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 * 24 hr data collection on the same set of vesicles (either microscopy or plate reader) * Efficient flow of small molecules on agar pads/cover slides * Modeling a whole synthetic cell model |
| **Objectives**   * Feb 12 - Add -hemolysin to vesicles and test membrane integration. * Feb 19 – Model previous beginning spike/data collected. Simulate bulk experiment w/ various initial conditions. Start creating whole syn cell model. * 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 – Hypothesize differences and test. | **Approach**   * Use purified -hemolysin to make membrane pores (help from Zoila) * Use agar pads or cover slips with nail polish to limit vesicle movement during imaging(help from Manisha) * Work with William to reduce the Vivarium model to a minimal synthetic cell |