A novel design strategy for industrially relevant, unnatural modular megasynthases

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Project Goal:

The project aims to develop a strategy for designing unnatural polyketide synthases (PKSs) that produce industrially relevant molecules in *E. coli*. As descendants of fatty-acid synthases (FASs), PKSs are known for synthesizing natural products with complex structures from simple building blocks such as malonyl-CoA. Many examples of natural PKSs and a few examples of engineered PKSs have been successfully expressed in *E. coli* but no universal approach to engineering PKSs has been elucidated. This project attempts to leverage new computational tools and the power of synthetic biology to solve this problem and thus expand the set of molecules produced at industrially relevant scale by *E. coli*.

Abstract:

One of the most significant challenges facing industrial biotechnology as a field is selecting the proper target molecules for strain development. The easiest molecules to produce in industrially relevant strains, such as ethanol in *S. cerevisiae* or fermentation products in *E. coli*, also tend to be the most competitive. This makes it incredibly difficult to balance the cost of strain development with the low-value of the target molecule, and has forced industrial biotechnology into high-value molecules. Generally, these high-value molecules are structurally complex and more synthetic steps from a commodity chemical, increasing the difficulty of strain development. Thus, technologies that simplify the process of strain development for more complex, valuable molecules are in high demand.

To this end, we have developed a new approach to designing modular megasynthases such as Type I Polyketide Synthases (PKSs) or Non-ribosomal Peptide Synthases (NRPSs). This design approach starts with a computational pipeline that searches publically available bacterial genomes for design rules specific to PKSs and NRPSs. This pipeline outputs amino acid sequences for linking the modular catalytic domains. These amino acid linker sequences are then combined with the requisite catalytic domains to create a complete amino acid sequence for an unnatural PKS/NRPS that should synthesize the target molecule. Codon harmonization is applied to this amino acid sequence to generate a nucleotide sequence that is then incorporated into the *E. coli* genome using a CRISPR/Cas9-based technique reported by our lab.[1] This gene is assembled via TAR cloning in *S. cerevisiae*. Here we report the first complete implementation of this design strategy to develop an *E. coli* strain that produces the fragrance ingredient delta-hexalactone via an unnatural Type I PKS. This strategy is universally applicable to all Type I PKSs and NRPSs and theoretically can be used to expand the biosynthetic capabilities of *E. coli* to increasingly complex molecules.

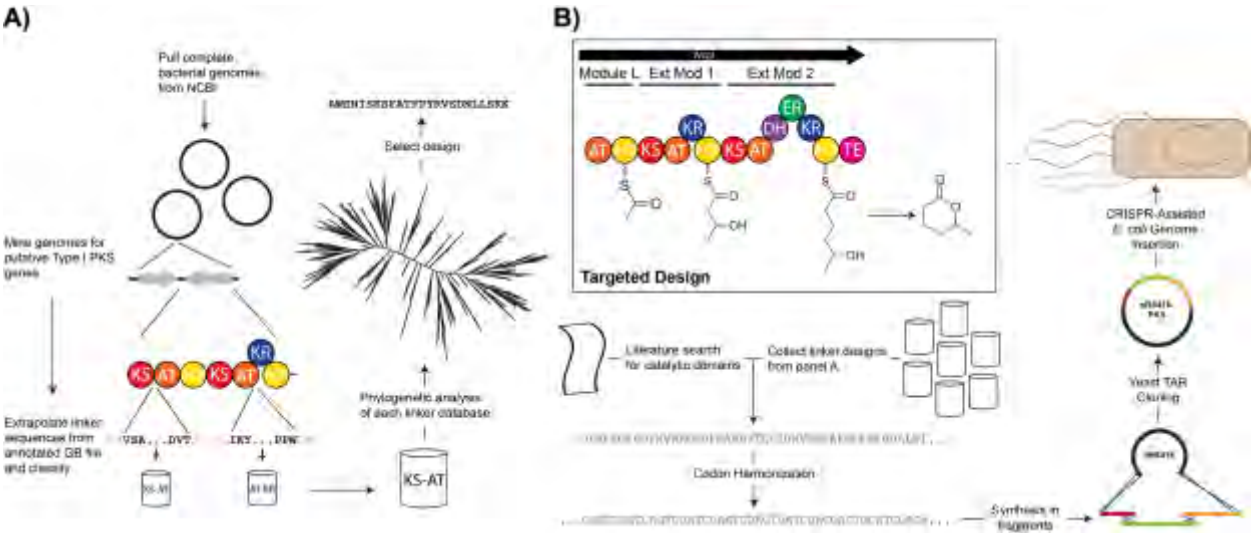

Figure 1. Outline of approach for the design of unnatural Type I PKS producing delta-hexalactone in *E. coli.*

[1]Bassalo, M.C., Garst, A.D., Halweg-Edwards, A.L., Grau, W.C., Domaille, D.W., Mutalik, V.K., Arkin, A.P., Gill, R.T.. Rapid and Efficient One-Step Metabolic Pathway Integration in E. coli. ACS Synth. Biol. 2016. doi:10.1021/acssynbio.5b00187

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