

Identifying and applying design rules to engineer polyketide synthases for production of biofuels and bioproducts**Jay D. Keasling (PI)¹, Michael C. Jewett (Co-PI)²****¹Lawrence Berkeley National Laboratory, ²Northwestern University**

Abstract: Polyketide synthases (PKSs) produce numerous secondary metabolites in soil dwelling bacteria. For over two decades, researchers have known that portions of these enzymes can be recombined to produce modified natural products and other molecules that are not found in nature. However, it has been extremely challenging to refactor PKSs to produce chemicals (biofuels and bioproducts) that they do not naturally produce and achieve the high activities of natural PKSs. Because of the difficulty in refactoring these enzymes and the low activity of the resulting enzymes, relatively few new products have been produced using recombinant PKSs. Given the vast array of chemical functional groups that are found in natural polyketides, these enzymes have the potential to produce millions of different chemicals, including biofuels and bioproducts, if we could only refactor the enzymes easily and retain high activity. To use PKSs to produce many different biofuels and bioproducts, it is essential that we reduce the time it takes to build PKSs and improve the success rate of PKS designs.

The goal of this research is to discover the design principles for PKSs so that they can be readily constructed for producing many different biofuels and bioproducts. In turn, this information will also inform the scientific community about the rational design of PKSs and will enable more rapid use of these incredibly flexible and important enzymes. The specific aims are as follows. 1) We will learn the design rules for substituting reducing loops within PKS modules by substituting 84 PKS reducing domain donor cassettes into three engineered PKS acceptors. 2) We will identify domains for four different types of termination (lactonization, hydrolysis to acid, reduction to alcohol, and reduction/dehydration to alkene) that will be important for producing a variety of biofuels and bioproducts. 3) We will develop a machine learning software pipeline which systematically analyzes these data to infer the rules of successful PKS design, including modeling important structural features of PKSs that enable predicting stable and functional chimeric PKSs. 4) Based on the information we will obtain from the above specific aims, we will develop optimized chimeric PKSs to expand chemical space and produce one specialty chemical (a γ -lactone), one commodity chemical (caprolactam) and a gasoline replacement molecule. Constructs will be expressed in *Streptomyces* sp. and analyzed for production. 5) We will publish the data in JBEI public databases for the community to use in their designs of PKSs for various products.

This proposal will provide the data to construct unnatural PKSs that can produce advanced biofuels, such as branched alkanes, ketones and lactones. This proposal will also provide the data to construct unnatural PKSs for polymer intermediates and other commodity chemicals that might otherwise be produced from petroleum. It is not possible to produce most of these products using existing enzymes or microorganisms. The ability to produce them in a biological system will greatly enhance the US bioeconomy.