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| **Goals**  The development of artificial cells can be a useful tool for engineering within biology. By using genetic elements of a cell to create a completely non-living, synthetic cell, a wide variety of experiments and applications can exist.  We have shown 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 molecular rheostat that maintains ATP levels. We also see that the TX/TL system can last for longer periods of time with the regeneration of ATP. We have identified a set of robust, efficient parameters for longer-lasting energy production. With this technology, synthetic cells will be able to perform energetic processes for longer time periods. | **Technical Challenges**   * Implement a system that requires energy into liposomes. * There are no metabolic pathways that function in liposomes. * Implement an ATP regenerating pathway that will increase rate of activity within a synthetic cell * Implement an ATP regenerating pathway that will increase the lifetime of activity within a synthetic cell * We do not know what specific parameters affect ATP production rate. We do not know how/if these can be optimized. |
| **Objectives**   * Develop liposomes that can successfully produce proteins with the TX/TL system by week 2 of SURF * Develop liposomes that can successfully adopt ATP rheostat machinery from prokaryotes by week 4.   + ATP rheostat machinery from doi: 10.1038/nchembio.2418 * Show that the liposomes with the ATP regeneration machinery can cause more TX/TL activity by week 6. * Show that the liposomes with the ATP regeneration machinery can cause extended lifetime of TX/TL activity by week 8. * Understand, identify, and attempt to optimize parameters that affect the rate of ATP production by week 10. | **Approach**   * Implement the TX/TL system into liposomes. Use the output (protein production) to quantify activity and lifetime. * Use the ATP rheostat machinery to attempt to create a functioning metabolic pathway that works with the TX/TL system in liposomes. Adjust as needed. * Study protein production with the TX/TL system and ATP rheostat machinery to quantify the rate of activity. Detect the target proteins by using fluorescent protein and quantify the fluorescence with microscopy. * Study protein production with the TX/TL system and ATP rheostat machinery to quantify the lifetime of activity * Attempt to change experiment conditions, protocols, and other parameters in order to optimize protein production rate and activity lifetime. |

Things to Change

Goals – narrow down (seems like 6 years not 10 weeks)

Objectives –

* Can everything I am claiming really work?
* Recreating metabolism might be too hard for 2 weeks
  + Look what has been done close to this
  + Metabolism doesn’t work with Tx-TL
  + Why oxidative phosphorylation
* Mention specific pathway for ADP 🡪 ATP reaction
  + And paper reference
* Study what **Noireaux** has done to extend working time of TXTL
  + They do 12,24 hrs – not 3-10 days
* It’s not 2 liposomes lol

Technical Challenges

* Written as activities but should be technical challenges to be overcome
* Ex) no known metabolic pathways for ATP regeneration that work in tx-tl

Refine approach once everything else is fixed

Comments

* Energy regeneration is good idea but hard to find in the summer
* Have specific ideas about ADP 🡪 ATP regeneration demonstrated in the literature
* More realistic path – take mechanism from Altamura paper or from Bowie paper and get that working in liposomes
* Choose and implement machinery (ATP synthase etc) from prokaryotic cells and test efficient, robust functionality in liposomes
* Identify advantages and drawbacks of machinery from various cell types (test efficiency, robustness to perturbations, etc)
* Identify a metric of ATP production (fluorescence detection assays etc)
* Ensure there are no toxic effects or buildup so the functionality of the machinery does not decline over time

Identify methods to quantify metabolism