

Advances in the Applications of Metabolic Engineering in Synthetic Biology

Shilu Lin

Southwest University

Abstract. Metabolic engineering, a core pillar of synthetic biology, enables the precise reprogramming of cellular metabolism for efficient production of valuable compounds. This review outlines recent advances in genome editing, pathway construction, and dynamic regulation strategies that underpin modern metabolic engineering. Emerging tools such as CRISPR-derived editors, machine learning-guided design, and automated high-throughput workflows have accelerated the design–build–test–learn cycle and enabled predictive strain optimization. Applications span microbial cell factories, mammalian and plant hosts, cell-free biosynthesis systems, synthetic consortia, and non-traditional chassis such as photosynthetic microbes, extremophiles, and filamentous fungi. These engineered systems have facilitated the biosynthesis of fuels, pharmaceuticals, specialty chemicals, and nutraceuticals, often under environmentally sustainable conditions. Challenges remain in scaling processes, overcoming metabolic bottlenecks, and ensuring biosafety in complex systems. Nevertheless, the integration of systems biology, artificial intelligence, and synthetic design is ushering in a new era of intelligent and modular biomanufacturing. Continued innovation in chassis development, global metabolic rewiring, and closed-loop automation will further expand the capabilities and impact of metabolic engineering across biotechnology and industrial applications.

Keywords: Synthetic biology; Metabolic engineering; Genome editing; Biomanufacturing.

1. Introduction

Synthetic biology has advanced rapidly, establishing a paradigm of standardization and modular design in engineering biology. In this context, metabolic engineering – the technology of modifying cellular metabolic pathways to optimize product synthesis – has become a key application area of synthetic biology [1]. From early trial-and-error approaches (e.g., gene knockouts and pathway enhancements) to today's rational designs guided by systems biology and artificial intelligence, metabolic engineering has shifted from experience-driven to theory-guided and automated methods. Advances in metabolic engineering have spurred innovation in traditional industries (pharmaceuticals, energy, chemicals, food) and extended synthetic biology into new frontiers such as cell-free synthesis systems, multi-species consortia, and novel chassis cells [1]. Microbial 'factories' have been reprogrammed to efficiently produce amino acids, fuels, and drug precursors, and applying metabolic engineering concepts to mammalian and plant cells is showing great potential. Moreover, combining metabolic engineering with other synthetic biology tools enables global metabolic network reprogramming, overcoming inherent cellular metabolic constraints. This review summarizes the development of metabolic engineering technologies and toolkits, highlights recent advances in their application across various areas of synthetic biology, and provides an outlook on future trends and challenges.

2. Development of Metabolic Engineering Technologies and Toolkits

2.1 Genome Editing and Pathway Construction

Modern metabolic engineering applies high-precision genome editing and modular pathway assembly to rewire cellular metabolism (Figure 1). CRISPR/Cas9 allows multiplex genome modifications in microbes and eukaryotic cells [2]. Derived tools such as CRISPRi/a employ catalytically inactive dCas9 fused to transcriptional repressors or activators for tunable gene regulation. Base editors (e.g., dCas9–cytidine deaminase) enable single-nucleotide substitutions without introducing double-strand breaks, while prime editors utilize Cas9 nickase, a reverse transcriptase, and pegRNA to mediate accurate insertions and deletions without donor templates.

Advanced DNA assembly techniques such as Gibson Assembly and Golden Gate cloning facilitate seamless, scar-free construction of multi-gene pathways[3]. Modular design using standardized parts—promoters, coding sequences (CDSs), and terminators—supports rapid iteration. Dynamic control circuits improve metabolic performance: irreversible metabolic switches delay product formation until sufficient biomass is reached, separating growth and production phases; feedback-regulated loops respond to metabolite levels to autonomously adjust enzyme expression, balancing flux distribution. These advances enable flexible and scalable rewiring of metabolic pathways for efficient biosynthesis.

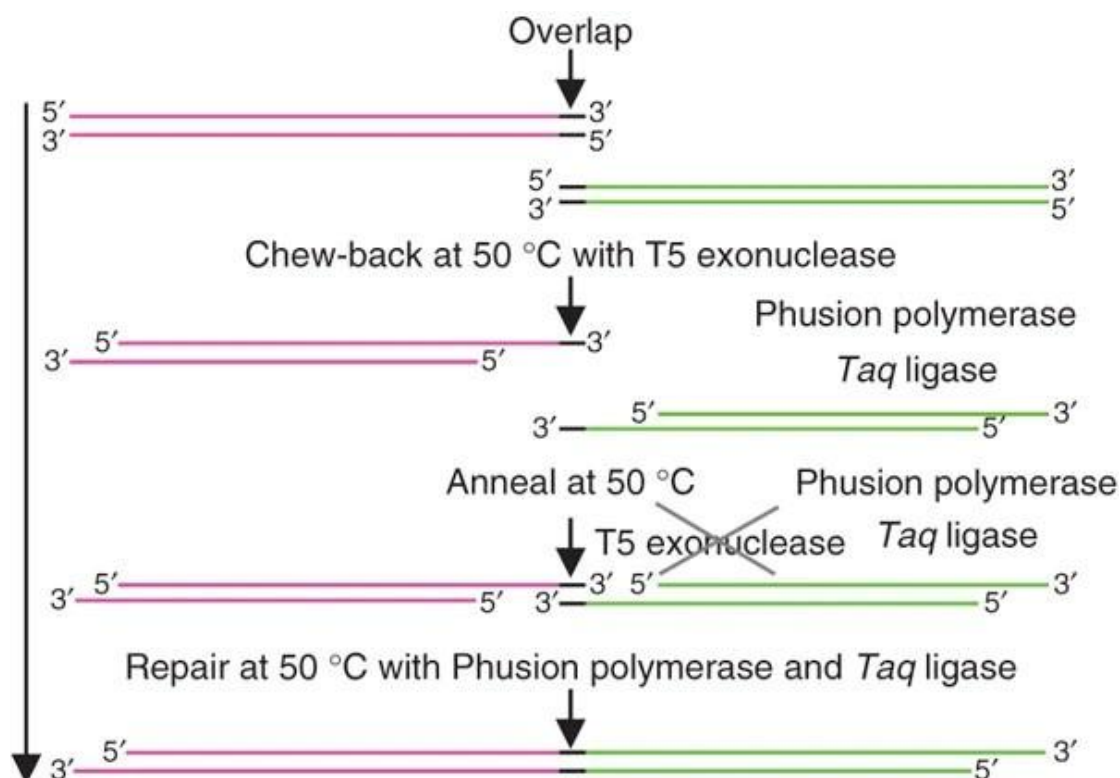


Figure 1. One-step isothermal in vitro recombination

2.2 Metabolic Flux Control and Systems Biology Approaches

Quantitative analysis of metabolic fluxes is essential for rational strain optimization. ¹³C-metabolic flux analysis (MFA) tracks isotopic labeling to map *in vivo* carbon flow and identify

rate-limiting steps. Flux balance analysis (FBA), using genome-scale metabolic models, predicts flux distributions and the impact of genetic perturbations. Multi-omics profiling (transcriptomics, proteomics, metabolomics) further enables systems-level identification of key regulatory nodes; comparative analyses between high-yield and wild-type strains reveal non-obvious engineering targets.

Beyond pathway-specific edits, global reprogramming tools such as global transcription machinery engineering (GTME) introduce mutations in components like RNA polymerase σ or α subunits, broadly shifting gene expression to enhance product titers. Directed evolution of global regulators, e.g., *crp* in *E. coli*, has also been used to amplify biosynthetic flux, significantly boosting tryptophan production[4].

Machine learning (ML) models trained on genotype–phenotype and time-series omics data are increasingly employed to predict beneficial genetic modifications. Compared to traditional kinetic models, ML approaches offer greater accuracy in forecasting dynamic responses to perturbations, enabling data-driven pathway optimization.

2.3 High-throughput and Automation Technologies

Accelerating the design–build–test–learn (DBTL) cycle in metabolic engineering relies on high-throughput tools and automation[5]. Robotic platforms and microfluidic systems enable parallel construction of large mutant libraries. An automated DBTL pipeline integrating *in silico* pathway design, combinatorial DNA assembly, strain construction, and phenotype screening significantly increased rutin titers within two iterations.

High-throughput screening technologies are critical for evaluating large variant libraries. Metabolite-responsive biosensors translate intracellular product concentrations into fluorescent or luminescent signals for real-time detection. Combined with fluorescence-activated cell sorting (FACS) or microfluidic droplet systems, these platforms facilitate screening at single-cell resolution across millions of variants per hour.

Self-driving optimization platforms integrate machine learning (ML) with robotic execution. ML algorithms analyze prior experimental data to predict beneficial genetic designs; automated handlers construct and test the predictions, and results feed back into the model. Industrial “lights-out” biofoundries and academic intelligent platforms implement these closed-loop workflows, enabling continuous operation and compressing optimization timelines from months to days.

3. Advances in Applications of Metabolic Engineering in Synthetic Biology

3.1 Microbial Cell Factories

Model microorganisms such as *Escherichia coli* and *Saccharomyces cerevisiae* have been extensively engineered as microbial chassis for biosynthesis. *E. coli* mutants can accumulate amino acids at titers >10 g/L, while *S. cerevisiae* strains overproduce compounds like tryptophan. Heterologous pathways allow *E. coli* to synthesize higher alcohols (e.g., isobutanol), and yeast enables high-yield production of ethanol and terpene-derived pharmaceuticals such as artemisinic acid.

Beyond traditional hosts, non-model microbes offer specialized metabolic capabilities. *Streptomyces* spp. are manipulated to enhance antibiotic biosynthesis via activation of silent gene clusters. *Bacillus* spp., known for protein secretion, are optimized for enzyme production, while

lactic acid bacteria like *Lactococcus* and *Bifidobacterium* are reprogrammed to convert plant substrates into functional polyphenols (e.g., catechin, resveratrol). Synthetic biology further enables custom-designed chassis. The minimal *Mycoplasma* cell JCVI-syn3.0 (473 genes) provides a simplified platform for pathway integration. Additionally, fully recoded *E. coli* genomes—with synonymous codon reduction from 64 to 61—free up codon space for incorporation of non-standard amino acids and enhance biosafety via viral resistance.

Overall, metabolic engineering across model strains, non-model organisms, and synthetic chassis underpins the growing versatility and efficiency of microbial cell factories in biomanufacturing.

3.2 Mammalian and Plant Cells

Mammalian cells, particularly Chinese hamster ovary (CHO) cells, are widely used for therapeutic protein production due to their capacity for human-like glycosylation. To reduce glycan heterogeneity, metabolic engineering strategies include knockout of specific glycosyltransferase genes and expression of human *ST6GAL1* (α 2,6-sialyltransferase), resulting in more uniform N-glycan profiles on antibodies. Additionally, reprogramming CHO metabolism to reduce by-products such as lactate and NH_4^+ has significantly improved productivity, with monoclonal antibody titers increasing from <100 mg/L in the 1990s to several g/L today[6]. With advances in synthetic biology, intelligent CHO systems capable of self-optimization are on the horizon.

Plant cell cultures provide a renewable platform for high-value natural product synthesis. For example, *Taxus* cell suspension cultures are used to industrially produce paclitaxel, an anti-cancer compound, with yields enhanced by overexpression of biosynthetic enzymes and elicitor treatments (e.g., methyl jasmonate). Metabolic engineering of other plant systems enables production of pigments (e.g., carotenoids from *Daucus carota*) and nutraceuticals (e.g., resveratrol from *Vitis vinifera* cells). While partial pathways can be transferred to microbial hosts, plant cells provide unique organelles and cofactors essential for complex biosynthesis. Combined plant–microbial systems may offer synergistic production platforms.

3.3 Cell-free and Artificial Cell Systems

Metabolic engineering extends beyond living organisms through *in vitro* cell-free systems that reconstruct biochemical pathways using crude extracts or purified enzymes[7]. The timeline of cell-free gene expression systems is shown in Figure 2. Free from cellular constraints, these systems permit enzyme reactions under optimal conditions, tolerating otherwise toxic environments (e.g., high substrate load, solvents, extreme pH), and have been applied to synthesize metabolites and biofuels such as ethanol from *E. coli* extracts. Purified multi-enzyme cascades can be assembled in a single pot to convert simple substrates into complex products, avoiding competing *in vivo* reactions. For example, reconstituted enzyme systems have produced plant-derived drug intermediates entirely *in vitro*, eliminating host burden. Artificial compartmentalization further enhances pathway control. Lipid vesicles (liposomes) encapsulating enzyme sets act as minimal “protocells,” where embedded transport proteins enable substrate–product exchange. Sequential vesicle fusion allows multi-step cascade reactions. Similarly, engineered protein scaffolds—such as enzyme cages or bacterial microcompartments—spatially co-localize enzymes, increasing catalytic efficiency and product yield.

Overall, cell-free and artificial cell platforms complement microbial factories by enabling rapid prototyping and optimization of metabolic pathways prior to *in vivo* implementation. Coupling

these systems with automated, high-throughput biofoundries is expected to accelerate next-generation biomanufacturing development.

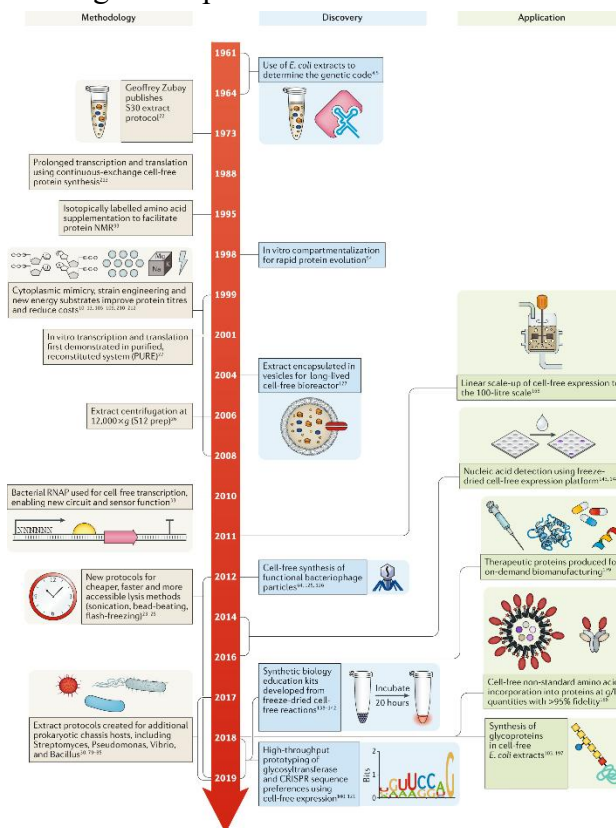


Figure 2. Timeline of cell-free gene expression systems

3.4 Co-culture and Synthetic Microbial Communities

Dividing metabolic tasks among multiple engineered species allows biosynthetic pathways to be modularized across microbial consortia. For example, a natural product pathway split between *Escherichia coli* and *Saccharomyces cerevisiae*—with *E. coli* producing an intermediate and *S. cerevisiae* completing synthesis—achieved significantly higher titers than monoculture approaches^[8]. Each species can operate under its preferred conditions, and system performance is tunable through species ratios, nutrient inputs, and signal exchange.

Applications span medicine, agriculture, and environmental remediation. Engineered probiotic *E. coli* expressing phenylalanine ammonia-lyase (PAL) has been used to lower phenylalanine levels in phenylketonuria patients. In agricultural systems, soil microbes are modified to enhance nitrogen fixation or phosphate solubilization, supporting crop productivity. Synthetic consortia are also deployed for pollutant degradation, with sequential microbial steps converting toxic compounds into inert by-products.

Maintaining stability in multi-species systems presents a key challenge. Engineered interdependencies—such as metabolic cross-feeding or synthetic “kill switches”—can constrain population imbalances. Additionally, quorum-sensing circuits facilitate synchronized growth and metabolic flux across community members.

By leveraging the complementary strengths of distinct microbial species, co-culture platforms provide enhanced flexibility and robustness for complex bioprocesses. Their integration with synthetic regulatory networks and ecological control strategies continues to push the boundaries of precision metabolic engineering.

3.5 Emerging Non-traditional Chassis

Non-traditional and extremophilic organisms are gaining traction as alternative chassis for metabolic engineering. Photosynthetic microbes such as cyanobacteria and microalgae harness solar energy and fix CO₂, enabling carbon-neutral bioproduction of ethanol, isobutanol, and organic acids[9]. Although limitations like low light conversion efficiency and diel cycling persist, strategies including enhanced photosynthetic pathways and synthetic energy storage modules are under development.

Extremophilic archaea provide metabolic functions under harsh conditions. Methanogens, for example, utilize H₂ and CO₂ to produce CH₄, offering a biological “power-to-gas” route for renewable energy storage. Engineering these pathways could enable scalable methane bioreactors.

Filamentous fungi, notably *Aspergillus* spp., are being repurposed to synthesize complex metabolites. Through rewiring the aromatic amino acid pathway, *A. niger* was engineered to convert lignocellulosic biomass into vanillic acid. Additionally, fungal genomes harbor numerous cryptic biosynthetic gene clusters that, when activated via CRISPR tools and omics-guided strategies, can yield novel bioactive compounds. Their innate ability to secrete large molecules efficiently makes fungi ideal for industrial-scale fermentation.

Expanding the repertoire of host organisms enhances both the functional and environmental dimensions of biomanufacturing. With the development of specialized genetic toolkits, these unconventional chassis can be systematically optimized to complement or surpass traditional hosts in yield, resilience, and sustainability.

4. Conclusion and Perspectives

The integration of metabolic engineering with synthetic biology has greatly expanded our ability to reprogram biological systems for production. We can now redesign metabolism not only in single microbes, but also in cell-free systems, multi-species consortia, and even minimal synthetic cells[7]. Metabolic engineering has spurred innovations in medicine, energy and materials, demonstrating immense practical value.

Despite these advances, several challenges remain. First, cellular metabolic networks are highly complex, so purely rational design is difficult—model predictions often deviate from biological reality, meaning iterative experimentation and optimization are still required. Second, host organisms have inherent limitations (e.g., substrate uptake rates, product toxicity tolerance, metabolic trade-offs) that can bottleneck production and must be alleviated through measures like adding transporters or increasing stress resistance. Third, many processes that work in the lab encounter issues at industrial scale, such as metabolic burden in high-density cultures, instability of engineered consortia, feedstock costs, and purification difficulties. These scale-up concerns need to be addressed early in the design process. Finally, as engineered biological systems grow more complex (for example, artificial organelles or living therapeutic cells), biosafety and ethical issues become critical—engineered genes must be prevented from escaping into ecosystems, and engineered organisms must be kept under control and safe in their intended environments.

Looking forward, metabolic engineering is poised to become more intelligent and more holistic. Machine learning and artificial intelligence are beginning to guide strain design by predicting which genetic modifications will boost target fluxes. We envision an “AI designer” that can propose

optimal engineering strategies for a given molecule, greatly reducing the need for trial-and-error. Another trend is multi-scale integration: future metabolic engineering will consider gene regulation, enzyme activity, whole-cell physiology, and even interspecies interactions together, enabling coordinated design across the gene–enzyme–pathway–cell–community spectrum. In the era of Industry 4.0, next-generation “bio-robot” factories may autonomously execute the entire design–build–test cycle (from strain construction to product recovery) under computer control.

Sustainable biomanufacturing will likewise be a major focus. Engineering photosynthetic microbes (cyanobacteria, microalgae) to convert CO₂ into fuels and materials holds great promise. Improving photosynthetic efficiency and stress tolerance in these organisms will enable renewable production of fuels and chemicals while sequestering carbon. In summary, metabolic engineering is becoming increasingly smart, efficient, and eco-friendly. We expect that in the coming years, this field will continue to deliver breakthroughs, allowing even greater contributions to sustainable development and progress for humanity.

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