Biosensor-based Strategies for Improving Pathway Production in *Escherichia coli*

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Microbial production of chemicals and fuels is an attractive renewable alternative to petroleum-based processes. D-glucaric acid, a Department of Energy top value-added chemical from biomass, is a precursor to polymers such as nylons and used as a chelating agent in detergents. An engineered metabolic pathway requiring three heterologous enzymes to convert glucose into glucaric acid in *Escherichia coli* was previously demonstrated by the Prather lab. Glucaric acid production has been shown to be limited by the two downstream enzymes *myo*-inositol-1-phosphate synthase (MIPS) and *myo*-inositol oxygenase (MIOX). This work develops and deploys a biosensor that recognizes a pathway intermediate in order to overcome both limitations.

A biosensor for *myo*-inositol (MI) was developed using the transcriptional regulator IpsA from the organism *Corynebacterium glutamicum*. A hybrid promoter was designed to enable function in the desired host organism *E. coli*. The modular design of the biosensor permitted the behavior and MI dose response to be adjusted for the pathway applications.

The MI biosensor was used to regulate expression of *Miox*, the enzyme that consumes MI, such that *Miox* was transcribed only in the presence of its substrate. Controlled expression of *Miox* led to a 2.5-fold increase in glucaric acid titer compared to the static case where *Miox* was constitutively expressed. This dynamic regulation scheme was then paired with a system that dynamically knocked down glycolysis, which independently improved glucaric acid production by relieving competition of glycolysis with MIPS, the first pathway enzyme. The layered dynamic regulation scheme improved glucaric acid production by up to 9-fold compared to the static strain. The layered regulation strain produced nearly 2 g/L glucaric acid, representing the highest titer observed in *E. coli* K strains to date.

Next, the MI biosensor was used as a high-throughput screen for mutants of MIPS generated by directed evolution. By coupling fluorescence intensity to MI production, the biosensor provided a rapid readout for mutants of MIPS. The biosensor enabled 1 million-member libraries of MIPS generated by error-prone PCR to be screened by fluorescence-activated cell sorting (FACS). The biosensor-based screen identified MIPS mutants with up to 20% improvement in MI production.

This work used a biosensor to tackle two pathway limitations and improve glucaric acid production, showcasing the biosensor as a powerful metabolic engineering tool.

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