Explanation of the Temperature Ladder Selection Approach

Overview

The temperature ladder selection in this workflow ensures efficient sampling in Replica Exchange Molecular Dynamics (REMD) simulations. It dynamically computes the number of replicas and generates an **exponentially spaced temperature ladder**, optimizing exchange acceptance rates and computational efficiency.

Mathematics of the Approach

1. Dynamic Temperature Spacing

The acceptance rate **P***accept* between replicas in REMD is determined by the Metropolis criterion:

$$P_{\text{accept}} \sim \exp\left(-\frac{\Delta E}{k_B T}\right)$$

Where:

- ΔE: Difference in potential energy between replicas.
- kBT: Thermal energy, with kB being Boltzmann's constant and T the temperature.

To achieve a user-defined target acceptance ratio **P***target:

$$P_{\text{target}} = \exp\left(-\frac{\Delta E}{k_B T}\right)$$

By approximating ΔE as proportional to the number of atoms (**N***atoms*), the **temperature spacing factor** (δ) is derived as:

$$\delta = \sqrt{\frac{2}{P_{\text{target}} \cdot N_{\text{atoms}}}}$$

```
delta = (2 / (acceptance_target * natoms)) ** 0.5
```

2. Calculating the Number of Replicas

The number of replicas (**n**replicas) required to span the temperature range while maintaining the target acceptance ratio is computed logarithmically:

$$n_{\text{replicas}} = \left\lceil \frac{\log\left(\frac{T_{\max}}{T_{\min}}\right)}{\log(1+\delta)} \right\rceil$$

Where:

- Tmin: Minimum temperature.
- Tmax: Maximum temperature.
- δ: Temperature spacing factor.

```
n_replicas = int(math.ceil(math.log(Tmax / Tmin) / math.log(1 + delta)))
```

Additionally, the user has the flexibility to specify the number of replicas directly, bypassing the first two options and proceeding directly to the subsequent step.

3. Exponential Temperature Ladder

Once **n**replicas is determined, the temperatures are distributed exponentially across the range:

$$T_i = T_{\min} \cdot \left(\frac{T_{\max}}{T_{\min}}\right)^{\frac{i}{n_{\text{replicas}}-1}}$$

Where:

- Ti: Temperature of the i-th replica.
- i: Index of the replica (0 to **n**replicas−1).

```
temperatures = [Tmin * (Tmax / Tmin) ** (i / (n_replicas - 1)) for i in
range(n_replicas)]
```

This ensures a **constant ratio** between adjacent temperatures:

$$\frac{T_{i+1}}{T_i} = \text{constant}$$

Relationship Between Temperature Spacing and Exchange Acceptance Rates

1. Temperature Difference and Acceptance Rates

The temperature difference (ΔT) between neighboring replicas directly influences the acceptance rates:

- **Small ΔT**: Leads to higher acceptance rates but requires more replicas, increasing computational cost.
- **Large ΔT**: Reduces the number of replicas but results in lower acceptance rates, leading to poor sampling.

By dynamically calculating δ, the script balances this trade-off to achieve the desired acceptance ratio.

2. Uniform Acceptance Rates

Exponential temperature spacing ensures that the acceptance rates remain nearly uniform across all replicas:

P*accept* ≈ constant across all replica pairs

This prevents bottlenecks where some pairs have poor exchange rates, ensuring efficient sampling throughout the temperature range.

Advantages of This Approach

1. **Dynamic and Adaptive**:
   - The number of replicas is calculated based on the system size (**N***atoms*) and the desired acceptance ratio (**P***target*).
2. **Efficient Sampling**:
   - Exponential spacing enhances the overlap of energy distributions, improving the probability of exchanges.
3. **Computational Efficiency**:
   - Reduces unnecessary replicas while maintaining robust sampling.
4. **Avoidance of Bottlenecks**:
   - Uniform acceptance rates ensure smooth and efficient sampling across all replicas.

Summary

The temperature ladder selection approach dynamically computes the number of replicas using:

$$\delta = \sqrt{\frac{2}{P_{\text{target}} \cdot N_{\text{atoms}}}}$$

and determines the temperatures via exponential spacing:

$$T_i = T_{\min} \cdot \left(\frac{T_{\max}}{T_{\min}}\right)^{\frac{i}{n_{\text{replicas}}-1}}$$

This method ensures uniform exchange rates and minimizes computational costs, making it superior to static or linearly spaced approaches. It balances efficient sampling with computational resources for optimal performance in REMD simulations.

Resources for Temperature Ladder and Parameters

1. **Virtual Chemistry REMD Temperature Generator**:
    - https://virtualchemistry.org/remd-temperature-generator/
    - This tool provides insights into generating temperature ladders dynamically for REMD simulations. It highlights the role of system-specific parameters like temperature ranges, acceptance ratios, and number of replicas.
2. **RSC Article on Optimized Replica Exchange Simulations**:
    - https://pubs.rsc.org/en/content/articlelanding/2008/cp/b716554d
    - This publication discusses the optimization of REMD simulations and the impact of temperature spacing on exchange probabilities. It serves as a theoretical basis for designing efficient temperature ladders.
3. **GitHub Repository for REMD Temperature Generator**:
    - https://github.com/dspoel/remd-temperature-generator
    - This repository offers a practical implementation of temperature ladder generation, demonstrating the importance of factors such as exponential spacing and target acceptance ratios in determining optimal temperature distributions.

These resources were instrumental in understanding the parameters influencing temperature ladders, the mathematical approaches to optimizing them, and the practical implementations for REMD simulations. They informed the design and logic behind the script and its dynamic replica calculation.

Description of Exchange Attempt Scheduling

In our Replica Exchange Molecular Dynamics (REMD) simulation, exchange attempt scheduling is implemented to ensure efficient sampling of conformational space while balancing computational cost and simulation accuracy. The strategy considers the current performance metrics, such as exchange success rates and temperature mixing, and incorporates plans for optimization based on observed outcomes.

Current Implementation

1. Exchange Frequency

- The interval between exchange attempts is determined by the `-replex` **parameter** in GROMACS.
  - **Current Value**: `-replex 1000` (exchanges every 1000 steps).
  - **Simulation Step Size**: `dt = 0.002 ps`, translating to an exchange frequency of **2 ps**.

Reason for `-replex 1000`:

- Provides a balance:
  - **Equilibration**: Ensures replicas have sufficient time to equilibrate at their assigned temperatures.
  - **Sampling**: Exchange attempts occur frequently enough to promote temperature mixing.
- Suitable as a baseline for the current system, given its size and complexity.

2. Exchange Criteria

- Exchanges are based on the **Metropolis criterion**: $P_{\text{accept}} = \min\left(1, \exp\left(-\frac{\Delta E}{k_B \Delta T}\right)\right)$
  - ΔE: Potential energy difference between replicas.
  - ΔT: Temperature difference between adjacent replicas.
  - kB: Boltzmann constant.
- Neighboring replicas attempt exchanges at each interval, and the acceptance is determined probabilistically based on their potential energy overlap.

3. Temperature Ladder

- **Current Setup**:
  - **Temperature Range**: 300 K to 400 K.
  - **Number of Replicas**: Initially 8 replicas.
  - **Spacing**: Exponential spacing for consistent energy overlap.

Performance Observations:

- Exchange success rates ranged from **0% to 5%**, particularly low for lower-temperature replicas.
- Limited temperature mixing across the ladder indicates suboptimal sampling.

Performance Analysis

1. **Exchange Success Rates**:
   - Observed success rates are significantly lower than the optimal range of **20%–40%**.
   - Limited energy overlap between replicas, especially at lower temperatures, is likely the cause.
2. **Temperature Mixing**:
   - The temperature trajectories show that replicas are largely confined to their initial temperature ranges.
   - Poor mixing hinders the sampling of diverse energy landscapes.
3. **Equilibration Time**:
   - `-replex 1000` ensures reasonable equilibration time between exchanges but may require fine-tuning for specific system sizes.

Planned Improvements

1. Increase the Number of Replicas

- **Proposed Change**: Increase from **8 to 12 replicas**.
- **Impact**:
  - Reduces the temperature spacing (ΔT) between replicas.
  - Improves energy overlap, enhancing exchange success rates, particularly for lower-temperature pairs.

2. Adjust the Acceptance Ratio Target

- **Proposed Change**: Increase the acceptance ratio target from **0.2 (20%)** to **0.25 (25%)**.
- **Impact**:
  - Promotes better energy overlap between neighboring replicas.
  - Encourages more frequent exchanges.

3. Fine-Tune `-replex` Parameter

- **Current Setting**: `-replex 1000`.
- **Future Plans**:
  - Test shorter intervals (e.g., `-replex 500`) to allow for more frequent exchange attempts, particularly for short simulations.
  - Alternatively, test longer intervals (e.g., `-replex 2000`) to provide additional equilibration time between exchanges for larger or more complex systems.

4. Validate Changes with Trial Runs

- Conduct trial simulations with the updated parameters to:
  - Monitor exchange success rates, aiming for **20%–40%**.
  - Evaluate temperature mixing, ensuring replicas traverse the full temperature range.

Future Goals

1. **Achieve Optimal Exchange Success Rates**:
   - Target a uniform range of success rates across all replica pairs to ensure consistent energy overlap and efficient sampling.
2. **Improve Temperature Mixing**:
   - Ensure replicas mix effectively across the entire temperature range, enhancing sampling diversity and reliability of results.
3. **Enhance Sampling Efficiency**:
   - Balance the trade-off between computational cost and sampling efficiency through iterative parameter optimization.

Summary

Our exchange attempt scheduling currently uses:

- `-replex 1000` for a balance between equilibration and exchange attempts.
- An **8-replica exponential temperature ladder** over the range of 300 K to 400 K.

Based on performance metrics, we plan to:

- Increase the number of replicas to 12.
- Raise the acceptance ratio target to 0.25.
- Fine-tune `-replex` based on trial simulations.

These adjustments aim to improve exchange success rates and temperature mixing, ensuring robust and efficient sampling for REMD simulations. This iterative approach ensures the system achieves optimal performance while maintaining computational feasibility.

References

The approach is supported by insights from the following resources:

1. Tutorial on Setting Up the REMD Simulation Using Alanine Dipeptide as a Toy Model
2. PMID: 29744830 - Factors Affecting Exchange Frequencies in REMD Simulations
3. AIP: Exchange Frequency in REMD Simulations

Discussion of Convergence Assessment Approach

Our convergence assessment for REMD simulations employs a comprehensive methodology that ensures the system has adequately sampled conformational space. By analyzing metrics such as **Potential Energy (PE)**, **Radius of Gyration (Rg)**, and **Root Mean Square Deviation (RMSD)** across multiple time intervals, we can dynamically monitor and validate the simulation's convergence. This is achieved through a combination of Bash and Python scripts, with results consolidated into `convergence_metrics_results.txt`.

Implementation Details

1. Data Extraction and Analysis

Time Interval Splitting

- The simulation time is divided into **four equal intervals** for dynamic convergence evaluation:

$$\text{Interval} = \frac{\text{Total Simulation Time}}{4}$$

- Time intervals are calculated using the total simulation steps (`SIMULATION_STEPS`) and time step size (`dt`).

Metrics Computation

For each replica, the following metrics are calculated at every interval using the Bash script `calculate_convergence.sh`:

- **Potential Energy (PE)**: Extracted using `gmx_mpi energy`.
- **Radius of Gyration (Rg)**: Computed via `gmx_mpi gyrate`.
- **Root Mean Square Deviation (RMSD)**: Derived from `gmx_mpi rms`.

Output Storage

Results are stored in the `output/convergence_metrics_xvg/` directory, with subdirectories organized by metric, replica, and time interval.

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2. Result Parsing and Statistical Analysis

File Parsing

The Python script `parse_convergence_metrics.py` processes `.xvg` files for PE, Rg, and RMSD, located in `output/convergence_metrics_xvg/`.

Statistical Analysis

For each metric, the script computes:

$$\text{Mean} = \frac{\sum x_i}{n}, \quad \text{Standard Deviation} = \sqrt{\frac{\sum (x_i - \text{Mean})^2}{n}}$$

`parse_convergence_metrics.py`

```
data = np.loadtxt(file, comments=["@", "#"])

        return np.mean(data[:, 1]), np.std(data[:, 1])
```

This provides detailed insights into system behavior across replicas and time intervals.

Summary Output

The analyzed results are written to `output/data_files/convergence_metrics_results.txt`.

Convergence Analysis Results

Key Observations

From `convergence_metrics_results.txt`, we observe the following for Replica 001:

Potential Energy (PE):}

$$\begin{aligned} &\text{Interval 1:} \quad -81256.416 \pm 349.470\,\text{kJ/mol} \\ &\text{Interval 4:} \quad -81242.210 \pm 314.522\,\text{kJ/mol} \end{aligned}$$

Radius of Gyration (Rg):

$$\text{Interval 1: } 0.717 \pm 0.008\,\text{nm} \\ \text{Interval 4: } 0.703 \pm 0.007\,\text{nm}$$

RMSD:

$$\text{Interval 1: } 0.159 \pm 0.022\,\text{nm} \\ \text{Interval 4: } 0.181 \pm 0.011\,\text{nm}$$

Convergence Insights

1. **Energy Stabilization**:
    - PE values stabilize across intervals, reflecting consistent sampling of low-energy conformations.
2. **Structural Compactness**:
    - Minor Rg fluctuations suggest consistent structural compactness.
3. **Structural Equilibration**:
    - RMSD gradually stabilizes, indicating the system has equilibrated relative to the reference structure.

Enhancements to Precision

Custom Index Groups

We improved the precision of Rg and RMSD calculations by creating a custom index group focusing on protein heavy atoms:
`center.sh`

```
gmx_mpi make_ndx -f em.gro -o "$INDEX_FILE" <<EOF
1 & ! a H*
name 17 protein_heavy
q
EOF
```

- **Advantages**:
    - Heavy atoms are less sensitive to thermal fluctuations, ensuring more accurate structural metrics.

Usage in Calculations

The custom index group was employed in Rg and RMSD computations:
`calculate_convergence.sh`

```
gmx_mpi gyrate -s ${REPLICA}/remd.tpr -f ${REPLICA}/final.xtc -b $TIME_3 -e
$LAST_TIME -o "$RG_3" -n ${REPLICA}/index.ndx <<< "17"

gmx_mpi rms -s $REF_STRUCTURE -f ${REPLICA}/final.xtc -b 0 -e $TIME_1 -o
"$RMSD_0" -n ${REPLICA}/index.ndx <<< "4 17"
```

4 is the backbone index group and 17 is the protein_heavy index group

Significance:

- Enhances the accuracy of structural metrics by focusing on stable, structurally relevant atoms.

Reference Molecule

We used `em.tpr` (energy-minimized structure) as the reference molecule for RMSD calculations:

- **Rationale**:
  - Represents the most stable conformation after energy minimization.
  - Ensures consistent and unbiased baseline comparisons.
- **Benefit**:
  - Helps identify whether structural deviations are due to equilibrated dynamics or simulation artifacts.

Future Improvements

1. **Enhanced Sampling**:
   - Increase the number of replicas to improve temperature mixing.
2. **Extend Simulation Time**:
   - Allow for longer sampling periods to validate convergence further.

Conclusion

The convergence assessment dynamically evaluates key metrics (PE, Rg, RMSD) across intervals, providing robust insights into system stability and sampling efficiency. The use of custom index groups and a stable reference molecule ensures precision and reliability in structural assessments. Future enhancements aim to build on this foundation to improve sampling and validation further.

Expanded Discussion on Potential Energy Overlap

Concept of Potential Energy Overlap

Potential energy overlap between neighboring replicas is essential for achieving effective exchanges in REMD simulations. It quantifies the similarity of energy distributions between adjacent replicas, which directly impacts the exchange acceptance rate. Greater overlap ensures smoother transitions between temperature states, enabling efficient sampling of the conformational space.

The key principle is based on the **Boltzmann factor**:

$$e^{-\Delta E/k_BT}$$

This governs the exchange probability and is highly sensitive to the energy distributions of neighboring replicas. When these distributions overlap sufficiently, the exchange likelihood increases, facilitating better exploration of the system's energy landscape.

Process Overview

1. **Data Extraction**:
   - Potential energy values are extracted for each replica using the `Epot_rmsd.sh` script:

```
gmx_mpi energy -f ${ENERGY_FILE} -o ${ENERGY_OUTPUT} <<EOF Potential EOF
```

The extracted energy data is saved in `output/data_files/energy_replica_*.xvg`, which serves as input for further analysis.
2. **Analysis**:

```
- The `plot_energy_overlap.py` script calculates and visualizes energy
overlap using **Kernel Density Estimation (KDE)**:
```

```
sns.kdeplot(energy_values, label=f"Replica {replica_index}", linewidth=2,
fill=False)
```

The resulting KDE plot shows the probability density of potential energy values for each replica. This allows direct visualization of overlap between energy distributions (e.g., `potential_energy_overlap_kde.png`).

Kernel Density Estimation (KDE)

Probability Density Calculation: The script uses **KDE** to compute the probability density function (PDF) of potential energy values. KDE is a non-parametric method that smooths the data to create a continuous density curve.

Key Parameters:

- `energy_values`: Input data representing potential energy values for each replica.
- `linewidth`: Defines the thickness of the KDE curve for better visibility.
- `fill=False`: Ensures that only the curve is displayed without shaded areas, making it easier to compare replicas.

KDE Function in the Script:

```
sns.kdeplot(energy_values, label=f"Replica {replica_index}", linewidth=2,
fill=False)
```

Insights Gained from Energy Overlap

1. **Visual Assessment**:
    - KDE plots provide a clear visualization of the overlap between neighboring replicas. If the overlap is narrow or absent, it indicates suboptimal replica spacing, which could reduce exchange efficiency.
2. **Improving Simulation Parameters**:
    - If insufficient overlap is observed, several adjustments can be made:
        - **Increase the number of replicas**: This reduces the temperature spacing, leading to better alignment of energy distributions.
        - **Adjust the temperature range**: Narrowing or expanding the range can enhance overlap.
        - **Tune the exchange frequency (`-replex`)**: Optimizing this value can improve sampling efficiency.
3. **Guiding Future Adjustments**:
    - The results provide actionable insights for tuning simulation parameters in future REMD runs. By iteratively improving overlap, both sampling and convergence can be enhanced.

Conclusion

This script offers a robust method to calculate and visualize potential energy overlaps in REMD simulations. The use of KDE ensures smooth and accurate representation of energy distributions, while automated file detection and dynamic replica identification streamline the workflow. Key benefits include:

- **Clear Visualization**: The KDE plot directly highlights energy overlaps between replicas.
- **Actionable Insights**: Results guide the optimization of simulation parameters, improving both efficiency and convergence.
- **Reliability**: Robust error handling and efficient data processing ensure accurate and dependable analysis.

By leveraging these tools, the REMD simulation setup can be fine-tuned for optimal performance.

Discussion of Temperature Mixing Assessment

Purpose

Temperature mixing assessment is crucial in Replica Exchange Molecular Dynamics (REMD) to ensure replicas traverse the full temperature ladder. Effective temperature mixing enables efficient sampling of both high-energy and low-energy conformational spaces, ensuring proper thermodynamic sampling.

Assessment of Temperature Mixing

1. Extraction of Temperature Trajectories

- Using the Bash script `temperature_traj.sh`, temperature trajectories are extracted from the energy output files (`remd.edr`) of each replica.
- The extracted data is saved as `.xvg` files in the `output/temperature_xvg` directory.
- Example command:

```
gmx_mpi energy -f "$ENERGY_FILE" -o "$OUTPUT_FILE" <<EOF 15 EOF
```

Output:
- Files like `replica_001_temperature.xvg`, containing time (ps) and temperature (K), are generated for each replica.

2. Visualization of Temperature Mixing

- The Python script `plot_temperature_mixing.py` reads these `.xvg` files and plots the temperature trajectories for all replicas.
- **Key Steps**:
  1. **Load Data**:
     - Data for each replica is dynamically loaded:

```
data = np.loadtxt(file_path, comments=["@", "#"])
time = data[:, 0]  # Time in ps
temperature = data[:, 1]  # Temperature in K
```

2. **Plot Trajectories**:
   - Each replica's temperature trajectory is plotted:

   ```
   plt.plot(time, temperature, label=replica)
   ```

3. **Output Visualization**:
   - A single plot (`temperature_mixing.png`) displays the movement of all replicas across the temperature range over time.

Interpreting the Temperature Mixing Plot

The `temperature_mixing.png` plot provides a direct visual representation of replica movement across temperatures:

- **Good Mixing**:
  - If the trajectories of replicas cross multiple temperature states throughout the simulation, it indicates effective mixing.
  - This suggests sufficient energy overlap and proper exchange rates between neighboring replicas.
- **Poor Mixing**:
  - If some replicas remain confined to a specific temperature range, it indicates suboptimal mixing. Possible causes include:
    - Inadequate energy overlap between replicas.
    - Large temperature spacing in the ladder.
    - Insufficient exchange attempts (`-replex` set too high).

Insights from the Current Plot

1. **Replica Transitions**:
   - The plot demonstrates that replicas are transitioning across the temperature ladder, but some may exhibit limited movement, indicating room for improvement in mixing efficiency.
2. **Evaluation Metrics**:

- The trajectory crossings between replicas and the distribution of temperature values over time are key indicators of mixing efficiency.

Planned Improvements

1. **Increase Number of Replicas**:
   - Adding replicas will reduce temperature spacing and improve energy overlap.
2. **Adjust Exchange Frequency (`-replex`)**:
   - Decreasing `-replex` will increase the frequency of exchange attempts, enhancing temperature mixing.
3. **Reevaluate Temperature Ladder**:
   - Refine the temperature range or spacing to align energy distributions better.

Conclusion

The temperature mixing plot provides valuable insights into the behavior of replicas across the temperature ladder. While the current results indicate reasonable mixing, further tuning of the number of replicas, temperature spacing, and exchange frequency can significantly enhance the simulation's sampling efficiency.

This approach ensures that REMD simulations achieve comprehensive thermodynamic sampling and robust convergence.

Assumptions Made and Potential Improvements

1. **Virtual Environment Integration**:
   The current workflow relies on exporting GROMACS binaries for execution, but integrating the entire setup into a virtual environment (e.g., Conda) would improve isolation and reproducibility. Future efforts could focus on creating a unified base image that incorporates both Conda and CUDA environments, though this requires significant adjustments and debugging.
2. **Automation of `install_gromacs_binaries.sh`**:
   The current `install_gromacs_binaries.sh` script requires manual export of GROMACS to the system PATH after execution. Optimizing the script to handle this step automatically would streamline usability and allow GROMACS to run immediately upon script completion.
3. **MDP File Parameter Fine-Tuning**:
   While the MDP files are configured based on GROMACS documentation and prior

experimentation, further refinement through systematic testing could improve equilibration and overall simulation performance, particularly for complex systems.

4. **Enhanced Script Error Handling and Parallel Processing**:
Current workflow scripts handle errors on a basic level. Implementing more robust error handling would allow the workflow to manage unexpected issues more gracefully. Additionally, optimizing the scripts to process multiple inputs concurrently would enhance efficiency and scalability, especially for large-scale simulations.

Resources for Temperature Ladder and Parameters

1. **Virtual Chemistry REMD Temperature Generator**:
https://virtualchemistry.org/remd-temperature-generator/
This tool provides insights into dynamically generating temperature ladders for REMD simulations.
2. **RSC Article on Optimized Replica Exchange Simulations**:
https://pubs.rsc.org/en/content/articlelanding/2008/cp/b716554d
Discusses temperature spacing optimization and its impact on exchange probabilities.
3. **GitHub Repository for REMD Temperature Generator**:
https://github.com/dspoel/remd-temperature-generator
Offers practical implementations of temperature ladder generation.

References for Exchange Scheduling

1. **Tutorial on Setting Up the REMD Simulation Using Alanine Dipeptide as a Toy Model**:
https://www.researchgate.net/publication/335453547
A comprehensive guide to setting up and running REMD simulations for alanine dipeptide.
2. **PMID: 29744830 - Factors Affecting Exchange Frequencies in REMD Simulations**:
https://pubmed.ncbi.nlm.nih.gov/29744830/
Provides an analysis of factors influencing exchange frequencies and their optimization.
3. **AIP: Exchange Frequency in REMD Simulations**:
https://pubs.aip.org/aip/jcp/article-abstract/128/2/024103/899639/Exchange-frequency-in-replica-exchange-molecular
Discusses the theoretical framework and practical considerations for optimizing exchange frequencies.

GROMACS and Enhanced Sampling Resources

1. **GROMACS User Manual**:
   https://manual.gromacs.org/current/index.html
   Comprehensive documentation for GROMACS, including setup, commands, and enhanced sampling techniques.
2. **Enhanced Sampling Methods Tutorials by NNairIITK**:
   https://github.com/NNairIITK/Enhanced_Sampling_Methods_Tutorials/tree/master/REST2
   Offers tutorials on advanced sampling methods, including REST2, complementing REMD workflows.
3. **Snakemake Documentation**:
   https://snakemake.readthedocs.io/en/stable/index.html
   Provides guidance on implementing scalable workflows using Snakemake, which can improve automation and parallelism in simulations.
4. **MD Alanine Dipeptide Repository**:
   https://github.com/sbrodehl/MD-AlanineDipeptide/tree/master
   Contains resources and scripts for alanine dipeptide simulations, useful for benchmarking and method validation.