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), Jonathan Strutz,[1] Sara Wixon,[1] Matthew T. Robey,[2] Valerie J. Winton,[3,4] Neil L. Kelleher,[2,3,4] Paul M. Thomas,[4] **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

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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 work has shown 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 leverage this capacity for valuable unnatural 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 provides opportunity to assemble new favorable biochemical pathways to targets of interest. One-step condensations could allow for 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 our characterization workflow with proof-of-concept enzymes and reactions.

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