BioGlass Crystallization of Type III Polyketide Synthase
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Abstract
Type III polyketide synthases such as benzophenone synthase (BPS) synthesize long carbon chain molecules. Having large families of substrates, these proteins are of special interest for biorenewable fuels. Previously, the structure of BPS was solved. This research seeks to expand on previous work by providing higher resolution data on this family of proteins with porous nucleating agent crystallization, potentially lending to better understanding of structural functionalities and, thus, better understanding of biorenewable fuel development.

Introduction
Dr. Stewart’s previous work on BPS resulted in diffraction data at 2.8 angstroms. Aiming to increase the data resolution, porous nucleating agents (PNA) were looked into. The conjecture behind PNA crystallization is that the porosity will help keep proteins in place and stabilize them, assisting in crystallization. BioGlass, the agent of interest, was and will be used in crystallization trials. Figure 1 is the backbone of BPS.

Methods
The proteins were produced in specialized E. Coli cells which were lysed and pelleted. After lysing, the proteins were run through nickel resin columns (figure 2) and an FPLC column to purify. To verify the purity of the proteins, an electrophoresis gel was used (figure 3). The existence of only single bands in the fractions indicates that the proteins are pure.

Results & Discussion
At this point in time, no substantial results have been found. However, it was observed that BioGlass does facilitate crystallization. Other proteins were also tested with BioGlass, showing some similar results. Currently, no crystals have been found that are viable for diffraction analysis. Figure 7 is an ideal crystal solution. Figure 8 is an actual BPS-BioGlass crystal solution.

Further Work
This work will be continued by Dr. Stewart. With the lack of viable crystals, a technique called “seeding” will be used. Already crystallized BPS will be broken up and then deposited into crystal trials to further facilitate crystallization. Additional techniques will be done as needed in order to optimize the crystals for diffraction analysis. Figure 9 shows BPS in protein analysis software, PyMol.

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References