Dynamic regulation of metabolic flux using orthogonal quorum sensing

By Michael J. Ream

Dynamic regulation allows engineers to direct metabolic flux and cellular resources towards target pathways, improving production of value-added chemicals. One dynamic regulation strategy is quorum sensing (QS), a cell-to-cell communication that allows populations of cells to function as a collective. By applying QS to engineered pathways, the diversion of metabolic resources can be coupled to the population of the culture, thereby ensuring sufficient growth is achieved. These circuits can then be layered to allow for fine-tuned control of the cell.

Previous research has focused on QS systems that utilize acyl homoserine lactones (AHL) as signaling molecules. These systems are well characterized, but pairing them in layered systems is difficult due to similarities in signals, which can cause unintended switching of the opposing control system. Here, we first confirmed the functional orthogonality of the previously identified Tra and Rpa systems within a single strain of *Escherichia coli* MG1655 by analyzing the pairwise interactions of several AHL systems. The orthogonality of the systems allowed for independent tuning of two control strategies, which were utilized to increase the production of the natural products naringenin and bisnoryangonin. Tra was used to activate expression of TAL and 4CL in the production pathway, while Rpa dynamically downregulated competing pathways of native metabolism via CRISPRi. To our knowledge, the resulting regulations led to the highest extracellular titers at the flask scale with a final naringenin titer of 1251.2 ± 59.6 mg/L. This regulation strategy was then adapted to the parallel pathway of bisnoryangonin, leading to a naringenin equivalent titer of 597.7 ± 18.3 mg/L, which, to our knowledge, is the first time this pathway has been dynamically regulated and the largest reported titer from microbial biosynthesis.

In a parallel effort to obtain orthogonal QS-based regulations, we also focused on expanding the available QS systems for the model organism *E. coli*. Specifically, the Gram-positive QS circuits of Agr from *Staphylococcus aureus* and Com from *Bacillus subtilis* were implemented and subsequently improved for functionality in *E. coli*. These systems were implemented and improved for functionality by modifying the expression strength of circuit components, leading to the final iteration of the Com system reaching a dynamic range of 2.27 ± 0.05, while the Agr system was improved to a dynamic range of 4.05 ± 0.43. Both systems displayed tight control of expression, making it ideal for the control of toxic proteins or highly active enzymes such as Cas12a. This was demonstrated by applying the Agr system to downregulate endogenous genes *tyrA, pheA, trpE, ppc*, and *pabB* via CRISPRi, which diverted flux to the production pathway for salicylic acid.

Overall, this work developed and applied QS-based regulation systems to improve microbial biosynthesis of value-added natural products in *E. coli*.

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