# CALIFORNIA STATE SCIENCE FAIR
# 2014 PROJECT SUMMARY

**Name(s)**

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**Project Number**

34765

**Project Title**

## Illuminating Disease Pathways: Developing Bright Fluorescent Proteins to Improve FRET Biosensing

**Abstract**

**Objectives/Goals**

The discovery and development of fluorescent proteins, recognized by the 2008 Nobel Prize in Chemistry, enabled a revolution in biological microscopy and sensing. Biosensors employing fluorescence resonance energy transfer (FRET) between fluorescent proteins are powerful tools to non-invasively report biochemical events within living cells. The development of new FRET sensors remains difficult, however, often due to low FRET dynamic range. The objective is to develop a brighter green fluorescent protein (GFP) and a brighter red fluorescent protein (RFP) which can function as an effective FRET pair.

**Methods/Materials**

Using random and site-directed mutagenesis and standard cloning techniques (ligation, transformation), I created libraries of GFP and RFP mutants, which were screened for photostability, brightness, and performance in a FRET-based calcium sensor. To screen for brightness, upon taking fluorescence images of bacterial colonies, I identified proteins with the greatest brightness values via ImageJ software, where the brightest proteins were incorporated into a FRET pair. Mutants with high FRET to GFP fluorescence ratios were selected. To measure extinction coefficient and quantum yield, I used the base-denaturation method and integrated the emissions, respectively. To screen for photostability, colonies and protein lysates were photobleached with a LED array, where brightness levels were quantified using the software ImageJ or a plate reader. I purified the fluorescent proteins using cobalt-chelating affinity chromatography, and I used an inverted microscope to continuously photobleach proteins through time-lapse imaging. To evaluate dynamic range, mutants were used to construct TN-XXL calcium sensors.

**Results**

I engineered a new green fluorescent protein Clover3, which is the brightest monomeric fluorescent protein to date. Clover3 confers increased FRET dynamic range onto biosensors and shows improved photostability and quantum yield. Moreover, I developed a new red fluorescent protein mRuby3, which is the brightest red fluorescent protein to date.

**Conclusions/Discussion**

With superior optical characteristics, Clover3 and mRuby3 are expected to benefit diverse biomedical applications, including the imaging of neural structures, visualization of cancer metastases, and monitoring of signaling pathways to elucidate disease mechanisms.

**Summary Statement**

I engineered the brightest monomeric green and red fluorescent proteins to date, which may allow us to visualize biological activities with greater clarity than before.

**Help Received**

This independent research project was carried out by Emily Wang under the supervision and guidance of Dr. Jun Chu and Professor Michael Lin at Stanford University.