

Metabolic flux measurement in *I. orientalis* and *R. toruloides*

Yihui Shen^{1,2*} (yihuis@princeton.edu), Tianxia Xiao^{1,2}, Xi Xing^{1,2}, Sarat Gopalakrishnan³, Costas D. Moranas³, and **Joshua D. Rabinowitz**^{1,2}

¹Lewis-Sigler Institute for Integrative Genomics, Princeton University, Princeton, NJ;

²Department of Chemistry, Princeton University, Princeton, NJ;

³Department of Chemical Engineering, The Pennsylvania State University, University Park, PA

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

Project Goals: Measure metabolic flux in *I. orientalis* and *R. toruloides* using isotope tracers and mass spectrometry, in combination with quantitative modeling

Understanding of native metabolic pathway activity and regulation is valuable for accelerating metabolic engineering efforts. One reason for the great success of metabolic engineering in *S. cerevisiae* is decades of accumulated knowledge from its use as a model laboratory organism. Other organisms are likely intrinsically superior for many metabolic engineering objectives; however, their utilization is hindered by lack of knowledge. We are engaged in an effort to rapidly advance understanding of metabolism in two non-model yeasts, *I. orientalis* (which is desirably acid tolerant) and *R. toruloides* (which desirably synthesizes lipids). To this end, we have initially focused on mapping their metabolic pathway activity in batch culture and various nutrient limitations. Here, we will present data using different ¹³C-glucose tracers to explore central carbon metabolic activity in these two organisms (relative to *S. cerevisiae*) in batch culture. In addition, we will present data on how nutrient uptake, waste excretion, and biomass composition varies across carbon, nitrogen, and phosphorus limitation. Finally, early efforts to computationally integrate these data to achieve experimentally informed quantitative flux maps will be described. The overarching goal is to first quantitatively determine metabolic pathway fluxes across a diversity of conditions, and then to use integrative ‘omic approach to determine how these fluxes are controlled¹. In this manner, we are optimistic that, within a few years, we can bring knowledge of these organisms’ metabolic regulation up to a level approaching *S. cerevisiae*, dramatically increasing their value as metabolic engineering platforms.

References

1. Hackett, S. R. et al. Systems-level analysis of mechanisms regulating yeast metabolic flux. *Science*, 354, (2016).

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