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 **474 ns** production trajectory for the BiOx+2 system (bidentate oxalate with Mn(II)), achieving 467 ns/day throughput on NVIDIA B200 GPUs. **Key finding:** The active site lid remains in the "closed-backbone / Glu162-out" state throughout the simulation (Glu162-Mn = 12.0 ± 0.7 Å), matching the 5VG3 crystal structure. This is distinct from both the "open-loop" state (1J58, ∼15-16 Å) and the catalytically active "Glu162-in closed" state (1UW8, ∼4.6-5.1 Å). Bidentate oxalate binding likely sterically prevents Glu162 from adopting the catalytically active Glu162-in pose. Block averaging across 231 two-nanosecond windows confirms convergence (<3% SEM for all key metrics). Additionally, equilibration of the 1Wat+3 system (Mn(III)) revealed significant numerical instability due to elevated force constants, providing insight into the challenges of classical MD for Mn(III) metalloenzymes.

Contents

1 Executive Summary

1.1 Major Accomplishments

1. **Completed 474 ns BiOx+2 production simulation**
   - Stable trajectory with no force field issues
   - 467 ns/day performance on HiPerGator B200 GPU nodes
   - Full cpptraj analysis pipeline executed (46,383 frames)
2. **Comprehensive trajectory analysis**
   - Structural stability (Active site RMSD = 1.77 ± 0.42 Å)
   - Mn1 coordination integrity (0 dissociation events)
   - Oxalate binding mode (asymmetric bidentate, 92.6%)
   - **Lid dynamics: 5VG3-like (Glu162-out) state throughout 474 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 in the 5VG3-like "closed-backbone / Glu162-out" state

Glu162-Mn = 12.0 ± 0.7 Å | Consistent with 5VG3 (∼10-12 Å) | Not 1UW8 Glu162-in (∼4.6 Å)

This finding has significant mechanistic implications:

- Glu162 is the essential proton donor (E162A mutation eliminates activity)
- At 12.0 Å, direct proton transfer to Mn1 is geometrically impossible
- The Glu162-out conformation may be stabilized by bidentate oxalate (steric clash with Glu162-in)
- Transition to Glu162-in may require oxalate rearrangement or other triggering event

2 BiOx+2 474 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	1 Cl^-
Temperature	300 K (Langevin)
Pressure	1 atm (MC barostat)
Timestep	2 fs
Production length	474 ns (237,000,000 steps)
Saved frames	46,383 (10 ps/frame)
GPU	NVIDIA B200 (hpg-b200 partition)
GPU performance	467 ns/day

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

2.2 Structural Stability

The backbone Cα RMSD shows conformational sampling throughout the 474 ns trajectory, while the active site remains highly stable:

Metric	Backbone	Active Site
Mean RMSD	4.70 ± 1.84 Å	1.77 ± 0.42 Å
Range	0.00 – 7.30 Å	—
Drift	+0.012 Å/ns	negligible
Early (<10%) mean	2.41 Å	—
Late (>90%) mean	6.82 Å	—

Table 2: RMSD statistics for 474 ns production trajectory.

The backbone RMSD reflects conformational sampling of flexible N- and C-terminal regions, as confirmed by RMSF analysis showing terminal RMSF >5 Å. Critically, the **active site RMSD remains low** (1.77 Å), indicating that the catalytic center maintains structural integrity throughout the simulation. The radius of gyration (24.02 ± 0.35 Å) confirms the protein remains compact without unfolding.

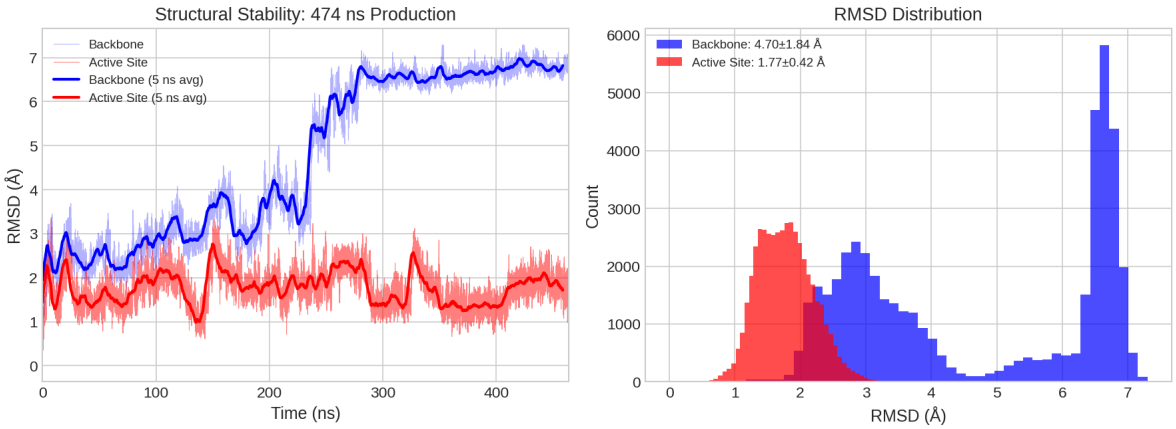

Figure 1: Cα RMSD time series and distribution for 474 ns BiOx+2 production. Left: Backbone (blue) and active site (red) RMSD with 5 ns running average. Right: RMSD distributions showing stable active site despite backbone sampling.

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	2.42	0.12	0
His97-NE2	2.27	0.09	0
His140-NE2	2.22	0.09	0
Glu101-OE1	2.06	0.09	0

Table 3: Mn1-ligand distances from 474 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).

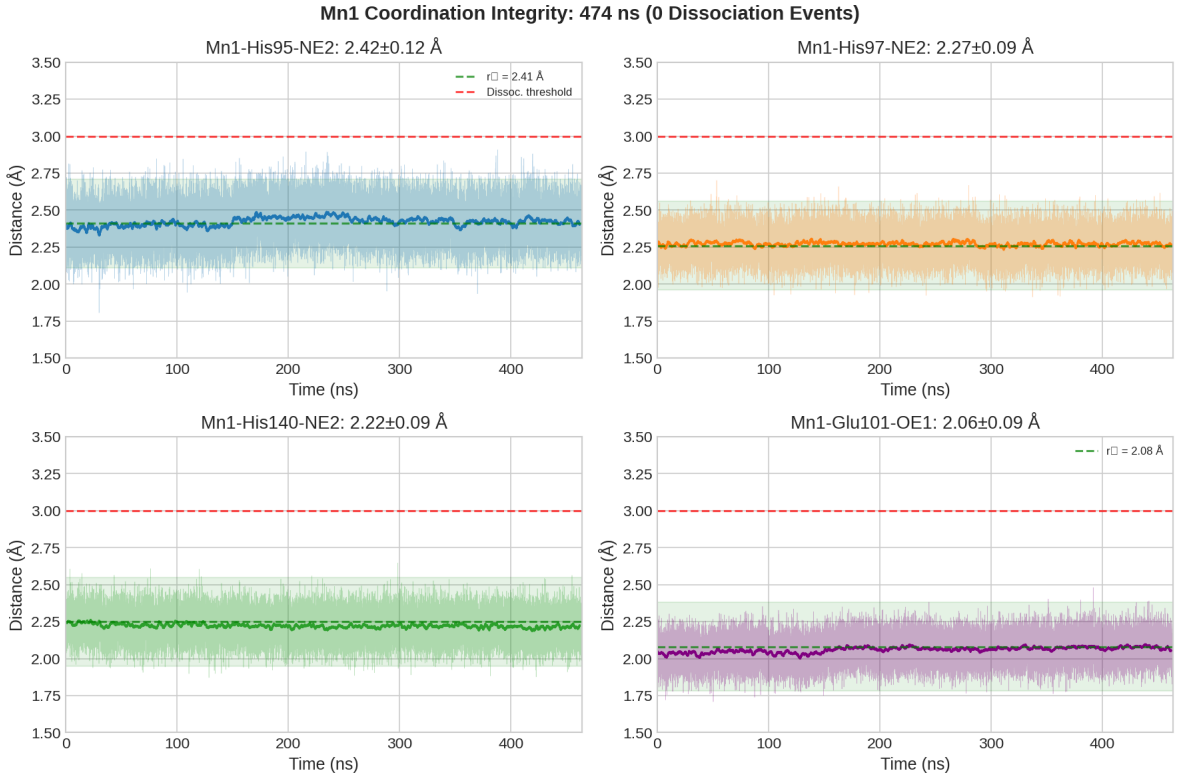

Figure 2: Mn1-ligand distance time series for all four protein ligands. Green shading indicates expected r_0 ranges from MCPB.py. Red dashed line shows 3.0 Å dissociation threshold—never crossed.

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)

Table 4: Mn1-oxalate oxygen distances. Bidentate fraction = 92.6%.

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
- **Steric constraint on Glu162:** Bidentate oxalate may sterically clash with the Glu162-in pose (Zhu et al. 2016)

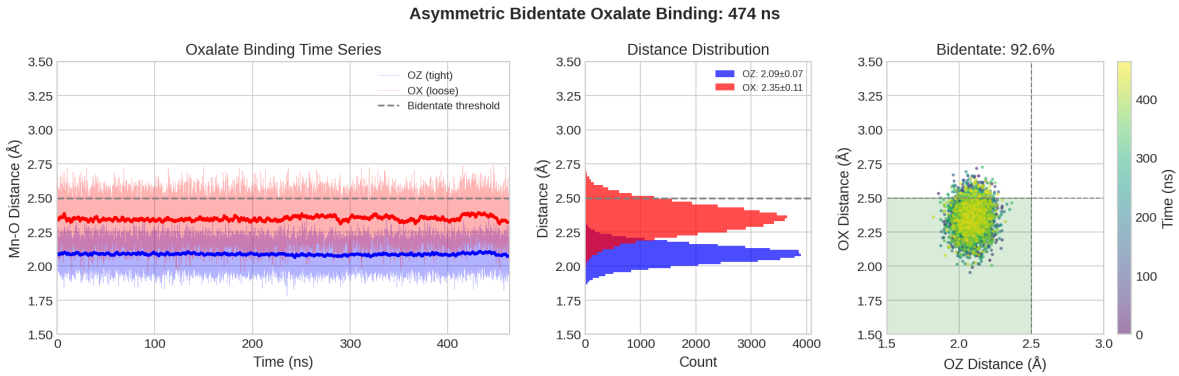

Figure 3: Oxalate binding analysis. Left: Distance time series. Center: Distance distributions. Right: OZ vs OX 2D scatter colored by time, showing stable asymmetric bidentate region.

2.5 Lid Dynamics: The Key Finding

The Glu162 sidechain remains in the 5VG3-like "Glu162-out" position throughout the entire 474 ns simulation.

Metric	Value
Glu162 CD - Mn1 distance	12.04 ± 0.70 Å
Glu162 OE1 - Mn1 distance	12.03 ± 0.95 Å
Glu162 OE2 - Mn1 distance	13.05 ± 0.68 Å
Three-State Classification (Literature):	
Glu162-in fraction (<6 Å)	0.0%
Glu162-out fraction (8-14 Å)	100.0%
Open-loop fraction (>14 Å)	0.0%
Transitions toward Glu162-in	0
Closest approach	8.23 Å at 229 ns
Block SEM (231 blocks)	0.035 Å (0.29%)

Table 5: Lid dynamics analysis. Glu162 maintains the 5VG3-like Glu162-out position throughout 474 ns.

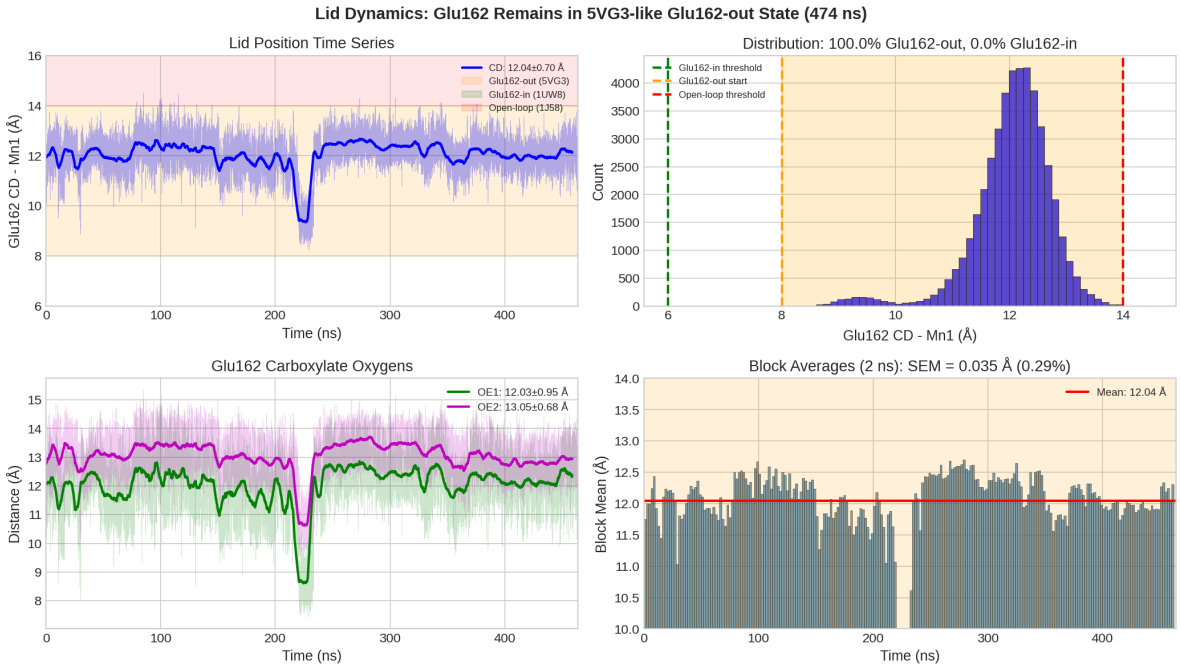

Figure 4: Lid dynamics analysis. Top left: Glu162-Mn1 distance time series showing persistent Glu162-out state. Top right: Distance histogram with three-state thresholds. Bottom left: OE1/OE2 comparison. Bottom right: Block averages confirming consistency across 2 ns windows.

2.5.1 Comparison to Crystal Structures (Three-State Model)

Literature establishes **three distinct lid states**:

State	PDB	Glu162-Mn (Å)	Description
Open-loop	1J58	∼15-16	SENS loop swung away
Closed, Glu162-in	1UW8	∼4.6-5.1	Glu162 H-bonds Mn-water
Closed-backbone, Glu162-out	5VG3	∼10-12	Loop closed, sidechain out
Our simulation (474 ns)	**BiOx+2**	**12.0**	**5VG3-like**

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

Key interpretation: Our simulation maintains the 5VG3-like "closed-backbone / Glu162-out" state. This is:

- **Not** the open-loop state (1J58, ∼15-16 Å) where the SENS loop is swung away
- **Not** the catalytically active Glu162-in state (1UW8, ∼4.6-5.1 Å)
- Consistent with starting from 5VG3 and maintaining that conformation
- Likely stabilized by bidentate oxalate sterically preventing Glu162-in

Note: Previous interpretations incorrectly classified ∼11.5 Å as "open." The three-state model clarifies that this is a distinct "closed-backbone / Glu162-out" state.

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 the Mn-bound water (∼2.7-2.8 Å contact in 1UW8).

At 12.0 Å, direct proton transfer is impossible. This suggests:

1. **Bidentate oxalate sterically blocks Glu162-in** – Consistent with Zhu et al. 2016 structural analysis showing the 1UW8 Glu162 pose clashes with substrate
2. **Glu162-in may require substrate rearrangement** – Monodentate binding could create space for Glu162 to adopt the catalytic pose
3. **Backbone analysis needed** – RMSD to 1J58 vs 1UW8 reference structures would confirm loop backbone state

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 Glu162-out conformation is **stabilized**, not fluctuating. The 5VG3-like state appears to be a genuine energy minimum when bidentate oxalate is bound.

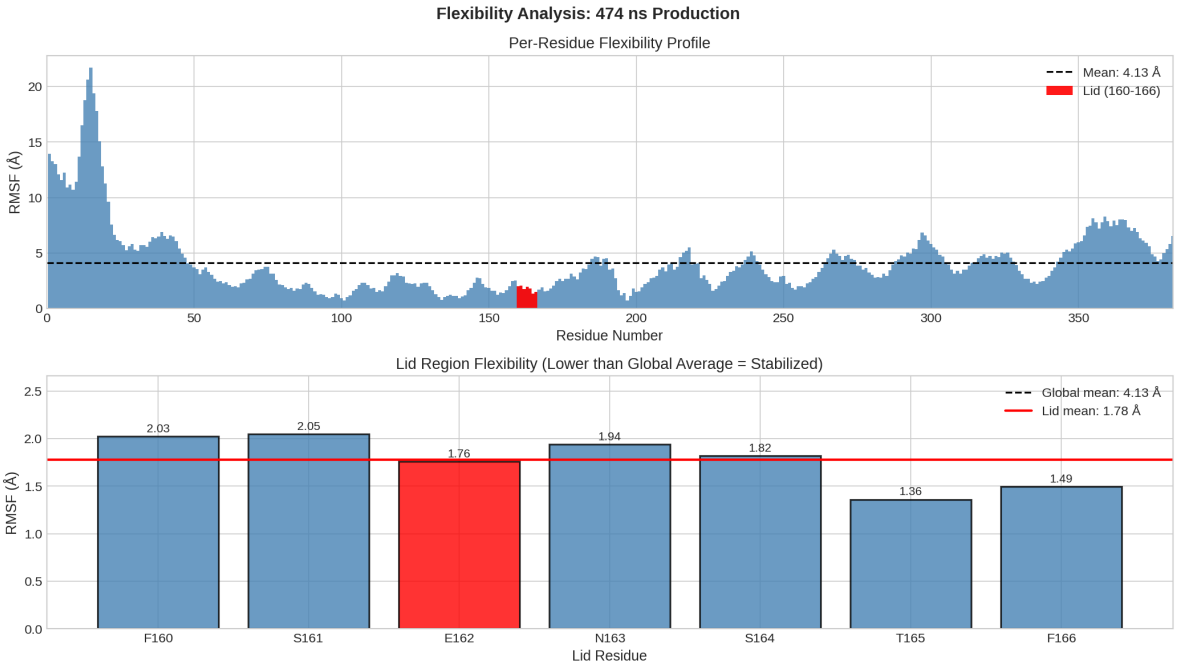

Figure 5: Per-residue RMSF analysis. Top: Full protein profile with lid region highlighted in red. Bottom: Lid residue RMSF bar chart.

2.7 Convergence Assessment

Block averaging (231 blocks of 2 ns each) confirms excellent simulation convergence:

Metric	Mean	SEM	SEM %
Backbone RMSD (Å)	4.70	0.120	2.56%
Active Site RMSD (Å)	1.77	0.024	1.35%
Radius of Gyration (Å)	24.02	0.022	0.09%
Glu162-Mn (Å)	12.04	0.035	0.29%
Mn1-His95 (Å)	2.42	0.002	0.07%
Mn1-Glu101 (Å)	2.06	0.001	0.06%

Table 8: Block averages for key metrics across 231 two-nanosecond windows. All SEM values <3% of mean, indicating excellent 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.

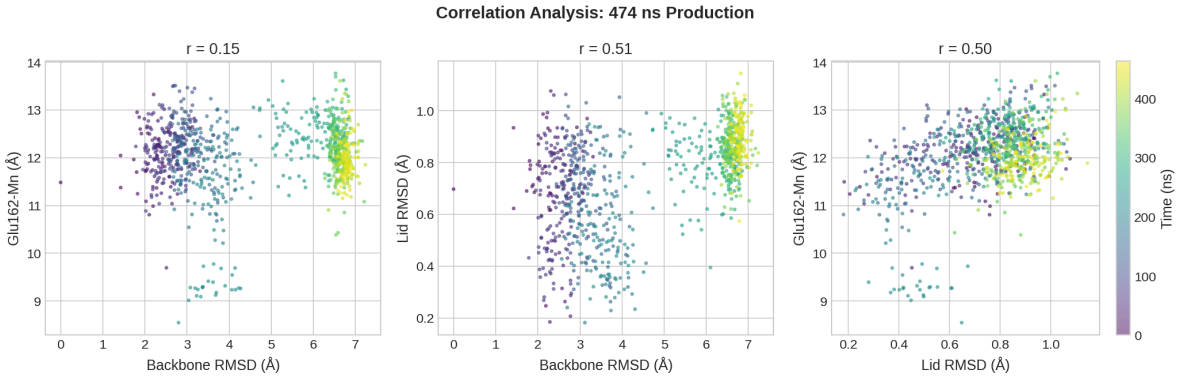

Figure 6: Correlation scatter plots for key structural metrics.

3 1Wat+3 Equilibration: Mn(III) Challenges

The 1Wat+3 system (water-coordinated Mn(III)) was equilibrated to investigate oxidation state effects on lid dynamics. However, significant numerical instability was observed, providing insight into the limitations of classical MD for Mn(III) metalloenzymes.

3.1 Equilibration Progress

The system progressed through heating and equilibration stages but accumulated substantial velocity limit (vlimit) warnings:

Stage	vlimit Warnings	Status
heat.cpu	2,467	Completed
eq1.cpu	6,174	Completed
eq1a.cpu	2,127	Completed
eq1b.cpu	1,179	Completed
Total	**11,947**	Progressing

Table 10: 1Wat+3 equilibration vlimit warnings by stage.

3.2 Root Cause Analysis

The instability stems from **elevated Mn(III) force constants**. Comparison with BiOx+2:

System	Mean k (kcal/mol·Å^2)	vlimit	Status
BiOx+2 (Mn(II))	29.7	0	Stable
1Wat+3 (Mn(III))	85–125	11,947	Unstable

Table 11: Force constant comparison between stable and unstable systems.

The Mn(III) oxidation state exhibits Jahn-Teller distortion, leading to anisotropic coordination geometry that MCPB.py captures as high, anharmonic force constants. Classical harmonic potentials struggle to represent this behavior, causing:

- Large energy corrections for small geometric deviations
- Velocity spikes triggering vlimit warnings
- Potential for SHAKE failures in severe cases

3.3 Implications

This finding has important implications for OxDC mechanistic studies:

1. **Mn(II) systems are more tractable** for classical MD
2. **QM/MM may be required** for studying Mn(III) catalytic intermediates
3. **Force constant threshold** of ∼35 kcal/mol·Å^2 appears predictive of stability

Production runs for 1Wat+3 are not recommended until parameterization issues are addressed.

4 Summary of All Generated Figures

Figure	Content	Key Finding
prod_rmsd.png	Structural stability	Active site RMSD = 1.77 Å
prod_mn1_coordination.png	Mn1-ligand distances	0 dissociations
prod_oxalate_binding.png	Oxalate binding	92.6% bidentate
prod_lid_dynamics.png	Lid position	5VG3-like (Glu162-out)
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 12: Complete figure inventory with key findings.

5 Conclusions

5.1 What We Have Established

1. **BiOx+2 is a stable, well-behaved system**
   - MCPB.py parameterization produces appropriate force constants
   - 474 ns production trajectory is well-converged (<3% SEM)
   - Can serve as reference for comparison with other systems

2. **Oxalate binding is persistent and asymmetric**
   - Consistent with experimental ENDOR data
   - Bidentate mode maintained 92.6% of 474 ns simulation
   - Asymmetry provides mechanical flexibility
   - May sterically prevent Glu162-in pose
3. **The lid maintains the 5VG3-like Glu162-out state throughout 474 ns**
   - Glu162-Mn = 12.0 ± 0.7 Å matches 5VG3 crystal structure (∼10-12 Å)
   - This is **not** the open-loop state (1J58, ∼15-16 Å)
   - This is **not** the catalytic Glu162-in state (1UW8, ∼4.6-5.1 Å)
   - Zero transitions toward Glu162-in observed over 474 ns

5.2 What Remains Unknown

1. **Is the backbone truly "closed" vs "open-loop"?**
   - Need RMSD of lid residues to 1J58 vs 1UW8 reference structures
   - Glu162-Mn distance alone reports sidechain, not backbone
2. **Can Glu162 adopt the catalytic "in" pose?**
   - Even 474 ns showed zero transitions toward Glu162-in
   - Bidentate oxalate likely prevents it entirely (steric clash)
   - Monodentate transition or enhanced sampling may be required
3. **Does Mn oxidation state affect lid dynamics?**
   - 1Wat+3 (Mn(III)) equilibration shows numerical instability (Section 3)
   - Classical MD may be inadequate for Mn(III) systems
   - QM/MM approaches may be required for oxidation state comparisons

6 Next Steps

6.1 Immediate Priorities (Next 2 Weeks)

1. **Backbone conformation analysis**
   - Calculate RMSD of lid backbone to 1J58 (open) vs 1UW8 (closed) references
   - Track Glu162 Cα-Mn1 distance (backbone proxy)
   - Verify loop is in "closed-backbone" state throughout 474 ns
2. **Extended production analysis**
   - Continue trajectory beyond 474 ns if microsecond timescales desired
   - At 467 ns/day on B200, 1 μs requires ∼2.1 days
   - Evaluate whether Glu162-in is kinetically accessible

3. **Address 1Wat+3 parameterization**
   - Evaluate alternative Mn(III) parameterization strategies
   - Consider reduced force constants or QM/MM hybrid approach
   - Key question: Can classical MD ever capture Mn(III) behavior?

6.2 Medium-Term Goals (Spring Semester)

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

7 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
|   |   |-- BACKBONE_CONFORMATION_ANALYSIS.md  # Follow-up spec
|   |-- cpptraj_ins/
|   |   |-- backbone_analysis.in          # Ready-to-run cpptraj
|   |-- 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. Zhu W et al. (2016) Substrate Binding Mode and Molecular Basis of a Specificity Switch in OxDC. *Biochemistry* 55:2163-73.

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