# Computational Docking of Small Molecules as Potential Inhibitors of Xanthine Oxidase

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## Abstract

Excess amounts of uric acid in the body can cause a variety of health issues including chronic gout and hyperuricemia. Xanthine Oxidase (XOD) is an enzyme involved in the catabolism of purines that ultimately produces uric acid. XOD aids in the breakdown of purines to hypoxanthine, to xanthine, and lastly to uric acid. Allopurinol, Febuxostat and Topiroxostat are three known XOD inhibitors taken as oral medication to prevent or reduce uric acid production, thus reducing pain caused by gout, hyperuricemia, and other uric acid related diseases but side effects do occur. In this project, we are interested in the discovery of more XOD inhibitors that have equal or enhanced potency by performing docking of inhibitors with different structures using high performance commuters. The inhibitor structural classes to be docked will have similar skeletal structures to the known ligands (uric acid structure), the known inhibitors (allopurinol structure), and entirely different structures. The project will first present binding results ($K_d$ values) of inhibitors that have similar skeletal structures to known ligands and inhibitors. Inhibitor docking uses the reduced form of the monomer crystal structure of XOD from the Protein Data Bank. The $K_d$ values of uric acid (~0.028 µM) docked to the crystal structure and allopurinol (~0.15 µM) docked to the reduced form were used as baseline values to establish and relate the binding affinities of known inhibitors, ligands, and manipulated ligands or structures.

## Introduction

Uric acid is a metabolic waste product (**see Figure 1.**) that increases when the activity of XOD is accelerated. When an excessive amount of uric acid is produced, urate crystals form causing a buildup in the joints. Most commonly, this result leads to chronic gout, a form of arthritis.

**Figure 1. Oxidation of purines to produce uric acid (8)**

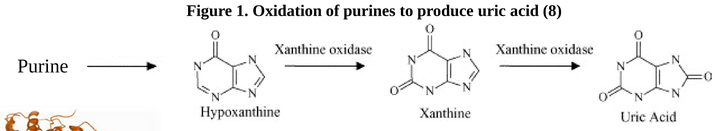



**Figure 2. Enzyme 3AMZ, bovine xanthine oxidase (7)**

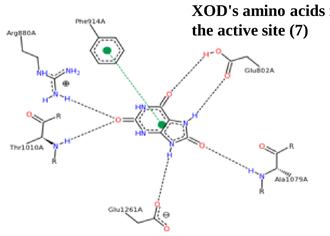



**Figure 3. Interactions of uric acid with XOD's amino acids in the active site (7)**

Our project used a bovine xanthine oxidase enzyme, 3AMZ from the Protein Data Bank (1). 3AMZ is a two-chain protein, with each chain containing 1332 amino acids, plus the coenzymes and the native ligand (uric acid). For this project, since both chains are the same, we only needed to use one (chain A) to run the experiments.

In XOD, uric acid bonds through its peripheral oxygen and polar hydrogen atoms. The active site is characterized by six surrounding amino acids side chains; Glu-802, Phe-914, Arg-880, Thr-1010, Ala-1079, and Glu-1261. With these amino acids, our experiments test for how well small molecules bond (comparing bonding affinities) to the active site and what effect each molecule has on the entire enzyme (RMSD values).

## Objective

The purpose of this study is to use computational methods to discover new XOD inhibitors and/or derivatives of known inhibitors using the enzyme 3AMZ.

## Methods

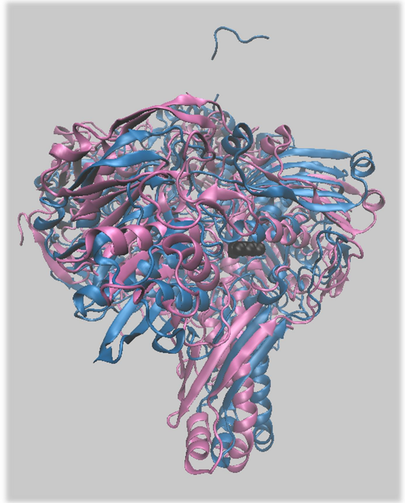
1. Separate the enzyme into chain A and B, removing the coenzyme and ligand
2. 'Fill' in the amino acid sequence at the breaks
   - Original analysis of 3AMZ used X-Ray Diffraction (1), a method where the entire sequence was known but not all amino acids were seen. To best replicate the enzyme's behavior, we had to fill in the missing sections and connect the chain (2).

**Breaks in the 3AMZ Amino Acid Sequence**

*N-terminus* *C-terminus*

{1-2} {166-191} {530-537} {1321-1325}

3. Add a solvation box, ionize, minimize, and run molecular dynamics (3,4).
   - This was done to imitate normal cell conditions and derive the most stable form of the protein.
4. Superimpose the backbone of the reformed version of the enzyme (after steps 2 & 3) to the crystal structure (3).
   - Computationally, step 3 alters the original coordinates that specifies the active site. Fixing this makes docking small molecules in the accurate position possible.



5. Build new or inhibitor-derivative small molecules (6) and dock at the active site (4).
6. To determine the binding effectiveness, compare the $K_d$ values and RMSD values to uric acid (~0.028µM, 0.00 Å) and to allopurinol (~0.15µM, 0.30 Å)

**Figure 4. Superimposed structures**

The backbone of the original polypeptide chain A (blue) superimposed with the backbone of the altered polypeptide chain (pink). Uric acid is black and docked in the active site.

## Results

**Table 1. Inhibitor trials with higher bonding affinities than uric acid with changes of the inner atoms.**

| Inhibitor/Trial | ΔG° | Kd (µM) | RMSD (Å) |
|---|---|---|---|
| Uric Acid | -6.2 | 0.028 | 0.00 |
| Allopurinol (8) | -5.2 | 0.15 | 0.30 |
| 56 | -6.5 | 0.017 | 0.10 |
| 60 | -6.8 | 0.010 | 0.20 |
| 62 | -6.7 | 0.012 | 0.20 |
| 69 | -7.5 | 0.0032 | 0.10 |
| 72 | -7.1 | 0.0062 | 0.10 |
| 73 | -6.4 | 0.020 | 0.10 |
| 74 | -7.1 | 0.0062 | 0.20 |
| 75 | -7.0 | 0.0073 | 0.50 |
| 102 | -6.3 | 0.024 | 0.00 |
| 103 | -6.3 | 0.024 | 0.20 |
| 104 | -6.4 | 0.020 | 0.10 |
| 106 | -7.1 | 0.0062 | 0.10 |
| 107 | -6.5 | 0.017 | 0.10 |
| 109 | -6.6 | 0.014 | 0.30 |
| 110 | -6.9 | 0.0087 | 0.40 |
| 112 | -6.8 | 0.010 | 0.40 |

**Table 2. Inhibitor trials with bonding affinities of –3.2 and higher with changes of the inner atoms.**

| Inhibitor/Trial | ΔG° | Kd (µM) | RMSD (Å) |
|---|---|---|---|
| Uric Acid | -6.2 | 0.028 | 0.00 |
| Allopurinol (8) | -5.2 | 0.15 | 0.30 |
| 23 | -3.0 | 6.3 | 0.10 |
| 26 | -3.2 | 4.5 | 0.40 |
| 27 | -3.0 | 6.3 | 0.50 |
| 31 | -3.2 | 4.5 | 0.50 |
| 37 | -2.5 | 15 | 0.30 |
| 39 | -2.8 | 8.8 | 0.40 |
| 85 | -1.9 | 40. | 0.80 |

$K_d$ values were calculated using $\Delta G° = RT ln(K_d)$
R= 0.001987 kcal/K.mol, T= 298K

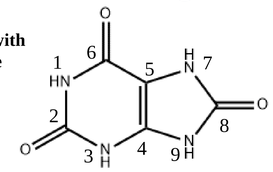
**The more negative the ΔG° value and the lower the $K_d$ value, the better. These values equate to how well the small molecule bonded in the active site.

## Results (Continued)

### Visualizing Small Molecule Inhibitors

**Figure 5. Uric Acid with the skeletal structure numbered (8)**

$K_d$: 0.028 µM
RMSD: 0.00 Å



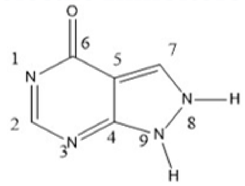
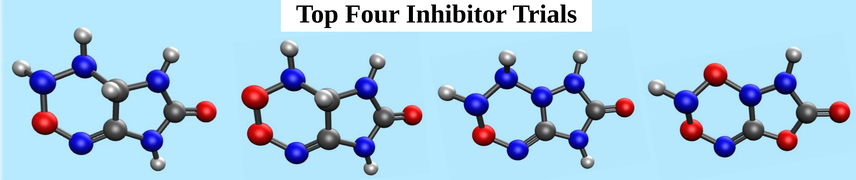


**Figure 6. Allopurinol with the skeletal structure numbered (8)**

$K_d$: 0.15 µM
RMSD: 0.30 Å

**Top Four Inhibitor Trials**



| #69 | #72 | #74 | #106 |
|---|---|---|---|
| $K_d$: 0.0032 µM | $K_d$: 0.0062 µM | $K_d$: 0.0062 µM | $K_d$: 0.0062 µM |
| RMSD: 0.10 Å | RMSD: 0.10 Å | RMSD: 0.20 Å | RMSD: 0.10 Å |

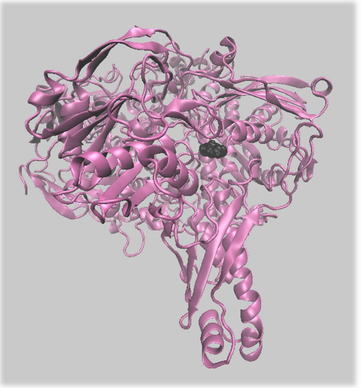


**Figure 6. Small molecule trial #69 (black) docked in the active site of Xanthine Oxidase (pink)**

## Conclusions

Computational methods, such as the methods used in this project, can enhance the proficiency of drug-design. Our project focused on the very beginning stages of investigation towards new solutions to treat diseases caused by excess uric acid in the body, through defining new XOD inhibitors. By utilizing computational methods and high-performance computers, our project was able to access a high volume of molecular structures, successfully discovering 16, previously unknown small molecule inhibitors for XOD that bond better than known inhibitors. Although these initial results show a level of promise, more testing an analysis is needed for result confirmation.

## Future Directions

- Continue to explore for more small molecule inhibitors of XOD
- Synthesis the small molecule inhibitors from this project in a wet-lab and perform the binding experiments.

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## Acknowledgements

**Commitment to equity, diversity, and inclusion**

- We acknowledge that the University of Wisconsin-Eau Claire occupies the sacred and ancestral lands of Indigenous Peoples. We honor the traditional land of the Ojibwe and Dakota Nations.
- We commit to a teaching and learning environment that ensures a culture of equity, diversity, and inclusion.

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