CALIFORNIA STATE SCIENCE FAIR
2017 PROJECT SUMMARY

Name(s)
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Project Number
S0501

Project Title
Optimizing Light-Controllable Proteins for Optogenetics Experiments

Abstract

Objectives/Goals
The objective of this project was to develop a diverse library of Light-Oxygen-Voltage sensing (LOV2) domain variants that could permit for more precise protein manipulation. The LOV2 domain is a plant blue light receptor that, when inserted into a protein of interest, makes the protein light controllable. This technique had not been previously refined to permit for exact protein control. In order to control proteins precisely, the domain must switch quickly between active and inactive states upon blue light illumination. LOV2 mutants which undergo this change quickly were thus screened for.

Methods/Materials
Wild type LOV2 domain, bacteria, Alpha-Imager, Image-J, Excel. LOV2 mutants were generated by random mutagenesis and were transformed into bacteria. The domains were activated by a blue light pulse. Since each bacterial colony#s fluorescence increases as its LOV2 domain returns to its inactivated state, recovery rate was determined by recording the domains# fluorescence over time. The data were normalized and recovery halftimes were calculated.

Results
Recovery halftime calculations lead to the identification of several fast LOV2 mutants, which switch from the active to the inactive state in seconds, as well as slow mutants, which do so in hours. The fastest recovering mutants were x462, x485, x482, and x480 and had halftimes as low as 3s, whereas the slowest mutant, x219, had a halftime of 16m.

Conclusions/Discussion
These results provide a means to optimize the LOV2 domain#s function as a tool in optogenetics experiments. The fast-recovering domains identified are advantageous for precise protein manipulation, while the slow-recovering domains identified are optimal for lengthy experiments, since they remain active with minimal illumination. By expanding the LOV2 domain library, I hope to encourage others to utilize these specialized mutants to improve the quality of their own research.

Summary Statement
I helped to develop a diverse library of mutated light-controllable protein domains that permit for optimized protein manipulation in cells.

Help Received
I worked on this project at Wittmann Lab at UCSF Parnassus. Torsten Wittmann (Principal Investigator) oversaw my work, and Jeffrey van Haren (Postdoctoral researcher) guided me through the project and taught me how to work in a lab.