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# From titre to Batch Record: Making Manufacturability A Design Objective in Synthetic-Biology-Enabled Therapeutics

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## Abstract

Synthetic biology has changed what molecules, proteins and cells can be built; it has not yet changed what can be reliably released as medicine. Two decades of pathway engineering have delivered microbial syntheses of artemisinic acid, opioids, cannabinoids, tropane alkaloids and monoterpene indole alkaloids, with titres spanning micrograms to tens of grams per litre, yet almost none of these strains has become a licensed manufacturing process. We argue that this gap is less biological than epistemic: the field optimises titre in shake flasks, whereas pharmaceutical translation rewards control of critical quality attributes, process robustness and cost of goods. Here we propose treating manufacturability as a design variable rather than a downstream problem, through five coupled layers: design-stage feasibility scoring, burden-aware chassis control, cell-free prototyping used as a quantitative bridge, genetically encoded quality-attribute sensing, and a digital-twin loop that closes the design–build–test–learn cycle with manufacturing data. To make the proposal operational we define a Biosynthetic Manufacturability Index that can be computed before a strain is built, specify how its weights could be elicited and validated, and state the observations that would falsify it. The framework reframes strain construction as process construction and gives molecular bioscientists, process engineers and regulators a shared vocabulary for deciding which pathways deserve scale-up.

**Keywords:** synthetic biology; biomanufacturing; quality by design; cell-free prototyping; metabolic burden; genetically encoded biosensors; design–build–test–learn; critical quality attributes

## Introduction

Molecular biosciences and therapeutics have converged on a single object: the molecule that is simultaneously understood and made. Programmable nucleases turned genome editing into routine engineering [1–3]; structure prediction and generative protein design have made the folding landscape navigable in silico [4,5]; and the cost of synthetic DNA continues to fall. In parallel, the therapeutic repertoire has widened from small molecules to recombinant proteins, mRNA–lipid-nanoparticle medicines, engineered bacteria and edited autologous cells [6–8]. Synthetic biology occupies precisely this junction, and its founding promise was economic as much as intellectual: anything that can be specified chemically can, in principle, be produced biologically, sustainably and at a cost compatible with global access [9].

That promise has been partly redeemed. Microbial routes now deliver precursors of antimalarial, anticancer, analgesic and psychoactive molecules

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that were previously accessible only through extraction or total synthesis [10–15]. Nevertheless, the number of licensed medicines whose active pharmaceutical ingredient (API) is manufactured by a purpose-built engineered pathway remains small compared with the number of pathways reported in the literature. The usual explanation is that titres are too low, and the usual remedy is more pathway engineering. We argue that this diagnosis is incomplete and that the remedy has become self-limiting.

The mismatch is epistemic before it is metabolic. Academic strain engineering is evaluated by a scalar readout — titre, yield, rate — measured in a controlled, well-mixed, short-duration experiment. Pharmaceutical translation is evaluated by a batch record: a document attesting that a defined process reproducibly yielded material meeting pre-specified critical quality attributes (CQAs), with validated analytical methods, a characterised impurity profile, a stability package and a defensible cost of goods [16,17]. Between the two evaluation regimes lies a gap that no amount of additional titre optimisation will close, because titre and manufacturability are not monotonically related. This article develops that claim and converts it into a working design framework.

## The titre trap

### What has been achieved

The landmark syntheses are well known and deserve their reputation. Artemisinic acid was produced in engineered yeast at industrially relevant concentrations after two decades of incremental work on the mevalonate pathway, cytochrome P450 chemistry and host tolerance [10,18]. Taxadiene overproduction in *Escherichia coli* demonstrated that balancing a two-module isoprenoid pathway could raise flux by orders of magnitude [11]. Complete biosynthesis of thebaine and hydrocodone in yeast established that a 20-plus-step opioid pathway could be reconstructed in a heterologous host [12]. Cannabinoid and tropane alkaloid pathways followed, together with unnatural analogues that do not exist in the plant [13,14]. Most recently, a microbial supply chain delivered monoterpene indole alkaloid precursors of vinblastine, one of the most structurally demanding anticancer agents in clinical use [15]. Reviews of plant natural product biosynthesis and of genome-mining pipelines document how general these strategies have become [19,20].

### Three reasons titre misleads

First, cost of goods is not titre-dominated. For intracellular products in microbial hosts, recovery and purification frequently dominate the cost structure once titre exceeds a threshold, and the marginal value of further increases falls sharply. Techno-economic assessments of cell-free and cell-based routes show that capital intensity, media and chromatography capacity enter the cost model as strongly as volumetric productivity [21,22]. Continuous processing can compress capital and improve product quality simultaneously, but only if the process is designed for it from the outset, not retrofitted onto a fed-batch recipe [23].

Second, complex natural products are chemical neighbourhoods, not single compounds. A pathway that yields 5 g $L^{-1}$ of a target alkaloid may also yield a family of congeners, regioisomers and deoxygenated shunt products, each of which is a potential impurity requiring identification, quantification and toxicological qualification. The number of CQAs therefore grows with molecular complexity, and a high-titre strain producing a broad impurity spectrum can be less developable than a moderate-titre strain producing a clean one. Quality by design formalises this point: process understanding, not endpoint performance, is what supports a control strategy [16,17]. Regulatory expectations for drug substance manufacturing, including the requirement to define starting materials and control the process across its lifecycle, were written for chemistry and have no ready analogue for a strain whose genome continues to evolve in the seed train [24, 25].

Third, biological manufacturing is embedded in a supply chain. Semi-synthetic artemisinin is the canonical case: a technically triumphant route that entered industrial production, yet whose commercial durability remained contingent on the price of plant-derived artemisinin and on the agricultural economics of a crop grown by smallholders [26, 27]. A manufacturing technology can be scientifically excellent and commercially fragile at the same time. Titre is silent on this dimension. Table 1 lists the decisions for which a titre value provides no guidance at all.

The consequence is a selection pressure that operates on the wrong phenotype. Students are trained to maximise a number that reviewers reward, and the resulting strains are rarely exposed to the questions that determine whether a process exists: does the production phenotype survive 200 generations in a stirred tank? Does the plasmid-free subpopulation take over? Is the product exported, or does it crystallise inside the cell? Can the CQA be measured at-line within the batch window? Each of these is an engineering question with a measurable answer, and each is currently asked after, rather than during, strain construction.

## Five layers of manufacturability-aware design

We propose to insert manufacturability into the design loop at five points, ordered by the stage at which they act.

### *Layer 1: feasibility scoring before construction*

Pathway selection is usually driven by retro-biosynthesis and chemical intuition, then tested empirically. Constraint-based modelling offers a cheap prior: flux balance analysis can test whether a pathway is stoichiometrically and cofactor-

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**Table 1:** What a titre value does not tell you.

| Decision | Information a titre conveys | Information actually required | Layer |
|---|---|---|---|
| Whether to pursue the pathway | That flux is achievable in one medium, in one vessel | That the required flux is thermodynamically and catalytically attainable at all | 1 |
| Choice of host | Yield in the host tested | Proteome budget and evolutionary stability over production timescales | 2 |
| Process design and scale-up | Nothing | Sensitivity of the production phenotype to mixing, oxygen transfer and pH gradients | 5 |
| Analytical control strategy | Nothing | Whether each critical quality attribute can be measured inside the batch window | 4 |
| Purification route | Product concentration entering downstream processing | Number, similarity and abundance of co-purifying congeners | 3–4 |
| Business case | One cost input | Cost of goods, capital intensity and supply-chain exposure | Index |

*Layer numbers refer to the five layers described in Section 3; "Index" refers to the Biosynthetic Manufacturability Index (Box 1).*

balanced compatible with growth-coupled production [28], thermodynamic constraints can eliminate routes that are infeasible at physiological metabolite concentrations [29], and enzyme-constrained models can show where catalytic capacity, rather than precursor supply, limits flux [30]. The binding constraint in most failed pathways is enzymatic: the required flux exceeds what the available catalysts can deliver at tolerable enzyme abundance. Machine-learning estimates of turnover numbers are now accurate enough to flag such cases before a plasmid is ordered [31], particularly when anchored to measured in vivo catalytic rates [32] and validated by isotope-based flux analysis [33].

### *Layer 2: burden as a design constraint*

Heterologous expression consumes resources that the host also needs for growth, maintenance and stress defence. Burden is measurable — capacity monitors quantify the fraction of the proteome diverted to a synthetic construct [34] — and it is predictive of evolutionary instability [35,36]. Treating burden as a first-class objective changes design choices: lower-copy integration instead of high-copy plasmids, inducible or dynamic expression instead of constitutive overexpression, and pathway-independent control circuits that redirect flux only when biomass accumulation is complete [37,38]. Dynamic control is attractive precisely because it decouples the growth phase from the production phase, which is also how industrial fed-batch processes are organised; a strain that requires constitutive overexpression to reach a high titre in a microplate is often a strain that cannot be run in a tank.

### *Layer 3: cell-free systems as a quantitative bridge*

Cell-free transcription–translation has matured from an analytical curiosity into an engineering platform with an expanding application space [39]. For pathway work, crude-lysate systems allow enzyme ratios, cofactor regeneration and intermediate channeling to be titrated independently of host physiology, which makes them ideal for diagnosing whether a bottleneck is catalytic or regulatory [40,41]. Cell-free systems have also been scaled to manufacturing-relevant volumes and used to produce therapeutic proteins on clinically relevant timelines [42], and their economics have been examined in techno-economic terms [21]. Their diagnostic value, however, is only realised if the results are calibrated against the intracellular case. We suggest that every cell-free prototyping study report an explicit in vitro-to-in vivo transfer coefficient for the pathway under study: the fraction of the cell-free flux retained on transfer to the host, measured under matched conditions. Publishing this coefficient would convert cell-free work from an anecdotal screening tool into a quantitative predictor, and would allow the community to learn where the two environments diverge systematically.

### *Layer 4: making the product report on itself*

If CQAs could be observed continuously inside the bioreactor, several of the difficulties above would soften. Genetically encoded sensors make this plausible: transcription-factor-based and RNA-based devices can report intracellular metabolite concentrations in real time and with single-cell resolution [43], and the same devices can be coupled to selection or to closed-loop control. Optical methods already provide in-line chemical information in bioprocesses, with Raman spectroscopy combined with indirect hard modelling now capable of quantitative monitoring and control of multiple analytes simultaneously [44]. The gap is not instrumentation but addressability: most CQAs of complex natural products are not spectroscopically resolvable in a fermentation broth. A production strain engineered to express a product-responsive reporter converts an unmeasurable attribute into a measurable signal, at the cost of additional burden and of the engineering required to keep the sensor orthogonal to the pathway. That cost is worth paying when the alternative is a 14-day off-line analytical turnaround, and it aligns naturally with the process analytical technology logic of quality by design [16,17].

### *Layer 5: closing the loop with manufacturing data*

Design–build–test–learn cycles have been automated and accelerated by biofoundries and machine learning [45–49], and digital twins — executable models of a process that are updated with plant data — are beginning to be applied in biopharmaceutical manufacturing [50]. The weakness of

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most current loops is the provenance of the "learn" data: they come almost exclusively from microplates and shake flasks, which differ from production reactors in mixing time, oxygen transfer, pH gradients and shear [51]. A model trained on such data extrapolates into a regime it has never seen. We therefore propose extending the canonical cycle to design–build–test–learn–**manufacture**, in which lot release data, deviations, stability results and scale-down model qualifications are treated as first-class training data. This is not a semantic addition: it changes which experiments are worth running, because it rewards designs whose behaviour is predictable across scales rather than designs that win on a single platform.

## A Biosynthetic Manufacturability Index

To make these layers actionable we need a number that can be computed before construction and audited afterwards. We propose a Biosynthetic Manufacturability Index (BMI) defined over five axes, each scored 0–1 against an explicit rubric and combined as a weighted geometric mean so that a near-zero score on any axis cannot be compensated (Box 1). The axes correspond to the layers above and to the failure modes documented in the literature: thermodynamic and catalytic feasibility; host burden and genetic stability; product toxicity and localisation; downstream separability; and quality-attribute controllability.

The index is deliberately not a measure of scientific merit. Its purpose is triage: to identify, at the proposal stage, pathways for which de novo microbial supply is the wrong answer, and for which a hybrid route — microbial production of an advanced intermediate followed by chemical completion — is likely to be superior. The semisynthetic artemisinin and vinblastine programmes are both, in effect, hybrid designs, and both were chosen for reasons of chemical tractability rather than ideological commitment to total biosynthesis [15,18].

Two features distinguish BMI from existing techno-economic or quality metrics. First, it is ex ante and cheap: it requires only sequence, kinetics and physicochemical property data, all obtainable within days. Second, it is falsifiable. Retrospective application to a set of published pathways with known outcomes would immediately test it: if BMI computed from the original publications does not separate the pathways that reached pilot scale from those that did not, the framework should be discarded or re-weighted. We regard this as the single most important experiment implied by this article, and it is one that a consortium of laboratories could complete within a year using published data alone.

## Where the framework bites differently

The five layers apply unevenly across therapeutic modalities, and being explicit about this prevents the framework from becoming a slogan.

For complex natural products and their analogues — alkaloids, terpenoids, polyketides — all five layers are active, and downstream separability is often decisive [13,15,19]. Here the most valuable design decision is frequently whether to make the final molecule or an advanced intermediate, a decision BMI is designed to inform.

For mRNA and recombinant protein therapeutics, pathway engineering per se is less relevant, but the framework's later layers are central. Nucleoside modification and lipid nanoparticle formulation are manufacturing-defined quality attributes as much as discovery achievements [6,52–54], and integrated continuous processing has already demonstrated that platform design, not titre, sets the economics of antibody production [23].

For engineered living therapeutics, the framework needs one amendment: genetic stability and containment are not process parameters but CQAs, because the product is a replicating organism whose genome is part of the specification [8,55]. Burden-aware design and long-term stability testing therefore move from layer 2 to the release specification.

For cell and gene therapies, the binding constraint is logistics rather than metabolism: decentralised manufacturing models exist precisely because shipping a patient's cells across a continent is a quality risk [7,56,57]. The recent approval of the first CRISPR-edited therapy marks the arrival of editing as a licensed manufacturing technology [58], and the accompanying analytical burden — off-target assessment, vector copy number, potency assays — is a preview of what pathway-derived products will eventually face.

## Objections and limits

The most likely objection is that this is one more metric in a field already over-supplied with them. The response is that BMI is a screening instrument, not a figure of merit, and that its value lies in what it forbids: it discourages the construction of strains whose downstream and analytical requirements have not been considered. A second objection concerns the weights. They are, initially, expert judgements, and expert judgements are noisy. We would elicit them by structured Delphi process, publish them with uncertainty, and update them as retrospective validation accumulates; the geometric form ensures that weight uncertainty cannot rescue a pathway that fails a single axis catastrophically.

A third objection is sociological: academic incentives reward novelty of molecule and height of titre, not robustness of process, and no index will change that unless journals and funders ask for manufacturability evidence alongside strain performance. A fourth is technical: genetically encoded sensors impose burden and can drift, and cell-free systems can

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be systematically unrepresentative of intracellular conditions. Both are empirical matters, and both are quantifiable with the transfer coefficients proposed above.

We should also be explicit about what would falsify the framework. If, across a well-curated set of pathways, post-hoc BMI fails to predict which programmes advanced beyond the laboratory, or if the dominant predictor turns out to be a single axis (most plausibly downstream separability), then the multi-axis structure is unjustified and a simpler decision rule should replace it. Frameworks that cannot fail are not frameworks.

## Governance and equity

Manufacturability is also a governance question. Distributed, small-footprint production — including cell-free and paper-based formats that can be deployed outside centralised facilities [59,60] — could widen access to biologics, but the same flexibility lowers barriers to misuse. Screening of synthetic DNA orders is the principal current control point, and it remains unevenly implemented across providers and jurisdictions [61]. A framework that encourages the design of easily manufactured therapeutic pathways should be paired with a norm of proportionate biosecurity review at the design stage, not only at the point of synthesis. There is an equity dimension too. The artemisinin case shows that a biologically superior route does not automatically deliver cheaper medicine; price is negotiated in a market that includes farmers, generic manufacturers and procurement agencies [26]. Claims that synthetic biology will democratise access should therefore be tested against supply-chain evidence rather than asserted.

## Outlook

Three concrete steps would move the argument from proposal to practice. First, journals and funders should require a short manufacturability statement — covering burden, genetic stability, impurity expectations and analytical accessibility — whenever a new microbial production system is reported; the cost to authors is a paragraph, and the effect on design culture would be disproportionate. Second, the community should adopt minimal reporting standards for the two quantities that connect discovery to manufacturing: the cell-free-to-cell transfer coefficient, and burden measured under production-relevant conditions. Third, a public reference dataset linking strain genotype, process conditions and lot release outcomes should be assembled from industry and academic partners; without it, machine-learning models will continue to learn from flasks and be surprised by tanks [48,50].

None of this diminishes the achievements of the last twenty years. The pathways that were reconstructed opioids, cannabinoids, tropanes, vinblastine precursors are extraordinary pieces of molecular bioscience, and they have permanently changed what is considered synthesizable [12–15]. The argument here is that the next decade's constraint is different in kind. The molecules are, in principle, makeable. What remains is to make the making of them a pharmaceutical act: specified, controlled, auditable and affordable. Tilting the field's objective function from titre toward the batch record is, we suggest, the most direct way to get there — and it can begin with a score computed before the first plasmid is designed.

**Box 1. The Biosynthetic Manufacturability Index (BMI)**

**Definition.** For a proposed pathway–host–product triplet, score five axes on 0–1 rubrics, then combine:

BMI = $(s_1^{w_1} \times s_2^{w_2} \times s_3^{w_3} \times s_4^{w_4} \times s_5^{w_5})^{(1/\Sigma w)}$, with default weights w = (0.20, 0.20, 0.15, 0.25, 0.20)

**Axes and rubric anchors**

1. *Feasibility* ($s_1$): thermodynamic feasibility at physiological concentrations; enzyme-constrained maximum flux; fraction of required reactions with characterised catalysts.

2. *Burden and stability* ($s_2$): predicted heterologous proteome fraction; number of genome edits; measured plasmid or circuit loss rate over ≥100 generations.

3. *Toxicity and localisation* ($s_3$): product inhibition constants relative to target intracellular titre; export or storage route; predicted membrane and solvent stress.

4. *Downstream separability* ($s_4$): structural distance from host metabolome; number of chiral centres and congeners; predicted number of chromatographic steps to specification.

5. *Quality-attribute controllability* ($s_5$): number of CQAs lacking an in-process measurement; availability of an orthogonal, burden-neutral sensor; expected analytical turnaround time.

**Interpretation.** BMI ≥ 0.65: proceed to strain construction. 0.45–0.65: proceed only with a hybrid or cell-free route under evaluation in parallel. < 0.45: de novo supply is unlikely to be the best manufacturing answer; consider semisynthesis, enzymatic late-stage functionalisation or a different target.

**Validation.** Retrospective scoring of published pathways with documented outcomes; blinded prospective scoring by independent groups; sensitivity analysis over weights; publication of the rubric and scores as supplementary data.

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