CALIFORNIA STATE SCIENCE FAIR
2013 PROJECT SUMMARY

Name(s)

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Project Number

S0527

Project Title

A New Light In Biosensing: Engineering Photostable GFP Mutants for FRET

Abstract

Objectives/Goals

Biosensors employing fluorescence resonance energy transfer (FRET) between fluorescent proteins (FPs) are powerful tools for non-invasively monitoring intracellular processes. Clover, the brightest existing FP, suffers from low photostability, reducing its utility for time-lapse imaging. The goal of this experimentation is to improve the photostability of Clover, while preserving its other optimized properties.

Methods/Materials

After mutagenesis and cloning, libraries of Clover mutants were screened for several rounds to evaluate resistance to photobleaching in three contexts: colonies via LED array, lysates via LED array, and purified protein using a microscope. Results were quantified using ratiometric photo analysis on FIJI/ImageJ or a plate reader. Mutants were characterized and further screened for efficient FRET donation to a red FP acceptor and performance in a FRET-based calcium sensor.

Results

Clover2S, a new mutant that differs from Clover by N149Y and G160S, shows increased photostability, quantum yield, and maturation, and confers increased dynamic range onto the calcium biosensor. Mapping the mutations onto the Clover structure implies that the mechanism of increased photostability may involve preventing oxygen from diffusing into Clover and reacting with the chromophore.

Conclusions/Discussion

Clover2S is the now the brightest existing fluorescent protein to date, as well as the most photostable Clover variant. The incorporation of Clover2S into a variety of biosensors is a promising avenue to elucidate the mechanisms behind cancer and neurological pathways.

Summary Statement

I developed a photostable fluorescent protein Clover2S, which is now the most photostable variant of Clover and the brightest existing fluorescent protein.

Help Received

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