Light-based Mammalian Cell Signaling

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Introduction

Cells can signal to each other through countless mechanisms. Paracrine and endocrine signaling is typically conducted through chemical means, by molecules such as hormones, neurotransmitters, and growth factors. Such signaling is limited by several factors. Diffusion speed limits all forms of cell-cell signaling; a molecule can only travel as fast as the medium it flows in. The species of the cells is also limiting; interkingdom signaling is difficult to coordinate, and consequently occurs only in relatively unique conditions, such as in the small intestine. Finally, scientists are limited in their ability to control cell signaling in vivo. Precise, temporal control of signaling is extremely difficult and has only been recently demonstrated by a technique called optogenetics, where light-activated ion channels are expressed in neurons, allowing neuroscientists to drive or silence action potentials with application of light. With this in mind, we set out to demonstrate a new method of cell signaling: signaling with light. If cells were able to communicate with light, it would overcome the aforementioned limitations because light is transmitted instantaneously, it is nonselective so it can signal between different species, and researchers can precisely and easily mediate this signaling by applying external light. Our goal was to create a line of "Sender Cells" and "Receiver Cells", where the Sender Cells produce light, and the Receiver Cells receive the signal and produce an instantaneous response.

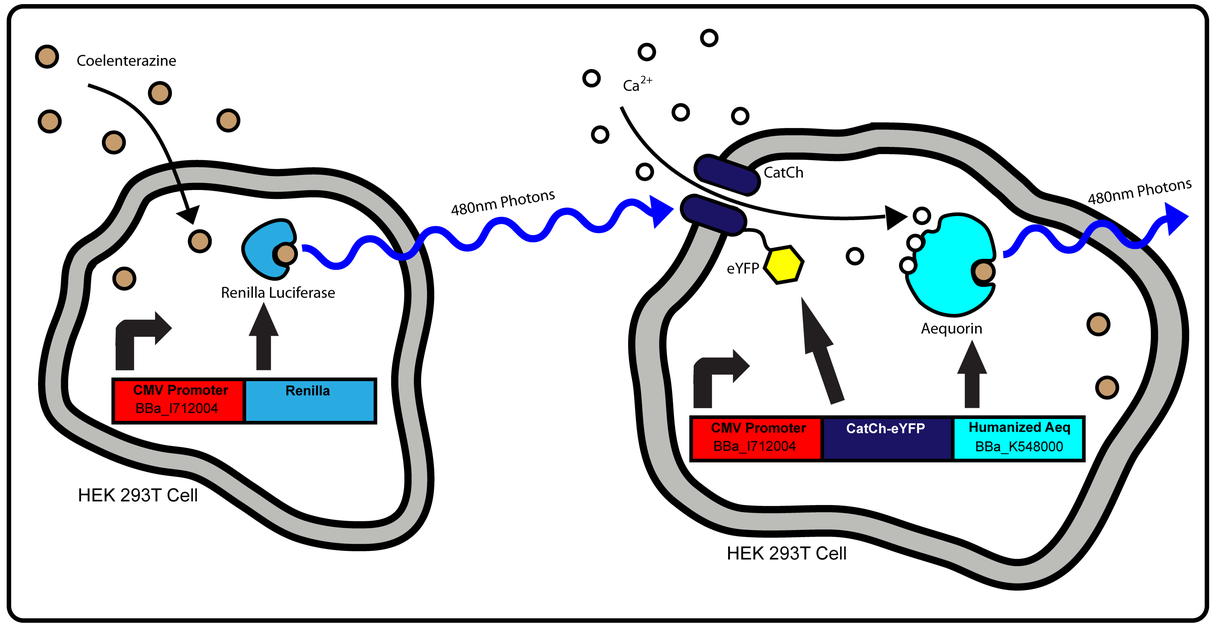
Molecular Biology

Both Sender and Receiver cells required us to create constructs for delivery into Human Embryonic Kidney (HEK) 293T Cells. The majority of our time this summer was devoted to creating our constructs and understanding the molecular biology behind the protocols.

We had planned to create lentivirus of our plasmids to create stable cell lines and therefore chose the pRRL backbone (provided by the Chen Lab at Penn) for all of our constructs. While we never wound up making stable cell lines, our basic protocol to create our constructs was still as follows:

1. Obtain pENTR5-CMV promoter plasmid from Chen Lab
2. Place reporter gene into pENTR/dTOPO (Invitrogen) using a directional topoisomerase I.
   A. Transform plasmid into bacteria, grow bacteria, then purify DNA
   B. Perform essential diagnostic restriction digests and gels
3. Perform Gateway Cloning using Clonase II Plus (Invitrogen) to clone CMV promoter and chosen reporter gene into pRRL backbone.
   A. Transform plasmid in bacteria, grow bacteria, then purify DNA
   B. Perform essential diagnostic restriction digests and gels
4. Sequence constructs
5. Experiment as needed

Our reporter genes were obtained from a variety of sources. Renilla luciferase was obtained from the Chen Lab, CatCh was obtained from Professor Ernst Bamberg at the Max Planck Institute of Biophysics, and Aequorin was synthesized through Invitrogen. This human-optimized Aequorin was then converted to BioBrick format using the BioBrick Assembly Kit (NEB) and submitted as BBa_K548000 (characterization data is on the Registry). Unfortunately, due to Materials Transfer Agreements (MTA's), we were unable to submit BioBricks for our Sender Cell protein (Renilla) and our Receiver Cell light sensor (CatCh).



The Sender Cell

To create a blue light sender cell, we needed cells to produce a bioluminescent protein such as a luciferase. Gaussia luciferase and Renilla luciferase both catalyze the same reaction:

Coelenterazine + O_2 → coelenteramide + CO_2 + 480 nm photons

pRRL-CMV-Gaussia was transfected into HEK 293T cells and collected from supernatant of the media. pRL-CMV-Renilla was transfected into HEK 293T cells and ultimately chosen because it was brighter than our Gaussia supernatant (see Figure 1). We also characterized Renilla's luminescence time course (see Figure 2).

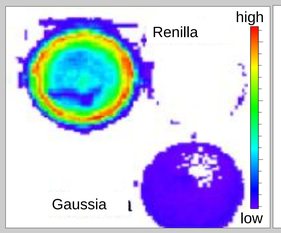


Figure 1: Luminescence heat map of Renilla and Gaussia.

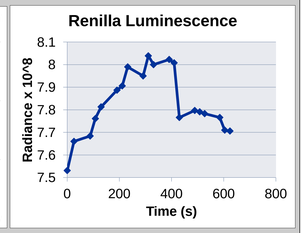


Figure 2: Luminescence of Renilla with Coelenterazine (CZ)

We also tried to make our system entirely genetically encoded with what we thought could be a precursor to coelenterazine (PCZ). We created pRRL-CMV-PCZ-mODC using site-directed mutagenesis to introduce the necessary amino acid substitution in eGFP-mODC (S65Y). However, PCZ was not a successful replacement for coelenterazine (see Figure 3).

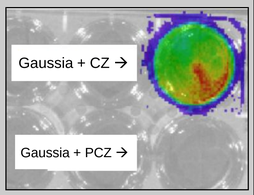


Figure 3: Luminescence of CZ and of PCZ with Gaussia supernatant

The Receiver Cell

To construct our receiver cell, we aimed to create a cell that could take a light input at approximately 480 nm and translate it to an instantaneous downstream response. Channelrhodopsin-2 is a light-dependent calcium channel that is activated by this wavelength. For our experiments, we used CatCh, which is a Channelrhodopsin-2 protein with a single amino acid substitution (L132C) that greatly improves Ca^{2+} permeability while retaining the activation profile of Channelrhodopsin-2. We hoped to use the calcium-influx to trigger luminescence from Aequorin, a protein isolated from jellyfish that cleaves Coelenterazine and emits 480nm photons when calcium binds to it. We characterized CatCh through Fura-2AM calcium imaging. We found some cells that, under excitation by a 473 nm diode-pumped solid state laser, exhibited a step-wise increase in intracellular calcium. However, our sample size was small due to time and equipment constraints (See Figure 4).

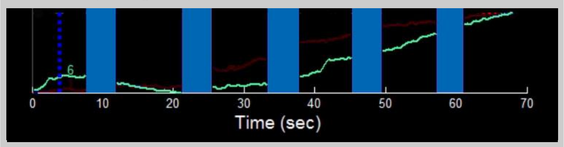


Figure 4: Characteristic plot of Fura-2AM ratio-based Ca^{2+} concentrations vs. time. Blue rectangles are laser pulses. Cell 1 (red, CatCh positive) exhibits stepwise increases in calcium at the 2nd and 3rd pulse, compared to Cell 6 (turquoise, CatCh negative).

After these positive results, we used Fura-2AM imaging on a co-culture of our sender and receiver cells, hoping to activate CatCh with luminescence from Renilla. While we did not see activation, we did not have time to optimize the system and manipulate the numerous variables such as Renilla:CatCh cell ratio, amount of CZ added, and total cell density.

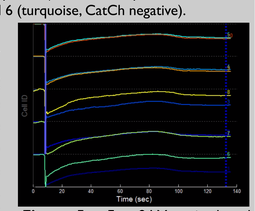


Figure 5: Fura-2AM ratio based Ca^{2+} for Renilla-CatCh co-culture. CZ added at 8s.

Human Practices

What is the effect of the news media in the perception and progression of Synthetic Biology? Our team decided to investigate this issue after hearing some adverse reactions to our project on engineering human cells to signal with light. We now believe that this is one of the most important issues facing the field. We interviewed Valerie Bonham, J.D., the Executive Director of the Presidential Commission for the Study of Bioethical Issues, and analyzed data from a survey of over 200 respondents investigating the role of the media in their perception of Synthetic Biology. Most were Penn students from non-biology related fields. We found that they learned almost all of their information on Synthetic Biology and genetic engineering from large, non-science focused media outlets. We also found that many people were already aware of genetic engineering, but synthetic biology has not garnered nearly as much public exposure.

So what should Synthetic Biologists do? Is it even possible to change the perspective of a public that obtains the majority of its information on science from the news media? We asked this question while interviewing the Director of the Presidential Commission. Ms. Bonham said that we need to "recognize, respect, and understand the fear surrounding Synthetic Biology, but at the same time, bring thoughtful analysis to the concerns behind those fears." Our team believes that this is an important point—if Synthetic Biologists simply dismiss the lay public's fears as irrational, it will most likely do nothing to help allay these fears. But we also believe that synthetic biologists should take an active role in public perception. Since we have shown that the public currently has very little knowledge on Synthetic Biology, we believe that researchers are in a strong position to improve the lay public's perception of the field. Currently, Synthetic Biologists write opinion pieces in very science-focused media outlets and academic reviews, which we showed are accessed by a minority of the public. Their voices are almost never heard in the most widely disseminated and accessed news forms. We believe that, going forward, synthetic biologists need to respect the fears of the public and make their voices heard, effectively using the media to improve the public's opinion of synthetic biology, instead of hurting it.

Discussion

As a first year team, we believe that we have accomplished a great deal. We created and optimized a gene originally found in jellyfish for mammalian cells, bringing mammalian cells one step closer to achieving completely self-contained bioluminescence. We contributed to the synthetic biology community by submitting a BioBrick (BBa_K548000) to the Registry of Standard Biological Parts, and characterized an innovative light dependent calcium channel that has potential to be a potent light sensing element for future iGEM projects.

Although we were not able to successfully transmit a signal between our sender and receiver cells as we had originally planned, we have shown that the individual parts of our system work as expected. However, there remains a great deal of work to be done. We believe that by carefully optimizing the sender and receiver cells, we can create a successful proof-of-concept system in the near future. Furthermore, as part of our human practices project, we have highlighted the important relationship between the field of Synthetic Biology and the media, and provided suggestions for how future groups and researchers should approach this sensitive and important issue.