Computational Prediction of Mutagenesis in *Glycine max* Rubisco Activase Monomer for Increased Thermal Stability

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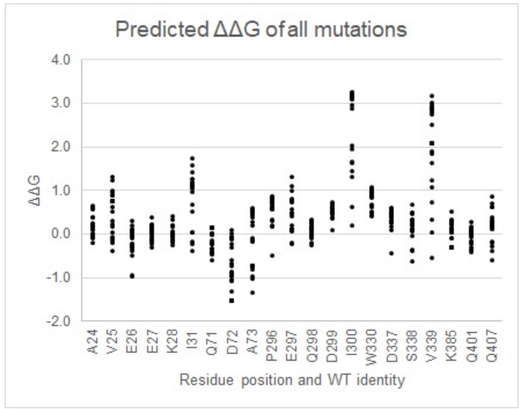
Background

- Rubisco activase has been found to be a crucial target for genetic engineering projects to increase photosynthetic thermostability
- Multiple computational programs exist for predicting stabilizing mutations, but are not all consistent
- Existing data from Argonne National Laboratory can be used to test which program is most accurate for the desired thermostability mutations and can then be used to produce more predictions

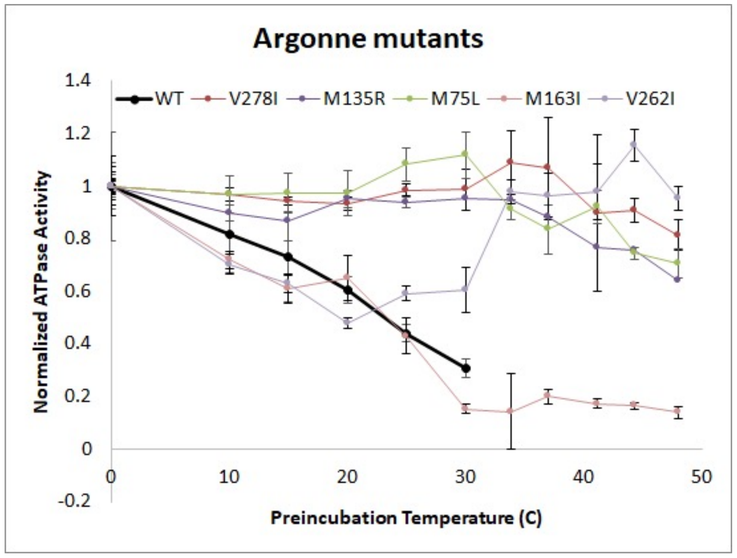
Why PremPS?

- $\Delta\Delta G$ serves as a measure of the changes in folding of the protein, indicating protein stability
- Depending on the software, the mutation's impact on stability is either a positive or negative $\Delta\Delta G$ value
- We utilized *Glycine max* mutations discovered in previous research from Argonne National Laboratory to compare computational software programs and find the most reliable tool for predictive mutations by comparing the established stabilizing and destabilizing mutations to the predictions from each software



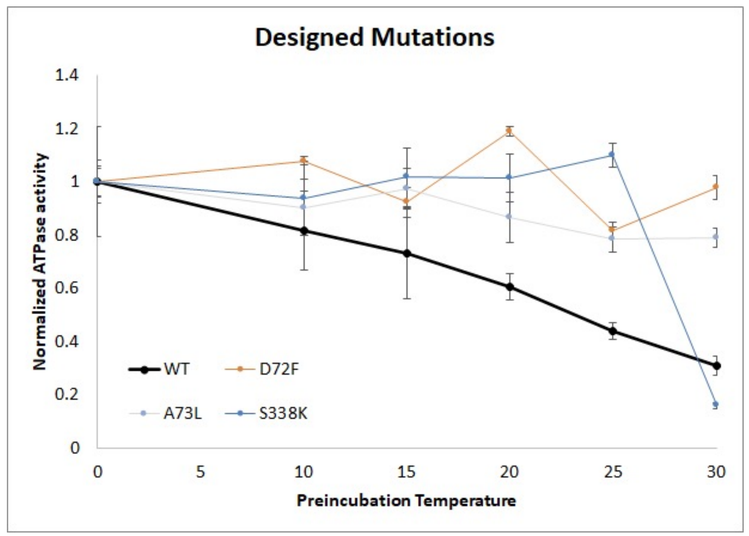
- Based on the consistency with our control mutations, PremPS was found to be the most accurate and our choice for future predictions
- Increased $\Delta\Delta G$ = less stable
- Decreased $\Delta\Delta G$ = more stable

Methods



1. Utilized BLAST alignments to construct an amino acid sequence for our organism of choice, *Glycine max*
2. Created a 3-D protein model of *Glycine max* RCA with hypothesized R-groups and folds using Phyre2 and RefineD
3. Used existing protein melt curve from Argonne National Lab to choose the most accurate predictive program for our experiments
4. Selected the 3 mutations predicted to have the largest increase in stability
5. Performed QuikChange PCR to induce single point mutations and create the 3 mutants in *E. coli*
6. Purified the proteins from each mutation
7. Pretreated the enzymes at 0˚C, 5˚C, 10˚C, 15˚C, 20˚C, 25˚C, and 30˚C for 1 hour before performing ATPase assays to monitor enzymatic efficiency post-treatment
8. Normalized and graphed the assay results

Results



- Provides evidence for PremPS as a promising program for thermostability predictions
- Protein melt curve results closely match ATPase activity results
- Found that each of the three mutations significantly improved thermostability

References

Pokkuluri P, Schiffer M, Joachimiak A, Wilton R. 2018. Stabilization of Rubisco Activase for Enhanced Photosynthesis and Crop Yields. United States UChicago Argonne, LLC (Chicago, IL, US) US Patent #20180094328" "Chen Y, Lu H, Zhang N, Chen Y, Zhu Z, Wang S, Li M. 2020. PremPS: Predicting the impact of missense mutations on protein stability. **PLoS Computational Biology"**

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