Summary

Applicant: University of California, Berkeley
Project Director: Jay D. Keasling, PhD
Project Title: Accelerating polyketide synthase engineering for high TRY production of biofuels and bioproducts
Major Participants: Lawrence Berkeley National Laboratory, National Renewable Energy Laboratory, Argonne National Laboratory, Pacific Northwest National Laboratory

Microbial cells have been engineered to produce several products. However, the range of products has been limited by the metabolic pathways that can be engineered into cells and the lack of engineered enzymes, particularly those that can form carbon-carbon bonds. Polyketide synthases (PKSs) have the ability to produce a nearly infinite number of organic molecules, including biofuels, commodity chemicals, and specialty chemicals. The modularity of PKSs render them as excellent targets for engineering (at the DNA level) to produce novel compounds with predicted structures. However, it has been challenging to program PKSs for production of any desired molecule, in part due to the lack of an adequate Design-Build-Test-Learn (DBTL) cycle to assemble and express PKSs in a suitable host and produce the desired final product. The availability of a rapid, high throughput (HT) DBTL cycle for polyketide synthases would greatly reduce the time to engineer PKSs to produce a desired product.

Project Objectives. The goal of the proposed work is to develop a rapid, HT DBTL cycle for PKSs and demonstrate its utility for production of materials precursors. The objectives of the proposed work are 1) to develop a rapid, HT DBTL cycle for PKSs that will enable production of a large number of unnatural, organic molecules on demand at high titer, rate, and yield (TRY); 2) to demonstrate the utility of the PKS DBTL cycle to produce three molecules: one commodity chemical (caprolactam) and two novel materials precursors (2-allylcaprolactam and 2-benzylcaprolactam); and 3) to demonstrate the utility of the PKS DBTL cycle to produce one molecule (caprolactam) at high TRY. When fully developed, the DBTL cycle will have the following components: *Design:* PKS design software that will enable the design of PKSs to produce a desired target molecule and that is fully integrated with *Build* and *Learn*; *Build:* automated (robotic) construction of PKS genes, directly integrated with *Build*, and transformation into a suitable host engineered with acyl-CoA precursor biosynthesis pathways for production of the target molecules; *Test:* proteomic and metabolomic analysis of the PKS expressing host to troubleshoot protein engineering and identify bottlenecks in the biosynthetic pathway; and *Learn:* machine learning to determine which natural PKS components will work together to improve future designs.

Potential Impact. The PKS DBTL cycle developed through this grant will allow engineers to produce a large number of biofuels, commodity chemicals and specialty chemicals, including high performance materials with properties that cannot be found in petroleum-derived materials, that otherwise could not be synthesized using a biological system and to produce those molecules at high titers, rates, and yields. We will use the PKS DBTL cycle to produce the nylon precursor, caprolactam, which has been difficult to produce using any other biological system, and novel materials precursors that cannot be easily produced using any biological or chemical process. The PKS DBTL cycle will also allow scientists to better understand how natural PKSs function, which will further expand the number of molecules that can be synthesized by these complex systems.