

Engineering *Escherichia coli* for improved production of FA and FAEE

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Project Goals:

Developing synthetic biology tools to increase titers and conversion yields for the microbial production of fatty acids and fatty acid-derived biofuels.

Microbial production of chemicals is now an attractive alternative to chemical synthesis. However, there are very few strategies for engineering regulatory components and co-factor manipulation to improve product titers and conversion yields of heterologous pathways¹. Nature has evolved sensors for a variety of intracellular and exogenous molecules, however the cognate regulators are rarely optimal for modulating engineered biosynthetic pathways. To demonstrate the utility of assimilating natural sensors and engineering regulators, we have developed a dynamic sensor-regulator system (DSRS) for the production of fatty acid ethyl esters (FAEEs) in *Escherichia coli*. DSRS detects a key intermediate in the fatty acid biosynthetic pathway and dynamically regulates expression of enzymes involved in FAEE production. The engineered DSRS optimized the host's metabolism, improved the genetic stability of the producing strain, and significantly enhanced the FAEE conversion yield. Manipulation of enzyme cofactor-specificity is an alternative engineering approach, especially in strategies that involve overexpression of cofactor-dependent enzymes. For operation and cost efficiency in an industrial context, anaerobic culture conditions would be preferred, but this raises the issue of NADH becoming more readily available than NADPH within the cell and poses a challenge for a key step in the fatty acid biosynthetic cycle: reduction mediated by the NADPH-dependent FabG enzyme. Through sequence alignment analysis and mutagenesis, we have identified *E. coli* FabG variants that potentially have a greater specificity for NADH than for NADPH. Here we describe our efforts in manipulating cofactor dependence of a highly conserved step in fatty acid biosynthesis.

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