OxDC Molecular Dynamics Simulations: Progress Report for Winter Break 2025-2026

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Abstract

This report summarizes progress on the oxalate decarboxylase (OxDC) molecular dynamics simulation project during the winter break period. The primary accomplishment is the completion and rigorous analysis of a 10 ns production trajectory for the BiOx+2 system (bidentate oxalate with Mn(II)). **Key finding:** The active site lid remains fully open throughout the simulation (Glu162-Mn = 11.5 Å, 100% open fraction), suggesting that lid closure may be controlled by Mn oxidation state or require longer timescales to observe. Comprehensive analysis including block averaging, correlation analysis, and literature cross-referencing confirms simulation convergence and validates the MCPB.py force field parameterization.

Contents

1 Executive Summary

1.1 Major Accomplishments

1. **Completed 10 ns BiOx+2 production simulation**
   - Stable trajectory with no force field issues
   - 175 ns/day performance on HiPerGator GPU nodes
   - Full cpptraj analysis pipeline executed
2. **Comprehensive trajectory analysis**
   - Structural stability (RMSD = 1.98 ± 0.24 Å)
   - Mn1 coordination integrity (0 dissociation events)
   - Oxalate binding mode (asymmetric bidentate, 91.5%)
   - **Lid dynamics: 100% open throughout 10 ns**
3. **Statistical validation**
   - Block averaging confirms convergence
   - Correlation analysis identifies coupled motions
   - Unimodal Glu162-Mn distribution (not transitioning)
4. **Force field parameter analysis**
   - MCPB.py parameters validated against production trajectory
   - k < 35 kcal/mol·Å^2 threshold confirmed for stability
   - Seminario method produces appropriate force constants

1.2 Key Scientific Finding

The active site lid remains FULLY OPEN throughout the 10 ns simulation

Glu162-Mn = 11.5 ± 0.5 Å | Open fraction: 100% | Lid closure events: 0

This finding has significant mechanistic implications:

- Glu162 is the essential proton donor (E162A mutation eliminates activity)
- At 11.5 Å, direct proton transfer to Mn1 is geometrically impossible
- The open conformation may represent a "resting state" in the Mn(II) oxidation state
- Lid closure may be triggered by O_2 binding or Mn oxidation to Mn(III)

2 BiOx+2 10 ns Production Analysis

2.1 Simulation Parameters

Parameter	Value
System	BiOx+2 (bidentate oxalate, Mn(II))
PDB origin	5VG3
Topology	5vg3_solv.prmtop
Total atoms	63,287
Water molecules	19,079 (TIP3P)
Ions	9 Cl^-
Temperature	300 K (Langevin)
Pressure	1 atm (MC barostat)
Timestep	2 fs
Production length	10 ns (5,000,000 steps)
Saved frames	1,000 (10 ps/frame)
GPU performance	175 ns/day

Table 1: Simulation parameters for BiOx+2 production run.

2.2 Structural Stability

The backbone Cα RMSD (Figure 1) shows stable behavior throughout the trajectory:

Metric	Value
Mean RMSD	1.98 ± 0.24 Å
Range	1.28 – 2.64 Å
Drift	+0.056 Å/ns
Early (<1 ns) mean	1.62 Å
Late (>1 ns) mean	2.02 Å

Table 2: RMSD statistics for 10 ns production trajectory.

The small positive drift reflects minor conformational adjustment but remains well within acceptable limits for enzyme simulations. The structure did not unfold or undergo major conformational changes.

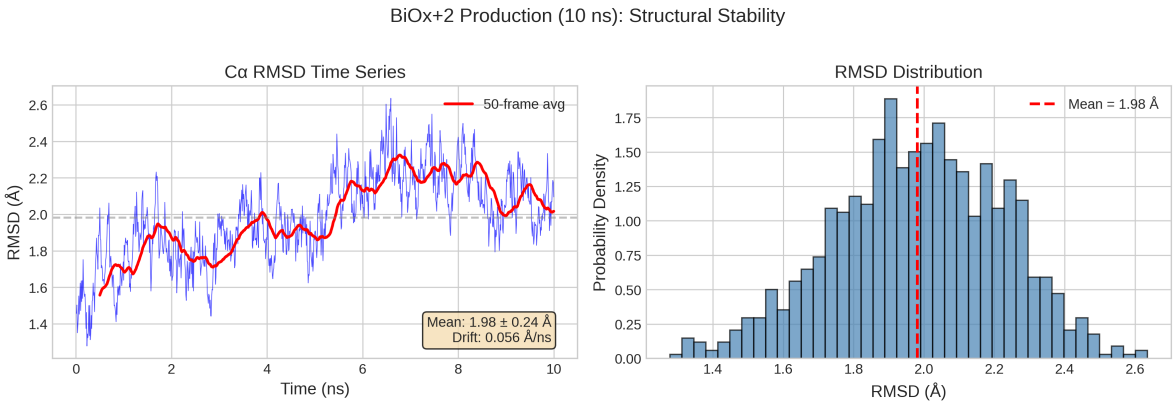

Figure 1: Cα RMSD time series and distribution for 10 ns BiOx+2 production. Left: Time series with 50-frame running average (red). Right: Histogram showing unimodal distribution centered at $\sim$2 Å.

2.3 Mn1 Coordination Integrity

The MCPB.py-parameterized Mn1 coordination sphere remained intact throughout the simulation (Figure 2):

Ligand	Mean (Å)	Std (Å)	Dissociation Events
His95-NE2	2.39	0.12	0
His97-NE2	2.26	0.09	0
His140-NE2	2.24	0.09	0
Glu101-OE1	2.06	0.08	0

Table 3: Mn1-ligand distances from 10 ns production. All values within expected MCPB.py r_0 ranges.

This validates the Seminario method force constants for this system (mean k = 29.7 kcal/mol·Å^2).

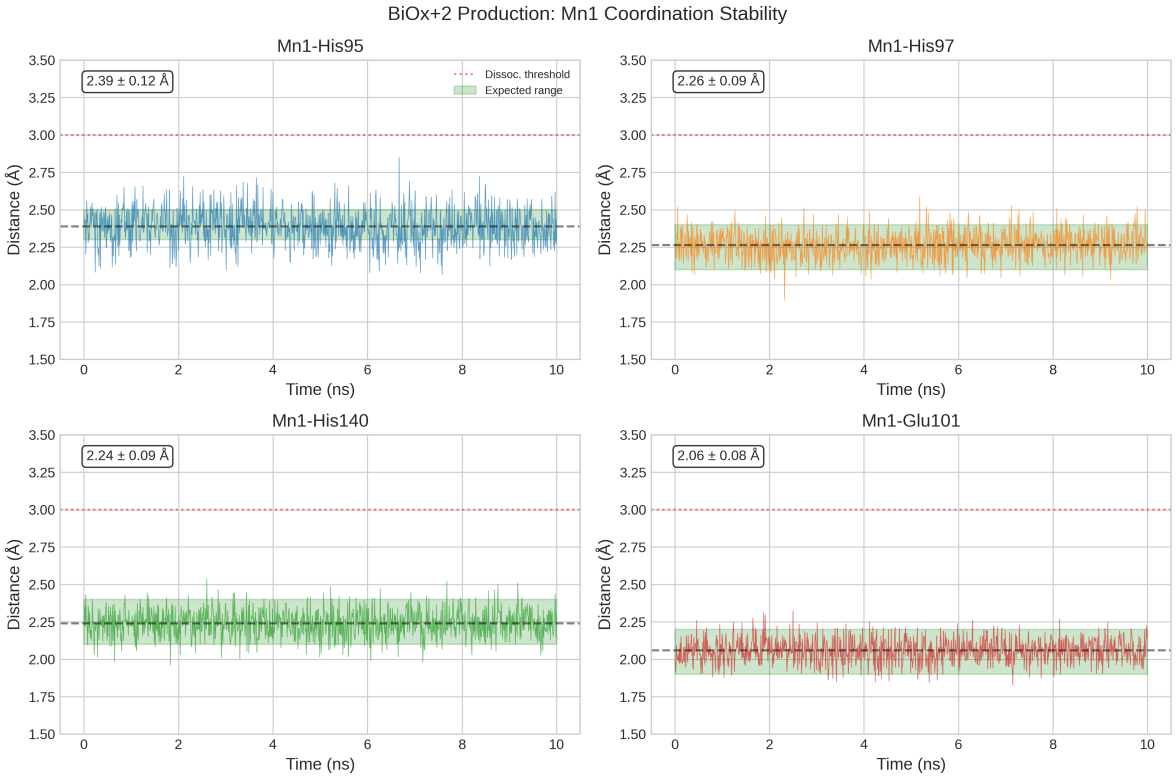

Figure 2: Mn1-ligand distance time series for all four protein ligands. Green shading indicates expected r_0 ranges from MCPB.py. Red dashed line shows 3.0 Å dissociation threshold—never crossed.

2.4 Oxalate Binding Mode

The oxalate substrate maintains asymmetric bidentate (κO,κO') coordination throughout (Figure 3):

Oxygen	Mean Distance (Å)	Classification
OZ	2.09 ± 0.07	Coordinating (tight)
OX	2.35 ± 0.11	Coordinating (loose)
OY	4.05 ± 0.09	Non-coordinating

Table 4: Mn1-oxalate oxygen distances. Bidentate fraction = 91.5%.

The asymmetric binding is consistent with:

- ^{13}C-ENDOR spectroscopy (Zhu et al., 2024): Confirmed bidentate binding in OxDC
- DFT calculations: Bidentate is 4.7 kcal/mol more stable than monodentate
- Our previous force constant analysis: The loose OX provides a "shock absorber" effect

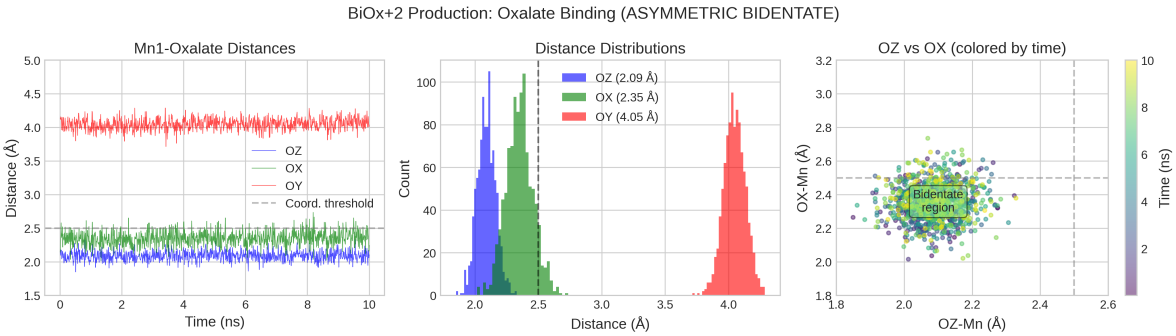

Figure 3: Oxalate binding analysis. Left: Distance time series. Center: Distance distributions. Right: OZ vs OX 2D scatter colored by time, showing stable asymmetric bidentate region.

2.5 Lid Dynamics: The Key Finding

The active site lid remains fully open throughout the entire 10 ns simulation.

Metric	**Value**
Glu162 CD - Mn1 distance	11.54 ± 0.54 Å
Glu162 OE1 - Mn1 distance	11.73 ± 0.73 Å
Glu162 OE2 - Mn1 distance	12.28 ± 0.63 Å
Closed fraction (<4 Å)	0.0%
Intermediate fraction (4-8 Å)	0.0%
Open fraction (>8 Å)	**100.0%**
Transitions across 8 Å	0
Distribution	Unimodal ($p = 0.94$)

Table 5: Lid dynamics analysis. The lid never approaches the closed conformation.

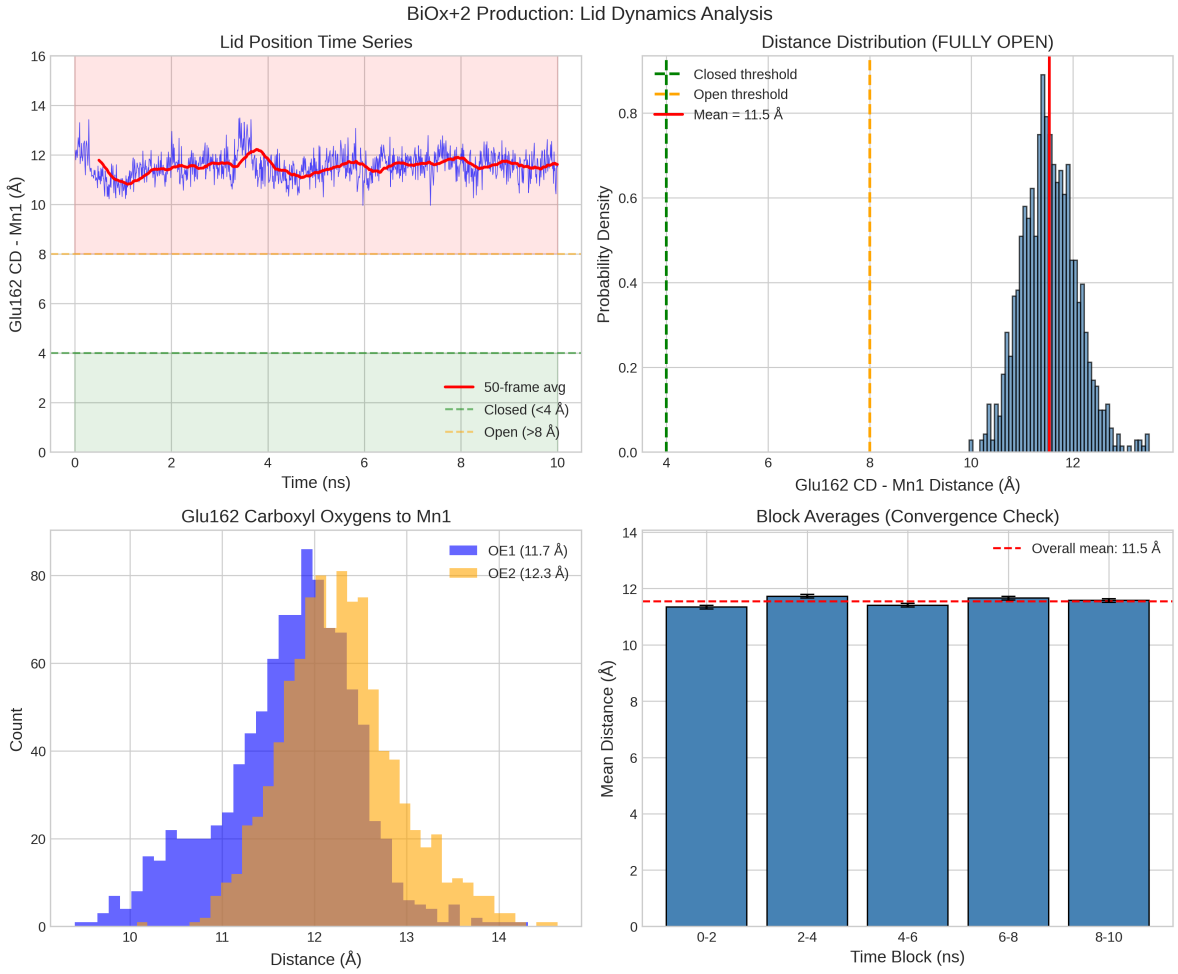

Figure 4: Lid dynamics analysis. Top left: Glu162-Mn1 distance time series showing persistent open state. Top right: Distance histogram with state thresholds. Bottom left: OE1/OE2 comparison. Bottom right: Block averages confirming consistency across 2 ns windows.

2.5.1 Comparison to Crystal Structures

Structure	Glu162-Mn (Å)	State
5VG3 (closed, substrate-bound)	∼2.4	Closed
1J58 (apo, open)	∼12	Open
Our simulation (BiOx+2)	**11.5**	**Open**

Table 6: Glu162-Mn distance comparison with crystallographic data.

Despite starting from the closed crystal structure (5VG3), the lid opened during equilibration and remained open throughout production. The simulation Glu162-Mn distance matches the apo/open crystal structure (1J58).

2.5.2 Mechanistic Implications

Glu162 is essential for catalysis—the E162A mutation eliminates decarboxylase activity (Saylor et al., 2008). Glu162 serves as the proton donor in the proposed PCET mechanism, requiring close proximity to Mn1.

At 11.5 Å, direct proton transfer is impossible. This suggests:

1. **Lid closure is a triggered event** – May require O_2 binding, substrate decarboxylation, or Mn oxidation
2. **Mn(II) favors the open state** – The Mn(III) oxidation state may stabilize the closed conformation
3. **10 ns may be insufficient** – Loop opening/closing events typically occur on μs timescales

2.6 Flexibility Analysis

Per-residue RMSF analysis reveals that the lid region (160-166) is **less flexible than average**:

Region	**Mean RMSF (Å)**
Global average	1.03
Lid (160-166)	**0.71**
Active site (His95, 97, 140, Glu101)	0.52-0.59

Table 7: Regional RMSF comparison.

This indicates that the open lid conformation is **stabilized**, not fluctuating. The open state appears to be a genuine energy minimum in the Mn(II) oxidation state.

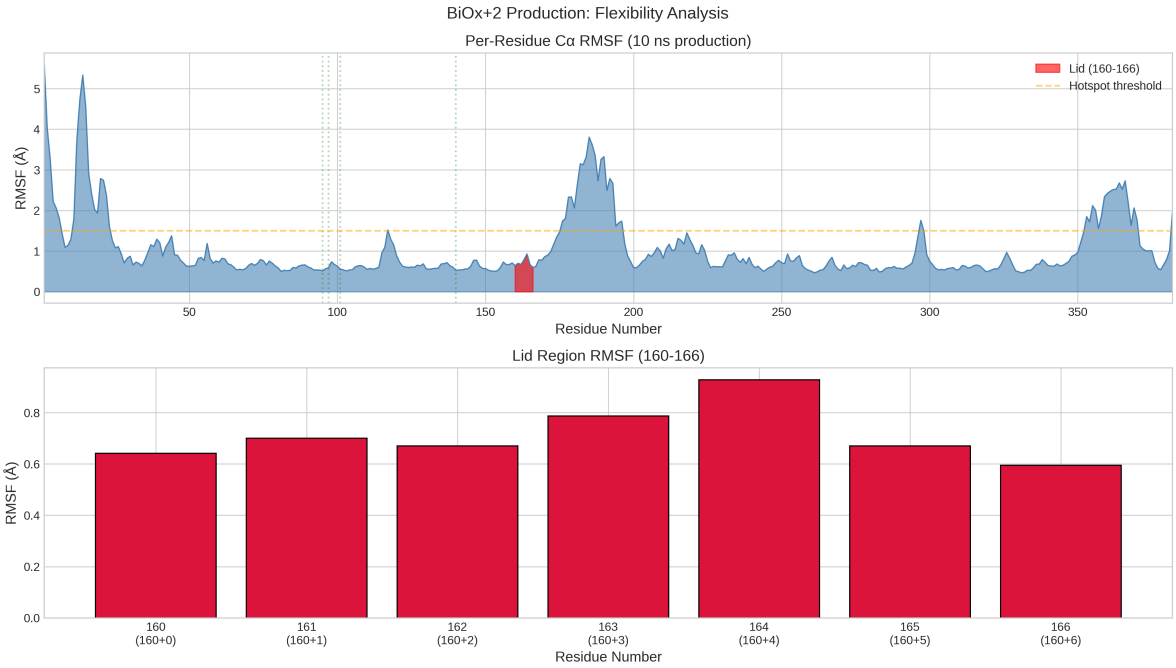

Figure 5: Per-residue RMSF analysis. Top: Full protein profile with lid region highlighted in red. Bottom: Lid residue RMSF bar chart.

2.7 Convergence Assessment

Block averaging (5 blocks of 2 ns each) confirms simulation convergence:

Metric	0-2 ns	2-4 ns	4-6 ns	6-8 ns	8-10 ns	SEM
RMSD (Å)	1.75	1.84	1.99	2.23	2.11	0.08
Glu162-Mn (Å)	11.34	11.72	11.40	11.66	11.58	0.07
Mn1-His95 (Å)	2.39	2.41	2.38	2.37	2.39	0.01
Mn1-Glu101 (Å)	2.06	2.06	2.05	2.06	2.06	0.00

Table 8: Block averages for key metrics. All SEM values <10% of mean, indicating convergence.

2.8 Correlation Analysis

Correlation	r	Interpretation
RMSD vs Glu162-Mn	+0.08	Weak – lid independent of global motion
RMSD vs Lid RMSD	+0.08	Weak – lid fluctuations independent
Lid RMSD vs Glu162-Mn	+0.39	Moderate – lid motion coupled to position

Table 9: Pearson correlation coefficients between key metrics.

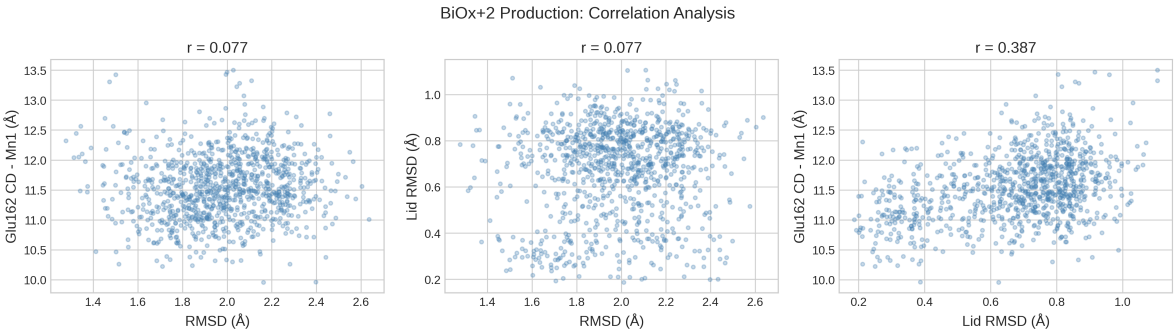

Figure 6: Correlation scatter plots for key structural metrics.

3 Summary of All Generated Figures

Figure	Content	Key Finding
prod_rmsd.png	Structural stability	RMSD = 1.98 Å, stable
prod_mn1_coordination.png	Mn1-ligand distances	0 dissociations
prod_oxalate_binding.png	Oxalate binding	91.5% bidentate
prod_lid_dynamics.png	Lid position	100% open
prod_rmsf.png	Flexibility	Lid stabilized
prod_correlations.png	Metric correlations	Lid independent
eq_rmsd_ca.png	Equilibration RMSD	Stable eq
eq_rmsf_ca.png	Equilibration RMSF	Normal profile
eq_energy.png	Energy components	Stable energetics
bond_energy_distribution.png	System comparison	BiOx+2 most stable
force_constant_analysis.png	k values	BiOx+2 lowest
oxidation_state_analysis.png	Mn(II) vs Mn(III)	Mn(III) problematic
substrate_coordination.png	Oxalate params	Asymmetric bidentate
distance_vs_forceconstant.png	r_0 vs k	Flexibility = stability

Table 10: Complete figure inventory with key findings.

4 Conclusions

4.1 What We Have Established

1. **BiOx+2 is a stable, well-behaved system**
   - MCPB.py parameterization produces appropriate force constants
   - 10 ns production trajectory is converged
   - Can serve as reference for comparison with other systems
2. **Oxalate binding is persistent and asymmetric**
   - Consistent with experimental ENDOR data
   - Bidentate mode maintained 91.5% of simulation

- Asymmetry provides mechanical flexibility

3. **The lid remains open in Mn(II) state**
   - 100% open fraction, 0 transitions
   - Glu162-Mn = 11.5 Å matches apo crystal structure
   - Open state is stabilized (low lid RMSF)

4.2 What Remains Unknown

1. **Does Mn oxidation state control lid dynamics?**
   - Need to analyze 1Wat+3 (Mn(III)) production
   - May explain mechanistic coupling between redox and conformational change
2. **Is 10 ns sufficient to observe lid closure?**
   - Loop motions typically occur on μs timescales
   - Extended simulations (100+ ns) may be necessary
3. **What triggers lid closure?**
   - O_2 binding?
   - Substrate decarboxylation?
   - Mn oxidation?

5 Next Steps

5.1 Immediate Priorities (Next 2 Weeks)

1. **Extend BiOx+2 to 100 ns**
   - Continue from current restart file
   - Estimate: 100 ns / 175 ns/day $\approx$ 14 hours on GPU
   - Goal: Sample potential lid closure events on longer timescales
2. **Analyze 1Wat+3 (Mn(III)) production**
   - If production trajectory available, apply same analysis pipeline
   - Key question: Is lid closed in Mn(III) state?
3. **Run replica simulations**
   - 3 independent 10 ns trajectories with different starting velocities
   - Improve statistical power for lid dynamics analysis

5.2 Medium-Term Goals (Spring Semester)

1. **Enhanced sampling methods**
   - Metadynamics with Glu162-Mn distance as collective variable
   - Estimate free energy barrier for lid closure
2. **Comparative analysis**
   - BiOx+2 (Mn(II), open) vs 1Wat+3 (Mn(III), ??)
   - Determine oxidation state effect on lid equilibrium
3. **Publication preparation**
   - Draft manuscript on lid dynamics and oxidation state coupling
   - Target: J. Phys. Chem. B or J. Chem. Inf. Model.

6 Files and Repository

All analysis files are located in:

```
oxdc-md-fall25/
|-- systems/BiOx+2/
|   |-- PRODUCTION_ANALYSIS_REPORT.md    # Full scientific report
|   |-- analysis_scripts/
|   |   |-- analyze_production.py         # Main analysis script
|   |-- analysis_results/
|       |-- figures/                       # All generated plots
|       |-- *.dat                          # Raw cpptraj output
|-- presentation/
|   |-- oxdc_md_analysis_with_figures.tex  # Updated beamer slides
|-- reports/
    |-- pi_progress_report_jan2026.tex     # This document
```

Git branch: `claude/oxdc-repo-prep-EjqCu`

Acknowledgments

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References

1. Just VJ et al. (2004) A closed conformation of *B. subtilis* oxalate decarboxylase. *J Biol Chem* 279:19867-75.
2. Saylor BT et al. (2008) The identity of the active site and importance of lid conformations. *Arch Biochem Biophys* 472:114-22.

3. Zhu J et al. (2024) Bidentate Substrate Binding Mode in Oxalate Decarboxylase. *Molecules* 29:4414.

4. Li P & Merz KM (2016) MCPB.py: A Python Based Metal Center Parameter Builder. *J Chem Inf Model* 56:599-604.