Analysis of the H178C Mutation on β-glucosidase (BglB) Protein

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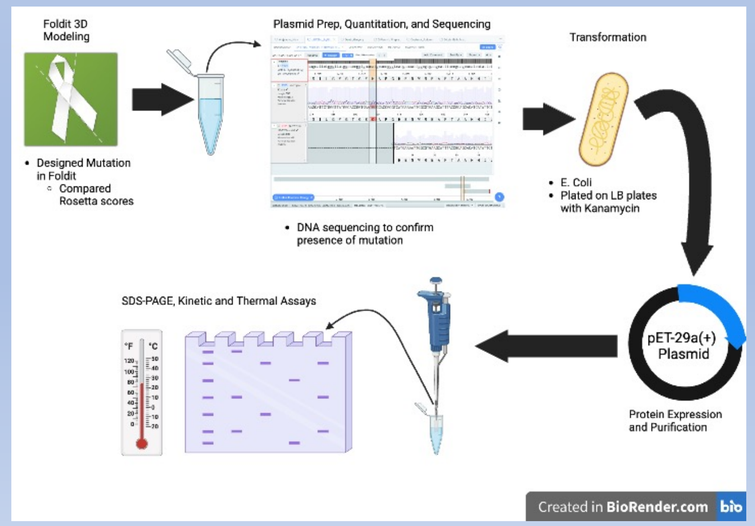
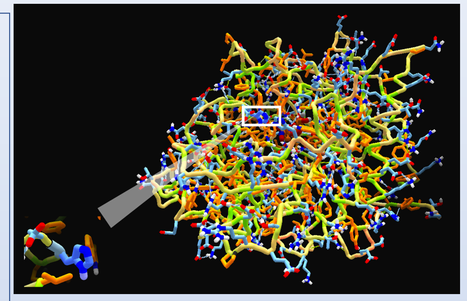
Introduction

- The goal of Design2Data (D2D) is to train artificial intelligence algorithms to predict protein functionality based on mutations in the amino acid sequence.
  - Currently have no way to accurately predict how amino acid sequence changes of a protein will affect the function.[1]
  - Having AI algorithms that can accurately predict function allows us to analyze a mutant pool without having to test them all.
  - BglB is an enzyme that catalyzes hydrolysis of cellulose and other β-1,4 linked sugars.[2]
- We hypothesize that BglB mutant H178C will demonstrate decreased catalytic efficiency (k_{cat}/K_m) and thermal stability (T_m) in comparison to the wild type (WT) because its overall Foldit score suggests a lower likelihood of expression, and it had minimal effect on local hydrogen bonds and hydrophobic interactions. Furthermore, previously published data on similar mutations H178A and H178S support this hypothesis.[3]





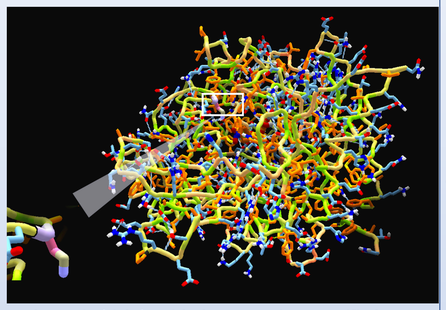


Figure 1: **Foldit 3D modeling of the Wild Type BglB protein (left) and the mutated protein (right). With the respective magnified section of the Histidine residue and Cysteine residue. The right photo shows the point mutation of Histidine to Cysteine in the BglB protein, with the mutation being magnified.**

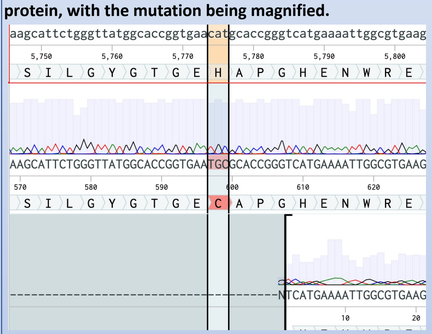


Figure 2: **Amino Acid sequence to confirm that the mutation was present, with the WT sequence on top and the mutated sequence on the bottom.**

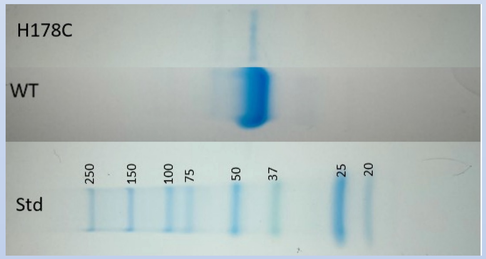
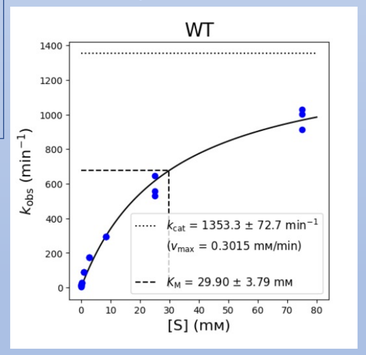
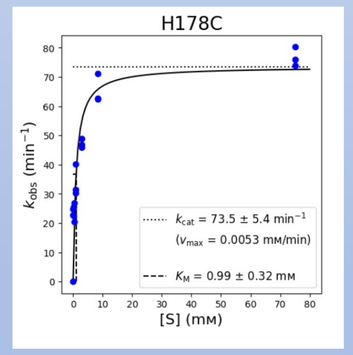
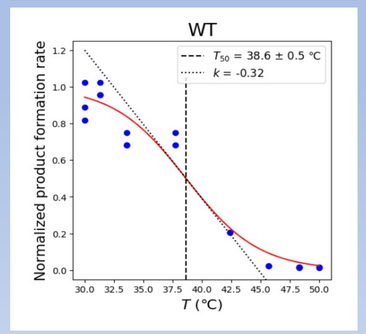


Figure 3: **SDS-PAGE of BglB mutation H178C to determine the expression and purity of the protein under consideration.**







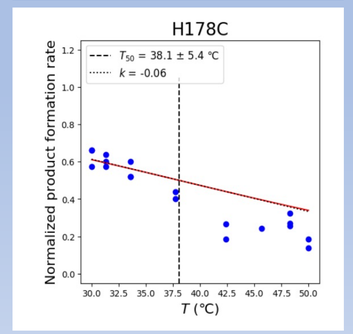


Figure 4: **Graphs from D2D representing kinetic activity of the WT (top left), as well as mutated BglB (top right) are shown. Also pictured are the graphs from the Thermostability assay results with the WT (bottom left) and mutated protein (bottom right) pictured. Concentrations for the samples were diluted 1:10. Kinetic assay graphs show k_{obs} being plotted compared to substrate concentration. For the thermal assay, we measured the T_{50} value which can indicate when the enzymatic activity is decreased by 50 percent, as well as being an indirect measure of how denatured the protein is.**

Discussion

We did express and purify our protein as seen by the appropriate band present in the SDS-PAGE.

Our initial hypothesis was not supported by the data.

The specificity constant (catalytic efficiency) of BglB WT was 45.26 $mM^{-1}min^{-1}$ and 74.24 mM^{-1} min^{-1} for BglB mutant H178C. So, catalytic efficiency increased by nearly 30 mM^{-1} min^{-1}, and we had hypothesized that it would decrease.

- K_m went down, meaning increased affinity between substrate and mutant enzyme compared to substrate and WT enzyme.

The thermal stability (measured with T50) of BglB WT was 38.6 ± 0.5 °C and was 38.1 ± 5.4 °C. For future work, it is detrimental to perform the experiments with a high level of precision. Since we are using such low concentrations throughout this experiment, even minimal error can cause drastic changes to the data.

References

1. Design2Data, https://www.d2dcure.com/about/#D2D
2. Singh, G, et al. 2016. Catalytic properties, functional attributes and industrial applications of β-glucosidases. *Vol 6, PMID: 28330074*
3. Carlin DA, Hapig-Ward S, Chan BW, Damrau N, et al. (2017) Thermal stability and kinetic constants for 129 variants of a family 1 glycoside hydrolase reveal that enzyme activity and stability can be separately designed.

Acknowledgements

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