Evidence for Metabolic Channeling of Glucose into the Oxidative Pentose Phosphate Pathway to Drive NADPH Production in *Rhodosporidium toruloides*

Tianxia Xiao[1*](tianxiax@princeton.edu), Yihui Shen[1], Carl Scultz[2], Mingfeng Cao[2], Hoang V. Dinh[3], John I. Hendry[3], Anshu Deewan[2], Sujit Jagtap[2], Costas Maranas[3], Christopher Rao[2], Huimin Zhao[2], and **Joshua Rabinowitz**[1]

[1]Princeton University, Princeton, New Jersey; [2]University of Illinois at Urbana-Champaign, Urbana-Champaign, Illinois; [3]The Pennsylvania State University, University Park, Pennsylvania

https://www.igb.illinois.edu/DOEcenter

Project Goal: Understand the metabolic pathways in R.toruloides.

Fatty acid synthesis enables production of biodiesel from renewable sources. However, efficiently supplying enough NADPH for fatty acid synthesis is challenging. How is this achieved in natural systems? To explore this, here we utilize stable isotope tracing in R.toruloides IFO0880, a yeast strain that engages in copious fatty acid and lipid production. Metabolic flux analysis (MFA) based on 1,2-^{13}C tracing reveals high metabolic flux through the oxidative pentose phosphate pathway (oxPPP) in both nitrogen-rich and nitrogen-scarce environments. ^{2}H-glucose tracing confirms substantial NADPH and fat labeling mediated by transfer of ^{2}H to NADPH at the G6PD step of the oxPPP. Strikingly, quantitative calculations show fat labeling *in excess* of that expected if all NADPH was made via the oxPPP in a well-mixed system. The data can be explained by channeling between hexokinase and G6PD to drive oxPPP flux. Implications and future research directions will be discussed.

This work is supported by U. S. Department of Energy Office of Science, Office of Biological and Environmental Research under Award Number DE-SC0018260.