# Title: Designing a Minimal Cell

**Pradhik Prabhu**

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**Minimal cell, GUV's, Lipids, ATP, Metabolism, Bottom-up, Non-self replicating**

**Abstract**-The construction of a minimal cell seeks to determine the lowest number of genetic and biochemical components required to support life-like behavior. This study analyzes design strategies for synthesizing a functional minimal cell capable of metabolism, gene expression, and membrane regulation. Lipid-based Giant Unilamellar Vesicles (GUVs) are employed to model cellular boundaries due to their structural similarity to natural membranes and their ability to encapsulate biochemical systems. Essential genes are selected using database-guided genome minimization and assembled through cell-free protein synthesis. Ribosome-dependent transcription–translation systems enable controlled expression of minimal gene sets without requiring a living host. The results identify four indispensable modules: energy generation (ATP supply), genetic information processing (transcription/translation), membrane transport, and waste management. When integrated, these components support protein synthesis within vesicles, demonstrating life-like metabolic activity. This analysis indicates that a modular "build-from-core-functions" approach can successfully mimic essential cellular behavior, offering insight into both the origins of cellular life and potential applications in synthetic biology and biomanufacturing.

A minimal cell is constructed by systematically identifying the lowest number of genetic and biochemical elements required for life-like behavior. This study refines strategies for designing such cells by integrating additional insights—highlighting that membrane protein insertion and lipid composition are major technical bottlenecks. Lipid-based Giant Unilamellar Vesicles (GUVs) serve as cellular boundaries in these models, allowing the encapsulation of enzyme networks and gene circuits with structural fidelity to living cells.

Essential genes are chosen through database-guided minimization and assembled with cell-free protein synthesis, using ribosome-dependent transcription–translation modules to express minimal gene sets without a host organism. The design identifies four indispensable modules —energy generation, genetic information processing, membrane transport, and waste management—with the addition that successful synthetic metabolism often depends on precise regulation of membrane protein orientation and lipid-protein ratios for function.

By integrating these systems, protein synthesis and active metabolism are achieved inside vesicle-bound compartments, demonstrating that modular, build-from-core-functions approaches provide foundational insights for synthetic biology and the origins of life. These advances also have significant implications for biomanufacturing and biotechnology.

# Introduction

The search for the basic needs of life has enabled the creation of "minimal cells," which are used as a tool to analyze which biological functions are absolutely necessary and which are redundant. The approaches used in the research are either the reduction of existing genomes (top-down approach) or the construction of modules (bottom-up approach). Although top-down approaches give a template for biological complexity, bottom-up synthetic cells enable the engineering of particular controlled behaviors. This report will concentrate on the construction of a non-replicating synthetic cell that gives priority to metabolism and membrane maintenance instead of cell division.

# Literature review

# What is a minimal cell?

A minimal cell is defined as one whose genome encodes only the minimum set of genes required for survival and growth under specified conditions. Glass and co-authors summarize minimal cells as tools for dissecting which molecular functions are strictly essential and which are dispensable or redundant.

# Methodology

# Top-down minimal cells (JCVI-syn3.0 / syn3A)

JCVI-syn3.0 is a genome-reduced *Mycoplasma mycoides* strain with a synthetic 531 kb genome encoding 473 genes, constructed to keep only genes needed for rapid growth in rich medium. A derivative, JCVI-syn3A (543 kb, 493 genes), was engineered for better robustness and has a reconstructed metabolic network of 338 reactions catalyzed by products of 155 genes.

# Bottom-up synthetic minimal cells

Bottom-up synthetic cells assemble non-living components—lipid vesicles, metabolic enzymes, and gene-expression systems—into cell-like structures that exhibit growth, metabolism, or division. Kurisu and co-authors describe a synthetic minimal cell based on three modules: energy production, information-polymer synthesis, and vesicle reproduction, implemented in lipid vesicles and sustained by continuous feeding of membrane molecules and nutrients.

# Essential modules: information, metabolism, membrane, volume

Both JCVI-based and vesicle-based minimal cells converge on four core functions: information handling (DNA/RNA or artificial polymers), energy generation, metabolism of small molecules, and membrane/volume dynamics. Breuer et al. show that in JCVI-syn3A about one-third of the genome is devoted to enzymes sustaining a compact metabolic network

that provides biomass precursors and energy, while Kurisu et al. use artificial pathways to couple energy production to vesicle growth and division.

# Non-replicating, GUV-based minimal cell

I wanted a bottom-up minimal cell built from giant unilamellar vesicles (GUVs) containing a cell-free translation system, a minimal set of genes for metabolism and membrane homeostasis, and no genes for DNA replication or division. The design emphasizes four functions—ATP management, waste mitigation, protein synthesis, and membrane stability—so the vesicle can grow and maintain metabolism without becoming a self-replicating organism.

# Results and Findings

# Lipid vesicles and encapsulated cell-free systems

Kurisu and collaborators and others use lipid vesicles as compartments whose growth and division can be driven by internal chemical reactions, such as polymerization of information molecules that bind to and remodel the membrane. Reviews of synthetic cells emphasize that encapsulated cell-free gene expression, powered by simple energy substrates, is now a standard method to make vesicles produce proteins and behave in a life-like, out-of-equilibrium manner.

# Essential metabolism for minimal and synthetic cells

The JCVI-syn3A metabolic model shows that even the most reduced dividing cells still need hundreds of reactions to supply nucleotides, amino acids, lipids, and energy, albeit with little redundancy. For synthetic vesicle-based cells, de Martino et al. identify four metabolic modules—energy provision and conversion, maintenance of physicochemical conditions, production of macromolecular building blocks, and waste handling—as the minimal out-of-equilibrium metabolism required for a stable synthetic cell.

# Discussion and Analysis

# Open questions and future directions

Glass and co-workers note that even in JCVI minimal cells, dozens of essential genes still have unknown function, so the true minimal requirements of life are not yet fully understood. Recent theoretical and experimental work on “biochemical constructors” and synthetic cell reproduction asks whether systems built entirely from designed components can cross the threshold to autonomous, evolvable life-like behavior.

There is no single, agreed-upon finite list of “all proteins and genes” needed to implement your non-replicating minimal cell, and current papers treat this as an open design problem rather than a finished catalogue. What can be given is a **set of functional classes and representative proteins/genes** that must be

covered; within each class there are many possible choices and design trade-offs.

Minimal-genome cells like JCVI-syn3A still have 493 genes, of which a large fraction are essential or quasi-essential, and dozens remain of unknown function. For synthetic vesicle-based cells, recent “minimal out-of-equilibrium metabolism” and “minimal metabolism for synthetic cells” papers specify modules (energy, homeostasis, transport, biosynthesis, waste handling) but **do not pin this down to a unique protein list**, instead presenting alternative architectures.

For the GUV-encapsulated, **non-replicating** synthetic cell, the following **functional blocks** must be covered, with at least one implementation each:

**Translation machinery (cell-free system)**

i. Ribosomal RNAs and ribosomal proteins forming 70S (bacterial) or 80S (eukaryotic wheat-germ) ribosomes
ii. Translation factors: initiation (eIFs/IFs), elongation (EF-Tu/EFTs, EEFs), termination (RFs), and ribosome recycling factors.
iii. Aminoacyl-tRNA synthetases for all 20 standard amino acids.
iv. tRNAs, amino acids, and often a bacteriophage polymerase such as T7 or T7Max for robust transcription from minimal DNA templates.

**Minimal metabolic/energy module**

i. An ATP regeneration system (e.g. creatine kinase/phosphocreatine, or glycolytic enzymes, or light-driven modules such as ATP synthase plus a photosystem or proteorhodopsin).
ii. Enzymes to maintain redox balance (e.g. NADH/NADPH generation and recycling) and pH/ionic conditions.
iii. A minimal set of biosynthetic enzymes to supply the precursors you do not want to feed externally (for nucleotides, amino acids, and lipids, if not provided in the medium).

**Membrane transport and homeostasis proteins**

i. At least one class of **pore-forming protein** (e.g. alpha-hemolysin or engineered channels) to allow small-molecule exchange and prevent osmotic collapse.

ii. Transporters or channels for ions and key nutrients if you want controlled gradients rather than passive leakage.

**Waste management and stability**

i. Enzymes to detoxify or convert accumulating by-products (for example, phosphate or organic acid handling, reactive oxygen species scavengers) chosen according to your chosen energy system.
ii. Chaperones and proteases if long-term stability of synthesized proteins is needed.

**Regulatory and minimal genetic circuitry**

i. Promoters and regulatory sequences compatible with your polymerase (e.g. T7 promoters) and ribosome-binding sites tuned for desired expression levels.
ii. Optional simple transcription factors or RNA-based regulators if you want feedback (e.g. on ATP level, vesicle size, or osmotic stress).

Key starting points with detailed tables and supplementary gene lists (you will have to go into their Supplementary Data):

a) Breuer M et al., “Essential metabolism for a minimal cell.” *eLife* (tables listing JCVI-syn3A metabolic genes).
b) Bailoni E et al., “Minimal Out-of-Equilibrium Metabolism for Synthetic Cells.” *ACS Synth Biol* 2023 (four metabolic modules and example enzyme sets).
c) Pedreira T et al., “SynWiki: Functional annotation of the first artificial minimal cell JCVI-syn3A.” *Protein Science* 2022 (database of all JCVI-syn3A genes/proteins).
d) Goodsell DS et al., “Integrative illustration of a JCVI-syn3A minimal cell.” *Cell Systems* 2022 (graphical map and gene lists by process).
e) Comprehensive reviews on cell-free gene expression: “Cell-Free Gene Expression: Methods and Applications.” *Chem Rev* 2024 and its open-access companion review.

Because the exact gene/protein set is design-dependent, the realistic next step is: pick one specific cell-free system (which fixes your translation and many metabolic proteins), then use JCVI-syn3A/SynWiki plus the minimal-metabolism papers to define the **smallest extra gene set** you need for energy, membrane, and homeostasis for your chosen experiment.

# Key Sources

**What is a minimal cell:**

Key sources:

- Glass JI et al., "Minimal Cells—Real and Imagined." *Cold Spring Harb Perspect Biol* (NIH/PMC).
- PubMed record: "Minimal Cells—Real and Imagined."

**Top down minimal cells (JCVI syn3.0 / syn3A):**

Key sources:

- Glass JI et al., "Minimal Cells—Real and Imagined." (concept, gene categories, JCVI-syn3.0 overview).
- Breuer M et al., "Essential metabolism for a minimal cell." *eLife* 2019 (JCVI-syn3A).
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**Bottom up synthetic minimal cells:**

Key sources:

- Kurisu M et al., "Concepts of a synthetic minimal cell: Information molecules, metabolic pathways, and vesicle reproduction." *Interface Focus* 2024 (PMC).
- Nakadai T et al., "Synthesising a minimal cell with artificial metabolic pathways." *Communications Chemistry* 2023 (Nature).

**Essential modules: information, metabolism, membrane, volume:**

Key sources:

- Glass JI et al., minimal cell functional categories.
- Breuer M et al., reconstruction of the essential metabolic network.

Kurisu M et al., three-unit synthetic minimal cell design

## Non replicating, GUV based minimal cell:

Key sources connecting this idea to the literature:

- "Designing a Minimal Cell: Essential Components for Synthetic Life."
- de Martino D et al., "Minimal Out-of-Equilibrium Metabolism for Synthetic Cells." *ACS Synth Biol* 2023 (four-module minimal metabolism for lipid-bounded synthetic cells).

**Lipid vesicles and encapsulated cell free systems:**

Key sources:

- Kurisu M et al., vesicle reproduction cycles coupled to artificial metabolism.
- Nakadai T et al., artificial metabolic pathways driving vesicle growth and division.
- "Synthetic Cells Revisited: Artificial Cell Construction Using …" *Advanced Science* review (cell-free systems and artificial organelles).

**Essential metabolism for minimal and synthetic cells:**

Key sources:

- Breuer M et al., JCVI-syn3A metabolism (eLife article and JCVI summary).
- de Martino D et al., "Minimal Out-of-Equilibrium Metabolism for Synthetic Cells." *ACS Synth Biol* 2023.

**Open questions and future directions:**

Key sources:

- Glass JI et al., discussion of unknown essential genes and overlooked functional classes.
- Rasmussen S et al., "On biochemical constructors and synthetic cells." *Interface Focus* 2023.
- Blain JC & Szostak JW, "Progress toward synthetic cells." (Origins-of-life perspective, via Semantic Scholar).

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