We determined the location and observed the effect of adding 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 models. Unlike the 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. This data is also not suitable for accurate modeling of cellular processes influenced by amounts of proteins.

Luciferase Reporters

To test the luminescent, the lux genes from GloFish were codon optimized for E. coli and expressed by a strong constitutive promoter.

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

The bio sensor model was written in the BioNetGen language, a rule-based modeling language used for generating differential equations from a description of how various biological reactions and components interact with one another. The model was constructed from both data found in the literature and our own experiments.

Biosensor Emission Analysis Machine

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Throughout the study, the CMU iGEM team was able to measure and characterize fluorescent proteins and make improvements to the last year’s sensor. However, we noticed a key problem: the lack of a broadening access to synthetic biology, future teams of citizens and scientists will need affordable and open software 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.

The lumimometer is a simple photodiode detector with the signal integration from an Arduino and output data processed with open source software. The fluorescence reader is an extension that includes an LED light source and emission/excitation filters appropriate for the fluorimeter. The sensor 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 living estrogen receptor alpha (ER-LBD) inserted into T7 RNA polymerase (T7 RNA) and YFP for normalization. When the ER-LBD binds estrogen, a conformational change brings together the sense orientation of T7 RNA and the activity of the sensor. When T7 Rnap is reconstituted, T7 Rnap is a strong single strand 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.

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