ATP Regeneration in Synthetic Cells

**Introduction/Background**

Synthetic biology focuses on the engineering of devices, pathways, networks, and systems that utilize tools already seen in biology. There is a new movement to figure out how to create and use genetically-programmed synthetic cells for future use. These cell-free systems can be used as environments in which more complex engineered systems can be carried out and designed.

The problem I have chosen to tackle is the metabolic processes and energy lifetime. I aim to extend the lifetime of synthetic cells derived from liposomes. Since their only source of energy is solely from the initial cell extract provided, the lifetimes of these artificial cells ranges from 4-6 hours. I would ideally like to get the lifetime to 8-10 hours. This problem arises from current experimental setups seen in the literature. One of the issues to consider during the construction of these synthetic cells is metabolism. We notice, from reading papers, that lifetimes of synthetic cells are limited. Extending the lifetime is crucial since it will allow for more complex, sustainable experiments. We will be able to dramatically broaden the possibility of experiments done when we can measure responses, production, etc for longer time periods.

I plan to pursue this project in the Richard Murray Lab. In Murray Lab, there is a special focus on biomolecular feedback circuits. People in Murray Lab work on improving the performance, robustness, and modularity of engineered biological circuits. There is a special focus on the use of synthetic cells to study and engineer improved circuits.

Given the success of this research, the possibilities of experiments with synthetic cells will be significantly affected. We will be able to understand how to extend lifetimes more. We will also be able to understand how metabolism truly works in cells. We can discern what components are crucial for energy regeneration. With longer lifetimes, we will be able to do more – such as more synthesis of bio-compatible materials, more accurate environmental monitoring and remediation, more self-assembly of complex multi-cellular machines, etc.

**Objectives**

In my project, I aim to show that synthetic cells, constructed from liposomes, are capable of generating greater absolute amounts of protein when the TX/TL system is in the presence of a molecular rheostat that maintains ATP levels. TX/TL is a transcription/translation system that creates protein from linear DNA templates. I want to show that, with added molecular machinery, we can extend the lifetime and increase the rate of TX/TL activity. I also aim to identify a set of robust, efficient parameters for longer-lasting energy production.

I will measure rate of protein production by measuring fluorescence. Each target protein will have an attached fluorescent protein, so we can effectively correlate fluorescence with protein production. The experiments will occur within liposomes with accepted concentrations of buffer and cell extract. The conditions that these experiments will be conducted in have been refined in the synthetic biology community or in Murray Lab (expand?). There are various starting assumptions I will have to make (?? expand?? Necessary?).

More specifically, I plan to develop liposomes that can successfully produce proteins with the TX/TL system. Then, I plan to introduce the prokaryotic ATP rheostat machinery to the liposomes and get a functioning metabolic system. (ATP rheostat machinery adopted from doi: 10.1038/nchembio.2418). Then I want to show that the liposomes with the ATP regeneration machinery can cause higher rates and extended lifetimes of TX/TL activity. Finally, I plan to understand, identify, and attempt to optimize parameters that affect the rate of ATP production.

**Approach**

There are some technical challenges that have to be tackled in order to have a successful project. We will have to implement a system into liposomes that requires energy, such as the processes of transcription and translation. Following previous experiment, we will make sure that the TX/TL system can be functional in liposomes. We will use the output of this machinery (protein production) to quantify activity and lifetime. The target proteins will be detected and quantified by using fluorescent proteins and microscopy. Further, there are no established metabolic pathways that function in liposomes. We plan on using the ATP rheostat machinery to attempt to create a functioning metabolic pathway that works with the TX/TL system in liposomes. We will study protein production in liposomes with and without machinery to understand its affect, if any. There are no ATP regenerating pathways that have shown to increase the rate or lifetime of activity within a synthetic cell. To combat this, we plan on studying and optimizing protein production with the TX/TL system and the ATP rheostat machinery. We are not sure what parameters affect ATP production rate or how to optimize them. By changing experimental conditions and doing some statistical modeling, we will attempt to optimize protein production rate and activity lifetime.

Both the TX/TL system and the chosen ATP rheostat machinery are inherited systems.

We will have to procure functioning liposomes that have both these systems together.

There are about five other undergraduates and some graduate students working on studying different parts of synthetic cells. Collaborating with them will key to the success of the synthetic cell project as a whole.

**Work Plan**

Week 2 – Develop liposomes that can successfully produce proteins with the TX/TL system

Week 4 – Develop liposomes that can successfully adopt ATP rheostat machinery from prokaryotes. The machinery is adopted from doi: 10.1038/nchembio.2418

Week 6 – Show that liposomes with the ATP regeneration machinery can cause more TX/TL activity

Week 8 – Show that liposomes with the ATP regeneration machinery can cause extended lifetime of TX/TL activity

Week 10 – Understand, identify, and attempt to optimize parameters that affect the rate of ATP production

**References**

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