

Expanding the Scope of Biofuels and Chemicals Produced by Microorganisms through Design of Artificial Enzymes Containing Unnatural Amino Acids and Non-native Cofactors

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Project Goals: Microorganisms are increasingly used to produce biofuels and chemicals. However, developing robust microorganisms for the economical production of biofuels and bioproducts from low-cost, often-recalcitrant feedstocks at large scale with high titers, rates, and yields remains a significant challenge, especially when the products are not natural compounds. Compared to the vast number of chemicals and fuels produced by chemical processes, the number of biofuels and bioproducts that have been produced by microorganisms is rather small. While progress has been made in biotransformation of novel compounds using native enzymes and their variants, it becomes increasingly difficult to use native enzymes for many other biotransformations that produce novel molecules that are unprecedented in nature, or even in chemical or biological fields. We hypothesize that a major reason for such a limitation is that native enzymes using 20 natural amino acids and a limited number of biosynthesized cofactors, such as protoporphyrin IX, for structures and functions. To overcome the limitation in order to meet the challenge, we plan to incorporate unnatural amino acids and non-native cofactors into native enzymes to expand the functional groups and exert control over reactivity and selectivity.

To incorporate unnatural amino acids into a protein, we plan to use genetic codon expansion by engineering a pair of orthogonal amino acyl tRNA synthetase (aaRS) and tRNA.¹⁻⁵ We choose this method because it has been demonstrated to work well in many organisms ranging from bacteria like *E. coli* to the multicellular organism *Caenorhabditis elegans* and *Arabidopsis thaliana*, and most recently to mammalian cells. To incorporate non-native cofactors into proteins, we will first replace native cofactors, such as heme, with non-native cofactors of similar structure, such as another planer molecule, MnSalen or other metalloSalens (Salen =N,N'-bissalicylidene-1,2-ethanediamino anion)⁶⁻⁷ and organometallic catalysts.⁸ The resulting artificial enzymes will first be characterized by spectroscopic methods, such as UV-vis and EPR, followed by enzymatic activity measurements to assess the effectiveness of this approach and its products.

While the above method works well in test tubes, it becomes very difficult to apply the above method for living cells. To overcome this limitation, we use iBioFAB in the Carl R. Woese Institute for Genomic Biology at the University of Illinois at Urbana-Champaign to explore different combinations of cofactors, enzymes and reaction conditions. This use of iBioFAB for high throughput cloning and enzyme characterization will allow us to design artificial and select metalloenzymes with not only high efficiencies, but also high selectivity.

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