Modeling a Glucose Pathway and an ATP Synthase Mechanism shows ATP Life Extension in Synthetic Cells

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Abstract

In synthetic cell protein synthesis, a potential limiting factor is the energy supply for transcription and translation. By computationally studying mathematical models of various ATP regeneration mechanisms in synthetic cells, we aim to propose experimental methods for ATP life extension. We use available software tools to study two models. These allow us to develop and study mass-action models by implementing simple chemical reaction networks. Our simulations show that a glucose metabolic pathway can extend lifetime of ATP up to about 60 hours. Integrating ATP synthase can also lengthen the lifetime of ATP to various times depending on the implemented proton gradient mechanism. These simulations will help us understand if ATP is truly the limiting factor. To ensure prolonged synthetic cell protein synthesis, either the glucose pathway or ATP synthase mechanism can be used. In the future, it will be useful to perform wet-lab experiments in order to compare our model to data.

Introduction

An ATP Rheostat and ATP Synthase Model were chosen as regeneration mechanisms for synthetic cells

There is a growing interest in the development and application of genetically-programmed synthetic cells, cells which do not replicate or divide. We want to explore whether ATP life extension mechanisms, as depicted in Figure 1, can extend ATP levels in synthetic cells with TX/TL, a transcription/translation system that creates protein from linear DNA templates [3,4,5]. See Figure 2 for a representation.

An efficient, longer-lasting method to provide energy required for internal reactions will allow us to carry out more complex, sustainable experiments.

The two mechanisms we studied are a glucose metabolic pathway, also known as the ATP rheostat (from Bowie Lab at UCLA), and an ATP synthase mechanism [5]. See Figure 3 below for the reaction pathway of the ATP rheostat. A schematic for the ATP synthase model is shown in Figure 4.

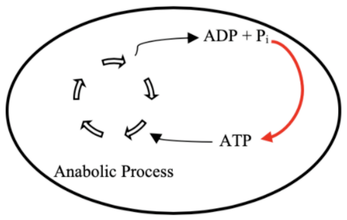

Figure 1. A diagram representing our overall goal. By implementing some mechanism (red arrow), we would like to regenerate ATP to longer support an anabolic process.

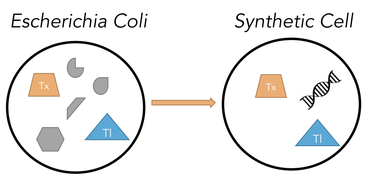

Figure 2. A depiction of a synthetic cell. The transcriptional (orange) and translational (blue) machinery from *E. Coli* is extracted and placed into a liposome with the desired DNA template.

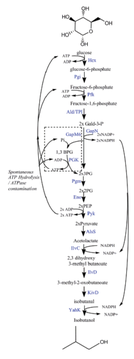

Figure 3. Entire Rheostat pathway as shown in the Opgenorth et al paper [5].

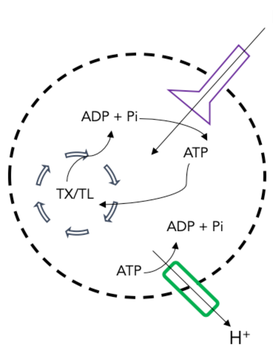

Figure 4. ATP Synthase (purple) model schematic. We include a proton pump (green) to maintain the proton gradient necessary for ATP synthesis.

Methods and Materials

Different software packages were used for model simulation

We used multiple software packages in order to test the hypothesis *in silico*. In particular, we used BioCRNpyler, bioscrape, SBML, and sub-sbml [7,8,9]. Biocrnpyler is an object-oriented framework (written in Python). Given simple descriptions, the software can generate chemical reaction networks (CRNs). These are outputted as SBML files. SBML is a model representation format, which uses the language XML and is commonly used in systems and synthetic biology. We then used bioscrape as a CRN simulator. Given an SBML file, bioscrape can solve the system and return an output of results that can be visualized. Sub-sbml was also used because it allows for the creation of subsystems, the ability to combine multiple subsystems, and the ability to model interactions such as molecule transport and membrane diffusion. A typical workflow is shown in Figure 5.

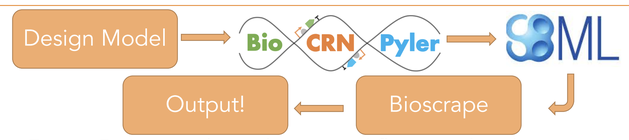

Figure 5. A typical simulation workflow used in this project.

Results

ATP Life Extension can be modeled with BioCRNpyler and Bioscrape

For both models, we were able to show that ATP life extension can be achieved. After choosing parameters based on literature for the ATP rheostat, we simulated the pathway, shown in Figure 6 below. We note that there is isobutanol production and glucose consumption, as expected (Fig 6a). The lifetime of ATP is extended when the rheostat is implemented compared to when we only model ATP use (Fig 6b). The ATP use case is included in all simulations to represent ATP consumed by TX/TL.

The three main components of this ATP synthase are ATP synthesis via ATP synthase, proton gradient maintenance via a proton pump, and ATP use. When we include the proton pump, ATP is completely regenerated and can reach a higher steady state (Fig 7 ab), confirming that a proton gradient is necessary for the success of the ATP synthase model.

The ability to combine different models is one of the challenges of synthetic biology. Thus, we attempted to integrate our ATP life extension models with others, such as the one shown in Figure 8.

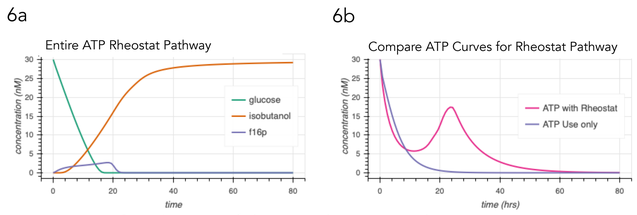

Figure 6. Simulations of the ATP rheostat pathway. We see stoichiometric production of isobutanol (6a) and extended ATP production with the rheostat (6b).

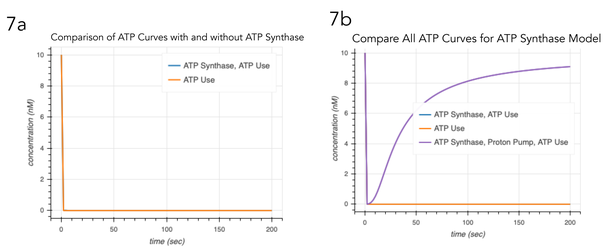

Figure 7. Simulations of the ATP synthase model. Without the proton pump, there is not enough regeneration (7a). When the proton gradient is maintained, we note a higher steady state of ATP (7b).

Figure 8. VirE2 membrane integration and ssDNA export model schematic

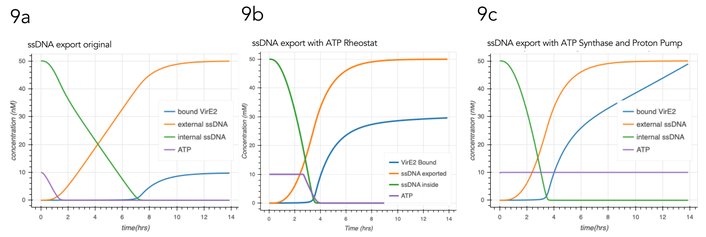

Figure 9. Combination of ssDNA export and ATP life extension models. 9a) Original ssDNA export model. 9bc) ssDNA export model with ATP Rheostat and ATP synthase model shows quicker ssDNA export and more bound VirE2.

Discussion

Our models can be integrated with others

It is important to combine these models with others to understand and confirm their effects. This was done with Agrima Deedwania's (IIT Delhi) single-stranded DNA (ssDNA) export model [10], schematic shown in Figure 8. We notice that when both ATP regeneration models are separately combined with the export model, there is more bound VirE2 and faster ssDNA export (Fig 9 bc). We also studied the effects of temperature on our model as a potential method to control the rates of ATP regeneration – Ayush Venkatesh Bindlish's (IIT Delhi) model (Figure 10). Results are shown in Figure 11. Going forward, it will be useful to validate and test these claims with experiments.

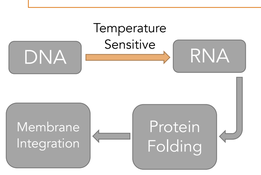

Figure 10. The DNA to RNA step of the modelling is temperature sensitive.

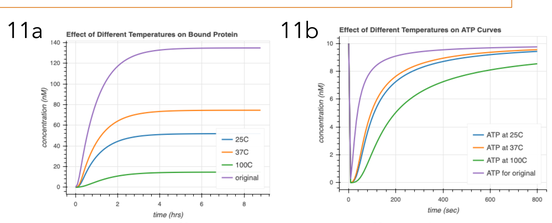

Figure 11. Different temperatures affects the amount of bound protein (11a) and ATP regeneration rates (11b) of the ATP synthase model.

Conclusions

Experimental data will be necessary to validate our claims

In conclusion, we have been able to model two mechanisms for ATP regeneration in synthetic cells; the ATP rheostat and ATP synthase model. These *in silico* results have allowed us to understand what parameter sets ensure desired ATP levels. A designer may choose a model based on the desired timescale and complexity of their experiment.

Validation of this project with experimental data will offer new information. The parameters implemented in this model are all presumed to be on the correct order of magnitude however there is minimal available data to confirm further accuracy. To continue, maintenance of a proton gradient mechanism for the ATP synthase model may be more challenging than depicted. Others have been able to use light-activated bacteriorhodopsin to develop a lasting gradient. Further, there are 15 additional enzymes that need to be expressed for the ATP rheostat pathway. This could lead to harmful side effects and rapid resource depletion *in vitro*. Experimental data will offer answers to these questions and more.

Samuel P. and Frances Krown SURF Fellow
8.19.2020
Note: All source code can be found at [11]

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