Experimental Characterization of Allosterically Relevant Mutations in Beta-Glucosidase Identified Using a Dynamics-Guided Computational Approach

Lau, Michael (School: Arizona College Prep High School)
Lakamsani, Sneha (School: Hamilton High School)
Igwe, Maxwell (School: ASU SCENE)

Enzymes, known informally as the workhorses of the cell, are proteins that quicken chemical reactions. Traditional research on improving enzyme efficiency focuses on the active site or binding location. However, the entirety of an enzyme is constantly moving and shifting, and mutations in locations other than the active site may have a profound effect on enzymatic function. This principle is known as allostery. With this principle in hand, we aim to identify, mutate, and experimentally characterize allosteric mutations in ß-glucosidase through a dynamic lens. ß-glucosidase was chosen as the protein of interest because of its burgeoning potential in the biofuel industry. First, ß-glucosidase undergoes dynamics analysis to identify amino acids dynamically coupled with but far from the active site. This location is mutated using Foldit software to observe its effects on the protein. Once a residue is selected for mutation, we use PCR mutagenesis to molecularly clone our gene of interest, insert the gene into plasmids, and transform E. coli cells with these mutant plasmids. Finally, the protein is expressed, purified, and characterized using a colorimetric assay to ascertain the mutations' effect on the protein's function. Ultimately, the allosteric mutation, N160E, decreased the efficiency of ß-glucosidase even though it lies ~18 Å away from the active site. A second control mutation near the active site, T355N, was highly deleterious to enzyme function. Simple dynamics analysis and Foldit mutations allowed us to find mutations far from the active site that affect enzyme function, suggesting the promise of this approach in further understanding enzyme dynamics and function. Additionally, these methods may help better optimize enzymes utilized in industrial applications.

Awards Won: