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Biosynthesis of Bi-Functional Products from the FASII System

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Biosynthesis of Bi-functional Products from the FASII System

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This project explored synthesizing long chain carbon structures with two functional groups using E. coli. We utilized the FASII system that normally produces long chain fatty acids and a previously engineered system that we created that allows us to modify the products of the FASII system. There were two methods that we explored in our research: attaching a second functional group after synthesizing a mono-functional molecule using pathways that we already have engineered using the genes pimA, alkJ, aftA, and LACS2, and introducing a precursor molecule that already contains a functional group to the previously engineered system. In addition, we explored an alternative method to synthesize bio-molecules called the cell-free system. This system takes advantage of the cell lysate that contains functional enzymes to bypass the theoretical constraints of working with living cells. Our results showed that the endogenous FabH does not tolerate precursor molecules that contain a functional group. Gas Chromatography Mass Spectrometry (GC-MS) data indicated that no molecules of interest were produced, however the cells continued to grow, showing that the FabH only took in endogenous precursor into the FASII system. In addition, the results showed that the LACS2 gene does not function well in our transformed cells, inhibiting the addition of a second functional group after the FASII system. Finally, the cell-free system also did not produce any fatty acids, signifying that there are still technical problems with the preparation of the lysate. Although the experiments were not successful, we were able to pinpoint the problems of each system, allowing us to return to this project at a later time to trouble shoot the issues.