Analysis of the Effects of H328N Mutation on β-Glucosidase B Catalytic Efficiency and Thermostability

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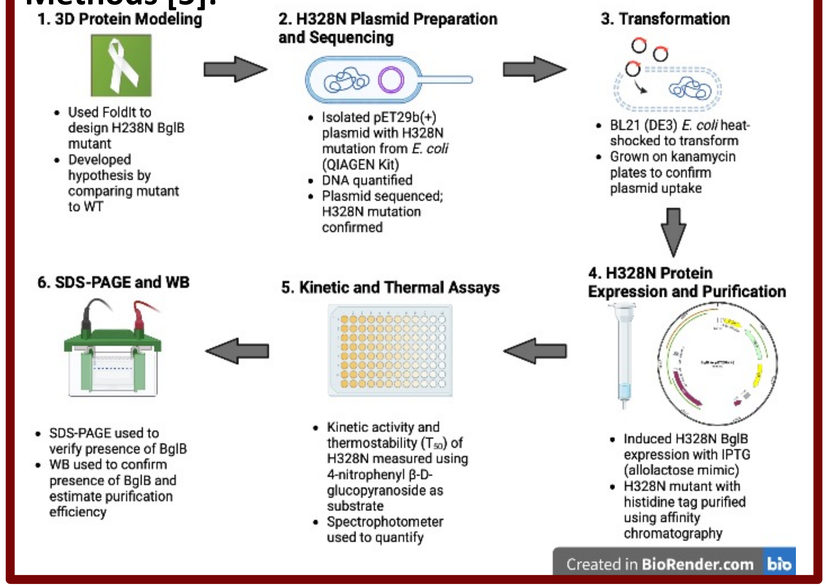
Introduction:

In silico protein modeling is a useful tool to predict tertiary or quaternary protein structure from a string of amino acids. However, we are not yet able to reliably predict a protein's function using its final structure [1]. Predicting protein function from structure would allow researchers to design and create novel catalysts, drugs, and other useful proteins more easily. Design 2 Data (D2D) is a nationwide project based out of Dr. Justin Siegel's lab at UC Davis attempting to address this problem [2]. The D2D database collates data from different β-glucosidase B (BglB) point mutations from labs nationwide, looking for patterns that can be used to train artificial intelligence algorithms in finding protein function from structure. BglB is a 53kD glucose hydrolase enzyme isolated from the Gram-positive bacterium *Paenibacillus polymyxa* [3]. Along with cellulase, BglB converts cellulose to free β-glucose monomers [4]. Here, we characterized one BglB point mutant, histidine to asparagine at amino acid position 328, or H328N. We collected data on protein expression, thermostability, and Michaelis-Menten kinetic constants (K_M, k_{cat}, and k_{cat}/K_M). We hypothesize that BglB mutant H328N will demonstrate similar catalytic efficiency and thermal stability in comparison to the wild type. Comparing the mutant overall Foldit score of -1089.075 to the wild-type score of -1089.697 suggests a high likelihood of expression. Based on intermolecular modeling analysis, there is little change in local interactions because both histidine and asparagine are both hydrophilic, and the local score changes from 1.252 to a mutated score of 1.625, which is a small increase. Furthermore, previously published data on the similar mutation H119N support this hypothesis, as this mutation demonstrates similar expression levels, slightly decreased catalytic efficiency, and slightly increased thermal stability.

Methods [5]:



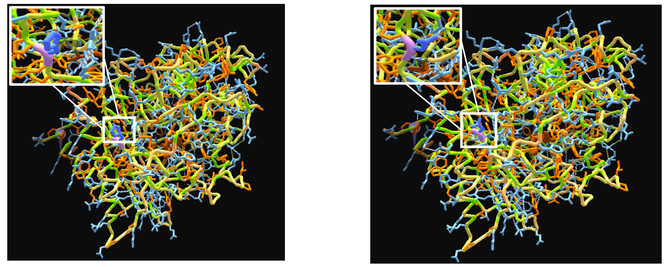


Figure 1. Foldit-generated 3D models of Wild-type (WT) BglB (left) and its H328N variant (right). The left image features a highlighted Histidine residue at the mutation site, while the right image showcases the H328N mutation. The molecular surroundings include various residues with hydrogen bonds shown in blue, disulfide bridges in yellow, polar residues in cyan, and hydrophobic residues in orange.

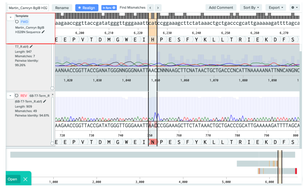


Figure 2. Sequence comparison confirming H328N point mutation. Wild-type amino acid sequence displayed on top while the H328N variant sequence is presented below.

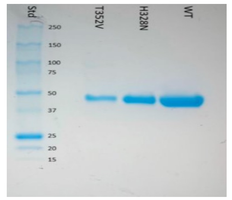


Figure 3. SDS-Page analysis of BglB H328N Variant. Assessing expression and purity of the mutated protein.

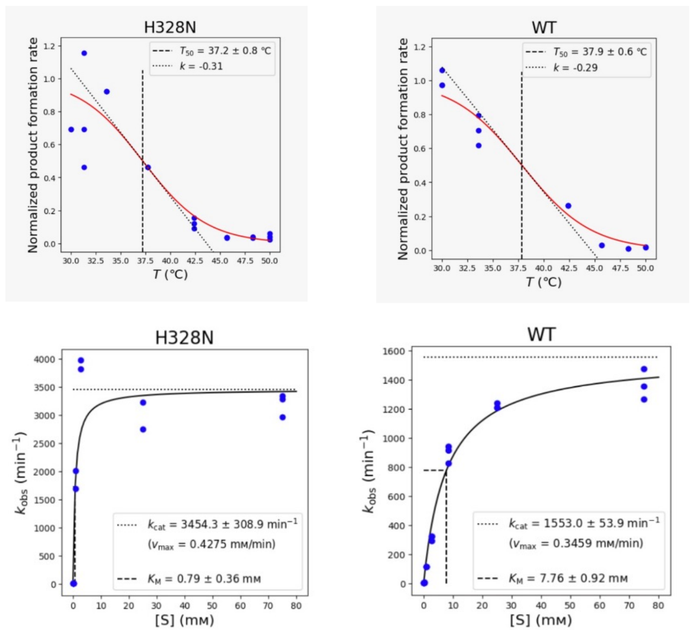


Figure 4. Comparative analysis of kinetic and thermostability assays for wild-type BglB (right) and H328N variant (left). For the kinetic assay, a 1:100 dilution of protein concentration was used. The graph represents a modified Michaelis-Menten plot, displaying K_{obs} values, which measure the direct rate of product formation over time normalized for enzyme concentration. The thermostability assay employs the T_{50} method, an indirect measure of protein denaturation, to determine the temperature at which the enzyme loses 50% of its initial activity. A temperature gradient of 30°C to 50°C was used, with the wild-type BglB having a T_{50} of 40°C.

Discussion:

We aimed to characterize and analyze the BglB H328N mutant through various experimental approaches, including SDS-PAGE, kinetic assays, and thermostability assays. Our results provided insights into the catalytic efficiency, thermostability, and expression of H328N mutant compared to the wild-type BglB enzyme.

- Our kinetic assay data indicated that the H328N mutant exhibits higher catalytic activity than the wild-type enzyme, as evidenced by the higher k_{cat} and V_{max} values. However, we must acknowledge that the precision of our kinetic assay data was not optimal. To confirm these findings, it would be beneficial to repeat the assays and further investigate the effect of the H328N mutation on the enzyme's catalytic efficiency.
- The thermostability assay results revealed that the H328N mutant exhibits thermostability similar to the wild-type enzyme, having a T_{50} value merely 0.7° C lower than the wild-type. This observation implies that the point mutation does not substantially impact the protein's overall stability.
- The SDS-PAGE analysis confirmed the presence and high expression of the H328N mutant protein, indicating successful protein expression and purification. Additionally, the Western Blot analysis further validated the presence of our protein of interest.
- Our findings highlight the need for further investigation and improvement of the Foldit algorithm to enhance the accuracy of protein functionality predictions. Although some limitations were observed in the precision of the kinetic assay data, our findings contribute to a better understanding of the H328N mutant's properties and highlight the need for further research to improve protein modeling algorithms like Foldit.

References:

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5. Created with BioRender.com.

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