## Computational Analysis of the Peptide Hydrolysis Pathway of Serine Proteases: Novel Reaction Engineering Perspectives

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Comprising more than one third of all proteases, serine proteases are the largest known category in the protease enzyme subclass. These enzymes are ubiquitous in nature, are present in all known organisms, and can be active under temperatures ranging from 4°C to 65°C. Additionally, serine protease-based products occupy over 90% of the current global protease market, spanning household detergents, waste management, leather processes, food processing, biomedical applications, and more. However, issues behind the hydrolysis reaction mechanism via this enzyme have arisen recently in literature—particularly regarding a high-energy transition state called the "histidine (His) flip." We propose an alternative, charge-neutral (with no charge separation) step that potentially allows for a more favorable reaction pathway. Better understanding this new hydrolysis reaction pathway will allow us to develop more accurate reaction rate law expressions to increase the efficiency of industrial processes involving these enzymes.

Investigation of the serine protease reaction pathway involves implementing a quantum mechanics (QM) cluster analysis on the enzyme active site, employing density functional theory (DFT) methodology. QM-cluster calculations allow for a more accurate and higher theoretical level and free transition state (TS) determination, as opposed to just a free energy surface, for example. To choose how big of a cluster to include, we decided to include all amino acid residues that are directly involved in the catalytic process, as well as all neighboring amino acids that are important to maintain the active site topology and those participating in hydrogen bonding with the reacting atoms. We propose that the inclusion of all these factors provides a robust model with minimal geometry restriction (i.e., freezing

of the atoms at the boundary of the cluster).

This project is still ongoing, but we have discovered certain preliminary results regarding the serine protease reaction pathway. Our reaction coordinate results are in accordance with the historical reaction profile shape but seem to be overestimating the activation barriers as compared to literature. We expect that increasing the cluster size will result in lowering these energies. Our proposed alternative mechanism has indeed yielded a more favorable reaction profile, and we will continue to investigate this new profile by calculating its corresponding transition states, along with comparing against profiles calculated with other density functionals.

The impact of this study is to provide more efficient reaction parameters for chemical processes using serine proteases, from length scales ranging from industrial bioreactors to drug design. From our calculations, we will soon be able to determine the reaction rate constants, which could eventually be used in reaction rate law expressions. An effective reaction rate law would enable better prediction of many reaction properties, such as how much reactant is needed, what concentrations of reactants is ideal, or how long it would take for the reaction to proceed—all highly important factors when optimization large-scale bioreactors or targeted drug delivery dosages.

## Statement of Research Advisor

Dylan Pollard has contributed to ongoing research efforts in computational modeling of catalysis, working closely with Ashraf Ali and group members. The project gave him the opportunity to connect fundamental chemical

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engineering and chemistry concepts to real-world applications.

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**Fig. 1.** QM-cluster active site of serine protease used in this study.

## References

[1] Chelulei Cheison, S., Brand, J., Leeb, E., Kulozik, U., "Analysis of the Effect of Temperature Changes Combined with Different Alkaline Ph on the B-Lactoglobulin Trypsin Hydrolysis Pattern Using Maldi-Tof-Ms/ Ms," Journal of Agricultural and Food Chemistry, 59, pp. 1572–1581, (2011).

[2] Gudmundsdóttir, Á., Pálsdóttir, H. M., "Atlantic Cod Trypsins: From Basic Research to Practical Applications," Marine Biotechnology, 7, pp. 77–88, (2005).

[3] Salwan, R., Sharma, V., "Trends in Extracellular Serine Proteases of Bacteria as Detergent Bioadditive: Alternate and Environmental Friendly Tool for Detergent Industry," Archives of Microbiology, 201, pp. 863–877, (2019).

[4] Sundus, H., Mukhtar, H., Nawaz, A., "Industrial Applications and Production Sources of Serine Alkaline Proteases: A Review," J. Bacteriol. Mycol. Open Access., 3, pp. 191–194, (2016).

## Authors Biography



Dylan Pollard is a senior at Auburn University majoring in chemical engineering and physics. He will attend the University of California, Santa Barbara for their PhD program in chemical engineering. His undergraduate research included heterogeneous/ enzymatic catalysis and machine learning (REU).





Dr. Andrew Adamczyk is an assistant professor in the Dept. of Chemical Engineering at Auburn University. He has been at Auburn since 2018 and conducts research using computation chemistry and physics, kinetic modeling, and reaction engineering principles to better understand complex reacting systems.

Dr. Ashraf Ali is a postdoctoral researcher in the Kieslich Lab in the Dept. of Chemical Engineering at Auburn University. He recently received his PhD at Auburn University. Ashraf uses mathematical optimization, and data science to predict how molecular changes affect their therapeutic properties.