

Biosensor and Optogenetics for Systems Biology of Yeast Branched-Chain Higher Alcohol Production and Tolerance

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Project Goals:

The goal of this project is to carry out a comprehensive systems biology study of branched-chain higher alcohol (BCHA) production and tolerance in yeast. We will leverage a genetically encoded biosensor of BCHA production to screen various yeast genomic libraries to measure the effects of different genetic perturbations (gene deletion, overexpression, or mutation) on BCHA production or tolerance. In addition, we will carry out transcriptomic studies leveraging again our BCHA biosensor, as well as optogenetic circuits to control BCHA production with light. This combination will allow us to establish closed-loop control systems that we can use to measure transcriptomic changes in well-controlled steady state or dynamic production settings. Ultimately, we will use these genomic and transcriptomic data to discover the key cellular networks involved in BCHA production and tolerance, which will be instrumental in developing better producing strains.

Branched-chain higher alcohols (BCHAs), including isobutanol, isopentanol, and 2-methyl-1-butanol, are some of the most promising advanced biofuels in development. These alcohols have better fuel properties than bioethanol, including higher energy density and better compatibility with current gasoline use and distribution infrastructure. Furthermore, BCHAs can be upgraded to jet fuel, making them excellent renewable fuels for ground as well as air transportation. Existing efforts to commercialize these types of biofuels are challenged by limited productivities, as well as the high toxicity that these alcohols have on strains engineered to produce them. Significant progress has been made in boosting yields and titers, particularly of isobutanol, through extensive metabolic and enzyme engineering based on detailed knowledge of branched chain amino acid metabolism and the structure and function of the enzymes involved. In contrast, virtually nothing is known about the interplay between different cellular networks and BCHA production and tolerance, leaving two basic questions unexplored: i) What are the key cellular networks that influence BCHA production? ii) What cellular networks are most affected by BCHA production, accounting for cellular sensitivity and tolerance to these alcohols?

In this presentation I will describe and demonstrate two new enabling technologies, which we will use to carry out the first systems biology studies on the production, sensitivity, and tolerance to BCHAs in yeast. The first is a genetically encoded biosensor that can monitor the activity of BCHA biosynthesis. We have used this biosensor not only to identify high BCHA producing strains, but also to develop selection screens, which we have used to engineer several enzymes in

the BCHA biosynthetic pathway to enhance their activity. The second enabling technology is a set of optogenetic circuits used to control the expression of different metabolic enzymes with light. Using this platform, we can dynamically control cell ethanol production and growth, as well as BCHA production through light pulses, allowing us to achieve record-breaking yields of these advanced biofuels [1]. Combining this genetically encoded BCHA biosensor with established yeast genomic collections, as well as with our optogenetic control platform to established closed-loop control systems, we will carry out first-in-class systems biology studies, including genomic and transcriptomic measurements to address the two fundamental questions above. These studies will provide new fundamental insights into the production and tolerance of BCHAs in yeast, which will open new avenues for strain development.

References

1. Zhao EM, Zhang Y, Mehl J, Park H, Toettcher JE, Avalos JL. Optogenetic regulation of engineered cellular metabolism for microbial chemical production. *Nature* 29; 555 (7698):683-87 (2018).

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