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Molecular Dynamics Investigation of Alanine: Enthalpic Control of Dihedral Free Energy

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Abstract: This project investigates the molecular dynamics of the amino acid alanine and examines the relationship between Gibbs free energy, dihedral angles, and bond lengths. The primary objective is to determine whether entropy or enthalpy plays a more significant role in governing the free energy landscape. Our findings indicate that the magnitude of free energy exhibits minimal variation with temperature, suggesting that the torsional barrier is predominantly enthalpic in origin.

Keywords: Molecular dynamics (MD); Alanine dihedral analysis; Free energy landscapes; Enthalpy vs. entropy CP2K force field; AmberTools Simulations.

1. Introduction

Molecular simulation is a powerful computational tool used to validate theoretical models and interpret experimental observations. Various simulation methods exist, each operating on different spatial and temporal scales, ranging from the atomic level at ~0.1 nm or femtoseconds (fs) to macroscopic phenomena on experimental timescales. At the smallest scales, quantum mechanical (QM) methods, such as semi-empirical and *ab initio* approaches, are employed to simulate electronic structures and processes involving bond formation and cleavage.

At a larger scale, atomistic simulation techniques, such as molecular dynamics (MD)—the method adopted in this project—are widely applied. MD[1][2][3] typically operates at spatial scales from 1 nm to 0.1 μm and temporal scales from 1 ps to 1 μs. It is extensively used across academic disciplines: in physical chemistry, MD enables the investigation of molecular properties, modeling the fine-grained motions of atoms under force fields to study mechanical properties such as elasticity and tensile strength; in biological sciences, it allows researchers to analyze the structure and dynamics of plasma membranes, including properties like fluidity and permeability. For example, phospholipid bilayers can be simulated to observe phenomena such as tail conformational changes and membrane protein diffusion.

The MD simulations used in this study are based on the Verlet integration algorithm, which relies on Newton's equations of motion to compute atomic displacements and velocities at each timestep. The Verlet algorithm is widely adopted because of its excellent energy conservation and high numerical accuracy.

For phenomena involving larger spatial and temporal scales, mesoscale methods—such as Lattice Monte Carlo, Brownian Dynamics, and Dissipative Particle Dynamics—are applied. These techniques balance computational efficiency with physical fidelity by incorporating microstructural details while avoiding the prohibitive cost of fully atomistic calculations.

Finally, at the macroscopic level, continuum methods grounded in classical Newtonian mechanics are used to model large-scale systems, such as the stress distribution within a metal block or the aerodynamics of a car.

The purpose of this research is to identify the most stable dihedral angles of alanine and investigate their relationship to molecular free energy using molecular dynamics simulations. By tracking the temporal evolution of specific dihedral angles, we generate histograms representing their probability distributions. Higher probabilities correspond to more stable, energetically favorable conformations. Using the equation

$$F(\theta) = -k_{\mathrm{B}} T \ln P(\theta) + C,$$

where $F(\theta)$ is the free energy, $P(\theta)$ is the probability of observing angle θ, and k_B is the Boltzmann constant, we determine the free energy profiles. These results allow us to quantify the variation in free energy as a function of dihedral angle.

2. Theory

2.1. Operating Principle of Molecular Dynamics

The process of molecular dynamics (MD) begins by defining the interaction potential and the molecular topology, which describes how atoms are connected within a molecule. Next, the initial positions and velocities of all atoms are specified, typically based on experimental structures or theoretical models. Using these initial conditions, interatomic forces—including van der Waals interactions, electrostatic forces, and bonded terms—are calculated.

According to Newton's equations of motion, these forces are then used to update the positions and velocities of the atoms over discrete time steps. Constraints or corrections may be applied when necessary to maintain molecular stability or enforce specific conformations. After each time step, system properties such as total energy, temperature, and pressure are computed, and the resulting configurations are saved for further analysis. This cycle is repeated iteratively to simulate the temporal evolution of the molecular system.

2.2. Verlet Algorithm

The Verlet algorithm is widely used in molecular dynamics to integrate Newton's equations of motion. It updates atomic positions based on their current and previous positions as well as the accelerations derived from interatomic forces.

The basic position update formula is:

$$r_i(t + \Delta t) = 2r_i(t) - r_i(t - \Delta t) + a_i(t)\Delta t^2$$

Derivation Using Taylor Expansion:

$$r_i(t + \Delta t) = r_i(t) + v_i(t)\Delta t + \frac{1}{2} a_i(t)\Delta t^2 + (1/6)b_i(t)\Delta t^3 + O(\Delta t^4)$$

$$r_i(t - \Delta t) = r_i(t) - v_i(t)\Delta t + \frac{1}{2} a_i(t)\Delta t^2 - (1/6)b_i(t)\Delta t^3 + O(\Delta t^4)$$

Adding the two expansions gives:

$$r_i(t + \Delta t) + r_i(t - \Delta t) = 2r_i(t) + a_i(t)\Delta t^2 + O(\Delta t^4)$$

Rearranging yields the Verlet formula:

$$r_i(t + \Delta t) = 2r_i(t) - r_i(t - \Delta t) + a_i(t)\Delta t^2$$

Velocity Approximation:

$$v_i(t) = [r_i(t + \Delta t) - r_i(t - \Delta t)] / (2\Delta t) + O(\Delta t^2)$$

This approximation is sufficient for computing thermodynamic properties such as temperature and kinetic energy.

2.3. Temperature Control

Temperature in MD simulations is closely linked to the kinetic energy of the system. The average kinetic energy per particle is:

$$\langle K \rangle = (3/2)Nk_B{*}T$$

Because kinetic energy is related to atomic velocity via $K = \frac{1}{2}mv^2$, temperature is proportional to the mean squared velocity:

$$T \propto \langle v^2 \rangle$$

To regulate the system temperature to a desired target value T_target, velocity rescaling is often applied:

$$v' = v \times \sqrt{T_{target} / T_{current}}$$

This approach adjusts the system's kinetic energy without disturbing the relative velocity distribution among particles. It is especially useful during the equilibration phase of simulations to reach the desired thermodynamic conditions. However, simple velocity rescaling suppresses natural fluctuations and can affect energy conservation. Therefore, in production runs, advanced thermostats such as Nosé–Hoover or Langevin dynamics are commonly used for more accurate sampling.

2.4. Gibbs Free Energy and Statistical Mechanics

The Gibbs free energy (G) describes the amount of energy available to perform useful work at constant temperature and pressure:

$$G = H - T{*}S$$

The change in Gibbs free energy (ΔG) determines the spontaneity of a process:

- $\Delta G < 0$: spontaneous process
- $\Delta G > 0$: non-spontaneous process
- $\Delta G = 0$: system at equilibrium

In statistical mechanics, Gibbs free energy connects to the Boltzmann distribution, relating the probability P of occupying a microstate of energy E at temperature T:

$$P \propto \exp(-E / (k_B{*}T))$$

Summing over all states leads to the partition function Q, from which the Gibbs free energy is derived:

$$G = -k_B{*}T{*}\ln(Q)$$

Thus, minimizing G corresponds to favoring the most probable molecular configurations, linking thermodynamics to the stability of dihedral conformations.

3. Force Field and Simulation Details

We used the CP2K-derived molecular dynamics force field [4][5] within AmberTools to simulate alanine. Each simulation timestep was set to 1 femtosecond, and we performed production runs for 500 picoseconds. Two independent simulations were carried out at 298 K and 500 K to assess temperature effects.

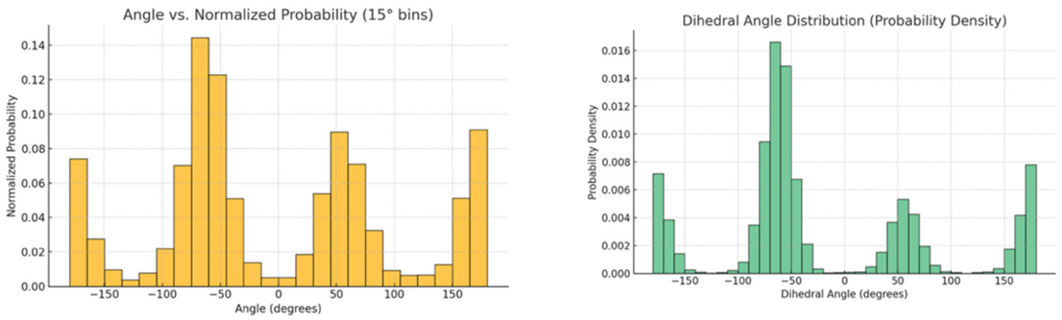
The force field is derived from the gradient of the potential energy:

$$F = -dE/dr$$

Simulations were performed under NVT ensemble conditions (constant number of particles, volume, and temperature). Prior to production, energy minimization was conducted to relax the system and remove unfavorable steric interactions.

4. Results

In this study, we investigated the relationship between dihedral angles, free energy, and temperature for alanine using molecular dynamics simulations. To analyze the torsional motion, we computed histograms of the dihedral angle distributions at two different temperatures: 298 K and 500 K that are shown in Figure 1.



500k

298k

Figure 1. Probabilities at different temperatures

The histograms above illustrate the probability density of specific dihedral angles across the simulation trajectory. Peaks in these distributions correspond to energetically favorable conformations, where the system spends most of its simulation time. At both temperatures, the locations of these peaks are highly consistent, indicating that the preferred conformations of alanine are stable and temperature-independent within the investigated range.

Using the probability distribution $P(\theta)$ for each dihedral angle θ, we calculated the free energy profile $F(\theta)$ using the

Boltzmann relation: $F(\theta) = -k\_B T \ln P(\theta)$

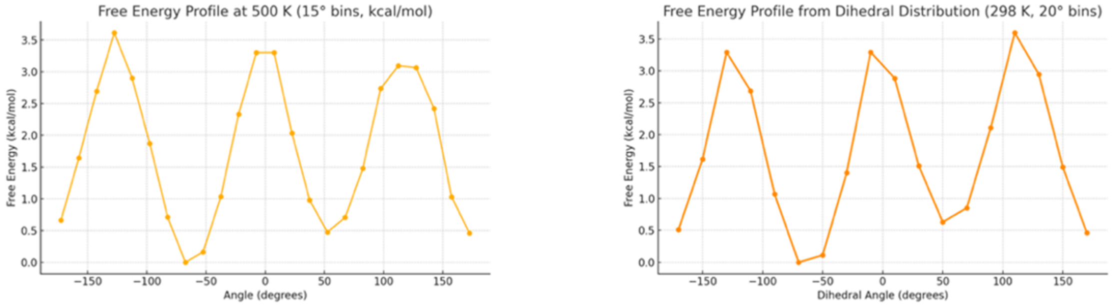


Figure 2. Free energies at different temperatures

Figure 2 displays the resulting free energy landscapes at 298 K and 500 K. Despite the large temperature difference, the shapes and relative energy barriers of the free energy curves remain nearly identical, suggesting that enthalpy dominates the torsional energy barrier for alanine.

This observation implies that the stability of specific dihedral conformations arises mainly from intramolecular energetic contributions (e.g., steric interactions and hydrogen bonding) rather than from entropic effects. The minor variations seen in the peak heights are attributed to thermal fluctuations rather than changes in the underlying free energy surface.

To further validate our methodology, we analyzed the ψ dihedral angle (N–Cα–C–N) in a two-alanine polypeptide. The computed ψ angle was plotted against a Ramachandran map, which illustrates the sterically allowed regions for polypeptide backbone dihedrals, which is shown in Figure 3.

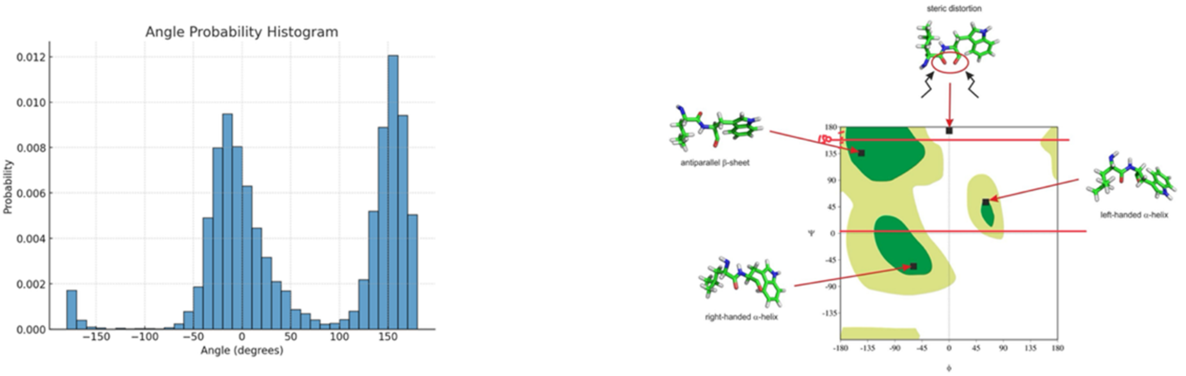


Figure 3. Angle-dependent probabilities at different temperatures

The resulting ψ value falls within a highly populated and sterically accessible region of the Ramachandran plot. This agreement with established protein conformational data indirectly confirms the reliability of our MD simulation methodology and suggests that the chosen force field and simulation parameters adequately reproduce realistic molecular conformations.

5. Discussion

Our results demonstrate that alanine's torsional free energy surface is highly robust to changes in temperature across the simulated range. This indicates that the torsional barrier is primarily enthalpic in nature, controlled by the potential energy surface rather than entropic effects.

In biological systems, torsional flexibility is a critical determinant of protein folding, enzyme activity, and conformational dynamics. Although our study focuses on a single amino acid, the insights gained suggest that temperature-independent dihedral stabilization may be a general feature for certain classes of small molecules. However, we also recognize the limitations of our approach:

1. Force Field Limitations: We used the CP2K-derived force field within AmberTools. While accurate for alanine, it may not fully capture long-range interactions in larger peptides or proteins.

2. System Size: Our study focuses on a single amino acid and a small dipeptide model. Extending this approach to longer chains or complex proteins may introduce additional entropic contributions and cooperative effects.

3. Simulation Timescale: We performed 500 ps simulations, sufficient for exploring dihedral equilibrium distributions but potentially insufficient for sampling rare conformational transitions.

6. Conclusion

In this project, we used molecular dynamics simulations to investigate the relationship between dihedral angles, Gibbs free energy, and temperature for alanine. By computing probability distributions of dihedral angles and transforming them into free energy landscapes, we demonstrated that:

- Dihedral conformations of alanine remain stable across 298 K and 500 K.
- The torsional energy barrier is dominated by enthalpic contributions, while entropy plays a minor role.
- The calculated ψ dihedral angles of a two-alanine polypeptide agree with the sterically allowed regions on a Ramachandran plot, confirming the validity of our simulation methodology.

This work establishes a computational framework for analyzing dihedral stability and free energy landscapes in biomolecules. Future studies could extend this methodology to larger peptides and proteins, test different force fields and solvent models, and apply enhanced sampling techniques such as metadynamics or umbrella sampling to capture rare conformational transitions.

Overall, our findings confirm that molecular dynamics provides a powerful and reliable approach for exploring the thermodynamics of dihedral conformations and free energy surfaces in biomolecular systems.

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