Carnegie Mellon iGEM presents

BEAM

Biosensor Emission Analysis Machine

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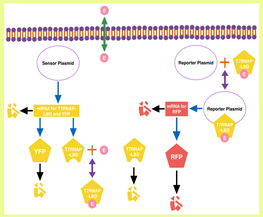
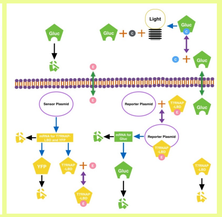
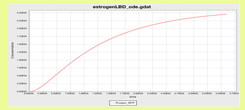
Modeling the Biosensors





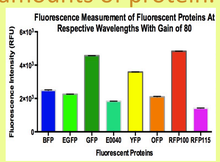
The biosensor model was written in the BioNetGen Language, a rule-based modeling language used for generating differential equations from a description of how various biological components interact with one another. The model was constructed from both data found in the literature and experimental data from the lab. This allows the model to run various simulations in order to optimize conditions. If experiments are not producing measurable results, the model can be used to identify which component could be the problem. The model was run in Rule-Bender, an environment that is dedicated to running, analyzing, visualizing, and debugging BioNetGen Language models. Unlike last year's sensor, the amount of RFP produced is significantly greater than the minimum threshold detection of 100 µm. Thus the model is able to be validated by our results from the wet lab.

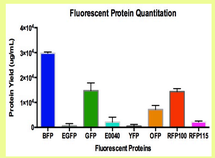


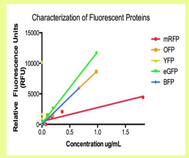
Measurement

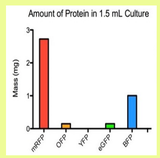
Fluorescent Protein Reporters

Fluorescent proteins allow kinetic measurements to be made in live cells. However, measurements are of a qualitative nature instead of an SI measure, so it is difficult to standardize across labs. Data is also not suitable for accurate modeling of cellular processes influenced by amounts of protein.



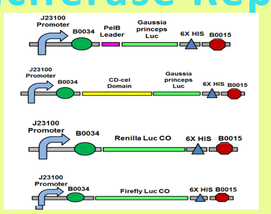






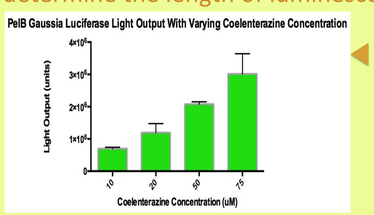
▲The first two graphs show RFU readings produced from proteins. The last two graphs show linear relationships to fluorescence.

Luciferase Reporters

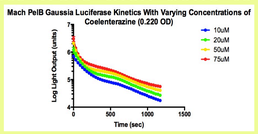


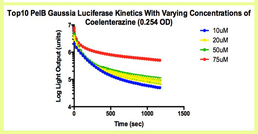
To test the luminometer, the luciferases from *Gaussia princeps*, *Renilla reniformis*, and *Photinus pyralis* were codon optimized for *E. coli* and expressed from a strong constitutive promoter.

We determined the location and observed the effect of adding increasing amounts of substrate (coelenterazine/luciferin) with respect to light output to characterize luciferase. Kinetics were then done to determine the length of luminescence.



◀Light output vs. concentration and time for PelB Gaussia Luciferase with Coelenterazine.





Special Thanks

Dr. Marcel Bruchez, Dr. Natasa Miskov-Zivanov, Dr. Cheryl Telmer, Dr. Diana Marculescu, Dr. Fred Lanni, Dr. Chris Szent-Gyorgyi, Dr. Andre Samuel, Dr. Jason Lohmueller, Dr. Yi Wang, Taylor Canady, Dr. Ming Zhang

References

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Routledge 1996. *Environ. Toxicol. Chem.* 15, 241–248.

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Project Description

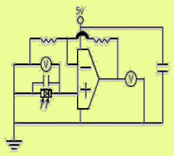
Throughout the summer, the CMU iGEM team was able to measure and characterize fluorescent proteins and make improvements to the last year's estrogen sensor. However, we noticed a key problem in broadening access to synthetic biology, future teams of citizens and scientists will need affordable ways to analyze common reporters. Luminescence and fluorescence readers can cost upwards of thousands of dollars. The focus this year was to improve access to synthetic biology by providing the data, and more importantly the low cost fluorimeter, necessary to bring a large component of synthetic biology to the broader public.

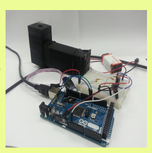
Maker Movement

To exemplify the power of the Maker Movement and its capabilities, we printed a 3D model of the Fort Pitt bridge, one of the iconic bridges of Pittsburgh, Pennsylvania, and used it to house the Gaussia luciferase.

▲LED Lit Model of Fort Pitt Bridge

Fluorimeter & Luminometer

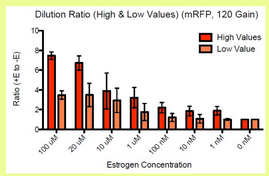




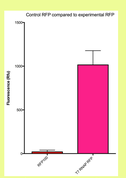
The luminometer is a simple photodiode detector with the signal integration using an Arduino and output data processed with open source software. The fluorimeter is an extension that includes an LED light source and emission/excitation filters appropriate for the fluorescent protein to be analyzed. The entire device is encased in a 3D printed shell.

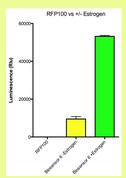
Estrogen Sensor

To test the reporters and BEAM, we improved the estrogen sensor from last year. It is a two-plasmid bacterial cell. The sensor plasmid is a high-copy plasmid with the ligand binding domain of human estrogen receptor alpha (ER-LBD) inserted into T7 RNA polymerase (T7 RNAP) and YFP for normalization. When the ER-LBD binds estrogen, a conformational change brings together the separated domains of T7 RNAP and the activity of the T7 RNAP is reconstituted. T7 RNAP is a strong phage RNA polymerase that requires no additional factors. The second plasmid, the reporter plasmid, has the T7 promoter driving expression of RFP. When the T7 RNAP is reconstituted upon binding to estrogen, it allows for binding to the T7 promoter on the reporter plasmid and transcription of the RFP mRNA which then is translated to produce RFP.
A TECAN plate reader was used to measure red and yellow fluorescence after overnight exposure to various concentrations of 17-beta-estradiol.

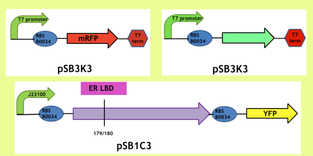


▲Response to estrogen concentrations. RFU of RFP ratio with RFU of YFP. There was a bimodal response to estrogen.





▲Data for controls.



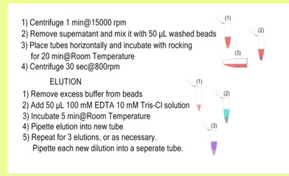
▲Schematic of estrogen sensor plasmid.

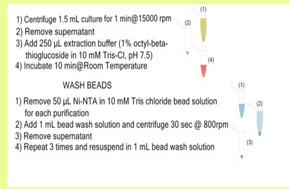
iGEM 2015

Community Involvement

Citizen Science Lab

The team worked to strengthen ties to the Citizen Science Lab in Pittsburgh, Pennsylvania. The program is run by Dr. Andre Samuel who is a strong believer in accessible science. We provided them with fluorescent proteins and the luciferases that we have cloned as part of our project for use in their after-school programs and weekend workshops.





Talks & Interviews

To get an understanding of the broader aspect of synthetic biology that is already in our community, we conducted several interviews with various professionals in the field: Dr. Christopher Szent-Gyorgyi, Dr. Andre Samuels, and Dr. Frederick Lanni.

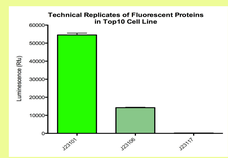
We gave several talks over the summer to various groups including the Pitt iGEM team, TechBio students, and CMU Summer Research Institute to garner interest in the larger field of synthetic biology

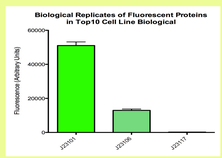
Collaborations

University of Pittsburgh: Provided them with last year's estrogen sensor. **University of Georgia:** Helped obtain mCherry fluorescence readings for an archaeal RBS library they prepared. **University of Eindhoven:** Participated in a cloning guide for new iGEM teams. **Also**, team members participated in many surveys and questionnaires conducted by other iGEM teams.

Interlab Study

The purpose of the Interlab study was to quantify the fluorescence data for three specific genetic devices expressing GFP across all iGEM teams that participate in the Interlab study. The devices contained promoters J23101, J23106, and J23117.





Future Directions

Using the luminometer as a backbone, we will attempt to 3D print a fluorimeter which can excite and read the fluorescent proteins. The models can be made robust by incorporating more wet lab data.

Future testing on our improved estrogen sensor would include testing our sensor with estrogenic compounds other than 17-beta-estradiol. We would also like to test our sensor with reporters other than RFP and Gaussia Luciferase.

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