CALIFORNIA STATE SCIENCE FAIR
2016 PROJECT SUMMARY

Name(s)

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Project Number

36622

Project Title

Genetically Encoded Bioluminescence Resonance Energy Transfer-based Ca2+ Indicator for in vivo and Deep Tissue Imaging

Abstract

Objectives/Goals

Fluorescent calcium indicators have given scientists much insight into roles of calcium in the body. However, fluorescent indicators must be externally excited, which causes complications such as photobleaching or autofluorescence. Bioluminescence resonance energy transfer (BRET) resolves these problems by eliminating the need for external excitation. In this project, I genetically insert CaM and M13, two calcium-sensing domains, into a BRET-based protein system called Antares in order to create a series of autofluorescent calcium indicators called CaM-Ant.

Methods/Materials

I used the Antares as the base for my cloning and inserted the calcium-sensing domain CaM-M13. I designed my constructs and primers using Geneious, ordered primers through Integrated DNA Technologies, and sequenced my constructs through Sequetech. I transformed and tested my indicators in E. coli and Hela cells.

Results

Of the seven original constructs, CaM-Ant 132 and 133 were selected as the best candidates for their high calcium sensitivity and BRET efficiency. Additional optimization steps resulted in four CaM-Ant SW constructs and twenty linker substitution constructs. The latter, of which CaM-Ant 133 F and I showed particular improvement, contain a deletion that make them the structural median of CaM-Ant 132 and 133. Overall, CaM-Ant 132 displays 5.7 times greater signal in the presence than in the absence of calcium. CaM-Ant 133, F, and I all have high BRET efficiencies, and CaM-Ant 133 has the highest signal emission. All CaM-Ant constructs can stably maintain light emissions for very long periods of time, surpassing 30 minutes in vitro.

Conclusions/Discussion

In conclusion, the CaM-Ant constructs boast high calcium sensitivities and signal intensities: CaM-Ant 132 has the highest calcium sensitivity of any similar, BRET-based indicator currently published. The CaM-Ant indicators can be applied to observe a variety of phenomena. For example, they can be applied to studies of the brain for easier and more detectable neuronal imaging. They can also aid in the stem cell treatment of heart disease by visually reporting whether the transplanted heart tissue has been integrated into the patient's body.

Summary Statement

I created a series of 31 genetically encoded, BRET-based calcium indicators that have the highest sensitivity of any similar indicators currently published within the scientific community.

Help Received

My research mentor, Dr. Younghee Oh, helped me in my research by teaching me experimental procedures and prompting me in the next step. Our lab PI, Professor Michael Lin, guided the project by suggesting new ideas and paths.