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Advances in metabolic pathway and strain engineering paving the way for sustainable production of chemical building blocks

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Bio-based production of chemical building blocks from renewable resources is an attractive alternative to petroleum-based platform chemicals. Metabolic pathway and strain engineering is the key element in constructing robust microbial chemical factories within the constraints of cost effective production. Here we discuss how the development of computational algorithms, novel modules and methods, omics-based techniques combined with modeling refinement are enabling reduction in development time and thus advance the field of industrial biotechnology. We further discuss how recent technological developments contribute to the development of novel cell factories for the production of the building block chemicals: adipic acid, succinic acid and 3-hydroxypropionic acid.

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Introduction

Chemicals are an integral part of our daily life and play an important role in the world economy. The global chemical industry has grown rapidly since 1970, and the revenue it has generated increased about threefold over the last decade, growing to USD5000B in 2011 (according to the American Chemistry Council, URL: http://www.americanchemistry.com/Jobs/EconomicStatistics/Industry-Profile/Global-Business-of-Chemistry). Although providing a variety of useful products, the chemical industry is inherently non-sustainable as it heavily relies on crude petroleum and its environmentally damaging production processes [1]. The desire to reduce dependency on petroleum and an increased concern for the environment are leading drivers for the sustainable production of both fine and bulk chemicals from renewable resources. The high productivity of the current chemical industry has contributed to efficient conversion of platform petrochemicals into a broad array of industry chemical products. It is therefore of great interest to produce a number of bio-based building block chemicals from biomass as a renewable resource, to fit into the highly optimized chemical manufacturing processes [2].

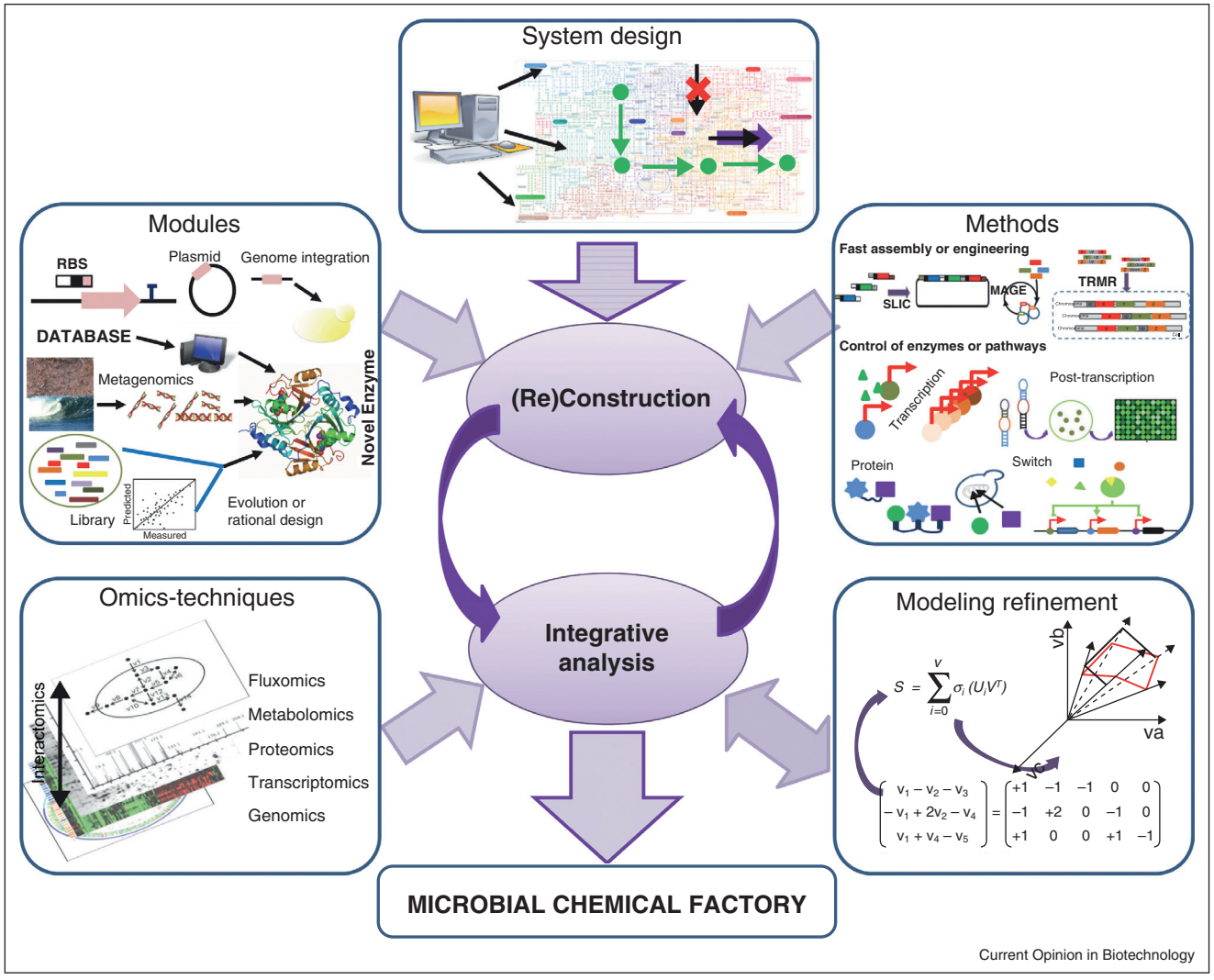
Microorganisms are increasingly used as platform cell factories by industrial biotechnology to produce a whole range of compounds that find numerous applications, such as alcohols, organic acids, amino acids, pharmaceuticals and polymers. However, the cost and required platform techniques dictate that currently only a few platform cell factories are preferred in industry, including (but not limited to) the bacteria *Escherichia coli*, *Corynebacterium glutamicum* and *Bacillus subtilis*, the filamentous fungi *Aspergillus niger*, *Aspergillus oryzae*, *Penicillium chrysogenum* and the yeast *Saccharomyces cerevisiae*. Of these, *S. cerevisiae* proves to be an attractive cell factory due to its robustness and remarkable tolerance against various stresses at industrial conditions. This cell factory has been extensively used in food and beverage production, and is also exploited for the production of fine and bulk chemicals such as nutraceuticals and pharmaceuticals.

By far the most important reason for industry not to shift from petroleum to bio-based production of chemicals is the higher production cost. To cope with these constraints, an integrated approach to constructing robust microbial chemical factories and improved production processes would be required (Figure 1). Available computational algorithms allow systems level design [3]. Emerging tools and methods in synthetic biology open up the possibility to make metabolic pathway and strain engineering easier and faster. Integrative analysis of comprehensive omics data with modeling refinement facilitates multiple-objective optimization. In this review, we will discuss how progress in fundamental techniques advances metabolic pathway and strain engineering, thereby affording the potential to substantially reduce research and development time and to decrease time to market. As examples of building block chemicals, adipic acid, succinic acid and 3-hydroxypropionic acid, which represent a near-term opportunity for the replacement of petrochemicals with renewable resources, will be discussed in detail.

Computational platforms guide systematic design

The use of powerful computational tools can lead to better-informed and more rapid design and optimization.

Figure 1



Overview of integrated approach for construction of robust microbial chemical factories (MCF). Computational platforms guide systematic design. Discovering and engineering novel modules and devices afford great potential for robust pathway construction. Emerging methods for fast assembly and engineering, and tools for modulating gene expression and regulating metabolic pathway facilitate efficient construction. Systematic analysis of comprehensive omics-data with modeling refinement enhances capability for global optimization. Iterative engineering cycles are needed to achieve the desired MCF.

A range of computational pathway prediction algorithms has been generated providing a systematic framework for metabolic pathways (re)design, which is not only limited to changing existing pathways through introduction of gene knockouts or amplifications, such as OptKnock [4], OptGene [5], OptForce [6] or FSEOF [7], but also aids in identifying possible pathways from first principles based on known enzyme reactions, for example, DESHARKY [8] or based on possible biotransformations of functional groups by known chemistry, for example, BNICE [9]. A similarly principled predictor has identified more than 10 000 possible pathways for the synthesis of 1,4-butanediol from common central metabolites [10]. Therefore, it is of primary importance not only to predict a wide range of possible routes, but also to rank them based on discriminative criteria. A prioritization scoring algorithm based on binding covalence, chemical similarity, thermodynamic favorability and pathway distance has been suggested [11•], and the recently published web server RetroPath [12] offers a way to retrieve reactions varying in number from the large numbers of reactions found using BNICE to the small numbers of reactions that are presented in the KEGG database.

In addition, computer-aided design software for designing regulatory circuits and aided protein engineering has