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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 into liposomes that requires energy, such as the processes of transcription and translation. * There are no metabolic pathways that function in liposomes. * There does not exist an ATP regenerating pathway that has shown to increase rate of activity within a synthetic cell. * There does not exist an ATP regenerating pathway that has shown to 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 adopted 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. Detect the target proteins by using fluorescent protein and quantify the fluorescence with microscopy. * Use the ATP rheostat machinery to attempt to create a functioning metabolic pathway that works with the TX/TL system in liposomes. Study protein production in liposomes with and without the machinery. Adjust as needed. * Study protein production with the TX/TL system and ATP rheostat machinery to quantify the rate of activity. * 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. |