Characterization and Analysis of β-Glucosidase B (BglB) Mutant M114S

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Background

BglB is a protein component of the cellulase complex that assists in breaking down cellulose and other β(1→4) linked sugars. The goal of the Design to Data (D2D) project is to improve the ability of modeling software to predict protein function from structure.[1] We will generate a single point mutation in BglB, M114S, and perform kinetic and thermal assays to gather empirical data, including catalytic efficiency (K_{cat} /K_m), turnover rate (K_{cat}), and substrate affinity (K_m). Based on previously published data on similar mutations, such as M261D, and an overall change in Foldit score greater than 1, we hypothesize that M114S will demonstrate decreased catalytic efficiency (K_{cat}/K_m) and thermal stability (T_{50}) in comparison to the wild-type.[2]

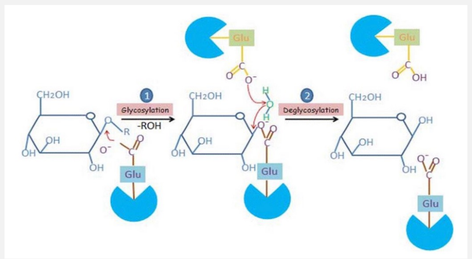

Figure 1. The Proposed Two-Step Hydrolysis Mechanism of BglB. [2]

Methods

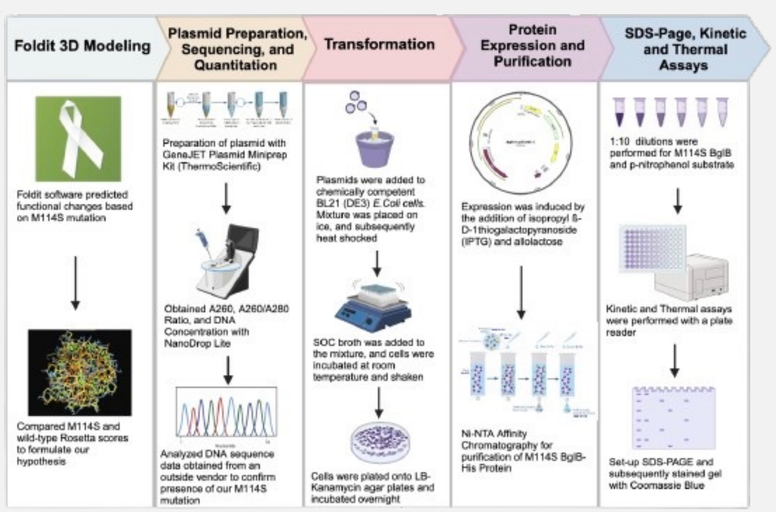

Figure 2. Overview of Methods

Results

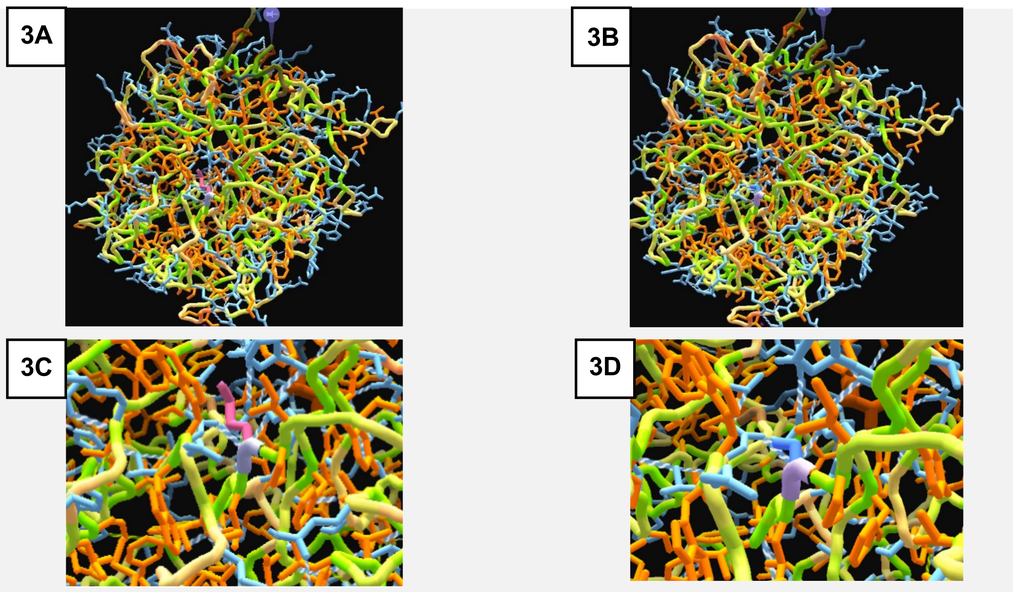

Figure 3. Foldit 3D Predictive Models of Wild-Type (WT) BglB and M114S Mutant.
Key: ideal hydrogen bonds, non-ideal hydrogen bonds, disulfide bridges, polar residues, and hydrophobic residues. **3A)** WT BglB protein structure. **3B)** M114S protein structure. **3C)** Position 114 in WT BglB, in which **methionine** is present. **3D)**Position 114 in M114S, in which **serine** is present.

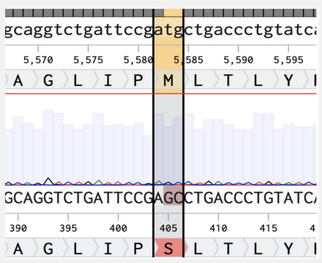

Figure 4: Sequence Alignment of WT BglB (top) and M114S (bottom).

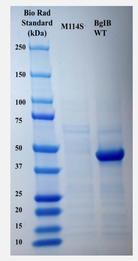

Figure 5: SDS-PAGE of BglB M114S mutant. Lane 1 – BioRad Standard Ladder, Lane 2 – M114S, Lane 3 – BglB WT.

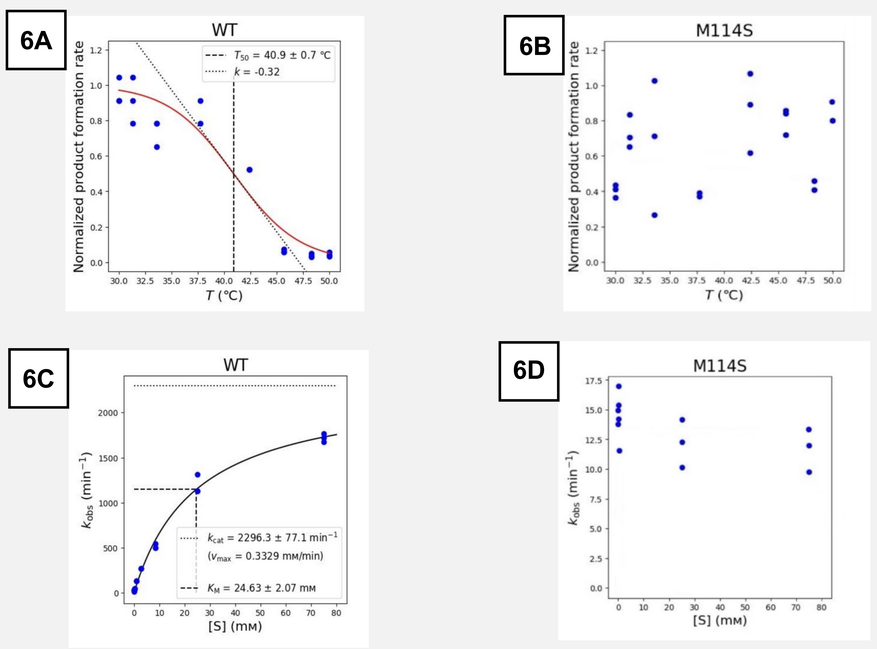

Figure 6: D2D Generated Graphical Representations of Kinetic and Thermostability Assay Data for BglB WT (left) and M114S mutant (right). 6A and 6B) Thermal assay data yielded an indirect measurement of thermostability (T_{50} value). 6C and 6D) Kinetic assay data is plotted as a variation of Michaelis- Menten graph, plotting the K_{obs} (min -1) versus substrate concentration (mM).

Conclusion

Our data neither supports nor negates our hypothesis.

- Concentration of expressed M114S had a value of 0.086 mg/mL, indicative of low expression
- Mutant band was not present at the approximate distance of the wild-type band (51 kDa) on the SDS-Page gel
- D2D application considered some kinetic assay data points to be outliers (Fails to demonstrate a decrease in Kobs with a decrease in substrate concentration)
- Assay graphs demonstrate a lack of correlation.

In general, this data indicates that M114S was **not** expressed. This could have resulted from systematic error or the unstable nature of M114S. As a result, M114S could not be obtained possibly due to inadequate purification, degradation, etc.

Next steps:

- Retest M114S mutant and upload more data to the D2D database § Collect and analyze samples throughout purification to limit systematic error and evaluate whether protein was sequestered by bacteria to its insoluble form

Acknowledgements

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