Determining the enzymatic efficiency and thermodynamic stability of BgIB-H223A
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Introduction
• β-Glucosidase (BgIB): 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: BgIB-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 BgIB 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)

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

References/Acknowledgement
- We thank Ashley Vater and the Justin Siegel Lab at UC-Davis and National Science Foundation for helping us with this project