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)

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)

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 -1089.076).) 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).

RESULTS

Figure 3. (Top is wildtype, bottom is mutant)

Sanger Sequencing data verifies Histidine 328 Asparagine (H328N) mutation located at 6226

Figure 4a. Amino acid Histidine Close-up (location 328 on backbone) highlighted in pink & blue (Wildtype)

Figure 4b. Full pET29b_Bglb_His protein with Histidine at 328. (Energy Score: -1089.697)

Figure 5a. Close-up of amino acid mutation from Histidine to Asparagine (H328N): energy increase can be visualized.

Figure 5b. Full view of BglB protein after the mutation (Histidine-328 Asparagine). A few voids appeared

Km also decreased (higher enzyme substrate affinity) (Huang X. et al. 2009. Struct Biol. 2009. 145)

Vmax, and decreased Km

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

DISCUSSION

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 efficient. Higher kcat also means the enzyme is more effective; since it increased, that means that it also does not follow initial hypothesis.

REFERENCES

2) Huang X. et al. Hindawi. Wireless Communications and Mobile Computing Volume 2018, Article ID 1843083, 8 pages