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The emerging impact of cell-free chemical biosynthesis

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Biomanufacturing has emerged as a promising alternative to chemocatalysis for green, renewable, complex synthesis of biofuels, medicines, and fine chemicals. Cell-free chemical biosynthesis offers additional advantages over *in vivo* production, enabling plug-and-play assembly of separately produced enzymes into an optimal cascade, versatile reaction conditions, and direct access to the reaction environment. In order for these advantages to be realized on the larger scale of industry, strategies are needed to reduce costs of biocatalyst generation, improve biocatalyst stability, and enable economically sustainable continuous cascade operation. Here we overview the advantages and remaining challenges of applying cell-free chemical biosynthesis for commodity production, and discuss recent advances in cascade engineering, enzyme immobilization, and enzyme encapsulation which constitute important steps towards addressing these challenges.

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Introduction

Biomanufacturing presents a renewable alternative to petroleum-based chemocatalysis for commodity production. In contrast to conventional industrial manufacturing, living cells utilize serially organized enzymatic cascades to drive many vital physiological processes. Such enzyme cascades are a compelling means for biomanufacturing, as they are: (1) highly selective and specific, (2) biodegradable and non-toxic, and (3) optimized to function at similar conditions, minimizing unit ops and the need for intermediate purification. Harnessing these strengths, microbial cells with engineered pathways have been applied towards pharmaceutical and fine chemical manufacturing [1–3]. Despite these successes, it is still challenging to engineer and optimize synthetic pathways in live cells. In particular, mass transfer and pathway optimization are constrained by cell membranes and intracellular processes [4–6]. A promising alternative is 'cell-free' chemical biosynthesis (CFCB) for biomanufacturing, which seeks to exploit the advantages of whole-cells outside of the constraints of live cells.

In CFCB, enzymes and cofactor components are assembled and optimized towards enzymatic pathways with great engineering freedom, thanks to the absence of cell membranes and cellular processes. Such *in vitro* networks have been used to produce a variety of products, such as hydrogen [5,7], electricity [8], medicines [9,10], and fine chemicals [9]. Several excellent reviews have discussed cell-free metabolic engineering and its many applications [6,11–13]. Here we present an overview of CFCB as a platform for industrial chemical biosynthesis, discussing its advantages, challenges and current applications.

Advantages of CFCB

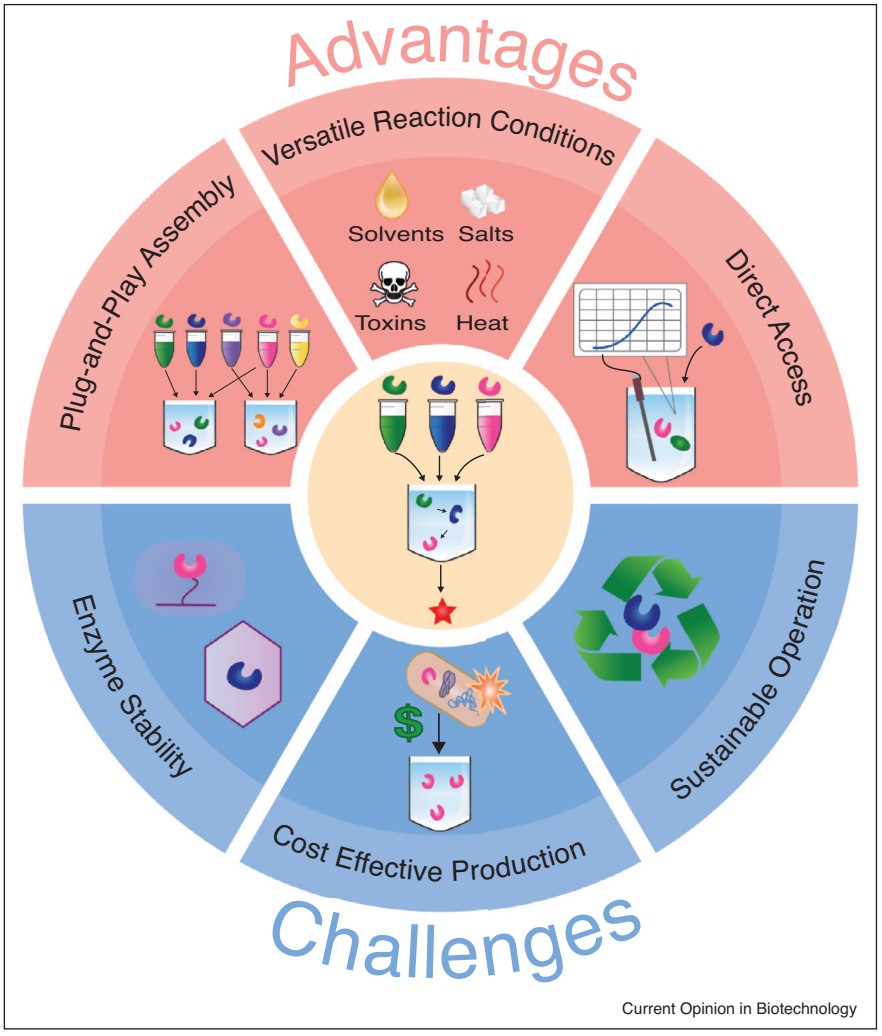
As a chemical production platform, CFCB provides several key advantages over *in vivo* synthesis, including (1) facile plug-and-play cascade assembly, (2) versatile reaction conditions, and (3) direct access to the reaction environment for monitoring, manipulation, and maintenance, as depicted in Figure 1.

Plug-and-play assembly

The CFCB system simplifies cascade production because each enzyme can be produced separately using various hosts and growth conditions, simplifying the optimization of overall biocatalyst production efficiency [14]. Thereafter, enzyme concentrations and combinations can be screened in a modular and high throughput manner [15,16], improving yield and overall titers [16].

These optimized designs can then be scaled up to larger production volumes with minimal re-optimization, as demonstrated recently [17]. Individual enzyme building blocks can also be grouped into modules with known functions, which can be mixed and matched to produce a variety of products [8,18]. Recently, crude lysates have been demonstrated as a useful tool in cascade design [11,19••], enabling rapid iteration through design-build-test cycles by eliminating enzyme purification and allowing biocatalyst synthesis from linear PCR templates [19••,20,21]. Finally, the simpler, more linear setup of CFCB promises simpler computational modeling,

Figure 1



Advantages and challenges of cell-free chemical biosynthesis. Advantages include (1) plug-and-play cascade assembly for construction and optimization of novel cascades, (2) versatile reaction conditions due to the absence of cell viability constraints, and (3) direct access for monitoring and manipulation of the reaction environment. Challenges include (1) cost-effective biocatalyst production, (2) improving enzyme stability, and (3) enabling economically sustainable cascade operation through cofactor and biocatalyst recycling.

broadening the potential for computational design of cascades [5,7,22,23].

Versatile reaction conditions

A primary challenge of *in vivo* biomanufacturing is the balance between product synthesis and the constraints of cellular viability. These constraints introduce two primary limitations: (1) a portion of the energy supplied must be used in cellular growth and metabolism, reducing system efficiency, and (2) the scope of acceptable reaction conditions is restricted to environments tolerated by the cell, limiting solvent options, ionic strength, titers of toxic products or intermediates, and reaction temperatures. Because CFCB eliminates the constraints of cellular viability, CFCB expands available reaction conditions [24–26], enables high reaction rates [8], and allows higher titers of some products and intermediates [9,16,27••], providing greater opportunity for cascade optimization. For example, a CFCB process enabled saccharification of chitin to pyruvate at a higher optimal temperature of 70 °C, which is difficult to achieve *in vivo* [24]. Similarly, the absence of cellular toxicity and crossover with native metabolism recently allowed CFCB processes to exceed *in vivo* titers of various monoterpenes [27••] and fructose 1,6-diphosphate [9].

Direct access to reaction environment

The absence of cell walls in CFCB also eliminates many transport limitations. This enables greater control over the reaction environment and more affordable product