SENSITIVE CELLS: ENABLING TOOLS FOR STATIC AND DYNAMIC CONTROL OF MICROBIAL PATHWAYS

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For the purpose of reprogramming the cellular network we employ *in silico* model of the genome-wide metabolism in order to predict genetic modifications that lead to increases in carbon fluxes of interest. Such Systems Biology approaches, in combination with traditional genetic engineering have resulted in robust production levels that can result in the commercially viable processes for the synthesis of important molecules. In addition to such approaches, we will also describe the engineering of both positive and negative feedback controls for dynamic tuning of metabolic flux around intracellular metabolites, such as malonyl-CoA in microorganisms using a dual transcriptional regulator. We will also demonstrate that such dynamic regulation can also be accomplished through the construction of orthogonal variants of the classic T7lac promoter using site-directed mutagenesis, generating a panel of inducible hybrid promoters regulated by both LacI and dCas9 and covering a wide expression range. Remarkably, dCas9 orthogonality in our system is mediated by only 23 nucleotide mismatches in a narrow window of the RNA:DNA hybrid, neighboring the protospacer adjacent motif (PAM). Finally, the presentation will also cover our work on the development and optimization of polycultures (three or more strains in co-culture) for the extension of recombinant pathways, such as the flavonoid branch pathway, *in vivo*. This technology has enabled, for the first time, the *de novo* production of flavan-3-ols and anthocyanins in *E. coli*. Utilizing a computationally guided optimization approach, we were able to demonstrate up to a 970-fold improvement over previously published monoculture titers.