**Prospecting thiamine diphosphate-dependent carboligases and characterizing their promiscuity to create novel metabolic pathways from primary metabolites**

Bradley W. Biggs[1]* (bradleybiggs2014@u.northwestern.edu), Tracey Dinh[1], Matthew T. Robey,[2] Catherine Majors[1], Lindsay Caesar[2], Neil L. Kelleher,[2,3,4] Paul M. Thomas,[4] Linda J. Broadbelt[1], **Keith E.J. Tyo**[1]

[1]Department of Chemical and Biological Engineering, Northwestern University, Evanston, IL; [2]Department of Molecular Biosciences, Northwestern University, Evanston, IL; [3]Department of Chemistry, Northwestern University, Evanston, IL; and [4]Proteomics Center of Excellence, Northwestern University, Evanston, IL

https://pamspublic.science.energy.gov/CCBond

**Project Goals: The goal of this project is to characterize a library (>100) of thiamine-diphosphate dependent carboligase enzymes against a diversity of α-ketoacid substrates to determine the reaction landscape of this family of enzymes using machine learning, to identify ideals candidate enzymes from this family for biosynthesis applications, and then to use this information to assembly favorable enzymatic pathways to target bioproducts.**

Abstract. Recent studies have demonstrated that enzyme promiscuity, the ability of an enzyme to accept non-native substrates and perform non-native chemistries, is widespread in nature. This provides an opportunity for biological engineers to both leverage this capacity for valuable chemical transformations and to hone desired activities. One particularly interesting family of enzymes to this end is thiamine-diphosphate dependent carboligases, which condense two α-ketoacids (or aldehydes) to form new carbon-carbon bonds. Because of an abundance of α-ketoacids in the central metabolism of common metabolic engineering hosts like *Escherichia coli*, this allows for possible assembly of new and favorable biochemical pathways to targets of interest. One-step condensations could generate more efficient routes to desired targets and access to novel molecules, including chiral compounds. Our goal is to characterize a library (>100) of carboligases, map their reactivity on a diversity of α-ketoacid substrates using machine learning, and then utilize promising enzyme candidates for biosynthesis applications. Here, we demonstrate ability to characterize this class of enzymes against a library α-ketoacids on our way to mapping the catalytic promiscuity of this enzyme family.

*This work is supported by DOE grant DE-SC0019339.*