**ATP Life Extension in Synthetic Cells**

Ankita Roychoudhury (SURF student), Richard M. Murray (mentor)

3 July 2020

*Keywords: synthetic biology, TX/TL system, ATP regeneration, rheostat*

**Background/Motivation**

Synthetic biology focuses on the engineering of devices, pathways, networks, and systems that utilize tools which already exist in biology. There is a growing interest in the development and application of genetically-programmed synthetic cells for future use. These cell-free systems can be used as environments in which more complex engineered systems can be implemented and designed [9].

When building synthetic cells, there are five main subsystems to be considered. These are: spatial organization, metabolic subsystems, sensing and signaling, regulation and computation, and actuation. The problem we have chosen to tackle involves the metabolic subsystems, specifically the power supply and energy lifetime [11]. We aim to extend the lifetimes of synthetic cells derived from liposomes by implementing an ATP life extension mechanism. This mechanism can be a biochemical ATP regeneration pathway, a directed transporter, etc. An efficient, longer-lasting method to provide energy required for internal reactions will allow us to carry out more complex, sustainable experiments. We will be able to broaden the range of possible research in synthetic cells if we can measure responses, production, etc. for longer time periods.

Given the success of this research, the possibilities of experiments with synthetic cells will be positively affected. We will be able to understand how to extend their lifetimes. By discerning what components are crucial for energy regeneration, we can understand how metabolism truly works in cells. Longer lifetimes will allow for more synthesis of bio-compatible materials, accurate environmental monitoring and remediation, self-assembly of complex multi-cellular machines, etc. [8].

This project has been pursued in the Murray Lab, due to its focus on the application of feedback and control in biology. Members of 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]. Ongoing wet lab research involving synthetic cells includes the expression of the MsbA transporter and Superfolder Green Fluorescent Protein (sfGFP), a search of different extracts from various extremophiles, as well as the use of ATP synthase as a motor for synthetic cell actuation. There is also ongoing dry lab research related to modeling and simulations of synthetic biology. Various software packages, such as BioCRNPyler, bioscrape, and autoReduce are being actively developed by members of Murray Lab. BioCRNPyler and bioscrape are packages that allow for simulations of chemical reaction networks [13, 14]. This software has been integral to our research. autoReduce is a Python-based tool that is used for model reduction of input-controlled biological circuits [15]. This tool will help with parameter extraction that is relevant to biological experiments with the assumptions made (such as time-scale separation with the quasi-steady state assumption). There are complementary projects ongoing in Murray Lab indirectly related to synthetic cells, such as dosage control and the 3D segmentation of encapsulated cells.

**Problem**

In this project, we aim to find a solution to the ATP problem since we cannot get long-running reactions due to a lack of energy. We want 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 mechanism that is able to maintain ATP levels, as shown in Figure 1. TX/TL is a transcription/translation system that creates protein from linear DNA templates [1, 6, 7]. Since the only source of energy for synthetic cells is from the initial cell extract provided, the peak lifetimes of these artificial cells range from 4-6 hours [6, 8]. We would ideally like to increase this lifetime to at least 10-12 hours. This problem arises from current experimental setups seen in the literature. One of the factors to consider during the construction of these synthetic cells is metabolism [8]. We notice, from literature that lifetimes of synthetic cells are limited [4, 7].

Our initial proposed mechanism for ATP lifetime extension is the rheostat machinery published by James Bowie Lab (UCLA) [7]. The rheostat is able to maintain ATP concentrations for up to 70 hours in buffer. We want to explore whether or not the rheostat can extend ATP levels in synthetic cells with TX/TL. See Figure 2 for a schematic of the TX/TL system in conjunction with the rheostat pathway. It is able to selectively choose different metabolic pathways depending on the amount of free phosphate present in the reaction environment. At low Pi concentrations, ATP levels are high, so the GapN pathway is preferred. The GapN pathway does not make any additional ATP. At higher Pi concentrations, ATP levels are low, so the mGapDH-Pgk pathway is preferred. This pathway allows for the regeneration of ATP [7].