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Research Brief: Design to Data for mutants of β -glucosidase B from *Paenibacillus*

polymyxa: E26K, I170Y, and V398N

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Faculty Sponsor: Dr. Daniel Kaluka

Background

The greatest challenge in protein engineering is creating stable and efficient proteins for application in various industries. Modeling algorithms like Alpha Fold have predicted over 200 million protein structures based on primary sequences. However, algorithms have limited protein-function predictive capabilities due to the lack of experimental data sets.

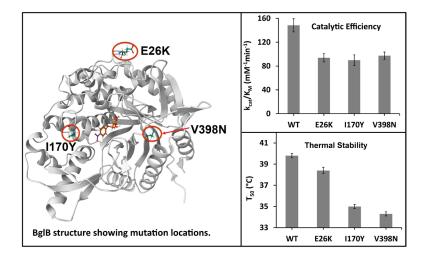
Purpose

The Design to Data program developed in the Siegel lab at UC Davis aims to generate large protein structure-function data sets by including undergraduate researchers in designing, building, and testing single-point mutations of β -glucosidase B (BglB). The student-generated data will train protein modeling algorithms like the Rosetta Commons to enhance their predictive capabilities.

Methodology

Mutants E26K, I170Y, and V398N were designed using Foldit. Foldit calculates a Total System Energy score (TSE) to predict each mutant's folding potential. Mutants were generated using the Kunkel Mutagenesis protocol, followed by protein expression and purification. A calorimetric assay was used to obtain the kinetic and thermostability parameters for the BglB variants.

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Results and Conclusions

Based on the increase in TSE, I170Y and V398N were correctly predicted to have decreased catalytic efficiency and thermal stability. However, despite a decrease in TSE, E26K exhibited a reduction in thermostability and catalytic efficiency, suggesting Foldit's limited reliability in predicting protein stability. The three mutant data were uploaded to the growing D2D database. Additional variants are presently being developed and characterized to enhance the database.