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Co-evolution of strain design methods based on flux balance and elementary mode analysis

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ABSTRACT

More than a decade ago, the first genome-scale metabolic models for two of the most relevant microbes for biotechnology applications, *Escherichia coli* and *Saccaromyces cerevisiae*, were published. Shortly after followed the publication of OptKnock, the first strain design method using bilevel optimization to couple cellular growth with the production of a target product. This initiated the development of a family of strain design methods based on the concept of flux balance analysis. Another family of strain design methods, based on the concept of elementary mode analysis, has also been growing. Although the computation of elementary modes is hindered by computational complexity, recent breakthroughs have allowed applying elementary mode analysis at the genome scale. Here we review and compare strain design methods and look back at the last 10 years of *in silico* strain design with constraint-based models. We highlight some features of the different approaches and discuss the utilization of these methods in successful *in vivo* metabolic engineering applications.

Contents

1. Introduction

Computational modeling has emerged as a fundamental tool for unraveling the complexity of biological processes. There are currently many different mathematical formalisms that can be used to model biochemical reaction networks (Machado et al., 2011). Among these formalisms, the constraint-based modeling approach has become widely adopted for large-scale modeling of metabolism (Bordbar et al., 2014). Constraint-based models have been used for a multitude of applications from guiding biological discovery to the improvement of industrial bioprocesses (McCloskey et al., 2013).

Constraint-based models can be used to simulate the cellular phenotype at steady-state using different methods. The most common approach, flux balance analysis (FBA), is a linear programming formulation that relies on the maximization of a cellular objective, such as growth or ATP generation, to determine the steady-state flux distribution through a metabolic network (Orth et al., 2010). Other methods, typically used for simulation of mutant strains, are based on principles of minimization of metabolic and regulatory adjustments (MOMA, ROOM) (Segrè et al., 2002; Shlomi et al., 2005). These kinds of methods are usually classified as biased, since they rely on the assumption of some evolutionary optimization principle to determine a biologically

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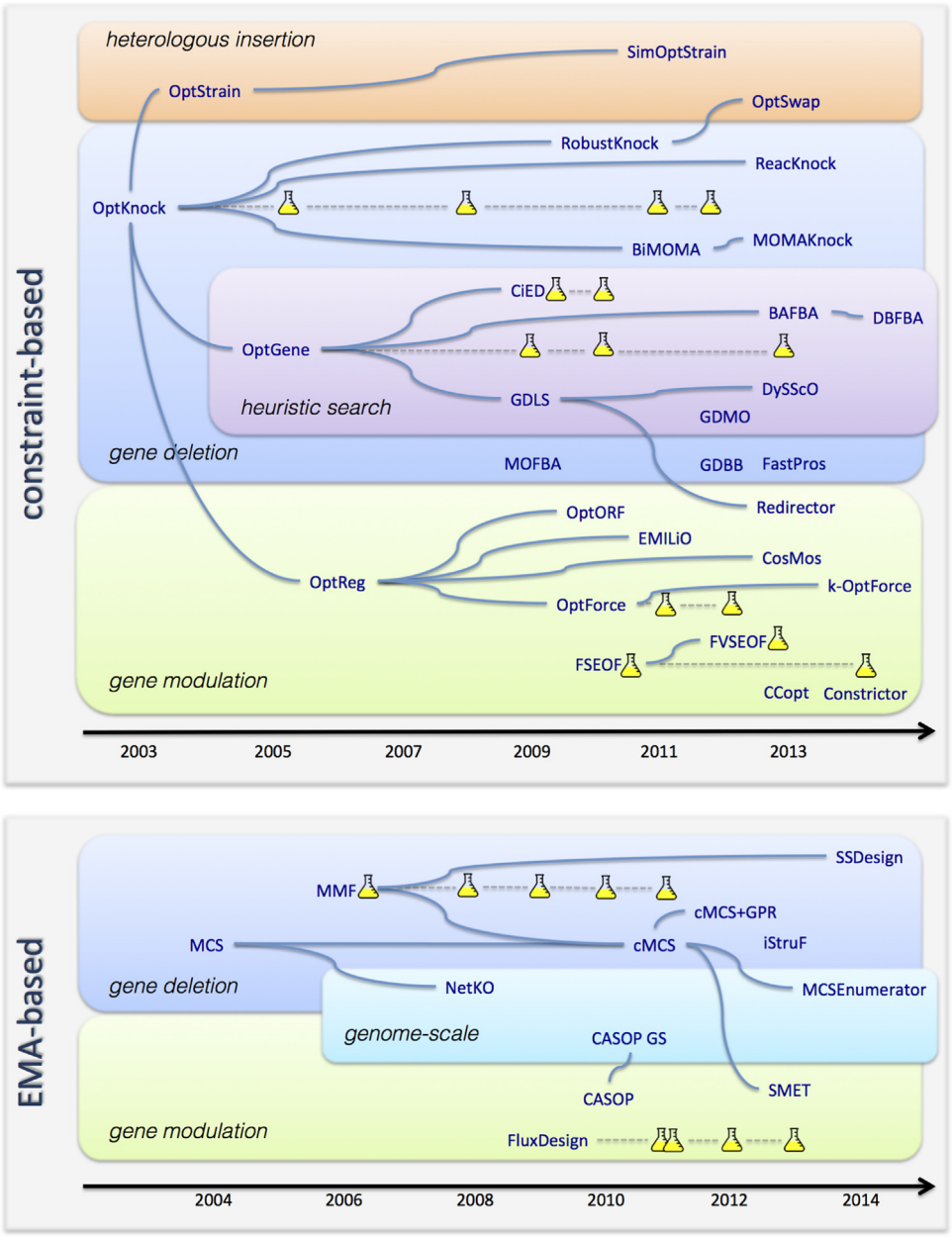

Fig. 1. Chronological perspective of the evolution of strain design methods using constraint-based analysis and elementary mode analysis (EMA). Connections represent common features between methods, not necessarily a direct extension of the previous method. The shake flask symbol represents experimental applications of the respective methods.

meaningful and physicochemically valid steady-state flux distribution.

There are also unbiased approaches to analyze feasible flux distributions in large-scale metabolic networks, including Monte Carlo sampling and metabolic pathway analysis (Lewis et al., 2012). Elementary mode analysis (EMA) is one of the most popular approaches for metabolic pathway analysis. It provides an unbiased description of the metabolic solution space in terms of minimal sets of reactions that operate in steady-state (Schuster and Hilgetag, 1994). These so-called elementary (flux) modes (EMs) are the basis for several methods to analyze the properties of metabolic networks, including robustness and fragility, as well as to calculate the theoretical yields of all metabolic routes (Trinh et al., 2009).

Both biased and unbiased methods have been used for strain design since the first genome-scale metabolic models of two industrially relevant microbes, *Escherichia coli* and *Saccaromyces cerevisiae*, were published in the early 2000s (Edwards and Palsson, 2000; Förster et al., 2003). From a metabolic engineering perspective, such models can be used for computer-aided design of optimal genetic and culture condition manipulation strategies to improve the production of industrially relevant compounds. However, given the size of metabolic networks, the exhaustive analysis of multiple simultaneous genetic manipulations becomes computationally infeasible. In order to address this challenge, a variety of methodological solutions have been proposed (Fig. 1).

2. Constraint-based methods

The first systematic optimization-based method for strain design was the OptKnock approach introduced by Burgard et al. (2003). OptKnock is a bilevel optimization approach that determines reaction deletion strategies to couple the production of a