**Why is there a hump in the atp for the rheostat?**

This represents the flux through the atp generating part of the pathway. ATP is consumed and then, once the system is able to react to the higher free phosphate concentrations, it activates the atp generating part of the rheostat. That initial consumption going low could also be due to the fact there is an atp use step near the beginning of the rheostat pathway.

**Speak about the parameters you’ve chosen. How and why?**

So there were a multitude of parameters that had to be decided. Unfortunately, it was quite difficult to find a substantial amount of these parameters online so I started with some guesses- things like a typical enzyme carries out 10 reactions per second.

Then I started to do some parameter searches in attempt to learn what sets will give me the results that I want (which is part of the beauty of modeling, doesn’t take much effort to do this). If I wanted to mimic those parameter sets in experiments, I could perhaps introduce competitive binding for slower rates and/or use temperature as a way to control the binding rates.

**Why did you choose the rheostat?**

The rheostat was able to show ATP life extension up until about 70 hours in buffer. I wanted to see if those changes could be translated to a liposome. I tried to match my data to their experimental data and see for what parameters can I get my rheostat to last up until arount 70 hours as well (even with atp use).

**Did you use Tx/TL parameters / initial conditions?**

As far as Tx/Tl initial conditions go, I wasn’t super picky about keeping things exact, but I tried to remain around the correct order of magnitude. Sometimes it was off and I was told to fix it by members of lab and I haven’t gotten to do any experiments yet so my intuition of how things work was more naïve at times.

**Why does the ATP curve eventually go down in the ssDNA export model? What is the limiting factor there?**

At the point when the ATP is being used too fast and there is not enough left to power the first steps of the rheostat pathway, regeneration shuts down. A way to mitigate that is to make the engineered enzyme that responds to free phosphate concentration more sensitive and trigger flow through the atp generating pathway quicker – not sure how I would do that but if we could that could make this model more sustainable.

**Why is the ATP curve at a lower timescale for the export model compared to the original simulation?**

Yes! So this was one of the first obstacles encountered. When I used the chosen parameters that you see in Figure 6b in conjunction with the export model, I didn’t see any effects because the timescale of regeneration was so slow that the atp was entirely consumed in her model by the time the rheostat pathway was even activated. In attempt to solve this problem, I made my reaction much quicker, so that we could visualize effects in her model. What the true parameters are I am not sure but we are able to learn what parameters will positively affect the export model. This is something that I would be able to fix and learn from with experimental data.

**What proton pump are you using? How did you choose how much ATP is consumed by the pump?**

Plasma membrane H+-ATPase aka P-type proton ATPase

This is often found in the plasma membrane of [plants](https://en.wikipedia.org/wiki/Plants), [fungi](https://en.wikipedia.org/wiki/Fungi), [protists](https://en.wikipedia.org/wiki/Protists) and many [prokaryotes](https://en.wikipedia.org/wiki/Prokaryotes). Made an estimate as to how much atp is being used, less than what is being used in txtl bt enough to power a proton gradient.

**How did you model ATP use in the atp synthase model? Why is different from the other simulations?**

I tried to make an order of magnitude of estimate for how much energy is used for transcription and translation of one protein per 10 seconds on average. The number was around 75 ATP used per sec.

For the rheostat simulation, the goal was to mimic existing experimental data. But since I didn’t have that for the ATP synthase I took a more order of magnitude approach. With experimental data the results might be different.

**Why is the ssDNA export model with atp synthase different than with atp rheostat?**

This is because the rheostat model is not self-sufficient for as long as the atp synthase model.

**Why does ATP continue indefinitely for ATP synthase model?** **Should there be degradation modeling there?**

Yeah! I didn’t account for protein degradation so that would be something to add to the model.

**Why did you model without proton pump for atp syn model?**

Initially I wasn’t going to include one and then through modelling I learnt that a proton pump will be necessary because I couldn’t get much life extension using parameters typically found in literature.

**Why is only that step temperature sensitive? Do you know that is true?**

This was Ayush Bindlish’s summer project! From my understanding he did a lot of reading and found some papers that said the process of rna polymerase finding and binding to the promoter region was the limiting step for different temperatures. So I included all those parts in the model and the purpose was to understand and study if different models could be combined – so we were glad to see that worked!

**SBML vs subsbml?**

SBML is used as language communication. Sub sbml has added functionality to combine compartments or different sbml files and pool resources and also model things such as liposome merging and membrane transport.

**Why can’t biocrn and bioscrape be one software?**

They are basically used as one software but they are each optimized for different things…

**What is massaction? What are CRNs?**

Massaction is the idea that a chemical reaction (like binding, a step in a pathway) is directly proportional the product of the activities or concentrations of the reactants.

A CRN is a set of reactants, set of products, and reactions. And you can generate ordinary differential equations for each specie in the system.

**What is Tx/Tl?**

Tx/Tl is a cell extract from e coli that is used in murray lab for synthetic cell experiments. It basically has all transcriptional and translational machinery from e coli so you can supply a dna template and get proteins.

**What determines the timescale of regeneration for atp syn model?**

The proton gradient mechanism! The proton pump functions to regenerate atp by maintaining the proteon gradient which allows atp synthase to function.

**What is the point of this project?**

Since more complex projects are being pursued in synthetic cells, more energy is required. However, simply inputting more energy systematically may not be the best way to solve that problem. For this reason, I wanted to find a way that energy could be consistently regenerated so processes aren’t limited by ATP but instead by other things, like degradation, that may need other solutions.

**What did you learn from modelling that you wouldn’t have learnt experimentally?**

I learnt how to deal with things from a molecular and more fundamental viewpoint. I also learnt that the rheostat is very sensitive to rate constants like binding and unbinding. So a system I’m studying may have the potential to have the effects that I want but perhaps those effects aren’t naturally occurring – perhaps there needs to be some engineering of parameters so I can use the same system that may be naturally occurring to do something that I want it to do.

**What experiments will supplement this?**

For one – I am considering trying the atp rheostat by expressing each enzyme.

I most likely will pursue the atp synthase project and learn how the proton gradient holds up and if atp regeneration is fast enough. I haven’t considered modelling of multiple atp synthases, I kind of grouped them all into one. SO once I have more experimental data I can make better guesses at what is actually happening.

**Was there value in combining models? What did you learn?**

For sure! From a computational standpoint, typically combining models in synthetic biology is difficult. But with the techniques we learnt it was really simple and doable and I think that’s noteworthy.

I learnt that sometimes the timescale of what I’m looking at is different than what someone else may be looking it. SO it’s really important to factor those things when you want to do this experimentally.