Genome-scale metabolic rewiring to achieve predictable titers, rates and yields of non-native products at scale

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Project Goals: Establish the scientific knowledge and new technologies to transform the maximum amount of carbon available in bioenergy crops into biofuels and bioproducts.

The grand challenge facing synthetic biologists today is understanding how any microorganism can be engineered to produce any desired final product. To meet this challenge, we have developed a new paradigm for host engineering, termed PrOSE (Product Obligatory Strain Engineering). Using genome scale metabolic models, we select a host which has the highest theoretical maximum yield with the added biochemical reactions from a given heterologous multi-gene pathway. Computational models predict gene targets for repression, which are realized using multiplex CRISPR interference (CRISPRi). We demonstrate that PrOSE successfully optimized production of the renewable dye, indigoidine, when produced using the emerging industrial host, *Pseudomonas putida* KT2440. Using PrOSE, production of the desired final product was shifted from stationary phase to exponential phase under optimized conditions, and close to 50% maximum theoretical yield indogidine was realized. In the absence of genome scale models, other systems biology methods can be used to query the solution space. Our results indicate that the careful selection of host/product pair along with computationally guided methods for rational strain engineering is possible. With the advent of facile tools for genetic engineering in nearly any organism, these methods may be generally applicable for any favorable host/product pair.

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