β-glucosidase Mutation T352V Catalytic Efficiency and Thermal Stability

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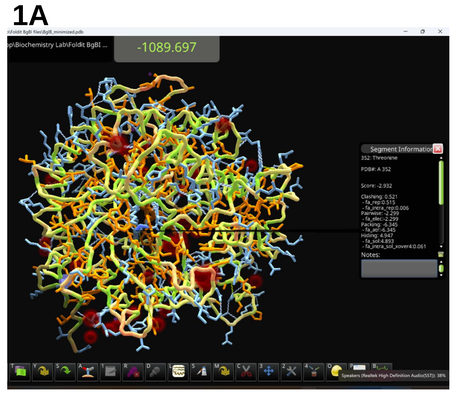
Introduction

- Design to Data (D2D) aims to produce data to improve the predictive restrictions of protein function for modeling software.
- β-glucosidase (BglB), an enzyme that completes the final step during cellulose hydrolysis (Xia, 2021).
- Structural and functional algorithms are important in aiding research regarding the function of β-glucosidase, such as its role in Gaucher's Disease (Michelin, 2004).
- Hypothesis: β-glucosidase (BglB) mutant T352V will demonstrate decreased catalytic efficiency and thermal stability compared to the wildtype because its overall Foldit score suggests expression and points to decreased local hydrogen bonding interactions. Furthermore, previously published data on mutant T352A shows decreased catalytic efficiency and thermal stability (Carlin, 2017).

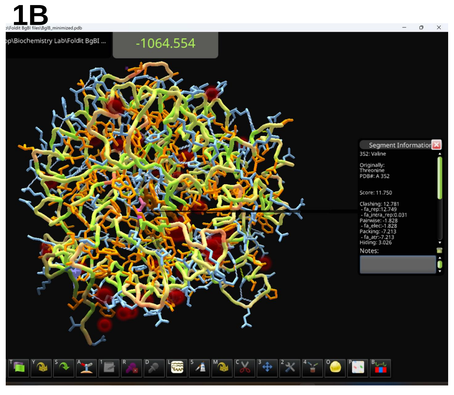
Methods

- Mutant and Hypothesis Design
- Plasmid Preparation, DNA Quantitation & Sequencing
- Transformation of Bacteria
- Expression & Purification of Mutant BglB
- Enzyme Kinetic Assay
- Thermal Stability Assay
- Characterization of Affinity Purified Protein Using SDS-PAGE
- Data Analysis

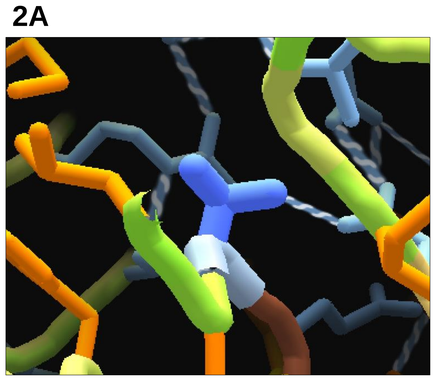
1A



1B



2A



2B

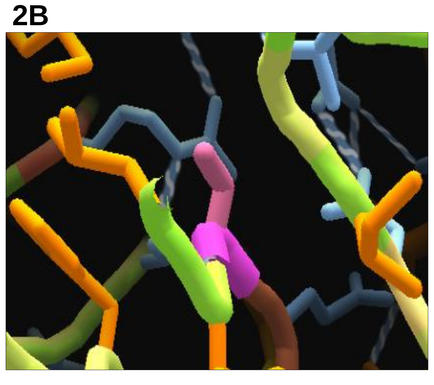


Figure 1A & 1B: Foldit modeling software images with the overall scores of the BglB wild type (left) and mutant T352V (right).

Figure 2A & 2B: Foldit modeling software images showing the mutation of Threonine (left) to Valine (right).

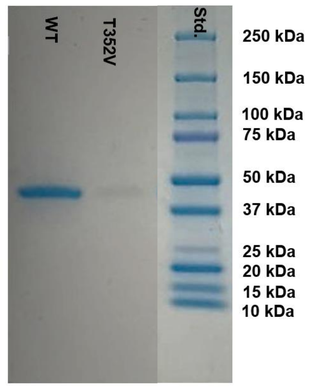
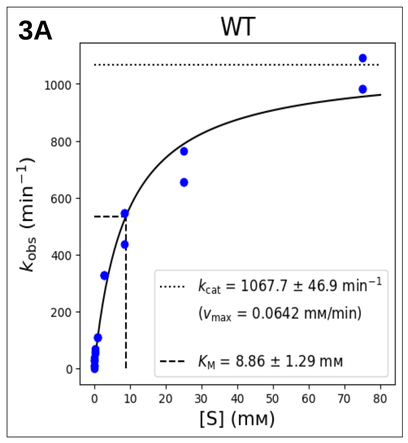


Figure 2: Analysis of protein expression. The protein was expressed, purified, and loaded onto polyacrylamide gel according to the BioRad manual. Using the Beer Lambert equation, a calculated quantity of 0.045 mg/mL was obtained.



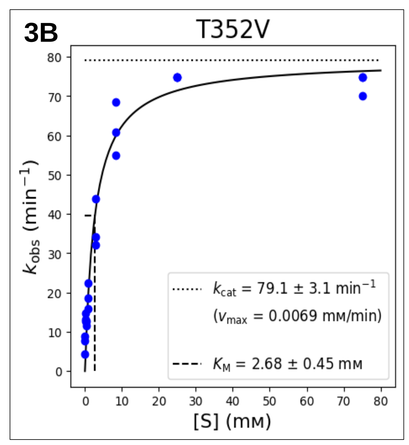
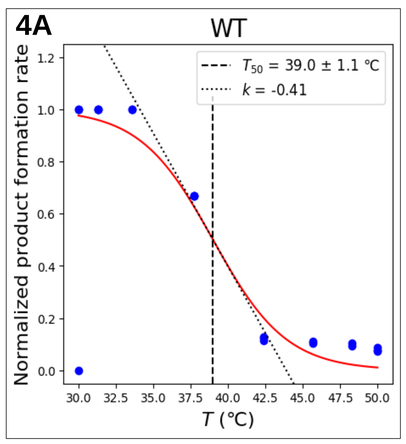


Figure 3A and 3B: Catalytic activity of T352V (B) compared to the wildtype (A). Purified protein and substrate were mixed. The color change in the enzyme-substrate-product solution at a constant temperature was measured. The catalytic activity of T352V and the wild type were calculated using Michaelis Menten's equation.



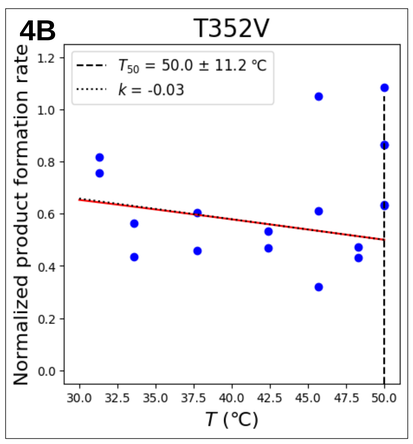


Figure 4A and 4B: Thermal stability of T352V (B) compared to the wild type (A). Purified protein was incubated in a thermal cycler with a gradient set from 50 ºC to 30 ºC. The heated enzyme and substrate were mixed, and the color change in the enzyme-substrate-product solution was measured. The thermal stability of T352V and the wild type were calculated using Michaelis Menten's equation.

Discussion

- After implementing the mutation, the overall Foldit score (Figure 1) decreased and there was a loss of hydrogen bond, suggesting unlikely expression.
- SDS-PAGE analysis (Figure 2) confirms the expression and purity of BglB mutation T352V, but the faint line suggests low expression.
- A calculated quantity of 0.045 mg/mL further confirms the expression and purity of BglB mutation T352V.
- The kinetic assay (Figure 3 A & B) shows a decrease in Kcat/Km values from the WT to the mutant T352V, indicating a decrease in catalytic efficacy, supporting the hypothesis.
- The thermal assay (Figure 4 A & B) shows no activity, suggesting an error or the temperature range is too broad, meaning the protein loses its function and denatures below 30 C°. A second thermal assay should be conducted with a narrower temperature range of 30-25 C°.
- Further research should be conducted on how this mutation affects secondary structure formation and substrate binding.

Acknowledgements

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References

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Michelin, K et al. 2004. International Journal of Clinical Chemistry vol. 343(1) pgs. 145–153. PMID: 15115687
Xia, w et al. 2021. Biomolecules vol. 11(12) pgs. 1882-2021. PMID: 34944526