ATP Regeneration in Synthetic Cells

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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 [9].

The problem we have chosen to tackle involves the metabolic processes and energy lifetime. We aim to extend the lifetimes of synthetic cells derived from liposomes. Since their only source of energy is from the initial cell extract provided, the lifetimes of these artificial cells range from 4-6 hours [6, 8]. We would ideally like to increase this lifetime to 10-12 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 [8]. We notice, from reading papers, that lifetimes of synthetic cells are limited [4, 7]. Extending the lifetime will be an integral component of being able to carry out 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.

This project will be pursued 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. Specifically, we will be involved with the group that focuses on the use of synthetic cells to study and engineer improved circuits [8, 9].

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 their lifetimes. By discerning what components are crucial for energy regeneration, we will be able to understand how metabolism truly works in cells. With longer lifetimes, we will be able to have more

synthesis of bio-compatible materials, more accurate environmental monitoring and remediation, more self-assembly of complex multi-cellular machines, etc. [8].

Objectives

In this project, we 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 [1, 6, 7]. We want to show that, with added molecular machinery, we can extend the lifetime and increase the rate of TX/TL activity. We also aim to identify a set of robust, efficient parameters for longer-lasting energy production.

We 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 (TX/TL activity). 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 by the synthetic biology community or in Murray Lab. For example, it is already established that allowing liposomes to function in HEPES buffer provides the best reaction conditions [6]. Techniques and protocols such as these will be adopted. There are various starting assumptions we will have to make. We will assume that previously adopted protocols are optimized for our desired setup. We also assume that it is possible for the synthetic cells to be able to function at higher rates and for longer lifetimes. They will not degrade or buckle to physical or chemical stresses. Going forward, we will adjust project goals and methods based on how well these assumptions hold.

More specifically, we plan to develop liposomes that can successfully produce proteins with the TX/TL system. Then, we plan to introduce the prokaryotic ATP rheostat machinery to the liposomes and get a functioning metabolic system [8]. After, we want to show that the liposomes with the ATP regeneration machinery can cause higher rates and extended lifetimes of TX/TL activity. Finally, we plan to understand, identify, and attempt to optimize parameters that affect the rate of ATP production.

Approach

In order to accomplish our objectives, we have hypothesized some specific methods and approaches. First, we will have to implement a system into liposomes that requires energy, such as the processes of transcription and translation. By following previously published papers, we will develop a functioning TX/TL system in liposomes [1, 5, 6]. We will use the output of this system (protein production) to quantify activity and lifetime. The target proteins will be detected and quantified by using fluorescent proteins and microscopy. Further, we plan on using ATP rheostat machinery developed by Opgenworth et al. to create a functioning metabolic pathway that works alongside the TX/TL system in liposomes [7]. See Figure 1 for a schematic of the TX/TL system in conjunction with the ATP regeneration pathway. It is able to selectively choose different metabolic pathways depending on the amount of phosphate present in the reaction environment. At low P_i concentrations, ATP levels are high, so the GapN pathway is preferred. The GapN pathway does not make any additional ATP. At higher P_i concentrations, ATP levels are low, so the mGAPDH-PGK pathway is preferred. This pathway allows for the regeneration of ATP [7].

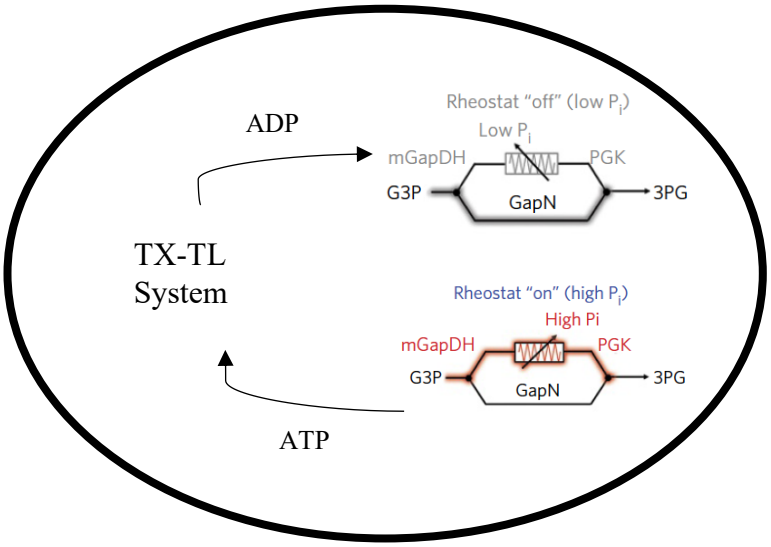

Figure 1 Simplified schematic of our proposed liposome design. The TX-TL system will be the metabolic process using ATP and the ATP rheostat machinery will be able to re-energize the system. Image of the ATP rheostat machinery adapted from Opgenworth et al. [7]

We will study protein production in liposomes with and without machinery to understand its effect. There are no established 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. By testing experimental conditions and doing some statistical modeling, we also plan on identifying and optimizing the parameters the affect ATP production rate.

Both the TX/TL system and the chosen ATP rheostat machinery are inherited systems. We will have to create functioning liposomes that have both of these systems.
There are about five other undergraduates and some graduate students working on projects related to the study and optimization of synthetic cells. Collaborating with them will be key to ensure 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 Opgenworth et al. [7]
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

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