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Intro Draft

# Understanding the difference between encapsulated and bulk protein synthesis experiments while maximizing energy use

## Introduction

*Synthetic Biology*

Synthetic biology focuses on the engineering of devices, pathways, networks, and systems that utilize tools pre-existing in biology. In this field, principles from engineering and biology can be combined to design new, multifunctional biomolecular systems for unique purposes[10]. These include DNA computers for cancer treatment, targeted therapies for antibiotic resistance, creation of synthetic cells, and more. The objective is to seek solutions for improving and utilizing biomimetic processes to address existing engineering challenges.

*Build-A-Cell*

There is a growing interest in the development and application of genetically-programmed synthetic cells, which do not replicate or divide. This initiative is part of the Build-A-Cell project, which aims to construct a cell using a bottom-up approach[11]. To uncover the components of a cell, we must study and gain a comprehensive understanding of their building blocks.

By developing a programmable chassis which we understand completely, it will be easier to repurpose biochemical circuitry to do a variety of diverse tasks. We can build machines that will die after performing a desired task. Constructing synthetic cells allows us to program their destruction and to control whether the organisms enter our biosphere. Synthetic cells can be customized to contain the bare-minimum components. They can be used as environments in which more complex engineered systems can be implemented with less off-target interactions and undesired noise. In this process, we can uncover what we really know about cellular processes. By supplementing experiments with simulations, we add an additional level of confidence in our knowledge. We are able to easily test hypotheses for underlying mechanisms, such as enzymatic mechanisms or transcription, by implementing and solving them as chemical reaction networks.

There are various commonly used parts in the Build-A-Cell community. To make vesicles, we use and oil-emulsion technique with phosphatidylcholine (POPC) lipids. For small scale transcription and translation, TXTL is used. TXTL is an extract composed of parts and proteins from *Escherichia Coli* that are necessary for transcription and translation[6]. A schematic is shown in Figure 1. Given a GFP DNA template with TXTL within a vesicle, we are able to visualize protein production, a vital step for the success of the Build-A-Cell project.

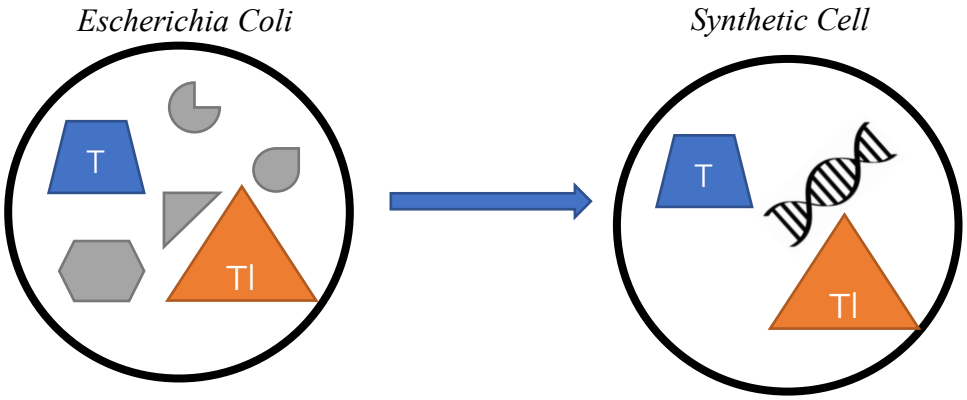


**Figure 1**. A depiction of a synthetic cell. The transcriptional (orange) and translational (blue) machinery from *E. Coli* is extracted and placed into a liposome with the desired DNA template.

*Limitations*

Current synthetic cell technologies can be used in both batch (bulk) and compartment mode (encapsulated). They are used for optimal biofuel production, since controlling which molecules are in which compartments can result in higher efficiency and yield by reducing off-target interactions. Synthetic cells can also be used to sense environments and release/remove molecules as desired.

However, power for transcription and translation is limited. There is opportunity to address this issue and find new ways to optimize energy use. Various metabolic pathways have been shown to replenish energy sources for a longer time period, although it is difficult to investigate and quantify the toxic side effects these metabolites could have on protein production. There are also proposals to integrate proteins observed naturally, such as ATP synthase and proton pumps, that are capable of regenerating ATP with minimal side effects. However, ATP synthase is a large, complex membrane protein. Spontaneously integrating this into a vesicle from purified protein or genetic code *in vitro* is a unique challenge on its own. Currently, encapsulated protein production with TXTL lasts only 10-12 hours, varying with different TXTL batches[12]. There is a need to find a complementary mechanism for energy lifetime extension that is not overcomplicated while mitigating unnecessary crowding or toxic effects.

Further, different dynamics are often observed in batch and encapsulated experiments. It remains quantitatively unexplained how and why protein production rates and steady states differ. Uncovering this issue and attempting to develop an automated, universal platform for cell-free systems will significantly reduce variability.

*Research Objective*

When building synthetic cells, there are five main subsystems to be considered: spatial organization, metabolic subsystems, sensing and signaling, regulation and computation, and actuation. The problem we have chosen to tackle involves the metabolic subsystems and spatial organization[2]. More specifically, we choose to study how to maximize ATP lifetime and to uncover the differences between bulk and vesicle dynamics[2].

We aim to extend the lifetime of processes within cells derived from liposomes by exploiting compartments and membrane channels. Currently, processes last about 10-12 hours.

We aim to increase this to at least 24 hours. ATP, or adenosine triphosphate, is an organic compound that acts as the energy source for many different processes in cells. It has been repeatedly shown that ATP is the limiting factor in bulk and encapsulated protein synthesis, which we will confirm[12]. We will explore whether taking advantage of compartments will allow us to optimize energy use within a vesicle, offering opportunity for molecules that may cause toxic side effects to leave the cell.

Previous work has shown that vesicles with passive membrane channels ($\alpha$-hemolysin) surrounded by energy buffer has extended the steady state values of deGFP production[13]. When integrated into the membrane, it allows components from energy buffer, such as ATP or NTPs, to enter the cell and revive or extend protein production[13]. Further, these pores also give toxic molecules a chance to escape the vesicle. For example, more free phosphate may leave the cell, resulting in less random, toxic phosphorylation, as indicated in Figure 2. However, adding additional energy buffer in bulk experiments does not always guarantee greater protein production. By adding reagents, such as energy buffer or extract, in the beginning or middle (spike) of protein production in bulk and vesicle, we will be able to collect sufficient data to develop models in attempt to explain any observed differences.

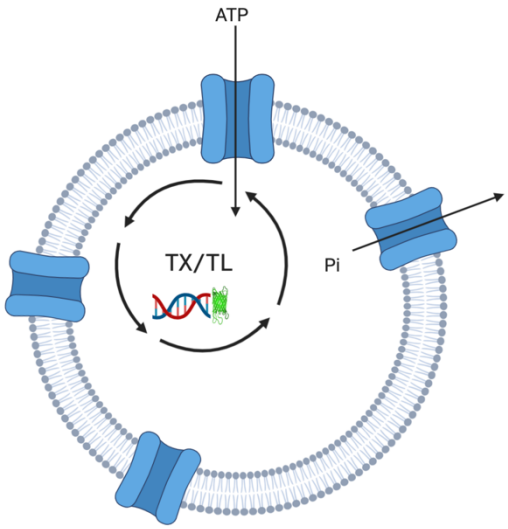


**Figure 2**. Schematic of $\alpha$-hemolysin membrane pores on a vesicle with DNA and TXTL inside. Ideally, we would see some outward diffusion of toxic molecules, such as free phosphate (Pi), and inward diffusion of desired molecules, like ATP.

We use TXTL in vesicles so we may control the creation of desired protein from DNA templates. We hypothesize that models and experiments will reveal the different dynamics in bulk vs encapsulated reaction, and allow us to design ways to maximize energy use. 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 and how encapsulation affects cellular processes. By discerning what components are crucial for energy regeneration, we can understand how metabolism truly works in cells. We can also understand if

energy is the limiting factor for many existing experiments. 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]

*Membrane Integration*

In previous work, $\alpha$-hemolysin has been used to extend lifetimes in synthetic cells. $\alpha$-hemolysin is a membrane pore that allows the passive transport of small molecules around 3kDa in size. We use a simple genetic circuit to verify and test whether $\alpha$-hemolysin membrane integration has occurred, as shown in Figure 3. IPTG is membrane impermeable. When added, it allows for the constitutive activation of a fluorescent protein, such as mScarlet. When absent, LacI will repress mScarlet transcription.

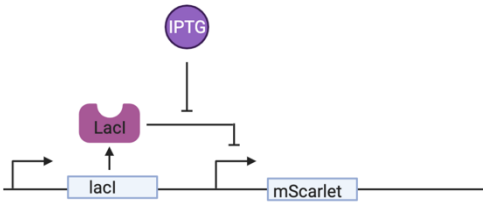


**Figure 3.** Genetic circuit schematic. IPTG, a membrane impermeable transcription factor, is used to repress LacI such that mScarlet production can be transcribed, translated and measured.

*Modeling and Simulations*

We will model our data via chemical reaction networks (CRNs). Software, such as BioCRNpyler, allow us to choose enzymatic mechanisms, as well as rate constants and initial conditions. We can model bulk experiments simply by including our assumptions about degradation, leak, what processes use ATP, etc. Similarly, we can model vesicle and $\alpha$-hemolysin by including parameters which code for the concentration of $\alpha$-hemolysin and how long it may take for transport to occur.

Additionally, there is an existing challenge to easily combine models in synthetic biology. Because ATP extension mechanisms will be studied *in silico*, this gives us an opportunity to link these models with others (such as DNA export or temperature sensitivity). This will allow us to better understand the effects ATP extension may have on these processes as well as studying methods by which model combination can be achieved easily.

*Methods Overview*

Moving forward, we will (1) use chemical reaction network simulators to model bulk and experimental data, (2) perform experiments with TXTL and collect fluorescence data on a plate reader, and (3) collect microscopy data of vesicles with fluorescent reporter proteins.

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