Characterization Of Enzymatic Activity And Thermostability Of β-glucosidase B (BglB) Histidine-328-Asparagine For D2D Database Contribution

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INTRODUCTION

Design2Data (D2D): Through Siegel Lab collaborating with students, we are able to contribute and generate databases big enough to improve protein design algorithms *(Huang 1)* [2]

Advantages of Prediction Algorithms: The function of a protein is mostly dependent on its 3D structure, which predictive algorithms can accurately guess. Protein structure prediction/modeling is very useful because we are able to learn theoretical protein structure and dynamics, by utilizing these algorithms. Without them, the experiments are costly, complex, and time consuming. *(Zhang)* [4]

Beta-glucosidase B (BglB): β-Glucosidase occurs in bacteria and even highly evolved animals. It catalyzes the hydrolysis/ degradation of glucosylceramide into ceramide and glucose, and it is beneficial to study to better understand the cellulose degradation mechanism. *(Wade 250)* [3]

Hypothesis: β-glucosidase (BglB) mutant H328N will demonstrate a decreased catalytic efficiency (Kcat/KM) and thermostability (Tm) in comparison to wild type [Foldit score suggests increase in energy (from -1089.697 to -1089.078)]. It will be slightly less stable after the mutation, even though no new clashes and only few voids form. Intermolecular modeling analysis (local score) also points to increased local interactions (from 1.250 to 1.625).

OVERALL METHODS

1) Identify H328N mutation to characterize (using Foldit) & hypothesize
2) Confirm H328N presence (Sanger Sequencing)
3) Express normal and mutant BglB and purify them through Affinity Chromatography
4) Characterize enzymatic activity (Kinetic assay: to measure binding rate) and Thermostability of normal and mutant BglB
5) SDS-Page (Gel to visualize protein components separated by molecular weight)
6) Put data into D2D database, conclude if hypothesis was supported

RESULTS

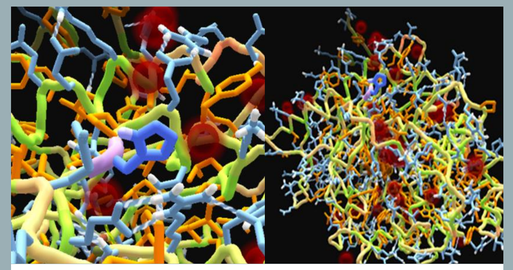


Figure 1a. Amino acid Histidine Close-up (location 328 on backbone) highlighted in pink & blue (Wildtype)
Figure 1b. Full pET29b_Bglb_His protein with Histidine at 328. (Energy Score: -1089.697)

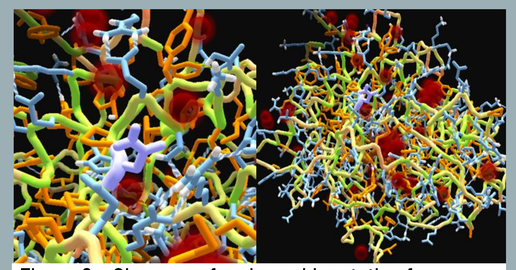


Figure 2a. Close-up of amino acid mutation from Histidine to Asparagine (H328N): energy increase can be visualized.
Figure 2b. Full view of BglB protein after the mutation (Histidine-328-Asparagine): a few voids appeared

Figure 3. (Top is wildtype, bottom is mutant) Sanger Sequencing data verifies Histidine 328 Asparagine (H328) mutation located at ~6226

Protein Quantitation Data

	Sample	A280	Concentration (mg/mL)
1	H328N	0.127	0.0578

Figure 4. Amount of protein yield was lower than desired, but protein still detected. Absorbance at 280 nm through NanoDrop Spectrophotometer.

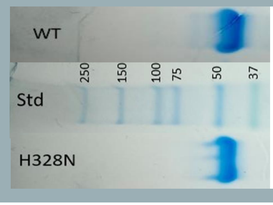
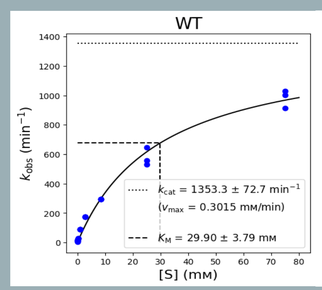


Figure 5. In gel, WT BglB & H328N mutated proteins are present & active. If bands were not present, that would indicate there is no activity happening.



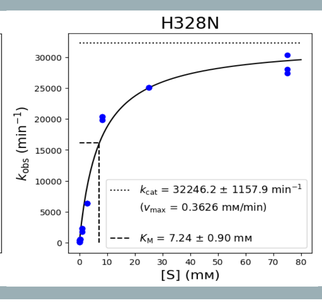
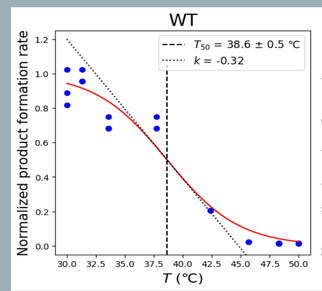


Figure 6a. The graph on the left displays the WT kinetic assay graph, Kcat (turnover rate), Vmax (maximal velocity), and Km (substrate concentration needed to give Vmax) values.
Figure 6b. The graph on the right displays the Histidine 328 Asparagine mutation of BglB protein, its increased kcat and Vmax, and decreased Km values.



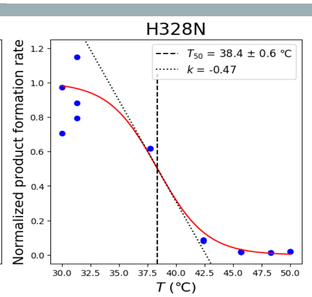
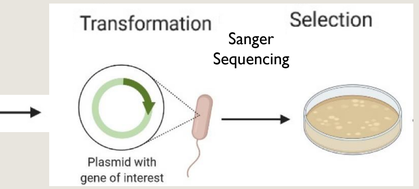


Figure 7a. The graph on the left displays the Beta-Glucosidase wild-type thermostability assay. It also displays T50 (indirect measure of destabilized protein structure) and k values.
Figure 7b. The graph on the right displays the thermostability assay of the mutated BglB gene (H328N), its decreased T50 range, and displays its increased k value as well.

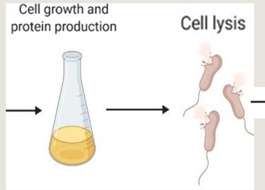
METHODS IN THE LAB *(Gomes 2)* [1]

Foldit Program

Protein Expression & Purification



Components Separated & Purified



Nanodrop Spectrophotometer (Measured Absorbance)

Kinetic & Thermal Assays

DISCUSSION

Figure 3: The reading frame shifted from 2nd to 1st (due to poor quality sequence data), but H328N was still found.
Figures 4 & 5: Mutation is present, though at low quantity
Figures 6: Since there is activity, H328N protein is present. Line would be flat (not increasing) if there was no activity.
Km also decreased (higher enzyme-substrate affinity)
Figures 7: Some points from the thermostability assay were removed (outliers & extremely low numbers). This could indicate pipetting issues, but data improved after removal. (Need to be more precise)

Overall, Our mutant H328N was successfully expressed & purified. Thermostability (measured in T50), and it is seen to decrease after mutation, which supports the initial hypothesis.
Results that do not support initial hypothesis: Km decreased after mutation occurred (indicating higher substrate-enzyme affinity, which makes stability of protein more effective). Higher kcat also means the enzyme is more efficient; since it increased, that means that it also does not follow initial hypothesis.

REFERENCES

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2) Huang X. et al. Hindawi. Wireless Communications and Mobile Computing Volume 2018, Article ID 1843083, 8 pages
3) Wade AA, et al. 2009. ScienceDirect. Page 520
4) Zhang Y. Curr Opin Struct Biol. 2009. 145-55. PMID: 19327982

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