Investigating the Impact of Protein Dynamics and Molecular Crowding on the Inhibitors Binding to Prolyl-tRNA Synthetases Using High-Throughput Docking

Eduardo Miranda[1,2], Sanchita Hati[1], and Sudeep Bhattacharyay[1]

[1]Department of Chemistry and Biochemistry, University of Wisconsin-Eau Claire, Eau Claire, WI, United States, [2]University of South Florida, Tampa, FL, United States

Abstract

Prolyl-tRNA synthetases (ProRSs) are multi-domain proteins crucial for protein biosynthesis in all organisms, making them prime targets for anti-microbial drug development. These enzymes exhibit unique variations in domain architecture across species, suggesting potential for species-specific inhibitor development; inhibitors are molecules that bind to the active site of a protein and inhibit its function. The relationship between protein dynamics and inhibitor binding is not completely understood for these enzymes. Currently, we aim to understand i) how a molecule binds to various active site conformations of an enzyme that result because of the dynamic nature of the target protein and how that varies from species to species and ii) how binding affinity changes in a crowded cell-like environment. This methodology, which includes both protein dynamics and molecular crowding effects, has not been explored thoroughly. The present study could shed light on how conformational dynamics and crowding affect inhibitor binding affinity and aid in developing more selective and potent inhibitor screening. We are performing high-throughput docking to calculate binding affinity values using high-performance computing (HPC). The initial results with docking poses of several inhibitor molecules and interactions with the active site residues will be analyzed to assess how the conformational dynamics are altering the binding affinity of the inhibitors for various ProRSs.

Background

Cells are inherently crowded, and the crowded nature changes various intramolecular interactions. Enzymes, which are proteins, functions in these crowded environments. Their conformations and catalytic activities are expected to be influenced by the surrounding environments. The multi-domain *Escherichia coli* ProRS is our model enzyme and Polyethylene glycol (PEG 600) is our model crowder for exploring the impact of crowding on protein dynamics and inhibitor binding.

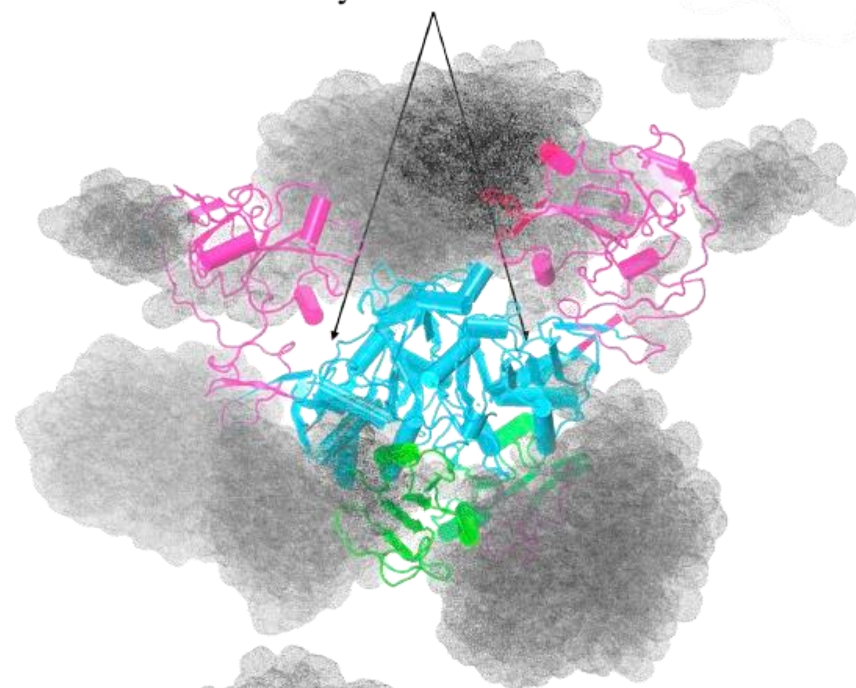
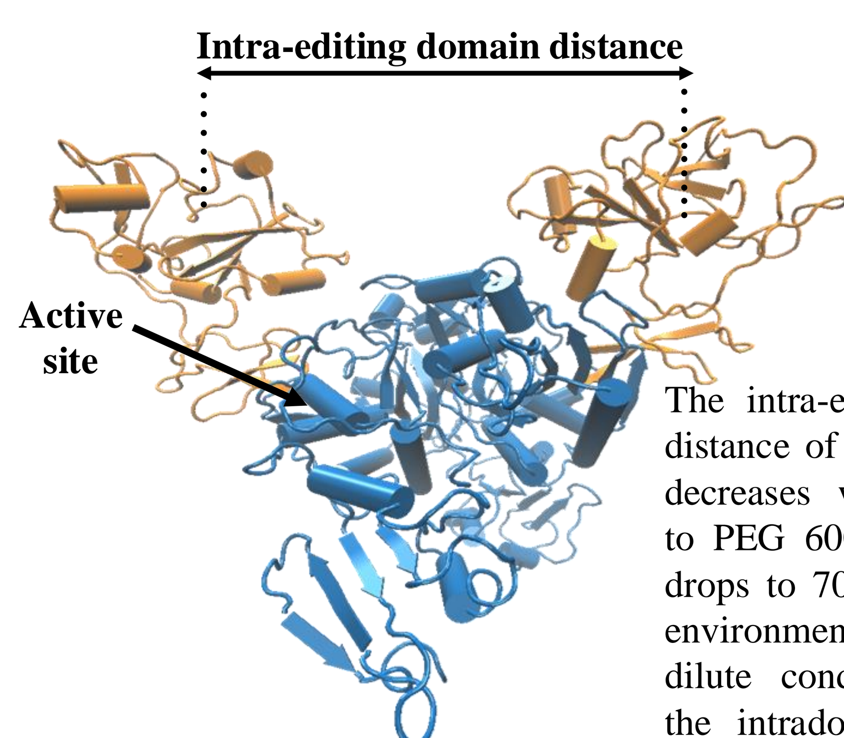


Figure 1. Active site imaging on ProRS in the presence of PEG 600.



The intra-editing domain distance of *E. coli* ProRS decreases when exposed to PEG 600 crowders. It drops to 70Å in crowded environments from that in dilute conditions, where the intradomain distance is 110Å.

Methods

AutoDock- This software was used to perform molecular docking simulations. It uses a combination of algorithms and free energy values to explore the best inhibitor for the target protein.

Visual Molecular Dynamics (VMD)- VMD was employed to model, simulate, and visualize the structural dynamics of the target protein and inhibitors.

ZINC- A database and tools used for screening inhibitors for the target protein.

IQMol- This program was utilized to edit molecules. It provided an easy method to create, manipulate, and visualize molecular structures. Additionally, it's computational tools ensured efficient editing.

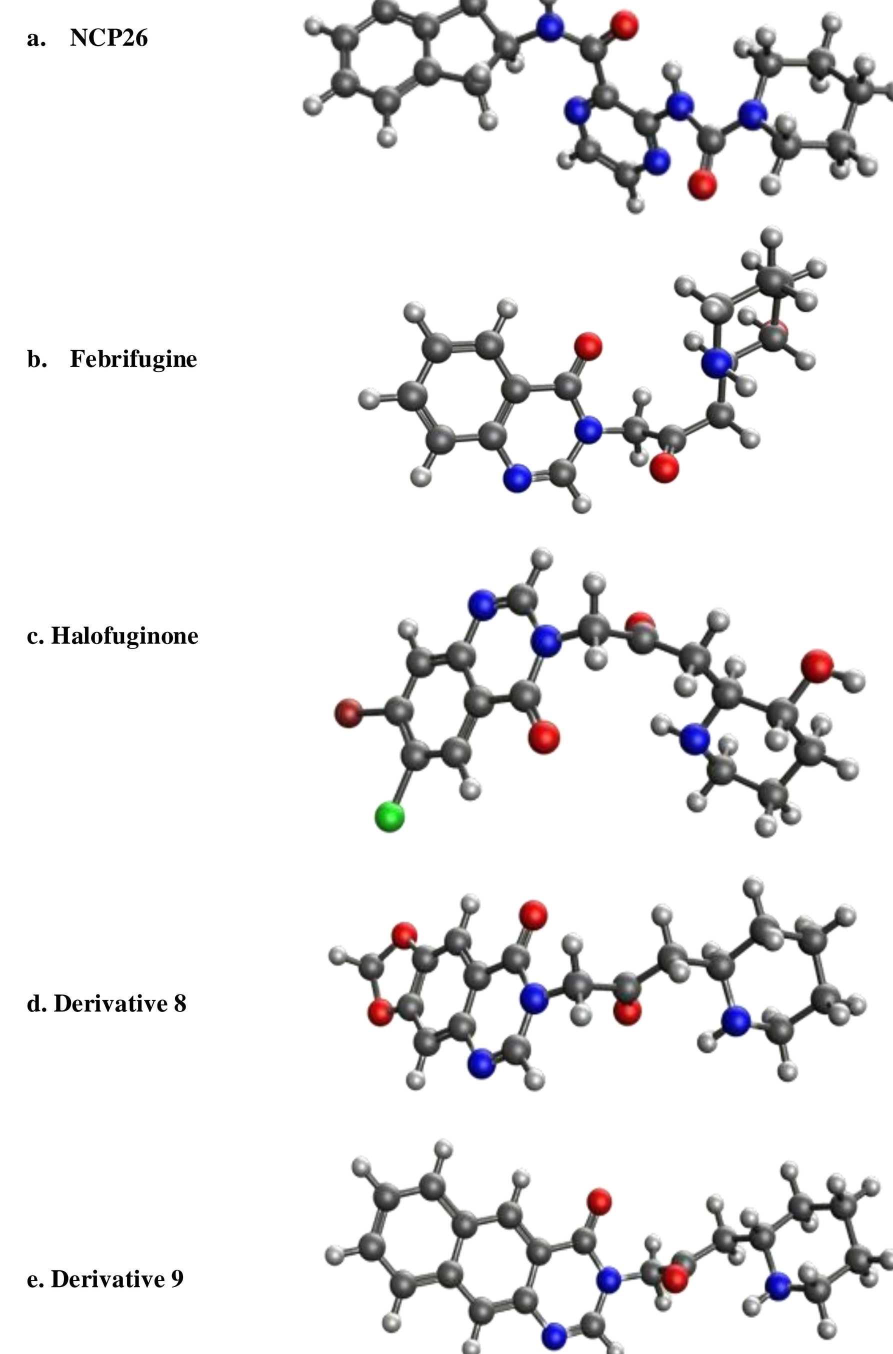
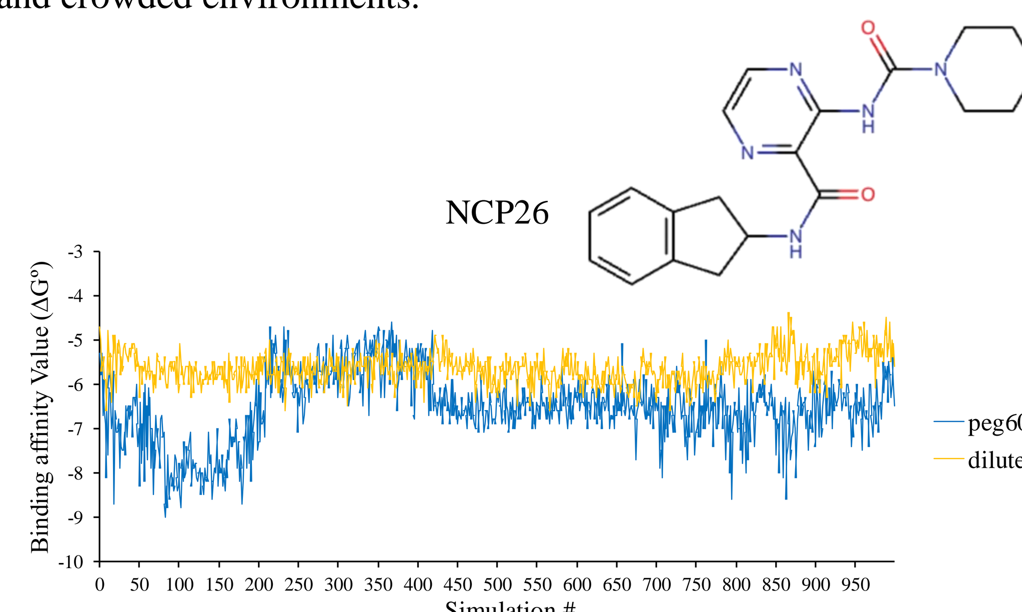


Figure 2. Ball and stick 3D models of inhibitors of ProRS; the white, gray, blue, red colors represent hydrogen, carbon, nitrogen, and oxygen atoms, respectively.

Results

The comparative analysis of binding energies for the above five inhibitors into the active site pocket of *E. coli* ProRS in dilute and crowded environments.



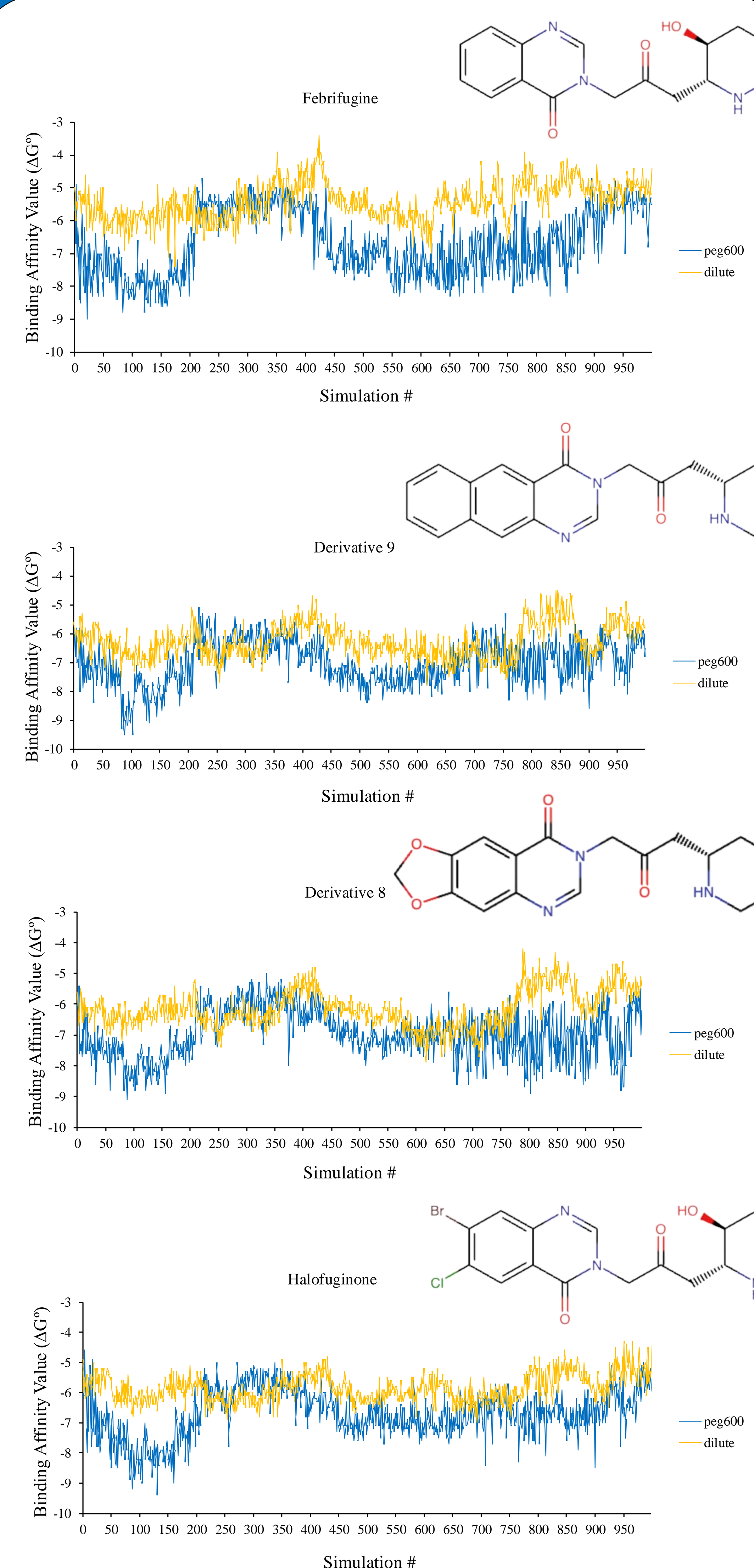


Figure 3. Docking results of NCP26, Febrifugine, Halofuginone, Derivative 8, and Derivative 9 inhibitors. These inhibitors were docked, and the above graphs are the fluctuation of binding energy during the simulated time in dilute and in the presence of PEG 600 crowders.

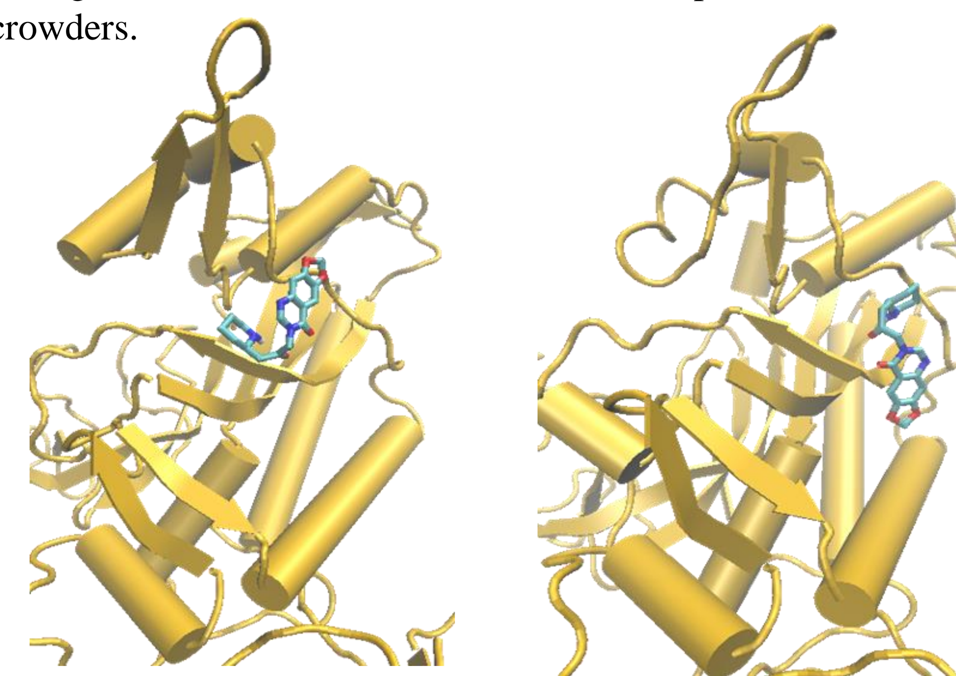


Figure 4. Inhibitors (Derivative 8 docked in E. coli ProRS as depicted by Visual Molecular Dynamics (VMD)

Conclusions

- Most inhibitor screening studies are conducted in dilute condition using the static structure of the target protein. This study involves the active site dynamics in response to the solvated and crowded surroundings.
- This is the first report of high-throughput docking used to probe protein dynamics and molecular crowding, and their impacts on small molecule binding.
- The results obtained from this study demonstrate that the inhibitors bind with Gibbs' binding free energies ranging from -6 to -9 kcal/mol.
- For all five inhibitors, the presence of PEG 600 crowders favored stronger binding as compared to the dilute environment.

Future Directions

This study provided a new insight into the docking of inhibitors in the dynamic active site of target protein. It shows potential to further understand active site orientation and how binding study can be performed by taking into accounts for protein dynamics and crowding effects for screening potent inhibitors. Future works include performing detailed docking and interaction analysis at the active site.

References

1. Cheng, B., Cai, Z., Luo, Z., Luo, Z., Cheng, Y., Yu, Y., Guo, J., Ju, Y., Gu, Q., Xu, J., Jiang, X., Li, G., & Zhou, H. (2022). Structure-guided design of Halofuginone derivatives as ATP ... ACS Publications. https://pubs.acs.org/doi/10.1021/acs.jmedchem.2c01496
2. Keller TL, Zocco D, Sundrud MS, Hendrick M, Edenius M, Yum J, Kim YJ, Lee HK, Cortese JF, Wirth DF, Dignam JD, Rao A, Yeo CY, Mazitschek R, Whitman M. Halofuginone and other febrifugine derivatives inhibit prolyl-tRNA synthetase. Nat Chem Biol. 2012 Feb 12;8(3):311-7. doi: 10.1038/nchembio.790. PMID: 22327401; PMCID: PMC3281520.
3. Kurata, K., James-Bott, A., Tye, M. A., Yamamoto, L., Samur, M. K., Tai, Y.-T., Dunford, J., Johansson, C., Senbabaoglu, F., Philpott, M., Palmer, C., Ramasamy, K., Gooding, S., Smilova, M., Gaeta, G., Guo, M., Christianson, J. C., Payne, N. C., Singh, K., … Oppermann, U. (2023, January 12). Prolyl-tRNA synthetase as a novel therapeutic target in multiple myeloma. Nature News. https://www.nature.com/articles/s41408-023-00787-w
4. Ravindranath, P. A., Forli, S., Goodsell, D. S., Olson, A. J., & Sanner, M. F. (2015). AutoDockFR: Advances in protein-ligand docking with explicitly specified binding site flexibility. *PLOS Computational Biology*, *11*(12). https://doi.org/10.1371/journal.pcbi.1004586

Acknowledgements

- We acknowledge that the University of Wisconsin-Eau Claire occupies the sacred and ancestral lands of Indigenous Peoples. We honor the land of the Ojibwe and Dakota Nations.
- Office of Research and Sponsored Programs, UW-Eau Claire
- Blugold Center for High-performance Computing, UW-Eau Claire
- The National Science Foundation, Award number: OAC 2150191