Script: Today I’m going to talk about two mechanisms that I modeled this summer in attempt to study ATP life extension in synthetic cells.

The overall motivation of this project was to extend the lifetime of anabolic processes in synthetic cells, or liposomes. An efficient, longer-lasting method to provide energy required for internal reactions will allow us to carry out more complex, sustainable experiments which will broaden the range of possible research in synthetic cells.

First, we looked at a metabolic pathway that can create isobutanol from glucose. This was termed the ATP rheostat pathway and was developed by James Bowie Lab from UCLA. When there are low free phosphate concentrations in the surrounding environment, the original pathway is preferred, which does not create any net ATP. However, when there are high free phosphate concentrations, a different enzyme of the rheostat is activated and preferred, through which there is a net gain of ATP ~~(and still stoichiometric isobutanol production).~~

After choosing parameters believed-to-be on the correct order of magnitude we performed simulations by using biocrnpyler and bioscrape, two softwares that work in conjunction to output results from simple descriptions of chemical reaction networks.

After simulating, we were able to see the production of isobutanol and the consumption of glucose as expected. We also model ATP use in these simulations, which is representative of the all the energy that is used in an anabolic process within a synthetic cell, such as transcription and translation. We notice that ATP can last in the system up until hour 70 for a particular parameter set. It is experimentally seen that ATP is depleted by 20 hours for this particular setup. Thus, we were able to visualize atp life extension!

Next, we looked at an ATP synthase model. In this model we include gene expression and membrane integration of ATP Synthase, which is a membrane protein that synthesizes ATP from ADP and free phosphate when there is a proton gradient. ~~Then, protons rush into the liposome, causing rotation of the protein, which triggers ATP production.~~ However, in the absence of this proton motive force, ATP synthase cannot create energy. For this reason, we also included a proton pump in this model. This proton pump uses some energy to pump out h+ ions, thereby maintaining the proton gradient. When we include the proton pump, we see that ATP levels can be maintained at their initial concentration for a much longer timescale than the rheostat. This is due to the self-sufficient nature of this model.

To test both models, we combined it with a VirE2 membrane protein integration and single-stranded DNA export model developed by Agrima Deedwania, another summer student in the Murray lab. We found that the ATP life extension models had positive effects and caused faster export and more membrane protein integration in her model. We also studied the effects of temperature on our model as a potential method to control the rates of ATP regeneration.

In conclusion, we have been able to study simulations for two separate mechanisms of ATP regeneration in synthetic cells. ~~These~~ *~~in silico~~* ~~simulations 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.

For the future steps, validation of this project with experimental data will offer new information. For example, 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. Other questions, such as the viability of our proton gradient maintenance mechanism and harmful side effects from the rheostat enzymes, can be also answered with experimental data.

~~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.~~

Thank you and I look forward to answering your questions!