Option 1: Understanding flux of molecules in encapsulated protein synthesis to maximize energy use

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| **Goal**  For use of synthetic cells *in vitro*, it is important to implement various ATP regeneration or maximization pathways to guarantee robust, long-lasting processes1. With experiments and simulations, we have gained a better understanding of the flux of molecules in encapsulated protein synthesis, such as ATP, to maximize energy use. By utilizing compartments, we are able to encourage the outward flow of toxins, such as free phosphate, and inward flow of energy buffer components. Current encapsulated protein synthesis protocols in Murray lab plateau around 6 hours\*. | **Technical Challenges**   * Making membrane pores on vesicles * Time course microscopy on the same set of vesicles * Efficient flow of small molecules on agar pads * Modeling a whole synthetic cell model |
| **Objectives**   * Feb 5 – Model previous beginning spike/data collected. Simulate bulk experiments with various initial conditions (of ATP, DNA, 3PGA, etc.) to find efficient or unexpected combinations. * Feb 12 – Add -hemolysin to vesicles and test membrane integration. Perform bulk experiments of any combinations from simulations (with a focus on ATP / energy buffer). * Feb 26 – Add ATP / energy buffer to outside of vesicles with membrane pores to see if toxic effects are mitigated. Compare data to bulk experiments. * Mar 12 – Include phosphatases in vesicles with -hemolysin. Model vesicles with membrane pores in a whole cell model. * Mar 26 – Test other combinations (from simulations) in vesicles. | **Approach**   * Use purified -hemolysin to make membrane pores (help from Zoila) * Use agar pads to ensure imaging of same vesicles over time (help from Manisha) * Test flow of molecules on agar pads by comparing addition of reagents to vesicle outer solution vs to dried vesicles on pads * Work with William to reduce the Vivarium model to a minimal synthetic cell |

\*Murray lab note included because Noireaux lab data plateaus around 10 hours

Option 2: Understanding the difference between encapsulated and bulk protein synthesis experiments when maximizing energy use

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| **Goal**  Often in encapsulated protein synthesis, there is a discrepancy between bulk and vesicle dynamics. Previous work has shown that vesicles with membrane channels (-hemolysin) surrounded by energy buffer has extended the steady state values of deGFP production1. We were able to recreate this data and develop models to explain the difference in dynamics between bulk and encapsulated experiments. | **Technical Challenges**   * Making membrane pores on vesicles * Time course microscopy on the same set of vesicles * Efficient flow of small molecules on agar pads * Modeling a whole synthetic cell model |
| **Objectives**   * Feb 12 - Add -hemolysin to vesicles and test membrane integration. * Feb 26 – Add ATP / energy buffer to outside of vesicles with membrane pores to see if protein steady state can be increased (recreate Noireaux work1). * Mar 12 – Create a synthetic cell model of vesicle experiments. Create simple model of bulk experiments. * Mar 26 – Highlight any discrepancies between models and experiments / between vesicle and bulk. * April 9 – Find reasons for differences and test them. | **Approach**   * Use purified -hemolysin to make membrane pores (help from Zoila) * Use agar pads to ensure imaging of same vesicles over time (help from Manisha) * Test flow of molecules on agar pads by comparing addition of reagents to vesicle outer solution vs to dried vesicles on pads * Work with William to reduce the Vivarium model to a minimal synthetic cell |

Option 3: Discovering the optimal concentrations of reagents for encapsulated protein synthesis

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| **Goal**  ATP is considered the limiting factor in encapsulated protein synthesis. We have performed experiments and simulations to dispute (or validate) this claim by testing a variety of reagent concentrations. By exploring different ratios of reagents, we have also discovered optimal concentrations for protein synthesis in vesicles. | **Technical Challenges**   * Making membrane pores on vesicles * Time course microscopy on the same set of vesicles * Efficient flow of small molecules on agar pads * Modeling a whole synthetic cell model |
| **Objectives**   * Feb 5 - Model previous beginning/spike data collected. Simulate bulk experiments with various initial conditions (of ATP, DNA, 3PGA, NTP’s etc.) to find more efficient or unexpected combinations. * Feb 12 –Perform bulk experiments of any interesting combinations from simulations. * Feb 26 – Model those experiments, highlight and explain discrepancies from simulations. * Mar 12 – Iterate through another set of interesting simulations/bulk experiments(?). * April 9 – Recreate chosen experiments in vesicles. Perform spike experiments by using -hemolysin. * April 30 – Model vesicle experiments. Highlight/explain any discrepencies | **Approach**   * Use purified -hemolysin to make membrane pores (help from Zoila) * Use agar pads to ensure imaging of same vesicles over time (help from Manisha) * Test flow of molecules on agar pads by comparing addition of reagents to vesicle outer solution vs to dried vesicles on pads * Work with William to reduce the Vivarium model to a minimal synthetic cell |

References

1. Vincent Noireaux and Allen P. Lui, “The New Age of Cell-Free Biology”, *Annual Review of Biomedical Engineering*, Mar 9 2020, https://www.annualreviews.org/doi/pdf/10.1146/annurev-bioeng-092019-111110