Multiplex Genome Engineering for Bioproduction of 3-Hydroxypropionic Acid and 1,3-Propanediol from Waste Gases

Fungmin (Eric) Liew (eric.liew@lanzatech.com)[1*], Nick Fackler[1], James Daniell[1], Steven D. Brown[1], Michael Köpke[1], and **Howard M. Salis**[2]

[1]LanzaTech Inc, Skokie, IL; [2]The Pennsylvania State University, University Park, PA.

http://www.lanzatech.com; https://salislab.net/

Project Goals: Gas fermentation is a commercially scalable platform for the sustainable biomanufacturing of valuable chemicals from abundant, low cost C1 feedstocks. We have engineered improved *Clostridia* strains that produce 3-hydroxypropionic acid (3-HP) from biomass syngas or industrial waste gas, characterized during continuous culture with online monitoring. 3-HP is an ideal bio-renewable precursor to acrylates and polymers (acrylonitrile, acrylamide, acrylic acid and acrylate esters) with a global market estimated as 3.63 million tons per year. To do this, we carried out genome-scale modeling and genome engineering to evaluate and introduce 3-HP biosynthesis pathways into an industrial *Clostridium* strain. We also developed new genetic tools that are capable of controlling the expression of many enzymes simultaneously, without introducing repetitive DNA. By combining these genetic tools with system-wide modeling, we are systematically redirecting metabolic flux towards 3-HP biosynthesis and eliminating byproduct formation with overall improved titers, yields, and productivities.

Gas fermentation has emerged as a promising biorenewable platform for manufacturing valuable chemicals from gaseous, non-food feedstocks that would normally be considered pollutants or waste. These gases include carbon dioxide (a greenhouse gas) and carbon monoxide (a harmful pollutant that will be oxidized to CO_2 when released in the atmosphere). LanzaTech is a world-wide leader in gas fermentation having commercialized and scaled up the production of ethanol from CO/CO_2 gas mixtures using *Clostridium autoethanogenum* as the whole-cell biocatalyst. Gas feedstocks are sourced from lignin-derived syngas, steel mill waste gas, and biorefinery waste gas, providing ample commercial opportunities for upgrading negative value pollutants into valuable co-products.

In this project, we are engineering industrial *C. autoethanogenum* strains to manufacture 3-hydroxypropionic acid (3-HP) with commercially relevant metrics (volume, titer, yield, productivity) from a syngas feedstock. As a first step, we applied a customized genome-scale metabolic model to evaluate the yield and thermodynamic feasibility of 25 different 3-HP biosynthesis pathways. *In silico* optimization revealed that high 3-HP yields can be achieved, but

require a significant level of strain engineering: from 5 to 30 enzyme expression levels need to be modified to yield significant improvements.

This challenge motivated the development of new genetic tools that can tunably control many enzyme expression levels in *C. autoethanogenum* to avoid slow iterative cycles and growth defects or genetic instabilities observed for knock-out of specific target genes. We developed the first dynamic CRISPRi knock-down system for *C. autoethanogenum* and are currently scaling up the number of enzymes that can be simultaneously targeting by leveraging Extra Long sgRNA Arrays (ELSAs), which are capable of expressing up to 20 CRISPR sgRNAs within a compact, non-repetitive DNA cassette (Reis et al., 2019).

By introducing the malonyl-CoA reductase from *Chloroflexus aurantiacus* we have demonstrated *de novo* biosynthesis of 3-HP in *C. autoethanogenum*. Surprisingly, we also observed considerable production of 1,3-propanediol (1,3-PDO); by itself, 1,3-PDO is an important chemical with market size of $490 million in 2019. We confirmed that the conversion of 3-HP to 1,3-PDO in *C. autoethanogenum* occurs via aldehyde::ferredoxin oxidoreductase (AOR) enzymes (Liew et al., 2017). Knocking-out or knocking-down AOR expression eliminates 1,3-PDO production and increases 3-HP production. The ultimate goal of this project is to introduce model-designed ELSAs into the *C. autoethanogenum* genome, guided by genome-scale modeling as well as techno-economic analysis, to deliver strains that convert syngas into 3-HP with improved titers, yields, and productivities.

References

Liew, F., Henstra, A. M., Köpke, M., Winzer, K., Simpson, S. D., & Minton, N. P. (2017). Metabolic engineering of Clostridium autoethanogenum for selective alcohol production. *Metabolic Engineering*, *40*, 104–114. https://doi.org/https://doi.org/10.1016/j.ymben.2017.01.007

Reis, A. C., Halper, S. M., Vezeau, G. E., Cetnar, D. P., Hossain, A., Clauer, P. R., & Salis, H. M. (2019). Simultaneous repression of multiple bacterial genes using nonrepetitive extra-long sgRNA arrays. *Nature Biotechnology*, *37*(11), 1294–1301. https://doi.org/10.1038/s41587-019-0286-9

We acknowledge the U.S. Department of Energy Office of Science, Biological and Environmental Research Division (BER), Genomic Science Program for funding of this project under Contract DE-SC0019090 for funding of this project.