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Editorial

Engineering Microbes and Cellular Circuits Using Synthetic Biology: Current Status and Future Prospective

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Introduction

Synthetic biology seeks to apply principles of engineering to reprogram and repurpose cells for useful applications. By engineering modifications to the genomes and biochemical pathways inside microorganisms like bacteria, yeast, and algae, these cells can transform into tiny manufacturing platforms or sensors. Major advances in synthetic biology tools and capabilities in the last two decades have enabled numerous real-world applications using engineered microbes in areas spanning medicine, biomanufacturing, environmental protection, and fundamental research. This review will explore foundational and emerging synthetic biology approaches enabling the engineering of microbial systems and some promising directions for further progress. We highlight case studies across diverse engineered microbes deployed to produce commodity chemicals and smart living diagnostics, therapies, biosensors, and bioremediation agents.

Brief History of Engineered Microbes and Emergence of Synthetic Biology

Humans have been engineering microbes for thousands of years for applications like fermentation in brewing, wine making, and baking even without understanding the underlying biological mechanisms.

In the 1970s it became possible to directly manipulate the DNA of organisms like E. coli using recombinant DNA technology, marking the birth of genetic engineering and biotechnology. Early genetic engineering focused on introducing foreign genes into microbes to produce proteins, antibiotics, and compounds of interest like insulin and growth hormone. Progress was challenging and adhoc, involving trial-and-error tweaking of one or a few genes at a time[1].

In the early 2000s, advances in understanding gene regulation and adoption of engineering principles led to the emergence of synthetic biology. Synthetic biology aimed to create standardized modular DNA parts that act as building blocks to engineer increasingly complex and sophisticated biological systems. The focus shifted from genetic engineering's direct DNA tweaking to rational, model-driven design and testing cycles[1].

Synthetic biology has enabled revolutionary progress in programming microbes for expansive new functions related to manufacturing, environmental protection, biomedicine and more. Synthetic biology built upon early genetic engineering but transformed the potential scale and complexity at which we can engineer living cells like microorganisms by applying systematic engineering approaches.

Synthetic Biology Principles and Tool Development[2]

Standardized BioBricks

- Concept of BioBrick parts - standard biological parts with defined functions that can be reliably assembled
- Enable modular assembly by focusing on part interfaces and abstraction
- Registry serves as collection of thousands of BioBrick parts encoding devices like promoters, ribosome binding sites, terminators, signaling proteins

Advanced DNA Assembly

- Methods like Gibson assembly greatly improved ease of seamlessly fusing DNA fragments
- Automated liquid handlers and robotic platforms enable massively parallel DNA construction
- Reduced time from design to testing functional genetic constructs in cells from months to days

Genome Editing

- Site specific nucleases like CRISPR-Cas allow targeted insertion, deletion or mutation of genes
- Expanded synthetic biology capabilities for programming cells by writing circuits into genomes

DNA Synthesis

- Rising efficiency of custom gene and full genome synthesis techniques
- Allows bootstrapping and booting up cells with entirely human designed DNA
- *Computational Modeling*
- Applying principles from electrical engineering and computer science to model biological system behavior
- Mathematical models and simulations used to guide designs before finalizing testing

Standardized parts coupled with advanced assembly methods, genome editing tools and computational models have transformed capacities to rationally construct increasingly sophisticated systems using synthetic biology workflows to convert digital sequences into biological outputs[3].

Engineering Microbes with Synthetic Gene Circuits[3]

- Microorganisms like E. coli and yeast can be engineered as chassis for synthetic gene circuits to carry out designed functions in cells.
- Synthetic gene circuits are constructed by combining regulatory, signaling, and output genes/proteins to create genetic programs inside cells.

Regulatory Parts

- Promoters, transcription factors, terminators to control expression
- RNA regulators that control translation
- Scaffolding proteins to recruit circuit components

Signaling Parts

- Sensory domains linked to promoters to detect signals
- Two component systems for signal transduction cascades
- Receptor proteins activated by ligands, metabolites

Output Parts

- Metabolic enzymes to synthesize chemicals
- Fluorescent proteins for visual readout
- Pores for release of compounds or signals

Circuit Design Principles:

- Use BioBrick standard DNA parts
- Model behavior computationally
- Iterate through design-build-test cycles
- Multiplex circuits with other cellular pathways

By programming gene circuits, we can engineer microbes to process information and produce useful outputs for technological, industrial, agricultural and medical applications.

Applications of Engineered Microbes[5, 6]

1. Biomanufacturing

- Microbes engineered to synthesize valuable chemical products
  - Biofuels - Ethanol, butanol, biodiesel
  - Pharmaceuticals - Artemisinin (antimalarial), opioids
  - Polymers - Bioplastics from organic feedstocks
- Benefits include renewable sources, less waste, and reduced costs.
- Fine chemical production possible by engineering biosynthetic pathways.

2. Bioremediation

- Bacteria engineered to break down environmental pollutants:
  - Petroleum and oils spills in oceans.
  - Pesticides, heavy metals in contaminated soils
  - Salinized soils and industrial discharge
- Microbe biosensors also deployed to identify contamination
- Environmental cleanup and renewal applications

3. Medicine

- Engineered probiotic bacteria designed to function in gut:
  - Metabolic functions - Synthesis of vitamins
  - Immune modulation - Anti-inflammatory effects
  - Pathogen blocking - Preventing adhesion of harmful bacteria
  - Toxin degradation
- Diagnostics - Engineered cell biosensors that identify disease biomarkers
- Cell therapies - T cells or stem cells engineered to treat diseases like cancer

Engineered microbes hold enormous potential as tiny self-replicating factories for producing renewable fuels, chemicals, pharmaceuticals, and smart living medicines while also protecting environmental ecosystems. Continued progress in synthetic biology will lead to greater adoption[4].

Case studies of organisms engineered by academia and companies[7, 8]

Here are some case studies highlighting organisms that have been engineered using synthetic biology approaches from both academic research and companies:

- *Academic Labs:*

1. E. coli engineered to produce renewable biofuels, plastics, and commodity chemicals like glucaric acid.
2. Yeast redesigned to produce the anti-malarial compound artemisinin.
3. Cyanobacteria modified to excrete sucrose into the media as a test case for producing exportable fuels or chemicals.

4. Gut probiotic E. coli engineered to detect and destroy pathogens for antimicrobial therapy applications.
5. Agrobacterium for plant-engineering used as a chassis to test modular logic gates and circuits.

- Companies[8]:

1. *Ginkgo Bioworks* engineering yeast to detect flavors in end products for food optimization.
2. *Intrexon* optimizing an E. coli strain to mass produce isobutanol for fuel blending.
3. *Evolva* developing yeast that converts sugar to nootkatone, an orange oil for the cosmetics industry.
4. *Solugen* using engineered enzyme pathways in Pseudomonas putida host for chemical manufacturing.
5. *Synlogic* programming probiotic E. coli Nissle to treat diseases by synthesizing therapeutic metabolites.

The case studies highlight bacteria, yeast and algae engineered with synthetic circuits, biosynthetic pathways, sensors, export systems and other novel functions. Both startups and established leaders are contributors across diverse industrial and medical markets[9, 10].

Future Outlook and Conclusions

Synthetic biology has already delivered major advances in engineering microbes for useful applications, yet we are likely still only scratching the surface of what will become possible as tools and techniques continue advancing.

Some promising directions include:

- Moving beyond bacteria and yeast to engineer eukaryotic microbes like algae, protozoa, and multi-cellular fungi which offer capabilities like photosynthesis, motility, complex development and extracellular structures[10].
- Expanding beyond metabolic engineering and biomolecule production to integrate more complex phenomena like microbe-microbe signaling, biofilm formation, symbiosis and tissue development.
- Increased adoption of fully automated iterative design cycles between computational models and robotic rapid prototyping platforms for accelerated strain improvement.
- Leveraging machine learning for predictive modeling and designing increasingly sophisticated sensation, decision making and control circuits to yield more autonomous living systems.
- Exploring alternate microbial chassis or minimal genomes that stretch definitions of traditional organisms[11].
- Commercial scaling of microbe engineering efforts as technologies mature from proof of concept stages to

robust and competitive manufacturing processes.

Overall, the field is shifting faster than our imaginations can keep up. While domains like healthcare, agriculture, materials, energy and the environment will be disrupted in the coming decades, perhaps the most exciting opportunities are discoveries not yet conceived. Just as synthetic chemistry and engineering created industries and products that defined the modern world, synthetic biology promises a new revolution.

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