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ADVANCES IN CELL-FREE SYNTHETIC BIOLOGY PLATFORMS, ENGINEERING STRATEGIES, AND BIOLOGICAL APPLICATIONS

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ABSTRACT

Cell-free synthetic biology has evolved into a widely applicable engineering tool for the reconstruction, manipulation and optimization of biological functions in vitro. This review examines the foundational principles, major platform architectures, engineering strategies, expanding applications, translational barriers, and emerging directions shaping the field. There are currently a number of cell-free systems available, all of which have different advantages for the synthesis of proteins, pathway engineering, biosensing, biomaterials, and biomanufacturing, including bacterial, yeast, mammalian, plant, insect, and reconstituted platforms. Their performance is increasingly enhanced through regulatory sequence design, metabolic rewiring, synthetic gene circuits, microfluidic integration, and high-throughput automation. All these advances have been extended to a wide range of therapeutics, peptide antibiotics, natural products, diagnostics, biomaterials, and agricultural and industrial biotechnology applications. While this is progress, there are still significant challenges in terms of reproducibility, scalability, longevity of the reaction, cost, biosafety, and regulatory standardization. Future development is expected to depend on deeper integration of predictive modeling, artificial intelligence, automated design–build–test–learn workflows, synthetic genomics, and lab-on-a-chip technologies. Platform standardization, interoperable workflows, and application-specific system engineering will be important elements to help bring cell-free systems from the lab to reliable, scalable, and deployable biotechnology platforms.

Keywords: Cell-free synthetic biology, cell-free protein synthesis, synthetic biology platforms, biomanufacturing, biosensing

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1. Introduction

Synthetic biology has grown into an interdisciplinary science that involves the engineering principles of designing, building and manipulating biological systems. It's built on the idea of being able to build a functional system of biological components that can be predicted and designed to do things that the cell cannot by itself. It has been sped up in recent years by the development of DNA synthesis, genome engineering, computational modeling and standardized biological parts (Ausländer et al., 2017). The principles of modularity, abstraction, standardization, and iterative design are then used to create biological functions with greater and greater accuracy, systematically (Garner, 2021). The developments also have implications for biomanufacturing, with engineered biological processes that are considered a flexible manufacturing alternative for chemicals, fuels, materials, enzymes and other high-value products (Zhang et al., 2017).

The standard synthetic biology approach is to focus on engineering pathways and genetic circuits in living cells representing the physical environment where they function. While cellular systems are characterized by biochemical machinery that can be engineered to function as self-replicating units, they are, in turn, complex, and this complexity can limit the engineering properties due to the requirement to grow the systems, competing metabolic pathways, toxicity issues, membrane transport, and regulatory interactions. A few of these limitations are overcome in cell-free synthetic biology by moving necessary biological functions out of the cell and into more manageable in vitro settings. This open reaction architecture allows for manipulation of transcription, translation, metabolism and biochemical conditions without the need for cellular viability (Lu, 2017). The accessibility thus created has resulted in the value of cell-free systems for rapid prototyping, mechanistic investigation, and engineering of biological functions that may be hard to implement in living systems (Tinafar et al., 2019).

The boundaries of cell-free biology have been greatly broadened from traditional protein synthesis to programmable platforms that can accommodate genetic circuits and metabolic pathways, biosensors, and more complex biomolecular functions. Modern cell-free gene-expression systems are optimized for extract preparation, reaction formulation, energy reconstitution and molecular engineering and offer great flexibility in experimental design, making them applicable in basic and biotechnology research (Hunt et al., 2025). Reconstituted and extract-based platforms also allow for studying the organization of a biological system under controlled conditions and for building systems with certain properties of living systems (Noireaux & Liu, 2020). This ability could be used in addition to the other synthetic biology goals of understanding how the regulatory network and interactions in molecules can create complex behaviors in biology, such as development (Davies, 2017). Another example of the relevance of programmable biology is the development of engineered diagnostics, vaccines and therapeutic technologies: biomedical translation (Tan et al., 2021).

But this has so far not been enough, as the field is still divided into platform development, reaction engineering, molecular design, and application-specific optimization. Comparisons between cell-free systems are hindered by differences in biological source, preparation protocols, reaction composition, scale-up, reproducibility and performance metrics. An integrated evaluation that includes the features of the platform, engineering strategies, application needs and requirements is thus required to explain existing capabilities and provide guidance on potential translation challenges.

The purpose of this review is to critically analyse the design principles and technological developments of the leading platforms of cell-free synthetic biology, to compare/evaluate engineering strategies for enhanced performance and functional complexity of these platforms and to summarise and compile their evolving biological and biotechnological applications, highlighting key limitations and needs for future developments.

2. Foundations and Design Principles of Cell-Free Synthetic Biology

Cell-free synthetic biology is based on the controlled reconstruction of biological processes outside living cells and the ability to access and manipulate cellular machinery without constraints due to

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growth, membrane transport or viability. The central technological foundation is cell-free protein synthesis (CFPS), a technology that links transcription and translation in an open biochemical environment, allowing for quick translation of genetic information into functional protein products (Yue et al., 2023). This design exemplifies the broader principles of synthetic biology, such as modularity, abstraction, standardization, and rational design, which enable the construction of robust, functional, and modular synthetic biology systems (Zhang et al., 2023). There are two main types of cell-free systems that are the foundation of modern cell-free systems: crude cell extract-based systems and purified molecular component-based systems. Extract-based platforms include all the biochemical machinery, enzymes, translation factors and ribosomes of the source organism, and provide biochemical richness with relatively simple preparation. The advantage of purified systems is that the composition of individual components can be modified or substituted depending on the requirement for the experiment. Both of them allow for fast prototyping and allow direct control of DNA templates, substrates, cofactors, inhibitors, and regulatory molecules (Hong, 2019). The cell-free transcription–translation systems can thus link molecular-scale biochemical interactions with larger engineered structures, creating a general platform for the engineering and study of multiscale biological processes (Garenne & Noireaux, 2019).

To design effective systems, the processes of transcription, translation, provision of energy, availability of precursors, and reaction conditions must be coordinated. These controllable elements can be used to build up increasingly complex biomolecular systems from characterized components, thus establishing an experimental platform that allows for systematic variations of the interactions and network composition (Laohakunakorn et al., 2020). Regeneration of energy is especially significant because many of the reactions of transcription, translation, and metabolism use up significant amounts of ATP and related cofactors. Better utilization and resource re-use can increase the reaction time and productivity and decrease reliance on costly reaction components (Rasor et al., 2021). Cell-free design principles are not limited to protein expression but extend to metabolic and material-producing systems. The cells do not have to be maintained for cell growth; biochemical resources can be channelled to desired products, and reaction conditions may be different from those that would affect cell physiology. This has paved the way for the creation and assembly of functional biological materials by programmable in vitro processes (Kelwick et al., 2020). How reaction engineering, extract optimization and pathway reconstruction can turn cell-free systems into production platforms is further illustrated by the progressive development of cell-free biomanufacturing (Vilkhovoy et al., 2020).

Another key design dimension is that of multi-enzyme reaction networks. Enzymes can be grouped into specific pathways where the concentration of substrates, the ratio of enzymes, cofactors, the thermodynamic balance and competing reactions can be optimized separately. Pathway stoichiometry, enzyme kinetics, cofactor balance and intermediate stability are therefore necessary to consider to rationally construct an efficient pathway that can convert a variety of interconnected reactions (Morgado et al., 2018).

Figure 1 highlights the fundamental components of cell-free synthetic biology, which include the biological sources, molecular machinery (including enzymes), reaction resources, and engineering controls that all play a role in determining functional outputs.

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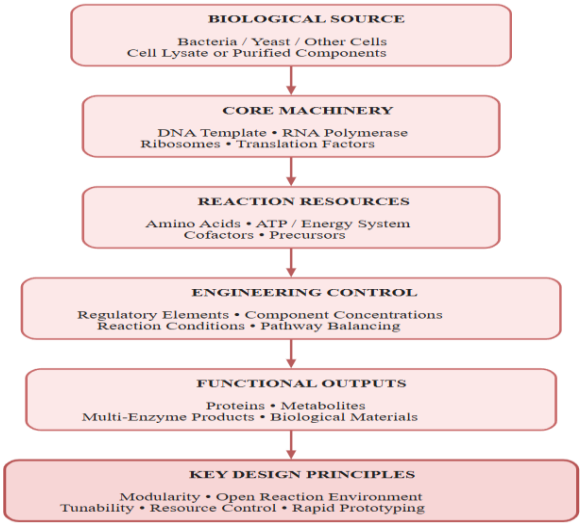

Figure 1. Core architecture and design principles of cell-free synthetic biology systems

In the end, cell-free synthetic biology gains in versatility from the open and modular architecture of its biochemical machinery, which makes it possible to measure, modify, and recombine elements in as unusual a way as possible. The design principle of increasingly complex cell-free platforms is the careful integration of the expression machinery, energy systems, pathway components and reaction environments.

3. Diversity and Evolution of Cell-Free Platforms

The genetic engineering of protein-expression systems on cell-free platforms has grown from a rather limited range of systems to a variety of experimental systems that meet particular molecular and biotechnological demands. Platform selection is now based on the amount of protein that can be expressed, the complexity, post-translational processing requirements, biochemical accessibility, scalability, and compatibility with downstream applications. The diversification has bolstered the application of cell-free protein synthesis in various fields, such as the design-to-product cycle, which is important for rapid production of proteins or in the field of drug discovery (Caschera, 2025). Despite the advantages of bacterial cell-free systems, such as their high productivity and relatively low cost and engineering flexibility, they have not yet become the platform of choice for many. The genetic manipulation of extracts is particularly easy for Escherichia coli-based extracts due to the availability of multiple cores for genetic modification of the source organism prior to the preparation of the extract. Bacterial systems can be genetically recoded to incorporate multiple noncanonical amino acids into proteins, which has extended this capability, thus providing opportunities to engineer proteins with chemical functionalities beyond that of the standard genetic code (Martin et al., 2018). Beyond the traditional use of using proteins for synthesis, cell-free biocatalytic systems containing enzymes and metabolic machinery can be used for the production of platform chemicals from renewable resources (Bergquist et al., 2020).

The alternative eukaryotic platform, such as yeast, offers characteristics that are important for the production of proteins that are more difficult in bacterial systems. The potential of this yeast for biotechnology and the fact that a cell-free reaction format is accessible made Pichia pastoris an interesting eukaryotic host for cell-free engineering (Spice et al., 2022). There are also some advantages of mammalian cell-free systems for studying regulatory elements and proteins in a biochemical system environment closer to mammalian cells. Their advantage as rapid prototyping platforms is that genetic constructs can be tested before implementation in living mammalian systems, potentially speeding up the iterative synthetic biology workflows (Kopniczky et al., 2020).

The diversity of plant-derived systems is also growing. The chloroplast-derived cell-free platform generated from various plant species offers a platform to rapidly test genetic parts and expression

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behavior without the need for time-consuming plant transformation processes. Variations in the biological source also demonstrate the potential for a species-specific optimisation of platform performance when using chloroplast extracts, and the impact different biological sources can have on platform performance (Bohm et al., 2024). The other niche that insects fill is specialized systems for structurally complex membrane proteins. Their suitability for proteins that need eukaryotic folding and an environment within the membrane has been demonstrated by successfully producing G protein-coupled receptors (Sonnabend et al., 2017). Reconstituted platforms are an alternative platform trajectory in cell-free technology. Because the PURE system uses defined components for transcription and translation, there is exceptional control of the reaction composition. Ongoing efforts have enhanced its functionality and made PURE a key platform for mechanistic studies, the construction of synthetic cells, and controlled biological engineering (Cui et al., 2022). Platform evolution is now associated more and more with automation and standardized engineering infrastructure. The biofoundries approach brings together computational design, automated construction, high-throughput experimentation and iterative testing, and provides an organizational structure to enable faster design–build–test–learn cycles for cell-free platforms (Heo et al., 2025). This integration can change the platform selection from the universally best to the best for biochemical capability and design and use. Table 1 shows the main cell-free platforms and their biological origin, distinguishing characteristics and major application areas.

Table 1. Comparative Characteristics of Major Cell-Free Synthetic Biology Platforms

Platform	Biological Source	Key Strength	Application	References
Bacterial CFPS	E. coli and related bacteria	High yield; easy engineering	Protein production, recoding	Martin et al., 2018; Bergquist et al., 2020
Yeast CFPS	Pichia pastoris	Eukaryotic expression	Complex protein synthesis	Spice et al., 2022
Mammalian CFPS	Mammalian extracts	Native-like regulatory context	Mammalian circuit prototyping	Kopniczky et al., 2020
Plant CFPS	Chloroplast extracts	Rapid plant prototyping	Plant genetic-part testing	Bohm et al., 2024
Insect CFPS	Insect-cell extracts	Membrane-protein compatibility	GPCR production	Sonnabend et al., 2017
PURE system	Purified components	Precise compositional control	Mechanistic studies, synthetic cells	Cui et al., 2022

The variety of cell-free platforms has taken protein expression technology from a bacterial technique to a range of prokaryotic, eukaryotic, organelle-derived and reconstructed platforms. The complementary nature of these capabilities will make the application-driven choice of platform even more critical, and automation and high-throughput workflows will likely drive even greater specialization of platforms.

4. Engineering Strategies for Enhanced Cell-Free Performance

Optimization of molecular regulation, reaction composition, metabolic activity, and physical reaction environment must be coordinated when engineering cell-free systems. Protein synthesis can be optimized by modifying the template concentration, composition of the extract, energy regeneration, ionic conditions and availability of substrates and systematic optimization can help identify combinations that would result in high protein yield and stability of the reaction (Batista et al., 2021).

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The other important level of control involves regulatory sequences, as the architecture of the promoter and the 5' regulatory region can affect the transcriptional and translational activity of an engineered system (Tietze & Lale, 2021). In addition, the rational modification of promoters and ribosome binding sites can be used to further tune gene expression and enhance pathway performance (Sun et al., 2020). Cell-free engineering can be programmed to go beyond constitutive protein production. Synthetic gene circuits can be engineered into a set of known circuits that are able to regulate biological responses and orchestrate multiple functionalities (Xie & Fussenegger, 2018). The external inputs offer external control of the reaction behavior. Light-responsive cell-free expression enables temporal regulation of transcription and translation, providing a non-invasive approach for activating or modulating biochemical processes during a reaction (Zhang et al., 2020). These methods improve the ability of cell-free systems to sustain dynamic and not just static, biological programs.

Another approach to enhancing biosynthetic efficiency is metabolic engineering. The source organisms can be metabolically engineered prior to the extract preparation in order to provide the biochemical machinery more compatible with a desired pathway in the extract. In vivo engineering has been integrated with in vitro optimization to show the potential for improving cell-free biosynthesis using metabolically modified yeast extracts (Rasor et al., 2021). Synthetic metabolism is an extension of this concept, where metabolic engineering and enzyme design are used to create biochemical pathways that can alter substrate and pathway flux to desired products (Erb et al., 2017).

In addition to improved performance and throughput, control of the reaction environment is important. Microfluidic systems enable the miniaturization, compartmentalization and precise control of cell-free reactions with reduced reagent consumption and capability for parallel experimentation (Damiati et al., 2018). The advent of microfluidics integration with synthetic biology also allows for automated handling, high-throughput screening, and precise control of biological reactions at the microscale level (Leal-Alves et al., 2024). Advances in programmable molecular recognition and signal-processing architectures also benefit cell-free biosensor engineering, such that synthetic biology can spur advances in biosensor design, while biosensing can generate synthetic biology tools to evaluate engineered biological functions rapidly (Liu et al., 2026). Table 2 presents the main engineering strategies employed to improve the control of expression in cell-free systems, bolster biosynthetic efficiency, programmability and experimental throughput.

Table 2. Major Engineering Strategies for Improving Cell-Free System Performance

Engineering Strategy	Primary Target	Main Purpose	Expected Benefit	References
Regulatory sequence design	Promoters and RBSs	Control gene expression	Higher expression efficiency	Tietze & Lale, 2021; Sun et al., 2020
Genetic circuit engineering	Regulatory networks	Programmable control	Dynamic biological responses	Xie & Fussenegger, 2018
Light-based regulation	Transcription/translation	Temporal control	Tunable expression	Zhang et al., 2020
Metabolic rewiring	Source-cell metabolism	Redirect pathway flux	Improved biosynthesis	Rasor et al., 2021
Synthetic metabolism	Enzymes and pathways	Create optimized routes	Enhanced product formation	Erb et al., 2017
Microfluidic integration	Reaction environment	Miniaturize and automate	High-throughput screening	Damiati et al., 2018; Leal-Alves

				et al., 2024

Regulatory engineering, dynamic gene circuits, metabolic rewiring, microfluidic control and biosensor integration are complementary approaches to enhancing cell-free performance. Their simultaneous use can enhance expression efficiency, reaction control, biosynthetic productivity, and experimental throughput, and can facilitate ever more complex, programmable cell-free systems.

5. Expanding Biological and Biotechnological Applications

The field of cell-free synthetic biology is maturing from a protein-expression technology to a powerful platform for developing therapeutics, biomanufacturing, molecular sensing, and biological discovery. Its open reaction environment allows direct manipulation of its substrates, enzymes, genetic templates and its pathway intermediates enabling rapid design–build–test cycles, and production strategies that are challenging in living cells. Such capabilities will also enhance the broader developments in synthetic biology that have revolutionised industrial biotechnology in the last few years by the advent of programmable production hosts and increasingly complex manufacturing processes (Katz et al., 2018). A very promising field is biomedical and pharmaceutical applications. Engineered systems for the production of therapeutic proteins, vaccines, drug precursors and other biologically active molecules have been developed using synthetic biology, and programmable systems are emerging as novel tools for diagnosis and treatment (Yan et al., 2023). Cell-free platforms have further benefited when the products to be screened are toxic to a living host and/or when rapid screening is needed. Cell-free systems offer great promise in this regard, as peptide-based antibiotics can be discovered, characterized, and synthesized in these systems, and sequences can be diversified and even biosynthetic strategies can be modified (Park et al., 2024). Another emerging opportunity is medical biomaterials, in which controlled synthesis of biomaterials for therapeutic and biomedical applications is achieved using synthetic biological methods (Xu et al., 2025).

Modular biological engineering has also helped in the biosynthesis of natural products. Reconstructed biosynthetic pathways can be used to produce complex plant-derived compounds, minimizing reliance on slow-growing natural sources, and systematically modifying the pathway (Cravens et al., 2019). A cell-free implementation will also allow pathway interrogation by easily changing the concentration of the enzyme, substrate and cofactor. This versatility is in line with the general trend towards industrial biomanufacturing, where alternative approaches to the production of fuels, chemicals and value-added compounds from renewable feedstocks are being developed using biological conversion (Clomburg et al., 2017). Another area of application is biosensing. Synthetic biological sensors allow the integration of molecular recognition with measurable output, enabling a biological system to sense a signal in the environment, its metabolism, or a clinically relevant signal. Programmable Biosensor Architectures have set the rules for the integration of sensing modules with genetic signal-processing and reporting (Hicks et al., 2020). Implementation of these principles in the cell-free domain can remove the need for maintaining viable sensor organisms and enable mobile and possibly deployable diagnostic configurations.

Engineered Biological Systems have more applications in the real world outside of controlled laboratory and manufacturing environments, through environmental and agricultural applications. Interspecies interactions, metabolic cooperation and functional specialization can be manipulated in microbial communities by synthetic biology tools to produce biotechnology (McCarty & Ledesma-Amaro, 2019). As for other plant-associated microbiomes, there are approaches that attempt to enhance nutrient acquisition, stress resistance, and sustainable agriculture practices by harnessing engineered microbial functions (Ke et al., 2021). These approaches can be complemented by cell-free technologies that enable quick characterization of the genetic components and biochemical pathways prior to their application in more complex biological communities. Ultimately, however, it is the ability to link molecular engineering to scalable and economically viable production that is key to industrial translation. The adoption of synthetic biology is becoming increasingly important in

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industrial biotechnology as the ability to rapidly develop standardized biological systems and improved biological design, coupled with automation, can shorten development times and expand the variety of products available (Clarke & Kitney, 2020). Engineered biological systems have already progressed beyond proof-of-concept studies with commercially deployed synthetic biology products when the technical performance, coupled with explicit manufacturing and market requirements, is matched (Voigt, 2020). Table 3 shows the most prominent application areas of cell-free SB and the unique benefits that enable its translation to the biomedical and industrial fields.

Table 3. Major Biological and Biotechnological Applications of Cell-Free Synthetic Biology

Application Area	Main Function	Cell-Free Advantage	Translational Potential	References
Therapeutics	Produce bioactive molecules	Rapid and flexible synthesis	Medicines and vaccines	Yan et al., 2023
Peptide antibiotics	Discovery and production	Supports sequence diversification	Antimicrobial development	Park et al., 2024
Natural products	Pathway reconstruction	Direct pathway manipulation	Drug and chemical production	Cravens et al., 2019
Biosensing	Molecular detection	Portable reaction formats	Diagnostics and monitoring	Hicks et al., 2020
Biomaterials	Functional material production	Programmable synthesis	Biomedical materials	Xu et al., 2025
Biomanufacturing	Chemical and biomolecule production	Redirected biochemical resources	Industrial biotechnology	Clarke & Kitney, 2020; Clomburg et al., 2017

Today, the cell-free synthetic biology field encompasses therapeutics, antibiotics, natural products, biosensing, biomaterials, agriculture and industrial biomanufacturing. It is most important as a means of making a link between the ability of being readily accessible in the laboratory and the ability of being readily programmed within biological design, thus connecting fast prototyping in the laboratory with the growing variety of practical biotechnology applications

6. Critical Challenges and Translational Barriers

Rapid progress of cell-free synthetic biology has not yet been paralleled by similar advances in reproducibility, scalability, cost efficiency and translational readiness. Laboratory systems frequently use very well-optimized reaction conditions, with special reagents and workflows, which can be challenging to replicate in other laboratories or to scale up for production systems. In addition to this, robust standards, reliable performance metrics, scalable preparation methods, and consistency in the preparation across biological components and experimental conditions are required in translation beyond research environments (Brooks & Alper, 2021). The cost of the project is another significant constraint. The use of expensive enzymes, nucleotides, cofactors, energy substrates, and extract-processing procedures may be necessary for cell-free reactions, and purification of the downstream products can add to the manufacturing costs. In addition to high biochemical performance, commercial development depends upon the development of production models that provide a practical advantage to the existing cell-based production. Successful deployment requires meeting market needs, scalable processes, IP strategies and paths to commercial products from the lab (Clarke, 2019). The more complex the cell-free system, the more prominent the technical challenges become. Reaction activity over long periods, balancing the use of resources, controlling the pathway intermediates and reproducing complex cellular functions are still challenging. Other synthetic

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biology challenges remain in the field, such as the challenges of biological predictability, engineering scalability, standardization of measurements, automation, and bridging various levels of technological sophistication, which are particularly relevant to the maturation of sophisticated cell-free systems (Gallup et al., 2021).

Given the rising availability of cell-free technologies and their programmability, biosafety and biosecurity need to be considered simultaneously. Cell-free systems can enable fast biological molecule and genetic construct synthesis and testing, though there are some containment concerns that can be mitigated by removing living cells. Risk assessment should therefore consider unintended biological consequences and potential misuse while preserving legitimate scientific and industrial innovation (Wang & Zhang, 2019). Additionally, with improved synthetic biology technologies, new tools are becoming available to redesign and synthesize living organisms that increase the scope and availability of potential biological threats (National Academies of Sciences et al., 2018). In addition, regulatory frameworks provide another obstacle to translation since new technologies in synthetic biology are not necessarily classified in a manner that makes sense for traditional organisms, pharmaceuticals or manufacturing processes. As these engineered systems become more complex, product characterization, risk assessment, oversight and regulatory consistency between jurisdictions may become an issue. Regulatory strategies will then need to be flexible and adaptable as technologies develop, and proportionate to the nature and purpose of the specific products (Sheahan & Wieden, 2021). All the barriers to translational readiness are summarized in Figure 2, which displays how these barriers are interconnected.

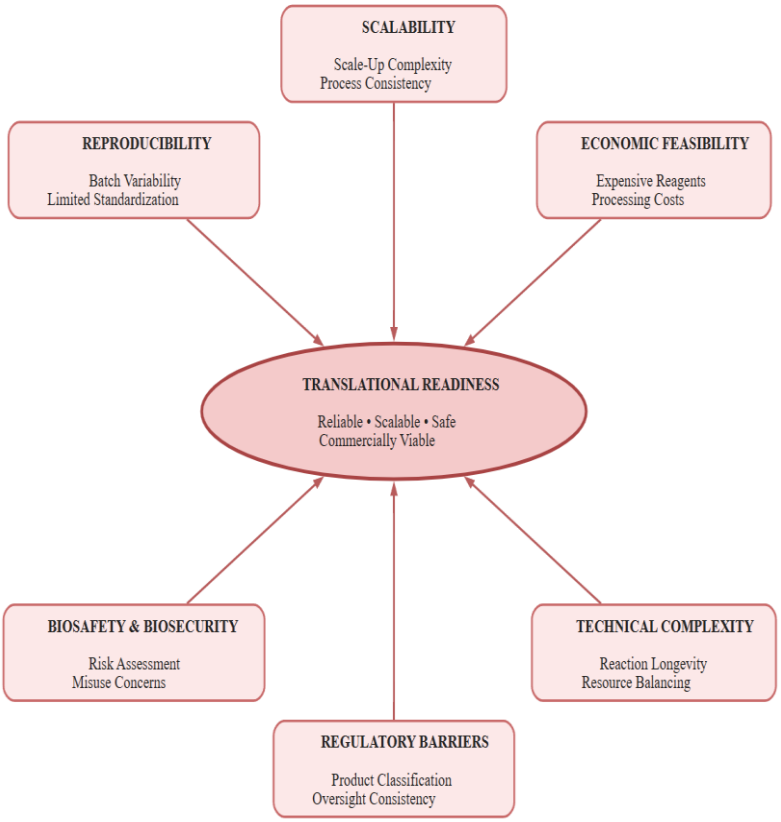

Figure 2. Critical challenges influencing the translational readiness of cell-free synthetic biology

The challenges to get cell-free synthetic biology from the laboratory to widely adopted technology are interrelated and include technical, economic, standardization, safety, and regulatory hurdles. Overcoming such constraints in concert with advances in biological capability will be critical for making increasingly complex cell-free systems viable research, clinical and industrial platforms.

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7. Emerging Frontiers and Future Research Priorities

The next phase of cell-free synthetic biology is likely to move beyond isolated reaction optimization toward integrated, programmable, and increasingly autonomous biological engineering. Future platforms are expected to combine improved molecular control with miniaturization, automation, computational prediction, and scalable manufacturing. Broader trends in synthetic biology indicate growing convergence among engineering biology, advanced computation, genome-scale design, and distributed biological production, creating opportunities for cell-free systems to function as rapid development platforms rather than solely as alternatives to cellular expression (El Karoui et al., 2019). Predictive control represents a major research frontier. Cell-free reactions contain interconnected transcriptional, translational, and metabolic processes whose behavior can change as resources are consumed and products accumulate. Systems and control engineering offers mathematical frameworks for predicting these interactions, designing feedback mechanisms, and improving the robustness of engineered biological networks (Del Vecchio et al., 2018). Developing dynamic models specifically calibrated for cell-free environments could enable rational adjustment of resource allocation, reaction kinetics, pathway flux, and circuit behavior before extensive experimental testing. Artificial intelligence is expected to accelerate this transition toward predictive engineering. Machine-learning and deep-learning approaches can identify complex relationships between biological sequences, molecular features, reaction conditions, and functional outputs, potentially reducing the experimental space required for optimization (Goshisht, 2024). Their integration with cell-free platforms could support promoter and regulatory-sequence design, protein engineering, pathway optimization, reaction formulation, and performance prediction. High-quality standardized datasets will remain critical because model reliability depends strongly on consistent experimental measurements and representative training data.

Automation provides a complementary route to faster optimization. Design–build–test–learn workflows increasingly integrate computational design, robotic experimentation, high-throughput measurement, and iterative model refinement into coordinated engineering cycles (Matzko & Konur, 2024). Cell-free systems are particularly compatible with this framework because reactions can be miniaturized and experimentally manipulated without maintaining viable organisms. Closed-loop workflows combining automated experimentation with algorithmic learning could therefore enable rapid exploration of large biological design spaces. Synthetic genomics may further expand the functional boundaries of cell-free engineering by enabling systematic redesign of genetic information and biological machinery. Genome-scale recoding and construction approaches provide routes for repurposing biological systems toward engineered functions, including altered genetic codes, specialized biosynthetic capabilities, and redesigned molecular components (Fu & Shen, 2024). Coupling such engineered source systems with cell-free technologies could generate extracts and reconstituted platforms with properties deliberately optimized for particular applications. Lab-on-a-chip technologies offer another important direction by integrating biological reactions with microscale fluid handling, sensing, and analytical operations. Their convergence with synthetic biology is supporting next-generation biosensors with greater portability, reduced reagent consumption, multiplexing potential, and rapid response characteristics (David & Cai, 2025). Figure 3 illustrates the convergence of predictive control, artificial intelligence, automated design–build–test–learn workflows, synthetic genomics, lab-on-a-chip technologies, and standardized data toward next-generation cell-free systems and their future applications.

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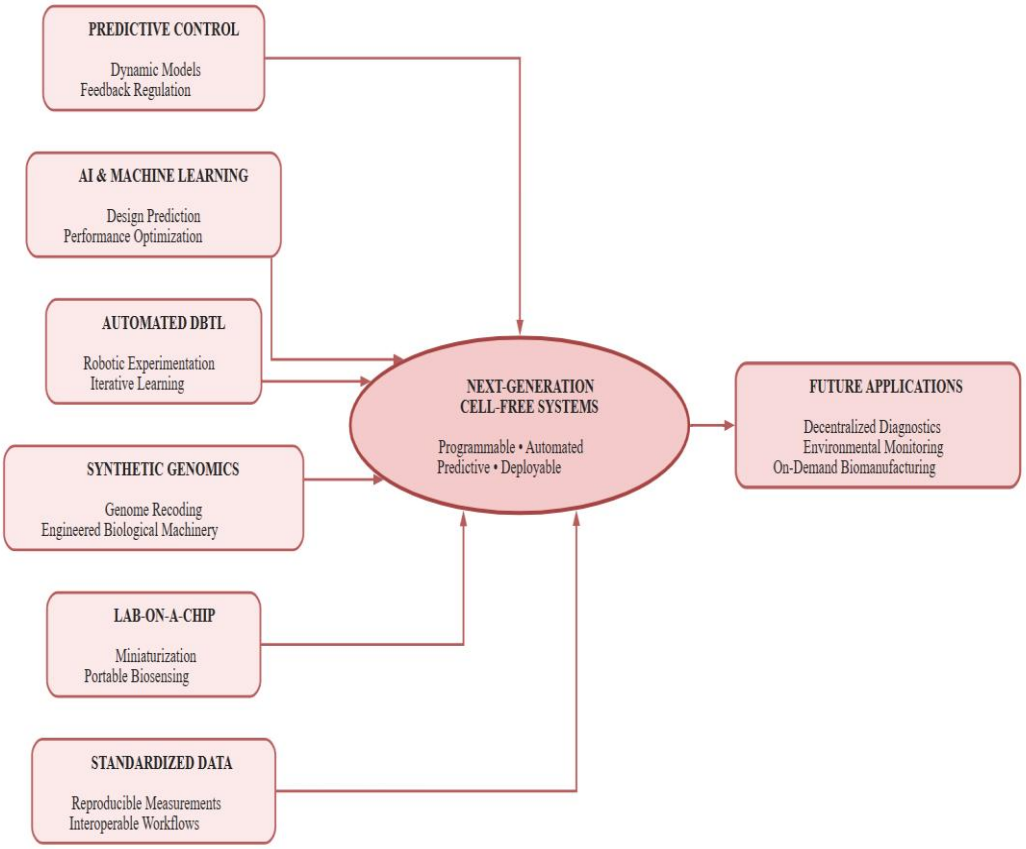

Figure 3. Emerging technological frontiers shaping next-generation cell-free synthetic biology

Future cell-free devices could consequently support decentralized diagnostics, environmental monitoring, field-deployable biosensing, and on-demand biological production. Future progress will depend on integrating cell-free experimentation with predictive modeling, AI-guided optimization, automated design–build–test–learn cycles, synthetic genomics, and miniaturized devices. Establishing standardized datasets, interoperable workflows, and scalable platform architectures will be equally important for translating these emerging capabilities into reproducible and deployable technologies.

8. Conclusion

The field of cell-free synthetic biology has moved beyond a specialized protein-expression strategy to become a more general method for the reconstruction, control, and use of biological functions outside of living cells. It now includes all types of bacterial, yeast, mammalian, plant, insect and reconstituted platforms with unique advantages for production of proteins, pathway engineering, biosensing, biomaterials, and biomanufacturing. Significant progress has been made in regulatory design, metabolic rewiring, genetic circuits, microfluidics, and high-throughput automation which has greatly enhanced the programmability and functional capabilities of these systems. Reproducibility, scalability, reaction durability, cost, biosafety and regulatory compatibility will need to be overcome for broader translation to become a reality. The review also clearly shows a trend towards integrated platforms targeting predictive control, artificial intelligence, automated design–build–test–learn workflows, synthetic genomics and lab-on-a-chip technologies. Future steps will thus rely more on platform standardization, interoperability of workflows and engineering of applications. These advancements have paved the way for the growing significance of cell-free systems in the realm of fast biological design, decentralized diagnostics, sustainable biomanufacturing, and next-generation biotechnology.

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