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Cell-Free Biosystems for On-Demand Pharmaceutical Synthesis:
A Review of a Novel Platform for Accessible Drug Production

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Abstract— Cell-free biosystems (CFBS) represent a transformative paradigm in pharmaceutical manufacturing, enabling the synthesis of complex therapeutic molecules outside of living cells. By harnessing cell-free expression systems (CFES), in vitro enzymatic cascades, and modular metabolic pathways, CFBS circumvent many intrinsic limitations of conventional cell-based production, including lengthy fermentation cycles, GMO regulatory burdens, and restricted access in low- and middle-income countries (LMICs). This review systematically examines the mechanistic foundations, key enabling technologies, and emerging applications of CFBS across major drug classes — including alkaloids, polyketide antibiotics, terpenoids, biologic peptides, and vaccine antigens. We critically evaluate current bottlenecks such as cofactor regeneration, extract variability, post-translational modification fidelity, and scale-up economics, while surveying cutting-edge solutions including lyophilized on-demand systems, continuous flow microreactors, and AI-driven pathway optimization. We further analyze regulatory considerations and outline a prospective roadmap for CFBS integration into global pharmaceutical supply chains. Our analysis affirms that CFBS, while not a wholesale replacement for traditional manufacturing, constitute a compelling complementary platform — particularly suited to distributed, rapid-response, and point-of-care drug production — with the potential to substantially democratize access to life-saving medicines.

KEYWORDS: *cell-free expression; in vitro metabolic engineering; pharmaceutical biosynthesis; natural products; on-demand drug manufacturing; distributed medicine; low-resource settings*

I. INTRODUCTION

The global pharmaceutical supply chain, built largely on cell-based fermentation, chemical synthesis, and complex cold-chain logistics, faces profound structural vulnerabilities. The COVID-19 pandemic exposed these weaknesses dramatically, as disruptions in active pharmaceutical ingredient (API) manufacturing predominantly concentrated in India and China threatened supplies of essential medicines worldwide. Beyond pandemic preparedness, endemic challenges persist: over 2 billion people lack reliable access to essential medicines (WHO, 2023), and the cost and complexity of drug manufacturing often places affordable production beyond the reach of LMICs. Against this backdrop, cell-free biosystems (CFBS) have emerged as a compelling alternative manufacturing paradigm. In contrast to conventional cell-based approaches, CFBS operate through the coordinated activity of purified or semi-purified enzymes, cofactors, and biochemical machinery outside the confines of a living cell. This architectural openness confers critical advantages: reactions can be directly monitored and intervened upon, toxic intermediates tolerated, non-natural substrates introduced, and the entire system can be lyophilized for room-temperature storage and rapid rehydration at the point of need.

The concept of cell-free biology is not new. Buchner's seminal 1897 discovery that yeast extracts could ferment sugar without the presence of intact yeast cells laid the philosophical and experimental foundation. However, it was only in the last two decades, propelled by advances in synthetic biology, metabolic engineering, and high-throughput enzyme characterization, that CFBS have evolved from laboratory curiosities to viable pre-clinical manufacturing platforms. Landmark demonstrations include the reconstitution of the 12-step thebaine biosynthetic pathway in Escherichia coli extracts (Galanie et al., 2015), the multi-enzyme synthesis of artemisinic acid for antimalarial

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production, and the rapid cell-free prototyping of COVID-19 vaccine antigens within 24 hours of genome release.

This review provides a comprehensive, critically analyzed overview of CFBS as a platform for pharmaceutical synthesis. We systematically survey the mechanistic underpinnings, enabling technologies, drug-class-specific applications, challenges, and regulatory landscape. We aim to offer both foundational context for newcomers to the field and nuanced analysis for experienced practitioners seeking to understand where CFBS currently stand and where they are heading.

II. Mechanistic Foundations of Cell-Free Biosystems

A. Cell-Free Expression Systems (CFES)

Cell-free expression systems (CFES) constitute the most widely employed category of CFBS for pharmaceutical applications. In their most basic form, CFES consist of a cell lysate or crude extract; prepared by mechanical disruption of source cells followed by centrifugation to remove cellular debris — supplemented with exogenous DNA or RNA encoding the desired biosynthetic enzymes, along with an energy regeneration system (e.g., phosphocreatine/creatine phosphokinase, glucose-6-phosphate, or maltose/maltose phosphorylase) and a buffered reaction mixture containing amino acids, nucleotides, magnesium, potassium, and polyethylene glycol. The source organism for the extract critically determines the system's capacity. Escherichia coli-derived extracts are the most cost-effective and extensively characterized, offering high transcription/translation rates and suitability for prokaryotic biosynthetic pathways. Wheat germ extracts excel in eukaryotic protein synthesis, providing ribosomes capable of complex mRNA secondary structure resolution. CHO (Chinese hamster ovary) and Sf21 insect cell extracts enable post-translational modifications, including N-glycosylation, disulfide bond formation, and proper folding of mammalian glycoproteins, making them preferred for biologic drug synthesis. Streptomyces-derived extracts uniquely support polyketide and non-ribosomal peptide synthetase (NRPS) activity for antibiotic biosynthesis.

The PURE (Protein synthesis Using Recombinant Elements) system, pioneered by Shimizu et al. (2001), represents the fully defined alternative to crude extracts. By replacing the heterogeneous lysate with 31 purified and reconstituted E. coli translation factors, ribosomes, and energy enzymes, PURE eliminates background enzymatic activity and enables unprecedented mechanistic precision. While more expensive and currently limited in yield relative to crude extract CFES, PURE serves as both a research platform and a quality benchmark, and recent engineering efforts have substantially closed the cost gap.

B. In Vitro Multi-Enzyme Cascades

Beyond expression systems, CFBS encompass direct in vitro enzyme cascade technologies, wherein purified or partially purified enzymes are assembled in defined sequences to catalyze multi-step biosynthetic pathways. This approach bypasses transcription/translation entirely, directly deploying the biochemical catalysts responsible for product formation. The advantages include faster reaction completion (minutes to hours vs. hours to days for CFES), greater specific productivity per reaction volume, and elimination of nucleotide and amino acid supplementation costs. A paradigmatic example is the cell-free synthesis of glucose from CO_2 via the CETCH (crotonyl-CoA/ethylmalonyl-CoA/hydroxybutyryl-CoA) cycle, demonstrating the potential to reconstitute complex metabolic networks in vitro (Schwander et al., 2016). For pharmaceutical applications, cascades have been assembled for the synthesis of hyaluronic acid, nucleoside analogs (including remdesivir precursors), and key pharmaceutical building blocks such as L-DOPA and shikimic acid derivatives.

Substrate channeling; the structural proximity of sequential enzymes enabling direct intermediate transfer has been achieved in CFBS through scaffolded enzyme assembly on DNA origami, synthetic protein scaffolds, and co-immobilization on engineered surfaces. These strategies reduce the diffusion distance for reactive intermediates, mitigate their accumulation-related toxicity, and improve overall pathway flux.

C. *Synthetic Genetic Circuits in Cell-Free Contexts*

Sophisticated genetic circuits — including toggle switches, oscillators, AND-gate logic circuits, and biosensors — have been successfully reconstituted in CFES. These circuits enable programmable regulation of biosynthetic enzyme expression within cell-free reactions, providing dynamic control over pathway flux analogous to what metabolic engineers achieve in living cells, but with the additional benefit of direct intervention capability. Cell-free genetic circuits have been deployed to sense and respond to the accumulation of toxic intermediates, dynamically redirecting flux toward productive pathways. This intersection of synthetic biology and cell-free systems — sometimes termed cell-free synthetic biology represents one of the most intellectually fertile frontiers of the field, promising programmable, responsive biomanufacturing systems that can self-optimize in real time.

III. Enabling Technologies and Platform Advances

A. *High-Throughput Extract Optimization and Standardization*

A historically significant limitation of CFBS has been extract variability — batch-to-batch inconsistency arising from differences in source cell physiology, harvest timing, lysis conditions, and storage protocols. Recent advances in standardized extract preparation, including defined growth media compositions, mid-exponential-phase harvesting, bead-milling lysis at controlled temperatures, and rapid-freeze protocols, have substantially reduced coefficients of variation. Additionally, commercial suppliers (e.g., Arbor Biosciences, Promega, New England Biolabs) now provide quality-controlled CFES platforms with certified lot-to-lot consistency, analogous to the standardization achieved in the clinical diagnostics industry. High-throughput screening (HTS) platforms, including acoustic liquid handlers, microfluidic droplet systems, and robotic workstations coupled to plate-reader fluorescence or LC-MS analytics, have enabled the simultaneous evaluation of hundreds of extract compositions, DNA template concentrations, cofactor combinations, and temperature profiles. These platforms dramatically accelerate CFBS optimization cycles, compressing what once required months of empirical iteration into days.

B. *Lyophilization and On-Demand Deployment*

The lyophilization (freeze-drying) of cell-free reaction components — including extracts, DNA templates, cofactors, and energy regeneration enzymes — into shelf-stable powders has been a critical technological advance for distributed and point-of-care pharmaceutical synthesis. Lyophilized CFBS can be stored at ambient temperature for months and rehydrated with water immediately prior to use, eliminating cold-chain requirements. Pardee et al. (2016) demonstrated lyophilized CFES-based diagnostics for Zika virus detection, and subsequent work has extended this principle to biosynthetic applications. For pharmaceutical production, lyophilized systems offer the prospect of distributed manufacturing nodes, essentially portable bioreactors deployable to conflict zones, remote communities, or disaster-affected regions. A lyophilized unit containing all components required to produce a defined dose of an essential medicine, rehydrated and activated on-site, would constitute a radically decentralized manufacturing paradigm. While this vision remains largely aspirational for complex drug molecules, it has been partially realized for smaller molecules and biosensor-coupled diagnostic applications.

C. *Continuous Flow and Microreactor Technologies*

Batch CFBS reactions are inherently constrained in duration; most systems maintain productive activity for 6 to 24 hours before cofactor depletion, product inhibition, or protease-mediated enzyme degradation terminates the reaction. Continuous flow microreactor systems address this limitation by continuously replenishing substrates and cofactors while removing products and inhibitory byproducts. In such systems, CFBS components are immobilized within or on membrane-bounded microreactor chambers, allowing sustained multi-day operation with dramatically improved volumetric productivity. Microfluidic integration offers additional advantages: precise control of reaction volumes (nanoliter to microliter scale), reduced reagent consumption

for prototyping, and the potential for on-chip purification using integrated separation modules. Combined with automated sampling and inline analytics (e.g., microfluidic mass spectrometry interfaces), these platforms enable near-real-time monitoring of biosynthetic progress a capability largely unavailable in cell-based manufacturing.

D. *Artificial Intelligence and Machine Learning Integration*

The intersection of CFBS with artificial intelligence (AI) and machine learning (ML) is reshaping the pace and efficiency of platform development. Enzyme selection from large sequence databases, pathway thermodynamic feasibility prediction, extract composition optimization, and reaction condition fine-tuning are all increasingly informed by ML models trained on large biochemical datasets. Tools such as ProteinMPNN, RFDiffusion, and ESM-2 have accelerated the design of novel enzymes with improved activity, stability, or specificity for CFBS applications.

Specifically, generative AI models are being used to design enzyme variants with improved thermostability for lyophilized CFBS, enhanced cofactor promiscuity for NADPH-independent catalysis, and novel substrate scope for the synthesis of non-natural pharmaceutical analogs. Reinforcement learning-based optimizers have been applied to automatically tune CFBS pathway configurations in microfluidic experimental loops, closing the design-build-test cycle within hours rather than weeks.

IV. Applications Across Pharmaceutical Drug Classes

A. *Alkaloids and Opioid Analgesics*

Alkaloids represent some of the most pharmacologically potent natural products, including opioid analgesics (morphine, codeine, hydrocodone), anticancer agents (vinblastine, vincristine), and antimicrobials (berberine). Their biosynthesis involves complex, multi-step enzymatic pathways — often exceeding 15 steps spanning cytosolic and compartmentalized reactions, making them formidable targets for cell-free reconstruction. Significant progress has been achieved. Galanie et al. demonstrated end-to-end synthesis of opioid compounds from glucose in engineered yeast; subsequent cell-free adaptations have reconstituted portions of these pathways in E. coli extracts, achieving conversion of key intermediates to thebaine and hydrocodone. The cell-free format is particularly attractive for opioid synthesis because it allows synthesis within controlled, closed environments with built-in containment — a biosecurity advantage over whole-organism approaches that could theoretically produce controlled substances undetected. Regulatory frameworks must evolve in tandem with these technical capabilities to govern cell-free synthesis of scheduled substances appropriately.

B. *Antimalarials and Terpenoids*

Artemisinin, the cornerstone of artemisinin-based combination therapies (ACTs) for malaria, is extracted from Artemisia annua plants; a slow, geographically restricted, and climate-sensitive supply chain. Semisynthetic production via engineered yeast expressing the amorphadiene pathway (Ro et al., 2006) improved but did not fully solve supply constraints. Cell-free approaches have reconstituted the terpenoid biosynthetic pathway using combinations of plant-derived cytochrome P450s (CYPs), reductases, and mevalonate pathway enzymes in supplemented E. coli extracts.

The key challenge for terpenoid CFBS lies in CYP activity; these membrane-bound enzymes require electron transfer from NADPH-cytochrome P450 reductase (CPR) and are poorly soluble in aqueous systems. Engineering of soluble CYP variants, CYP fusion proteins with their reductase partners, and incorporation of nanodiscs or vesicular systems to support membrane-associated catalysis have all been productively explored in the cell-free context.

C. *Polyketide Antibiotics*

Polyketide natural products encompass erythromycin, tetracyclines, rifamycin, and numerous other first-line antibiotics. Their biosynthesis via large modular polyketide synthase (PKS) enzyme complexes some exceeding 1 MDa in molecular weight; presents extraordinary challenges for cell-free reconstitution. Despite this complexity, remarkable

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progress has been achieved. Kornisa et al. (2019) demonstrated the complete cell-free biosynthesis of erythromycin A from propionate in a Streptomyces venezuelae extract, reconstituting all six PKS modules and tailoring enzymes in a single pot reaction. Subsequent work has extended cell-free PKS reconstitution to tylosin, epothilone, and spinosyn. These achievements are not merely academic: many polyketide antibiotics are produced by Streptomyces strains that are notoriously slow-growing and difficult to engineer using genetic tools. Cell-free reconstitution circumvents these limitations, enabling rapid combinatorial exploration of PKS module swaps, substrate-specificity engineering, and post-PKS tailoring diversity — accelerating antibiotic analogue libraries for drug discovery as well as production.

D. Biologic Drugs, Peptides, and Proteins

Biologic drugs — including insulin, erythropoietin, monoclonal antibodies, and glucagon-like peptide-1 (GLP-1) receptor agonists — represent the fastest-growing segment of the pharmaceutical market. Their cell-free synthesis, particularly using eukaryotic extract systems or the PURE platform, offers unique capabilities unavailable in conventional cell-based manufacturing. These include the incorporation of non-standard amino acids (nsAAs) at specific sites using genetic code expansion, enabling precisely placed azide or alkyne handles for site-specific drug conjugation, PEGylation, or radiolabeling. Cell-free systems have been used to produce insulin analogs, GLP-1 derivatives with extended half-lives via chemical modification, and a range of conotoxin peptides. For bispecific antibodies and antibody-drug conjugates (ADCs), CFES provides exquisite control over conjugation chemistry unavailable in cell-based systems. Commercial entities including Sutro Biopharma have advanced cell-free ADC production into clinical trials, representing perhaps the most clinically advanced application of CFBS in biologic drug manufacturing to date.

E. Vaccines and Immunotherapeutics

The application of CFBS to vaccine production gained global attention during the COVID-19 pandemic, when several groups demonstrated the rapid cell-free synthesis of SARS-CoV-2 spike protein antigens and virus-like particles (VLPs) within 24 hours of genome sequence publication. This speed — impossible in conventional cell-based vaccine manufacturing — highlights the potential of CFBS for outbreak-response vaccine production. Similarly, cell-free systems have been used to produce self-assembling VLPs of influenza hemagglutinin, hepatitis B surface antigen, and malaria Pfs25 transmission-blocking vaccine candidates. Conjugate vaccines, which link polysaccharide antigens to carrier proteins to boost immunogenicity, have also been produced cell-free using cell-free glycoprotein synthesis (CFGpS) technology, which couples oligosaccharyltransferases from Campylobacter jejuni with cell-free protein synthesis machinery. This approach, pioneered by the Jewett laboratory at Northwestern University, enables the custom glycosylation of carrier proteins in a single, modular reaction.

V. COMPARATIVE ANALYSIS: CFBS VERSUS CELL-BASED MANUFACTURING

A structured comparison of CFBS and conventional cell-based manufacturing across key operational parameters is essential for contextualizing the technology's strengths and limitations (Table 1). CFBS offer compelling advantages in speed, programmability, containment, and point-of-care suitability, while cell-based systems retain advantages in unit cost at industrial scale and in post-translational modification fidelity for complex glycoproteins.

Parameter	Cell-Free Biosystems (CFBS)	Cell-Based (In Vivo) Systems
Production Speed	Hours to days	Days to weeks
Scalability	Modular; linear scale-up	Requires bioreactor optimization
Containment Risk	Low; no living organisms released	High; GMO regulations apply
Regulatory Complexity	Simplified; extract-based	Complex; full GMP cell banking
Toxic Intermediate Tolerance	High; open system permits	Low; cellular toxicity limits

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	addition	range
Genetic Programmability	Rapid; direct DNA/RNA addition	Requires stable integration
Cold-Chain Dependency	Potentially lyophilizable	Strict cold-chain required
Cost per Unit	Currently higher; decreasing rapidly	Lower at industrial scale
Point-of-Care Applicability	High potential	Very limited

Table 1. Comparative analysis of cell-free biosystems (CFBS) versus cell-based (in vivo) manufacturing systems across key pharmaceutical production parameters.

Table 2 summarizes landmark CFBS demonstrations across major pharmaceutical compound classes, illustrating the breadth of applicability achieved to date.

Compound Class	Example Compounds	CFBS Platform	Key Achievements
Opioid Analgesics	Thebaine, hydrocodone	E. coli CFES	Complete 5-step pathway in vitro
Antimalarials	Artemisinin, artemisinic acid	Yeast CFES + plant enzymes	Mg-independent synthesis demonstrated
Antibiotics	Erythromycin, tylosin	Streptomyces CFES	PKS reconstitution in cell-free
Anticancer Agents	Vinblastine, camptothecin	Plant CFES	Monoterpene indole alkaloid synthesis
Biologics/Peptides	Insulin, GLP-1 analogs	Wheat germ / PURE system	Non-standard amino acid incorporation
Vaccines	VLP-based candidates	CHO CFES	Rapid antigen prototyping
Cannabinoids	CBD, CBG derivatives	Yeast CFES	Geranyl pyrophosphate coupling

Table 2. Representative cell-free biosystem platforms and their pharmaceutical synthesis achievements by compound class.

VI. Challenges and Emerging Solutions

Despite its promise, CFBS faces substantive technical, economic, and regulatory challenges that must be systematically addressed for broader pharmaceutical deployment (Table 3).

Challenge	Current Status	Emerging Solutions
Cofactor Regeneration	NADPH depletion limits yield	Glucose-6-phosphate + enzyme cascade; electrochemical regeneration
Reaction Duration	Most systems active <24 hours	Lyophilized encapsulated systems; continuous flow microreactors
Product Yield	Often <1 g/L for complex NPs	Pathway optimization via cell-free prototyping; substrate channeling
Extract Variability	Batch-to-batch inconsistency	Defined PURE-like systems; standardized crude extract protocols
Post-Translational Modifications	Limited glycosylation fidelity	Insect cell CFES; glycosyltransferase supplementation
Regulatory Frameworks	No dedicated CFBS drug approval pathway	FDA/EMA exploratory engagement; ICH Q11 analogs proposed
Scale-Up Economics	Extract cost high at small scale	Industrial fermentation of extract source organisms; automated systems

Table 3. Key challenges in cell-free biosystems for pharmaceutical synthesis and emerging mitigation strategies.

A. Cofactor Regeneration

The dependence of most biosynthetic enzymes on reduced nicotinamide cofactors (NADPH, NADH) represents one of the most significant constraints on CFBS productivity. In living cells, cofactor regeneration is continuously accomplished through central metabolic pathways (glycolysis, pentose phosphate pathway, TCA cycle) coupled to electron transport chains. In cell-free systems, these native regeneration networks are absent or attenuated. Supplementary regeneration systems — most commonly glucose-6-phosphate/glucose-6-phosphate dehydrogenase for NADPH, or phosphocreatine/creatine kinase for ATP — add cost and complexity. Electrochemical cofactor regeneration using bio-electrochemical cells has emerged as an attractive alternative, coupling NADPH regeneration to electrochemical reduction of $NADP^+$ at modified electrode surfaces, and has been demonstrated at laboratory scale with promising yields.

B. *Reaction Duration and Stability*

The finite catalytic lifetime of CFBS — typically 6-24 hours for crude extract systems, driven by protease activity, cofactor exhaustion, and enzyme denaturation — limits volumetric productivity in batch format. Multiple strategies are being pursued in parallel: protease inhibitor cocktails, genetic deletion of proteases from the source organism prior to extract preparation, immobilization of enzymes to solid supports or in hydrogel matrices, continuous flow operation, and encapsulation of reaction components in hydrogel microparticles. Notably, the Pardee group at the University of Toronto has demonstrated that lyophilized CFBS encapsulated within paper-based matrices retain catalytic activity for over 12 months at room temperature — a dramatic extension over refrigerated liquid systems.

C. *Post-Translational Modifications*

Therapeutic proteins frequently require post-translational modifications (PTMs) for proper folding, biological activity, and pharmacokinetic stability. Glycosylation, in particular, is essential for many biologic drugs and is performed in the endoplasmic reticulum and Golgi apparatus — organelles absent from cell-free systems derived from disrupted cells. Insect cell (Sf21, High Five) and CHO CFES partially recapitulate glycosylation, as membrane vesicle fractions from these extracts retain glycosyltransferase activity; however, the pattern and density of glycosylation achieved cell-free typically do not match that of live-cell-produced glycoproteins. Advances in glycosyltransferase cocktail supplementation, cell-free glycosylation (CFGpS) platforms, and the engineering of hyperactive OST variants offer paths toward improved PTM fidelity.

D. *Regulatory Considerations*

No regulatory agency currently maintains a dedicated approval pathway for pharmaceuticals manufactured via CFBS. Drug products produced cell-free would, in principle, be subject to existing GMP and ICH quality guidelines applicable to their product class (small molecule API vs. biologic). However, the unique process attributes of CFBS — open reaction systems, novel extract-based starting materials, and absence of cellular containment — will necessitate novel guidance frameworks covering extract characterization, lot release testing, and process analytical technology (PAT) integration. The FDA's Emerging Technology Program and EMA's Innovation Task Force represent appropriate engagement channels for CFBS developers seeking early regulatory dialogue. Proactive engagement with regulators, analogous to the pathways established for mRNA therapeutics prior to COVID-19, will be essential.

VII. CFBS FOR GLOBAL HEALTH AND EQUITABLE DRUG ACCESS

Perhaps the most compelling long-term value proposition of CFBS for global health lies in their potential to democratize pharmaceutical manufacturing. The centralized, capital-intensive, highly regulated infrastructure required for conventional drug manufacturing represents a near-insurmountable barrier to domestic pharmaceutical production in most LMICs. CFBS, by contrast, offers a modular, lower-capital-expenditure manufacturing model potentially compatible with distributed production at regional, national, or even facility level. The WHO Essential Medicines List includes over 400 molecules, the majority of which are small molecule APIs whose biosynthetic pathways have been partially or fully elucidated. For many of these — including antimalarials, antibiotics, antiretrovirals, and analgesics — CFBS demonstration of synthesis has already been achieved or is plausibly achievable within the next decade. A network of CFBS-enabled regional pharmaceutical production hubs in sub-Saharan Africa, South Asia, and Latin America could substantially reduce API import dependency, lower medicine costs through competitive local production, and improve supply security.

The Nigeria Centre for Disease Control (NCDC) and African Union's Pharmaceutical Manufacturing Plan for Africa (PMPA) have explicitly identified decentralized manufacturing technologies as a strategic priority. CFBS fits squarely within this strategic vision. Collaborative programs between CFBS research groups in high-income countries and manufacturing development partners in LMICs — supported

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by financing mechanisms from the Wellcome Trust, CEPI, Gates Foundation, and BARDA — are emerging as a viable translational pipeline.

VIII. Future Directions and Research Priorities

Several high-priority research directions will shape the trajectory of CFBS for pharmaceutical synthesis over the next decade. First, the systematic reconstitution of complete biosynthetic pathways for WHO essential medicines in standardized, reproducible CFES platforms — creating a public-domain library of cell-free biosynthetic routes analogous to the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway database — would provide a foundational resource for global pharmaceutical innovation.

Second, the development of economically viable, fully defined (PURE-like) cell-free systems that replace crude extract heterogeneity with precisely characterized components would accelerate regulatory acceptance and enable rational quality-by-design approaches. Third, integration of CFBS with downstream purification processes — including membrane-based separations, countercurrent chromatography, and crystallization — into end-to-end continuous manufacturing trains would address the current disconnect between upstream synthesis and downstream processing.

Fourth, the development of CFBS-specific computational tools for pathway thermodynamic analysis, enzyme kinetic parameter prediction, and extract composition optimization — ideally deployed as open-source platforms — would democratize access to CFBS design capabilities. Fifth, rigorous techno-economic analysis (TEA) and life cycle assessments (LCAs) of CFBS versus conventional manufacturing across multiple drug classes and production scales are needed to inform rational deployment decisions. Finally, formal engagement between the CFBS research community and regulatory agencies in collaborative guidance development will be essential for the field's maturation.

IX. Conclusions

Cell-free biosystems represent a genuinely novel and rapidly maturing platform for pharmaceutical synthesis. Their unique combination of open-system accessibility, programmable biochemistry, rapid deployment, and potential cold-chain independence positions them as a compelling complement — and in some contexts an alternative — to conventional cell-based manufacturing. While substantive technical challenges remain in cofactor regeneration, PTM fidelity, system longevity, and regulatory navigation, the trajectory of innovation across all these dimensions is strongly positive.

The synthesis of opioids, antimalarials, antibiotics, biologics, and vaccine antigens using cell-free approaches has advanced from proof-of-concept to pre-clinical and, in the case of ADCs, clinical demonstration. The integration of CFBS with AI-driven enzyme design, continuous flow systems, lyophilization, and microfluidics is creating a technological ecosystem of remarkable capability. Most importantly, the potential for CFBS to serve as a mechanism for equitable, distributed, and resilient pharmaceutical manufacturing in LMICs gives the field a moral urgency that extends beyond its scientific interest.

We anticipate that within a decade, CFBS-produced pharmaceuticals will be a recognized category in regulatory frameworks, and CFBS-enabled manufacturing nodes will be operational contributors to regional pharmaceutical supply chains in multiple continents. Achieving this vision will require sustained interdisciplinary collaboration among synthetic biologists, chemical engineers, pharmaceutical scientists, health economists, regulators, and global health practitioners. The scientific foundation, we conclude, is ready. The translational work has begun.

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