

## 37. Flux balance analysis constraint modification to locate metabolic engineering targets for ethylene production in *Escherichia coli*

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<https://sites.google.com/site/gillgroupcu/>  
<http://www.colorado.edu/UCB/chatterjeelab/>

**Project Goals:** This project aims to cultivate a genome-scale technology platform that allows for prognostic design and optimization of biological systems. One obstacle to successful manipulation of genome-scale systems is that the combinatorial space is intractable (i.e.  $\gg 10^{15}$ ). To address this challenge, we are developing computational and synthetic biology tools that allow for more effective search of the design space.

The use of *in silico* methods has become standard practice to correlate the structure of a biochemical network to the expression of a desired phenotype. Flux balance analysis (FBA) is one of the most prevalent techniques for modeling cellular metabolism. FBA models range from genome-scale reconstructions to simple minimal reaction sets, and have been successfully applied to obtain predictions of growth, theoretical product yields from heterologous pathways, and location of engineering targets to maximize product yield or design antibiotics. We take inspiration from high-throughput recombineering techniques, which show that combinatorial exploration can reveal optimal mutants, and apply the advantages of computational techniques to analyze these combinations. We introduce Constrictor, an *in silico* tool for flux balance analysis that allows gene mutations to be analyzed in a combinatorial fashion by applying simulated constraints accounting for down-regulation of gene expression. We apply this algorithm to study ethylene production in *Escherichia coli* through the addition of the heterologous ethylene-forming enzyme from *Pseudomonas syringae*. Targeting individual reactions as well as sets of reactions results in theoretical ethylene yields that are as much as 25% greater than yields calculated without constraint modification. We demonstrate additional functionality that allows Constrictor to scan a network for possible alternate metabolite products. Constrictor is an adaptable technique that can be used to generate and analyze disparate populations of *in silico* mutants, select gene expression levels, and troubleshoot metabolic networks.

### Publications:

Erickson KE, Gill RT, Chatterjee A. 2014. CONSTRUCTOR: Constraint Modification Provides Insight into Design of Biochemical Networks. PLoS ONE 9(11): e113820. doi:10.1371/journal.pone.0113820.

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