Team: Balachandran N., Bhatt A., Chandasir N., Chang E., Cleland S., Franklin A., Harris J., Hong L., Kwon J., Leveille J., Minjur N. Orberger L., Standeven D., Shih N., Tao K.
Advisors: Cantrell B., Cochran S., Standeven J.

SWITCH

Characterization of Non-Lysosomal Proteolysis

Acknowledgements: Georgia Tech Styzynski Research Lab, National Science Foundation, Building With Biology, Lambert High School, New England BioLabs, Coli Genetic Stock Center23, Georgia State University iGEM

Human Practices

- Maker Faire – Building with Biology
- Next Generation Focus
- Atlanta Science Festival
- Congenital Heart Defect Walk
- Collaboration with Monica McNerney and Dr. Styczynski
- Twitter iGEM Group Chat

Abstract

Biosensors rely on precise control of reporter protein expression which can be regulated through cellular transcriptional mechanisms. Our project focused on protein degradation and specifically a non-lysosomal mechanism called ClpXP. The system is composed of two subunits - ClpX, which unfolds the protein marked with a degradation tag, and ClpP, which degrades the protein. We created an inducible genetic construct to regulate the transcription of ClpXP, allowing us to characterize the degradation of reporter proteins that were tagged with DAS and LAA. The aim of our project was to characterize green fluorescent protein, GFP, as a baseline and expand our work to chromoproteins; building on the work of Uppsala 2013 and Edinburgh 2014.

Objectives

- **Engineer and characterize an inducible switch for more precise control in biosensors**
- **Design and build a device to standardize chromoprotein expression measurement**
- **Further characterize the well known protease mechanism, ClpXP using GFP and TsPurple**

Methodology

Assemble Constructs

tsPurple → tsPurple, tsPurpleDAS, tsPurpleLAA

GFP → GFP, GFP DAS, GFP LAA

Sara Cleland (left) and Jackson Harris (right) working in the lab to assemble our constructs.

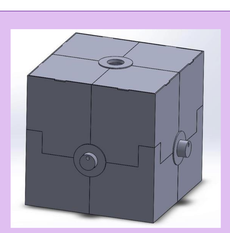
Grow in Keio strains wild, ClpP, ClpX

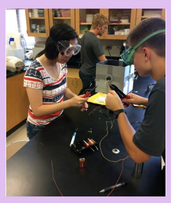
We grew the constructs in the Keio wild strain to see if ClpXP degrades the tagged reporter. We grew the constructs in the Keio knockout strains, ClpP and ClpX, to see how ClpXP would behave if one of its composite proteins was inhibited.

Induce in IPTG

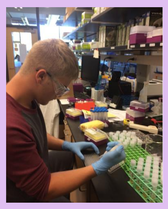
Quantification with Camera

Quantification with Plate Reader





Elynna Chang (left) and Jackson Harris (right) assembling the ChromQ chamber.



David Standeven preparing samples for the plate reader at Georgia Institute of Technology.

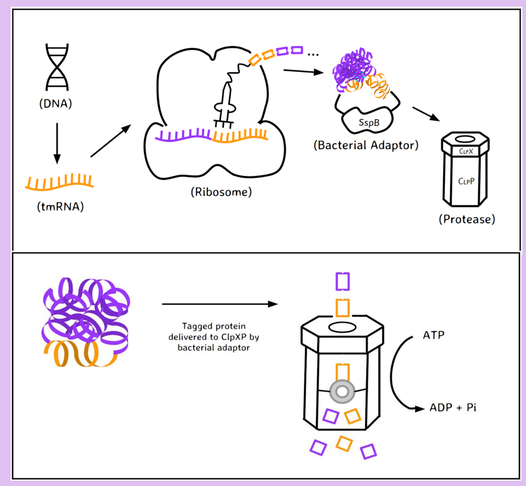
Sponsors

- **Chick-fil-A biscuit sales**
- **GoFundMe - tax deductible fundraiser**
- **Franklin Technologies**
- **Siemens**
- **AGCO**
- **Inlighta Biosciences**
- **Snapgene**

Lauren Hong (right) and Julia Leveille (left) holding liquid cultures from successful transformations of GFP constructs induced with different IPTG levels in Keio Wild cells

Background Information

- **SsrA is a monocistronic gene that codes for a specialized strand of RNA known as tmRNA; tmRNA is responsible for rescuing ribosomes from abnormally shortened mRNA templates that lack terminator codons.**
- **ClpXP - proteolysis mechanism that degrades a chromoprotein upon induction**
- **Data is quantified with the ChromQ Light Chamber or a standard fluorescent plate reader.**
- **Main goal: to measure the relative strength of degradation of a reporter and consequently further characterize a well known protease mechanism.**



ChromQ Light Chamber

- **3D-printed imaging measurement system used to quantify results of nutrient deficiency**
- **Quick and inexpensive measurement of relative protein degradation through quantification of the color in chromoprotein expression**
- **Designed to be easily assembled and taken apart leading to ease of transportation**
- **Colors can be normalized using RGB values/OD**
- **On-site application and data collection**
- **Relative strength of the degradation of tsPurple cells can be quantified by using a smartphone camera lens and color controls**

Color	Decimal Code (R,G,B)
	rgb(230,230,250)
	rgb(216,191,216)
	rgb(221,160,221)
	rgb(238,130,238)
	rgb(218,112,214)
	rgb(255,0,255)
	rgb(255,0,255)
	rgb(186,85,211)
	rgb(147,112,219)
	rgb(138,43,226)
	rgb(148,0,211)
	rgb(153,50,204)
	rgb(139,0,139)
	rgb(128,0,128)
	rgb(75,0,130)

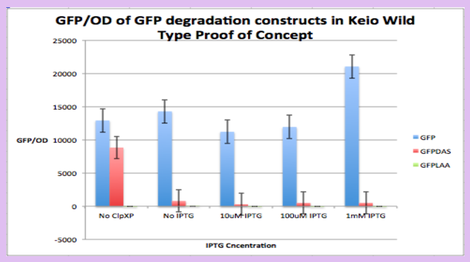
Achievements

Gold - integrated human practices, improve a previous part or project, proof of concept

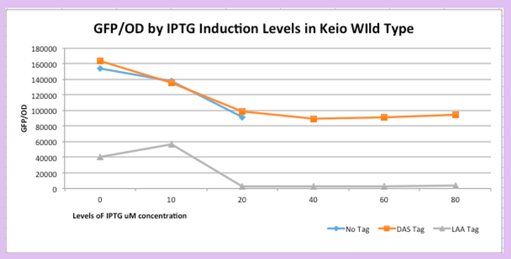
Silver - validated part/validated contribution, collaboration, human practices

Bronze - register and attend, deliverables, attribution, part/contribution

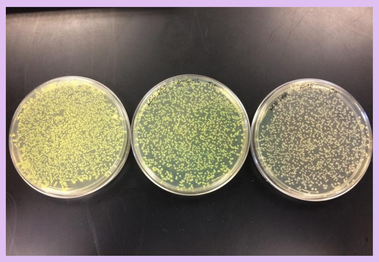
Results



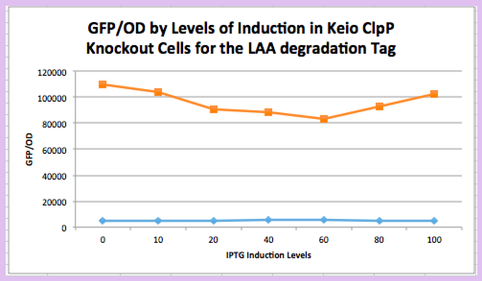
Results from our advisor, Janet Standeven's work over the summer of 2016 were unexpected in that GFP values increased with increasing levels of IPTG in the GFP-no tag constructs, but showed dramatic decreases with the DAS and LAA tags. This data inspired our team to extend Standeven's work through further characterization.



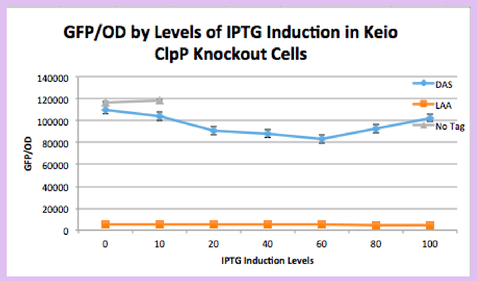
As levels of IPTG in Keio Wild *E. coli* cells containing each of the constructs with degradation tags DAS and LAA increase, the GFP/OD values decrease due to our "Switch" degrading the fluorescent protein. The Keio Wild strain with the plasmid GFP-no tag failed to grow in 40-80uM leading to incomplete results.



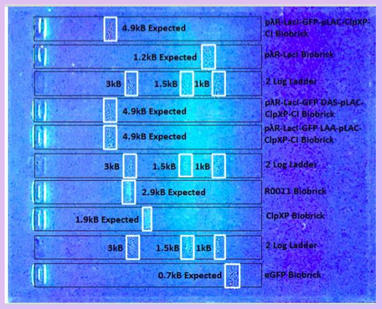
Keio ClpP *E. coli* cells on overnight LB Plates with no IPTG. From left to right: GFP-no tag, GFP with DAS, GFP with LAA. Note the visible difference in fluorescence.



Effect of IPTG induction at different concentrations of the GFP/OD in Keio ClpP knockout *E. coli* cells with a LAA degradation tag. As the IPTG concentration increased, the GFP/OD showed a small decrease, but much less than those observed in the Keio Wild strain. We theorize this is due to the knocked out protease ClpP.



The graph indicates the effect of varying levels of IPTG on GFP/OD levels in Keio ClpP knockout cells with different GFP constructs containing no degradation tag, DAS, or LAA. As seen on the graph, LAA degrades GFP significantly more than the other degradation tags across all IPTG levels. The levels of degradation did not follow the same trend present in the Keio Wild strain.



Colony PCR results for the BioBricks submitted to the registry. Our team assembled pλRlacIGFP (Tag) and placClpXP CI constructs from 1)pλRlac 2) pLac ClpXClpP 3) CI 4- GFP DAS, GFP LAA and eGPF plasmids obtained from Monica McNerney at the Styczynski Lab at Georgia Tech.