.gro) and topology (asa.top), selecting the CHARMM27 force field and the TIP3P water model.

```
gmx pdb2gmx -f asa.pdb -o asa.gro -p asa.top -ter
```

This produces asa.gro and asa.top, which contain all atomic coordinates, charges, bonds, angles, and dihedrals needed to evaluate the potential energy.

2. **Define a cubic unit cell.** To ensure there is solvent around the peptide, we center the structure and create a cubic box of side length 3.0nm in each dimension:

```
gmx editconf -f asa.gro -o asa_box.gro -bt cubic -box 3.0 3.0 3.0
```

The output file asa_box.gro positions the peptide at the center of a 3 nm cube.

3. **Solvate the system.** Finally, we fill the box with TIP3P water, automatically updating the topology file with the added solvent molecules:

```
gmx solvate -cp asa_box.gro -cs -o asa_solv.gro -p asa.top
```

The resulting file asa_solv.gro contains the peptide together with the surrounding water molecules as shown in Figure 2.

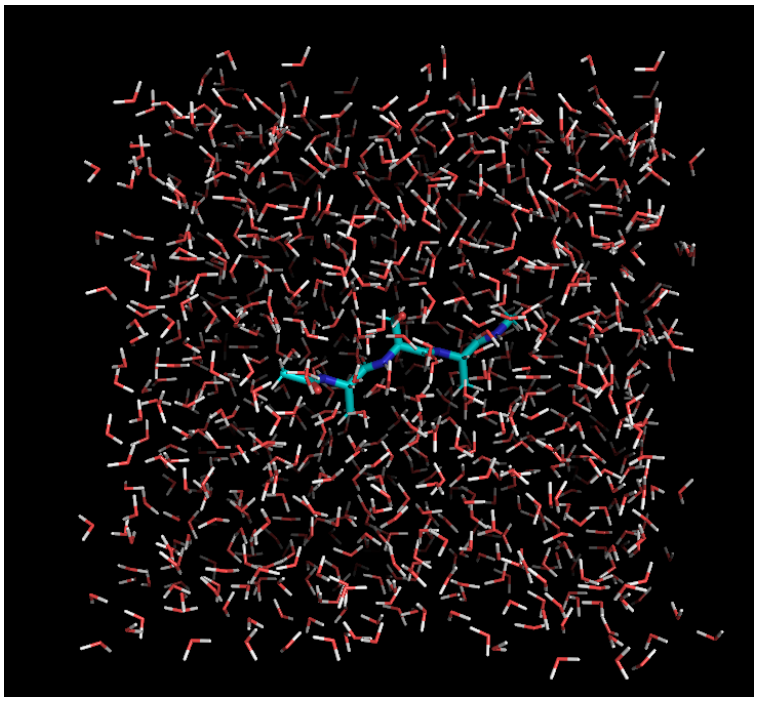

Figure 2: Structure of the tripeptide (asa) solvated with water molecules.

Equilibration

1. **Check system neutrality.** We preprocess the NVT equilibration input using the solvated structure and its topology:

   ```
   gmx grompp -f equiNVT.mdp -c asa_solv.gro -p asa.top -o asa.tpr -maxwarn 1
   ```

   The output indicates the system is neutral, so no ion addition is required.

   The file equiNVT.mdp defines all parameters for a 4ps NVT equilibration at 298K. It is organized in three main sections:

2. **Run NVT equilibration.** We perform a short constant-volume, constant-temperature run to equilibrate the solvent around the peptide. The final coordinates and velocities are written to asa.g96:

   ```
   gmx mdrun -deffnm asa -c asa.g96 -ntomp 24
   ```

   Here, -deffnm asa is used to make all output files (log, edr, etc.) share the name asa, and -c asa.g96 writes the equilibrated positions and velocities to asa.g96.

Production Run

1. **Generate the input file for production MD.** We take the equilibrated coordinates and velocities from asa.g96 along with the topology, and preprocess using the file `runNVT.mdp`, which defines all parameters for a short production NVT run, in this case 4000 steps of 0.5 fs suming a total of 2 ps of simulation

   ```
   gmx grompp -f runNVT.mdp -c asa.g96 -p asa.top -o asa.tpr
   ```

2. **Execute the production molecular dynamics.** With the tpr just created, we launch the full production run, writing the final coordinates and velocities again to asa.g96 and naming all output files with asa:

   ```
   gmx mdrun -deffnm asa -c asa.g96 -ntomp 24
   ```

Trajectory Analysis

After the production run, we extracted the time evolution of one bond length, one bond angle, and the ϕ/ψ dihedrals for Serine residue 3 as follows:

1. **Side-chain bond length measurement.** Using the index file `distances.ndx` (defining the Cα–Cβ pair of Ser 3), we ran:

```
gmx distance -f asa.trr -s asa.tpr -n distances.ndx -o ser3_ca_cb.xvg
-xvg none
```

The output `ser3_ca_cb.xvg` contains the Cα–Cβ distance of Ser 3 at each frame.

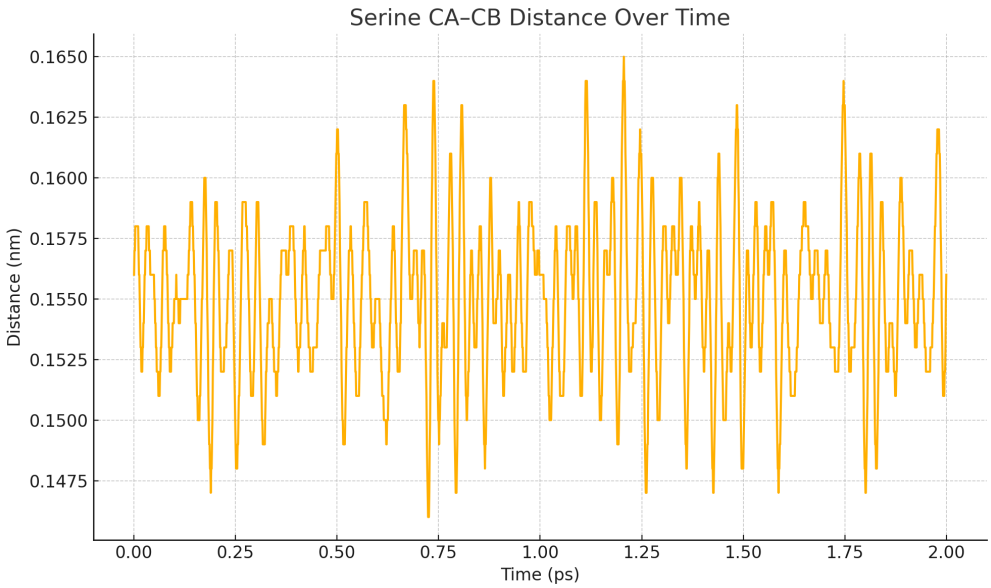

Figure 3: Time evolution of the Cα–Cβ distance in Serine 3 over the production run.

As shown in Figure 3, the Cα–Cβ bond distance oscillates around its equilibrium value of 0.155 nm, which corresponds to a typical carbon–carbon single bond length, it oscillates as a harmonic oscillator.

2. **Side-chain bond angle measurement.** With an index file `angles.ndx` defining the three atoms Cα(Ser 3)–Cβ(Ser 3)–Oγ(Ser 3), we executed:

```
gmx angle -f asa.trr -s asa.tpr -n angles.ndx -ov ser3_sc_angle.xvg
-xvg none
```

We selected group 0 and pressed Enter. The file `ser3_sc_angle.xvg` records the Cα–Cβ–Oγ angle (orientation of the hydroxyl) over time.

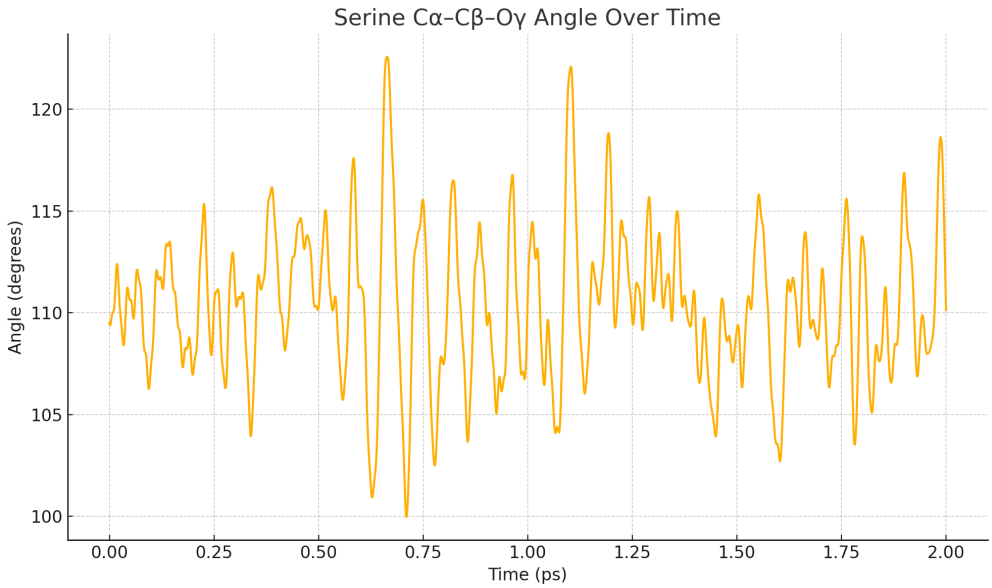

Figure 4: Time evolution of the C_α–C_β–O_γ angle in Serine 3.

As shown in Figure 4, the C_α–C_β–O_γ angle oscillates between approximately 100° and over 120°. This range of motion reflects the bending of the serine hydroxyl group relative to the backbone, illustrating the side-chain's flexibility in aqueous solution.

3. **Backbone dihedral (ϕ/ψ) calculation.** We used the Ramachandran analysis tool to extract ϕ and ψ angles for residue 3:

```
gmx rama -f asa.trr -s asa.tpr -xvg none
grep SER-3 rama.xvg | awk '{print $1}' | cat -n > phi_ser3.dat
grep SER-3 rama.xvg | awk '{print $2}' | cat -n > psi_ser3.dat
```

The files `phi_ser3.dat` and `psi_ser3.dat` contain the time series of the ϕ and ψ dihedral angles for Ser 3, respectively.

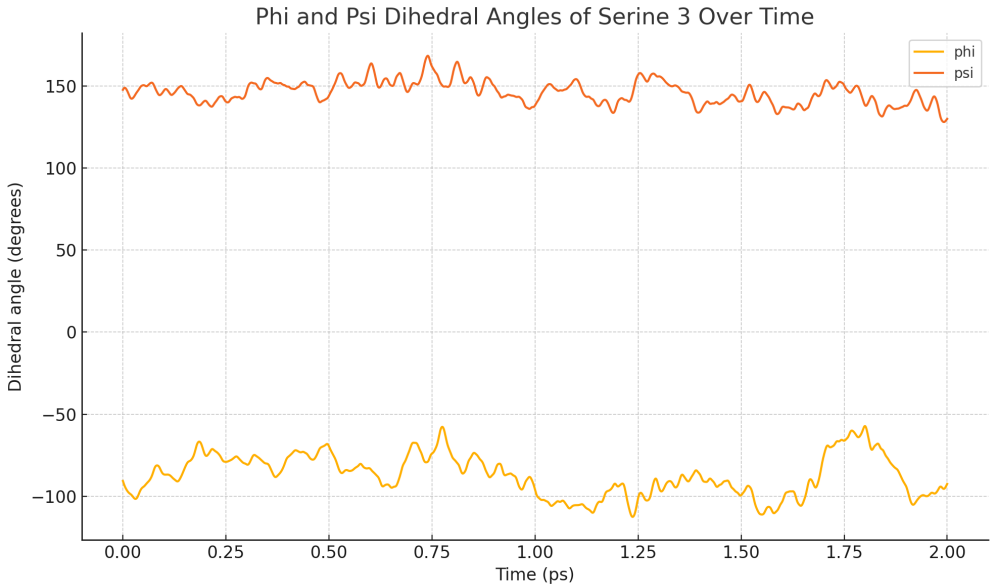

Figure 5: Time evolution of the backbone dihedral angles ϕ (yellow) and ψ (orange) for Serine 3.

Figure 5 shows the evolution of the backbone dihedral angles in Serine 3 over the course of the simulation. The ϕ angle remains centered near $-90°$ and the ψ angle near $+145°$, with both exhibiting fluctuations of approximately $\pm 15°$. The observed oscillations represent intrinsic thermal fluctuations of the peptide backbone at physiological temperature.

4. **Temperature and energy calculation.**

   To monitor the system temperature and total potential energy over the production run, we used the following GROMACS commands:

```
gmx traj -f asa.trr -s asa.tpr -xvg none -ot
```

   At the "Select a group:" prompt we entered `0` (System), yielding `temp.xvg`, which contains the temperature (K) at each saved frame.

```
gmx energy -f asa.edr -s asa.tpr -xvg none -o energy.xvg
```

   After choosing "Total Energy" from the list, `energy.xvg` was produced, giving the system's total energy (kJ/mol) as a function of time.

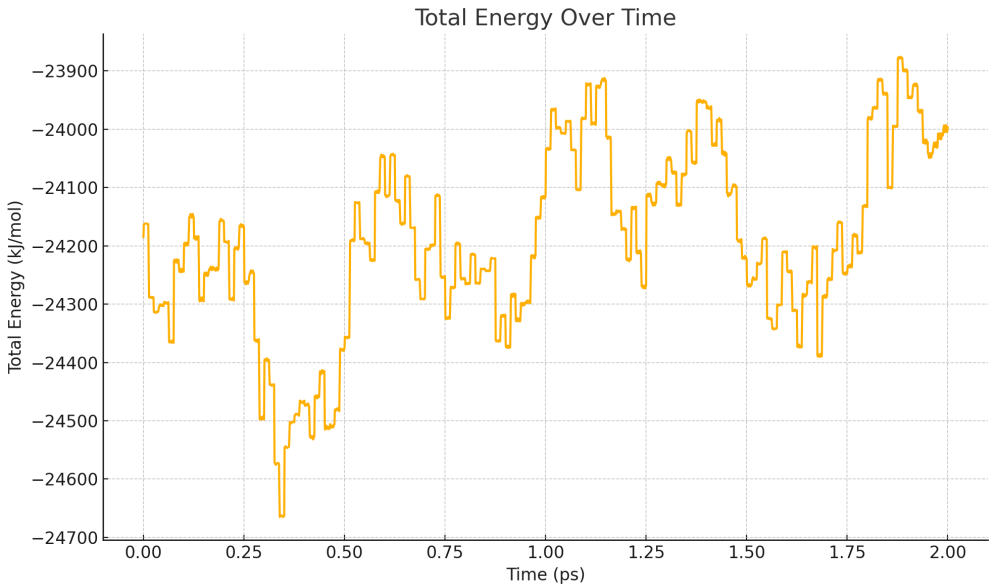

Figure 6: Total energy of the solvated peptide as a function of simulation time.

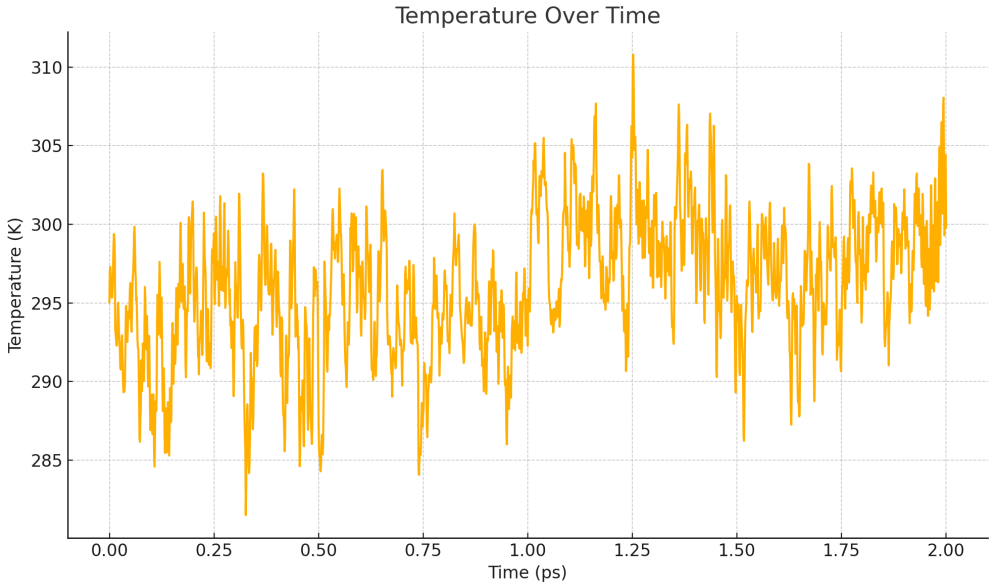

Figure 7: Temperature of the system as a function of simulation time.

Figure 6 shows that the total potential energy fluctuates between approximately -2.47×10^4 and $-2.39\times 10^4\,\mathrm{kJ/mol}$, with an average near $-2.43\times 10^4\,\mathrm{kJ/mol}$ and no systematic drift, indicating that the system has reached a stable energetic equilibrium. Figure 7 demonstrates that the temperature remains tightly controlled around 298 K, oscillating by roughly $\pm 10\,\mathrm{K}$, confirming effective thermostat coupling and maintenance of physiological conditions throughout the run.

4 Conclusions

I performed MD simulations of ACE–Ala–Ser–Ala–NME in TIP3P water with the CHARMM27 force field. The Ser3 C_α–C_β bond stayed at about 0.155nm. The C_α–C_β–O_γ angle varied between 100° and 120°. The backbone dihedrals ϕ and ψ remained near –90° and +145° (±15°). The total energy was around -2.43×10^4kJ/mol and the temperature around 298K (±10K). These results indicate the system was well equilibrated and stable.