Usage Report for Fiscal Year 2022

Project Title:

Designing a Bioremediator: Mechanistic Models Guide Cellular and Molecular Specialization

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1. Background and purpose of the project, relationship of the project with other projects

Density Functional Theory (DFT) is a popular quantum mechanical method for predicting the properties of molecules and materials. While DFT is a flexible approach that allows for accurate modelling of a variety of systems, its application has long been limited to systems of only a few hundreds of atoms by its high computational cost. Recently, advances in algorithms and computer power have made it possible to apply DFT to much larger systems (several thousands of atoms).

In this study, we apply those recent developments to the study of the bioremediation of aflatoxins by means of laccase enzymes. Aflatoxins are carcinogenic natural pollutants which can contaminate crops such as maize. Laccase is an environmentally friendly enzyme which has shown promise for degrading aflatoxins, but its efficiency is too low for practical application.

2. Specific usage status of the system and calculation method

BigDFT is a program for performing large scale DFT calculations. Its unique wavelet formalism allows it to be applied to large systems. BigDFT was used to perform calculations on the full toxin-enzyme system as well as for the ΔSCF study of aflatoxins in the gas phase.

PyBigDFT is a python wrapper for BigDFT that can be used to manage the complex workflows required to simulate large systems. It contains unique functionality (called the Complexity Reduction Framework) that allows it to partition systems into chemically meaningful fragments and analyze inter-fragment interactions.

OpenMM is a python framework for performing classical molecular dynamics simulations. Molecular dynamics simulations were used to generate a diverse set of binding poses which were scored using DFT calculations.

XTB is a program for performing semi-empirical quantum chemistry calculations. XTB was used to preprocess our structures.

3. Result

We experimentally assessed the efficiency of laccase from *Trametes versicolor* (TV) on two congeners: aflatoxin B_1 (AFB1) and aflatoxin G_2 (AFG2). The lactone ring of aflatoxin is responsible for both its toxicity and natural fluorescence, and thus the degradation process can be fluorescently assayed. Our experimental results showed a surprising and significant difference between the two congeners; while AFG2 was thoroughly degraded within 48 hours, AFB1 degradation quickly stalled.

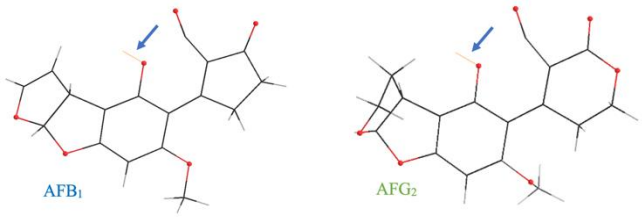

Figure 1: The two congeners and the opening of the lactone ring (arrow) required for detoxification.

To understand the origin of these differences, we utilized ΔSCF to study the properties of the two congeners in the gas phase. This model suggested that while both congeners can be oxidized at the lactone ring, AFB1 also had a second oxidation site on its furan ring. We hypothesized that this could potentially lead to side products with the ring

remaining closed (still toxic); this hypothesis was supported by investigation of the reaction products using LC-MS.

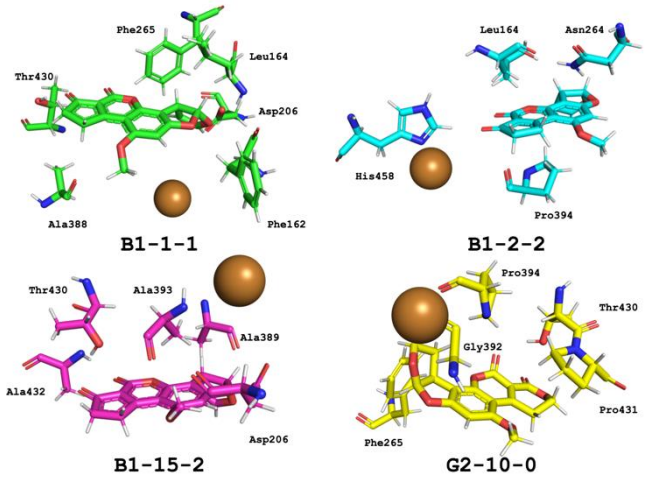

Figure 2: Favorable binding poses and the relevant amino acids for binding as identified by our Complexity Reduction Framework.

To understand better the binding of the two congeners to TV laccase, we performed docking and molecular dynamics simulations of the toxin-enzyme systems. We extracted the most energetically favorable binding poses (assessed using cluster models at the PBE+D3 and B97M-V levels of theory). We performed large scale DFT calculations of the full toxin-enzyme systems, and applied our Complexity Reduction Framework to extract the amino acids which play an important role in binding. By identifying these amino acids, we were able to make suggestions for future engineering of TV laccase for higher efficiency.

4. Conclusion

Using a combination of experimental and multi-scale theoretical modeling, we examined the use of TV laccase for the bioremediation of aflatoxins. Our results showed that significant tuning will be required before it can be deployed in industrial settings. Importantly, the tuning will have to be highly substrate specific, as even two structurally similar congeners show significant differences in degradation dynamics. Our analysis has elucidated the most relevant amino acids for binding, which can be used as a starting point for enzyme engineering.

5. Schedule and prospect for the future

The PyBigDFT framework proved useful for this study. We were able to use it to encapsulate the entire calculation and analysis process within a set of Jupyter notebooks. It enabled the coupling together of calculations performed using different codes and high throughput workflows. This framework was further used in our other studies during the period. For our benchmarking of density functionals, it was useful for automatically generating protein-ligand cluster systems. For the study of lignin, it was used to couple a python-based machine learning framework with high level quantum mechanical calculations. In the next fiscal year, we plan to further develop and assess these capabilities.

6. If no job was executed, specify the reason.

N/A

Fiscal Year 2022 List of Publications Resulting from the Use of the supercomputer

[Paper accepted by a journal]

Chan, Bun, William Dawson, and Takahito Nakajima. "Searching for a Reliable Density Functional for Molecule–Environment Interactions, Found B97M-V/def2-mTZVP." *The Journal of Physical Chemistry A* 126, no. 15 (2022): 2397-2406.

Chan, Bun, William Dawson, and Takahito Nakajima. "Modeling the Conformational Preference of the Lignocellulose Interface and Its Interaction with Weak Acids." *The Journal of Physical Chemistry A* 126, no. 13 (2022): 2119-2126.

Zaccaria, Marco, William Dawson, Darius Russel Kish, Massimo Reverberi, Maria Carmela Bonaccorsi di Patti, Marek Domin, Viviana Cristiglio et al. "Experimental–theoretical study of laccase as a detoxifier of aflatoxins." *Scientific Reports* 13, no. 1 (2023): 860.

[Conference Proceedings]

[Oral presentation]

William Dawson, Luigi Genovese, Takahito Nakajima. “Density functional theory calculations of large systems: interplay between observables and computational complexity.” Challenges and Advances in Solving Eigenproblems for Electronic-Structure Theory. November 2022. Laussane, Swizterland.

William Dawson, Luigi Genovese, Takahito Nakajima. “Productive Large Scale DFT Calculations on the Fugaku Supercomputer.” PSI-K. August 2022. Laussane, Swizterland.

[Poster presentation]

[Others (Book, Press release, etc.)]