Determining the enzymatic efficiency and thermodynamic stability of BglB-H223A

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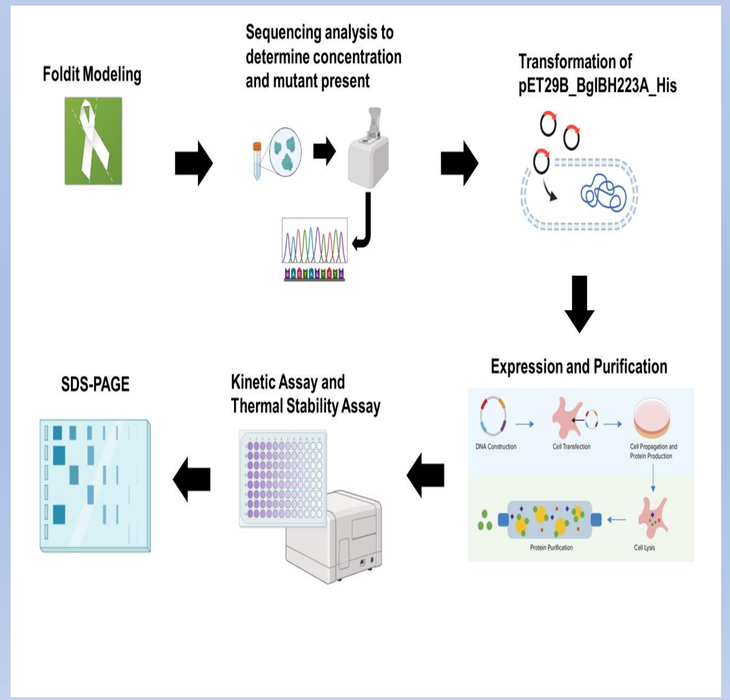
Introduction

- β-Glucosidase (BglB): a component of cellulase enzyme complex that is important for hydrolysis of cellulose and other (β1→ 4)-linked sugars.
- Design2Data (D2D)'s Goal: create and improve an AI program to predict the function of a protein based on the given amino acid sequence.
- Mutation: H223A which replaced Histidine by Alanine at 223, which remove the hydrogen bond with R338
- Hypothesis: BglB-H223A will decrease catalytic efficiency and thermal stability compared to the wild type based on Foldit score
- To support our hypothesis, we using:
  + Enzyme Kinetic Assay
  + Thermal Stability Assay
  + SDS-PAGE

Method



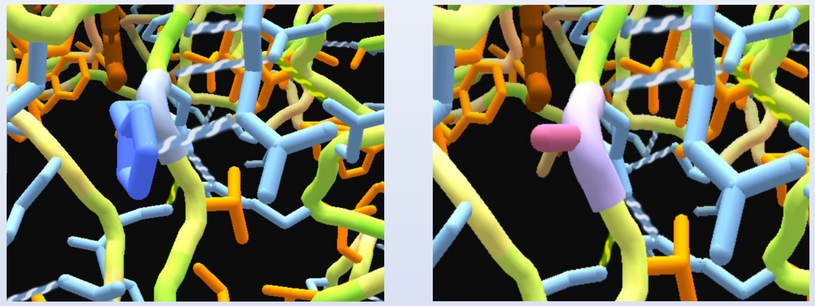


Figure 1: Wild type of BglB protein (left) at location 223 with Histidine and energy: -1089.697. Mutation H223A (right) made the energy increased to -1086.492 → protein less stable

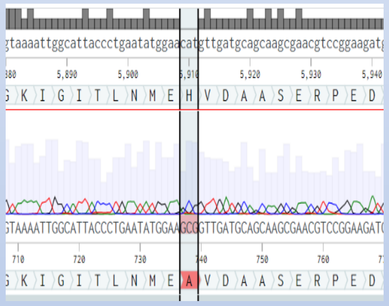


Figure 2: Sequence Analysis Data confirm the H223A mutation (red)

Figure 3: SDS-PAGE to determine the H223A is expressed and purified (between 50 to 37 kD)

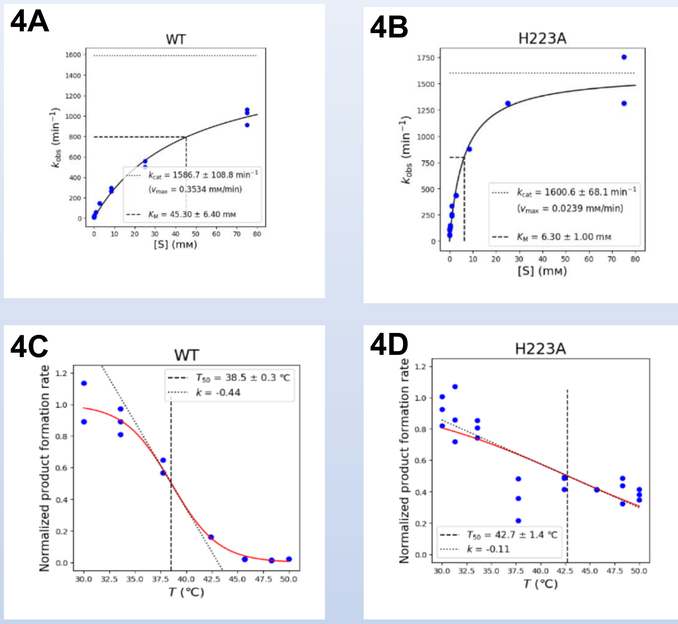


Figure 4: D2D graph of Kinetic and Thermostability Assay for BglB wild type (left) and H223A (right). Substrate concentration were diluted by 1:100 dilution factor. H223A have higher Kcat/Km and T50 than the WT

References/Acknowledgement

- Carlin DA, 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. PLoS ONE 12(5): e0176255
- Naraian R, et al. (1970) Chapter 6 – Penicillium enzymes for the saccharification of lignocellulosic feedstocks. Semantic Scholar
- Design2Data. (n.d.). Retrieved April 15, 2023, from https://d2d.ucdavis.edu/
- We thank Ashley Vater and the Justin Siegel Lab at UC-Davis and National Science Foundation for helping us with this project

Discussion

- Based on Kcat/Km data the mutant H223A have higher catalytic efficiency than the wild type and it also have higher T50 which is higher thermodynamic stability than the wild type → opposite with predicted hypothesis
- Low protein yield (0.08) → incorrect during the experiment, and/or insufficient protein would be produced
- SDS-PAGE → protein was expressed and purified

→ Put data in D2D → help improving the AI in predicting protein function