EBRC Engineering Biology Research Consortium

Engineering Biology for Space Health

An Innovative Research Roadmap

October 2024

roadmap.ebrc.org

1900 Powell St., Suite 1200
Emeryville, CA 94608
https://ebrc.org | https://roadmap.ebrc.org

This material is based upon work supported by the Translational Research Institute for Space Health at the Baylor College of Medicine (NASA Cooperative Agreement NNX16AO69A) under award #INN0013. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the author(s) and do not necessarily reflect the views of NASA, the Translational Research Institute for Space Health, or Baylor College of Medicine.

Contributors

Any opinions, findings, and conclusions or recommendations expressed in this material are those of the author(s) and do not necessarily reflect the views of the authors' affiliations or the Baylor College of Medicine Translational Research Institute for Space Health.

Roadmap Leadership

Emily R. Aurand, EBRC Director of Roadmapping and Education | *Executive Editor*
Kaitlyn Duvall, EBRC Program Research Assistant | *Project Coordinator*
Michael Köpke, LanzaTech | *EBRC Roadmapping Working Group Chair*
Kate Adamala, University of Minnesota
Adam Arkin, University of California, Berkeley
Mark Blenner, University of Delaware
Rihana Bokhari, Translational Research Institute for Space Health/Baylor College of Medicine
Nicole Buan, University of Nebraska-Lincoln
Karmella Haynes, Emory University
India Hook-Barnard, EBRC
Stephen Mayo, California Institute of Technology
Tae Seok Moon, J. Craig Venter Institute

Roadmap Contributors

Chris Anderson, University of California, San Diego
Ludmilla Aristilde, Northwestern University
Yvonne Armitage, CPI (Centre for Process Innovation)
Nils Averesch, Stanford University/University of Florida
Ania Baetica, Drexel University
Aaron J. Berliner, University of California, Berkeley
Edgar Cahoon, University of Nebraska
Ryan Cardiff, University of Washington
Goncagul Cengiz Baris, The University of Queensland
Liyam Chitayat, Massachusetts Institute of Technology
Patrick Cirino, University of Houston
Devin Coleman-Derr, USDA ARS/University of California, Berkeley
Marc-André D'Aoust, Aramis Biotechnologies
Kaitlin Dailey, University of Nebraska Medical Center, Eppley Institute for Research in Cancer and Allied Diseases, Fred & Pamela Buffett Cancer Center
Leah Davis, Rowan University
Gozde Demirer, California Institute of Technology
Garrett Dunlap, UK Science and Innovation Network
Chris Dupont, J. Craig Venter Institute
Ian Ehrenreich, University of Southern California
Jennifer Fogarty, Translational Research Institute for Space Health/Baylor College of Medicine
Paul Freemont, Imperial College London

Andrea Garza Elizondo, Rice University/Oak Ridge National Lab
Erin Garza, J. Craig Venter Institute
Simon Gilroy, University of Wisconsin-Madison
John I. Glass, J. Craig Venter Institute
Rahul Goel, Axiom Space
Jimmy Gollihar, Houston Methodist Research Institute
Alex Harkess, HudsonAlpha Institute for Biotechnology
Matt Hotze, The Good Food Institute
Jacques Izard, University of Nebraska Medical Center
Miguel Jimenez, Boston University
Robert Jinkerson, University of California, Riverside
Lauren Junker, BASF
Ava Karanjia, University of Washington
Fathi Karouia, BioServe Space Technologies, University of Colorado Boulder; Blue Marble Space Institute of Science, NASA ARC
Cholpisit (Ice) Kiattisewee, University of Washington
Cameron Kim, Duke University
Jonathan A. Kirk, Loyola University Chicago
Ross Klauer, University of Delaware
Jon Klonowski, University of Pittsburgh
Ursula Koniges, NASA
Kok Zhi Lee, Washington University in St. Louis
Ying-Chiang Jeffrey Lee, SPA Liaison, EBRC Security Working Group
Peter Ling, Ohio State University
Arren Liu, Arizona State University
George Lu, Rice University
Becky Mackelprang, EBRC
Karen A. McDonald, University of California, Davis
Matt McNulty, Tufts University
June Medford, Colorado State University
Amor Menezes, University of Florida
Rebecca Mickol, US Naval Research Laboratory
Jenny Mortimer, University of Adelaide
Cynthia Ni, EBRC/Beam Circular
Gene Olinger, MRIGlobal/UTMB GNL National Laboratory
John W. K. Oliver, ZBiotics
Meagan Olsen, Northwestern University
Elizabeth Onderko, Capra Biosciences
Pamela Peralta-Yahya, Georgia Institute of Technology
Yu “Max” Qian, J. Craig Venter Institute
Elibio Rech, NIST Synthetic Biology/EMBRAPA
Camilo Riquelme-Guzmán, Tufts University
Eugenia (Jane) Romantseva, National Institute of Standards and Technology
Rebecca L. Roston, University of Nebraska-Lincoln

Amy Rowat, University of California, Los Angeles
Kate Rubins, NASA Astronaut Office
Bruno Sacchetto Paulo, University of Illinois at Chicago
Rosa Santomartino, University of Edinburgh
Aditya Sarnaik, Arizona State University
Aaron Schaller, MeliBio, Inc.
Julietta Sheng, EBRC
Michael Smanski, University of Minnesota
Kevin Solomon, University of Delaware
Ranjan Srivastava, University of Connecticut
Taylor Stallings-Pinnick, Space Tango
Gil Stamm, University of Washington
Patrick Stover, Texas A&M University
Rahul Subramaniam, National Institutes of Health/University of Oxford
Molly Sullivan, Space Tango
Gene Tan, J. Craig Venter Institute
Yinjie Tang, Washington University in St. Louis
Deepti Tanjore, Lawrence Berkeley National Lab
Christopher G. Taylor, Ohio State University
Sheela Vemu, Waubonsee Community College
Beth Vitalis, Life Science Alive, LLC
Cătălin Voiniciuc, University of Florida
Ray Westenberg, Georgia Institute of Technology
Phil Williams, University of Nottingham
Ben Woolston, Northeastern University
Feilun Wu, J. Craig Venter Institute
Yan Zhang, California Institute of Technology
Cristal Zuniga, San Diego State University

Special thanks to EBRC staff members Mary Tomagan and Julietta Sheng for their support with this project.

The EBRC Roadmapping Working Group provides technical guidance and oversight to EBRC Roadmapping activities. Working Group members can be found at https://ebrc.org/focus-areas/roadmapping/#wg

Table of Contents

Overview and Introduction

Overview

This EBRC technical research roadmap, Engineering Biology for Space Health, provides a detailed evaluation of opportunities for engineering biology to improve human health and well-being during space exploration missions and help solve societal challenges here on Earth. Keeping humans alive on ever-longer and ever-further missions into space will require the sustainable production and access to food, new and more efficient and effective health and medicine capabilities, and enabling and ensuring resources to support life and control the local environment, particularly when those resources are limited. This roadmap is intended to guide technical research and development, investment, and programmatic decisions into engineering biology tools and technologies that will help overcome the challenges of extended space travel.

The roadmap has been created through the contributions of over 100 diverse stakeholders with broad scientific expertise, including academic and industry experts, government leaders, and exceptional trainees. The planning, production, and publication of this roadmap was led by the science policy experts at the Engineering Biology Research Consortium (EBRC) with input from the leadership of the Translational Research Institute for Space Health (TRISH). Three in-person workshops were held for content development, with additional virtual workshops to further edit and refine the roadmap's content.

The roadmap serves to provide a diverse audience, including the research community, burgeoning private space industry, government funders, and policy experts, with an inclusive, but not exhaustive, landscape of potential engineering biology advancements towards solving known and anticipated challenges facing human health and well-being in space. The roadmap consists of five cascading *Elements*, further detailed below (see **About the Roadmap**), in three overarching themes: Health & Medicine, Food & Nutrition, and Environmental Control & Life Support. This roadmap's broad Goals are delineated by Breakthrough Capabilities to which engineering biology can directly contribute. The Breakthrough Capabilities are broken down into short-, medium-, and long-term milestones for engineering biology advancement, each with relevant technical bottlenecks and potential solutions. This *Element* structure makes the roadmap accessible to audiences with different levels of technical expertise or topical interest. The roadmap also includes a Fictional Narrative and a contextual Glossary to help orient readers.

Each field of science and engineering will have its own advancements and approaches to the challenges discussed in this roadmap, and the tools and technologies identified here are only a small portion of possible solutions to advance human health during long-term spaceflight. Advances in other disciplines must be supported and researchers should remain actively engaged in a way that fosters multi-disciplinary collaboration. The ethical and social considerations of the tools and technologies described in this roadmap and beyond should be meaningfully incorporated at the early stages of research, including proper training for researchers to consider potential ethical, security, and legal implications from the start. The opportunities described in this roadmap are only a small part of a broader body of work but present the great potential engineering biology holds for future advancements in space health.

Introduction

The expansion of human capabilities in Low Earth Orbit and beyond will catalyze advancement in scientific discovery and capabilities, with far-reaching implications. We can leverage advancements in engineering biology to expand both human spaceflight capabilities and on-Earth technological capabilities for a better future. Identified in the roadmap are novel engineering biology approaches to research in space health that will improve environmental control and life support systems, provide enhanced nutrition, and ensure crewmember health and safety. Some of the potential solutions identified in the roadmap include bio-based sensors for improved environmental monitoring, waste bioremediation and recycling bioprocesses, bioproduction of micronutrients, protective microbiomes, and the bio-enabled on-demand production of pharmaceuticals.

Technological breakthroughs in biomedicine have significantly increased the understanding of the effects of long-term space travel and microgravity on health outcomes. Applications in engineering biology are critical to improve surveillance and diagnostics to health outcomes, with real-time monitoring and on-demand therapeutic interventions tailored to the specific needs of each patient. Rapid response to illness and injury will be achieved with cross-cutting technologies that enable on-demand production and long-term stability and storage of biopharmaceuticals and biologics. Additional advancements in engineering biology research will also help to support long-term maintenance of the physical health and well-being of astronauts. Research opportunities include innovations in enzyme biosensors for health biomarkers, cell-free production cascades for small molecule pharmaceuticals, and coating materials with bioactive proteins or other biomolecules for wound care.

The health of astronauts additionally depends on the availability of highly nutritious and palatable food that meets dietary needs. Future efforts in engineering biology to support food and nutrition systems will likely include: on-demand, in-Space production of nutrients and nutrient-rich food products; engineered food crops with high harvest index, pathogen resistance, and a short harvest time; and innovative efforts to improve palatability and stability of food and advance biobased food preservation. Combinations of genetic, biomolecular, and circuit and pathway engineering can enable platform organisms to generate needed vitamins and minerals for consumption, or macronutrients, including proteins, carbohydrates, and fats. Advancements in cell culturing and three-dimensional bioprinting can enable the production of whole foods (e.g., meats) in space, while crop-focused engineering tools can produce wholly-consumable plants or enable plant tissues that serve as food or pharmaceutical; and creative enzyme engineering can help to generate stronger, desirable food flavor experiences otherwise lost in space.

Rapid advancements in environmental control and life support systems over the last 70 years have catalyzed human presence in space, from the launch of the first unmanned Sputnik satellite in 1957, to the International Space Station (ISS), which has been continuously occupied since November of 2000. Advancements in engineering biology tools and technologies are uniquely suited to further support these systems through air and water filtration and recycling, enabling circularity with biotic and abiotic wastes transformed into newly usable materials and feedstocks, and enhanced living and bio-derived materials for infrastructure to protect and support human health. These capacities will be achieved with advancements in enzyme

engineering, expanded uses for cell-free systems, tighter direction and control of engineered microbes and microbial consortia, and new capabilities in fungi, insects, and plants.

Across all themes and applications, there is a distinct need for analogs on Earth that replicate elements of space, including microgravity, radiation exposure, thermal changes, and the controlled and restricted nature of the environment. Space analogs allow researchers to economically and efficiently test potential tools and technologies before sending them to space, greatly increasing the number of solutions able to be tested. For the application and implementation of many of the engineering biology capabilities described in the roadmap, there are certain biological processes that need to be better understood and explored, and certain foundational tools and technologies that need to be tested and adapted, to work in the space environment. Examples of such include characterizing the dynamics of microbial systems and consortia, where (at minimum) cell-to-cell signaling could be impacted by changes in aerosol and fluid dynamics, and the need to develop compact or modular, highly-efficient and high-fidelity bioreactors. Some of these needed capacities are captured in the roadmap explicitly, while others are understood to be solved with further research in space; however, better analogs for the space environment here on Earth would accelerate this innovation.

During creation of the roadmap, contributors acknowledged and discussed biosecurity in engineering biology advancements, highlighting the importance of maintaining a controlled, but adaptable environment, and understanding the impact engineering biology can have in new contexts where evolutionary or physical controls and mitigations are not always present. Throughout the roadmap, contributors identified strategies and opportunities for biocontainment, such as genetic kill-switches and enhanced quorum sensing, to enable technological redundancy and fail-safe measures. The highly controlled nature of space environments, like the International Space Station, creates inherent containment, which is ideal for certain types of research where biocontainment is of the utmost importance; however, it is important that any technologies, particularly those impacting human health and well-being, are developed considering security implications.

Engineering biology offers novel, scalable solutions that integrate well with current and future economies and have broad applications in a variety of sectors, including the expansion of human spaceflight capabilities. The unique challenges posed by the space environment can be overcome, and in some cases, leveraged by engineering biology to establish new tools and technologies. Engineering biology can help to enable environmental complexity and propel systems towards circularity. In-space research will enable the further development and engineering of systems that move toward black box research models, which promote user independence and are particularly helpful in the early stages of research. The space environment also challenges researchers to engineer for austere and low-resource environments, with potential applications extending to health and medicine, resource utilization, and sustainability on Earth.

Roadmapping Process and Project Development

Engineering Biology for Space Health: An Innovative Research Roadmap is EBRC's sixth technical research roadmap. The topic and focus of the roadmap were developed in coordination with the Translational Research Institute for Space Health (TRISH), with scoping

and oversight from the Roadmap Leadership Team (see **Contributors**) and the EBRC Research Roadmapping Working Group. Working within NASA's Human Research Program and Baylor College of Medicine, TRISH is dedicated to supporting research aimed at solving the many challenges of deep space exploration, including high-risk, high-reward projects (TRISH, 2024).

Roadmap stakeholders include the engineering biology research and space health science communities. The roadmap aims to bring these communities together to better understand, and spur ideas towards, what engineering biology can enable. It is also intended for use by those in industry, government, non-profit, and nongovernmental organizations and institutions to provide a statement on opportunities in the field and identify milestones and targets to consider in program and infrastructure development and investment.

EBRC's roadmapping is an interactive process of brainstorming, drafting, discussion, review, and revision. Over 100 individual contributors with a broad variety of expertise (see **Contributors**) helped draft *Engineering Biology for Space Health*. Initial scoping began in October 2023, followed by three in-person writing workshops held in December 2023, March 2024, and May 2024. A number of virtual workshops and collaborative writing sessions were held June-July 2024 to further refine and clarify roadmap content. The roadmap was reviewed, edited, and revised by contributors and stakeholders from May through August 2024 and prepared for publication in September 2024.

The EBRC Roadmapping Working Group, chaired by Dr. Michael Köpke (Chief Innovation Officer, LanzaTech) with staff direction from Dr. Emily Aurand, leads EBRC roadmapping efforts. This work received substantial support from Kaitlyn Duvall, EBRC Program Research Assistant, with editing assistance from EBRC Science Policy Postdoc, Dr. Julietta Sheng, and event coordination support from EBRC Senior Administrator, Mary Tomagan. EBRC is led by Executive Director, Dr. India Hook-Barnard.

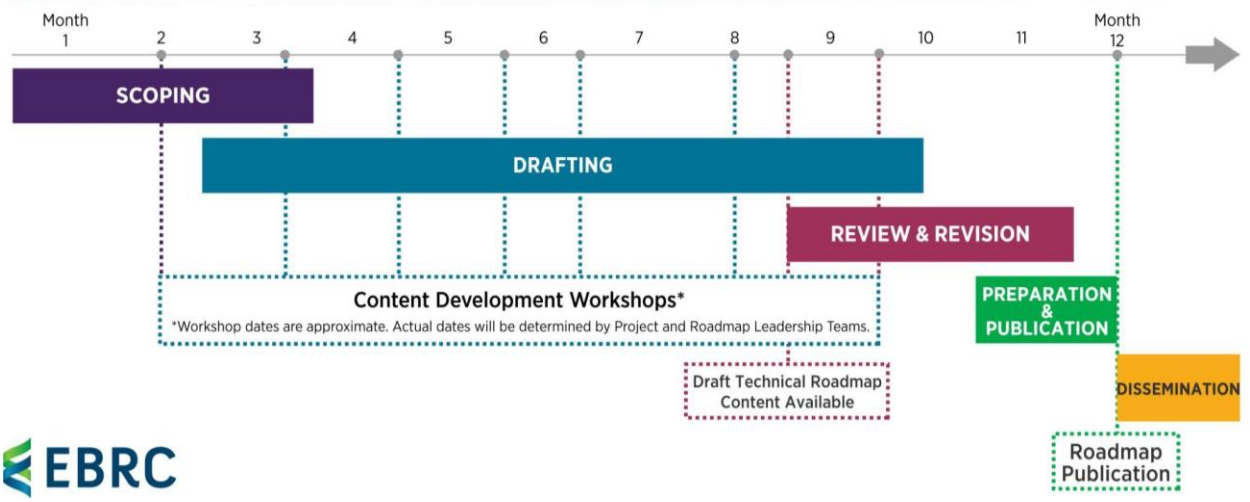

Figure 1. Timeline of *Engineering Biology for Space Health* development. The EBRC roadmapping process consists of scoping, drafting, revision, and review processes. Development of the roadmap began in October 2023 and the roadmap was published in October 2024.

About the Roadmap

This technical research roadmap includes three major themes which detail significant breakthroughs and milestones for engineering biology for space health. The **Health & Medicine** theme addresses opportunities to improve the health and well-being of crewmembers while ensuring access to necessary diagnostics and therapeutics. The **Food & Nutrition** theme focuses on opportunities to sustainably produce and maintain palatable and nutritious food and supplements. The **Environmental Control & Life Support** theme explores the application of engineering biology tools and technologies to improve environmental control and life support systems for long-term spaceflight, including clean air and water, waste remediation and recycling, and materials synthesis and adaptation. Each theme is broken down into a series of increasingly technical roadmap *Elements*:

Goals – The roadmap Goals are the "big-picture" objectives, what we hope to accomplish through science and technology to improve human health and well-being in space. Written in a way that is accessible to non-technical audiences, the Goals are intended to convey some of the biggest issues and opportunity areas in tackling challenges and enabling long-term solutions.

Current State-of-the-Art – Each Goal is followed by a short summary of where we are today, including some of the biggest challenges for both the Space context and engineering biology.

Breakthrough Capabilities – The Breakthrough Capabilities identify how we can contribute to the Goal with engineering biology and are representative of major aims across the field. Typically written as what you might see in a *Science* or *Nature* publication headline, the Breakthrough Capabilities are the engineering biology achievements towards their higher Goal.

Milestones (Short-, Medium-, and Long-term) – The Milestones represent advancements in engineering biology tools and technologies that make stepwise progress towards achieving the Breakthrough Capability. Short-term Milestones are expected to be about 3-5 years away from achievement, representing research that is currently funded (or where funding opportunities exist) or could be accomplished with existing resources. Medium-term Milestones are tools and technologies anticipated to be achieved in approximately 10-15 years; these research areas likely need funding (including new grant/award programs) or infrastructure development, and other support at the institutional or federal level. Long-term Milestones are anticipated to be 20-30+ years from realization and, in most if not all cases, would require new funding, infrastructure, or other resources (including significant tool and technology development). All of the milestones are intended to be ambitious and visionary, representative of what engineering biology could accomplish with unconstrained resources and congruent advancements in other fields, so as to spur investment and action across the science, engineering, social, and political enterprise. Many of the milestones are written with the expectation that they can be achieved in the Space

context, requiring extensive testing in microgravity environments and thus, more time than similar capacities on Earth, before the milestone is reached.

Bottlenecks and Potential Solutions – The Bottlenecks represent a specific technical challenge to achieving the milestone. Likewise, the Potential Solutions represent one or more ways in which we might overcome the bottleneck. These elements are not comprehensive, capturing only a few of the issues and approaches researchers may encounter and undertake.

We recommend the following citation when referencing this roadmap: Engineering Biology Research Consortium (2024). *Engineering Biology for Space Health: An Innovative Research Roadmap*. Retrieved from http://roadmap.ebrc.org. doi: 10.25498/E4D59R

Feedback or questions about this roadmap or EBRC's other roadmaps and roadmapping activities can be directed to roadmapping@ebrc.org.

About EBRC

EBRC is a non-profit, public-private partnership dedicated to bringing together an inclusive community committed to advancing engineering biology to address national and global needs. We showcase cutting-edge research in engineering biology, identify pressing challenges and opportunities in research and application, and articulate compelling research roadmaps and programs to address these challenges and opportunities. Our four focus areas, driven by member-led working groups, are Research Roadmapping, Education, Security, and Policy & International Engagement.

Fictional Narrative

The below mock journal entry[1] is included to provide informal context for some of the potential advancements detailed in the roadmap.

September 30th, 2050

We have been in transit for one hundred and twelve days, each moment bringing us closer to Mars, a planet that once felt impossibly distant. As I glide down the main corridor of the spacecraft, I think back to when the idea of space travel was grueling, little true joy could be found in the food, and concerns about staying healthy weighed on one's mind. But this time, thanks to the wonders of engineering biology, this mission has felt more like an exciting adventure.

During my morning float through our craft, I go by the hydroponics hub. It's a green oasis in the center of the ship, filled with vibrant plants and small streams that flow through nearly-invisible tubes against lunar regolith. Aside from providing solace and a reminder of home on Earth, the hydroponics hub is a major source of environmental control and life support aboard the spacecraft.

The plants and crops grow in soil that is created from human waste-derived nutrients and contains engineered bacteria to enhance growth and harvest rate, one of the largest successes in innovations to the wastestream remediation system. The plants themselves are engineered to use nitrogen and water from human urine for nutrients and growth. They also sense, filter, and decontaminate pollutants for clean, breathable air onboard the spacecraft. The fluid tubes of the hydroponics hub are made from materials that contain genetically engineered microbes that recover nutrients from wastewater to be reused for agriculture or human consumption. Potable water obtained from this system is further electrolyzed to generate hydrogen and oxygen for other environmental and life support uses.

The regolith we collected from our pit-stop on the Moon makes up the foundation of the hydroponics oasis. The rocks, with their specialized organic supplementations, not only help maintain healthy space crops but are also crucial for *in situ* synthesis of our biomaterials. Microbes engineered to be regolith-tolerant allow for extraction of minerals, heavy metals, and other essential elements for downstream biosynthesis of materials we use for (re)packaging, crop fertilizer, and bio-concrete for emergency repairs of the ship.

My next stop is the cafeteria for breakfast. Space food has also come quite a long way. We have successfully leveraged 3D-bioprinting technology and adaptive bioreactors to produce foods with optimized nutrient and dietary requirements that look more like what we would get in a restaurant on Earth. Engineered enzymes additionally allow us to enhance the taste and texture of our food to our own preferences, making it more palatable.

After I finish my coffee and "Starberry" waffles, I visit our doctor in the medical bay. They are currently attending to another crewmate who is undergoing analysis of their biomarker data so they can adjust the concentration of her weekly biologic that prevents bone and muscle density loss in microgravity environments. As I wait outside the patient room, I peer into the

[1] We provide no guarantees to the technical accuracy of this journal entry. It's just for fun!

medical testing lab where one of our scientists is adding in macromolecular sensors and thermo-stable enzymes to biological feedstocks. We don't have much refrigerated storage space onboard, so these sensors and enzymes allow us to monitor, store, and process feedstocks and samples at room temperature. In the case of a medical emergency, the spacecraft is equipped with the biotech data and infrastructure to rapidly diagnose health conditions and perform life-saving procedures, such as personalized gene therapies or blood transfusion, at nearly the push of a button.

I finally arrive at the command center of the ship and gaze out the main observation window. The twinkling stars seemed to dance together, as if welcoming us into these endless depths of Space as our ship hurtled towards the Red Planet. Thirty-eight more days, and we will truly test the lengths engineering biology can take us.

Health & Medicine

Introduction and Impact: The space environment poses unique challenges to the health and well-being of humans. Off Earth, the human body is subjected to a variety of unique environmental stressors that include musculoskeletal system effects, cardiovascular impacts, damage to the central nervous system, impaired cognitive function alteration, reduced motor function, increased cancer risk, psychological/behavioral changes, and many more (NASA HRP, 2016; Reynolds et al., 2020 Bushnell & Gross, 2023, Barratt et al., 2020). Although a significant body of work is dedicated to understanding the impacts of spaceflight on humans, there is still much to learn, particularly regarding long-term missions and extended flight time. The breadth and uncertainty of what diagnostics and therapeutics may be needed – particularly for missions that extend beyond Low Earth Orbit (LEO) and are longer in duration – make it difficult to provide immediate, accurate, and effective care (Averesch et al., 2023). To expand human capabilities in space, improved medical support and countermeasures (including advanced diagnostics and therapeutics for care and treatment of conditions) are necessary to support and maintain crewmember health and ensure mission success.

This technical theme considers engineering biology solutions to improve medical surveillance, diagnostics, and treatments to promote better understanding of health and enhance overall patient care. Engineering biology offers the opportunity to employ biosensors and bio-enabled devices that are capable of real-time monitoring and data collection. On an individualized level, biology-based diagnostics will enable rapid development of personalized medicine, specific to the health needs of each patient. With integration of biochemical analysis and metabolic monitoring techniques, the roadmap explores how these tools can be leveraged to better predict human health status and enhance treatment outcomes.

The roadmap addresses engineering biology opportunities to rapidly respond to illness and injury. Cross-cutting technologies will enable on-demand production of pharmaceuticals and biologics using cells, cell-free systems, plants and other biological platforms, allowing for more immediate and effective medical treatment. Long-term stability, storage, and quality monitoring of biopharmaceuticals and biologics will enable high efficacy of these treatments over time (Perez-Pinera et al., 2016; Adiga et al., 2018). Advanced hemostasis management and wound healing can reduce the recovery time in severe conditions. Together, these advancements in engineering biology will enable accessibility and availability for faster and more reliable health care.

Long-term spaceflight will require not only response to illness, but considerations for overall health and well-being over time. The roadmap considers engineering biology approaches to the maintenance of human health and physiology, through approaches such as engineered biomaterials to protect against radiation and the establishment of robust and stable human microbiomes to prevent illness and disease (Cao et al., 2020; Mortazavi et al., 2023). Another important capability is the prevention of bone density and muscle loss. There are also potential engineering biology capabilities that could complement approaches to addressing psycho-social well-being and performance, to ensure good physical and mental health of astronauts during prolonged space travel.

This roadmap presents only a few of the many possible engineering biology approaches to improving human health during long-term spaceflight. Further research in the pharmaceutical

and medical fields should accompany the achievements highlighted here and such research should be multidisciplinary and include a focus on the ethical and social implications of these technologies and their applications.

Goal	Breakthrough Capability	Milestone

Surveillance, monitoring, and diagnostics for human health and physiology.

Real-time, personalized and individualized modeling and prediction of health status and trajectory.		
Creation of digital twins from precision health AI/ML model training based on in vitro analysis of individual biomarker data.	A comprehensive model to monitor multi-modal health biomarkers.	Comprehensive modeling of personalized health analytics with capability to scale to variable environmental conditions.
	Holistic genome data and models of major human tissues to predict individualized environmental and lifestyle effects on health.	Personalized microbiome and environmental data to reliably predict dysbiosis and low perturbation prophylactic intervention.
Integrated, continuous, and autonomous sensing and monitoring of human metabolic and biochemical biomarkers.		
Shelf-stable biosensors for detection of specific human health-related chemicals or chemical groups.	Non-invasive, remote monitoring of gene expression and signaling states in human cells for major organs and tissue systems.	Biochemical sensors that monitor physiological baselines of individuals over time to predict health concerns before clinical presentation.
	Devices that provide point-of-care solutions from analysis of biological samples without the need for cold chain storage or processing.	
	Real-time metabolomic sensor arrays to monitor function in real-time.	
	Multi-biosensor system for biochemical surveillance that does not require specialized equipment.	Real-time monitoring of biological samples with in vivo and ex vivo biosensors to predict and treat disease, infection, and other health outcomes in space.
	Wearable or implantable biosensors to continuously monitor a range of metabolic markers, such as glucose and hormone levels.	
	Specific biosensors capable of monitoring immune status of the individual.	

Short-term → **Medium-term** → **Long-term**

Rapid engineering of biology-based or -enabled diagnostics and intervention decision-making.		
High-throughput, small volume liquid handling that is robust, reproducible, and requires minimal human intervention or troubleshooting.	Cell-based methods for detection of stress hormones in blood and tissue.	Wearable sensors to provide real-time indicators of health risk.
Miniaturization and combination of sample processing, metabolomic profiling, transcriptome and genomic sequencing, and AI/ML analysis.	Modular or combined diagnostic biosensors to screen for multiple pathogens or biomarkers in a single test.	
Detection tools for monitoring foodborne, waterborne, and airborne pathogens in the local environment.		
Computational models of illness and injury that provide therapeutic interventions based on disease severity and time course.	Genetically modify human cells or microbiota with feedback-control genetic circuits that activate desired immune pathways in response to specific diseases.	

Rapid response to illness or injury.

Long-term stability, storage, and quality monitoring of biopharmaceuticals.		
Low-resource stabilization (e.g., lyophilization, drying) and rehydration of biologics through iterative testing for the duration of space flight missions.	Engineer microbes or microbial consortia storage systems capable of rejuvenation in space.	Enable storage of complex cell factory systems (e.g., human or plant tissue culture) for further on-demand biosynthesis.
		Medical-grade responsive biomaterials to mitigate oxidative and radiative degradation of biopharmaceuticals.
	Point-of-care quality control and assurance tests to assess proper folding, glycosylation, and activity of manufactured or stored therapeutics.	Long-term storage of pre-synthesized DNA on solid substrate (e.g., paper strips) to enable rapid, cell-free synthesis of mRNA.

Short-term **Medium-term** **Long-term**

On-demand bioproduction of small molecule pharmaceuticals.		
On-demand enzymatic semi-synthesis of prodrugs.	Production of therapeutics using transient expression in edible plants.	Cell-free production of any pharmaceutical using DNA synthesized and stored onboard the spacecraft.
	Cell-free production of ready-to-use pharmaceuticals with minimal downstream processing steps.	Library of genetic constructs for on-demand production of most common small-molecules within appropriate chassis.
Demonstrate on-demand synthesis of pharmaceuticals in lyophilized cell-free reactions in a single tube/pot or via purified enzymes.	Engineer autotrophic microbes (e.g., cyanobacteria, halobacteria) or bryophytes that can withstand high radiation for the production of chemicals and pharmaceuticals.	Engineer heterologous production strains for major classes of small molecule therapeutics (antibacterial, antifungal, anti-inflammatory agents, etc.) that can be kept as lyophilic or freezer stocks in minute quantities for space flight applications.
	Regulate pharmaceutical release from organism or microbial systems with endogenous mechanisms.	

On-demand production of biologics.		
Target human cells or tissues for genetic manipulation to enable *in vivo* pathways for biologics production and therapeutic proteins.	Robust control of infection with viral vectors optimized for microgravity environments for drug and gene delivery in space.	Bacterial, living therapeutics capable of transient medicine production and essential nutrients in the human body.
	Protein-based biopharmaceuticals in viral vectors in space.	Adoptive T-Cell therapies to improve wound healing.
	Microbes or plants able to produce common classes of shelf-stable biologics, such as antimicrobials.	Transient viral delivery systems that are adaptable to various conditions for the host (i.e., the duration of therapeutic delivery, titers of therapeutic produced, side-effects in host cells, etc.).
	Allogeneic human cells with desired biological activity in any host (e.g., CAR-T cells that target a class of cancers).	Strain-specific phage chassis to target bacteria that commonly cause infections during space flight.
	Selectable system for transient skin colonization by an engineerable skin microbiome to combat infection or injury.	Selective delivery of mRNA drugs to tissues with smart-targeting particles.
	Selectable system for transient colonization of internal human microbiomes (GI tract, lung, vaginal, etc.) to address illness or infection.	On-demand production of therapeutics within 24-hours in plant hosts.
	DNA templates for production of all biological World Health Organization Essential Medicines relevant for space travel.	

Short-term **Medium-term** **Long-term**

Cross-cutting and enabling technologies for on-demand bioproduction of pharmaceuticals and biologics.		
Portable DNA synthesis machines to enable Earth-to-vehicle/habitat communicated synthesis.	Recycling of excess biopharmaceuticals excreted in waste streams (e.g., insulin in urine).	Reuse of source materials and reagents (NTPs, dNTPs, cofactors, etc.) from bioproduction processes in space/low-resource settings.
Drop-in, freeze-dried replacements of standard biochemical and biomolecular components (e.g., macromolecular sensors, thermo-stable enzymes) to enable room-temperature biological sample processing.		
Microfluidic and nanofluidic bioreactors that are functional in microgravity.		
Categorized common spaceflight ailments and associated treatments.		
Characterization of the adverse effects of microgravity on host cells utilized for biologic production.		
Advanced hemostasis management and wound healing.		
Use of natural pro- and anti-coagulants (i.e., activated protein C to treat thrombotic or bleeding emergencies from impaired blood flow or radiation.	Biomaterials and hydrogels for optimal production and material adaptation in spaceflight scenarios, to create a pipeline of first-aid and surgical supplies.	Ability to grow full or partial human skin from stem cells to treat severe skin damage.
Successful whole blood transfusions during spaceflight using transfusable artificial oxygen carriers or engineered blood product substitutes to replace blood products.		
Biomanufacturing of natural bioactive products effective for wound dressing and healing, such as cell-free or microbial production of synthetic antibacterial minerals to combat topical infections.	Engineered liver cells to create blood proteins or blood-like fluid on-demand.	Synthesized treatments to instantly adapt and enhance human production of hemostasis and coagulation proteins to recover hemostasis and wound healing.
Engineered plants to produce effective bioactive products for wound dressing and healing.		
Short-term	**Medium-term**	**Long-term**

Engineering biology for long-term maintenance of health.

Short-term	Medium-term	Long-term
Maintenance of sufficiently diverse and stable human microbiomes.		
Genetic toolkits and metabolic models for dominant commensal taxa of human microbiota (e.g., Escherichia coli, Enterococcus spp., Bacteroides spp., Cutibacterium).	Robust methods for engraftment and maintenance of persistent community levels of engineered microbes in diverse human environments.	Human microbiota communities synthesized *in situ* to promote astronaut health.
High-throughput screening of important microbiota gene targets with functional genomics technologies, including CRISPR libraries and transposons.	Biological reporters to quantify and detect abnormal fluctuations in the microbiome communities.	Engineered microbes to deliver specific and on-demand treatments to dysbiosis.
Established baselines for the composition, stability and evolution of the human microbiome (i.e., gut, skin, etc.) in space conditions.		Phage that optimize human microbiome to accomplish dynamic human physiological responses to disease, infection, and other health outcomes.
Prevention and treatment of long-term, cellular-level radiation damage and genetic mutation.		
Defined baseline genetic parameters to track mutations.	Efficient human DNA repair genes to repair the genome from radiation damage.	Effective delivery of improved human repair genes and implementation into human host.
On-demand production of radiation protection reagents (e.g., melanin, granulocyte colony-stimulating factor, entolimod, or resveratrol) as antioxidants.		
Radiotrophic fungi or selenomelanin analogs constructed or embedded into biomaterials to protect against radiation.		
Prevention and reversal of bone density loss.		
Produce drugs that can protect bone density in host (micro)organisms compatible with spaceflight.	Optimized enzymes engineered to improve biomineralization.	Inducible capacity to mitigate osteoporosis without harmful side effects (such as continued bone density increases above baseline, particularly when subjects return to Earth gravity).
Identification of key enzymes and bioprocesses associated with bone density maintenance that are downregulated or damaged in microgravity.		
Living biologic therapeutics to prevent bone loss as an alternative to daily vigorous exercise.		

Short-term	Medium-term	Long-term
Prevention and reversal of muscle loss.		
Neuronal activation of muscles during sleep with muscle stimulator or therapeutic that activates lower motor neurons or their receptors in muscle tissue.	Personalized diet (e.g., amino acid profiles, hormone supplements) to support muscle deposition without adverse complications.	Molecular simulation of the biochemical response to exercise in muscle tissue with biologics or biologically produced Muscle-targeted expression or delivery of myosin, actin, or other activating molecules.
Robust, cell-based theranostics (integrated diagnostics and therapeutics) for sustained, precision disease prevention.		
In silico techniques and open-source platforms to design and optimize precision medicine and live biologic therapeutics.	Human or human microbiota chassis that can sense and respond with therapeutics release to a range of relevant environmental conditions at clinically relevant concentrations.	Remote user control of behavior and therapeutic actions of engineered cells.
Suitable host chassis for human cells or microbiota to implement living therapeutics.	Microbial living therapeutics capable of transient medicine production and essential nutrients in the human body.	
Engineering biology opportunities to promote long-term psycho-social well-being and performance.		
Production of neuromodulatory molecules in gut microbiome members at clinically relevant concentrations.	Production and use of plant and fungal antipsychotic compounds in space.	Establish persistent neuromodulatory therapeutic production in gut microbiome members and identify factors affecting the longevity of engineered functions.
Relevant biological responses to neuromodulatory molecules produced by the gut microbiome at clinically relevant concentrations.		

Goal: Surveillance, monitoring, and diagnostics for human health and physiology.

Current State-of-the-Art: The capability to perform in-depth medical surveillance, monitoring, and diagnostics in spaceflight is essential to maintain crew health on long-term missions. Significant testing, monitoring, and diagnostics are currently completed pre-flight, during, and post-flight to ensure crewmember health and safety. The NASA-driven program, Complement of Integrated Protocols for Human Exploration Research on Varying Mission Durations (CIPHER), is a aimed at investigating how humans adapt to long-term space flight, particularly the five major hazards associated with space flight: radiation, reduced gravity, and distance from Earth, isolation, and hostile/closed environments (Oubre et al., 2024). Data collected from CIPHER will include a broad variety of medical testing (including but not limited to, blood tests, imaging, and functional evaluations completed pre- and post-flight) and will aid in the identification of ideal health indicators focused on preventative care and optimal health and well-being. Advanced human microbiome monitoring, including oral, lung, and gut microbiomes, may be made possible through the utilization of 16S rRNA sequencing techniques (Ames et al., 2018), though datasets should be expanded to include skin and olfactory microbiome data as additional health and wellness indicators. Microphysiological systems, like lab-on-a-chip and inflammation-on-a-chip devices, enable further study of cellular activities relevant to the spaceflight environment (Irmia & Wang, 2018). Radiation exposure characterization and innovative, non-invasive, capabilities to detect and characterize radiation exposure will be emerging from the Intelligence Advanced Research Projects Activity (IARPA) Targeted Evaluation of Ionizing Radiation Exposure (TEI-REX) experiment, and are likely to include bioengineering components (Patterson et al., 2021). Personalized microbiome and environmental data may be further used to predict and prevent dysbiosis.

Sensing and monitoring biomarkers of human health and physiology remains a highly active area of research, with the ultimate goal of allowing timely diagnostics and interventions to health events. To achieve this, knowledge about relevant biometrics, frequency of measurement, and their heterogeneity across human populations must be improved. Biometric monitoring devices must also be capable of generating the desired data at desired time points. Biomarkers using multi-omics approaches, integrated with environmental and behavioral data, remain an expanding area of research, with focus areas including oncology, rare diseases, and inherited genetic disorders. Currently, chemokine, cytokine, and cardiovascular biomarkers are captured for in-flight astronauts (Overbey et al., 2022). Accuracy and clinical relevance are inherent in current devices and rely on algorithms from large data sets that are often unstructured and not widely shared. Wearable device technology that monitors health and fitness is largely driven by consumer products (Alavi et al., 2021; Lim, 2020). While wearable sensors are used to monitor vital signs, exercise output, and some specific advanced health metrics, algorithms and data used to train the AI/ML aspects are proprietary and often are not standardized, making data interpretation difficult, and access to raw data is often impossible. Wearable devices also commonly rely on continuous internet access to process data, which is a challenge for low resource environments.

Advancements toward implantable biomonitors have been made, such as the FDA-approved Eversense E-3 implantable, continuous blood glucose monitor; although the approved lifespan of the device is 6 months, the manufacturer claims it may be viable for up to 1.5 years (Hoskins, 2022). The use of similar technologies would yield interesting data and may work well as a diagnostic tool on long-term missions, but equipment to collect and synthesize data enabled with the appropriate software to scan and/or monitor the device, would be needed. Advancements in technology, data sources, artificial intelligence (AI) and machine learning (ML) are rapidly evolving and generative AI large language models may be utilized for the capture and tracking of medical data (Lee et al., 2023). Personalized health based on ML models must be trained by *in vivo* biomarker data.

The International Space Station (ISS) does not currently have the capacity to perform basic diagnostics, like complete blood count and differential diagnostics. Various diagnostic instruments and assays have been tested aboard the ISS, but none are flight-certified and use is limited (Kilkenny & Russell, 2022). Engineering biology offers solutions to improve and expand monitoring and diagnostic capabilities. CRISPR-based diagnostics and toehold-switch technologies can be leveraged to rapidly identify disease indicators and provide effective therapeutic intervention (Ryung Kim et al., 2022; Kaminski et al., 2021; Pardee et al., 2016). Recent developments in these technologies reduce the need for specialized equipment and trained personnel, enabling point-of-care diagnostics (Kaminski et al., 2021). Paper-based point-of-care diagnostic tools, like lateral flow assays, also show great potential for rapid screening in low-resource environments (Omidfar et al., 2023). These devices enable sample-in/answer-out analysis of key biochemical markers from easily obtained samples (saliva, urine, blood) and often do not require cold storage or processing. The production and distribution of safe medicine also requires the ability to complete Pharmacokinetic-Pharmacodynamic (PK/PD) analysis of an individual at a particular point in space and time when the medicine is needed. In the long-term, this would be combined with on-site, on-demand manufacturing of tailored therapeutics to provide fully personalized medicines for anyone in resource-limited environments, on and off Earth (Aziz et al., 2022).

Breakthrough Capability: Real-time, personalized and individualized modeling and prediction of health status and trajectory.

- Short-term Milestone: Creation of digital twins from precision health AI/ML model training based on *in vitro* analysis of individual biomarker data (Katsoulakis et al., 2024; National Academy of Sciences, 2024).
    - Bottleneck: Insufficient data and validation frameworks for individualized genomics data for AI model training to detect susceptibility to health perturbations.
        - Potential Solution: Microphysiological systems as models (e.g., tissue chips, organ-on-chips) to increase validation frameworks for predictive health outcomes.
    - Bottleneck: Access to Low Earth Orbit (LEO) and LEO-based systems for high-throughput research to enable microgravity-based ML models to predict health trajectories.

        - Potential Solution: Automated high-throughput biomodel screening systems (e.g., microphysiological systems such as tissue chips and organs-on-chips) that can be deployed on a breadth of LEO platforms.
    - Bottleneck: Limited understanding of physiological adaptation to extreme environments (i.e., variable gravity environments).
        - Potential Solution: Collect physiological data from individuals exposed to extreme environmental conditions to enhance capability of ML models to detect abnormal patterns.
- Medium-term Milestone: A comprehensive model to monitor multi-modal health biomarkers.
    - Bottleneck: Lack of adequate biomarker sample sizes in variable gravity forces to establish “normal” parameters in these environments.
        - Potential Solution: Use “Organ-on-chip” and “Tissue Chip” microphysiological systems as synthetic mimics of human biological systems to increase the effective sample sizes for spaceflight-relevant studies (Shinde et al., 2023).
        - Potential Solution: Conceptualize variation based per individual to avoid transposing the general population data and evaluate deviation per astronaut.
    - Bottleneck: Lack of automated systems for biological data collection modules.
        - Potential Solutions: Automated data transformation modeled after systems on Earth to reduce required training.
        - Potential Solution: Generative AI methods for biological data collection, analysis, and interpretation.
- Medium-term Milestone: Holistic genome data and models of major human tissues to predict individualized environmental and lifestyle effects on health.
    - Bottleneck: Lack of individualized astronaut genomics data.
        - Potential Solution: Microphysiological system models (e.g., organ chips, tissue chips) to increase individualized and representative datasets (NASA Science Editorial Team, 2022).
    - Bottleneck: Limited understanding of which specific biological processes and traits result from genetic or epigenetic regulation.
        - Potential Solution: Investigate the relationship between genetic and epigenetic regulation that control phenotypic outcomes.
    - Bottleneck: The possibility of temporal changes over extended missions in space.
        - Potential Solution: Longitudinal studies examining physiological changes in biological models in space.
- Long-term Milestone: Comprehensive modeling of personalized health analytics with capability to scale to variable environmental conditions.
    - Bottleneck: Lack of statistical methods for longitudinal human health data modeling in varied gravity and radiation environments.
        - Potential Solution: Collect long-term data in simulated and real (e.g., ISS) microgravity environments.

        - Potential Solution: Power analysis for identification and prediction of longitudinal changes of personalized health data.
        - Potential Solution: Genomic sequencing of gametes from astronauts before and after long-term space missions to determine effects of radiation on mutation accumulation.
    - Bottleneck: Lack of population diversity in available ISS crew data.
        - Potential Solution: Data inclusion from more diverse crews exposed to variable gravity orbits (e.g., Commercial Low-orbit Destination crew).
    - Bottleneck: Paucity of facilities in which to study effects of LEO and Beyond Low Earth Orbit (BLEO) environments on health.
        - Potential Solution: Integrating LEO/BLEO parameters into existing *in silico* modeling platforms (Chowdhury & Fong, 2020).
        - Potential Solution: Use of smaller microgravity simulating reactors and devices to examine health outcomes.
- Long-term Milestone: Personalized microbiome and environmental data to reliably predict dysbiosis and low perturbation prophylactic intervention.
    - Bottleneck: Lack of data integration models and identification of appropriate microbiome biomarkers.
        - Potential Solution: Design a relevant microbiome diversity index with high-throughput sequencing.
        - Potential Solution: Use NIST Human Gut Microbiome reference material for benchmarking, assay analysis, and model verification (Jackson et al., 2024).
        - Potential Solution: Study microbiomes of humanized mouse models to better predict outcomes (NIH Human Microbiome Project, 2024; Park & Im, 2020).

Breakthrough Capability: Integrated, continuous, and autonomous sensing and monitoring of human metabolic and biochemical biomarkers.

- Short-term Milestone: Shelf-stable biosensors for detection of specific human health-related chemicals or chemical groups.
    - Bottleneck: Sensitivities of shelf-stable biosensors may not distinguish between small chemical moieties.
        - Potential Solution: Implantable biosensors within the host.
        - Potential Solution: Equipment that uses non-invasive sample collection (e.g., toilet that can measure biomarkers in urine and feces).
        - Potential Solution: Identifiable biomarkers integrated into microbiome biosensors that indicate health status.
    - Bottleneck: Activity and stability of biosensors may be limited under shelf-stable conditions.
        - Potential Solution: Hydrogels or other polymer-based media to sustain optimal conditions for activity (Li et al., 2023).

        - Potential Solution: Robust detection of biomolecules with shelf-stable lateral flow assays (Rink & Baeumner, 2023).
- Medium-term Milestone: Non-invasive, remote monitoring of gene expression and signaling states in human cells for major organs and tissue systems.
    - Bottleneck: Lack of non-invasive probes to monitor gene expression and cellular signaling in real-time.
        - Potential Solution: Enhanced imaging technologies, such as positron emission tomography (PET), near-infrared fluorescence (NIRF), and magnetic resonance imaging (MRI) for reporter genes, to visualize gene expression in major organs and tissue systems.
- Medium-term Milestone: Devices that provide point-of-care solutions from analysis of biological samples without the need for cold chain storage or processing.
    - Bottleneck: Achieving maximized sensitivity and specificity for clinical use.
        - Potential Solution: Development of biochemical assays that use synthetic biomarkers resistant to degradation in harsh environments.
- Medium-term Milestone: Real-time metabolomic sensor arrays to monitor function in real-time.
    - Bottleneck: Limited selection of metabolic signals to sample.
        - Potential Solution: Microchip arrays that can sense multiple signals and black box models to interpret them (Luo et al., 2023).
    - Bottleneck: Updated device technology and durability over time.
        - Potential Solution: Durable wearables for metabolic monitoring capable of software updates on long missions.
        - Potential Solution: Engineered synthetic biology sensors embedded into paper and textiles worn by astronauts that passively collect biochemical data (Nguyen et al., 2021).
- Medium-term Milestone: Multi-biosensor system for biochemical surveillance that does not require specialized equipment.
    - Bottleneck: Visible wavelengths for colorimetric readouts are limited.
        - Potential Solution: Machine processing of data with outputs delivered to a wearable device.
- Medium-term Milestone: Wearable or implantable biosensors to continuously monitor a range of metabolic markers, such as glucose and hormone levels.
    - Bottleneck: Current monitoring of most metabolic markers requires blood draws and *ex vivo* processing.
        - Potential Solution: Monitor saliva or urine for biomarkers indicative of abnormal organ function (e.g., ion fluctuations, urea or salt concentrations).
    - Bottleneck: Computational resources required for real-time collection and analysis directly at point-of-care.
        - Potential Solution: Pre-trained machine learning models for continuous monitoring and feedback.

- Medium-term Milestone: Specific biosensors capable of monitoring immune status of the individual.
    - Bottleneck: Complex manufacturing of biosensors for immune markers requires resources currently only found on Earth.
        - Potential Solution: Printable biomaterials based on DNA, proteins, or bioplastics to manufacture biosensors in space.
        - Potential Solution: Adaptable theranostic and diagnostic interventions to immune responses of the host.
- Long-term Milestone: Biochemical sensors that monitor physiological baselines of individuals over time to predict health concerns before clinical presentation.
    - Bottleneck: Validation of prognostic biomarkers and extensive longitudinal datasets to train AI models.
        - Potential Solution: Enable use of smaller datasets to identify and validate candidate predictive biomarkers.
    - Bottleneck: Physiological and anatomical variation between astronauts often prevents a standardized approach to correlating deviations with health concerns.
        - Potential Solution: Continuously monitor physiological states of individual astronauts prior to the space mission to create AI-generated digital twins.
- Long-term Milestone: Real-time monitoring of biological samples with *in vivo* and *ex vivo* biosensors to predict and treat disease, infection, and other health outcomes in space.
    - Bottleneck: Limited access to human samples (i.e., tissue, blood, urine).
        - Potential Solution: Urine monitoring or engineered bacteria on skin to report radioactivity response.
    - Bottleneck: Biological samples are small and difficult to obtain for complete metabolic panels to detect a wide range of metabolites.
        - Potential Solution: Use multiplexed CRISPR-based diagnostics to detect hundreds of metabolites simultaneously from a single clinical sample and report results using fluorescent or biophysical indicators (Roh et al., 2023).

Breakthrough Capability: Rapid engineering of biology-based or -enabled diagnostics and intervention decision-making.

- Short-term Milestone: High-throughput, small volume liquid handling that is robust, reproducible, and requires minimal human intervention or troubleshooting.
    - Bottleneck: Validation of fluidic behaviors and methods in microgravity environments.
        - Potential Solution: Utilization of dedicated suborbital or Low Earth Orbit (LEO) precursor flights to validate handling and production.
        - Potential Solution: Consult hardware providers that specialize in miniature technology for precision diagnostics to adapt for microgravity.
- Short-term Milestone: Miniaturization and combination of sample processing, metabolomic profiling, transcriptome and genomic sequencing, and AI/ML analysis.
    - Bottleneck: Current accuracy and reliability of portable or handheld GC-MS/LC-MS equipment hinders metabolomics.

        - Potential Solution: Continuous sequencing-based biosensing system for biomarkers of potential disease.
        - Potential Solution: Combine orthogonal biosensing systems into a single surveillance system (e.g., biomarker for inflammation, infection, etc.).
    - Bottleneck: Identification of specific metabolomic markers and integration with other metrics that are prognostic and predictive of health outcomes.
        - Potential Solution: Produce immunoassays specific for the most relevant targets.
        - Potential Solution: Create a compatible colorimetric assay to monitor fluctuation of metabolites as biomarker of microbiome dysfunction.
    - Bottleneck: One-dimensional binary analysis of current diagnostics approaches.
        - Potential Solution: Integration and algorithm-based analysis using personalized computational analysis of multiple data types that diagnose and inform prognosis for personalized inventions.
- Short-term Milestone: Detection tools for monitoring foodborne, waterborne, and airborne pathogens in the local environment (Patel, 2001; Clarridge, 2004).
    - Bottleneck: Limited diagnostics and detection methods available in space.
        - Potential Solution: Miniaturization of detection tools (i.e., LAMP amplification, miniON) for immediate sampling and pathogen identification in space.
- Short-term Milestone: Computational models of illness and injury that provide therapeutic interventions based on disease severity and time course.
        - Bottleneck: Limited understanding of therapeutic efficacy in space.
            - Potential Solution: Compare model-based treatments for specific illnesses *in vitro* and *in vivo* (e.g., rodent) models between spaceflight and ground studies.
            - Potential Solution: Compare studies conducted in microgravity and on Earth to parameterize metabolic function during different disease states and reverse vaccines to better predict outcomes in humans (Thumsi et al., 2024).
- Medium-term Milestone: Cell-based methods for detection of stress hormones in blood and tissue.
    - Bottleneck: Selection and characterization of safe and easily detectable biomarkers indicative of stress.
        - Potential Solution: Cell- or molecular-based assays that can detect stress-related proteins and enzymes in blood and tissue.
    - Bottleneck: Accurate and adequate collection of blood and tissue samples.
        - Potential Solution: Consistent time collections of blood and tissue samples to reduce influence of circadian fluctuations on the level of stress hormones.
- Medium-term Milestone: Modular or combined diagnostic biosensors to screen for multiple pathogens or biomarkers in a single test.
    - Bottleneck: Accurate and clear diagnosis of multiple outputs or output gradients.

        - Potential Solution: Regulation of biosensor signal processing and output with downstream endogenous mechanisms without the need for quantitative signal analysis.
        - Potential Solution: Microfluidic devices or ML algorithms capable of processing signals and providing clear and concise readouts.
- Medium-term Milestone: Genetically modify human cells or microbiota with feedback-control genetic circuits that activate desired immune pathways in response to specific diseases.
    - Bottleneck: Individualized medicine specific per person.
        - Potential Solution: Engineer stem cells for therapeutic intervention that can adapt to an individual's biological and immunological system to exert desired effects.
- Long-term Milestone: Wearable sensors to provide real-time indicators of health risk.
    - Bottleneck: Accuracy and sensitivity of data produced by sensors.
        - Potential Solution: Personalized wearable devices capable of measuring vital signs (e.g., heart rate, blood pressure, glucose levels).
        - Potential Solution: Integrate multi-sensor arrays that can measure multiple health parameters predictive of health risk (e.g., skin temperature, sweat composition).

Goal: Rapid response to illness or injury.

Current State-of-the-Art: Engineering biology offers the opportunity to improve rapid response to illness or injury in austere and low-resource environments like spaceflight. All therapeutics currently used in flight are produced and prepackaged on Earth. Medicines must go through prior testing and approvals before being certified for in-flight use (Blue et al., 2022). The current medical kit for the ISS includes almost only small-molecule drugs but no currently FDA-approved biologics or cell-based therapies (apart from natural antimicrobial peptides) (NASA, 2019b). Difficulties predicting demand, efficacy, and storage complicate which medications are reviewed and approved for spaceflight (Averesch et al., 2023). Many therapeutics are rejected because of issues with short shelf-life (Lutzmayer, 2023); some recent NASA studies have shown a longer shelf-life than those published for select drugs, but the same challenges with storage and demand remain (Diaz et al., 2024). The current NASA approach is to increase the stability of currently available drugs through advances in medical packaging and storage to increase the shelf-life and stability (NASA OCHMO, 2023c).

Engineering biology offers a host of solutions to address the impacts of the space environment on pharmaceutical longevity, including bio-based methods for mitigating oxidation and radiation damage. Biologics for in-flight use must be able to remain stable for long periods of time and be able to be rehydrated or (re)activated. A broad collection of biosynthetic pharmaceuticals can also be developed on Earth with an initial focus on the least stable compounds or those that require refrigeration. Biopharmaceuticals often utilize native polypeptides with rapid enzymatic and metabolic degradation, leading to significantly reduced half-lives. Lipidation techniques can further improve the stability and longevity of

biopharmaceuticals (Bech et al., 2018). Recent research has also demonstrated the long-term stability and enhanced resistance to extreme environmental conditions of material-based stabilization techniques for synthetic extremophiles (Jimenez et al., 2024). Hydrogel-encapsulated cell cultures may be used for on-demand production and long-term storage of therapeutic-producing cell lines (Johnston et al., 2020). In addition, engineered microbial consortia may be developed to act as storage systems for on-demand biosynthesis, with built-in quality control and assurance testing (Chua et al., 2024).

The range of medications needed to sustain long-term space travel is too large to prepare on Earth and would likely expire before use, so on-demand production is necessary (Chua et al., 2024). Engineering biology presents the opportunity to optimize nucleic acid, enzyme, and cell-free system platforms for use in low-resource environments, including the development of efficient methods for bacterial and eukaryotic cell-free protein synthesis and necessary co-factors, with an ultimate goal of cell-free production of ready-to-use pharmaceuticals with minimal downstream processing. The functionality of *in vitro* cell-free systems has been demonstrated on the ISS and can produce a variety of glycosylated therapeutics, including antibodies, vaccine carrier proteins, and hormones (DeWinter et al., 2023, Cai et al., 2015, Stark et al., 2021). In addition, microfluidic devices and scale-up technologies (up to 100 L) have been developed for cell-free reactions (Timm et al., 2016, Murphy et al., 2019, Cai et al., 2015, Zawada et al., 2022). Recent studies have demonstrated the stability of lyophilized cell-free reactions (up to 18 months) and cell-free systems that have undergone multiple freeze-thaw cycles (Rasor et al., 2023, Warfel et al., 2023).

Cell-based platforms can also be leveraged to offer solutions to some of these 'on-demand pharmaceutical' challenges. Biological treatments (like antimicrobials) may be produced and stored in cell factories. The identification of specific phages for bacteria and viruses that commonly cause infections during space flight would catalyze advancements in biologics therapeutics. For example, specific phages may be engineered to target pathogens that don't have natural phage predators or viral vectors may be engineered for drug/gene delivery for infection control. Efficient and effective non-intravenous delivery methods for RNA drugs (e.g., delivery via aerosolized drugs) and/or developing smart-targeting particles may be able to deliver mRNA drugs to tissues selectively. And generation of allogeneic ('off-the-shelf') human cells that can be administered to any person and show desired biological activity (e.g., CAR-T cells that target a class of cancers) will further catalyze in-flight treatments.

Blueprints for pharmaceuticals may be stored in the form of transgenic organisms (seeds/strains), purified DNA, or digital DNA sequences for on-demand synthesis. The capacity to produce and purify small amounts of therapeutics on-demand has been demonstrated through transient expression, for example, in plant-based platforms (Venkataraman et al., 2023). There is also much opportunity for advancements *in vivo*, low-cost, modular bioreactors for point-of-care production and purification of therapeutics (Perez-Pinera et al., 2016; Adiga et al., 2018). At-scale bioreactor and purification processes must be designed with consideration to environmental constraints of gravity, radiation, and containment, as well as energy, mass, and volume limitations in low-resource or otherwise constrained environments.

Engineering biology also offers a host of solutions to improve hemostasis management and wound healing in low-resource environments, through advancements in biomaterials and bioactive products for emergency medicine. Biomaterial-based solutions could include 3D-printed structures for casts, high-strength fibers such as natural and bioengineered spider-silk, and biological polyesters (Averesch et al., 2023; Mulinti et al., 2021; University of Applied Sciences Anhalt, 2020). Cellulose may also be produced and used for bandages, sutures, or slings (Odermatt et al., 2012; Utsunomia et al., 2020). Precursors for Nylons, Kevlar, and Vectran (liquid-crystal polymers) have been produced in plants and in microbes, with further applications for biomaterials production (Averesch & Rothschild, 2019). Genetically engineered protein-based adhesives could offer alternatives for wound healing (Jeon et al. 2023). Natural pro- and anti-coagulants (e.g., activated protein C) can be leveraged to treat thrombotic or bleeding emergencies from impaired blood flow or radiation (White & Wenthe, 2023; Bansal et al., 2024; Griffin et al., 2015). In the long term, synthesized treatments can be developed to instantly increase *in vivo* human production of hemostasis and coagulation proteins to recover hemostasis and wound healing.

Breakthrough Capability: Long-term stability, storage, and quality monitoring of biopharmaceuticals.

- Short-term Milestone: Low-resource stabilization (e.g., lyophilization, drying) and rehydration of biologics through iterative testing for the duration of space flight missions.
    - Bottleneck: Onboard infrastructure is insufficient for the storage and on-demand rehydration of required components.
        - Potential Solution: Modification of existing infrastructure or reprocessing of waste from other orbital processes.
        - Potential Solution: Production of biologics directly into gas or lipid environments inside cellular compartments to facilitate downstream isolation and preservation.
    - Bottleneck: Lyophilized biologics lose activity during storage.
        - Potential Solution: Identification of co-factors or cryoprotective formulations that improve stability during lyophilization.
        - Potential Solution: Include steps during sample preparation that minimize activity loss.
        - Potential Solution: Nanoparticle strategies to enhance analytical profile index storage and improve pharmacokinetic stabilization properties in space.
        - Potential Solution: Use biological by-products and excipient production to stabilize or shield existing therapeutics.
    - Bottleneck: Limited information on medicine stability and degradation rates in microgravity environments.
        - Potential Solution: Test the ability of existing stabilization methods on bio-derived medications to protect against excessive vibration and ionizing radiation.

- Medium-term Milestone: Engineer microbes or microbial consortia storage systems capable of rejuvenation in space.
    - Bottleneck: Optimal storage environments (i.e., temperature and humidity control) to prevent undesired mutations or loss of diversity in microbial consortia over time.
        - Potential Solution: Efficient freeze-drying methods or protectant systems for long-term storage at ambient temperature.
        - Potential Solution: Engineer microbes with metabolic activity controlled through rehydration or addition of a specific additive to promote desired growth.
        - Potential Solution: Engineer microbes to improve microdose storage and recovery for efficacy of starter culture.
- Medium-term Milestone: Point-of-care quality control and assurance tests to assess proper folding, glycosylation, and activity of manufactured or stored therapeutics.
    - Bottleneck: Therapeutics act through a wide variety of mechanisms and pathways that are not always conserved between hosts.
        - Potential Solution: Identify conserved mechanisms or motifs for which activity can be assessed across a variety of products.
    - Bottleneck: Glycosylation is primarily assessed via gel-based methods.
        - Potential Solution: Use mass spectrometry-based approaches or glycoproteomics to provide data on molecular mechanisms of glycans.
- Long-term Milestone: Enable storage of complex cell factory systems (e.g., human or plant tissue culture) for further on-demand biosynthesis.
    - Bottleneck: Reviving functional and living plant and mammalian cells after freeze-drying.
        - Potential Solution: Engineer antifreeze proteins into cells to protect cells from harmful effects of freezing.
- Long-term Milestone: Medical-grade responsive biomaterials to mitigate oxidative and radiative degradation of biopharmaceuticals.
    - Bottleneck: Effective degradation of bio-based encapsulations to release the stored biopharmaceutical.
        - Potential Solution: Programmable biopolymers that disassemble when needed (Odermatt et al., 2012).
    - Bottleneck: Decreased efficiency of biologics when exposed to galactic cosmic rays (GCR) and solar particle events (SPE).
        - Potential Solution: Quantitative analysis of the loss of activity and models to appropriately modify dosage to account for the drop-in activity.
- Long-term Milestone: Long-term storage of pre-synthesized DNA on solid substrate (e.g., paper strips) to enable rapid, cell-free synthesis of mRNA.
    - Bottleneck: High enough yields of DNA, mRNA, or protein from solid substrates for viable use.
        - Potential Solution: Engineer the substrate with properties that facilitate easy release of biological compounds.

Breakthrough Capability: On-demand bioproduction of small molecule pharmaceuticals.[2]

- Short-term Milestone: On-demand enzymatic semi-synthesis of prodrugs.
    - Bottleneck: Variability of the shelf-life across different active ingredients.
        - Potential Solution: Explore multi-step reaction cascades to enable biochemical conversion from stable forms of prodrugs.
- Short-term Milestone: Demonstrate on-demand synthesis of pharmaceuticals in lyophilized cell-free reactions in a single tube/pot or via purified enzymes (Korman et al., 2014).
    - Bottleneck: Components of cell-free extracts are often not recyclable.
        - Potential Solution: Engineer systems that can use *in situ* resources to regenerate energy, cofactor, and enzyme recycling.
    - Bottleneck: Unwanted side-effects of antibiotics on cellular genome editing mechanisms that alter synthesis of pharmaceuticals.
        - Potential Solution: Engineer antibiotics with mechanisms of action that target fatty acid and nutrient synthesis, the cell wall, or membrane integrity (antimicrobial peptides).
        - Potential Solution: Identify novel antibiotics with unique functions that can be manufactured biologically in cell-free systems.
        - Potential Solution: A stepwise system to separate enzyme synthesis and therapeutic synthesis to prevent unwanted interaction effects.
    - Bottleneck: Reproducibility of cell-free protocols due to unclear enzyme stability and activity timelines.
        - Potential Solution: Centralized production and packaging of modular cell-free systems.
        - Potential Solution: Extended stability tests of lyophilized cell-free reactions in a variety of environments, especially those that mimic the conditions found during space travel (Brookwell et al., 2023).
    - Bottleneck: Limited availability of high-purity and -quality reagents (amino acids, NTPs, PEP or 3PGA) for cell-free reactions in space.
        - Potential Solution: Design an efficient cell-free system able to use lower quality reagents.
    - Bottleneck: Cell-free systems are unable to perform certain complex cellular reactions due to the lack of critical membrane-bound organelles.
        - Potential Solution: Modular design of cell-free systems and genetic parts to function in cell-free or cell hosts.
    - Bottleneck: Recycling and reuse of reagents and materials in cell-free systems.
        - Potential Solution: Energy, cofactor, and enzyme recycling optimization, sustainable for more than three years.
        - Potential Solution: Engineer cell-free systems to efficiently extract critical components from cells grown on board the spacecraft (Mullin et al., 2022).

[2] Please see the Glossary for comparable definitions of “pharmaceuticals” and “biologics”.

        - Potential Solution: Additional techniques for screening for useful bacterial and plant membrane proteins in cell-free transcription-translation.
- Medium-term Milestone: Production of therapeutics using transient expression in edible plants.
    - Bottleneck: Correct dosage and bioavailability for oral administration.
        - Potential Solution: Develop high-throughput quantification methods to provide pharmacokinetic information for optimal dosage of specific plants and pharmaceuticals.
    - Bottleneck: Availability of expression systems for biologics production in edible plants.
        - Potential Solution: Develop expression technologies and engineered plants for safe consumption.
- Medium-term Milestone: Cell-free production of ready-to-use pharmaceuticals with minimal downstream processing steps.
    - Bottleneck: Immunogenicity from cell-free reactions.
        - Potential Solution: Non-immunogenic cell-free systems or simple removal of immunogenic compounds during downstream processing steps.
    - Bottleneck: Purification of therapeutics from cell-free matrix.
        - Potential Solution: Simplified, user-friendly, and modular protein purification procedures based on enzymatic cleavage, microfluidics, resin-exchange, etc.
- Medium-term Milestone: Engineer autotrophic microbes (e.g., cyanobacteria, halobacteria) or bryophytes that can withstand high radiation for the production of chemicals and pharmaceuticals (Averesch, 2021).
    - Bottleneck: High mortality rate of photosynthetic microbes in extreme environments (e.g., high radiation).
        - Potential Solution: Engineer plant compounds in organs such as the cell wall of mosses resistant to extreme environments.
- Medium-term Milestone: Regulate pharmaceutical release from organism or microbial systems with endogenous mechanisms.
    - Bottleneck: Certain microbial bioproduction pathways exist for the microbe's benefit and thus the products remain intracellular (i.e., lipids are naturally produced as mechanisms of carbon storage when faced with low nutrients (nitrogen, phosphorus).
        - Potential Solution: Determine internal signaling mechanism that initiates bioproduction and engineer control/switches or hijack signal.
    - Bottleneck: Commonly used model species (e.g., *Escherichia coli* or *Staphylococcus aureus*) have high water requirements and restrictive storage conditions.
        - Potential Solution: Leverage more robust, non-model organisms, such as spore-forming bacteria, to increase effectiveness and efficiency.
- Long-term Milestone: Cell-free production of any pharmaceutical using DNA synthesized and stored onboard the spacecraft.

    - Bottleneck: A limited number of DNA base pairs can be synthesized consecutively
        - Potential Solution: Harvest gene blocks from cell-based microsystems.
    - Bottleneck: Oligonucleotide production requires large volumes of unsustainable solvents that don't make sense in compact environments (Andrews et al., 2020).
        - Potential Solution: Cell-based production and purification of short oligos for sequence amplification and purification of gene blocks (Elbaz et al., 2016).
        - Potential Solution: One-pot cloning techniques in the pipeline.
    - Bottleneck: Drugs that require raw, organic materials, post translational modifications, and purification for functional and safe use.
        - Potential Solution: Recycling associated with environmental control and life support systems that can break down organic compounds to raw, organic materials.
        - Potential Solution: Pair multiple production platforms in a modular manner to enable production, post-translational modification, and purification of desired therapeutics.
- Long-term Milestone: Library of genetic constructs for on-demand production of most common small-molecules within appropriate chassis.
    - Bottleneck: *De novo* DNA or RNA synthesis is resource intensive.
        - Potential Solution: Develop phages to scale up and deliver nucleic acid cargo to produce DNA, RNA, or protein biologics by a microbial chassis.
    - Bottleneck: Nominal activity of higher organism derived enzymes for catalysis of complex metabolic pathways in microbes.
        - Potential Solution: Leverage developments in predictive structure determination to engineer enzymes with desired catalytic activity.
    - Bottleneck: Multiple chassis production organisms require specialized equipment to produce and purify drugs.
        - Potential Solution: Leverage export of microbial pathways to harvest drugs from supernatants of active culture.
    - Bottleneck: Limited number of resources are available aboard the spacecraft for drug purification.
        - Potential Solution: Develop non-toxic production hosts that produce sufficient titer to minimize purification needs.
- Long-term Milestone: Engineer heterologous production strains for major classes of small molecule therapeutics (antibacterial, antifungal, anti-inflammatory agents, etc.) that can be kept as lyophilic or freezer stocks in minute quantities for space flight applications.
    - Bottleneck: Limited storage available onboard the space station.
        - Potential Solution: Design highly concentrated, lyophilized stocks of small molecule therapeutics that can be diluted further upon use.

Breakthrough Capability: On-demand production of biologics.[3]

- Short-term Milestone: Target human cells or tissues for genetic manipulation to enable *in vivo* pathways for biologics production and therapeutic proteins.
    - Bottleneck: Genetic manipulation is more difficult in some human cells or tissues compared to others.
        - Potential Solution: Screen human cells to determine specific phenotypes that influence the capability of genetic manipulation.
    - Bottleneck: Many tissues are not easily accessed with non-invasive delivery methods (e.g., brain, marrow, etc.).
        - Potential Solution: Aerosol delivery to the lungs with trafficking of delivered virus to the tissue of interest or expression of tagged proteins in epithelial lung cells that are then transported to different tissues.
        - Potential Solution: Use of implantable scaffolds to deliver drugs (Kobsa & Saltzman, 2008, Aurand et al., 2012).
- Medium-term Milestone: Robust control of infection with viral vectors optimized for microgravity environments for drug and gene delivery in space.
    - Bottleneck: Generation of neutralizing antibodies against the delivery vehicle containing the drug in the human host.
        - Potential Solution: Deliver the drug through a series of modular phage or viral vectors to avoid adaptive immune effects of the host.
        - Potential Solution: Rapid, lateral-flow based assays for neutralizing antibodies to determine appropriate vehicle for use.
        - Potential Solution: Customizable protein coronas that can sheath vehicles to hide epitopes that would be recognized by host antibodies (Mahmoud et al., 2023; Oh et al., 2018).
    - Bottleneck: Rapid, single-step construction of stable viral vectors in space.
        - Potential Solution: Cell-free production of viral particles that can be produced in basic environments.
    - Bottleneck: Persistence and stability of viral vectors in space habitat conditions.
        - Potential Solution: Dose-time dependent testing of stability of viral particles in space-like conditions.
        - Potential Solution: Engineer viral vectors that remain stable and functional when lyophilized and reconstituted.
- Medium-term Milestone: Protein-based biopharmaceuticals in viral vectors in space (Petry et al., 2008; Sychla et al., 2024).
    - Bottleneck: Large proteins can't be packaged efficiently in a single viral particle.
        - Potential Solution: Encode large proteins as split-inteins.
    - Bottleneck: Serotype of viral vector could influence repeat-dosing or pre-existing immunity on a patient-by-patient basis.
        - Potential Solution: Characterize the antibody production capabilities of astronauts to determine pre-existing immunities.
    - Bottleneck: Varying expression levels of different protein biologics.

[3] Please see the Glossary for comparable definitions of "pharmaceuticals" and "biologics".

        - Potential Solution: Combine multi-parallel reporter screens and machine learning to determine gene expression levels of virus-encoded proteins.
- Medium-term Milestone: Microbes or plants able to produce common classes of shelf-stable biologics, such as antimicrobials.
    - Bottleneck: Many biologics require refrigeration for long-term storage.
        - Potential Solution: Engineer antimicrobial proteins and other biologicals encapsulated within protective microbial or plant polysaccharides (e.g., yeast capsules or plant seeds) to allow for storage at ambient temperatures.
        - Potential Solution: Produce antimicrobial peptides in plant plastids, possibility to produce antimicrobial peptides on demand with the advantage to be stored in a plant (Hoelscher et al., 2022).
- Medium-term Milestone: Allogeneic human cells with desired biological activity in any host (e.g., CAR-T cells that target a class of cancers).
    - Bottleneck: Long-term storage of the cell cultures from astronauts in space.
        - Potential Solution: Develop capabilities for room temperature storage of human cells and biologics.
    - Bottleneck: Introducing the correct genetic modifications to engineered cells to perform desired biological activities.
        - Potential Solution: Use systems modeling and AI/ML to predict the necessary genetic modifications for the desired biological activity.
        - Potential Solution: Strategic genome-scale perturbation experiments to identify genetic modifications that provide desired biological activities.
    - Bottleneck: Monitoring and analysis of allogeneic cell safety.
        - Potential Solution: Basic tests in humanized mice and clinical trials.
    - Bottleneck: Injury repair and lack of regenerative medical care.
        - Potential Solution: Allogeneic or autologous stem cell induction to repair damaged tissue or organ.
        - Potential Solution: Engineer harvested cells to revert back to stem or pluripotent cells for self-repair.
- Medium-term Milestone: Selectable system for transient skin colonization by an engineerable skin microbiome to combat infection or injury.
    - Bottleneck: Safe and efficacious technology to modify skin microbiome.
        - Potential Solution: Evaluate skin colonies *in vitro* and with animal models before testing in humans.
- Medium-term Milestone: Selectable system for transient colonization of internal human microbiomes (GI tract, lung, vaginal, etc.) to address illness or infection.
    - Bottleneck: Ensuring transient colonization without permanent changes to the microbiome.
        - Potential Solution: Engineer conditional circuits in microbial strains that rely on the supplement of a specific prebiotic.
    - Bottleneck: Treatment of illness or delivery of infection to specific organs or tissues with minimal systemic exposure.

        - Potential Solution: Engineer organ-specific expression systems, based on physiological cues or biomarkers, that can be activated only at the designated organs.
        - Potential Solution: Engineer microbes with biocontainment kill-switches that are tissue-selective with apoptotic mechanisms activated in alternative tissue or organ types.
- Medium-term Milestone: DNA templates for production of all biological World Health Organization Essential Medicines relevant for space travel.
    - Bottleneck: Limited materials and storage for engineered strains or DNA templates in space.
        - Potential Solution: Lyophilize microbial strains or utilize spore forming strains that can be stored long-term and revived.
        - Potential Solution: Leverage portable DNA synthesis machinery to produce DNA templates.
- Long-term Milestone: Bacterial, living therapeutics capable of transient medicine production and essential nutrients in the human body.
    - Bottleneck: Harmful conditions from unwanted colonization of bacteria in closed systems.
        - Potential Solution: Engineer quorum-sensing system to program colonization circuits in bacteria.
        - Potential Solution: Use minimized or synthetic genome organisms capable of close-loop programming.
        - Potential Solution: Implement induced kill-switches for all supplemented microbes.
    - Bottleneck: Delivery of sufficient amounts of bacteria without negatively impacting native gut flora.
        - Potential Solution: Better understanding of factors in bacterial colonization and their interaction with gut microbiome.
- Long-term Milestone: Adoptive T-Cell therapies to improve wound healing (Adusei et al., 2021; Baker et al., 2023).
    - Bottleneck: Limited understanding of tissue and immune T-cell interactions.
        - Potential Solution: Perform *in vivo* and *in vitro* studies to examine T-cell healing capabilities with various tissue types.
    - Bottleneck: Selectivity of T-cell therapies to specific tissue types.
        - Potential Solution: Engineer stable T-cells modulated to heal target tissue types.
- Long-term Milestone: Transient viral delivery systems that are adaptable to various conditions for the host (i.e., the duration of therapeutic delivery, titers of therapeutic produced, side-effects in host cells, etc.).
    - Bottleneck: Viruses that produce unwanted off-target or downstream effects in host cells.
        - Potential Solution: Engineer tissue-specific viruses that can induce desired therapeutic outcome with minimal side effects.

- Long-term Milestone: Strain-specific phage chassis to target bacteria that commonly cause infections during space flight.
    - Bottleneck: Unknown strain-specific phages for some bacterial species.
        - Potential Solution: Deeper metagenomics or ML/AI processing to understand the bacterial milieu.
        - Potential Solution: Engineer phage specificity for bacterial species (Jia et al., 2023).
    - Bottleneck: Protection against accidental targeting from horizontal gene transfer or homologous recombination events of phage.
        - Potential Solution: Engineer phage chassis with orthogonal, non-natural component to the specification protocol.
- Long-term Milestone: Selective delivery of mRNA drugs to tissues with smart-targeting particles.
    - Bottleneck: Non-specific control of surface functionalization (i.e., low control over ligand density, stoichiometry, patterning of multifunctional surfaces) of current mRNA delivery platforms, such as lipid nanoparticles (Maeki et al., 2022).
        - Potential Solution: Leverage evolved bacteria, viruses, and phage as active drug delivery vehicles capable of selectively targeting specific cell types and tissues.
        - Potential Solution: Engineer bacteria, viruses, or phage with ligands to mediate target recognition and uptake.
        - Potential Solution: Control phage:virus aspect ratio to tune systemic biodistribution within the body for enhanced tissue selectivity (Shukla et al., 2015).
- Long-term Milestone: On-demand production of therapeutics within 24-hours in plant hosts.
    - Bottleneck: Purification of plant-derived molecules is challenging in space habitat.
        - Potential Solution: Engineer edible plants or part of plants for biomolecule production to avoid downstream purification challenges.
    - Bottleneck: Current expression and purification processes take about 10 days once the desired growth stage is reached.
        - Potential Solution: Engineer plants for increased productivity of therapeutic.
        - Potential Solution: Eliminate the requirement for Agrobacterium infiltration.
        - Potential Solution: Engineer a chromosome to carry the genes needed to produce specific pharmaceuticals under induction conditions.

Breakthrough Capability: Cross-cutting and enabling technologies for on-demand bioproduction of pharmaceuticals and biologics.

- Short-term Milestone: Portable DNA synthesis machines to enable Earth-to-vehicle/habitat communicated synthesis.
    - Bottleneck: Miniaturized enzymatic synthesizer not publicly available.

        - Potential Solution: DNA biobrick libraries in orbit for on-demand production.
        - Potential Solution: Develop a phage-dependent approach capable of transfecting production strains of interest for overexpression of genes and pathways.
        - Potential Solution: Synthesis in cell-free systems to eliminate the need for strain engineering and cloning.
- Short-term Milestone: Drop-in, freeze-dried replacements of standard biochemical and biomolecular components (e.g., macromolecular sensors, thermo-stable enzymes) to enable room-temperature biological sample processing.
    - Bottleneck: Cold-chain requirement for sample processing workflows including genetic sequencing and processing blood samples.
        - Potential Solution: Lyophilize components to allow for better stabilization at ambient temperatures.
- Short-term Milestone: Microfluidic and nanofluidic bioreactors that are functional in microgravity.
    - Bottleneck: Chromatographic separation and dose dispensing of on-demand pharmaceuticals.
        - Potential Solution: Automated and configurable end-to-end microbial bioreactor with purification systems that are not product specific (i.e., enable affinity purification, tag cleavage).
    - Bottleneck: High resource requirements of chromatographic separations.
        - Potential Solution: Adapt self-cleaving inteins to function on triggers outside of solution conditions.
- Short-term Milestone: Categorized common spaceflight ailments and associated treatments.
    - Bottleneck: Lack of access to space health and medicine data.
        - Potential Solution: Develop databases (similar to NASA GeneLab) containing information from current spaceflight health outcomes and treatments.
        - Potential Solution: Health data and biometrics combined with post-mission medical follow up for ML-guided prediction of ailments.
- Short-term Milestone: Characterization of the adverse effects of microgravity on host cells utilized for biologic production.
    - Bottleneck: Lack of access to microgravity and reproducible methods to induce and characterize biologic production.
        - Potential Solution: Use AI/ML to analyze biological data from various hosts in microgravity environments to improve performance predictability.
- Medium-term Milestone: Recycling of excess biopharmaceuticals excreted in waste streams (e.g., insulin in urine).
    - Bottleneck: Potentially toxic reagents excreted into waste streams from bioproduction processes.
        - Potential Solution: Microbes that sequester or degrade toxic wastes leaving behind a more processable stream for recycling.

- Long-term Milestone: Reuse of source materials and reagents (NTPs, dNTPs, cofactors, etc.) from bioproduction processes in space/low-resource settings.
    - Bottleneck: Complicated purification of small molecules may limit isolation of reagents from reactions.
        - Potential Solution: Engineer biological production platforms capable of taking in undefined or toxic feedstocks.
        - Potential Solution: *In vitro* or *in vivo* platforms capable of regenerating key cofactors and reagents without the need for external supplementation of pure components.
    - Bottleneck: Finite supply of element sources (e.g., carbon, nitrogen) in biologically accessible formats.
        - Potential Solution: Engineer microbes capable of extracting key element sources from lunar or Mars regolith.

Breakthrough Capability: Advanced hemostasis management and wound healing.

- Short-term Milestone: Use of natural pro- and anti-coagulants (i.e., activated protein C, (Griffin et al., 2015)) to treat thrombotic or bleeding emergencies from impaired blood flow or radiation (White & Wenthe, 2023; Bansal et al., 2024).
    - Bottleneck: Controlled delivery that ensures hemostasis.
        - Potential Solution: Tissue- or cell-specific treatment to target affected areas.
- Short-term Milestone: Successful whole blood transfusions during spaceflight using transfusable artificial oxygen carriers or engineered blood product substitutes to replace blood products (Cap et al., 2021).
    - Bottleneck: Individuals have different blood types and will require different blood product substitutes to prevent unwanted clotting.
        - Potential Solution: Blood product substitute that could be universal for all blood types.
- Short-term Milestone: Biomanufacturing of natural bioactive products effective for wound dressing and healing, such as cell-free or microbial production of synthetic antibacterial minerals to combat topical infections (Morrison et al., 2024).
    - Bottleneck: Lack of data on bioactive products and their effectiveness for wound dressing and healing that are possible to produce via bioengineered processes.
        - Potential Solution: Screen library of bioactive products or protein therapeutics, such as fibrin or fibrin-activating fibers, in mouse or organoid model, to determine impact on coagulation and wound healing.
- Short-term Milestone: Engineered plants to produce effective bioactive products for wound dressing and healing (Deng et al., 2022).
    - Bottleneck: Plant-based expression requires downstream isolation and purification of the bioactive products, preventing rapid response to wound dressing.
        - Potential Solution: Engineer leaves or other partial plant organs that produce medicinal products to be applied directly to wounds.

- Medium-term Milestone: Biomaterials and hydrogels for optimal production and material adaptation in spaceflight scenarios, to create a pipeline of first-aid and surgical supplies.
    - Bottleneck: Fine control and tailoring of hydrogel structure and behavior.
        - Potential Solution: Increased tunability and simplified adaptability of medical polymers.
- Medium-term Milestone: Engineered liver cells to create blood proteins or blood-like fluid on-demand.
    - Bottleneck: Functional liver cells that can survive and function in a physiological relevant environment.
        - Potential Solution: Lab-on-a-chip testing with liver tissue cells in a sustained environment similar to the human liver.
- Long-term Milestone: Ability to grow full or partial human skin from stem cells to treat severe skin damage.
    - Bottleneck: Stem cell differentiation and scaffolding is complex and slow.
        - Potential Solution: Consortia capable of producing artificial extracellular matrices or other scaffolds to enhance proliferation and growth of skin cells.
- Long-term Milestone: Synthesized treatments to instantly adapt and enhance human production of hemostasis and coagulation proteins to recover hemostasis and wound healing.
    - Bottleneck: *In vivo* production and delivery of coagulation proteins.
        - Potential Solution: Engineer scaffolding matrices or mRNA therapies to enhance production of clotting proteins (Cano-Garridoet al., 2022).

Goal: Engineering biology for long-term maintenance of health.

Current State-of-the-Art: Maintaining human health and well-being during long-term space exploration will require maintaining the many tissues, organs, and systems of the human body with very few resources while countering the negative effects of being away from Earth. Engineering biology can help to maintain human microbiome systems, defend tissues against radiation and the harmful effects of microgravity and confined spaces, and help to sustain disease prevention and mitigation.

Recent advancements in living therapeutics research and the approval of various medications has enabled successful gut microbiome modulation solutions, including those from Vowst, Rebyota, and ZBiotics (Zhu et al., 2023; Cubillos-Ruiz et al., 2022; FDA, 2023; Ferring Pharmaceuticals, 2022; ZBiotics, 2020). *In situ* engineering of the human microbiota in their native systems (e.g., respiratory and gastrointestinal tracts) may be utilized to detect and treat dysbiosis. Engineered probiotics and phages could be used “as needed” to engineer the human microbiome to accomplish dynamic human physiological responses to health and infection. Monitoring of the gut microbiome may also be used to predict treatment outcomes (Lee et al., 2022). Baseline parameters must be established for human microbiome structure and diversity during spaceflight conditions to better understand the composition, stability, and evolution of the human microbiome in space conditions.

Cellular-level radiation damage and genetic mutation pose significant risks to long-term health outcomes, especially increased cancer risk. Engineering biology offers a variety of solutions to protect against radiation damage, including the on-demand production of radiation protection reagents, such as antioxidants like melanin or resveratrol, G-CSF (granulocyte colony-stimulating factor), and entolimod (toll-like receptor 5 agonist). Biomaterials derived from radiotrophic fungi or selenomelanin analogs may be used to shield against certain forms of radiation. Eventually, improved human repair genes may be directly delivered and implemented into a human host to improve DNA damaged by increased radiation (Beheshti et al., 2021; Sakama et al., 2021).

Spaceflight causes changes in the mineral composition of the human body, including decreased calcium absorption and altered phosphorus levels. Changes in the absorption and excretion of magnesium, iron, and zinc also occur and hormone imbalances, including testosterone, estrogen, are common. Reduced sun exposure often results in decreased vitamin D levels (Dakkumadugula et al., 2023). Crewmembers aboard the ISS currently maintain a highly regulated diet and strict exercise regime to reduce the impacts of the space environment on the loss of bone and muscle density. Efforts to improve in-flight bone density include the identification of bioprocesses and key enzymes that are responsible for bone density maintenance, as well as potential drugs to mitigate osteoporosis and protect bone density that are also good candidates for biomanufacturing (Yan et al., 2023). The use of bioengineered materials and living biologic therapeutics may also help to prevent bone and muscle loss (Chen et al., 2020).

Cell-based theranostics (i.e., integrated diagnostics and therapeutics) can be developed for sustained, precision disease prevention, with applications in a wide variety of disease treatments, including cancer, microbiome dysbiosis, autoimmune disease, and metabolic disease (McNerney et al., 2021). Cell-based theranostics represent the next generation of cell engineering in order to monitor and respond to the local disease state in real time, creating a “smart” approach to treatment. Much of this work is quite nascent, but is ideal for research and implementation for astronauts in space.

Maintenance of psycho-social well-being and performance is just as important as physical health indicators. Engineering biology offers solutions to achieve this, including the production of neuromodulatory molecules and therapeutics for use in the gut (Lynch & Hsiao, 2023; Gibbons et al., 2021). Plant and fungal antipsychotic compounds may also be produced for use (Meade et al., 2022). The social and ethical considerations of this research should be meaningfully incorporated and included at early stages of research. Research should be cross-disciplinary and researchers should be trained to consider this from the start.

Breakthrough Capability: Maintenance of sufficiently diverse and stable human microbiomes.

- Short-term Milestone: Genetic toolkits and metabolic models for dominant commensal taxa of human microbiota (e.g., *Escherichia coli*, *Enterococcus* spp., *Bacteroides* spp., *Cutibacterium*) (Dukovski et al., 2021; Zampieri et al., 2023; García-Jiménez et al., 2021).
    - Bottleneck: Selective genetic programming of specific microbes in a multi-species community.

        - Potential Solution: Develop host-agnostic genetic engineering workflows that retain specificity toward the designated strain (e.g., RNA-guided CRISPR systems).
        - Potential Solution: Bypass restriction-modification systems of each microbe by prior modification of the DNA used for genetic manipulation (Riley et al., 2023).
        - Potential Solution: Incorporate additional selection strategies based on the distinct bacterial cell membrane structure.
    - Bottleneck: Difficult to build accurate models from microbes (or metagenomes) with unknown function (Vanni et al., 2022).
        - Potential Solution: New functional genomics approaches for rapidly characterizing genes of unknown function.
- Short-term Milestone: High-throughput screening of important microbiota gene targets with functional genomics technologies, including CRISPR libraries and transposons.
    - Bottleneck: Delivery of CRISPR systems into non-model organisms can be challenging.
        - Potential Solution: Host-agnostic CRISPR screening platform for important genes (e.g., mobile-CRISPRi system) (Banta et al., 2020).
- Short-term Milestone: Established baselines for the composition, stability and evolution of the human microbiome (i.e., gut, skin, etc.) in space conditions (Mortazavi et al., 2023; Tesei et al., 2022).
    - Bottleneck: Collection and storage of microbiome samples during space flights.
        - Potential Solution: A space-flight compatible microbiome analysis platform based on novel sequencing technology, e.g., nanopore sequencing.
        - Potential Solution: Reference materials and calibrants for benchmarking and validation of changes in microbial composition (e.g., Jackson et al., 2024).
        - Potential Solution: Study effects of space-specific stressors (e.g., microgravity, galactic cosmic radiation, etc.) that potentially select for a simplified gut community.
    - Bottleneck: Variance in microbiome influenced by food and nutrients consumed by astronauts.
        - Potential Solution: Establish a reference database with microbiome data from multiple astronauts with different diets.
        - Potential Solution: Study microbiome changes and dynamics from space food consumption.
        - Potential Solution: Produce probiotic or therapeutic Kombucha, yogurt, kimchi, and other fermented foods in space.
- Medium-term Milestone: Robust methods for engraftment and maintenance of persistent community levels of engineered microbes in diverse human environments.
    - Bottleneck: Undesired or unexpected mutations in native microbes from the environment, infection, or other perturbations.

        - Potential Solution: Create probiotic regimens for reestablishing a healthy microbiome across all major microbiome sites.
        - Potential Solution: An engineering platform for *in vivo* microbiome editing to introduce healthy traits back into the microbiome.
- Medium-term Milestone: Biological reporters to quantify and detect abnormal fluctuations in the microbiome communities (Stirling et al., 2020).
    - Bottleneck: Certain locations in the body are more difficult to obtain a signal from biosensors.
        - Potential Solution: Utilize persistent biosensors (i.e., pigment production or luminescence) that can be easily detected in saliva, sweat, or urine samples.
        - Potential Solution: Non-invasive reporters (e.g., magnetic proteins, gas vesicles) that do not require sampling (Mimee et al., 2018, Hurt et al., 2023).
    - Bottleneck: Many metabolites of interest do not have associated regulators.
        - Potential Solution: *De novo* design or directed evolution of transcription factors or riboswitches.
        - Potential Solution: Large-scale bioprospecting to identify response regulators.
- Long-term Milestone: Human microbiota communities synthesized *in situ* to promote astronaut health.
    - Bottleneck: The health impact of specific microbiome compositions remains unclear.
        - Potential Solution: Monitor the human gut or skin microbiome (e.g.,16s MinION profiling) and correlate with key health indicators, including caloric expenditure, bone density, radiation exposure, etc.
    - Bottleneck: Real-time monitoring of microbiome composition.
        - Potential Solution: Identify metabolic biomarkers with AI/ML for easy sampling from human wastes, saliva, and urine.
        - Potential Solution: Develop probiotic bacteria to monitor and respond to undesired changes in the microbiome or general health status.
    - Bottleneck: Delivery systems must be host specific and host selective.
        - Potential Solution: Engineer co-opted lysogenic phage with host specificity.
        - Potential Solution: Genus-specific modification of common probiotics to reduce host selectivity.
- Long-term Milestone: Engineered microbes to deliver specific and on-demand treatments to dysbiosis.
    - Bottleneck: Supplying microbes to the host could result in dysbiosis of certain commensal microbes due to competition at a microbial community level.
        - Potential Solution: Incorporate necessary members to the delivered consortia to enable successful recovery of commensals or provide suitable replacement variants.

    - Bottleneck: Limited understanding of the impact of microbial therapeutics on long-term gut health on a personalized basis.
        - Potential Solution: Engineer probiotics to sense and rapidly respond to disease markers in the gut or skin environments.
- Long-term Milestone: Phage that optimize human microbiome to accomplish dynamic human physiological responses to disease, infection, and other health outcomes.
    - Bottleneck: Production of unwanted bacteria by phages that are not cleared by the human host.
        - Potential Solution: Design non-lysogenic phage to target potentially problematic taxa.
        - Potential Solution: Engineer phage lysogeny, reactivation and self-destruction circuits dependent on quorum sensing or number of replication cycles.
    - Bottleneck: Selective delivery of specific genetic or metabolic programming to a specific microbe in the community.
        - Potential Solution: Engineer phage from natural lysogens to deliver a genetic program.

Breakthrough Capability: Prevention and treatment of long-term, cellular-level radiation damage and genetic mutation.

- Short-term Milestone: Defined baseline genetic parameters to track mutations.
    - Bottleneck: Limited studies of microbial mutagenesis and response in relevant microgravity conditions.
        - Potential Solution: Conduct spaceflight experiments to examine real-time or continuous genetic mutations caused by radiation.
- Short-term Milestone: On-demand production of radiation protection reagents (e.g., melanin, granulocyte colony-stimulating factor, entolimod, or resveratrol) as antioxidants.
    - Bottleneck: Long-term production of protection reagents with a biomanufacturing system in space.
        - Potential Solution: Design radiation protection reagents based on a robust commensal microbial strain capable of colonizing human skin to ensure long-term effectiveness.
        - Potential Solution: Transient production (agrobacterium-, viral-, biolistic-, nanoparticle- mediated delivery) of compounds in plant systems for on-demand use (Sukenik et al., 2018).
        - Potential Solution: Develop biosynthesis of amifostine or other radiochelators with microbial chassis during spaceflight (Singh & Seed, 2019).
        - Potential Solution: Engineer microbiota expressing Tardigrade gene proteins that upregulate specific DNA repair mechanisms in response to radiation (Clark-Hachtel et al., 2024; Zimmer, 2024).
- Short-term Milestone: Radiotrophic fungi or selenomelanin analogs constructed or embedded into biomaterials to protect against radiation (Cao et al., 2020).
    - Bottleneck: Proper biocontainment solutions for radiotrophic fungi.

        - Potential Solution: Genetic kill switches that activate should the fungi escape the material or desired biosystem.
    - Bottleneck: Biomanufacturing of high radiation protection-per-mass molecules while maintaining efficacy.
        - Potential Solution: Engineer radiotrophic fungi with enhanced metabolic efficiency.
        - Potential Solution: Upregulate genes in selenomelanin or selenomelanin analogues to increase production without increasing biomass.
- Medium-term Milestone: Efficient human DNA repair genes to repair the genome from radiation damage.
    - Bottleneck: DNA repair genes must respond to radiation.
        - Potential Solution: Engineer Tardigrade or *Ataxia Telangiectasia* genes to introduce damage suppressor proteins that protect DNA from radiation (Clark-Hachtel et al., 2024).
- Long-term Milestone: Effective delivery of improved human repair genes and implementation into human host.
    - Bottleneck: Successful manipulation of somatic cells in tissue and organ systems are difficult to manipulate (Haasteren et al., 2020).
        - Potential Solution: Engineer human germline cells to improve delivery vectors (e.g., adeno-associated viruses) (Haasteren et al., 2020; Saha et al., 2021).
    - Bottleneck: Genotoxicity and immunologic response to gene-editing technologies and delivery vectors.
        - Potential Solution: Administer immuno-suppressants when implementing gene-editing tools.
        - Potential Solution: Utilize non-biological delivery methods, such as gold nanoparticles, to mitigate immunological response from the host.

Breakthrough Capability: Prevention and reversal of bone density loss.

- Short-term Milestone: Produce drugs that can protect bone density in host (micro)organisms compatible with spaceflight.
    - Bottleneck: *In silico* modeling platforms can require expert programming knowledge of data that may not exist because of space context.
        - Potential Solution: Test biological systems for effective production of candidate drugs (e.g., for osteoporosis) in space (Xiong et al., 2022; Asclepios, 2024).
- Short-term Milestone: Identification of key enzymes and bioprocesses associated with bone density maintenance that are downregulated or damaged in microgravity.
    - Bottleneck: Lack of relevant biological samples from astronauts during space flight to screen for bone density-related enzymes.
        - Potential Solution: Identification of bone mass-related biomarkers and physiological conditions that can be screened via urine, saliva, blood, or other relatively non-invasive methodologies.

- Short-term Milestone: Living biologic therapeutics to prevent bone loss as an alternative to daily vigorous exercise (Mulinti et al., 2021; Seely et al., 2021).
  - Bottleneck: Limited known compounds and biosynthetic pathways with bone loss prevention activity.
    - Potential Solution: Identify candidate therapeutics capable of preventing bone loss from other medical studies irrelevant to space flight.
    - Potential Solution: Utilize AI/ML models to suggest suitable biosynthetic pathways for drug candidates of bone loss prevention.
  - Bottleneck: Delivery of therapeutic or restorative proteins and biomolecules to bones.
    - Potential Solution: Targeted microbiome engineering for micro- and macronutrient production *in situ*.
    - Potential Solution: Gut or skin microbiome that release therapeutic compounds in response to bone loss.

Medium-term Milestone: Optimized enzymes engineered to improve biomineralization.

- Bottleneck: Indirect bioprocesses cannot reverse the bone density loss caused by lack of muscular and skeletal resistance in microgravity.
  - Potential Solution: Improved monitoring of diet and nutrient intake to remove nutrient deficiency and bioprocesses from contributing factors.
  - Potential Solution: Develop directly endogenous or exogenous active modalities to enhance bioprocesses.

- Long-term Milestone: Inducible capacity to mitigate osteoporosis without harmful side effects (such as continued bone density increases above baseline, particularly when subjects return to Earth gravity).
  - Bottleneck: Direct enhancement of bioprocesses for osteoblast recruitment, osteoclast aversion, and biomineralization.
    - Potential Solution: Bone-specific expression or delivery of osteoblast promoting or osteoclast inhibiting factors (e.g., RANKL activates osteoclasts).

Breakthrough Capability: Prevention and reversal of muscle loss.

- Short-term Solution: Neuronal activation of muscles during sleep with muscle stimulator or therapeutic that activates lower motor neurons or their receptors in muscle tissue.
  - Bottleneck: Identification of physical or genetic targets for neuronal activation in muscle tissue.
    - Potential Solution: Genomic and proteomic studies to identify targets and inform strategies for modification of neuronal motor circuits.
    - Potential Solution: Targeted activation of acetylcholine receptors in neuromuscular junction (e.g., Nicotinic Acetylcholine Receptor agonists).
- Medium-term Milestone: Personalized diet (e.g., amino acid profiles, hormone supplements) to support muscle deposition without adverse complications.

    - Bottleneck: Human metabolism and muscle deposition is complex and involves many interacting variables.
        - Potential Solution: Engineer personalized supplements based on metabolic needs of the individual.
- Long-term Milestone: Molecular simulation of the biochemical response to exercise in muscle tissue with biologics or biologically produced pharmaceuticals.
    - Bottleneck: Satisfying specific protein and nutrition requirements needed to maintain muscle mass.
        - Potential Solution: Inducible overexpression of a suite of synthetic muscle growth proteins in nutritional sources to meet specific metabolic requirements of each individual.
- Long-term Milestone: Muscle-targeted expression or delivery of myosin, actin, or other activating molecules.
    - Bottleneck: Targeted expression and delivery to skeletal and cardiac muscle tissue, without influencing other tissue and organ systems.
        - Potential Solution: Tissue-specific delivery of biomolecules that enhance muscle growth (Kobsa & Saltzman, 2008).
        - Potential Solution: Engineer enzymes that inhibit Adenosine Triphosphate (ATP)-dependent proteases responsible for disuse atrophy.

Breakthrough Capability: Robust, cell-based theranostics (integrated diagnostics and therapeutics) for sustained, precision disease prevention.

- Short-term Milestone: *In silico* techniques and open-source platforms to design and optimize precision medicine and live biologic therapeutics.
    - Bottleneck: Sufficient data collection to parameterize personalized models.
        - Potential Solution: Open source, free access platforms for submission of (depersonalized, HIPPA compliant) individual subject data.
    - Bottleneck: Current computational modeling software for applications in drug discovery does not incorporate complex membrane structures for cell-based therapeutics.
        - Potential Solution: Expand current modeling software to allow for larger protein structure modeling.
    - Bottleneck: Machines with high-processing capabilities and high-resolution graphics are required to run useful software applications (e.g., Molecular Dynamics, GROMACs or NAMD simulations).
        - Potential Solution: Develop a ML algorithm to analyze and overlap the trajectories and molecular dynamics of different components of the whole-cell based device to reduce atom per simulation.
- Short-term Milestone: Suitable host chassis for human cells or microbiota to implement living therapeutics.
    - Bottleneck: Limited genetically tractable hosts for engineering sensing and therapeutic production capabilities.
        - Potential Solution: Develop genetic toolkits for screening and modulation of individual members within the gut microbiome.

        - Potential Solution: Engineer food-associated or known probiotic strains to ensure transient colonization and defined therapy duration.
    - Bottleneck: Instability of engineered living therapeutics in microgravity and high radiation environments.
        - Potential Solution: Engineer biocontainment strategies (i.e., kill switches) to prevent mutation of genetic programs.
        - Potential Solution: Genetic engineering technologies to enhance stability and resilience of biologics in microgravity.
        - Potential Solution: Integrate additional DNA repair systems from tolerant microbes to prevent mutations from extreme and continuous radiation exposure (Puig et al., 2021).
- Medium-term Milestone: Human or human microbiota chassis that can sense and respond with therapeutics release to a range of relevant environmental conditions at clinically relevant concentrations.
    - Bottleneck: Lack of biosensing data across various growth stages, population, and environmental conditions.
        - Potential Solution: Research to build a database of chassis at different environmental conditions and concentrations.
- Medium-term Milestone: Microbial living therapeutics capable of transient medicine production and essential nutrients in the human body.
    - Bottleneck: Susceptibility of microbial communities to invasion by foreign microbes.
        - Potential Solution: Map potential environmental microbes and interactions with engineered microbial consortia.
        - Potential Solution: Use minimized or synthetic genome organisms capable of close-loop programming.
        - Potential Solution: Engineering robust kill switches (Cas9, synchronized lysis circuits, quorum-sensing) in bacteria (Chan et al., 2016; Din et al., 2016).
    - Bottleneck: Underdeveloped strategies to mitigate diseases caused by possible mutations of engineered microbes from spaceflight and radiation
        - Potential Solution: Incorporate genetic circuits and kill switches to sense and eliminate the mutated microbes.
- Long-term Milestone: Remote user control of behavior and therapeutic actions of engineered cells.
    - Bottleneck: Current technology for fully-remote control of cellular behavior is extremely limited.
        - Potential Solution: Implement laboratory techniques for cellular control (e.g., optogenetics, sonogenetics, and magnetogenetics) into clinical and space settings.

Breakthrough Capability: Engineering biology opportunities to promote long-term psycho-social well-being and performance.

- Short-term Milestone: Production of neuromodulatory molecules in gut microbiome members at clinically relevant concentrations.
    - Bottleneck: Identify suitable gut microbiome members for genetic engineering and pathway implementation.
        - Potential Solution: Leverage microbiome species known to produce mood-altering neuromodulators (Lynch & Hsiao, 2023; Gibbons et al., 2021).
- Short-term Milestone: Relevant biological responses to neuromodulatory molecules produced by the gut microbiome at clinically relevant concentrations.
    - Bottleneck: *In vitro* testing of biological responses of gut microbiome in a physiologically-relevant environment.
        - Potential Solution: Dosage experiments with a lab-on-a-chip device that enables the gut microbiome to stay intact.
- Medium-term Milestone: Production and use of plant and fungal antipsychotic compounds in space.
    - Bottleneck: Changes in properties of antipsychotic plants and fungi in a microgravity environment.
        - Potential Solution: Produce and store plant and fungal compounds on Earth to bring to space.
    - Bottleneck: Unknown effects of these compounds and edibles on astronauts.
        - Potential Solution: Regular mental and physiological assessments on astronauts to determine effects.
- Long-term Milestone: Establish persistent neuromodulatory therapeutic production in gut microbiome members and identify factors affecting the longevity of engineered functions.
    - Bottleneck: Potential for commensal or symbionts to over-colonize or out-compete natural microbes.
        - Potential Solution: Utilize non-colonizing organisms.
    - Bottleneck: Precise dose control across dynamic growth phases and population levels.
        - Potential Solution: Implement feedback control mechanisms to regulate bacterial growth, population size, and environmental drug concentration.

Food & Nutrition

Introduction and Impact: Optimized food and nutrition systems are essential for maintaining human health, particularly while operating under the severe environmental and physiological conditions presented in space. The Food & Nutrition theme focuses on opportunities to leverage engineering biology to enable further exploration by enabling on-demand nutrient production, optimizing food crop production, and increasing food stability and palatability.

Currently, most food available in space is pre-prepared, prepackaged as meals similar to the "Meals, Ready to Eat" (MRE) used by the military, with a few modifications (Kumar & Gaikwad, 2023; Lewis, 2023). Because of anticipated space restrictions, long-term missions will require *in situ* production of food, as opposed to pre-made meals. Engineering biology also enables the opportunity to extract nutrients or transform used materials and wastes into food and nutrition products; while not glamorous, this capability brings us closer to circularity in space travel. Techniques for on-demand biosynthesis can be leveraged to produce micro- and macronutrients in-flight or at-destination. Organisms can be engineered for optimized nutrient density and small-scale biomanufacturing, and 3D-printing technology leveraged to produce food. To make this a reality, we need to develop autonomous, continuous, and adaptable bioreactors for nutrient production that function in microgravity environments, which also have value in other onboard bioprocessing.

Growing traditional food crops in space is an area of active research (see, for example, NASA's Veggie System; NASA, 2019c); however, onboard agricultural capabilities must be expanded for long-term mission success. While future advancements in engineering biology could potentially enable total nutrition through microbial systems, growing plants in space provides valuable psychological and emotional stimulation, in addition to better understanding the biological underpinnings of life on Earth. Engineering biology can enable advanced crop-support biosystems, such as more complete soils and rhizosphere-plant interactions, that account for the stressors of space environments, microgravity, and flight limitations. Plants may also be engineered to grow more efficiently, such as increased biomass or adaptation for hydroponics, have improved nutrient content and density under these conditions, and be more resistant to radiation, pathogens, and environmental perturbations.

Diversity of flavors and the palatability of food is crucial to appease personal and cultural preferences. However, microgravity impacts the perceived taste and texture of most food items and creative engineering biology solutions can help to satisfy individual preferences while still abiding by safety standards. Engineering biology presents the opportunity for improved food textures through the use of recombinant plant platforms, precision fermentation, and 3D-bioprinting. Enzyme catalysis and fermentation techniques can generate unique flavor and smell compounds to complement pre-packaged rations and counter changes in taste caused by the spaceflight environment. The environmental conditions of space travel, including prolonged storage and exposure to radiation, degrade nutrients and present challenges in long-term food preservation (Zwart et al., 2009; Pittia et al., 2023). Engineering biology can help to stabilize food biology and can be leveraged to generate coatings, materials, and other biobased packaging to support long-term storage and preservation of food (and food precursors, such as seeds and cell lines), while maintaining freshness and mitigating nutrient and flavor loss.

Goal	Breakthrough Capability	Milestone

On-demand micro- and macro-nutrient production.

On-demand and in situ biosynthesis of labile micronutrients.		
Optimized microbial synthesis platforms for labile micronutrient production.	Assembly of critical pathways for microbial micronutrient production, intracellular storage, and secretion.	Engineered human gut microbiome with capability to digest/process food into specific micronutrients.
	Bioreactor-ready cells, including bacteria, fungi, plant, and mammalian cells, for food production to additionally synthesize bioavailable micronutrients for nutritionally more complete food.	
Enzyme-based biochemical reaction pathways for critical vitamin synthesis from shelf-stable pro-vitamin intermediates, towards *in situ* vitamin synthesis.	Engineer plants as a recombinant protein expression platform.	Transient production of terminal enzymes to facilitate labile vitamin (e.g., Vitamin C) production in human cells.
Nutritionally-dense fungi and microbes biosynthesized as primary food sources.		
Engineered microbial production of major nutritional components, including fiber, polysaccharides, fats.	Engineered consumable microbes with enhanced nutritional value, including increased vitamin content and altered amino acid profiles.	Automated growth, downstream processing, and packaging of microbial products for ease of micro- and macro-nutrient consumption.
Characterize nutrient content of autotrophic microbes.	Engineer increased nutrient fluxes in microbes.	
Determine bioavailability and nutrient profiles for microbes that could grow on electricity or H_2, CO_2, other C1 substrates, intermediates derived from those substrates like acetate or ethanol, or other atypical carbon sources, such as biodegradable plastics, arrested anaerobic digestate (VFAs), or other waste-carbon.	Enable *in situ* removal of toxins and antinutrients in microbial foods.	
Tailored autotrophic microbe single-cell protein production with added nutritional value, such as Omega-3 fatty acids.	Engineer controlled morphology of autotrophic microbes and microbiomes to produce structured or 3D-printed foods.	
Define the nutrient composition requirements to target for microbial food sources for: (i) human consumption, and (ii) augmentation of other living systems on spaceflight (plant or insect).	Tailor microbial cell factories to funnel metabolic flux and adjust composition profile for suitable ratio of starches, fats and proteins for human consumption.	

Short-term → **Medium-term** → **Long-term**

<table>
<tr><th colspan="3">Optimized cell lines from traditional food sources for macronutrient production.</th></tr>
<tr><td>Attain optimal growth of animal (bovine, poultry, fish) cells in minimal media, this may include the development of multi-organismal systems of phototrophs and heterotrophs that will sustain the protein production.</td><td rowspan="3">Assembly of critical pathways for optimal carbohydrate, fat, and protein production, intracellular storage, and secretion in stem cell lines.</td><td>Engineer “metabolically minimalist” cell lines which focus metabolism solely in relation to macronutrient production and storage, cell growth, and flavor optimization, as opposed to cellular functions originally related to animal tissue health.</td></tr>
<tr><td>Production of cultivated meat from immortalized animal cells using specialized growth media intended for application in low-resource environments.</td><td rowspan="2">Engineer animal and plant cells to grow on H_2, CO_2, or electricity.</td></tr>
<tr><td>Functional microbial protein production determined by physiological needs during travel.</td></tr>
<tr><th colspan="3">Adaptable, self-driving continuous bioreactor technology for diverse macronutrient production.</th></tr>
<tr><td rowspan="2">Engineer sensors that can identify variability in feedstocks for both macro- and micro-nutrients.</td><td>Bioreactor biosensors that can detect early stress indicators for adaptation of process conditions.</td><td>Measurement of unstable metabolites in real-time.</td></tr>
<tr><td>Biosensors to identify variability in feedstocks for both macro- and micronutrients and early stress indicators to allow for adaptation of process conditions and control systems.</td><td>Create new, and enhance existing, mathematical models for use in model-based biocontrol processes.

Self-cleaning and water recycling capabilities which minimize cross-contamination.</td></tr>
<tr><td rowspan="2">Workflows to capture bioreactor data and build artificial intelligence (AI) that can integrate with control systems on bioreactors with robotic arms.</td><td>Modular downstream processing units that can be integrated with bioreactors on an as-needed basis.</td><td>Consortia engineering to maximize production goals.</td></tr>
<tr><td>Efficient bioprocesses with zero waste, including recycling of water and excess nutrients and minimal energy requirements.</td><td>Low-mass, low-energy, automated growth and processing of biomass into final food form that is significantly nutrient-dense.

Advanced culture methods that include solid-state fermentation and biofilters.</td></tr>
<tr><th>Short-term</th><th>Medium-term</th><th>Long-term</th></tr>
</table>

Optimized food crop production.

Highly-efficient and controllable biosystems for food crop growth and development.

Short-term	Medium-term	Long-term
Characterization of the interactions between plants and microbes under microgravity and partial gravity conditions.	Genetic systems for controlling rhizobiome symbionts scope for growth and preferred niche space.	Resilient, continual plant-microbiome consortium for optimized space crop production.
Identified molecular circuits and genes that underlie known symbioses between plants and beneficial (nutrient fixing) microbes.	Support crop growth with synthetic soil or rhizosphere microbial consortia.	
	Plant-microbe symbiotic pairings to alleviate the need for nitrogen fertilizers.	Biosensors that continuously monitor and report plant health and needs (water, nutrients, etc.).
Crop-specific exudate compounds to control rhizobiome composition and the abundance of target beneficial strains.	Plant-microbe symbiotic pairings, such as epiphytic bacteria, to reduce abiotic stress.	Biosensors that continuously monitor and report plant health and needs (water, nutrients, etc.).
	Fungal or mycorrhizae communities for resilient, sustained, carbon-negative food and nutrient production.	Precision agriculture with miniaturized, automated farming machines for onboard plant health detection.
Automated adaptive laboratory evolution (ALE) tools for engineering of photosynthetic microbes, particularly cyanobacteria and algae, for space environmental conditions (e.g., toxins, sulfur, iron, magnesium, high CO_2 concentrations).	Biomaterials that support growth and maintenance of nitrogen-fixing bacteria.	
	Beneficial microbes that protect plants from pathogens.	

Optimized plant architecture and growth for space flight and habitation conditions.

Short-term	Medium-term	Long-term
Targeted plant varieties for compact growth spaces in microgravity environments.	Robust food crops tolerant to increased radiation of off-planet environments.	Sustainable food crop ecosystem with various identified and cultivated plant species and plant-associated microbial communities suitable for space flight.
Engineered food crop roots suited for hydroponic and aeroponic growth.	Food crops with high tolerance to increased planting densities while maintaining high harvest indices.	
Engineered plants to recycle human occupancy waste.	Additional plant varieties for closed-environment agriculture (CEA), particularly those with cultural significance.	Plants that can efficiently pollinate and produce seeds and fruits in the spaceflight environment.
Engineered plants that grow on fermentation intermediates.	Food crops with minimal media requirements to reduce excess weight added to vessels by plant growth supplies.	
High-nutrition crop species optimized for closed environment agriculture (CEA).	Genetic tools to engineer food crops ideal for microgravity environments.	Engineered plants and crops that can grow on planet regolith.

Engineered food crops with high nutrient biomass, high harvest index, pathogen resistance, and short harvest time.

Germplasm repository improvement and pan-genome sequencing of diverse potential crops to support genome-wide association study (GWAS)-like analyses.	Targeted genome editing in crop plants to enhance post-harvest stability and palatability.	Combine all space-flight environmental services provided by plants into five or fewer species of plants.
Edible plant parts with enhanced micronutrients.	Crop varieties with complete micronutrient profiles for human support.	
	Crops with high harvest index and short harvest time.	
Cultivated food crops with enhanced resistance to pathogens.	Plants with edible organs and tissues optimized for space flight that are compact, nutrient-dense, and mature quickly.	

Increased palatability and stability of food.

Biobased platforms for engineering structure and composition of on-demand foods.

Precision fermentation or plant recombinant protein expression platforms to generate precisely-controlled fat structures.	Biomanufactured matrices that add structure and texture to 3D-printed foods, for a range of food types.	Scalable approaches that enable 3D structuring of lab-grown meats.
Scalable approaches to cultivate adipose tissue from mammalian cells.		Synthetic microbial consortia that produce edible, 3D structures mimicking familiar foods.

Foods with specified texture profiles.

Defined range and importance of texture profiles required for long-term happiness for clear engineering goals.	Advancement in existing technical capabilities for additive manufacturing or 3D-printing of textures in foods for space application.	
Process engineering to generate a variety of desired textures that improve palatability; for example, engineering more surface area for spice deposition, popping bubbles, etc.	Optimized engineering biology-enabled texturizers (such as additives or coatings) that are lightweight, low cost, compact, and ideally nutritionally dense.	
Molecular gastronomy methods that are achievable in low-resource settings, including microgravity and below low Earth orbit environments.		

Short-term → **Medium-term** → **Long-term**

Short-term	Medium-term	Long-term
Foods with specified flavor profiles.		
Characterized metabolic pathways for fats and off-flavors produced by laboratory cultured foods.	Complex flavor profiles from microbial strains using multi-circuit or multi-pathway engineering.	Microbes that use waste-stream feedstocks to produce space-flight relevant flavors and fragrances.
Enzyme-based biochemical reaction pathways for flavor compounds.	Flavor compounds engineered to respond to altered tasting capacity.	Portfolio of flavors optimized for performance in space and low-gravity or altered atmospheric conditions available to all space missions.
Removal of metabolic pathways that produce off-flavors and smells from fermentation hosts.	Encapsulation of flavor and fragrance to be released at the time of consumption.	
Stabilized crops and food sources.		
Identified genes responsible for radiation tolerance tested in crops for environmental protection.	Replacement of error-prone replication machinery with higher-fidelity enzymes in space crop genomes.	Multi-generational crop propagation with minimal genetic mutations or phenotypic fluctuations from space-related environmental factors.
Assessment of the evolutionary capacity and risk of engineered microbes in the microgravity environment.		
Biobased food preservation.		
Commensal bacteria modified for the production of preservative, antimicrobial, or biocoatings on food substrates.	Biological materials synthesized in situ for food preservation during plant growth or processing of foods.	A space ecosystem of commensals and crops that provides adequate food supply for human survival for 2+ years.
	Engineered yeast cells for encapsulation of bioactive compounds in food products	

Goal: On-demand micro- and macro-nutrient production.

Current State-of-the-Art: Optimized macro- and micro-nutrient production is essential to improving and expanding human capabilities in resource limited environments (Smith et al., 2021). For spaceflight, crewmembers are currently prescribed a diet with a macronutrient composition of 55% carbohydrates, 30% fat, and 15% protein, based on the World Health Organization's energy requirement predictions for moderately active persons (Baba et al., 2020). No macronutrients (proteins, fats, carbohydrates) are currently produced during spaceflight, and all food and dietary supplements are prepared and packaged on Earth. Leveraging engineering biology, we can optimize biological platforms for the on-demand production of micro- and macronutrients.

Currently, biological and chemical synthesis methods are used to produce a range of micronutrients. Recent advancements in biosynthetic pathway engineering in plants and microalgae have resulted in the production of α-tocopherol, β-carotenes, and provitamin D3 (Li et al., 2022). The production of vitamin K and β-carotenes through microbial fermentation has also been explored (Yuan et al., 2020). Leveraging microbial cell factories and metabolic engineering, precision fermentation is capable of nutrient production with high yield and purity (Chai et al., 2022). Plant recombinant expression platforms, capable of protein, and small molecule production, can also be utilized (Kulshreshtha et al., 2022). The human microbiome, particularly the gut, may also be engineered to improve nutrient absorption and digestion processes. Production of Vitamin B within the gut microbiome has been demonstrated, but further research is needed (Albert et al. 1980). The BioNutrients Flight's first-generation small-scale bioreactor experiment produced various micronutrients, including beta-carotene and zeaxanthin (Keller, 2023a; Ball et al., 2021). The next generation tested four additional organism types for the production of carotenoids, follistatin, yogurt, and kefir products (Keller, 2023a).

Starch and sugars have been synthesized from carbon dioxide using whole-cell catalysis, cell-free techniques, and algae with higher efficiency than plants (Nangle et al., 2020; Cai et al., 2021; Zhang et al., 2021). Alternative proteins and single-cell proteins (SCP) can be efficiently produced with microbes at a high rate and yield with much lower resource input (e.g., direct utilization of CO_2) (Sillman et al., 2019; Marcellin et al., 2022; Graham & Ledesma-Amaro, 2023). Bovine, chicken, and fish cells may also be optimized for growth in minimal media, with the ultimate goal of producing meat from immortalized cells using specialized growth media tailored to low-resource environments. Stem cell lines are an ideal source for on-demand food generation, but there are some limitations. Maintenance of stem cells requires specific cytokines and substrates that are not currently well defined. The production of stem cells in space is being actively researched through the ongoing Stellar Stem Cells Mission 1, aimed at evaluating the potential benefits of microgravity on the cellular reprogramming process (Svendsen et al., 2024). Identification and deeper understanding of the critical pathways that create micronutrients, flavor, carbohydrates, fats, proteins, and intracellular storage will further catalyze advancement in this space.

Current needs for advancement and increased circularity include engineered organisms for the production of both micro- and macronutrients, utilization of renewable feedstocks (including CO_2, packaging waste, inedible plant components, and human waste),

and specialized bioreactors for stable and continuous high-yield production. Due to limited physical space and resources, processes must be optimized to be as efficient and circular as possible. Ideally, waste processors would provide feedstocks compatible with food production systems. Improvements in sensing and monitoring, including real-time measurements of metabolites and engineered sensors that identify any variability in feedstocks, are also needed. Automated growth, downstream modular processing, and packaging of microbial products for consumption will advance the production and utilization of nutrients in low resource environments. Improved methods of bioreactor data collection are needed to better inform mathematical modeling, workflows, and AI.

Breakthrough Capability: On-demand and in situ biosynthesis of labile micronutrients.

- Short-term Milestone: Optimized microbial synthesis platforms for labile micronutrient production.
    - Bottleneck: Microbial synthesis requires storage of microbes and feedstocks, which are limited in space habitats.
        - Potential Solution: Engineer tractable microbes capable of metabolizing more readily-available alternative feedstocks, including supplemented/transformed regolith, organic waste-streams, and carbon dioxide (CO_2).
    - Bottleneck: Carbon content gradually declines due to microbial growth and metabolism.
        - Potential Solution: Utilize or engineer in carbon-conserving metabolic pathways that can function in various microbes (Bogorad et al., 2013; Westenberg & Peralta-Yahya, 2023).
- Short-term Milestone: Enzyme-based biochemical reaction pathways for critical vitamin synthesis from shelf-stable pro-vitamin intermediates, towards *in situ* vitamin synthesis.
    - Bottleneck: Enzymes corresponding to such bioconversion might be unstable through spacecraft launch or extended environmental exposure.
        - Potential Solution: Shelf-stable cell-free protein/enzyme synthesis platform and shelf-stable DNA template for in-space production of enzymes.
- Medium-term Milestone: Assembly of critical pathways for microbial micronutrient production, intracellular storage, and secretion.
    - Bottleneck: Ability to engineer multiple pathways in a single organism is limited due to missing pathway components.
        - Potential Solution: Develop a bioinformatic platform that identifies necessary pathway components missing in the chassis of interest.
        - Potential Solution: Utilizing a division of labor strategy to engineer microbial consortia for synthesizing repertoire of nutrients in a single system.
    - Bottleneck: Ability to predictably engineer pathways is challenging due to lack of rapid, real-time feedback of metabolic flux with a spatial component (e.g., cell-type or subcellular localization for multi-cellular systems).

        - Potential Solution: Systems biology and modeling approaches for rational pathway engineering in candidate systems for space flight bioreactors.
- Medium-term Milestone: Bioreactor-ready cells, including bacteria, fungi, plant, and mammalian cells, for food production to additionally synthesize bioavailable micronutrients for nutritionally more complete food.
    - Bottleneck: Bioreactor currently used on Earth might not work in microgravity habitat.
        - Potential Solution: Design new aerobic continuous bioreactors with nanobubble functionality that can function in a microgravity environment specific to the spaceflight (Adam et al., 2020; Granata et al., 2021).
    - Bottleneck: Increased metabolic demand may decrease growth rate, particularly if multiple pathways are introduced or induce undesired mutations making engineered lines useless.
        - Potential Solution: Select and engineer fast growing microbes or consortia that have native pathways for micronutrient synthesis and thrive in continuous fermentations.
        - Potential Solution: Embedding quality control of strains as part of sustainability via transcriptomics during long space flights.
    - Bottleneck: Pathway introduction may introduce new byproducts with different taste or potential toxicity.
        - Potential Solution: Metabolomics before and after modification to identify differences in outputs and process byproducts.
        - Potential Solution: Models of cellular processes to predict unwanted metabolites.
        - Potential Solution: Divide micronutrient engineering between different strains.
- Medium-term Milestone: Engineer plants as a recombinant protein expression platform.
    - Bottleneck: Plants take a long time to grow and produce edible structures (i.e., fruit, large roots, and leaves).
        - Potential Solution: Engineer and cultivate fast growing plants like duckweed (Liu et al., 2020).
        - Potential Solution: Co-design plants and controlled-environment growth systems to maximize rapid vegetative growth (Mortimer & Gilliham, 2021).
        - Potential Solution: Use transient expression systems such as engineered plant viruses to produce recombinant proteins.
- Long-term Milestone: Engineered human gut microbiome with capability to digest/process food into specific micronutrients.
    - Bottleneck: Gut microbiome is a very complex and dynamic system and would be challenging to sustain a specific species composition.
        - Potential Solution: Creation of modular microbial guilds that enable stable assembly of microbial communities that retains function although specific species composition may vary (Burke et al., 2011).

        - Potential Solution: Assemble microbial ingestible and engraftable communities with comprehensive metabolic output based on the known food loaded at the start of the mission.
- Long-term Milestone: Transient production of terminal enzymes to facilitate labile vitamin (e.g., Vitamin C) production in human cells.
    - Bottleneck: Human cell modification, to incorporate these engineered enzymes, remains challenging, both technically and ethically.
        - Potential Solution: Engage multidisciplinary teams to explore regulatory pathways for further human system engineering specific to space exploration.
    - Bottleneck: Engineering human somatic cells usually require specific engineering for each individual and would take a long time to make each variant.
        - Potential Solution: Engineer a universal cell-line that can be implanted on any individual, like as a skin patch, for nutrient microproduction (Rothschild et al., 2021).

Breakthrough Capability: Nutritionally-dense fungi and microbes biosynthesized as primary food sources.

- Short-term Milestone: Engineered microbial production of major nutritional components, including fiber, polysaccharides, fats.
    - Bottleneck: Microbes do not have the necessary pathways to produce either the polymeric forms or the necessary precursors.
        - Potential Solution: Engineer microbial strains with synthetic metabolic pathways and enzymes to produce the necessary precursors and polymeric forms of fibers, polysaccharides, and fats, potentially using adaptive laboratory evolution and high-throughput screening to optimize and select efficient microbial producers for 3-D bioprinting of on-demand foods.
- Short-term Milestone: Characterize nutrient content of autotrophic microbes.
    - Bottleneck: Algal species contain many different types of nutrients. Some nutrients' concentrations are low and hard to be extracted and quantified.
        - Potential Solution: High-throughput and highly sensitive LC-MS tools to screen and quantify nutrient compounds.
    - Bottleneck: Metabolomics databases do not capture the majority of microbial and plant metabolites.
        - Potential Solution: Systematically characterize metabolomes of plant, microbe and microbial communities.
        - Potential Solution: Leverage a publicly-accessible organism-agnostic metabolomics database.
- Short-term Milestone: Determine bioavailability and nutrient profiles for microbes that could grow on electricity or H_2, CO_2, other C1 substrates, intermediates derived from those substrates like acetate or ethanol, or other atypical carbon sources, such as biodegradable plastics, arrested anaerobic digestate (VFAs), or other waste-carbon

feedstocks (Kerckhof et al., 2021; Barbosa et al., 2021; Ismail et al., 2024; Marcellin et al., 2022; Hann et al., 2022; Mishra et al., 2024).
    - Bottleneck: CO_2 fixation can be very slow leading to slow biomass accumulation.
        - Potential Solution: Engineer microbes for gas fermentation that utilize CO_2 from waste materials providing additional nutrients to generate protein (Liew et al., 2022; Marcellin et al., 2022; Molitor et al., 2019).
    - Bottleneck: RuBisCO, which fixes CO_2, is inefficient and trades off activity for specificity (activity on O_2 leads creates a toxic product which requires energy to detoxify, reducing yield).[4]
        - Potential Solution: Resurrect ancestral RuBisCOs evolved for low oxygen environments with much higher activity. Optimize oxygen levels for plant cultivation to improve crop yield. Create chimeric rubisco with associated accessory programs to more efficiently screen O_2 binding (Lin et al., 2022).
        - Potential Solution: Directed evolution of RuBisCO. (Schultz et al., 2022)
        - Potential Solution: Employ CO_2 concentrating mechanisms to increase RuBisCO activity (Flamholz et al., 2020) and generation of carbonic anhydrase (Reginato et al., 2023).
        - Potential Solution: Generative AI-improved RuBisCO (Eisenstein, 2023)
- Short-term Milestone: Tailored autotrophic microbe single-cell protein production with added nutritional value, such as Omega-3 fatty acids.
    - Bottleneck: To be viable in space, these bioprocesses will need to use alternative feedstocks.
        - Potential Solution: Utilization of different waste streams for nitrogen fixation or concurrent with CO_2 fixation.
- Short-term Milestone: Define the nutrient composition requirements to target for microbial food sources for: (i) human consumption, and (ii) augmentation of other living systems on spaceflight (plant or insect).
    - Bottleneck: Microbial food sources may contain toxins (e.g., microalgae produce cyanotoxin).
        - Potential Solution: Screen for toxins and toxic byproducts and engineer strains deficient in such compounds without compromising growth.
        - Potential Solution: In-depth genomic characterization of potential microbial food sources.
        - Potential Solution: Detoxify microbe-derived byproducts with post-production treatment of microbial fermentation *in vitro.*
- Medium-term Milestone: Engineered consumable microbes with enhanced nutritional value, including increased vitamin content and altered amino acid profiles.
    - Bottleneck: Metabolic burden on microbes, particularly cellular byproducts (toxins, anti-nutrients, imparting a change in flavor).

[4] For more, see Breakthrough Capability: "Improve CO_2 uptake by engineering more efficient photosynthetic organisms (plants, algae, cyanobacteria)." in EBRC's *Engineering Biology for Climate & Sustainability* (EBRC, 2022).

        - Potential Solution: Focus strain design on addressing macronutrients that are inaccessible or difficult to access via plant-based food sources, e.g., fats, simple carbohydrates, and proteins.
        - Potential Solution: Specialize strains for production of specific macronutrients.
- Medium-term Milestone: Engineer increased nutrient fluxes in microbes.
    - Bottleneck: Directing flux toward increased nutrient production can limit microbial growth.
        - Potential Solution: Characterize energy conservation pathways used by the organism to inform metabolic engineering strategies.
        - Potential Solution: Focus on engineering microbes with naturally high flux toward desired product.
- Medium-term Milestone: Enable *in situ* removal of toxins and antinutrients in microbial foods.
    - Bottleneck: Separation and purification of nutrients from other compounds can be difficult and resource intensive.
        - Potential Solution: Engineer algae to extracellularly produce nutrients.
        - Potential Solution: Systems biology research to identify and engineer enzymes and pathways that produce toxins and antinutrients in organisms of interest.
- Medium-term Milestone: Engineer controlled morphology of autotrophic microbes and microbiomes to produce structured or 3D-printed foods.
    - Bottleneck: Quorum sensing (QS) systems will need to be altered or deleted for many of the species involved.
        - Potential Solution: Construct *de novo* QS systems for orthogonal and programmable cell-to-cell communication.
        - Potential Solution: Design specific communities as planktonic or biofilm based on production need.
- Medium-term Milestone: Tailor microbial cell factories to funnel metabolic flux and adjust composition profile for suitable ratio of starches, fats and proteins for human consumption.
    - Bottleneck: Single strain tuning and maintaining specific ratios of products/compounds is challenging.
        - Potential Solution: Enable technologies to adjust starch, fat, and protein ratios in post-processing.
- Long-term Milestone: Automated growth, downstream processing, and packaging of microbial products for ease of micro- and macro-nutrient consumption.
    - Bottleneck: Stability of microbes, consortia, and microbial products remain challenging during space flight without temperature control.
        - Potential Solution: Focus on microbes that are capable of surviving under harsh conditions (e.g., endospore forming) so that it can be stored on the shelf.

        - Potential Solution: Engineer microbes to remain stable under dehydrated conditions (e.g., introduce sporulation in model microbes that usually do not readily form spores).
    - Bottleneck: Rejuvenating microbes during spaceflight requires a controlled environment to mitigate spillage/contamination to other areas.
        - Potential Solution: Install biocontainment circuits to all space-flight microbial products for conditional rejuvenation.
    - Bottleneck: Consumption of microbial byproducts could lead to adverse effects on human health.
        - Potential Solution: Isolate common undesired components from the microbial products using space-flight-compatible downstream processes.
        - Potential Solution: Engineer novel microbes that contain reduced common byproducts that are known to have adverse effects on human health so that downstream processing is simplified.

Breakthrough Capability: Optimized cell lines from traditional food sources for macronutrient production.

- Short-term Milestone: Attain optimal growth of animal (bovine, poultry, fish) cells in minimal media, this may include the development of multi-organismal systems of phototrophs and heterotrophs that will sustain the protein production.
    - Bottleneck: Availability of cell lines that are capable of using non-traditional mammalian cell culture media.
        - Potential Solution: Engineer stem cell lines/culture environment capable of routine maintenance in low gravity environments.
        - Potential Solution: Genetic engineering of endogenous expression of growth factors to stimulate cell expansion (Stout et al., 2024).
        - Potential Solution: Optimizing cell lines to grow both in low gravity situations in addition to creating non-adherent conditions (Lee et al., 2016; Pasitka et al., 2023).
- Short-term Milestone: Production of cultivated meat from immortalized animal cells using specialized growth media intended for application in low-resource environments.
    - Bottleneck: Chemically-defined media is necessary for each cell line.
        - Potential Solution: AI-based methods for media optimization (Nikkhah et al., 2023).
        - Potential Solution: Simplify growth media to have minimal components (Skrivergaard et al., 2023; Mitić et al., 2023; Messmer et al., 2022).
    - Bottleneck: Traditional growth media is weight-volume prohibiting for space flight, especially if the media cannot be desiccated and then dissolved.
        - Potential Solution: Engineer *in vivo*/*in situ* production of growth factors that have traditionally been added to the media.
        - Potential Solution: Growth media that can be recycled by replacing specific elements, thus increasing the efficiency of cell growth to media weight/volume (Good Food Institute, 2024; Stout et al., 2023).

        - Potential Solution: More efficient solid-state fermentation systems to produce food using less water.
        - Potential Solution: Engineer photosynthetic microbes to produce macronutrients from recycled waste materials.
- Short-term Milestone: Functional microbial protein production determined by physiological needs during travel.
    - Bottleneck: Understanding of relevant protein expression and mixed protein production for complete nutrition in microbes that matches those of animal proteins.
        - Potential Solution: Identify nutritional needs during travel and engineer microbes that satisfy those protein needs.
- Medium-term Milestone: Assembly of critical pathways for optimal carbohydrate, fat, and protein production, intracellular storage, and secretion in stem cell lines.
    - Bottleneck: Ability to predictably engineer multiple cellular pathways at once is limited due to poor understanding of sink-source relationships, missing pathway components and a limited ability to get rapid, real-time feedback of metabolic flux with a spatial component (e.g., cell-type or subcellular localization for multi-cellular systems).
        - Potential Solution: Engineer optimal stem cell lines for each nutrient production pathway to be processed together in a balanced way to form dietary components.
        - Potential Solution: Systems biology and modeling approaches for rational pathway engineering.
- Long-term Milestone: Engineer “metabolically minimalist” cell lines which focus metabolism solely in relation to macronutrient production and storage, cell growth, and flavor optimization, as opposed to cellular functions originally related to animal tissue health.
    - Bottleneck: Essentially requires redesigning metabolism and other functions in cells.
        - Potential Solution: Mathematical models that enable *in silico* characterization of principal nutritional requirements that enable flavor profiling by shaping lipid and carbohydrates content such as the previously developed for algae (Li et al., 2023; Li et al., 2019; Zuñiga et al., 2018).
- Long-term Milestone: Engineer animal and plant cells to grow on H_2, CO_2, or electricity.
    - Bottleneck: Cells grown in gaseous environments that can safely and efficiently produce macronutrients.
        - Potential Solution: Optimize metabolic pathways of animal and plant cells to convert H_2, CO_2, and electricity to nutrients.
        - Potential Solution: Engineer electro-genetic systems which control cell fate by electric currents (Huang et al., 2023).

Breakthrough Capability: Adaptable, self-driving continuous bioreactor technology for diverse macronutrient production.[5]

- Short-term Milestone: Engineer sensors that can identify variability in feedstocks for both macro- and micro-nutrients.
    - Bottleneck: Chemical composition measurements of feedstocks are currently done in batch mode.
        - Potential Solution: Continuous measurement systems for multiple feedstocks, including gaseous feedstocks.
        - Potential Solution: Continuous measuring of waste process products to maximize recycling of nutrients and minerals for microbial and plant growth.
    - Bottleneck: Measurements of micronutrients and micro-toxins at very low concentrations is challenging, especially in a turbid background.
        - Potential Solution: Continuous measurement systems for multiple feedstocks, including gaseous feedstocks.
        - Potential Solutions: Engineer cell-based electronic devices (biohybrid sensors) to monitor target nutrient components.
- Short-term Milestone: Workflows to capture bioreactor data and build artificial intelligence (AI) that can integrate with control systems on bioreactors with robotic arms.
    - Bottleneck: Bioreactor (meta)data is small and complex (numeric, images, comments, etc.).
        - Potential Solution: Robust workflows to capture substantial bioreactor data from government funded research to teach AI methods that can be applied in space.
- Medium-term Milestone: Bioreactor biosensors that can detect early stress indicators for adaptation of process conditions.
    - Bottleneck: Challenge of measuring Intracellular changes of bioreactor contents in real time.
        - Potential Solution: In-line sampling and lysing to measure intracellular metabolic activity in microfluidic devices.
        - Potential Solution: In-line Raman spectroscopy (or other analytical techniques) or quantum sensing for cellular and metabolite fingerprinting.
- Medium-term Milestone: Biosensors to identify variability in feedstocks for both macro- and micronutrients and early stress indicators to allow for adaptation of process conditions and control systems.
    - Bottleneck: Empirical data collection under a variety of conditions for sensor validation, calibration, and fingerprinting; potentially requires a number of multiplexed sensors.

[5] Enabling bioreactor technologies for space travel would have numerous applications, including and beyond nutrient and food production. While we highlight milestones towards bioreactor design here, these advancements have benefits and value to other Breakthrough Capabilities and Milestones throughout this roadmap.

        - Potential Solution: Use of ML and AI to improve data collection, analysis, and predictions.
        - Potential Solution: Identify the key processes and steps that can serve as a minimal but representative detection suite.
- Medium-term Milestone: Modular downstream processing units that can be integrated with bioreactors on an as-needed basis.
    - Bottleneck: Efficient downstream processing of multiple molecules or compounds at low volumes.
        - Potential Solution: Versatile downstream processing equipment, including tangential flow filtration in microgravity, that can be modular and switched in and out as needed.
    - Bottleneck: Requirement for entirely new or unique downstream processing unit design due to the space environment (i.e., low pressure, low gravity, etc.).
        - Potential Solution: Development of downstream modules simultaneously with the development of the bioreactor.
        - Potential Solution: Fermentation technology with reduced downstream processing (e.g., self-precipitation and -separation).
- Medium-term Milestone: Efficient bioprocesses with zero waste, including recycling of water and excess nutrients and minimal energy requirements.
    - Bottleneck: Current bioprocesses generate substantial waste, especially in the form of contaminated water.
        - Potential Solution: Robust microbes, such as halophiles, that can thrive in otherwise suboptimal bioreactor conditions.
        - Potential Solution: Continuous bioprocess with feedback data analysis and ML to understand exact nutritional needs of microbes, for precise media constitution to minimize contaminants in resulting spent media.
        - Potential Solution: Rapid monitoring of byproducts and excess nutrients with regulated offtake and inputs as a result.
        - Potential Solution: Faster solid-state fermentation to reduce water usage.
    - Bottleneck: Current bioprocesses are conducted with suspended cells that require mixing with aeration in large volume reactors.
        - Potential Solution: Biofilm-based processes to obtain desirable food textures along with the necessary macro- and micronutrients.
        - Potential Solution: 3D-printed edible matrix that supports food cell growth and maturation, to obtain required food textures and structures.
- Long-term Milestone: Measurement of unstable metabolites in real-time.
    - Bottleneck: Current measurements involve pre-determined samples of known molecules with established standards.
        - Potential Solution: Engineer *in vivo* biosensors for metabolites, such as ribozyme switches.
        - Potential Solution: Identify and measure molecules at nanomolar range.
- Long-term Milestone: Create new, and enhance existing, mathematical models for use in model-based biocontrol processes.

    - Bottleneck: Currently, insufficient data exists to properly parameterize model-based biocontrol processes.
        - Potential Solution: The integration of machine learning with bioprocess modeling and parameter estimations.
- Long-term Milestone: Self-cleaning and water recycling capabilities which minimize cross-contamination.
    - Bottleneck: Current sterilization methods involve high temperatures, pressures, and an acid or base.
        - Potential Solution: Engineered microbial community that can survive contamination-related perturbations.
        - Potential Solution: Application of extremophiles into volatile bioprocesses.
- Long-term Milestone: Consortia engineering to maximize production goals.
    - Bottleneck: Lack of control over community composition.
        - Potential Solution: Leverage mobile gene elements to achieve functional stability while the microbial community composition shifts over time.
        - Potential Solution: Engineer the spatial characteristics of microbial communities, including through encapsulation or use of solid phase bioreactors.
    - Bottleneck: Underutilization of quorum sensing tools and capabilities.
        - Potential Solution: Use quorum sensing-based systems to monitor community composition in real-time and respond to optimize environmental conditions.
        - Potential Solution: Consider other tools, such as engineered genetic circuits to respond to environmental changes, that utilize native metabolic pathways to allow microbes to better adapt to diverse bioprocess conditions.
    - Bottleneck: Some species are dominant in terms of growth but are less beneficial overall to the consortia.
        - Potential Solution: Identify quorum sensing molecules, measure them at low concentrations, and control their concentrations to manipulate community behavior as needed.
        - Potential Solution: Use engineering biology methods to modify quorum sensing circuits to introduce novel behaviors and activities (for example, to synthesize other signal molecules or bioproducts of relevance *in situ*).
        - Potential Solution: Engineer individual species and strains to proliferate in a predicted and reproducible way.
- Long-term Milestone: Low-mass, low-energy, automated growth and processing of biomass into final food form that is significantly nutrient-dense.
    - Bottleneck: Processing raw materials into food is energy intensive (Good Food Institute, 2024).
        - Potential Solution: Engineering fermenting microbes that produce *in situ* enzymes that aid the transition from raw material to food form under low energy conditions and in high yield.

- Long-term Milestone: Advanced culture methods that include solid-state fermentation and biofilters.
    - Bottleneck: Sensitivity of eukaryotic cells to mass transfer limitations.
        - Potential Solution: Adaptation/evolution of fungal species for similar applications.
        - Potential Solution: New solid-state fermentation bioreactor design and optimizations with small-scale footprints.

Goal: Optimized food crop production.

Current State-of-the-Art: Healthy and sustainable sources of food are essential to maintain crew health and safety on long-term missions. Furthermore, food systems must demonstrate high useability and reliability. The vast majority of food onboard the International Space Station (ISS) is currently prepackaged on Earth and delivered for reheating. Through engineering biology, biological processes can be leveraged as a mechanism for improved plant growth, resulting in optimized food crop production in austere environments.

There are a variety of plants grown onboard the ISS for experimentation purposes consisting of at least 40 different species, twelve of which are leafy greens (Johnson et al., 2021). Similarly, the most common indoor food crops grown on Earth are vegetables, herbs, tomatoes, and microgreens (Ampim et al., 2022). NASA maintains two permanent growth facilities aboard ISS, the Veggie Vegetable Production System (Veggie) (Bunchek et al., 2024; Khodadad et al., 2020) and the Advanced Plant Habitat (APH) (Monje et al., 2020). Veggie is less controlled and open to cabin air, whereas APH is highly controlled and managed. Following extensive safety testing completed in 2015, astronauts are now permitted to consume a very small portion of the plants grown on board (mostly lettuce, arugula, and chilies), although most are still reserved for experimentation (Sable et al., 2016). A larger growth facility is currently under development, OHALO III, and is the first crop system specifically designed for a Mars transit vehicle (NASA Techport, 2024). Other currently funded projects include MarsOasis, an Efficient Autonomously Controlled Martian Crop Production System, Microgravity Investigation for Thin Film Hydroponics, ARC Centre of Excellence in Plants for Space, and EDEN ISS.

Engineering biology can rapidly develop plants optimized to thrive in a controlled environment, whether on Earth, in transit vehicles, or in habitats, offering a host of solutions for the optimization of indoor and Closed Environment Agriculture (CEA) and vertical farming (VF) (Benke & Tomkins, 2017). VF and CEA systems on Earth are also great comparative systems for space crop production as they have similar goals of high efficiency, low water usage and recycling, and autonomous growth. Controlled environments are fixed growth conditions, more akin to the laboratory and providing intrinsic containment, enabling a route to on-Earth commercialization for these types of technologies. Automated systems for the growth and maintenance of organisms would further improve onboard agricultural capacity, including the maintenance of plant health and stress detection.

Plants and microbes demonstrate beneficial symbiotic relationships on Earth (Harman et al., 2021). Further understanding and identification of the specific microbes that drive these

interactions and how these relationships would potentially function under microgravity and the stressors of space flight would catalyze engineered solutions. Improved symbiotic pairing through engineering biology has the potential to reduce the need for fertilizers and reduce abiotic and biotic stressors of spaceflight environments. Engineered soil microbial consortia that are resilient and self-propagating may further improve plant growth in microgravity (Santomartino et al., 2023). Tool sets, like molecular plant breeding, CRISPR, site-directed nucleases, and Automated Adaptive Laboratory Evolution (ALE), may be used to engineer photosynthetic organisms to survive in microgravity.

The identification of additional plant species compatible with compact growth systems (or capable of genetic modification toward this aim) would further expand potential space crops. It is important to find the balance between time, effort, and resources necessary to domesticate a plant that may meet the nutritional or culturally necessary needs of a space traveler, with the value of engineering those potential traits into a currently utilized (already domesticated) plant (Rech & Arber, 2013). Gene circuit engineering can be leveraged to improve and optimize plant root growth, structure, and nutrient and water uptake in microgravity, where water uptake is a challenge (Castañón, 2022). Plant pillow technology has partially solved this problem, but hypoxia, drought, and water creep remain present and have the potential to lead to disease. Metabolic pathway engineering can be leveraged to allow seedlings optimized for growth in non-traditional media to thrive in low-light conditions (Service, 2023). Meanwhile, advancements in LED technology, namely defined wavelengths, limited heat production, and reduced power consumption, have increased plant production capabilities in controlled settings (Ma et al., 2020).

Plants may also be engineered to improve the nutrient density of edible parts and create crop varieties that provide complete nutrition. Targeted genome engineering of plants could enhance or expand the micronutrients food crops could provide, tailored to the needs of astronauts. Additionally, food crops can be engineered to be more efficient, with higher harvest indices and shorter time-to-harvest. Finally, onboard plants can also be engineered for pathogen resistance and post-harvest stability, to ensure both long-term agriculture system and human health.

Breakthrough Capability: Highly-efficient and controllable biosystems for food crop growth and development.[6]

- Short-term Milestone: Characterization of the interactions between plants and microbes under microgravity and partial gravity conditions.
    - Bottleneck: Limited understanding of how beneficial and pathogenic microbes interact with plants in microgravity and partial gravity environments.
        - Potential Solution: Perform plant-microbe interaction studies in spaceflight and on ground-based spaceflight analogs at a range of actual or simulated g-forces.

[6] This Breakthrough Capability focuses on opportunities to engineer the growth environment and conditions, including plant associated microbiomes, rather than the crops themselves. See other Breakthrough Capabilities in this Goal for opportunities in engineering food crops directly.

        - Potential Solution: Formulate optimized seed coating with the necessary microbiota for rhizome performance in low gravity.
- Short-term Milestone: Identified molecular circuits and genes that underlie known symbioses between plants and beneficial (nutrient fixing) microbes.
    - Bottleneck: Metabolomic and proteomic datasets need to be improved and expanded to better capture molecular communication between plants and microbes.
        - Potential Solution: Engineer beneficial symbiotic microbes to colonize and out-compete non-beneficial microbes.
- Short-term Milestone: Crop-specific exudate compounds to control rhizobiome composition and the abundance of target beneficial strains.
    - Bottleneck: Different biotic and abiotic conditions influence development and expression of crop-specific exudates (Zhalnina et al., 2018).
        - Potential Solution: Measure exudate composition from space crops.
- Short term Milestone: Automated adaptive laboratory evolution (ALE) tools for engineering of photosynthetic microbes, particularly cyanobacteria and algae, for space environmental conditions (e.g., toxins, sulfur, iron, magnesium, high CO_2 concentrations).
    - Bottleneck: Adaptability of organisms to constantly variable conditions (such as changes in sulfur content in regolith).
        - Potential Solution: Engineer microbial consortia that are more resilient to concentration changes than individual organisms.
        - Potential Solution: Microbial biosensors capable of dynamically sensing environmental conditions.
- Medium-term Milestone: Genetic systems for controlling rhizobiome symbionts scope for growth and preferred niche space.
    - Bottleneck: Crew health outcomes from unwanted release of rhizobiome symbionts into the air of the spacecraft
        - Potential Solution: Control growth of rhizobiome strains with genetic circuitry that uses metabolic addiction or activated kill switches in the absence of the host exudate.
- Medium-term Milestone: Support crop growth with synthetic soil or rhizosphere microbial consortia (Moon, 2022).
    - Bottleneck: Unknown molecular interactions between plants and microbes.
        - Potential Solution: Controlled, miniaturized platforms to characterize plant-microbe interactions and identify beneficial consortia and media for growth in environmental parameters found on LEO, lunar, or Martian regolith.
    - Bottleneck: Unknown interspecies microbe-microbe and microbe-host interactions within the rhizobiome.
        - Potential Solution: Use of host-mediated or environment-mediated selection base methods of rhizosphere “evolution’ to produce desired host phenotypic outputs (Styer et al., 2024).

- Medium-term Milestone: Plant-microbe symbiotic pairings to alleviate the need for nitrogen fertilizers (Lindsey et al., 2021).
    - Bottleneck: Nitrogenases needed for nitrogen fixation are highly sensitive to oxygen (Barney, 2020).
        - Potential Solution: Identify microbe symbionts that are less sensitive to oxygen but have desired plant-microbe interactions.
- Medium-term Milestone: Plant-microbe symbiotic pairings, such as epiphytic bacteria, to reduce abiotic stress.
    - Bottleneck: Identifying the impact of symbionts on the complex and dynamic microbiome communities.
        - Potential Solution: Identify individual bacterial symbionts and communities of symbionts with desired impacts on crop growth.
- Medium-term Milestone: Fungal or mycorrhizae communities for resilient, sustained, carbon-negative food and nutrient production.
    - Bottleneck: Complex genomes make engineering mycorrhizal fungi challenging (French, 2017).
        - Potential Solution: Genetic toolkits for one or more mycorrhizal fungi to enable their use as mycorrhizal chassis.
    - Bottleneck: Maintenance and administration of obligate, mycorrhizal strains.
        - Potential Solution: Identify non-mycorrhizal symbionts that perform similar services, including trichoderma and beneficial bacteria.
- Medium-term Milestone: Biomaterials that support growth and maintenance of nitrogen-fixing bacteria.
    - Bottleneck: Nitrogen-fixing bacteria require carbon supply and form native associations with a limited number of crops.
        - Potential Solution: Engineer extracellular matrices, such as designer cell wall polymers, to sustain the growth of nitrogen-fixing bacteria without the plants.
        - Potential Solution: Addition of bacterial nitrogen-rich biocapsules or films to space crop growth systems.
- Medium-term Milestone: Beneficial microbes that protect plants from pathogens (Berg & Koskella, 2018; Li et al., 2021).
    - Bottleneck: Survival of engineered microbes in complex microbial communities.
        - Potential Solution: Biodesign of a modular microbial chassis that is robust to environmental perturbation.
        - Potential Solution: Engineer stress resistance to limited nutrients, harsh temperatures, etc., into existing microbes (Szydlo et al., 2022).
- Long-term Milestone: Resilient, continual plant-microbiome consortium for optimized space crop production.
    - Bottleneck: Limited resource availability (nutrients, physical space) that enables large consortia to grow in non-native environments.
        - Potential Solution: Build synthetic communities with microbes from functional guilds in complex microbial consortium to probe non-native environmental cultivability.

- Long-term Milestone: Biosensors that continuously monitor and report plant health and needs (water, nutrients, etc.).
    - Bottleneck: Sensors and detection mechanisms that can operate at the low cost and mass setting of space applications.
        - Potential Solution: Engineer tissues of sentinel plants to manifest visible color changes in response to specific environmental deficiencies.
        - Potential Solution: Microbial devices that report real-time changes in the water, soil, and root chemical environment.
- Long-term Milestone: Precision agriculture with miniaturized, automated farming machines for onboard plant health detection (Kacira & Ling, 2001; Zermas et al., 2015).
    - Bottleneck: Susceptibility of plant health to labor requirements, resource limitations, and environmental perturbations.
        - Potential Solution: Machine learning algorithms and spectral analysis to predict and detect environmental stressors and identify corrective action.
        - Potential Solution: Genetically encoded sensors in plants or plant-associated microbial biosensors for early detection and reporting of plant health.
        - Potential Solution: Non-aerosol-based treatments that can fight plant pathogens.
        - Potential Solution: Inoculate growing systems with multifaceted, biopriming solutions, such as engineered consortia, to fight plant pathogens and abiotic stresses.

Breakthrough Capability: Optimized plant architecture and growth for space flight and habitation conditions.

- Short-term Milestone: Targeted plant varieties for compact growth spaces in microgravity environments.
    - Bottleneck: Ability of consumable plants and algae with sufficient baseline growth to grow in a microgravity environment.
        - Potential Solution: Characterize health and growth of plant and algae candidates in microgravity.
    - Bottleneck: Gravity requirements for specific pollination of plant species to produce fruit.
        - Potential Solution: Engineer plant species that are adaptable to microgravity pollination habitats.
    - Bottleneck: Seed weight and germination efficiency varies by plant species.
        - Potential Solution: Selection of species varieties with low seed weight, high germination efficiency, and strong growth potential.
        - Potential Solution: Seed coatings and optimized storage conditions to improve germination efficiency.
- Short-term Milestone: Engineered food crop roots suited for hydroponic and aeroponic growth (NASA, 2008; Eden Grow Systems & Comeaux, 2022).
    - Bottleneck: Restricted growth of species cultivated in water or aeroponic substrates.

        - Potential Solution: Transfer genes that are optimal for hydroponic and aeroponic growth conditions to non-amenable species.
- Short-term Milestone: Engineered plants to recycle human occupancy waste.
    - Bottleneck: Current plant technology cannot convert human waste to usable materials (Lockhart, 2018).
        - Potential Solution: Engineer plants to use nitrogen and water sources from human urine for growth.
        - Potential Solution: Algae and microbial-based wastewater filtration systems for space that recover nutrients from human waste.
        - Potential Solution: Monitor for potential disease transmission when wastewater is reclaimed using plant, algal, and microbial resources.
- Short-term Milestone: Engineered plants that grow on fermentation intermediates (Kasiviswanathan et al., 2022).
    - Bottleneck: Currently, many plants cannot use intermediates (such as acetate) as a carbon source or do so only at low rates.
        - Potential Solution: Engineer a variety of plants to integrate with other bioprocesses, such as CO_2-driven fermentations, and more efficiently and effectively use intermediates.
- Short-term Milestone: High-nutrition crop species optimized for closed environment agriculture (CEA).
    - Bottleneck: A CEA environment with ideal environmental parameters (e.g., light intensity, light wavelength mix, air movement, temperature) for optimized production of desired crop varieties.
        - Potential Solution: Engineer chambers with desired environmental parameters to test likely microbiomes and co-growth with other plants (Danforth Center, 2024).
        - Potential Solution: Engineer endogenous reporting mechanisms in crops to show health status.
        - Potential Solution: Rapid, automated screening of desired metrics to create predictable models for linking environmental conditions with optimal crop production.
- Medium-term Milestone: Robust food crops tolerant to increased radiation of off-planet environments (Ali et al., 2015; Jan et al., 2012; Majeed et al., 2018; Vanhoudt et al., 2014).
    - Bottleneck: Radiation tolerance across lifecycles is understudied in plants currently deployed in space (De Micco et al., 2002).
        - Potential Solution: Screen for DNA damage of samples from plants that exhibited radiation tolerance on earth across various highly radioactive sites (e.g. Chernobyl).
        - Potential Solution: Engineer plants to incorporate heterologous radiation tolerant machineries from other plants or algae.
- Medium-term Milestone: Food crops with high tolerance to increased planting densities while maintaining high harvest indices.

    - Bottleneck: Shading and competition for available lighting limit growth and harvest index for most plants to below acceptable limits as planting densities increase.
        - Potential Solution: Genetically engineer the light harvesting complex of plants to decrease chlorophyll content for improved light use-efficiency-per-unit-photon and increased tolerance of shading (Caddell et al., 2023).
- Medium-term Milestone: Additional plant varieties for closed-environment agriculture (CEA), particularly those with cultural significance.
    - Bottleneck: Current agricultural crops are bred for outdoor field conditions that are highly variable across diurnal and seasonal cycles.
        - Potential Solution: Identify genetics and phenotypes (e.g., high harvest index, short body plan and seed-to-seed time, and long fruiting) of plant species successfully grown in CEA.
        - Potential Solution: Accelerate the breeding of existing commercial crops for CEA or domesticate crops which are not currently widely commercialized, such as duckweeds or amaranth.
- Medium-term Milestone: Food crops with minimal media requirements to reduce excess weight added to vessels by plant growth supplies.
    - Bottleneck: Viable media that is lighter than soil, has appropriate footprint to support plant root architectures, and supports robust plant growth.
        - Potential Solution: Conduct hydroponics and aeroponics research to understand media and root architecture needs of food crops.
        - Potential Solution: Engineer traditional crop waste products (e.g., roots) for downstream use as alternative root support.
- Medium-term Milestone: Genetic tools to engineer food crops ideal for microgravity environments.
    - Bottleneck: Limited methods to manipulate protein production and activity of plant varieties.
        - Potential Solution: Engineer the plant species of interest with mechanisms for post-translational modifications for specific protein activity.
        - Potential Solution: Integrate synthetic gene circuits into the plant genome to regulate protein production.
- Long-term Milestone: Sustainable food crop ecosystem with various identified and cultivated plant species and plant-associated microbial communities suitable for space flight.
    - Bottleneck: High efficiency requirement with limited available resources on the spacecraft.
        - Potential Solution: Automated control and feedback system to maintain the highest efficiency of the plant ecosystem.
        - Potential Solution: Utilize the plant ecosystem to store all microbial strains required for use in the spacecraft to maximize efficiency-benefit over mass ratio.

    - Bottleneck: Sufficient biodiversity and feedback loops to sustain the plant ecosystem.
        - Potential Solution: Multifunctional microbial strains capable of plant symbiosis, filtration, and production chassis of micronutrients.
- Long-term Milestone: Plants that can efficiently pollinate and produce seeds and fruits in the spaceflight environment.
    - Bottleneck: Poor productivity from fruiting crops from reduced transfer of pollen and pollination efficiency in microgravity.
        - Potential Solution: Engineer plants with parthenocarpic fruit production or apomictic seed production.
- Long-term Milestone: Engineered plants and crops that can grow on planet regolith.
    - Bottleneck: Lack of nutrients for plant growth in regolith.
        - Potential Solution: Enhance elemental composition of regolith through microbial use and manure recycling.
        - Potential Solution: Engineer microbial processes to extract available nutrients from regolith to incorporate into nutrients for plant growth.
    - Bottleneck: Regoliths lack weathering and their sharp edges directly impede root growth.
        - Potential Solution: Engineer regolith controls, such as tumbling, for effective pre-planting remediation techniques.
        - Potential Solution: Use earthworm-based regolith remediation as effective pre-planting remediation techniques.
    - Bottleneck: Martian regolith has high levels of perchlorate salts, toxic to both plants and humans (Kasiviswanathan et al., 2022).
        - Potential Solution: Identify engineering mechanisms to reduce and reclaim Martian regolith for human use and as a plant growth substrate.

Breakthrough Capability: Engineered food crops with high nutrient biomass, high harvest index, pathogen resistance, and short harvest time.

- Short-term Milestone: Germplasm repository improvement and pan-genome sequencing of diverse potential crops to support genome-wide association study (GWAS)-like analyses (McNulty et al., 2021).
    - Bottleneck: Identification of optimal starting crops.
        - Potential Solution: Coordination with USDA and International Agricultural Entities for organizing and assessing the existing germplasm collections.
    - Bottleneck: Assessing the diversity of possible germlines for fair representation of relevant diversity.
        - Potential Solution: Identify new collections of standing diversity for core target plants followed by pan-genome sequencing for variation analysis.
- Short-term Milestone: Edible plant parts with enhanced micronutrients.
    - Bottleneck: Limited ability of current engineering efforts to boost nutrient levels due to metabolic bottlenecks, single gene approaches, and limited use of DNA parts.

        - Potential Solution: Identify metabolic bottlenecks to overcome in cycling plant systems grown in extreme environments, such as aquatic plants, bryophytes, and seed plants.
        - Potential Solution: Engineer fast-growing plant or cyanobacterial platforms to produce or enrich for a single micronutrient or macronutrient.
        - Potential Solution: Explore fungal engineering as an expedient alternative to plant engineering for macronutrient and micronutrient production.
- Short-term Milestone: Cultivated food crops with enhanced resistance to pathogens.
    - Bottleneck: Susceptibility of plant crops to rapidly evolving pathogens.
        - Potential Solution: Inoculate the plant with pathogen-resistant microbes and fungi.
        - Potential Solution: Enhance the natural plant immune system with probiotics, biostimulants, and biocontrol strains as a preventative measure.
    - Bottleneck: Development of new, opportunistic pathogens on the space station due to mutations.
        - Potential Solution: A continuous system to monitor indicators of the health of the root system, leaves, and stems for the presence of pathogens.
        - Potential Solution: Periodically analyze crop tissues for potential pathogens that may arise from environmental organisms, microbes, and fungi.
- Medium-term Milestone: Targeted genome editing in crop plants to enhance post-harvest stability and palatability.
    - Bottleneck: Efficient and site-specific editing of plant genomes and validation of stable plant lines.
        - Potential Solution: Efficient genetic manipulation of plants and tissues to optimize gene delivery systems and co-factors and increase transgene delivery through plant cell barriers.
        - Potential Solution: An effective Agrobacterium-mediated system to deliver CRISPR-directed recombinases to gametes.
        - Potential Solution: PAM-free programmable endonucleases to enable for flexible site-specific editing.
        - Potential Solution: Target cell wall enzymes and polyphenol oxidases that contribute to ripening and post-harvest losses.
- Medium-term Milestone: Crop varieties with complete micronutrient profiles for human support.
    - Bottleneck: Limited production of the vitamins, minerals, and balanced amounts of nutrients humans require by current plant technology.
        - Potential Solution: Engineer a plant or plant colony that can provide a complete diet (Li et al., 2022).
        - Potential Solution: Metabolic engineering to increase the quantity or density of nutrients produced by a plant.
        - Potential Solution: Use optogenetics or other inducible genetic circuitry to stimulate plants to make specific nutrients on-demand.

        - Potential Solution: Use leaf infiltration to introduce complex-multigene pathways for micronutrient production (Stephenson et al., 2018).
- Medium-term Milestone: Crops with high harvest index and short harvest time.
    - Bottleneck: Crops with a long lifecycle but slow and limited production of edible seeds and fruits.
        - Potential Solution: Engineer wholly-edible plants to vegetatively propagate and produce desired nutrition features, including high protein with required balance of amino acids, carotenoids, etc.
        - Potential Solution: Develop libraries of cryopreserved clonal plants (e.g., duckweeds) that don't need a life cycle involving seeds, and can be regenerated exponentially from cryopreserved material.
        - Potential Solution: New crop varieties with energy and nutrient dense leaves.
    - Bottleneck: Accumulation of toxic or unpalatable compounds from many plants with desirable bioactive compounds to treat human disease.
        - Potential Solution: Engineer an edible bryophyte, such as *Marchantia polymorpha*, rich in bioactive compounds and fiber for oral drug delivery (Chandra et al., 2018; Sauret-Güeto et al., 2020).
- Medium-term Milestone: Plants with edible organs and tissues optimized for space flight that are compact, nutrient-dense, and mature quickly.
    - Bottleneck: Microgravity influences plant stem cell differentiation, cell gravitaxis, and gravitropism.
        - Potential Solution: Engineer novel plant strains to function independent of gravity.
        - Potential Solution: Engineer plants able to be induced or controlled with an alternative input or tropism.
- Long-term Milestone: Combine all space-flight environmental services provided by plants into five or fewer species of plants.
    - Bottleneck: Identification and integration of multiple critical functions into single plant species.
        - Potential Solution: Modify or introduce genes into plants for various critical functions, such as enhancing oxygen or nutrient uptake.
        - Potential Solution: Engineer genetic components that can be easily incorporated or removed from the plant genome to customize overall function.
        - Potential Solution: Engineer a single highly-photosensitive plant, which can be activated with spectrally engineered LED wavelengths to synthesize on demand proteins and nutrients.

Goal: Increased palatability and stability of food.

Current State-of-the-Art: Food produced and consumed during a long-term mission must be safe, nutritious, and support optimal physiological and cognitive performance (Smith et al., 2021). Currently, meals provided to astronauts must meet strict safety standards and undergo extensive packaging and conservation processes to remain stable in the space environment. The Space Food Systems Laboratory produces prepackaged foods that are very similar to military MRE's, including beverage powders, cookies, candy, and other dried goods, specifically tailored to the nutritional needs of astronauts. Specialty packaging allows for the injection and mixing of water in orbit as well as cooking and heating where appropriate (Kumar & Gaikwad, 2023; Lewis, 2023). Food processing and packaging is dependent on the composition and shelf-life of the individual food item.

Quality and palatability are among some of the biggest challenges in the production of food for human consumption during space flight. Astronauts are only able to select up to 20% of their own food and beverage options, while the other 80% comes from shared, standard foods (Douglas et al., 2020). Although many astronauts prefer foods with pleasing aromas and spices, their perception of taste is altered during space flight due to microgravity changes in physiological conditions (Bychkov et al., 2021). Moreover, due to the enclosed nature of space environments, foods with strong aromas are often avoided even though they are preferred. Texture and flavor also play a role in the appeal of foods in space, with astronauts preferring diets varied in both. A sensory evaluation is also completed for each food item to determine palatability (Tang et al., 2022; Smith et al., 2021; Lewis, 2023). Decreased palatability often results in a reduction in calorie consumption. ISS astronauts have been reported to consume only 80% of the daily recommended intake, altering their nutritional status after space flight (Smith et al., 2005). This decreased energy intake, especially over longer duration space travel, could result in malnutrition and other health concerns. Ideally, a meal plan would reduce menu fatigue by offering a variety of pleasing tastes, smells, textures, and flavors.

Through the application of process engineering, we can engineer the mechanical properties of food to make them more appealing. For example, precision fermentation and plant expression platforms can be leveraged to produce much needed fats (Boukid et al., 2023; Kulshreshtha et al., 2022; Chai et al., 2022) to generate enhanced textures in multiple foods. Three-dimensionally engineered foods, particularly those grown or generated onboard, may increase the availability of palatable textures (Santhoshkumar et al., 2024; NASA, 2019a). Engineering biology can help to generate edible matrices and structures to generate optimized textures.

New efforts are underway to understand how olfaction and gustation work will further efforts to improve the perception of taste in flight, particularly in physical receptors and neurochemistry (Billesbølle et al., 2023; del Mármol et al., 2021; Parry, 2023). However, there are a variety of ways engineering biology tools and techniques may be used to improve the flavor profiles of food consumed in space. Leveraging enzymatic catalysis and enhancing existing flavor profiles in microbial, animal, and plant cells, can help to generate appealing flavor compounds (Dickey et al., 2024). Flavor compounds may also be developed that are specifically engineered to respond to altered tasting capacity, with a long-term goal of

developing a robust portfolio of flavors optimized for performance in space. Additionally, the identification of metabolic pathways that produce *unappealing* flavor and scent compounds is the first step in being able to remove them from foods consumed in space.

Engineering biology offers a host of solutions to improve food packaging and preservation. Biological materials can be synthesized specifically for food preservation and processing to better maintain nutrition levels for prepackaged foods for 3-5 years (or longer). The identification of genes responsible for radiation tolerance, for example, can help to enable crop and food stability (Lamprecht-Grandío et al., 2020). Eventually, technologies like low-mutation crops that are resistant to the harsh conditions of spaceflight will be able to propagate for years without genotypic and phenotypic fluctuations.

Breakthrough Capability: Biobased platforms for engineering structure and composition of on-demand foods.

- Short-term Milestone: Precision fermentation or plant recombinant protein expression platforms to generate precisely-controlled fat structures.
    - Bottleneck: Control of the ideal fatty acid composition.
        - Potential Solution: Metabolic and circuit engineering of fatty acid biosynthesis and degradation.
        - Potential Solution: Identify exogenous additives and target candidates that regulate the composition of fatty acids produced in fermentation or expression platforms.
    - Bottleneck: Underdevelopment of methodology for controlling lipids and other hydrophobics.
        - Potential Solution: Collection of hydrophobic precision fermented products with organogel- and oleogel-based platforms (Davidovich-Pinhas, 2016).
- Short-term Milestone: Scalable approaches to cultivate adipose tissue from mammalian cells (Lew et al., 2024).
    - Bottleneck: Limited yields of adipose tissue derived from animal cells.
        - Potential Solution: Enhance growth of adipose tissue *ex vivo* with ideal culturing and bioreactor conditions.
        - Potential Solution: Engineer induced pluripotent stem cells or fibroblast lines encoded with gene circuits that enable them to easily differentiate into brown or white adipose tissue (Pawlowski et al., 2017; Genovese et al., 2017).
        - Potential Solution: Identify media formulations or additives that accelerate the growth and differentiation of adipose tissue *ex vivo*.
- Medium-term Milestone: Biomanufactured matrices that add structure and texture to 3D-printed foods, for a range of food types.
    - Bottleneck: Required heterogeneous combination of multiple matrices to increase palatability and texture of current foods.
        - Potential Solution: Engineered control of matrix properties, such as polymer length, linkages, and branching.

        - Potential Solution: Engineer fibroblast cell lines to produce optimized extracellular matrix for texture mechanics and flavor.
- Long-term Milestone: Scalable approaches that enable 3D structuring of lab-grown meats.
    - Bottleneck: Complexity of 3D bioprinting lab-grown meats to replicate texture, appearance, and palatability of real meat.
        - Potential Solution: Control structure and spatial organization of 3D meats on a scalable level to enable rapid production on a scalable level.
        - Potential Solution: Increase capabilities of 3D-printing for higher volume production of 3D foods.
- Long-term Milestone: Synthetic microbial consortia that produce edible, 3D structures mimicking familiar foods.
    - Bottleneck: Cell-cell communication necessary for coordination among a community in a 3D structure.
        - Potential Solution: Spatial control over microbial consortia with synthetic morphogens or modified gene circuits that allow for precise formation of a 3D structure (Deter and Lu, 2022).
    - Bottleneck: Growing microbes in a 3D structure.
        - Potential Solution: Create a cascade of developmental steps within a microbial consortium that allow for ideal spatial organization of microbes.
        - Potential Solution: Grow cells in a microgravity environment for easier manipulation of their final placement within the structure.

Breakthrough Capability: Foods with specified texture profiles.

- Short-term Milestone: Defined range and importance of texture profiles required for long-term happiness for clear engineering goals.
    - Bottleneck: Preferences for future Mars travelers may be more varied than current astronauts.
        - Potential solution: Modern customer research approaches (interview-based research) on food texture and happiness.
    - Bottleneck: Food tastes and textures change based on consumption environment.
        - Potential solution: Increased food experience testing in the international space station.
- Short-term Milestone: Process engineering to generate a variety of desired textures that improve palatability; for example, engineering more surface area for spice deposition, popping bubbles, etc.
    - Bottleneck: Individuals have varying taste and texture preferences.
        - Potential Solution: Extensive consumer research to ensure textures satisfy consumer preferences.
    - Bottleneck: Maintaining good manufacturing practices (GMP) to generate palatable and safely-digestible food.
        - Potential Solution: Standard operating procedures and quality control regulations.

- Short-term Milestone: Molecular gastronomy methods that are achievable in low-resource settings, including microgravity and below low Earth orbit environments.
    - Bottleneck: Stability of ingredients in extreme environments.
        - Potential Solution: Test ingredients in microgravity conditions on earth for stabilization and functionality.
- Medium-term Milestone: Advancement in existing technical capabilities for additive manufacturing or 3D-printing of textures in foods for space application.
    - Bottleneck: Mainly starchy products and alternative meats have been explored with current state-of-the-art technology (Bugarin-Castillo et al., 2023).
        - Potential Solution: Investigate different ingredients or nutrients that can be used in 3D-printing of textures.
- Medium-term Milestone: Optimized engineering biology-enabled texturizers (such as additives or coatings) that are lightweight, low cost, compact, and ideally nutritionally dense.
    - Bottleneck: Achieving the desired texture without compromising cost, compactness, and nutritional value.
        - Potential Solution: Edible coatings that are lightweight, rich with nutrients, and provide the desired texture.

Breakthrough Capability: Foods with specified flavor profiles.

- Short-term Milestone: Characterized metabolic pathways for fats and off-flavors produced by laboratory cultured foods.
    - Bottleneck: Limited understanding of the connection between fat composition and resultant sensory experiences of laboratory cultured foods.
        - Potential Solution: Compare cultivated fat with traditional livestock-derived products for better characterization and potential catering of cell growth conditions for achieving similar sensory experiences (Lew et al., 2024).
    - Bottleneck: Cultivated fat profiles and off-flavors might be difficult to modify or remove.
        - Potential Solution: Develop chemically-defined cell culture media to enhance the production of specific fats involved in positive sensory experiences.
        - Potential Solution: Engineer metabolic pathways to promote the production of specific fats while reducing the presence of off-flavors.
- Short-term Milestone: Enzyme-based biochemical reaction pathways for flavor compounds.
    - Bottleneck: Instability of enzymes capable of bioconversion during spacecraft launch or long exposure to microgravity.
        - Potential Solution: Shelf-stable, cell-free protein and enzyme synthesis platform with on-board DNA template for in-space production of enzymes.
- Short-term Milestone: Removal of metabolic pathways that produce off-flavors and smells from fermentation hosts.
    - Bottleneck: May require multi-step processing.

        - Potential Solution: Enable fermentation broths that are palatable without downstream processing
- Medium-term Milestone: Complex flavor profiles from microbial strains using multi-circuit or multi-pathway engineering.
    - Bottleneck: Achieving flavor complexity with relatively simple compounds.
        - Potential Solution: A palette of simple compounds that each have a distinct flavor that can also be combined in different ways to create various other flavor profiles.
    - Bottleneck: Current biochemical protocols typically optimize for a single compound.
        - Potential Solution: Engineer more promiscuous enzyme chemistry that produces an acceptable range of products needed for a certain flavor profile.
- Medium-term Milestone: Flavor compounds engineered to respond to altered tasting capacity.
    - Bottleneck: Influence of spaceflight on the expression and sensitivity of taste receptors, taste bud microarchitecture, and mass and heat transfer of food.
        - Potential Solution: Physiological and molecular studies examining the effects of spaceflight on taste receptors.
        - Potential Solution: Novel G-protein coupled receptor agonists that activate specific taste receptor combinations experienced in space.
- Medium-term Milestone: Encapsulation of flavor and fragrance to be released at the time of consumption.
    - Bottleneck: Stability of flavors and fragrances during space flight.
        - Potential Solution: Engineered plant biopolymers with release mechanisms to control the timing and amount of flavor released when astronauts chew the food.
        - Potential Solution: Agonists that stimulate the umami receptor.
    - Bottleneck: Microgravity-induced fluid shifts to sinuses that alter olfaction and gustation (Olabi et al., 2002).
        - Potential Solution: Lower the viscosity to enhance the overall taste profile.
- Long-term Milestone: Microbes that use waste-stream feedstocks to produce space-flight relevant flavors and fragrances.
    - Bottleneck: Appropriate lightweight and sterile bioreactors.
        - Potential Solution: Novel, space-specific bioreactor and growth systems that enable recycling of waste stream feedstocks for flavor and fragrance synthesis.
    - Bottleneck: Lack of research on how microbes behave in space compared to on Earth.
        - Potential Solution: Optimize microbial colonization in space for enhanced flavor and fragrance production.
- Long-term Milestone: Portfolio of flavors optimized for performance in space and low-gravity or altered atmospheric conditions available to all space missions.
    - Bottleneck: Transport and shelf-life of many flavors to space.

        - Potential Solution: Engineer shelf-stable flavor compounds.

Breakthrough Capability: Stabilized crops and food sources.

- Short-term Milestone: Identified genes responsible for radiation tolerance tested in crops for environmental protection.
    - Bottleneck: Isolation of specific crop genes responsible for radiation tolerance.
        - Potential Solution: Localize specific genes of interest with -omics sequencing.
- Short-term Milestone: Assessment of the evolutionary capacity and risk of engineered microbes in the microgravity environment.
    - Bottleneck: Limited knowledge of the capacity of genes to evolve metabolic mechanisms based on exposure to selective pressures in a microgravity ecosystem, relative to the most similar stable ecosystem on earth.
        - Potential Solution: A focused systems -omics approach to assess sequence and function of the engineered DNA relative to the ideal ecosystem.
- Medium-term Milestone: Replacement of error-prone replication machinery with higher-fidelity enzymes in space crop genomes.
    - Bottleneck: Genetic drift may allow for adaptations in space that alter crop growth.
        - Potential Solution: Implement controlled breeding programs combined with genomic monitoring to ensure genetic stability.
        - Potential Solution: Utilize CRISPR/Cas technology to introduce targeted genetic safeguards that prevent unwanted mutations, maintaining the desired crop phenotype despite the adaptive pressures of space environments.
- Long-term Milestone: Multi-generational crop propagation with minimal genetic mutations or phenotypic fluctuations from space-related environmental factors.
    - Bottleneck: Influence of space-specific environmental stressors on genetic and phenotypic stability of crops over time.
        - Potential Solution: Advanced genetic editing tools to introduce robust genetic safeguards, such as DNA repair enhancement and anti-mutation mechanisms.
        - Potential Solution: High-throughput sequencing and bioinformatics to detect and correct mutations in real-time.
        - Potential Solution: Implement controlled environmental systems to regulate and mitigate space-specific stressors, ensuring crops remain genetically and phenotypically stable over extended periods.
        - Potential Solution: Engineer apomixis into crops to provide for stable, clonal seed production over multiple generations.

Breakthrough Capability: Biobased food preservation.

- Short-term Milestone: Commensal bacteria modified for the production of preservative, antimicrobial, or biocoatings on food substrates.

    - Bottleneck: Inability of many bacteria to produce antimicrobial compounds without autotoxicity.
        - Potential Solution: Use commensal yeasts for production.
        - Potential Solution: Engineer novel strains that can synthesize antimicrobial compounds with non-toxic analogs.
    - Bottleneck: Potential harm of commensal bacteria to the gut microbiome of astronauts.
        - Potential Solution: Titrating bacteria for specific-use cases.
        - Potential Solution: Screen candidate bacteria for strains that could increase permeability of the digestive lining and lead to leaky gut.
- Medium-term Milestone: Biological materials synthesized *in situ* for food preservation during plant growth or processing of foods.
    - Bottleneck: Multiple instruments required for cell lysis and extraction of target proteins and biomaterials for food preservation.
        - Potential Solution: Engineer *in situ* production pathways of biomaterials and proteins in plants during growth.
        - Potential Solution: Develop multi-functional bioprocessing systems that perform cell lysis, extraction, and purification to reduce the need for multiple toolsets.
- Medium-term Milestone: Engineered yeast cells for encapsulation of bioactive compounds in food products (Dadkhodazade et al., 2021).
    - Bottleneck: Ability of yeast cells to release contents after ingestion.
        - Potential Solution: Engineer yeast cells to respond to specific physiologically relevant conditions of the human digestive system, such as pH changes, temperature, and the presence of digestion-related enzymes.
- Long-term Milestone: A space ecosystem of commensals and crops that provides adequate food supply for human survival for 2+ years.
    - Bottleneck: Shelf-life and risk of bacterial contamination to food supply.
        - Potential Solution: Use antimicrobial agents or preservatives in the food to protect from unwanted microbes.
        - Potential Solution: Engineer genetically modified crops to be resistant to microbial contamination.

Environmental Control & Life Support

Introduction and Impact: Environmental control and life support (ECLS) systems are the foundation of human space flight. Fortunately, engineered and bio-based solutions are emerging as a new paradigm to meet the challenge of ever-longer and ever-further exploration. Engineering biology offers the opportunity to optimize system efficiency and improve functioning. This roadmap technical theme explores the opportunity to use engineering biology to improve and expand upon environmental control and life support systems – including air, water, waste, and infrastructure – for use in low-resource environments, both on and off Earth.

ECLS systems are exceedingly complex and must account for a multitude of environmental considerations, particularly those important to maintaining long-term well-being of more diverse space travelers. Clean and breathable habitat air is essential for crew health and safety during long-term space flight. Biobased systems, including engineered microbes, plants, and fungi, can be leveraged to improve the efficiency and effectiveness of air filtration systems, with the ultimate goal of establishing a robust and complex air microbiome (Maurya et al., 2023). And engineered microbial consortia and more advanced biomaterials may enable the production, capture, and conversion of air to efficiently produce oxygen and sequester carbon dioxide.

Fresh water is also a top priority for spaceflight, including continuous and on-demand water filtration, recycling, purification, and even generation. Advanced biosensing capabilities can continuously monitor and rapidly detect changes in water quality to provide timely and accurate bioremediation. Using engineering biology, wastewater treatment systems can be optimized for efficiency and effectiveness, with byproducts available for use in other life support or food systems. Engineering biology can also enable the utilization of *in situ* resources for the regeneration and conditioning of fresh water. These capabilities are particularly important on long-term missions, where resupply may be infrequent or unavailable. Water generation and purification technologies also have extensive Earth-side applications, particularly in places most impacted by climate change.

No waste processing techniques are currently being implemented in space, rather current procedures rely on storage instead (Simon et al., 2017). However, engineered enzymatic and microbial systems, along with higher-order organisms can be leveraged to improve waste remediation and recycling through bioprocessing of human, food, and material waste streams. For example, microbial strains and consortia, and even insects, can be engineered to process human waste for further use in hydroponic or food production systems, such as crop growth supplements. Engineered fungi and microbiomes may be capable of lignocellulose destruction and metabolism, aiding in upcycling and reuse of crop and plant waste. The management of abiotic materials, including plastic and electronic waste, can be accomplished through engineered metabolic and enzymatic degradation, with the additional possibility of elemental recovery and brine production to support a closed-loop system (Santomartino et al., 2023). Processing and recycling of waste streams will significantly help to make long-term spaceflight more efficient and sustainable, enabling circularity of onboard materials and systems.

Opportunities exist to leverage extremophiles and other unique capacities of biology to process and remediate regolith for use in other systems. Planetary regolith provides a resource

base for mineral extraction and generation of supplemental compounds, transformation to soils and other growth substrates, and downstream materials synthesis (Küppers et al., 2014; Meyer, 2003). While tangential to supporting human health and well-being in the present moment, the potential of regolith biomining and transformation represents utilization of *in situ* resources, coupled with engineering biology, to make long-term space exploration more feasible.

Habitat materials contribute greatly to the maintenance of human health in resource-limited environments, like spaceflight. These include structural components of the habitat, clothing/fabrics, and single-use consumables (such as medical supplies) and packaging, which are currently made primarily from plastics. Engineering biology can enable living materials that report on environmental health or with self-healing properties developed for use in space habitats to address damage and leaks.

Finally, engineering biology offers solutions to improve biosensing and monitoring tools and technologies to continuously monitor ECLS system health (Huang et al., 2023). For example, integrated and comprehensive environmental monitoring systems could detect, diagnose, and address abnormalities, such as small changes in air or water quality or system microbiome health. Expansion of monitoring technologies can also improve on-Earth monitoring capabilities, such as early pathogen detection.

Goal	Breakthrough Capability	Milestone

Continuous production, recycling, and remediation of habitat air.

Continuous biomonitoring of habitat air.		
A comprehensive understanding of microbial ecology of habitat air in various enclosed environments (e.g., ISS, lunar and Mars transit, lunar and Mars base habitats).	Engineer immobilized synthetic olfactory receptors to detect specific odorants or volatiles in recycled air.	Stable, functional, self-replicating biosensor arrays for air monitoring in microgravity environments.
Multi-component biomolecular signal relay systems and transcription factors to detect specific air contaminants of concern (e.g., volatiles).	Real-time biosensing of habitat air that overcomes slow biological response.	
Bio-based filtration of biotic and abiotic pollutants from circulating habitat air.		
Microbes able to sequester particulate matter and other contaminants.	Engineered microbes that employ new non-Calvin cycle carbon dioxide (CO_2) fixation pathways that more efficiently capture excess CO_2 and pollutants from the circulating air habitat.	Genetically engineered space crop plants and phyllosphere epibionts that sense, filter, and decontaminate biotic and abiotic pollutants.
	Plants and plant-derived structures able to filtrate pollutants.	
Enzymes capable of decontamination immobilized into porous structures built specifically for ISS or habitat module air filtration systems.	Fungi able to produce mycelium structures that can act as physical and chemical filters.	
Robust bioprocesses for production, capture, and conversion of environmental gasses to habitat air.		
Microbial consortia able to fix carbon dioxide (CO_2) and produce Oxygen (O_2).	Space-tolerant organisms with carbon sequestration pathways.	Autonomous biofiltration system capable of regulating CO_2 and O_2 concentrations in the space environment.
	Autotrophic organisms capable of rapid carbon fixation and interconversion to quickly balance environmental and energy demands in space habitats.	
A proof-of-concept modular or adaptable biochemical pathway that sequesters carbon monoxide (CO) that could be implemented across different cell types or in a cell-free system.	Microbial consortia capable of highly-efficient CO_2 capture and O_2 production.	
	Harness pathways characterized from organisms with desired metabolic activity to build cell-free bioreactors capable of CO capture at concentrations relevant to the space	

Short-term → **Medium-term** → **Long-term**

Robust, complex habitat air microbiome.		
A comprehensive understanding of microbial composition of habitat air in various enclosed environments (e.g., positive control planet-side that is beneficial for health, ISS, lunar transit, Mars transit, lunar base, Mars base).	Compose or engineer an air microbiome that is self-sustaining in a microgravity environment.	A spacecraft with engineered microbiomes integrated into spacecraft that enhance air and life support systems

Continuous, on-demand fresh water.

Biosensing and bioremediation of contamination in potable water systems.		
Identified potential water system microbial contaminants in space environments to target with biosensors.	Ability to detect and disrupt biofilms that may arise in water recovery and storage systems.	Microbial systems and consortia for decontamination that can adapt to dysbiosis and emerging pathogens.
Biological interventions to selectively target and eliminate microbes of concern in the water supply.		
Biomaterials that are resistant to biofilm formation for water filtration systems.	Capability to produce novel phages on space missions for rapid control of evolved biofouling microbes.	Decontamination of water by infiltration percolation through fungal, plant-soil, or plant-hydroponic substrates.

Highly-efficient biobased recycling and purification of wastewater.		
Plant barriers, combined with synthetic water pumps, specific for wastewater contamination in NASA systems.	Algal and cyanobacterial farming systems that provide water filtration.	Microbial consortia capable of total water purification and reclamation of ion, nitrates, and salts for other uses.
Engineered biological components that enhance self-regeneration of membrane filters.		
Identified biological processes that mimic current ISS chemical and physical distillation.	Microbial desalination cells (MDCs) used as a bio-electrochemical system to break down organic matter in wastewater while simultaneously generating electrical energy.	
Utilize urine distillation brine as a source of salts and minerals for defined culture medium for fermentations, plant fertilizer, or other uses.		
Expansion of state-of-the-art methods for the conversion of blood into potable water.	Recovery and reuse of desired non-microbial contaminants in wastewater for plant and microbial growth.	
Reduce footprint and complexity of the filtration system to meet mission requirements.		

Short-term → **Medium-term** → **Long-term**

Generation and conditioning of fresh water from *in situ* resources.		
Scaled plant systems that desalinate water for enhanced water recycling.	Engineered microalgae for biodesalination.	Self-generating, self-renewing organismal consortia for fresh water generation without long-term maintenance.
Map of water net-positive (chemically producing water) and water net-negative (chemically destroying water) metabolic processes known in current microbial strains to aid design choices for overall water balance.	Engineered edible microalgae for combined food production and water treatment.	
Highly-efficient ethanol bioproduction and bioreclamation cycle for salt fractionation.	Microbial consortia implemented for fresh water generation from brine.	Conversion of ambient and waste gases (e.g., methane, hydrogen, and oxygen) into water by microbesmaintenance.

Waste remediation and recycling.

Bioprocesses for human waste management.		
Engineered microbial strains or consortia with high tolerance to microbial inhibitors found in human waste (aromatic compounds, ammonia/salts).	Microbial production strains with consistent output in varying microbial communities, e.g., stool microbiomes.	
Human waste-delineated medium to support growth of a microbial community to process human waste.		
Defined human-waste derived hydroponic growth media that supports plant growth.		
Measurements of the mass-balance of carbon, nitrogen, oxygen, and micronutrients and minerals for human waste consumption in insect larvae (e.g., black soldier fly).		
Food-safe insect larvae grown on human waste.		

Short-term **Medium-term** **Long-term**

Bioprocesses for biotic material waste management, including food waste.		
Fully characterized metabolic fluxes of lignocellulose deconstruction and conversion by engineered microbiomes.	Waste processing reactors that provide compatible feedstocks for use in a food-producing bioreactor.	Efficient downstream processes for separation and formulation of edible products from non-edible components.
Engineer fungi suitable for degradation of space-related plant lignocellulosic materials that could also be used as a potential food source.	Efficient waste-to-gas (CO_2, CO, CH_4, N_2) conversions using engineered microbial communities on a defined timeline.	A complete closed-loop conversion of solid waste into food using biological systems.
Identification of optimal insect candidates for waste-to-protein production in space conditions	Biomaterial regeneration loops for chitin exoskeletons of insects used for waste recycling	
Bioprocesses for abiotic material waste management, including plastic and electronic waste.		
Effective genetic engineering tools for non-model microbes with valuable native metabolism for processing mission waste streams.	Enzymatic degradation and recycling of mixed hydrolyzable plastic polymers into tractable monomers.	Integration of plastic-waste degradation byproducts into life-support-system to improve loop-closure
Database with bioconversion efficiencies and limitations of platform systems (insect bioconversion, microbial bioconversion, etc.) for solid waste bioconversion in operationally-relevant environments (e.g., low gravity, low resource).	Robust biorecovery of major elemental components (N, P, K, S, trace elements) from abiotic waste streams.	Microbial and archaeal organisms able to convert brine into useful products for extended space travel.
Efficient metabolic degradation of single polymer plastics under space conditions.		

Short-term → **Medium-term** → **Long-term**

Regolith and rock biomining, bioremediation, and transformation.

Biological extraction of elements and compounds for material biosynthesis from regolith.		
Engineering tools for microbes robust or resistant to heavy metals and regolith solubles.	Recombinant microbial biosequestration strains that overproduce specific metallophores and uptake transporter systems for inorganic nutrients of interest.	Organisms capable of scavenging target micronutrients and minerals from regolith.
Bioconcentration of heavy metals through metal precipitation and sequestration pathways.	Downstream bioseparation method to convert internalized or sequestered heavy metals and rare earth metals into a purer form for transportation or use as raw material for industrial processes.	
	Biochemical pathways that convert heavy metal into usable forms.	
Biological platforms for heavy and rare metals detoxification.	Organisms that are able to secrete and tolerate large amounts of organic acid for heavy metal solubilization, conversion, or valorization.	Robust engineering toolkits that use various non-model organisms to extract (biological) elements and compounds from regolith.
Determined chemical structure and biosynthetic pathways for specific metallophores produced by microbes.	Engineer robust chassis or microbial consortia containing multiple heavy metal uptake and detoxification pathways.	
	Biological organization (e.g., DNA origami) of regolith-derived metals into semiconductor composites and wires.	

Bioconversion of regolith to agricultural soils and growth substrates.		
Growth of vascular plants in soil with specific regolith compositions of organic and inorganic matter.	A database of potential biocontaminant strains of off-planet agricultural spaces, with species or strain level taxonomic resolution.	Complete bioconversion of regolith to agricultural soils.
Robust control mechanisms for microbial composition, adaptable across complex soil types.	Inorganic compounds obtained in situ from regolith for proper “soil” generation.	
Healthy space crop plants maintained by supplementations (e.g., organic, inorganic, or biotic) to specific regoliths to allow growth and food production.	Microbes engineered with different metal requirements to enhance bioconversion of regolith (i.e., engineer pumps to use metal abundant in the regolith other than sodium or lithium).	
Strategies to create or transport the necessary supplementations for regolith conversion on site.	Engineer a minimal microbial consortium that can generate soil that supports plant growth starting from a regolith base.	

Short-term **Medium-term** **Long-term**

Biosynthesis of habitat materials supporting human health.

Biosynthesis of organic or engineered living materials for habitat construction and infrastructure.

Proof-of-concept biogenic and living acoustic damping materials.	Radiotrophic organism metabolism using gamma radiation as power-source to create photosynthetic analogues.	Integration of acoustic damping capability into multipurpose, self-generating construction materials for crew cabins.
Validate capacity and efficiency of select living organisms, such as melanized filamentous fungi, to transform ionizing radiation into a net-energy gain.	Blend engineered living materials with the materials of the spacecraft.	
Microbial production of bioplastics and biomaterials with high volumetric efficiency and yield from waste-streams.	Biomanufacturing systems to convert *in situ* resources into versatile and durable biopolymers with sufficient mechanical requirements for construction purposes.	
Construction materials which help to regulate habitat humidity and condensation.	Integrate healable biomaterials with biosensors to create autonomous in-flight repair of air leakages.	Microbes and plants that generate and self-assemble advanced polymers into habitat structures.
Construction materials and coatings that contain a beneficial microbiome for the habitat, such as to help condition the air or provide environmental biosensing capabilities.	Extremophiles and enzymes capable of synthesizing biopolymers that perform well under adverse conditions.	
	Efficient recovery of urea from biological waste streams for biocement applications for habitat construction.	
	Enable sustainable infrastructures with controlled formation of biofilms useful to life support systems.	

In situ synthesis and repair of clothing and personal protective equipment.

3D-printed bioplastics [e.g., polyhydroxyalkanoates (PHAs), including PLA, P3HB, P4HB] which mimic clothing (texture, pattern).	Clothing with embedded enzymes that resist soiling and dirt.	Cells that can be applied directly to the astronaut's body to generate coatings with various protective and adaptive traits, such as protection from radiation, temperature and moisture regulation, and antimicrobial and antifungal properties.
Assessment of novel biomaterials and biocomposites for use in space clothing.	Microbes able to produce different types of advanced, high-performance bioplastics for durable clothing, including space suits and flame-retardant clothing.	

Short-term → **Medium-term** → **Long-term**

Integrated and comprehensive environmental monitoring.

Real-time biosensing and reporting on life support systems and built-in environment.		
Microbe-based system that generates an electronic (wireless) signal for aggregation by a central monitoring system (vs. the more typical biological readout, such as luminescence).	Programmable apoptotic circuits for control of microbial biosensing organisms.	Living surfaces that integrate microbial biosensors with physical sensors to monitor habitat areas.
Efficient and autonomous sensing, sampling, and sequencing of biocomponents of life support systems.	Efficient colorimetric-based biosensors for built-in environments.	An autonomous environmental control module for air, water, and waste remediation to minimize energy and infrastructure requirements.
Microbial biosensors to monitor and report elemental balances on closed life support systems.	Biological data from life support systems converted into machine-readable information with an automated platform.	Characterization of optimal environmental microbiome for symbiotic human microbiome maintenance.
		Real-time, continuous biosurveillance of microbiome consortia within the environment and present humans.

Biobased tools and technologies for early environmental threat or anomaly detection.		
Engineered microbial or cell-free biosensors for the detection of pathogens and harmful compounds in liquid and solid waste.	Directed evolution of proposed microbial biosensors under on-Earth and Low Earth Orbit conditions to inform predictive models of functional loss or potential invasive organism traits.	Predictive AI/ML tools using data from past threats and anomalies to understand future mutation outcomes, i.e., fitness positive, neutral, or pathogenic, impact for the human and environmental ecosystems.
Biosensors (for chemistry, pH, pathogens) that can relay information electronically and wirelessly.		
Detection of evolved or invasive organisms that could threaten environment or health.	Early pathogen detection for bioreactors.	Achieve understanding of pathways and networks of directed evolution studies with species and consortium growth on Earth and Low Earth Orbit conditions to understand mutations and adaptation processes of microbes in different environments.
Biobased sensor within the interior shell of the spacecraft to identify the location of leakages or other damage with a cell-based or cell-free indicator (i.e., color change).		

Short-term **Medium-term** **Long-term**

Goal: Continuous production, recycling, and remediation of habitat air.

Current State-of-the-Art: Using engineering biology, we can leverage biological processes as a mechanism for air sensing, purification, and production, resulting in continuous access to breathable habitat air. For NASA purposes, cabin air must meet very specific quality standards to be considered acceptable, as per the Spacecraft Maximum Allowable Concentrations (SMACs), which define acceptable levels of air pollutants. Onboard air systems must also include the ability to monitor, control, and prevent air pollution (NASA OCHMO, 2023d). There are a variety of air purification and generation systems currently in use onboard the International Space Station (ISS) that work together to provide clean air. The Air Revitalization System (ARS) is responsible for temperature and humidity control and cleans circulating cabin air through the removal of carbon dioxide, and trace contaminants produced by, for example, onboard electronics (NASA, 2017; NASA OCHMO, 2023b; NASA OCHMO, 2023a). Cabin air flows through three filtration units: an activated charcoal bed, a catalytic oxidizer, and a lithium hydroxide bed; molecular sieves are used to remove carbon dioxide (NASA, 2017; NASA OCHMO, 2023b). The Oxygen Generation Assembly (OGA) generates oxygen for crewmembers aboard the ISS. Potable water from the Water Recovery System (WRS) is electrolyzed, producing oxygen and hydrogen as a by-product; oxygen is then delivered to the cabin for crew use, while hydrogen is utilized by the Carbon Dioxide Reduction Assembly (CDRA) to regenerate the molecular sieves (NASA, 2017; Dominguez et al., 2023; Jones, 2016). Captured carbon dioxide and hydrogen derived from the OGA are used in a Sabatier Reactor to recover water. Methane is produced as a byproduct and is vented into space (NASA, 2017). The CDRA is a closed-loop system, where power is the only consumable required for operation, aiding in water conservation. The system does require significant maintenance and replacement parts.

Engineering biology offers the opportunity to increase the air system efficiency, reliability, resilience, and sustainability. Biological systems can complete habitat air production, recycling, and remediation reactions at low temperatures using abundant materials and self-regenerating catalysts. Biosensing technologies can be applied to cabin air monitoring systems for stable, functional, and eventually self-replicating sensing networks. Protein engineering of relay systems and transcription factors can be leveraged to detect air contaminants in real time and synthetic olfactory receptors may be able to detect odorants in recycled air that may cause discomfort (Quijano-Rubio et al., 2021). Engineered microbes, plants, and fungi may be used to filter environmental contaminants, either through biological or physical means. Any carbon captured through these processes may then be used for biomanufacturing of food, pharmaceuticals, or materials (Santomartino et al., 2023). A continuous, self-sustaining, cell-free bioreactor could be developed that generates oxygen while actively scrubbing carbon dioxide. A self-sustaining air microbiome could be established that further promotes crew health and well-being, with the potential to integrate with the spacecraft ECLS system. Current needs for further development include, but are not limited to, a greater understanding of microbial ecology in enclosed habitats, and modeling that accurately captures parameters and operation space where biology would either be desired or meets energy and resource demands that cannot be met abiotically.

Breakthrough Capability: Continuous biomonitoring of habitat air.

- Short-term Milestone: A comprehensive understanding of microbial ecology of habitat air in various enclosed environments (e.g., ISS, lunar and Mars transit, lunar and Mars base habitats).
    - Bottleneck: Reference materials and calibrants for airborne microbial surveillance.
        - Potential Solution: Leverage solutions from DHS Biowatch program, DoD surveillance programs, and ARPA-H BREATHE program (ARPA-H, 2024; DHS, 2024).
- Short-term Milestone: Multi-component biomolecular signal relay systems and transcription factors to detect specific air contaminants of concern (e.g., volatiles) (NASA OCHMO, 2023d; Quijano-Rubio et al., 2021).
    - Bottleneck: Molecular properties of air contaminants may differ on Earth compared to extreme environments, such as in space.
        - Potential Solution: Identify and engineer two-component sensors to detect target contaminants at low nanoMolar (nM) concentration under varying humidity and temperature.
    - Bottleneck: Identification of transcription factors for each air contaminant.
        - Potential Solution: Large scale screening of transcription factors from environmental samples.
        - Potential Solution: Protein design using directed evolution, *de novo* design utilizing Alpha Fold and related design tools.
- Medium-term Milestone: Engineer immobilized synthetic olfactory receptors to detect specific odorants or volatiles in recycled air.
    - Bottleneck: Identification of olfactory receptors for all odorants.
        - Potential Solution: Bioinformatics to help predict interactions between receptors and odors.
        - Potential Solution: Engineer olfactory receptors that are able to detect all desired odorants.
    - Bottleneck: Incorporate hybrid materials and membranes that use biological olfactory receptors with electrical interfaces (“nose-on-a-chip”).
        - Potential Solution: A chip with all 400 olfactory receptor-based sensors can be used to combinatorially detect all the odorants/volatiles in the environment.
- Medium-term Milestone: Real-time biosensing of habitat air that overcomes slow biological response.
    - Bottleneck: Commonly used biosensors based on gene expression circuits require time for an input to activate or inhibit the production of output signal.
        - Potential Solution: Exploit rapid biophysical properties such as inducible biomolecular condensate formation to control reporter output.
        - Potential Solution: Engineer enzymatic sensors that activate a reporter through post-translational modification or biosynthesis of an indicator component.

        - Potential Solution: Fluorescent-based protein sensor switches that change conformation upon binding of the chemical.
    - Bottleneck: Biosensors with high sensitivity and reliable data output in real-time.
        - Potential Solution: Develop methods to record and report biosensor activation and signal dynamics to interface with computers.
        - Potential Solution: Optical scanning and colorimetric or fluorescence-based detection.
- Long-term Milestone: Stable, functional, self-replicating biosensor arrays for air monitoring in microgravity environments.
    - Bottleneck: Differing sensing and reporting requirements for biological systems (e.g., bacteria, fungi, or phages).
        - Potential Solution: Engineer biofilm communities to sense combinations and gradients of volatiles to report concentration and spatiotemporal dynamics.
        - Potential Solution: Engineered electroactive microbes and microbial consortia with unique sensing capabilities that relay an electronic signal in response to their environment.

Breakthrough Capability: Bio-based filtration of biotic and abiotic pollutants from circulating habitat air.

- Short-term Milestone: Microbes able to sequester particulate matter and other contaminants (NASA OCHMO, 2023d).
    - Bottleneck: Known library of particulates and contaminants and biological mechanisms to capture them.
        - Potential Solution: Analyze air samples from different flight and habitat environments to understand the major contaminants and concentrations.
- Short-term Milestone: Enzymes capable of decontamination immobilized into porous structures built specifically for ISS or habitat module air filtration systems.
    - Bottleneck: Enzymes exhibit high instability and turnover.
        - Potential Solution: Enzyme cascades or enzymes attached to quantum dots in close proximity can improve kinetics (Breger et al., 2023).
        - Potential Solution: Continuous directed evolution of enzyme or AI-driven design to enhance stability and activity in space environments.
    - Bottleneck: Enzymes may require re-activation, specific coenzymes, or other molecules for optimal or continuous function.
        - Potential Solution: Establish minimal requirements (e.g., through rational or *de novo* protein design) for enzyme function.
        - Potential Solution: Engineer suitable feedback loop for continuous use.
    - Bottleneck: Enzymes appropriate for the air contaminants must be selected.
        - Potential Solution: Identifying enzymes from biochemical reaction databases (e.g. BRENDA, KEGG, Expasy) that are compatible with common air pollutants.
        - Potential Solution: Utilize transcriptomics data to mine relevant enzymes for air contaminants decomposition.

    - Bottleneck: Liquid environments are typically required for enzyme activity.
        - Potential Solution: Bioengineer materials such as polysaccharides with tunable hydrophilicity and binding protein domains to support enzyme docking and activity.
        - Potential Solution: Coat immobilized enzymes with a material that retains moisture but is permeable to air contaminants.
        - Potential Solution: Utilize liquid flow-through technologies (similar to bubbling gasses through a liquid culture on Earth) for gas transfer.
    - Bottleneck: Enzyme-doped materials require expensive scale-up and purification to manufacture.
        - Potential Solution: Engineer extremotolerant enzymes that require minimal purification from production hosts (i.e., secreted or effective when used as crude lysate) and can withstand membrane or porous material manufacturing.
- Medium-term Milestone: Engineered microbes that employ new non-Calvin cycle carbon dioxide (CO_2) fixation pathways that more efficiently capture excess CO_2 and pollutants from the circulating air habitat (Maurya et al., 2023; Cao et al., 2019).
    - Bottleneck: Limited research that examines microbe-assisted phytoremediation in air pollutants.
        - Potential Solution: Identify relevant degradation enzymes and uptake and removal mechanisms of air pollutants by microbes with non-Calvin cycle CO_2 fixation pathways (Westernberg & Peralta-Yahya, 2023).
    - Bottleneck: Pollutants toxic to human health may also be toxic to bacteria/plants.
        - Potential Solution: Engineer or evolve organisms against toxicity.
        - Potential Solution: Maintain a microbial, organismal, gene, or plasmid library for specific applications against toxicity.
        - Potential Solution: Engineer bacteria or plants with improved detoxification capacity and the ability to sequester toxic chemicals.
    - Bottleneck: Microbial capacity to sequester CO_2 is limited so far.
        - Potential Solution: Combine plants and microbes together to improve CO_2 sequestration levels; the efficiency of phytoremediation could be improved by gene editing the plant themselves with genes from microbes (Pandey & Bajpai, 2019).
- Medium-term Milestone: Plants and plant-derived structures able to filtrate pollutants.
    - Bottleneck: Surface area and diffusion rate of plants and plant-derived structures are limited by space conditions.
        - Potential Solution: Engineer plants with increased surface area, biofilm adherence, and degradation capabilities for increased air filtration.
        - Potential Solution: Engineer plants for maximum air uptake through stomata and larger intercellular spaces.
    - Bottleneck: Unknown identity of air contaminants prevents targeted engineering to eliminate.
        - Potential Solution: Characterize typical air contaminants in the space environment.

- Medium-term Milestone: Fungi able to produce mycelium structures that can act as physical and chemical filters.
    - Bottleneck: Controlled assembly and composition of mycelium structures.
        - Potential Solution: Use genetic tools to implement new phenotypes based on fundamental structure-function relationships of mycelium
        - Potential Solution: Increase selectivity by adding a protein adsorbent as part of the mycelium structure (Urbina et al., 2019).
        - Potential Solution: Engineer fungi to produce mycelium with required biological functions to enable directed production of biomaterials for air filtration (Chen et al., 2024).
    - Bottleneck: Limited knowledge around metabolic pathways in mycelium.
        - Potential Solution: Conduct more research or use computerized modeling to increase understanding of fungi production of mycelium as a filter.
- Long-term Milestone: Genetically engineered space crop plants and phyllosphere epibionts that sense, filter, ***and*** decontaminate biotic and abiotic pollutants.
    - Bottleneck: Space crop plants with desired air filtration properties that can maintain stability and function in space.
        - Potential Solution: Engineer novel phyllosphere epibionts to stably colonize plants and provide desired air filtration functions.
    - Bottleneck: Ensure organisms are able to withstand extreme conditions while maintaining optimized air filtration capacities.
        - Potential Solution: Engineer relevant biological entities (plant seeds, rhizosphere components) to be radiation and temperature resistant with the ability to enter long-term stasis at room temperature through spores or lyophilization.
        - Potential Solution: Test the survival of organisms under accelerated selection pressure for traits that enable robust growth under known and predicted space ecosystems.

Breakthrough Capability: Robust bioprocesses for production, capture, and conversion of environmental gasses to habitat air.

- Short-term Milestone: Microbial consortia able to fix carbon dioxide (CO_2) and produce Oxygen (O_2).
    - Bottleneck: Potential organisms must be analyzed by evaluating large datasets to generate optimal consortia for conversion of CO_2 to O_2.
        - Potential Solution: Utilize AI and machine learning to generate and refine models of existing consortial organisms that fix CO_2 and produce O_2.
- Short-term Milestone: A proof-of-concept modular or adaptable biochemical pathway that sequesters carbon monoxide (CO) that could be implemented across different cell types or in a cell-free system.
    - Bottleneck: Optimizing enzymes with desired function in naturally-occurring carbon monoxide sequestering pathways.

        - Potential Solution: Prototype biochemical pathway candidate in cells or cell-free system to identify the plausible reconstruction of CO-sequestering pathway prior to installation to living organisms.
    - Bottleneck: Storage of sequestered CO and other compounds.
        - Potential Solution: Modular connection of CO-sequestration with CO-valorization pathways.
- Medium-term Milestone: Space-tolerant organisms with carbon sequestration pathways.
    - Bottleneck: Carbon sinks in microbes may reduce overall availability of carbon for spaceflight participants.
        - Potential Solution: Modular design of sequestration and carbon fluxes to useful products or feedstocks (i.e., commodity chemicals) (Gao et al., 2022).
        - Potential Solution: Robust high-flux secretion systems for microbial biomass (e.g., biofilms, exopolysaccharide, polymers, etc.).
        - Potential Solution: Programmable or inducible systems for controlling bioprocesses in non-model organisms capable of gas metabolism, including methanogens, methanotrophs, acetogens, chemolithoautotrophs, etc.
    - Bottleneck: Bioprocesses involving multiple components that need to be induced at the same time, such as multi-step enzymatic reactions, are under-developed.
        - Potential Solution: Use a common inducible regulator for all components or encode all components in a single operon.
        - Potential Solution: Harness endogenous systems for co-translation or alternative RNA splicing.
    - Bottleneck: Limited metabolic engineering tools to genetically manipulate non-model organisms capable of gas metabolism.
        - Potential Solution: Multiplexed CRISPR-Cas tools for relevant non-model organisms.
- Medium-term Milestone: Autotrophic organisms capable of rapid carbon fixation and interconversion to quickly balance environmental and energy demands in space habitats.
    - Bottleneck: Autotrophy is rate-limited by the concentration of the carbon-fixing mechanism's reactants and availability of energy to drive the reaction.
        - Potential Solution: Engineer bifurcated and dismutable metabolic pathways.
        - Potential Solution: Design synthetic energy-conserving electron bifurcating enzyme complexes (i.e., methanogenesis and PSII).
        - Potential Solution: Novel biochemical pathways with optimized rate-yield balance that are appropriate for the intended application.
        - Potential Solution: Engineer adaptable enzymes that operate with high efficiency in various environmental conditions (i.e., RuBisCO with high or low CO_2 atmosphere).
        - Potential Solution: Use AI/ML to predict carbon fixation and conversion of enzymes under extreme environmental conditions.

        - Potential Solution: Develop biological/electrical interfaces that can use electrical energy as a driving force for biochemical reactions.
- Medium-term Milestone: Microbial consortia capable of highly-efficient CO_2 capture and O_2 production (Fahrion et al., 2021).
    - Bottleneck: Existing consortia may not be able to capture and produce the amount of gasses necessary due to volumetric biomass, water requirements, and gas-liquid mass transfer limitations.
        - Potential Solution: Engineer hypersaline microbes capable of photosynthesis, methanogenesis, and acetogenesis under different water activity levels.
        - Potential Solution: Utilize metabolic engineering and directed evolution to enhance the gas metabolism in desired organisms.
        - Potential Solution: Develop bioprocesses that can rapidly (<12h) scale up and down to maintain habitation thresholds.
        - Potential Solution: Develop novel bioreactors with high-efficiency CO_2 delivery systems.
    - Bottleneck: Stability of multiple genetic programs across members of microbial consortia.
        - Potential Solution: Engineer kill-switches or inactivation under certain conditions.
- Medium-term Milestone: Harness pathways characterized from organisms with desired metabolic activity to build cell-free bioreactors capable of CO capture at concentrations relevant to the space context.
    - Bottleneck: Enzymatic sequestration outside of typical aqueous solution.
        - Potential Solution: Engineer surface-associated CO sequestration or transformation into biofilms, mycelia, and plant tissues.
        - Potential Solution: Use chemical scrubber cartridges to capture CO from air and regenerate through solvent release into liquid culture medium (e.g., fresh or saline water).
        - Potential Solution: Enzymes that work in solid state and micellar levels of water to convert CO.
- Long-term Milestone: Autonomous biofiltration system capable of regulating CO_2 and O_2 concentrations in the space environment.
    - Bottleneck: Integration of system with materials involved in ventilation, filtering, and central air (adsorbed or a DSP step).
        - Potential Solution: Incorporate engineered biosensors that provide electronic signals relaying status in real-time.
        - Potential Solution: Encapsulate cells or cell-free lysate in a membrane to sense and respond to contaminants.
    - Bottleneck: Creation of synthetic cells that are self-replicating and self-sustaining.
        - Potential Solution: Encapsulate synthetic chromosome(s) with necessary proteins and small molecules within artificial vesicles or natural membranes.

    - Bottleneck: Creation of biological organisms capable of monitoring and regulating CO_2 and O_2 levels through gas-consuming and -forming reactions.
        - Potential Solution: Biological systems that are engineered for a specific range of CO_2 and O_2 concentrations; lack of signaling from these pathways could trigger translation of enzymes or signal pathways that bring the system back to set point (Luo et al., 2022; Schwander et al., 2016).
    - Bottleneck: Biological systems exclusive of plants or photosynthetic organisms able to convert CO_2 in the environment into mission-important compounds, materials, or products.
        - Potential Solution: Engineer CO_2 consuming organisms for the production of value-added products (e.g., polymers, food).
        - Potential Solution: Engineer *Escherichia coli* to convert CO_2 into value-added products.
        - Potential Solution: *In vitro* cell-free systems for rapid bioconversion of CO_2 into value-added components without loss of carbon to biomass (Jack et al., 2022).

Breakthrough Capability: Robust, complex habitat air microbiome.

- Short-term Milestone: A comprehensive understanding of microbial composition of habitat air in various enclosed environments (e.g., positive control planet-side that is beneficial for health, ISS, lunar transit, Mars transit, lunar base, Mars base).
    - Bottleneck: Validation of new and existing technologies with current reference materials and calibrants.
        - Potential Solution: Leverage solutions from DHS Biowatch program and include metagenomic sequencing (DHS, 2022).
- Medium-term Milestone: Compose or engineer an air microbiome that is self-sustaining in a microgravity environment.
    - Bottleneck: Limited data on the effect of microgravity on the composition of healthy microbiomes that exist in the Earth gravitational environment.
        - Potential Solution: Establish a testbed for long-term stability test in microgravity environment.
    - Bottleneck: Evaluation of microbiome stability is difficult without high-throughput sequencing infrastructure.
        - Potential Solution: Develop a miniature sequencing system, such as MinION, that is capable of deep sequencing at the microbiome level in space (Oxford Nanopore Technologies, 2024).
        - Potential Solution: Tag individual microbial consortia members with a unique fluorophore that can be used to assess changes in abundance.
- Long-term Milestone: A spacecraft with engineered microbiomes integrated into spacecraft that enhance air and life support systems
    - Bottleneck: Limited understanding of the long-term stability and evolution of engineered microbiomes in the space environment.

- Potential Solution: Conduct long-duration studies on microbiome stability and function under simulated spacecraft conditions, including radiation, microgravity, low pressure, temperature fluctuations, and habitat cycling.

Goal: Continuous, on-demand fresh water.

Current State-of-the-Art: Access to clean water is essential for crew health and safety on long-term missions in space. About one gallon of water per person per day is used for consumption, food preparation, and hygiene (Gaskill, 2023; NASA OCHMO, 2023e). Sources of water for filtration on the International Space Station (ISS) include urine, air condensate, and excess water from payload or maintenance activities, for which filtering, chemical treatment, and vacuum distillation is relied on for recovery of water (Gaskill, 2023). The ISS Water Recovery System (WRS) consists of the Urine Processor Assembly and Water Processor Assembly (NASA, 2017; Williamson et al., 2023). Water purity is checked by electrical conductivity sensors within the systems. ISS water and environmental control systems also employ a microbial check valve (MCV), a device designed to prevent the transfer of viable organisms within onboard water systems, to avoid bacterial contamination (Colombo & Greenley, 1980). The recovered water must meet stringent purity standards before it can be used to support crew activities. Currently, the WRS can recover and recycle an average of about 93-94% of the water on the ISS. A major water source that is currently not recoverable, is solid human waste (which is about 75% water). While impressive, a long-term mission in the future will require at least 98% water recovery to maintain crew health. A Brine Processor Assembly (BPA) was recently tested aboard the ISS to evaluate its ability to operate in microgravity, helping bring the Water Recovery System closer to the goal of 98% recovery. Additional standardization and flight certifications are still needed for proper use (Gaskill, 2023). Limitations on available physical space for water purification systems introduce the additional challenge of reducing the footprint and complexity of onboard filtration systems.

Biology is uniquely suited to address the challenge of water purification and generation in low resource environments. Biological processes can be leveraged for improved water sensing, purification, and generation, enabling continuous and on-demand access to fresh water. Engineering biology presents the opportunity to further improve and refine the Water Recovery System's (WRS) capacity and efficiency. Water recycling on the ISS does not currently employ biological processes, even though this is standard for secondary sewage treatment on the Earth (Sravan et al., 2024). Identification of biological pathways and activities that mimic current ISS chemical and physical distillation processes will help to establish engineering biology-enabled technologies capable of total water purification.

The identification and classification of potential contaminants and possible biological interventions will catalyze advancements in biosensor and bioremediation solutions for contamination in potable water systems. Contamination of potable water occurs mainly through the contamination of ground-supplied water, in-flight contamination (human metabolic products, chemicals and materials transferred from the spacecraft atmosphere), and the intentional addition of compounds (iodine for biocidal properties and/or minerals for flavor and

dietary enhancement) (NASA OCHMO, 2023e). Engineered microbial consortia may be used for water decontamination and adaptation to emerging pathogens, including the development of novel pathogens for rapid control of evolved biofouling microbes.

Collectively, there are numerous opportunities to leverage engineering biology for onboard recycling and generation of fresh water, many of which can be leveraged for other uses as well. Bio-based or bio-enabled materials can help to monitor, purify, and generate water onboard spacecraft for the health and safety of crew members during long-term flight. For example, bioplastics have been used as water filters to remove microbes. Microbial desalination cells (MDC) may be leveraged to produce fresh water while breaking down organic matter, removing ions, and generating electricity. And trace elements may also be recovered through biological processes for further use in other onboard fermentation processes.

Breakthrough Capability: Biosensing and bioremediation of contamination in potable water systems.

- Short-term Milestone: Identified potential water system microbial contaminants in space environments to target with biosensors.
    - Bottleneck: Limited data collection and testing of potential microbial contaminants on the ISS.
        - Potential Solution: Study microbial physiology of human-associated and environmental microbes under microgravity and partial-gravity conditions to better understand and predict microbial phenotypes in space (Tender, 2024).
    - Bottleneck: Long-term, real-time monitoring of changes in microbial contaminants.
        - Potential Solution: Use of LAMP, miniPCR, or MinION technologies for targeted detection and sequencing onboard spacecraft of specific contaminants (O'Neil, 2024).
        - Potential Solution: Use human gut microbiome reference materials to increase accuracy of identification from sequencing data (NIST, 2024).
        - Potential Solution: DNA-based flow assays for rapid detection and identification of pathogens (e.g., SHERLOCK) (Kellner et al., 2019).
        - Potential Solution: Handheld Raman spectrometry or paper-based biosensor for pathogen detection.
- Short-term Milestone: Biological interventions to selectively target and eliminate microbes of concern in the water supply.
    - Bottleneck: High prevalence of antimicrobial resistance among waterborne pathogens.
        - Potential Solution: Engineer a suite of phages that can selectively target microbes of concern in the water supply.
        - Potential Solution: Introduction of bioengineered cells, such as yeast, that secrete antimicrobial peptides or glycosyl hydrolases to disrupt bacterial cells and biofilms.

        - Potential Solution: Filter the microbes from the water supply with plants.
        - Potential Solution: Engineer antimicrobials activated by exposure to specific microbes of concern.
    - Bottleneck: Phage contamination that could destroy other microbial production hosts onboard.
        - Potential Solution: Detection of pathogenesis biomarkers from microbes of concern.
    - Bottleneck: Evolution of phage resistance to antimicrobial treatments.
        - Potential Solution: Phage therapeutics to infer pathways and processes of evolution (Sentinel Environmental, 2024).
        - Potential Solution: Engineer synthetic phages to counter problematic phage strains.
    - Bottleneck: Ability to predict specific contaminants in the future water supply.
        - Potential Solution: Deploy high-throughput screening approaches, such as sequencing and mass spectrometry on the ISS, commercial space stations, and lunar habitat to catalog contaminants in current and future water recovery and storage systems.
        - Potential Solution: Use high-throughput screening techniques to identify natural or engineered organisms that can degrade specific contaminants.
        - Potential Solution: Reprogrammable CRISPR-Cas systems that secrete a short-lived microbial toxin to specific pathogens responsible for observed microbial contamination.
- Short-term Milestone: Biomaterials that are resistant to biofilm formation for water filtration systems (Jiang et al., 2024).
    - Bottleneck: Lack of microbial attachment to materials may compromise water treatment capability of the microbes.
        - Potential Solution: Embedded, fiber-based or self-contained units with proteins capable of binding bacterial surfaces to support engineered microbes for water treatment (Shiral Fernando et al., 2023).
- Medium-term Milestone: Ability to detect and disrupt biofilms that may arise in water recovery and storage systems.
    - Bottleneck: Limited detection systems for biofilms.
        - Potential Solution: Engineer production of small antagonists for quorum sensing of biofilms.
- Medium-term Milestone: Capability to produce novel phages on space missions for rapid control of evolved biofouling microbes.
    - Bottleneck: Limited cargo capacity to establish space-based infrastructure for phage engineering.
        - Potential Solution: Leverage cell-free expression systems for rapid, on-pot expression of phages to reduce infrastructure and reagent requirements (Levrier et al., 2024).
        - Potential Solution: Reduce infrastructure requirements for phage display by transitioning traditional screening methods to multi-well plate format (Ng et al., 2021).

- Long-term Milestone: Microbial systems and consortia for decontamination that can adapt to dysbiosis and emerging pathogens.
    - Bottleneck: Detecting unbalanced microbial systems and identifying the undesirable population.
        - Potential Solution: Nano-sequencing combined with a functional prediction platform to characterize populations, such as SeqScreen that includes both taxonomic and functional prediction for all microbes (Balaji et al., 2023).
    - Bottleneck: Rebalancing the microbial system with minimum impact on the active population.
        - Potential Solution: Engineered synthetic phage as microbiota modulators to target and destroy pathogenic species in a population (Singha et al., 2023).
- Long-term Milestone: Decontamination of water by infiltration percolation through fungal, plant-soil, or plant-hydroponic substrates.
    - Bottleneck: Lack of appropriate material for infiltration percolation.
        - Potential Solution: Systematic exploration of regolith (size and composition) as potential filtration material, to determine optimal packing properties (Benbouzid et al., 2024).
    - Bottleneck: Accumulation and concentration of inorganic salts and ions in the collected potable water.
        - Potential Solution: Biological ion exchange in bacteria to selectively remove specific ions and salts from water (Liu et al., 2020).
        - Potential Solution: Use halophilic microbes such as *Bacteroidetes* and *Chloroflexi* for desalination and bioremediation (Huang et al., 2020; Harpke et al., 2022).

Breakthrough Capability: Highly-efficient biobased recycling and purification of wastewater.[7]

- Short-term Milestone: Plant barriers, combined with synthetic water pumps, specific for wastewater contamination in NASA systems.
    - Bottleneck: Efficiency of plants to uptake a wide range of pollutants.
        - Potential Solution: Introduce beneficial microbes to enhance the ability to uptake or filter contaminants through synergistic relationships with plants.
    - Bottleneck: Achieving optimal growth conditions for plants in a microgravity environment.
        - Potential Solution: Engineer closed growth systems with controlled humidity, temperature, and light conditions.
        - Potential Solution: Use specialized soil that retains water and nutrients for plant uptake in microgravity.

[7] In comparison with the below Breakthrough Capability which focuses on remediation of contaminated water in rapid response to an unexpected event, this Breakthrough Capability focuses on normal, ongoing, and sustainable water system operations.

- Short-term Milestone: Engineered biological components that enhance self-regeneration of membrane filters.
    - Bottleneck: Ability to generate biological components that can be sustained in filters.
        - Potential Solution: Genetically modify biological components for persistence in continuous flow environments.
- Short-term Milestone: Identified biological processes that mimic current ISS chemical and physical distillation.
    - Bottleneck: Current ISS water systems are designed to minimize microbial growth whenever possible, limiting testing and implementation.
        - Potential Solution: Characterize any natural biofilms accumulating in ISS systems to understand limitations in current filtration systems and circumstances promoting biofilm growth and development.
        - Potential Solution: Study microbial community structure and physiology of wastewater management systems *in situ* and under low-gravity conditions and how they change with time and feedstock.
- Short-term Milestone: Utilize urine distillation brine as a source of salts and minerals for defined culture medium for fermentations, plant fertilizer, or other uses (Speight et al., 2024).
    - Bottleneck: Concentration of toxic compounds.
        - Potential Solution: Use of a microbial consortium in a medium that will tolerate and reduce concentration of toxic compounds.
        - Potential Solution: Selectively filter or sequester toxic compounds away from useful compounds with engineered microbes.
        - Potential Solution: Biochemically convert urea from urine into valuable products such as fatty acids, volatiles, or methane (Zhang et al., 2023).
        - Potential Solution: Engineer or use adaptive evolution to reduce toxicity to microbes.
    - Bottleneck: Urine will have a broad range of pH, osmolarity, specific gravity, etc.
        - Potential Solution: Include extremophile microbes with buffering properties, such acidophilic and alkaliphilic microbes, in the consortium.
- Short-term Milestone: Expansion of state-of-the-art methods for the conversion of blood into potable water.
    - Bottleneck: Existing methods are proprietary and have been demonstrated only with pig blood as the starting material (Veos, 2024).
        - Potential Solution: Partner industry experts and researchers to develop novel methods.
- Short-term Milestone: Reduce footprint and complexity of the filtration system to meet mission requirements.
    - Bottleneck: Purification process is costly and requires a large footprint that would be unrealistic in low-resource settings.
        - Potential Solution: Optimize a robust filtration system to utilize minimal resources.

- Medium-term Milestone: Algal and cyanobacterial farming systems that provide water filtration (Revellame et al., 2021; Mapstone et al., 2022).
    - Bottleneck: Algal growth requires sufficient light and tightly controlled CO_2 conditions for optimized metabolic functions.
        - Potential Solution: Use of purple non-sulfur bacteria for wastewater remediation and biofertilization (Cerruti et al., 2020).
        - Potential Solution: Engineering and use of algal strains, such as *Galdieria* and *Euglena*, with adaptable metabolism to physio-chemical changes likely in space, including low-light conditions.
- Medium-term Milestone: Microbial desalination cells (MDCs) used as a bio-electrochemical system to break down organic matter in wastewater while simultaneously generating electrical energy.
    - Bottleneck: Accumulation of salts and other highly concentrated, and potentially toxic, chemicals.
        - Potential Solution: Couple enzymes capable of sequestering or eliminating toxic chemicals with membranes to treat water without biofilm formation.
        - Potential Solution: Engineer microbes that are highly-efficient at biological ion exchange, denitrification, and salt-accumulation.
        - Potential Solution: Application of halophiles; for example, *Vibrio natriegens* or *Halomonas* species are salt-tolerant and fast-growth bacteria that are genetically tractable.
- Medium-term Milestone: Recovery and reuse of desired non-microbial contaminants in wastewater for plant and microbial growth.
    - Bottleneck: Limited quantity of materials and chemicals recovered for onward utilization.
        - Potential Solution: Bioaccumulation of trace elements through the biological secretion of gelling agents (e.g., hydrophilic polysaccharides such as hemicellulose or pectin) that sequester metal cations; these hydrogels could be reintroduced into the plant growth matrix.
    - Bottleneck: Re-capture of trace elements and contaminations after biosequestration.
        - Potential Solution: Harvest and physically separate cells using membranes or magnets.
- Long-term Milestone: Microbial consortia capable of total water purification and reclamation of ion, nitrates, and salts for other uses.
    - Bottleneck: Microbes that do not naturally coexist in the same ecological niche may be required for removal of NaCl, nitrates, and other salts.
        - Potential Solution: Create a structured or staged microbial consortium that can processively remove the necessary components.
    - Bottleneck: Lack of measuring capabilities of the metabolic exchange among microbes, driving negative interactions in consortia and decreased consortia biodiversity.

        - Potential Solution: Robust and scalable cross-feeding circuits based on synthetic communities.
        - Potential Solution: Quick design of interventions based on principal waste components with computational tools (Focil et al., 2024).

Breakthrough Capability: Generation and conditioning of fresh water from in situ resources.

- Short term Milestone: Scaled plant systems that desalinate water for enhanced water recycling (Medford et al., 2023).
    - Bottleneck: Slow time to engineer and edit plant platforms.
        - Potential Solution: Use AI and ML algorithms to predict most efficient conditions for rapid plant growth.
- Short-term Milestone: Map of water net-positive (chemically producing water) and water net-negative (chemically destroying water) metabolic processes known in current microbial strains to aid design choices for overall water balance.
    - Bottleneck: Characterization of metabolic water dynamics in microbes would generate extensive data.
        - Potential Solution: Modeling that buckets circuits and pathways with similar processes where a single engineering tool would be sufficient.
- Short-term Milestone: Highly-efficient ethanol bioproduction and bioreclamation cycle for salt fractionation.
    - Bottleneck: Lack of efficiency in processing waste feedstocks to final composition of ethanol brine.
        - Potential Solution: Engineer microbes to improve fermentation efficiency and resistance to conditions in brine.
- Medium-term Milestone: Engineered microalgae for biodesalination (Nadersha & Hassan, 2022).
    - Bottleneck: Lack of understanding in resource allocation of hyper-halotolerant microbes.
        - Potential Solution: Characterization of the metabolism of hyper halotolerant microalgae to high salinity (Wei et al., 2017; Tibocha-Bonilla et al., 2023; Rickard, 2023).
- Medium-term Milestone: Engineered edible microalgae for combined food production and water treatment.
    - Bottleneck: The leftover waste may have an imbalance of salt, nitrogen, carbon ratio, and residual contaminants.
        - Potential Solution: Engineered plant cell walls with required charge to sequester unwanted contaminants, such as heavy metals released from cadmium-plated components through corrosion (Khan-Mayberry et al., 2011; Parrotta et al., 2015).
- Medium-term Milestone: Microbial consortia implemented for freshwater generation from brine.
    - Bottleneck: Toxicity from accumulation of certain types of salt.
        - Potential Solution: Engineered microbes to remove specific salts.

- Long-term Milestone: Self-generating, self-renewing organismal consortia for freshwater generation without long-term maintenance.
    - Bottleneck: Genetic drift may reduce water filtration quality.
        - Potential Solution: Couple water-conditioning to growth in microbial consortia that utilize the undesired contaminants as energy-source (Ruff et al., 2023).
- Long-term Milestone: Conversion of ambient and waste gases (e.g., methane, hydrogen, and oxygen) into water by microbes (Ortiz et al., 2021; Siebert et al., 2021; Chu et al., 2020).
    - Bottleneck: Bioavailability of methane, hydrogen, and oxygen.
        - Potential Solution: Identify the most efficient species of microbes that can maximize water output with minimal gas input.

Goal: Waste remediation and recycling.

Current State-of-the-Art: Improved waste management, remediation, and recycling are necessary to support long-term human spaceflight. The waste management systems actively in use are adequate for current mission needs, but are not sufficient for missions longer in duration and further from Earth. Together with biological waste, a large portion of future payloads will include synthetic waste, such as electronics, packaging, and clothes. These materials are not currently considered in classic life support systems; however, they contain valuable carbon, oxygen, hydrogen, metals, and other elements. If recycled, they could become precious feedstock materials, particularly for long-term missions where supply-chains are limited or impossible (Santomartino et al., 2023). A focus on recycling rather than disposal will also increase the sustainability of all future human space exploration endeavors.

Waste remediation procedures for spaceflight do not currently include solid waste processing and instead focus on waste storage (Simon et al., 2017). Solid waste management on the ISS relies on astronauts to manually load waste into bags for short-term storage. The waste is eventually either returned to Earth or ejected from the aircraft, burning up in Earth's atmosphere. NASA's Trash to Supply Gas project (TtSG) investigated the effectiveness and efficiency of a variety of waste processing techniques (including incineration, steam reforming, ozone oxidation, pyrolysis, and catalytic wet air oxidation) for conversion to useful products (Caraccio & Hintze, 2013; Benvenuti et al., 2020). NASA's Waste to Base Materials Challenge, sought novel solutions to the conversion of waste from different streams (including general trash, fecal material, foam packaging, and exhaled carbon dioxide) into usable base materials for propellants and other products. Two additional challenges, the Trash-to-Gas Management Challenge and the Waste Jettison Mechanism Challenge were subsequently launched to further catalyze research and development (Douglas, 2022). These initiatives are all part of the broader Logistical Reduction and Repurposing (LRR) effort led by NASA to reduce logistical mass during long-term spaceflight and habilitation (Caraccio & Hintze, 2013; Benvenuti et al., 2020). Although not yet realized, NASA's Advanced Exploration Systems Mars Transit Habitat (MTH) Refinement Point of Departure Design includes plans for a human waste collection system and a compactor/storage system at a combined 67 kilograms (not

accounting for the mass of human waste, which can be estimated at a wet weight of 100-500 grams per person per day) (Simon et al., 2017).

The Advanced Life Support Sizing Analysis Tool (ALSSAT) is a computer model that performs analysis of conceptual designs for ECLSS systems for spacecraft and surface habitats. Intended for use on missions to the Moon and Mars, the program includes considerations for biological and physicochemical processes. The tool aids researchers in investigating and understanding combinations of advanced life support (ALS) systems to optimize waste processing efficiency (Yeh et al., 2005; Benvenuti et al., 2020). Waste management techniques are an area of great interest to space agencies and increased investment signals a need for advancement. Engineering biology offers a variety of solutions to improve waste management in low-resource environments.

Human waste is possibly one of the most accessible mediums for bioprocessing. Numerous microbial technologies are available and could be optimized for the processing of human waste, including the recovery of valuable nutrients, chemicals, and compounds. Recent research investigating the use of Black Soldier Fly larvae (BSFL) to recycle human waste offers an additional alternative to solid waste management (Banks et al., 2014; Purkayastha & Sarkar, 2023). Currently, human waste is not an ideal substrate for the production of BSFL biomass, as it has a low nutritional profile and is a volatile growth medium, but has the potential for optimization through the application of engineering biology technologies (Purkayastha & Sarkar, 2023). Engineering biology introduces the potential for human waste-derived nutrients and hydroponic growth media that support plant growth, further promoting the closed-loop conversion of solid waste into food precursors and other products.

Engineered biology can also be used to process other biotic and abiotic waste streams. On Earth, anaerobic digestion for agricultural and municipal waste has been extensively researched and has potential spaceflight applications with slight modification, as spaceflight waste streams will vary from those produced on Earth. Further studies should investigate the use of anaerobic digestion in space, particularly how the space environment will impact its function and operation (Benvenuti et al., 2020). Toward this end, the European Space Agency (ESA) spearheads the Micro Ecological Life Support System Alternative (MELiSSA), which aims to optimize digester design for use in space and improve biogas yield (Benvenuti et al., 2020; ESA, 2024).

Microbial strains and consortia can be engineered to have a high tolerance to the microbial inhibitors found in wastes. Unique biological traits, such as that of extremophiles, can further expand wastes usable as feedstocks, highlighting the importance of novel biological toolkits development that can function across a variety of organisms from model to non-model chassis. And microbial degradation of single polymer plastics and packing materials into polymers and/or monomers is a possibility with advancements in engineering biology. Advanced solid waste processing technology, like anaerobic digestion and the use of BSFL, also has possible applications in austere Earth environments, where resources and physical space are limited, and system efficiency is of the utmost importance.

Breakthrough Capability: Bioprocesses for human waste management.

- Short-term Milestone: Engineered microbial strains or consortia with high tolerance to microbial inhibitors found in human waste (aromatic compounds, ammonia/salts).
    - Bottleneck: Multiple genes and signaling pathways in microbial oxidative stress that may alter strain functionality.
        - Potential Solution: Multiplex knockout libraries or evolution strategies to build tolerance for common inhibitors (or mixture of inhibitors).
        - Potential Solution: Identification of pathways that detoxify the inhibitors and can be provided as heterologous genetic cassettes.
        - Potential Solution: Utilize a specific suite of strains to detoxify waste and a different set of strains for biomanufacturing.
- Short-term Milestone: Human waste-delineated medium to support growth of a microbial community to process human waste.
    - Bottleneck: Granular microbiome measurement to improve sample preparation and in-depth sequencing processes during space flight.
        - Potential Solution: Develop rapid sequencing capabilities that require minimal resources for relatively small-scale microbial communities.
- Short-term Milestone: Defined human-waste derived hydroponic growth media that supports plant growth.
    - Bottleneck: Human wastes, especially during spaceflight, may lack crucial components for hydroponic growth.
        - Potential Solution: Identification of missing or limited components and potential alternative sources to generate or harvest those from within the station or planetary surface systems.
    - Bottleneck: Human waste composition varies over time and by person.
        - Potential Solution: Compare human waste between individuals during spaceflight to determine changes in composition over time.
        - Potential Solution: Identify undesired compounds that may interfere with the hydroponic growth and develop a method to remove the potential contaminants.
- Short-term Milestone: Measurements of the mass-balance of carbon, nitrogen, oxygen, and micronutrients and minerals for human waste consumption in insect larvae (e.g., black soldier fly) (Banks et al, 2014; Purkayastha & Sarkar, 2023).
    - Bottleneck: Include all living systems (e.g., plants, microbes) on the space flight in the mass-balance equation to ensure 100% organic matter recycling is more realistic and predictable.
- Short-term Milestone: Food-safe insect larvae grown on human waste.
    - Bottleneck: Risk of transferring human fecal pathogens.
        - Potential Solution: Perform microbiome studies of larvae grown on human waste to determine the rate of pathogen carryover.
- Medium-term Milestone: Microbial production strains with consistent output in varying microbial communities, e.g., stool microbiomes.
    - Bottleneck: Microbial strains must be able to out-compete the microbial communities found in the waste-substrates.

        - Potential Solution: Leverage NIST Human microbiome reference material to benchmark complex microbial communities.
        - Potential Solution: Direct testing of liquid culturing of stool microbiomes.
        - Potential Solution: Database of isolated gut (human and animal) or sewage microbiota strains with characterized metabolisms.
    - Bottleneck: Toxic volatile organic compounds produced from microbial breakdown.
        - Potential Solution: Generation of absorptive systems suitable for volatile organic compounds.
        - Potential Solutions: Engineer strains that do not produce or break down volatile organic compounds.

Breakthrough Capability: Bioprocesses for biotic material waste management, including food waste.

- Short-term Milestone: Fully characterized metabolic fluxes of lignocellulose deconstruction and conversion by engineered microbiomes.
    - Bottleneck: Microbial hosts with optimized efficiency to intracellularly deconstruct and metabolize polymer byproducts.
        - Potential Solution: Engineer a strain to add to the microbiome that can increase metabolism of by-products.
- Short-term Milestone: Engineer fungi suitable for degradation of space-related plant lignocellulosic materials that could also be used as a potential food source.
    - Bottleneck: Identify fungi that can grow readily under Low Earth Orbit (LEO) conditions.
        - Potential Solution: Testing of different fungal mycelia growth and fruiting body formation on the ISS.
    - Bottleneck: Further breakdown of used mycelia waste to usable bioproducts
        - Potential Solution: Return of waste to plant growing or microbial platforms for further utilization or breakdown.
- Short-term Milestone: Identification of optimal insect candidates for waste-to-protein production in space conditions (Scoles, 2023).
    - Bottleneck: Limited understanding of insect motion in a microgravity environment, particularly impacting containment.
        - Potential Solution: Increase low gravity insect research.
        - Potential Solution: Growth ‘reactors’ with a microgravity environment that are suitable for insect production.
- Medium-term Milestone: Waste processing reactors that provide compatible feedstocks for use in a food-producing bioreactor.
    - Bottleneck: Different microbes possess a diverse degree of tolerance to contaminants and catabolic capability, leading to potential incompatibility in waste processing.
        - Potential Solution: Utilization of compatible microbial consortia to break down waste to simple feedstocks.

        - Potential Solution: Streamline microbial strains for each type of biological wastes with the most suitable processing capacity.
- Medium-term Milestone: Efficient waste-to-gas (CO_2, CO, CH_4, N_2) conversions using engineered microbial communities on a defined timeline.
    - Bottleneck: Variations in the feedstock can cause the microbial community to grow at different rates.
        - Potential Solution: Use a robust microbial community that is able to efficiently breakdown anything in the feedstock with few disturbances to the overall degradation and conversion process.
- Medium-term Milestone: Biomaterial regeneration loops for chitin exoskeletons of insects used for waste recycling (Tibbetts, 2023).
    - Bottleneck: Removal of minerals and other proteins from the chitin so that they are in a usable form.
        - Potential Solution: Use the exoskeletons as a feedstock for microbial cultures.
- Long-Term Milestone: Efficient downstream processes for separation and formulation of edible products from non-edible components.
    - Bottleneck: Multiple processes need to converge, which utilize high energy processing steps.
        - Potential Solution: Extracellular production of nutrients through cell wall manipulation of microbes.
- Long-term Milestone: A complete closed-loop conversion of solid waste into food using biological systems.
    - Bottleneck: Mechanical degradation of solid waste requires new technology in space habitat.
        - Potential Solution: Use insects as mechanical decomposers to liquify solid waste for downstream decomposition by microbes.
        - Potential Solution: Use fungi to decompose waste, particularly cellulosic wastes.
    - Bottleneck: Downstream processing and purification of bio-derived waste is necessary for capturing recyclable molecules.
        - Potential Solution: Use microbes or microbial consortia to catabolize organic waste into common bioproducts (e.g., proteins or polysaccharides) that can be easily isolated as food, material precursors, or feedstock nutrients.
        - Potential Solution: Engineer organisms specifically to purify bio-derived waste products.

Breakthrough Capability: Bioprocesses for abiotic material waste management, including plastic and electronic waste.

- Short-term Milestone: Effective genetic engineering tools for non-model microbes with valuable native metabolism for processing mission waste streams.
    - Bottleneck: Waste streams with carbon-rich components are under-utilized.

        - Potential Solution: Evolve native microbes that can utilize a variety of carbon and nitrogen sources.
- Short-term Milestone: Database with bioconversion efficiencies and limitations of platform systems (insect bioconversion, microbial bioconversion, etc.) for solid waste bioconversion in operationally-relevant environments (e.g., low gravity, low resource).
    - Bottleneck: Organism performance and gene expression differ vastly between ground conditions and analogs in space.
        - Potential Solution: Increased space-based testing (e.g., aboard the ISS, via small satellites) of the performance and gene expression of different organisms.
- Short-term Milestone: Efficient metabolic degradation of single polymer plastics under space conditions.
    - Bottleneck: Metabolic degradation is slow and requires high operating temperatures.
        - Potential Solution: Enzyme engineering and adapted laboratory evolution (ALE) to develop fast plastic degrading microbes.
        - Potential Solution: Opportunity to pair insect-microbe biosystems to accelerate degradation of hydrolyzable polymers.
    - Bottleneck: Limited knowledge of enzymes for non-hydrolyzable plastics deconstruction.
        - Potential Solution: Biomining and multi-omics enzyme discovery from alternative sources of plastics deconstruction, such as landfill consortia.
    - Bottleneck: Some polymers do not have known microbial degradation pathways.
        - Potential Solution: Search for new metabolic degradation pathways for plastics that are currently considered recalcitrant.
        - Potential Solution: Targeted enzyme engineering to evolve existing polymer degrading enzymes.
- Medium-term Milestone: Enzymatic degradation and recycling of mixed hydrolyzable plastic polymers into tractable monomers.
    - Bottleneck: Identifying the appropriate enzymes and their pathways for degradation and recycling of plastic polymers.
        - Potential Solution: Substantial research investment in searching for natural sources (insect microbiomes) of appropriate enzymes.
        - Potential Solution: Directed evolution and other protein design methods for efficient enzymes.
    - Bottleneck: Microbes often have regulatory pathways that restrict their ability to utilize multiple feedstocks (from mixed materials) simultaneously.
        - Potential Solution: Engineered consortia for plastic degradation and recycling (Bao et al., 2023).
        - Potential solution: Identify specialist microbes capable of deconstructing key polymers and additives (for example, through bioprospecting insect microbiota, landfills, etc.).
        - Potential Solution: Engineer microbes to remove catabolite repression.
    - Bottleneck: Secretion or separation of monomers from cell biomass.

        - Potential Solution: Engineered transport proteins to improve separation of monomers.
        - Potential Solution: Engineer solvent-tolerant microbes with plastic degrading pathways for organic overlay extraction (Rodrigues & Lindberg, 2020).
    - Bottleneck: Efficient multi-step deconstruction of polymers.
        - Potential Solution: Engineer 'plastisome' scaffolds analogous to fungal cellulosomes for rapid extracellular polymer deconstruction.
- Medium-term Milestone: Robust biorecovery of major elemental components (N, P, K, S, trace elements) from abiotic waste streams.
    - Bottleneck: Variability in the composition and nutrient content of waste streams.
        - Potential Solution: Develop flexible, modular nutrient recovery systems that can handle variations in influent composition.
        - Potential Solution: Use advanced sensing and control systems to monitor influent composition in real time and adjust process parameters accordingly.
- Long-term Milestone: Integration of plastic-waste degradation byproducts into life-support-system to improve loop-closure (Santomartino et al., 2023).
    - Bottleneck: Complete recycling efficiency is challenging.
        - Potential Solution: Engineer single or multi-enzyme systems to deconstruct plastics waste into products that can benefit life-support-systems.
- Long-term Milestone: Microbial and archaeal organisms able to convert brine into useful products for extended space travel.
    - Bottleneck: Archaeal halophilic organisms (e.g., *Haloferax*) are currently underutilized as tractable chassis.
        - Potential Solution: Continuous evolution of known halophiles for brine survival and upcycling.
        - Potential Solution: Engineer non-halophilic microbes with enhanced brine tolerance with proven space chassis.

Goal: Regolith and rock biomining, bioremediation, and transformation.

Current State-of-the-Art: Regolith from planetary and asteroid bodies in space presents the opportunity for (micro) nutrient extraction, remediation for agricultural purposes, and use as composite construction material. Lunar regolith is broadly characterized by fine-grained, sharp particles, much of which is derived from eroded anorthositic and basaltic minerals. Size, shape, and composition vary across the Lunar landscape and are largely dependent on exposure to solar wind and thermal weathering (Cool et al., 2023). Martian regoliths vary significantly in composition and oxychlorine compounds (perchlorates) and other radicals are present (Hecht et al., 2009).

NASA has a number of active efforts to investigate the utilization of Lunar regolith, including the use of microwave power in additive manufacturing methodologies (Watson et al.,

2024), pyrometer technologies for oxygen extraction (Engelhart et al., 2024), solidification of concrete in microgravity (Sperl et al., 2024; Mueller et al., 2023), and electrolysis of molten regolith for production of oxygen and metals (Werkheiser et al., 2024); however, these efforts are still in the very early stages (Manning, 2023). Regolith simulants have been developed to mimic the physical, chemical, or mineral properties of regolith native to the Moon, Mars, and asteroid rocks using geologic materials collected on Earth. There is no single Earth-derived regolith simulant that will mimic all physical and compositional properties, so often a selection of simulants is used for comprehensive testing of new measurements and technologies, with varying degrees of fidelity to true regolith (ARES, 2024; Metzger et al., 2019). Currently, no samples of Martian-sourced regolith have been collected and returned back to Earth, so most studies have used regolith simulants, materials from meteorites originating from Mars, and data collected during the Phoenix Mars Lander Mission in 2008 (Hecht et al., 2009; Naz et al., 2023).

Microbial biomining – the use of microbes to extract materials – offers a path to resource resolution and utilization from regolith (Gumulya et al., 2022; Santomartino et al., 2022), reducing payload and improving a mission's ability to operate independently. Recent advancements in space additive manufacturing have made microbial biomining a viable method of resource extraction (Gumulya et al., 2022; NASA, 2016). There is the opportunity for microbial extraction and remediation of heavy and rare metals. Combined with biological binders, Martian and Lunar regolith may even be utilized as sources of construction materials (Dikshit et al., 2022). To further advance these engineering biology capabilities, best practices and necessary tools for the creation of heavy metal and regolith-resistant engineered microbes must be identified and developed, with the eventual aim of microbial bioconcentration through biosequestration and heavy metal precipitation. The development of downstream biological methods for separation and conversion inside cells would also be of great significance and utility.

Opportunities also exist to transform regolith to support plant growth. This will likely require both bioremediation or bioconversion of regolith, as well as plant engineering, to make organisms robust to these non-Earth substrates. Recent work has explored plant growth on Apollo-era-collected lunar regolith (as compared to regolith simulant) (Keeter, 2022). Further study and lab modeling must be completed to demonstrate the required supplementations (nutrients, microbiome) necessary to support plant life. Recent studies have also demonstrated the potential for microbial growth in Martian regolith (derived from meteorites and fragments of Martian origin) (Naz et al., 2023).

Breakthrough Capability: Biological extraction of elements and compounds for material biosynthesis from regolith.

- Short-term Milestone: Engineering tools for microbes robust or resistant to heavy metals and regolith solubles.
    - Bottleneck: Model microbes are not tolerant to metals.
        - Potential Solution: High-throughput method of screening, engineering, and directed evolution of microbes from native mining environments for desired tolerance to metals or salt.

- Short-term Milestone: Bioconcentration of heavy metals through metal precipitation and sequestration pathways.
    - Bottleneck: A sustainable, circular, source of electrons to enhance metal precipitation and sequestration.
        - Potential Solution: Leverage artificial redox pathways.
        - Potential Solution: Utilize a microbial consortium containing both electrogenic and electrotrophic microbes for storage and recovery of electrons (Yates et al., 2017; Yates et al., 2024; Cockell et al., 2020).
- Short-term Milestone: Biological platforms for heavy and rare metals detoxification.
    - Bottleneck: High concentrations of heavy metals are usually toxic to life forms.
        - Potential Solution: Use cell-free systems or other pseudo-living systems with higher-tolerance for heavy metals instead of living organisms.
        - Potential Solution: Directed evolution of organisms to retain fitness in the presence of high concentrations of heavy metals.
        - Potential Solution: Identify extremophilic microbial species capable of surviving in conditions with high concentrations of heavy metals.
- Short-term Milestone: Determined chemical structure and biosynthetic pathways for specific metallophores produced by microbes.
    - Bottleneck: Limited understanding of chemical and biosynthetic properties of metallophores.
        - Potential Solution: Use bioinformatics to predict chemical and biosynthetic mechanisms based on prior genomic data.
- Medium-term Milestone: Recombinant microbial biosequestration strains that overproduce specific metallophores and uptake transporter systems for inorganic nutrients of interest.
    - Bottleneck: Microbial strains that maintain stability and biosequestration function in microgravity environments.
        - Potential Solution: Engineer microbes to overexpress genes involved in production of specific metallophores.
- Medium-term Milestone: Downstream bioseparation method to convert internalized or sequestered heavy metals and rare earth metals into a purer form for transportation or use as raw material for industrial processes.
    - Bottleneck: Scaling up separation methods to handle large quantities of liquid while maintaining a lightweight system suitable for space missions.
        - Solution: Develop novel separation methods that require minimal equipment and rely more on biological processes, such as sedimentation and flotation of biological structures.
- Medium-term Milestone: Biochemical pathways that convert heavy metal into usable forms.
    - Bottleneck: Identification of metal conversion pathways and associated genes for relevant protein products.
        - Potential Solution: Use integrative -omics studies and isotope labeling to discover native conversion pathways.

        - Potential Solution: Apply AI-based protein design to create non-natural pathways for metal conversion.
- Medium-term Milestone: Organisms that are able to secrete and tolerate large amounts of organic acid for heavy metal solubilization, conversion, or valorization.
    - Bottleneck: Soluble metals are highly toxic to organisms.
        - Potential Solution: Select organisms that are able to withstand the toxicity of heavy metals.
- Medium-term Milestone: Engineer robust chassis or microbial consortia containing multiple heavy metal uptake and detoxification pathways.
    - Bottleneck: Most bio-based heavy metal uptake and detoxification schemes require pathways specific to one type of metal.
        - Potential Solution: AI/ML algorithms that use prior data on metal uptake and detoxification collected on Earth to predict outcomes in space settings.
- Medium-term Milestone: Biological organization (e.g., DNA origami) of regolith-derived metals into semiconductor composites and wires.
    - Bottleneck: Accurate and precise incorporation of metals to maximize the function of the semiconductor.
        - Potential Solution: Engineer DNA origami with selective binding sites for desired metal ions.
- Long-term Milestone: Organisms capable of scavenging target micronutrients and minerals from regolith.
    - Bottleneck: Regolith may comprise high levels of elements that have the potential to contaminate the micronutrient extraction.
        - Potential Solution: Directed evolution of organisms to improve fitness when in the presence of high levels of elements.
    - Bottleneck: The acidified solution needed to convert insoluble elements in regolith to soluble forms is harmful to most microbes.
        - Potential Solution: Use microbes that are able to promote metal leaching from regolith particles and uptake micronutrients directly from the solid regolith particles.
        - Potential Solution: Utilize microbes isolated from acid-mine drainage sites or cavern sites with biotic-mineralization with natural capabilities.
        - Potential Solution: Engineer organisms or consortia with conversion mechanisms that are adaptable to different metals.
    - Bottleneck: Difficulty in engineering selective micronutrient scavenging without disrupting overall host metabolism and growth.
        - Potential Solution: Employ metabolic modeling and flux balance analysis to provide guidance for optimal genetic modifications for enhancing micronutrient scavenging while minimizing metabolic burden on the host.
- Long-term Milestone: Robust engineering toolkits that use various non-model organisms to extract (biological) elements and compounds from regolith.
    - Bottleneck: Lack of methods and resources for transforming non-model organisms in space.

        - Potential Solution: Identify focal non-model organisms and develop needed methods and resources for these species.
        - Potential Solution: Use genetic engineering approaches that do not rely on endogenous cellular machinery for integration (e.g., prime editing, transposons, exogenous CRISPR/Cas systems).
    - Bottleneck: Limited approaches to integrate genetic constructs into the genome that work across multiple species in space.
        - Potential Solution: Identify the cross-species modification systems.
        - Potential Solution: Data collection on the influence of codon bias and transcription factors across species on gene expression and functional capacity for DNA production.

Breakthrough Capability: Bioconversion of regolith to agricultural soils and growth substrates.[8]

- Short-term Milestone: Growth of vascular plants in soil with specific regolith compositions of organic and inorganic matter.
    - Bottleneck: Limited characterization of plant growth in high-fidelity regolith simulants.
        - Potential Solution: Better characterization of the physical and chemical properties of regolith simulants.
    - Bottleneck: Access to true lunar regolith is extremely limited and true Martian regolith is not yet available.
        - Potential Solution: Perform ground-based testing of plant growth in high-fidelity regolith simulants.
- Short-term Milestone: Robust control mechanisms for microbial composition, adaptable across complex soil types.
    - Bottleneck: Regolith and soil are complex environments that may introduce instability to engineered microbial communities.
        - Potential Solution: Perform longitudinal microbial community studies in high-fidelity regolith simulants with multiple plant species and generations to define initial microbial community that leads eventually to a sustainable optimal stable artificial microbial community structure.
- Short-term Milestone: Healthy space crop plants maintained by supplementations (e.g., organic, inorganic, or biotic) to specific regoliths to allow growth and food production.
    - Bottleneck: Limited understanding of supplementation to regoliths for food production.
        - Potential Solution: Pilot studies on earth in a microgravity environment to examine regolith supplementation.
- Short-term Milestone: Strategies to create or transport the necessary supplementations for regolith conversion on site.
    - Bottleneck: Efficient transport of these materials to a forward operational site in large quantities.

[8] For related opportunities, see also the Breakthrough Capability: “Highly-efficient and controllable biosystems for food crop growth and development.” in the **Food & Nutrition** theme of this roadmap.

        - Potential Solution: Use of local resources for on-site extraction of nutrients, water, and other organic matters from regolith.
- Medium-term Milestone: A database of potential biocontaminant strains of off-planet agricultural spaces, with species or strain level taxonomic resolution.
    - Bottleneck: Microbial contaminants will vary across missions and soil substrates.
        - Potential Solution: Collect samples from multiple missions for deep genome resolved metagenomic sequence-based characterization and strain resolution.
- Medium-term Milestone: Inorganic compounds obtained *in situ* from regolith for proper "soil" generation.
    - Bottleneck: Mass of carbon needed for organic matter may be limiting.
        - Potential Solution: Develop methods to extract CO_2 from the atmosphere, like a direct air capture system.
- Medium-term Milestone: Microbes engineered with different metal requirements to enhance bioconversion of regolith (i.e., engineer pumps to use metal abundant in the regolith other than sodium or lithium).
    - Bottleneck: Efficient transport systems for alternative metals in microbes.
        - Potential Solution: Engineer transport pumps that can exchange or transfer alternative metals in microbes (i.e., a pump similar to a Na+ / K+ ATPase but exchanges a different metal for potassium).
- Medium-term Milestone: Engineer a minimal microbial consortium that can generate soil that supports plant growth starting from a regolith base.
    - Bottleneck: Microbiome community composition and development will change depending on the kind of regolith the plants will be grown in.
        - Potential Solution: Characterize crop growth in supplemented high-fidelity regolith simulants to cross-reference with known potential consortia members.
- Long-term Milestone: Complete bioconversion of regolith to agricultural soils.
    - Bottleneck: Materials to construct and run process units may be difficult to procure (e.g., metal tanks, water, air, power).
        - Potential Solution: Efficient use of as many *in situ* resources as possible from the lunar environment (e.g., solar power, water-ice at lunar poles, electrolysis of water to produce oxygen).

Goal: Biosynthesis of habitat materials supporting human health.

Current State of the Art: As we work to improve long-term human spaceflight capabilities, the biosynthesis of habitat materials will play an important role to ensure mission longevity and sustainability. Currently, each item approved for use in space must meet specific requirements (weight and volume, durability, resilience, safety). Engineering biology could enable the production of biomaterials for manufacturing of durable and consumable goods to be used as small replacement parts, medical applications, or even spacecraft and habitat repair and expansion (Jemison & Olabisi, 2021; Averesch et al., 2023). Although potentially

very useful, biomaterials are not currently approved for use on the International Space Station or during spaceflight.

Biocement and bioconcrete, and other biologically engineered construction materials with self-healing properties, may be used as future habitat construction materials (Tyagi et al., 2023). Self-healing biomaterials with built-in (bio)sensors could be designed to detect and repair material damage autonomously, reducing the time needed for diagnosis and repair. For example, an air leakage in the Zvezda service module on the ISS in 2020 was hard to identify, let alone fix, during the mission (Garcia, 2020). The use of integrated biosensors may have, in this case, improved leak identification and sped-up the repair process. In addition to response and repair, biomaterials may increase crew health and comfort. Biogenic materials have vast potential and are capable of things like air conditioning, protection from radiation, energy generation, and acoustic dampening. These materials can be integrated into multipurpose, self-generating construction materials to provide health benefits all while improving cabin environmental conditions.

Biologically-produced materials may also replace the clothing, medical supplies, and fabrics used in spaceflight. Microbes may be engineered with adaptive traits for use on clothing (or directly on the skin) for further protection from extreme environmental conditions, in space or on Earth. Recent advancements in microbial engineering have enabled the production of advanced biopolyesters from traditional carbon sources as well as waste-derived substrates like carbon dioxide (Averesch et al,. 2023; Bhatia, 2023; Cestellos-Blanco et al., 2021). These materials can be produced with high volumetric efficiency and yield from various waste streams. To achieve this, microbial cell factories, able to synthesize advanced high-performance biomaterials that are useful for manufacturing of durable goods, must be developed.

Breakthrough Capability: Biosynthesis of organic or engineered living materials for habitat construction and infrastructure.

- Short-term Milestone: Proof-of-concept biogenic and living acoustic damping materials.
    - Bottleneck: Effective design of biomaterials that can dampen acoustic noise.
        - Potential Solution: Explore biomaterials that confer unique acoustic impedance different from liquids (i.e., solid or gaseous structures).
        - Potential Solution: Identify naturally occurring biomaterials, such as protein-based or protein composite, that could be manufactured in microbial cell factories.
- Short-term Milestone: Validate capacity and efficiency of select living organisms, such as melanized filamentous fungi, to transform ionizing radiation into a net-energy gain.
    - Bottleneck: Limited data for biological absorption pathways of ionizing radiation with a potential secondary net-energy gain.
        - Potential Solution: Quantitative characterization of the ability to use ionizing radiation in candidate radiotrophic organisms.
- Short-term Milestone: Microbial production of bioplastics and biomaterials with high volumetric efficiency and yield from waste-streams.

    - Bottleneck: Microbes that accumulate bioplastics from underused waste-streams (e.g., CO_2, CH_4, VFAs) can have long generation times (Angra et al., 2023).
        - Potential Solution: Develop genome engineering tools to utilize faster-growing recombinant and non-model organisms (Averesch et al., 2023; Woo et al., 2024).
    - Bottleneck: Existing bioplastics have insufficient material properties for application in the manufacturing of durable inventory items.
        - Potential Solution: Metabolic engineering for production of tailored biopolyesters with defined composition (i.e., fraction of monomers, molecular weight).
- Short-term Milestone: Construction materials which help to regulate habitat humidity and condensation.
    - Bottleneck: Ability of biomaterials to respond to humidity and condensation changes could change in space environments.
        - Potential Solution: Optimized biopolymer layers that attract and entrap moisture in a microgravity environment, which can then be used as a growth substrate and water source for plant propagation and microbial cultivation.
- Short-term Milestone: Construction materials and coatings that contain a beneficial microbiome for the habitat, such as to help condition the air or provide environmental biosensing capabilities.
    - Bottleneck: Biofilms evolve from a mixture of microbes.
        - Potential Solution: Robust microbiome development with mixtures of species that can be incorporated into habitat coatings.
- Medium-term Milestone: Radiotrophic organism metabolism using gamma radiation as power-source to create photosynthetic analogues.
    - Bottleneck: The process and mechanism of radiotrophic metabolism is unknown.
        - Potential Solution: Develop biotechnology toolboxes to characterize and engineer radiotrophic organisms.
- Medium-term Milestone: Blend engineered living materials with the materials of the spacecraft.
    - Bottleneck: Lack of research of how living materials can blend together with the aluminum alloy of the spacecraft.
        - Potential Solution: Engineer biopolymers to form composites with metallic structures while preserving cellular traits and desired biochemical activities.
- Medium-term Milestone: Biomanufacturing systems to convert *in situ* resources into versatile and durable biopolymers with sufficient mechanical requirements for construction purposes.
    - Bottleneck: Feedstock availability to scale production according to infrastructure needs.
        - Potential Solution: Utilize autotrophic organisms, such as cyanobacteria, to produce feedstocks for bioproduction from waste materials (Macário et al., 2022).

        - Potential Solution: Engineer lithoautotrophs for direct production of a range of advanced high-performance biopolyesters from inorganic carbon, water, and electricity (Averesch et al., 2023).
    - Bottleneck: Purification and recovery of products is water- and resource-intensive.
        - Potential Solution: Use alternative solvents, such as acetate or methanol byproducts, from other manufacturing processes (Berliner et al., 2021).
        - Potential Solution: Engineer autotrophs with halotolerance; or the ability to utilize halophiles for susceptibility to osmolysis (Adams et al., 2023).
        - Potential Solution: Engineer artificial membrane channels that secrete bioplastics or integrate polymerases into the membrane.
- Medium-term Milestone: Integrate healable biomaterials with biosensors to create autonomous in-flight repair of air leakages.
    - Bottleneck: Reliable sensor for healable biomaterial that activates when there is leak.
        - Potential Solution: Biosensors that detect temperature or pressure changes and induce damage repair within the healable biomaterial
- Medium-term Milestone: Extremophiles and enzymes capable of synthesizing biopolymers that perform well under adverse conditions.
    - Bottleneck: Difficult to evaluate robustness and efficiency of model organisms in extreme conditions.
        - Potential Solution: Bioprospecting and metabolomics for fast-growing psychrophiles.
- Medium-term Milestone: Efficient recovery of urea from biological waste streams for biocement applications for habitat construction.
    - Bottleneck: Limited usable carbon sources for biocement applications.
        - Potential Solution: Minimize carbonate that is needed for biocement so more urea can be retained and used elsewhere.
- Medium-term Milestone: Enable sustainable infrastructures with controlled formation of biofilms useful to life support systems.
    - Bottleneck: Slow rate-of-growth of biofilms compared to suspension cultures.
        - Potential Solution: Engineer microbial strains that form biofilms more rapidly.
        - Potential Solution: Engineer biofilm microbial communities that allow more efficient nutrient movement and faster growth.
    - Bottleneck: Adaptation of microbes to specific surface chemistries (e.g., hydrophobic, oleophobic, etc.).
        - Potential Solution: Develop uses for secreted biofilm capsule, exopolysaccharides, and biocellulose.
    - Bottleneck: Unwanted biofilms could form on infrastructure surfaces (e.g., electronics).
        - Potential Solution: Combat surface contamination with antimicrobial macromolecules such as peptides and anti-corrosion coatings (e.g., through bioengineered plants or their extracts).

        - Potential Solution: Engineer and embed proteins or bacteria that can prevent adhesion or biofilm formation into materials or coatings (Mayton et al., 2021).
- Long-term Milestone: Integration of acoustic damping capability into multipurpose, self-generating construction materials for crew cabins.
    - Bottleneck: Identifying the best chassis microbe or microbial consortia for construction materials.
        - Potential Solution: Design algae, plants, or cyanobacteria that can perform multiple functions.
    - Bottleneck: Integrating multiple functions into a single type of biogenic or living material.
        - Potential Solution: Leveraging the hierarchical assembly of living materials to encode multiple functions.
- Long-term Milestone: Microbes and plants that generate and self-assemble advanced polymers into habitat structures.
    - Bottleneck: Integration of advanced biomaterials with current infrastructure.
        - Potential Solution: Engineering living materials (ELM) containing designer polymers, such as (hemi)cellulosic fibers or polypeptides, that are decorated with multiplexed sensors and biocatalysts for habitat reinforcement, monitoring and self-healing.

Breakthrough Capability: In situ synthesis and repair of clothing and personal protective equipment.

- Short-term Milestone: 3D-printed bioplastics [e.g., polyhydroxyalkanoates (PHAs), including PLA, P3HB, P4HB] which mimic clothing (texture, pattern).
    - Bottleneck: Existing bioplastics have insufficient material properties for application in manufacturing of durable inventory items.
        - Potential Solution: Metabolic engineering for production of tailored biopolyesters with defined composition (fraction of monomers, molecular weight).
        - Potential Solution: Enable processing of biopolymers and bioplastics into fibers or non-woven materials.
- Short-term Milestone: Assessment of novel biomaterials and biocomposites for use in space clothing.
    - Bottleneck: Economically-viable application of traditional textile characterizations to novel materials.
        - Potential Solution: Incorporation of novel biopolymers into traditional materials for biocomposite clothing.
- Medium-term Milestone: Clothing with embedded enzymes that resist soiling and dirt.
    - Bottleneck: A stable way to incorporate enzymes into fabrics.
        - Potential Solution: Fusion of enzymes to fiber binding proteins.
- Medium-term Milestone: Microbes able to produce different types of advanced, high-performance bioplastics for durable clothing, including space suits and flame-retardant clothing.

    - Bottleneck: Current biology does not allow the formation of polymers with properties that approach incumbent synthetic materials.
        - Potential Solution: Develop novel enzymes for biosynthesis of non-natural bioplastics (Averesch et al., 2023).
    - Bottleneck: Bioproduction of starting materials (such as amine-containing polymer precursor) for high-performance polymers is difficult due to inherent toxicity (Baidin et al., 2021).
        - Potential Solution: Develop biosynthesis platform in non-model chassis that are resistant to high concentration of potentially toxic precursors.
    - Bottleneck: Bioplastics from microbial fermentation are difficult to isolate and purify.
        - Potential Solution: Engineer efficient bioplastic secretion in microbial chassis.
- Long-term Milestone: Cells that can be applied directly to the astronaut's body to generate coatings with various protective and adaptive traits, such as protection from radiation, temperature and moisture regulation, and antimicrobial and antifungal properties.
    - Bottleneck: Assembly and patterning of microbial coatings.
        - Potential Solution: Engineer cellular systems with sufficient adhesion properties and porosity to coat human skin or suits and augment function.
        - Potential Solution: Microbial peels or sticky plant exudates could be layered onto the desired surfaces with minimal processing.

Goal: Integrated and comprehensive environmental monitoring.[9]

Current State-of-the-Art: Engineered biological systems may be leveraged as mechanisms for integrated and comprehensive environmental monitoring. Microbial monitoring on the International Space Station (ISS) for the last twenty years has relied on culture-dependent methods that require analysis on Earth and limitations of this method include a bias toward culturable organism detection and a significant delay between sample collection and analysis. A recent increase in molecular-based tools on board the ISS, including the MinION™ sequencer and the miniPCR™ thermal cycler has made real-time microbial monitoring a possibility, but not the standard (Oxford Nanopore Technologies, 2024; miniPCR bio, 2024; Stahl-Rommel et al., 2020).

There are a variety of studies currently underway to gain a greater understanding of microbial communities on the ISS, monitoring samples for genetic changes to bacteria and fungi that may increase antibiotic resistance and disease potential and to better understand interactions of coexisting microbial communities (Tabor, 2021; Sengupta et al., 2024; Keller, 2024b). Genomic adaptations are theorized to be driven by the singular nature of stressors in space, which are distinct from any on-Earth stressors. A greater understanding of optimal and

[9] See also earlier Goals and Breakthrough Capabilities in this theme on biosensing and monitoring of air and water.

pathogenic environmental microbiomes for humans aboard spacecraft would further improve efforts toward ensuring a safe and secure environment.

Recent developments in reporter bacteria and biosensor technologies have presented the opportunity for both the in-space application of biosensors, including pathogen detection, air and water quality monitoring, and plant-based biosensors engineered for environmental monitoring (Atkinson et al., 2022; Sears et al., 2023; Ostrov et al., 2017; van der Meer & Belkin, 2010). Advancements in cell-free protein synthesis (CFPS) also enable the development of diagnostics for environmental monitoring, including biosensors and testing platforms. CFPS requires minimal maintenance and equipment, making it the ideal choice for use in austere environments. A recent study assessed the performance of the cell-based system BioBits on the ISS, with RNA-based apromers and fluorescent proteins as biological indicators. Both performed well under space conditions (Kocalar et al., 2024).

Despite this, additional research is needed to develop stable, robust, long-term biosensors for use in microgravity, including optical and electrical sensor readouts that can be integrated into computerized cabin monitoring. Microbes can be engineered to be compatible with microbial-machine interfaces that convert biological data to an electronic signal for further aggregation by a central monitoring system to inform predictive models of potentially invasive organisms or functional loss. Advancements in this field would catalyze efficient and autonomous sensing, sampling, and sequencing in low-resource environments.

Breakthrough Capability: Real-time biosensing and reporting on life support systems and built-in environment.

- Short-term Milestone: Microbe-based system that generates an electronic (wireless) signal for aggregation by a central monitoring system (vs. the more typical biological readout, such as luminescence).
    - Bottleneck: Keeping microbial cells viable inside the device.
        - Potential Solution: Identify microbes that can be immobilized in compatible devices and maintain optimal performance.
        - Potential Solution: Navigating engineering traits of model organisms which enhance viability in encapsulated environments that could be transferred to other microbes.
    - Bottleneck: Ensuring stability of microbial cells inside the device.
        - Potential Solution: Engineer synthetic differentiation circuits to support biological complexity within a closed system.
- Short-term Milestone: Efficient and autonomous sensing, sampling, and sequencing of biocomponents of life support systems.
    - Bottleneck: Flow cells are typically single-use and expensive to manufacture for nanopore sequencing.
        - Potential Solution: Design cost- and space-efficient flow cell protocols and reusable wash buffers.
- Short-term Milestone: Microbial biosensors to monitor and report elemental balances on closed life support systems.

    - Bottleneck: Identification of robust modules for environmental sensing at dynamic ranges in Low Earth Orbit (LEO).
        - Potential Solution: Continued sequencing efforts of extremophiles and evolved biosensors using rational and random mutagenesis.
        - Potential Solution: Use of clinostats for simulating low gravity environments to develop space-functional microbial biosensors.
- Medium-term Milestone: Programmable apoptotic circuits for control of microbial biosensing organisms (Din et al., 2016).
    - Bottleneck: Current “kill switch” technologies are not efficient enough for deployment in spaceflight.
        - Potential Solution: Design redundant kill switches to prevent escape by enacting multiple circuits simultaneously.
- Medium-term Milestone: Efficient colorimetric-based biosensors for built-in environments.
    - Bottleneck: Number of orthogonal reporters for multiplex detection.
        - Potential Solution: Expand the suite of aerobic and anaerobic colorimetric reporters.
    - Bottleneck: Interpretability of colorimetric readouts.
        - Potential Solution: Integrate biosensors with electrical readouts to minimize chance of interpretation errors.
- Medium-term Milestone: Biological data from life support systems converted into machine-readable information with an automated platform.
    - Bottleneck: Lack of standardized data formats and ontologies for representing complex biological information across different scales and systems.
    - Potential Solution: Collaborate with the broader scientific community to establish consensus-based data standards and ontologies that enable seamless integration and interpretation of diverse biological datasets.
- Long-term Milestone: Living surfaces that integrate microbial biosensors with physical sensors to monitor habitat areas.
    - Bottleneck: Required complexity of intra- and inter-species or consortial organisms with quorum sensing biosensors on living surfaces.
        - Potential Solution: Develop model organisms and consortia platforms to allow for generalization and understanding of fundamental interactions between living surfaces and microbial networks
        - Potential Solution: Centralize data collection on organism and consortium genotype, transcription and outcomes for analysis by ML/AI.
- Long-term Milestone: An autonomous environmental control module for air, water, and waste remediation to minimize energy and infrastructure requirements.
    - Bottleneck: Engineering technology with continuous-flow process(es) to support six people for 36 months.
        - Potential Solution: Extensive testing on Earth to create a highly integrated system with a scalable and adaptable design.
- Long-term Milestone: Characterization of optimal environmental microbiome for symbiotic human microbiome maintenance.

    - Bottleneck: Standardized sampling of diverse human populations or segmented populations that provide appropriate metadata (environmental microbiome data, demographic(s), health status and history).
        - Potential Solution: Centralized data collection and reporting on on-going efforts in microbiome and human health.
        - Potential Solution: Consortium development by experts on the minimum data collection requirements.
- Long-term Milestone: Real-time, continuous biosurveillance of microbiome consortia within the environment and present humans.
    - Bottleneck: Human and environmental biomarker (genomic, metabolic, immunological, and other biochemistry) -analysis currently requires extensive time and resources.
        - Potential Solution: Novel sensing technologies which can perform multiplexed characterization on small- and individualized-scales.
    - Bottleneck: Accurate integration and interpretation of microbiome data from complex microbial interactions and heterogeneity across individuals and environments.
        - Potential Solution: Build multi-scale computational models that integrate real-time sensing data with prior knowledge of microbiome ecology and host physiology to enable predictive analytics and adaptive interventions.

Breakthrough Capability: Biobased tools and technologies for early environmental threat or anomaly detection.

- Short-term Milestone: Engineered microbial or cell-free biosensors for the detection of pathogens and harmful compounds in liquid and solid waste.
    - Bottleneck: Low sensitivity and specificity of biosensors, which increase the potential for false positives and false negatives.
        - Potential Solution: Biosensor engineering campaigns to improve protein-based identification of analytes and signal amplification and discernment.
        - Potential Solution: RNA and DNA-based sensors that can connect to electronic interfaces for more complex computer processing.
    - Bottleneck: Detection of key analytes from hard-to-sample environments (e.g., solid waste).
        - Potential Solution: Mesh grids or filamentous sensors that extend throughout the waste container.
        - Potential Solution: Embedded biosensing microbes throughout the waste container.
    - Bottleneck: Biosystems work under a limited set of conditions (e.g., pH, salt composition, temperature).
        - Potential Solution: Adaptable biosystems to respond to and function in adverse conditions.
- Short-term Milestone: Biosensors (for chemistry, pH, pathogens) that can relay information electronically and wirelessly.

    - Bottleneck: Limited technology for sensors that detect certain biotic and abiotic signals.
        - Potential Solution: Develop genetically encoded colorimetric reporters or other non-invasive sensors as molecular diagnostics for different microbial components (e.g., glycoproteins to both detect and combat pathogens) (Hassan et al., 2020; Tsaneva & Van Damme, 2020).
- Short-term Milestone: Detection of evolved or invasive organisms that could threaten environment or health.
    - Bottleneck: Current methods of detection are not time-, cost-, or resource-efficient.
        - Potential Solution: Use prior evolutionary and biosystems data of pathogens to predict changes in the biome that could threaten the environment.
        - Potential Solution: -Omics and biomarker discovery within the microbiome or the host immune system to detect dysbiosis or pathogen evolution.
- Short-term Milestone: Biobased sensor within the interior shell of the spacecraft to identify the location of leakages or other damage with a cell-based or cell-free indicator (i.e., color change).
    - Bottleneck: Other technologies on the interior of the shell that may interfere with the biosensor.
        - Potential Solution: Integrate the biobased sensor with other insulation or shielding layers in the shell.
- Medium-term Milestone: Directed evolution of proposed microbial biosensors under on-Earth and Low Earth Orbit conditions to inform predictive models of functional loss or potential invasive organism traits.
    - Bottleneck: Lack of modeling for assessment of microbes engineered via directed evolution.
        - Potential Solution: Identify key model systems for detailed analysis of directed evolution.
- Medium-term Milestone: Early pathogen detection for bioreactors.
    - Bottleneck: Pathogens could be undetectable at low enough concentrations during early stages of contamination.
        - Potential Solution: Quantum sensing or other early-stage detection at picomolar or lower concentrations coupled with edge computing can support low detection rates.
- Long-term Milestone: Predictive AI/ML tools using data from past threats and anomalies to understand future mutation outcomes, i.e., fitness positive, neutral, or pathogenic, impact for the human and environmental ecosystems.
    - Bottleneck: Computational analysis is currently not automated or autonomous and missing potential correlative or associative predictive capability.
        - Potential Solution: Application of emerging ML and AI to understand genotype, -omic, and phenotype relationships between single organisms and within consortium communities (micro and macro ecological settings-biomes).

        - Potential Solution: Leverage the development of new strategies capable of training ML/AI on limited data, particularly ML techniques for computational material design.
        - Potential Solution: Use mechanistic or hybrid ML/mechanistic models in place of pure ML models.
- Long-term Milestone: Achieve understanding of pathways and networks of directed evolution studies with species and consortium growth on Earth and Low Earth Orbit conditions to understand mutations and adaptation processes of microbes in different environments.
    - Bottleneck: Mutations- inducing reagent (endonuclease targeting, genotoxic drug, microirradiation) elicit different DNA repair mechanisms.
        - Potential Solution: Whole genome or RNA sequencing to better understand DNA repair pathways and mutations caused by various reagents.
    - Bottleneck: Mutations of microbes from microgravity, oxidative stress, and epigenetic changes are difficult to predict.
        - Potential Solution: Use AI/ML algorithms based on sequencing data on Earth to predict outcomes of mutations and repair mechanisms for relevant reagents and environments.

Glossary

This glossary presents definitions and descriptions of some of the key terms and concepts found in and specific to the context of this roadmap.

Active Pharmaceutical Ingredient (API) is the constituent of a drug that causes the desired effect.

Adaptive Laboratory Evolution (ALE) is a technique that leverages natural evolution to alter organisms, using (often artificial) selection pressure to adapt a population and permanently change its genetic traits (Portnoy et al., 2011).

Air Revitalization System (ARS) is part of the life-support system in closed environments (e.g., a spacecraft) and is dedicated to reconditioning the environment atmosphere.

Artificial Intelligence (AI) uses computation to simulate human intelligence in order to perform tasks that typically require human cognition, such as learning, problem-solving, and decision-making.

Beyond Low Earth Orbit (BLEO) refers to space that extends beyond approximately 1200 miles above Earth's surface. It encompasses regions such as medium Earth orbit, geostationary orbit, and deep space, including *cis*-lunar space.

Bioassay is a process that can measure the functional activity of a biological substance in a living organism, tissue, or cell.

Bioastronautics is the study of the effects of spaceflight on biological systems, including physiological and psychological effects on humans to survive and prosper in extraterrestrial environments (Berliner et al., 2024).

Biobased (and *bio-derived*) processes and materials are those that function or occur through biological activity or are made of or derived from biological components, often through fermentation. Note: the United States Department of Agriculture's BioPreferred Program has a further definition of "biobased" that we find helpful, available at https://www.biopreferred.gov/BioPreferred/faces/pages/BiobasedProducts.xhtml

Biocement/bioconcrete is a biologically engineered construction material that incorporates living microbes to self-heal and repair cracks by producing minerals that fill and seal (Tyagi et al., 2024).

Biodesalination is a process that uses biological organisms to remove salts and other (inorganic) impurities from water to obtain fresh water (Nadersha & Hassan., 2022).

Bio-Electrochemical System (BES) integrates biological processes with electrochemical reactions to produce electricity, chemicals, or other valuable products. A BES harnesses the metabolic activity of microbes to catalyze electrochemical reactions at the interface between living organisms and electrodes.

Biofilm is a complex aggregation of microbes that adhere to various surfaces commonly embedded within a self-produced matrix of extracellular polymeric substances (Herrling et al., 2019).

Biologics are relatively large, heterogeneous (organic) molecules, compounds, or cells produced by living systems. See for comparison *Pharmaceuticals*.

Biomarker is a biological measurement (e.g., hormone, protein or other gene transcript) found in blood or tissue indicative of disease, infection, or other physiological condition in an organism or cell.

Biomass is the amount of biological material that can be used for a process; when used directly for energy production, the term “biofuel” is often used interchangeably. See also *Feedstocks*.

Biomaterial is any biological substance that has been engineered to interact with biological systems or derived from biological systems for non-biological use.

Biometrics is a system of measurement that uses physical characteristics or behavioral traits to recognize individuals or verify their identity (e.g., fingerprint mapping, facial recognition, retinal/iris scans).

Biomolecules are one of several major classes of biological molecules or complexes, such as proteins (including enzymes), nucleic acids, lipids, and glycans. For more about engineering biomolecules, please see EBRC’s *Engineering Biology: A Research Roadmap for the Next-Generation Bioeconomy* (EBRC, 2019b).

Bioplastics are plastic materials derived from renewable biological sources, such as plants or microbes, reducing reliance on fossil fuels and minimizing environmental impact. They can be designed to be biodegradable or used as water filters to remove microbes from water and air (i.e., polylactic acid, polyhydroxyalkanoate) (Marova et al., 2015).

Bioreactor is an apparatus with an optimized environment for biological reactions or processes to take place.

Biosensors use biological molecules or systems to detect and quantify (and in some cases, report) specific substances or changes in environmental conditions (Bhalla et al., 2016).

Biotechnology uses biological processes to produce new and innovative products that can improve human health and society (e.g., antibiotics, biofuels, biomaterials, etc).

Carbon Dioxide Removal Assembly (CDRA) is a closed-loop system that removes carbon dioxide and aids in water conservation onboard the International Space Station.

Chassis, in engineering biology, is a cell or organism that serves as a foundation to physically house and support the genetic material and other biomolecules and materials necessary for biological function.

Circularity focuses on keeping resources and materials in a production-and-consumption loop to promote sustainable production and use (Möslinger et al., 2023; Khatiwada et al., 2021).

Closed Environment Agriculture (CEA) is a method of crop cultivation that takes place within an enclosed structure. In CEA, environmental factors such as temperature, humidity, light, and CO_2 levels are tightly controlled to optimize plant growth and yield.

Commercial Low-Earth Orbit Destination (CLD) is a free-flying, commercially developed, owned, and operated spacecraft located in low-Earth orbit (NASA, 2023).

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) are involved in the defense mechanisms of prokaryotic organisms to viruses and are the underlying biological phenomena for a technology that allows the genetic manipulation of bacteria, archaea, eukarya, and viruses.

Design-Build-Test-Learn (DBTL) cycle is an iterative process used to develop a product or system where each phase informs and improves the next iteration to enhance functionality and effectiveness. DBTL cycles rely on data analytics and mathematical models to effectively characterize and control host responses (EBRC, 2019a).

Engineering Biology is the design and construction of new biological entities such as enzymes, genetic circuits, and cells, or the redesign of existing biological systems. Engineering biology builds on the advances in molecular, cell, and systems biology and seeks to transform biology in the same way that synthesis transformed chemistry and integrated circuit design transformed computing. The element that distinguishes engineering biology from traditional molecular and cellular biology is the focus on the design and construction of core components (e.g., parts of enzymes, genetic circuits, metabolic pathways) that can be modeled, understood, and tuned to meet specific performance criteria, and the assembly of these smaller parts and devices into larger integrated systems to solve specific problems. Unlike many other areas of engineering, biology is incredibly dynamic, non-linear, and less predictable, and there is less knowledge of specific parts and how they interact. Hence, the overwhelming physical details of natural biology (e.g., gene sequences, protein properties, interactive biological components) must be organized and recast via a set of design rules that hide information and manage complexity, thereby enabling the engineering of many-component integrated biological systems. It is only when this is accomplished that designs of significant scale will be possible. The term “engineering biology” is often used synonymously with “synthetic biology;” EBRC considers engineering biology to encompass the field of synthetic biology.

Environmental Control and Life Support System (ECLS System or ECLSS) provides for breathable air, clean water, food production, temperature regulation, pressure control, and waste management to support human life in isolated or hostile environments (NASA OCHMO, 2023a).

Equivalent System Mass (ESM) is a metric used to compare different life support systems in space missions. It evaluates the total mass required to achieve a specific function by incorporating not just the physical mass but also the mass of power, volume, cooling, and crew time. By converting these factors into a common mass metric, ESM provides a comprehensive assessment of the resource demands of different systems, helping to identify the most efficient and sustainable solutions for space missions (Ho et al., 2022).

Feedstocks are the raw or unprocessed (biological) materials that are used or consumed by an organism or reaction. Feedstocks can be abiotic, including gases and metals, or biotic, including proteins, carbohydrates, cells, and plants.

Figures of Merit (FOM) are the mass, volume, time, and power resources that are required from the spacecraft or mission personnel to operate the capability effectively.

Galactic Cosmic Radiation (GCR) consists of high-energy particles originating from outside the solar system. These particles, primarily protons, but also including heavier ions and electrons, travel at nearly the speed of light and pose a significant radiation hazard to astronauts and spacecraft. GCR exposure is a concern for long-duration space missions due to its potential health risks, such as radiation sickness due to cellular damage and increased cancer risk as well as permanent damage to the central nervous system.

Genome-Wide Association Study (GWAS) is a whole-genome sequencing technique that can, for example, be used to identify potential crops and plants with high nutritional value, harvest index, pathogen resistance, and short harvest time.

Good Manufacturing Practices (GMP) is a set of guidelines and regulations established to ensure the quality, safety, and consistency of pharmaceuticals, food, medical devices, and other products manufactured for human consumption or use. GMP standards are designed to minimize the risks associated with production processes and to ensure that products meet quality standards and regulatory requirements (WHO, 2024).

Host is an organism that serves as a chassis or contained system for biological activity; typically a microbe, such as bacteria, plant or animal cell. For more about host engineering, please see EBRC's *Engineering Biology: A Research Roadmap for the Next-Generation Bioeconomy* (EBRC, 2019b).

In silico is used to describe scientific experiments, research, or discoveries conducted with computer modeling or computer simulation.

In situ is used to describe scientific methods in biology or engineering biology that are conducted in the original location where a biological system or process naturally occurs.

International Space Station (ISS) is a large, permanently crewed spacecraft in low Earth orbit at an altitude of about 250 miles.

In vitro is used to describe experiments performed outside a living organism. *In vitro* experiments usually occur in a controlled laboratory environment.

In vivo is used to describe experiments conducted in a living host organism, tissue, or cells.

Limited Biodiversity is the limited variability among living organisms from all sources including, inter alia, terrestrial, marine, and other aquatic ecosystems and the ecological complexes of which they are part; this includes diversity within species, between species and of ecosystems (CBD, 2016). The lack of competition present in limited biodiversity environments (such as those currently experienced off-Earth) has been shown to promote pathogenesis in some microbes.

Lignocellulose is a complex biopolymer composed of polysaccharides (e.g., cellulose and hemicelluloses) and polymers (e.g., lignin) (Zoghlami & Paës, 2019; Dias et al., 2019). Lignocellulose is a common biomass feedstock.

Loop-Mediated Isothermal Amplification (LAMP) is a nucleic acid amplification technique that amplifies DNA in a rapid, efficient, and specific way, which can be used as a real-time monitoring system to identify microbial contaminants.

Low Earth Orbit (LEO) is defined as the area around Earth up to an altitude of approx. 1200 miles, which is close enough for routine transportation, communication, observation, and resupply on space missions.

Macronutrients are required by the human body to perform its metabolic functions and operate. These include proteins, fats, and carbohydrates (Baba et al., 2020).

Microbial Check Valves (MCV) are devices that prevent the water-borne transmission of viable microbes through water systems onboard the space station.

Microbiomes are communities of diverse microbes that are found in a given environment. For more, please see EBRC's *Microbiome Engineering: A Research Roadmap for the Next-Generation Bioeconomy* (EBRC, 2020).

Microgravity is a condition in which objects experience minimal gravitational forces, creating a sensation of weightlessness.

Micronutrients are dietary nutrients, such as vitamins and minerals, that are essential for proper function of the human body.

Modified Energy Cascade (MEC) Model is an advanced crop simulation model designed for use in controlled environment agriculture, particularly for space missions. It builds upon the original Energy Cascade model by incorporating more accurate and dynamic responses to environmental conditions such as light, carbon dioxide levels, temperature, and humidity. The MEC model allows for realistic exponential canopy growth, adjusts for seed emergence timing, and corrects harvest dates for crops like potatoes and tomatoes. This model is crucial for optimizing crop production in variable and resource-limited settings, such as Lunar or Martian greenhouses (Yates et al., 2024).

NASA Baseline Values and Assumptions Document (BVAD) contains a common set of values and minimum assumptions which can be used as a baseline in the design and study of space missions (Ewert et al., 2022).

Oxygen Generation Assembly (OGA) is a critical system that regenerates oxygen onboard the International Space Station.

Pharmaceuticals are relatively small molecule substances or drug products synthesized using chemical or biological processes. See for comparison *Biologics*.

Pharmacokinetic—Pharmacodynamic (PK/PD) is the analysis of the dose-dependent effect of a drug on the body (Negus & Banks, 2019).

Point-of-care testing is clinical laboratory testing close to or at the site of where care is being provided to the patient.

Positron Emission Tomography/Magnetic Resonance Imaging (PET/MRI) is a type of imaging using reporter genes and probes. PET/MRI imaging reporter genes and probes are a non-invasive technique for gene and cell kinetic therapy in Space.

Potable Water describes water of a quality that is safe for human consumption (Salehi, 2022).

Precision Medicine, sometimes referred to as “personalized medicine,” is an innovative approach to prevent, diagnose, or treat disease by integrating genetic and environmental information of a specific group of patients. “Individualized medicine” falls within the category of “precision medicine,” but is even more tailored towards a patient’s individual circumstances (i.e., genetics, physiology, anatomy) (NIH, 2022; Delpierre & Lefèvre, 2023).

Protospacer Adjacent Motif (PAM) is a specific DNA sequence recognizable by an enzyme that enables CRISPR-associated endonucleases to cleave the DNA.

Regolith is the rock dust that makes up the "soil" of planetary bodies, including Martian and Lunar bodies. Particle dimensions, compositional profiles, and extent of amorphization vary considerably between sources and have been shaped across millennia by thermal weathering, unabated bombardment from micrometeorites, and atom/ion implantation from the solar wind (Cool et al., 2023).

Single Cell Proteins (SCP) are proteins derived from the cultures of microorganisms (including bacteria, algae, yeast, and fungi) (Pérez-Santaescolastica et al., 2021).

Solar Particle Event (SPE) is a burst of energetic particles, mainly protons, emitted by the Sun during solar flares and coronal mass ejections. SPEs can deliver intense radiation doses over short periods, posing acute health risks to astronauts, such as radiation sickness, and can also disrupt spacecraft electronics and communications. These events are sporadic but can be forecasted to some extent, allowing for protective measures to be taken.

Space Bioprocess Engineering (SBE) is the part of space life sciences focusing on the design and deployment of biotechnology in service of sustainable human space exploration (Berliner et al., 2022; see also Figure 1 in Santomartino et al., 2023).

Spacecraft Maximum Allowable Concentrations (SMACs) are acceptable levels of cabin air pollutants as defined by NASA (NASA OCHMO, 2023d).

Synthetic biology (See Engineering biology).

Technology Readiness Level (TRL) is a method for estimating the maturity of technologies during the acquisition phase of a program. TRLs enable consistent and uniform discussions of technical maturity across different types of technology. TRL is determined during a technology readiness assessment in which program concepts, technology requirements, and demonstrated technology capabilities are reviewed (Manning, 2023).

Vegetable Production System ("Veggie") is a permanent crop growth facility aboard the International Space Station (Bunchek et al., 2024).

Vertical farming/vertical agriculture (or indoor farming) is the practice of growing crops, most often indoors and in or close to urban centers, in vertical layers in a controlled environment (controlling for temperature, light, CO_2, and water levels) to optimize crop yield while reducing resource use. Vertical farming aims to reduce the negative environmental impacts of agriculture, particularly by growing food closer to where consumers live. See also *Closed Environment Agriculture*.

References

Adam, J. A., Gulati, S., Hirsa, A. H., & Bonocora, R. P. (2020). Growth of microorganisms in an interfacially driven space bioreactor analog. *Npj Microgravity*, *6*(1), 1–7. https://doi.org/10.1038/s41526-020-0101-4

Adiga, R., Al-adhami, M., Andar, A., Borhani, S., Brown, S., Burgenson, D., Cooper, M. A., Deldari, S., Frey, D. D., Ge, X., Guo, H., Gurramkonda, C., Jensen, P., Kostov, Y., LaCourse, W., Liu, Y., Moreira, A., Mupparapu, K., Peñalber-Johnstone, C., … Zuber, A. (2018). Point-of-care production of therapeutic proteins of good-manufacturing-practice quality. *Nature Biomedical Engineering*, *2*(9), 675–686. https://doi.org/10.1038/s41551-018-0259-1

Adusei, K. M., Ngo, T. B., & Sadtler, K. (2021). T lymphocytes as critical mediators in tissue regeneration, fibrosis, and the foreign body response. *Acta Biomaterialia*, *133*, 17–33. https://doi.org/10.1016/j.actbio.2021.04.023

Advanced Research Projects Agency for Health (ARPA-H). (2024). *BREATHE | ARPA-H*. https://arpa-h.gov/research-and-funding/programs/breathe

Alavi, A., Bogu, G. K., Wang, M., Rangan, E. S., Brooks, A. W., Wang, Q., Higgs, E., Celli, A., Mishra, T., Metwally, A. A., Cha, K., Knowles, P., Alavi, A. A., Bhasin, R., Panchamukhi, S., Celis, D., Aditya, T., Honkala, A., Rolnik, B., … Snyder, M. P. (2022). Real-time alerting system for COVID-19 and other stress events using wearable data. *Nature Medicine*, *28*(1), 175–184. https://doi.org/10.1038/s41591-021-01593-2

Albert, M. J., Mathan, V. I., & Baker, S. J. (1980). Vitamin B12 synthesis by human small intestinal bacteria. *Nature*, *283*(5749), 781–782. https://doi.org/10.1038/283781a0

Ali, H., Ghori, Z., Sheikh, S., & Gul, A. (2015). Effects of Gamma Radiation on Crop Production. In K. R. Hakeem (Ed.), *Crop Production and Global Environmental Issues* (pp. 27–78). Springer International Publishing. https://doi.org/10.1007/978-3-319-23162-4_2

Ames, N. J., Ranucci, A., Moriyama, B., & Wallen, G. R. (2017). The Human Microbiome and Understanding the 16S rRNA Gene in Translational Nursing Science. *Nursing Research*, *66*(2), 184. https://doi.org/10.1097/NNR.0000000000000212

Ampim, P. A. Y., Obeng, E., & Olvera-Gonzalez, E. (2022). Indoor Vegetable Production: An Alternative Approach to Increasing Cultivation. *Plants*, *11*(21), Article 21. https://doi.org/10.3390/plants11212843

Andrews, B. I., Antia, F. D., Brueggemeier, S. B., Diorazio, L. J., Koenig, S. G., Kopach, M. E., Lee, H., Olbrich, M., & Watson, A. L. (2021). Sustainability Challenges and Opportunities in Oligonucleotide Manufacturing. *The Journal of Organic Chemistry*, *86*(1), 49–61. https://doi.org/10.1021/acs.joc.0c02291

Angra, V., Sehgal, R., & Gupta, R. (2023). Trends in PHA Production by Microbially Diverse and Functionally Distinct Communities. *Microbial Ecology*, *85*(2), 572–585. https://doi.org/10.1007/s00248-022-01995-w

ARC Centre of Excellence in Plants for Space. (2024). *Home*. Plants for Space ARC Centre for Excellence. https://plants4space.com

Asclepios. (2024). *Asclepios III Mission – Asclepios*. https://asclepios.ch/asclepios-iii-3/

Astromaterials Research and Exploration Science Division (ARES). (2024). *ARES | Exploration Science Projects | Simulants*. https://ares.jsc.nasa.gov/projects/simulants/

Atkinson, J. T., Su, L., Zhang, X., Bennett, G. N., Silberg, J. J., & Ajo-Franklin, C. M. (2022). Real-time bioelectronic sensing of environmental contaminants. *Nature*, *611*(7936), 548–553. https://doi.org/10.1038/s41586-022-05356-y

Aurand, E. R., Lampe, K. J., & Bjugstad, K. B. (2012). Defining and designing polymers and hydrogels for neural tissue engineering. *Neuroscience Research*, *72*(3), 199–213. https://doi.org/10.1016/j.neures.2011.12.005

Averesch, N. J. H. (2021). Choice of Microbial System for In-Situ Resource Utilization on Mars. *Frontiers in Astronomy and Space Sciences*, *8*. https://doi.org/10.3389/fspas.2021.700370

Averesch, N. J. H., Berliner, A. J., Nangle, S. N., Zezulka, S., Vengerova, G. L., Ho, D., Casale, C. A., Lehner, B. A. E., Snyder, J. E., Clark, K. B., Dartnell, L. R., Criddle, C. S., & Arkin, A. P. (2023). Microbial biomanufacturing for space-exploration—What to take and when to make. *Nature Communications*, *14*(1), 2311. https://doi.org/10.1038/s41467-023-37910-1

Averesch, N. J. H., & Rothschild, L. J. (2019). Metabolic engineering of Bacillus subtilis for production of para-aminobenzoic acid – unexpected importance of carbon source is an advantage for space application. *Microbial Biotechnology*, *12*(4), 703–714. https://doi.org/10.1111/1751-7915.13403

Averesch, N., Reginato, P., & Caiati, A. (2023). Polyester synthase for novel bioplastics with tunable properties. *Homeworld Collective*. https://homeworld.pubpub.org/pub/r7mh54i5/release/1

Averesch, N., Vince, V., Kracke, F., Ziesack, M., Nangle, S., Waymouth, R., & Criddle, C. (2023). *Towards high-performance polyesters from carbon dioxide: Novel polyhydroxyarylates from engineered Cupriavidus necator*. Manuscript submitted for publication. https://doi.org/10.21203/rs.3.rs-2719603/v1

Aziz, S., Raza, M. A., Noreen, M., Iqbal, M. Z., & Raza, S. M. (2022). Astropharmacy: Roles for the Pharmacist in Space. *INNOVATIONS in Pharmacy*, *13*(3), Article 3. https://doi.org/10.24926/iip.v13i3.4956

Baba, S., Smith, T., Hellmann, J., Bhatnagar, A., Carter, K., Vanhoover, A., & Caruso, J. (2020). Space Flight Diet-Induced Deficiency and Response to Gravity-Free Resistive Exercise. *Nutrients*, *12*(8), Article 8. https://doi.org/10.3390/nu12082400

Baidin, V., Owens, T. W., Lazarus, M. B., & Kahne, D. (2021). Simple Secondary Amines Inhibit Growth of Gram-Negative Bacteria through Highly Selective Binding to Phenylalanyl-tRNA Synthetase. *Journal of the American Chemical Society*, *143*(2), 623–627. https://doi.org/10.1021/jacs.0c11113

Baker, D. J., Arany, Z., Baur, J. A., Epstein, J. A., & June, C. H. (2023). CAR T therapy beyond cancer: The evolution of a living drug. *Nature*, *619*(7971), 707–715. https://doi.org/10.1038/s41586-023-06243-w

Balaji, A., Liu, Y., Nute, M. G., Hu, B., Kappell, A., LeSassier, D. S., Godbold, G. D., Ternus, K. L., & Treangen, T. J. (2023). *SeqScreen-Nano: A computational platform for rapid, in-field characterization of previously unseen pathogens* (p. 2023.02.10.528096). Manuscript submitted for publication. bioRxiv. https://doi.org/10.1101/2023.02.10.528096

Ball, N., Hindupur, A., Kagawa, H., Kostakis, A., Gresser, A. L., Sims, K., Sharif, S., Villanueva, A. G., Donovan, F., Settles, A. M., & Hogan, J. A. (2021). *BioNutrients-2: Improvements to the BioNutrients-1 Nutrient Production System*. https://ttu-ir.tdl.org/server/api/core/bitstreams/eb140a17-9b6a-4931-8330-d1ca2fb30582/content

Banks, I. J., Gibson, W. T., & Cameron, M. M. (2014). Growth rates of black soldier fly larvae fed on fresh human faeces and their implication for improving sanitation. *Tropical Medicine & International Health*, *19*(1), 14–22. https://doi.org/10.1111/tmi.12228

Bansal, S., Li, Y., Bansal, S., Klotzbier, W., Singh, B., Jayatilake, M., Sridharan, V., Fernández, J. A., Griffin, J. H., Weiler, H., Boerma, M., & Cheema, A. K. (2024). Genetic Upregulation of Activated Protein C Mitigates Delayed Effects of Acute Radiation Exposure in the Mouse Plasma. *Metabolites*, *14*(5), Article 5. https://doi.org/10.3390/metabo14050245

Banta, A. B., Ward, R. D., Tran, J. S., Bacon, E. E., & Peters, J. M. (2020). Programmable Gene Knockdown in Diverse Bacteria Using Mobile-CRISPRi. *Current Protocols in Microbiology*, *59*(1), e130. https://doi.org/10.1002/cpmc.130

Bao, T., Qian, Y., Xin, Y., Collins, J. J., & Lu, T. (2023). Engineering microbial division of labor for plastic upcycling. *Nature Communications*, *14*(1), 5712. https://doi.org/10.1038/s41467-023-40777-x

Barbosa, R. G., van Veelen, H. P. J., Pinheiro, V., Sleutels, T., Verstraete, W., & Boon, N. (2021). Enrichment of Hydrogen-Oxidizing Bacteria from High-Temperature and High-Salinity Environments. *Applied and Environmental Microbiology*, *87*(4), e02439-20. https://doi.org/10.1128/AEM.02439-20

Barney, B. M. (2020). Aerobic nitrogen-fixing bacteria for hydrogen and ammonium production: Current state and perspectives. *Applied Microbiology and Biotechnology*, *104*(4), 1383–1399. https://doi.org/10.1007/s00253-019-10210-9

Barratt, M. R., Baker, E. S., & Pool, S. L. (Eds.). (2019). *Principles of Clinical Medicine for Space Flight*. Springer. https://doi.org/10.1007/978-1-4939-9889-0

Bech, E. M., Pedersen, S. L., & Jensen, K. J. (2018). Chemical Strategies for Half-Life Extension of Biopharmaceuticals: Lipidation and Its Alternatives. *ACS Medicinal Chemistry Letters*, *9*(7), 577–580. https://doi.org/10.1021/acsmedchemlett.8b00226

Beheshti, A., McDonald, J. T., Hada, M., Takahashi, A., Mason, C. E., & Mognato, M. (2021). Genomic Changes Driven by Radiation-Induced DNA Damage and Microgravity in Human Cells. *International Journal of Molecular Sciences*, *22*(19), Article 19. https://doi.org/10.3390/ijms221910507

Benbouzid, M., Azoulay, K., Bencheikh, I., Al-Jadabi, N., Meryem, B., Aarfane, A., Nasrellah, H., El Hajjaji, S., & Labjar, N. (2024). Evaluation of natural porous material as media filters for

domestic wastewater treatment using infiltration percolation process. *Euro-Mediterranean Journal for Environmental Integration*. https://doi.org/10.1007/s41207-024-00588-y

Benke, K., & Tomkins, B. (2017). Future food-production systems: Vertical farming and controlled-environment agriculture. *Sustainability: Science, Practice and Policy*, *13*(1), 13–26. https://doi.org/10.1080/15487733.2017.1394054

Benvenuti, A. J., Drecksler, S. R., Gupta, S. S., & Menezes, A. A. (2020). *Design of Anaerobic Digestion Systems for Closed Loop Space Biomanufacturing*. https://syborgs.mae.ufl.edu/media/syborgsmaeufledu/syborgs-papers/ICES-2020-498.pdf

Berg, M., & Koskella, B. (2018). Nutrient- and Dose-Dependent Microbiome-Mediated Protection against a Plant Pathogen. *Current Biology: CB*, *28*(15), 2487-2492.e3. https://doi.org/10.1016/j.cub.2018.05.085

Berliner, A. J., Hilzinger, J. M., Abel, A. J., McNulty, M. J., Makrygiorgos, G., Averesch, N. J. H., Sen Gupta, S., Benvenuti, A., Caddell, D. F., Cestellos-Blanco, S., Doloman, A., Friedline, S., Ho, D., Gu, W., Hill, A., Kusuma, P., Lipsky, I., Mirkovic, M., Luis Meraz, J., … Arkin, A. P. (2021). Towards a Biomanufactory on Mars. *Frontiers in Astronomy and Space Sciences*, *8*. https://doi.org/10.3389/fspas.2021.711550

Berliner, A. J., Lipsky, I., Ho, D., Hilzinger, J. M., Vengerova, G., Makrygiorgos, G., McNulty, M. J., Yates, K., Averesch, N. J. H., Cockell, C. S., Wallentine, T., Seefeldt, L. C., Criddle, C. S., Nandi, S., McDonald, K. A., Menezes, A. A., Mesbah, A., & Arkin, A. P. (2022). Space bioprocess engineering on the horizon. *Communications Engineering*, *1*(1), 1–8. https://doi.org/10.1038/s44172-022-00012-9

Berliner, A. J., Zezulka, S., Hutchinson, G. A., Bertoldo, S., Cockell, C. S., & Arkin, A. P. (2024). Domains of life sciences in spacefaring: What, where, and how to get involved. *Npj Microgravity*, *10*(1), 1–10. https://doi.org/10.1038/s41526-024-00354-y

Bhalla, N., Jolly, P., Formisano, N., & Estrela, P. (2016). Introduction to biosensors. *Essays in Biochemistry*, *60*(1), 1–8. https://doi.org/10.1042/EBC20150001

Bhatia, S. K. (2023). Microbial Biopolymers: Trends in Synthesis, Modification, and Applications. *Polymers*, *15*(6), Article 6. https://doi.org/10.3390/polym15061364

Billesbølle, C. B., de March, C. A., van der Velden, W. J. C., Ma, N., Tewari, J., del Torrent, C. L., Li, L., Faust, B., Vaidehi, N., Matsunami, H., & Manglik, A. (2023). Structural basis of odorant recognition by a human odorant receptor. *Nature*, *615*(7953), 742–749. https://doi.org/10.1038/s41586-023-05798-y

Blue, R., Nusbaum, D., & Antonsen, E. (2022). *Development of an Accepted Medical Condition List for Exploration Medical Capability Scoping* (20190027540). https://ntrs.nasa.gov/citations/20220003136

Bogorad, I. W., Lin, T.-S., & Liao, J. C. (2013). Synthetic non-oxidative glycolysis enables complete carbon conservation. *Nature*, *502*(7473), 693–697. https://doi.org/10.1038/nature12575

Boukid, F., Ganeshan, S., Wang, Y., Tülbek, M. Ç., & Nickerson, M. T. (2023). Bioengineered Enzymes and Precision Fermentation in the Food Industry. *International Journal of Molecular Sciences*, *24*(12), Article 12. https://doi.org/10.3390/ijms241210156

Breger, J. C., Vranish, J. N., Oh, E., Stewart, M. H., Susumu, K., Lasarte-Aragonés, G., Ellis, G. A., Walper, S. A., Díaz, S. A., Hooe, S. L., Klein, W. P., Thakur, M., Ancona, M. G., & Medintz, I. L. (2023). Self assembling nanoparticle enzyme clusters provide access to substrate channeling in multienzymatic cascades. *Nature Communications*, *14*(1), 1757. https://doi.org/10.1038/s41467-023-37255-9

BRENDA. (2024). *BRENDA Enzyme Database*. https://www.brenda-enzymes.org/

Brookwell, A. W., Gonzalez, J. L., Martinez, A. W., & Oza, J. P. (2023). Development of Solid-State Storage for Cell-Free Expression Systems. *ACS Synthetic Biology*, *12*(9), 2561–2577. https://doi.org/10.1021/acssynbio.3c00111

Bugarin-Castillo, Y., Rando, P., Clabaux, M., Moulin, G., & Ramaioli, M. (2023). 3D printing to modulate the texture of starch-based food. *Journal of Food Engineering*, *350*, 111499. https://doi.org/10.1016/j.jfoodeng.2023.111499

Bunchek, J. M., Hummerick, M. E., Spencer, L. E., Romeyn, M. W., Young, M., Morrow, R. C., Mitchell, C. A., Douglas, G. L., Wheeler, R. M., & Massa, G. D. (2024). Pick-and-eat space crop production flight testing on the International Space Station. *Journal of Plant Interactions*, *19*(1), 2292220. https://doi.org/10.1080/17429145.2023.2292220

Burke, C., Steinberg, P., Rusch, D., Kjelleberg, S., & Thomas, T. (2011). Bacterial community assembly based on functional genes rather than species. *Proceedings of the National Academy of Sciences*, *108*(34), 14288–14293. https://doi.org/10.1073/pnas.1101591108

Bushnell, D. M., & Gross, L. P. (2023). *Technological and Medical Human Health and Well-Being Options in Deep Space*.

Bychkov, A., Reshetnikova, P., Bychkova, E., Podgorbunskikh, E., & Koptev, V. (2021). The current state and future trends of space nutrition from a perspective of astronauts' physiology. *International Journal of Gastronomy and Food Science*, *24*, 100324. https://doi.org/10.1016/j.ijgfs.2021.100324

Caddell, D., Langenfeld, N. J., Eckels, M. J., Zhen, S., Klaras, R., Mishra, L., Bugbee, B., & Coleman-Derr, D. (2023). Photosynthesis in rice is increased by CRISPR/Cas9-mediated transformation of two truncated light-harvesting antenna. *Frontiers in Plant Science*, *14*. https://doi.org/10.3389/fpls.2023.1050483

Cai, Q., Hanson, J. A., Steiner, A. R., Tran, C., Masikat, M. R., Chen, R., Zawada, J. F., Sato, A. K., Hallam, T. J., & Yin, G. (2015). A simplified and robust protocol for immunoglobulin expression in scherichia coli cell-free protein synthesis systems. *Biotechnology Progress*, *31*(3), 823–831. https://doi.org/10.1002/btpr.2082

Cai, T., Sun, H., Qiao, J., Zhu, L., Zhang, F., Zhang, J., Tang, Z., Wei, X., Yang, J., Yuan, Q., Wang, W., Yang, X., Chu, H., Wang, Q., You, C., Ma, H., Sun, Y., Li, Y., Li, C., … Ma, Y. (2021). Cell-free chemoenzymatic starch synthesis from carbon dioxide. *Science*, *373*(6562), 1523–1527. https://doi.org/10.1126/science.abh4049

Cano-Garrido, O., Serna, N., Unzueta, U., Parladé, E., Mangues, R., Villaverde, A., & Vázquez, E. (2022). Protein scaffolds in human clinics. *Biotechnology Advances*, *61*, 108032. https://doi.org/10.1016/j.biotechadv.2022.108032

Cao, W., McCallum, N. C., Ni, Q. Z., Li, W., Boyce, H., Mao, H., Zhou, X., Sun, H., Thompson, M. P., Battistella, C., Wasielewski, M. R., Dhinojwala, A., Shawkey, M. D., Burkart, M. D., Wang, Z., & Gianneschi, N. C. (2020). Selenomelanin: An Abiotic Selenium Analogue of Pheomelanin. *Journal of the American Chemical Society*, *142*(29), 12802–12810. https://doi.org/10.1021/jacs.0c05573

Cao, Y., Mu, H., Liu, W., Zhang, R., Guo, J., Xian, M., & Liu, H. (2019). Electricigens in the anode of microbial fuel cells: Pure cultures versus mixed communities. *Microbial Cell Factories*, *18*(1), 39. https://doi.org/10.1186/s12934-019-1087-z

Cap, A. P., Cannon, J. W., & Reade, M. C. (2021). Synthetic blood and blood products for combat casualty care and beyond. *Journal of Trauma and Acute Care Surgery*, *91*(2S), S26. https://doi.org/10.1097/TA.0000000000003248

Caraccio, A. J., Hintze, P., Anthony, S. M., Devor, R. W., Captain, J. G., & Muscatello, A. C. (2013). Trash-to-Gas: Converting Space Trash into Useful Products. *43rd International Conference on Environmental Systems*. 43rd International Conference on Environmental Systems, Vail, CO. https://doi.org/10.2514/6.2013-3440

Castañón, L. (2022). *Synthetic genetic circuits could help plants adapt to climate change*. https://news.stanford.edu/stories/2022/08/synthetic-genetic-circuits-help-plants-adapt-climate-change

Cerruti, M., Stevens, B., Ebrahimi, S., Alloul, A., Vlaeminck, S. E., & Weissbrodt, D. G. (2020). Enrichment and Aggregation of Purple Non-sulfur Bacteria in a Mixed-Culture Sequencing-Batch Photobioreactor for Biological Nutrient Removal From Wastewater. *Frontiers in Bioengineering and Biotechnology*, *8*. https://doi.org/10.3389/fbioe.2020.557234

Cestellos-Blanco, S., Friedline, S., Sander, K. B., Abel, A. J., Kim, J. M., Clark, D. S., Arkin, A. P., & Yang, P. (2021). Production of PHB From CO2-Derived Acetate With Minimal Processing Assessed for Space Biomanufacturing. *Frontiers in Microbiology*, *12*. https://doi.org/10.3389/fmicb.2021.700010

Chai, K. F., Ng, K. R., Samarasiri, M., & Chen, W. N. (2022). Precision fermentation to advance fungal food fermentations. *Current Opinion in Food Science*, *47*, 100881. https://doi.org/10.1016/j.cofs.2022.100881

Chan, C. T. Y., Lee, J. W., Cameron, D. E., Bashor, C. J., & Collins, J. J. (2016). “Deadman” and “Passcode” microbial kill switches for bacterial containment. *Nature Chemical Biology*, *12*(2), 82–86. https://doi.org/10.1038/nchembio.1979

Chandra, S., Chandra, D., Barh, A., Pankaj, Pandey, R. K., & Sharma, I. P. (2017). Bryophytes: Hoard of remedies, an ethno-medicinal review. *Journal of Traditional and Complementary Medicine*, *7*(1), 94–98. https://doi.org/10.1016/j.jtcme.2016.01.007

Chen, J.-F., Hsu, C.-Y., Yu, S.-F., Ko, C.-H., Chiu, W.-C., Lai, H.-M., Chen, Y.-C., Su, Y.-J., & Cheng, T.-T. (2020). The impact of long-term biologics/target therapy on bone mineral density

in rheumatoid arthritis: A propensity score-matched analysis. *Rheumatology*, *59*(9), 2471–2480. https://doi.org/10.1093/rheumatology/kez655

Chen, X.-R., Cui, Y.-Z., Li, B.-Z., & Yuan, Y.-J. (2024). Genome engineering on size reduction and complexity simplification: A review. *Journal of Advanced Research*, *60*, 159–171. https://doi.org/10.1016/j.jare.2023.07.006

Chowdhury, S., & Fong, S. S. (2020). Computational Modeling of the Human Microbiome. *Microorganisms*, *8*(2), Article 2. https://doi.org/10.3390/microorganisms8020197

Chu, N., Liang, Q., Jiang, Y., & Zeng, R. J. (2020). Microbial electrochemical platform for the production of renewable fuels and chemicals. *Biosensors and Bioelectronics*, *150*, 111922. https://doi.org/10.1016/j.bios.2019.111922

Chua, C. Y. X., Jimenez, M., Mozneb, M., Traverso, G., Lugo, R., Sharma, A., Svendsen, C. N., Wagner, W. R., Langer, R., & Grattoni, A. (2024). Advanced material technologies for space and terrestrial medicine. *Nature Reviews Materials*, 1–14. https://doi.org/10.1038/s41578-024-00691-0

Clark-Hachtel, C. M., Hibshman, J. D., De Buysscher, T., Stair, E. R., Hicks, L. M., & Goldstein, B. (2024). The tardigrade Hypsibius exemplaris dramatically upregulates DNA repair pathway genes in response to ionizing radiation. *Current Biology: CB*, *34*(9), 1819-1830.e6. https://doi.org/10.1016/j.cub.2024.03.019

Clarridge, J. E. (2004). Impact of 16S rRNA Gene Sequence Analysis for Identification of Bacteria on Clinical Microbiology and Infectious Diseases. *Clinical Microbiology Reviews*, *17*(4), 840–862. https://doi.org/10.1128/cmr.17.4.840-862.2004

Cockell, C. S., Santomartino, R., Finster, K., Waajen, A. C., Eades, L. J., Moeller, R., Rettberg, P., Fuchs, F. M., Van Houdt, R., Leys, N., Coninx, I., Hatton, J., Parmitano, L., Krause, J., Koehler, A., Caplin, N., Zuijderduijn, L., Mariani, A., Pellari, S. S., … Demets, R. (2020). Space station biomining experiment demonstrates rare earth element extraction in microgravity and Mars gravity. *Nature Communications*, *11*(1), 5523. https://doi.org/10.1038/s41467-020-19276-w

Colombo, G. V., & Greenley, D. R. (1980). *Advanced microbial check valve development* (NASA-CR-163577). https://ntrs.nasa.gov/citations/19800024586

Convention on Biological Diversity (CBD). (2016). *Convention Text*. Secretariat of the Convention on Biological Diversity. https://www.cbd.int/convention/articles/default.shtml?a=cbd-02

Cool, N. I., Perez-Beltran, S., Cheng, J., Rivera-Gonzalez, N., Bronner, D., Anita, null, Wang, E., Zakira, U., Farahbakhsh, M., Liu, K.-W., Hsu, J.-L., Birgisson, B., & Banerjee, S. (2023). Matrix transformation of lunar regolith and its use as a feedstock for additive manufacturing. *iScience*, *26*(4), 106382. https://doi.org/10.1016/j.isci.2023.106382

Cubillos-Ruiz, A., Alcantar, M. A., Donghia, N. M., Cárdenas, P., Avila-Pacheco, J., & Collins, J. J. (2022). An engineered live biotherapeutic for the prevention of antibiotic-induced dysbiosis. *Nature Biomedical Engineering*, *6*(7), 910–921. https://doi.org/10.1038/s41551-022-00871-9

Dadkhodazade, E., Khanniri, E., Khorshidian, N., Hosseini, S. M., Mortazavian, A. M., & Moghaddas Kia, E. (2021). Yeast cells for encapsulation of bioactive compounds in food products: A review. *Biotechnology Progress*, *37*(4), e3138. https://doi.org/10.1002/btpr.3138

Dakkumadugula, A., Pankaj, L., Alqahtani, A. S., Ullah, R., Ercisli, S., & Murugan, R. (2023). Space nutrition and the biochemical changes caused in Astronauts Health due to space flight: A review. *Food Chemistry: X*, *20*, 100875. https://doi.org/10.1016/j.fochx.2023.100875

Danforth Center. (2024). Our Core Facilities. *Danforth Plant Science Center*. https://www.danforthcenter.org/our-work/core-facilities/

Davidovich-Pinhas, M. (2016). Oleogels: A Promising Tool for Delivery of Hydrophobic Bioactive Molecules. *Therapeutic Delivery*, *7*(1), 1–3. https://doi.org/10.4155/tde.15.83

De Micco, V., Arena, C., Di Fino, L., & Narici, L. (2022). Radiation environment in exploration-class space missions and plants' responses relevant for cultivation in Bioregenerative Life Support Systems. *Frontiers in Plant Science*, *13*. https://doi.org/10.3389/fpls.2022.1001158

del Mármol, J., Yedlin, M. A., & Ruta, V. (2021). The structural basis of odorant recognition in insect olfactory receptors. *Nature*, *597*(7874), 126–131. https://doi.org/10.1038/s41586-021-03794-8

Delpierre, C., & Lefèvre, T. (2023). Precision and personalized medicine: What their current definition says and silences about the model of health they promote. Implication for the development of personalized health. *Frontiers in Sociology*, *8*. https://doi.org/10.3389/fsoc.2023.1112159

Deng, X., Gould, M., & Ali, M. A. (2022). A review of current advancements for wound healing: Biomaterial applications and medical devices. *Journal of Biomedical Materials Research Part B: Applied Biomaterials*, *110*(11), 2542–2573. https://doi.org/10.1002/jbm.b.35086

Department of Homeland Security (DHS). (2022). *The BioWatch Program Factsheet | Homeland Security*. https://www.dhs.gov/archive/publication/biowatch-program-factsheet

Department of Homeland Security (DHS). (2024). *Detecting Bioterrorism | Homeland Security*. https://www.dhs.gov/archive/detecting-bioterrorism

Deter, H. S., & Lu, T. (2022). Engineering microbial consortia with rationally designed cellular interactions. *Current Opinion in Biotechnology*, *76*, 102730. https://doi.org/10.1016/j.copbio.2022.102730

DeWinter, M. A., Thames, A. H., Guerrero, L., Kightlinger, W., Karim, A. S., & Jewett, M. C. (2023). Point-of-Care Peptide Hormone Production Enabled by Cell-Free Protein Synthesis. *ACS Synthetic Biology*, *12*(4), 1216–1226. https://doi.org/10.1021/acssynbio.2c00680

Dias, C., Santos, J., Reis, A., & Lopes da Silva, T. (2019). Yeast and microalgal symbiotic cultures using low-cost substrates for lipid production. *Bioresource Technology Reports*, *7*, 100261. https://doi.org/10.1016/j.biteb.2019.100261

Diaz, T. E., Ives, E. C., Lazare, D. I., & Buckland, D. M. (2024). Expiration analysis of the International Space Station formulary for exploration mission planning. *Npj Microgravity*, *10*(1), 1–10. https://doi.org/10.1038/s41526-024-00414-3

Dickey, R. M., Gopal, M. R., Nain, P., & Kunjapur, A. M. (2024). Recent developments in enzymatic and microbial biosynthesis of flavor and fragrance molecules. *Journal of Biotechnology*, *389*, 43–60. https://doi.org/10.1016/j.jbiotec.2024.04.004

Dikshit, R., Gupta, N., Dey, A., Viswanathan, K., & Kumar, A. (2022). Microbial induced calcite precipitation can consolidate martian and lunar regolith simulants. *PLOS ONE*, *17*(4), e0266415. https://doi.org/10.1371/journal.pone.0266415

Din, M. O., Danino, T., Prindle, A., Skalak, M., Selimkhanov, J., Allen, K., Julio, E., Atolia, E., Tsimring, L. S., Bhatia, S. N., & Hasty, J. (2016). Synchronized cycles of bacterial lysis for in vivo delivery. *Nature*, *536*(7614), 81–85. https://doi.org/10.1038/nature18930

Dominguez, J. A., Brown, B., Reidy, L., Crawford, K., Oliver-Butler, K., Black, C., Dennis, B., Chanmanee, W., McCall, S., & Burke, K. A. (2023). *Development of an efficient alternative to recovery O2 from metabolic CO2 via electrolysis operated at ambient temperature and driven by a highly selective catalysis*. https://ntrs.nasa.gov/api/citations/20230002360/downloads/ICES23%20Paper%20No%2051.pdf

Douglas, S. (2022). *NASA Names Winners in Waste to Base Materials Challenge*. https://www.nasa.gov/directorates/stmd/prizes-challenges-crowdsourcing-program/center-of-excellence-for-collaborative-innovation-coeci/coeci-news/nasa-names-winners-in-waste-to-base-materials-challenge/

Douglas, G. L., Zwart, S. R., & Smith, S. M. (2020). Space Food for Thought: Challenges and Considerations for Food and Nutrition on Exploration Missions. *The Journal of Nutrition*, *150*(9), 2242–2244. https://doi.org/10.1093/jn/nxaa188

Dukovski, I., Bajić, D., Chacón, J. M., Quintin, M., Vila, J. C. C., Sulheim, S., Pacheco, A. R., Bernstein, D. B., Riehl, W. J., Korolev, K. S., Sanchez, A., Harcombe, W. R., & Segrè, D. (2021). A metabolic modeling platform for the computation of microbial ecosystems in time and space (COMETS). *Nature Protocols*, *16*(11), 5030–5082. https://doi.org/10.1038/s41596-021-00593-3

Eden Grow Systems & Comeaux. (2022). *15 Reasons NASA Chose Aeroponics for Space Missions and Home Growing*. Eden Grow Systems. https://edengrowsystems.com/blog/eden-blog-1/post/15-reasons-why-nasa-chose-aeroponics-28

EDEN ISS. (2024). *EDEN ISS | Ground Demonstration of Plant Cultivation Technologies and Operation in Space*. https://eden-iss.net/

Eisenstein, M. (2023). AI-enhanced protein design makes proteins that have never existed. *Nature Biotechnology*, *41*(3), 303–305. https://doi.org/10.1038/s41587-023-01705-y

Elbaz, J., Yin, P., & Voigt, C. A. (2016). Genetic encoding of DNA nanostructures and their self-assembly in living bacteria. *Nature Communications*, *7*(1), 11179. https://doi.org/10.1038/ncomms11179

Engelhart, et al. (2024). *NASA TechPort—Project Data*. https://techport.nasa.gov/view/154330

Engineering Biology Research Consortium. (2019a). *Design-Build-Test-Learn (DBTL) | EBRC Research Roadmap*. https://roadmap.ebrc.org/dbtl/

Engineering Biology Research Consortium. (2019b). *Engineering Biology | EBRC Research Roadmap*. https://roadmap.ebrc.org/2019-roadmap/

Engineering Biology Research Consortium. (2020). Engineering Microbiomes—Looking Ahead. *ACS Synthetic Biology*, *9*(12), 3181–3183. https://doi.org/10.1021/acssynbio.0c00558

Engineering Biology Research Consortium. (2022). *Engineering Biology for Climate & Sustainability: A Research Roadmap for a Cleaner Future*. https://doi.org/10.25498/E4SG64

Ewert, M. K., Chen, T. T., & Powell, C. D. (2022). Life Support Baseline Values and Assumptions Document. *Life Support*. https://ntrs.nasa.gov/api/citations/20210024855/downloads/BVAD_2.15.22-final.pdf

European Space Agency (ESA). (2024). *ESA - Melissa*. https://www.esa.int/Enabling_Support/Space_Engineering_Technology/Melissa

Expasy. (2024). *Expasy—ENZYME*. https://enzyme.expasy.org/

Fahrion, J., Mastroleo, F., Dussap, C.-G., & Leys, N. (2021). Use of Photobioreactors in Regenerative Life Support Systems for Human Space Exploration. *Frontiers in Microbiology*, *12*. https://doi.org/10.3389/fmicb.2021.699525

Fernando, K. A. S., Thakuri, R., Barry Schroeder, A. L., & Ruiz, O. N. (2024). Chemical Method for Recovery and Regeneration of Graphene Oxide. *ACS Applied Bio Materials*, *7*(1), 315–324. https://doi.org/10.1021/acsabm.3c00911

Flamholz, A. I., Dugan, E., Blikstad, C., Gleizer, S., Ben-Nissan, R., Amram, S., Antonovsky, N., Ravishankar, S., Noor, E., Bar-Even, A., Milo, R., & Savage, D. F. (2020). Functional reconstitution of a bacterial CO2 concentrating mechanism in Escherichia coli. *eLife*, *9*, e59882. https://doi.org/10.7554/eLife.59882

Focil, C., Canto-Encalada, G., Campos, D.-T., Zuñiga, C., & Zepeda, A. (2024). Chapter 15—Applying genome-scale metabolic modeling tools to understand microbial communities in wastewater treatment. In M. P. Shah & N. Shah (Eds.), *Development in Waste Water Treatment Research and Processes* (pp. 297–332). Elsevier. https://doi.org/10.1016/B978-0-443-13609-2.00020-3

Food and Drug Administration (FDA). (2024). *FDA Approves First Orally Administered Fecal Microbiota Product for the Prevention of Recurrence of Clostridioides difficile Infection*. FDA; FDA. https://www.fda.gov/news-events/press-announcements/fda-approves-first-orally-administered-fecal-microbiota-product-prevention-recurrence-clostridioides

French, K. E. (2017). Engineering Mycorrhizal Symbioses to Alter Plant Metabolism and Improve Crop Health. *Frontiers in Microbiology*, *8*. https://doi.org/10.3389/fmicb.2017.01403

Gao, J., Zuo, Y., Xiao, F., Wang, Y., Li, D., Xu, J., Ye, C., Feng, L., Jiang, L., Liu, T., Gao, D., Ma, B., Huang, L., Xu, Z., & Lian, J. (2023). Biosynthesis of catharanthine in engineered Pichia pastoris. *Nature Synthesis*, *2*(3), 231–242. https://doi.org/10.1038/s44160-022-00205-2

Garcia, M. (2020). *Crew Continues Troubleshooting as Tests Isolate Small Leak – Space Station*. https://blogs.nasa.gov/spacestation/2020/09/29/crew-continues-troubleshooting-as-tests-isolate-small-leak/

García-Jiménez, B., Torres-Bacete, J., & Nogales, J. (2021). Metabolic modelling approaches for describing and engineering microbial communities. *Computational and Structural Biotechnology Journal*, *19*, 226–246. https://doi.org/10.1016/j.csbj.2020.12.003

Gaskill, M. (2023). *NASA Achieves Water Recovery Milestone on International Space Station—NASA*. https://www.nasa.gov/missions/station/iss-research/nasa-achieves-water-recovery-milestone-on-international-space-station/

Genovese, N. J., Domeier, T. L., Telugu, B. P. V. L., & Roberts, R. M. (2017). Enhanced Development of Skeletal Myotubes from Porcine Induced Pluripotent Stem Cells. *Scientific Reports*, *7*(1), 41833. https://doi.org/10.1038/srep41833

Gibbons Jr., W. J., McKinney, M. G., O'Dell, P. J., Bollinger, B. A., & Jones, J. A. (2021). Homebrewed psilocybin: Can new routes for pharmaceutical psilocybin production enable recreational use? *Bioengineered*, *12*(1), 8863–8871. https://doi.org/10.1080/21655979.2021.1987090

Good Food Institute. (2024). *Cultivating alternative proteins from commodity crop sidestreams—The Good Food Institute*. https://gfi.org/resource/cultivating-alternative-proteins-from-commodity-crop-sidestreams/

Graham, A. E., & Ledesma-Amaro, R. (2023). The microbial food revolution. *Nature Communications*, *14*(1), 2231. https://doi.org/10.1038/s41467-023-37891-1

Granata, T., Rattenbacher, B., & John, G. (2022). Micro-Bioreactors in Space: Case Study of a Yeast (Saccharomyces cerevisiae) Bioreactor With a Non-Invasive Monitoring Method. *Frontiers in Space Technologies*, *2*. https://doi.org/10.3389/frspt.2021.773814

Griffin, J. H., Zlokovic, B. V., & Mosnier, L. O. (2015). Activated protein C: Biased for translation. *Blood*, *125*(19), 2898–2907. https://doi.org/10.1182/blood-2015-02-355974

Gumulya, Y., Zea, L., & Kaksonen, A. H. (2022). *In situ* resource utilisation: The potential for space biomining. *Minerals Engineering*, *176*, 107288. https://doi.org/10.1016/j.mineng.2021.107288

Hann, E. C., Overa, S., Harland-Dunaway, M., Narvaez, A. F., Le, D. N., Orozco-Cárdenas, M. L., Jiao, F., & Jinkerson, R. E. (2022). A hybrid inorganic–biological artificial photosynthesis system for energy-efficient food production. *Nature Food*, *3*(6), 461–471. https://doi.org/10.1038/s43016-022-00530-x

Harman, G., Khadka, R., Doni, F., & Uphoff, N. (2021). Benefits to Plant Health and Productivity From Enhancing Plant Microbial Symbionts. *Frontiers in Plant Science*, *11*. https://doi.org/10.3389/fpls.2020.610065

Harpke, M., Pietschmann, S., Costa, F. S., Gansert, C., Langenhorst, F., & Kothe, E. (2022). Biomineralization by Extremely Halophilic and Metal-Tolerant Community Members from a Sulfate-Dominated Metal-Rich Environment. *Microorganisms*, *10*(1), Article 1. https://doi.org/10.3390/microorganisms10010079

Hassan, S., Donia, A., Sial, U., Zhang, X., & Bokhari, H. (2020). Glycoprotein- and Lectin-Based Approaches for Detection of Pathogens. *Pathogens*, *9*(9), Article 9. https://doi.org/10.3390/pathogens9090694

Hecht, M. H., Kounaves, S. P., Quinn, R. C., West, S. J., Young, S. M. M., Ming, D. W., Catling, D. C., Clark, B. C., Boynton, W. V., Hoffman, J., DeFlores, L. P., Gospodinova, K., Kapit, J., & Smith, P. H. (2009). Detection of Perchlorate and the Soluble Chemistry of Martian Soil at the Phoenix Lander Site. *Science*, *325*(5936), 64–67. https://doi.org/10.1126/science.1172466

Herrling, M. P., Lackner, S., Nirschl, H., Horn, H., & Guthausen, G. (2019). Chapter Four—Recent NMR/MRI studies of biofilm structures and dynamics. In G. A. Webb (Ed.), *Annual Reports on NMR Spectroscopy* (Vol. 97, pp. 163–213). Academic Press. https://doi.org/10.1016/bs.arnmr.2019.02.001

Ho, D., Makrygiorgos, G., Hill, A., & Berliner, A. J. (2022). Towards an extension of equivalent system mass for human exploration missions on Mars. *Npj Microgravity*, *8*(1), 1–10. https://doi.org/10.1038/s41526-022-00214-7

Hoelscher, M. P., Forner, J., Calderone, S., Krämer, C., Taylor, Z., Loiacono, F. V., Agrawal, S., Karcher, D., Moratti, F., Kroop, X., & Bock, R. (2022). Expression strategies for the efficient synthesis of antimicrobial peptides in plastids. *Nature Communications*, *13*(1), 5856. https://doi.org/10.1038/s41467-022-33516-1

Hoskins, M. (2022). *FDA Approves Eversense 6-Month Sensor: What People with Diabetes Need to Know*. Healthline. https://www.healthline.com/diabetesmine/eversense-e3-six-month-implantable-sensor

Huang, C.-W., Lin, C., Nguyen, M. K., Hussain, A., Bui, X.-T., & Ngo, H. H. (2023). A review of biosensor for environmental monitoring: Principle, application, and corresponding achievement of sustainable development goals. *Bioengineered*, *14*(1), 58–80. https://doi.org/10.1080/21655979.2022.2095089

Huang, J., Xue, S., Buchmann, P., Teixeira, A. P., & Fussenegger, M. (2023). An electrogenetic interface to program mammalian gene expression by direct current. *Nature Metabolism*, *5*(8), 1395–1407. https://doi.org/10.1038/s42255-023-00850-7

Huang, J., Yang, J., Jiang, H., Wu, G., Liu, W., Wang, B., Xiao, H., & Han, J. (2020). Microbial Responses to Simulated Salinization and Desalinization in the Sediments of the Qinghai–Tibetan Lakes. *Frontiers in Microbiology*, *11*. https://doi.org/10.3389/fmicb.2020.01772

Hurt, R. C., Buss, M. T., Duan, M., Wong, K., You, M. Y., Sawyer, D. P., Swift, M. B., Dutka, P., Barturen-Larrea, P., Mittelstein, D. R., Jin, Z., Abedi, M. H., Farhadi, A., Deshpande, R., & Shapiro, M. G. (2023). Genomically mined acoustic reporter genes for real-time in vivo monitoring of tumors and tumor-homing bacteria. *Nature Biotechnology*, *41*(7), 919–931. https://doi.org/10.1038/s41587-022-01581-y

Irimia, D., & Wang, X. (2018). Inflammation-on-a-chip: Probing the immune system ex vivo. *Trends in Biotechnology*, *36*(9), 923–937. https://doi.org/10.1016/j.tibtech.2018.03.011

Ismail, S., Giacinti, G., Raynaud, C. D., Cameleyre, X., Alfenore, S., Guillouet, S., & Gorret, N. (2024). Impact of the environmental parameters on single cell protein production and composition by *Cupriavidus necator*. *Journal of Biotechnology*, *388*, 83–95. https://doi.org/10.1016/j.jbiotec.2024.04.009

Jack, J., Fu, H., Leininger, A., Hyster, T. K., & Ren, Z. J. (2022). Cell-Free CO2 Valorization to C6 Pharmaceutical Precursors via a Novel Electro-Enzymatic Process. *ACS Sustainable Chemistry & Engineering*, *10*(13), 4114–4121. https://doi.org/10.1021/acssuschemeng.1c06746

Jackson, S., Servestas, S., and Forry, S. (2024). Human Gut Microbiome Reference Material. *NIST*. https://www.nist.gov/programs-projects/human-gut-microbiome-reference-material

Jan, S., Parween, T., Siddiqi, T. O., & Mahmooduzzafar. (2012). Effect of gamma radiation on morphological, biochemical, and physiological aspects of plants and plant products. *Environmental Reviews*, *20*(1), 17–39. https://doi.org/10.1139/a11-021

Jemison, M., & Olabisi, R. (2021). Biomaterials for human space exploration: A review of their untapped potential. *Acta Biomaterialia*, *128*, 77–99. https://doi.org/10.1016/j.actbio.2021.04.033

Jeon, J., Lee, K. Z., Zhang, X., Jaeger, J., Kim, E., Li, J., Belaygorod, L., Arif, B., Genin, G. M., Foston, M. B., Zayed, M. A., & Zhang, F. (2023). Genetically Engineered Protein-Based Bioadhesives with Programmable Material Properties. *ACS Applied Materials & Interfaces*, *15*(49), 56786–56795. https://doi.org/10.1021/acsami.3c12919

Jia, H.-J., Jia, P.-P., Yin, S., Bu, L.-K., Yang, G., & Pei, D.-S. (2023). Engineering bacteriophages for enhanced host range and efficacy: Insights from bacteriophage-bacteria interactions. *Frontiers in Microbiology*, *14*. https://doi.org/10.3389/fmicb.2023.1172635

Jiang, M., Jing, C., Lei, C., Han, X., Wu, Y., Ling, S., Zhang, Y., Li, Q., Yu, H., Liu, S., Li, J., Chen, W., & Yu, G. (2024). A bio-based nanofibre hydrogel filter for sustainable water purification. *Nature Sustainability*, *7*(2), 168–178. https://doi.org/10.1038/s41893-023-01264-9

Jimenez, M., L'Heureux, J., Kolaya, E., Liu, G. W., Martin, K. B., Ellis, H., Dao, A., Yang, M., Villaverde, Z., Khazi-Syed, A., Cao, Q., Fabian, N., Jenkins, J., Fitzgerald, N., Karavasili, C., Muller, B., Byrne, J. D., & Traverso, G. (2024). Synthetic extremophiles via species-specific formulations improve microbial therapeutics. *Nature Materials*, 1–8. https://doi.org/10.1038/s41563-024-01937-6

Johnson, C. M., Boles, H. O., Spencer, L. E., Poulet, L., Romeyn, M., Bunchek, J. M., Fritsche, R., Massa, G. D., O'Rourke, A., & Wheeler, R. M. (2021). Supplemental Food Production With Plants: A Review of NASA Research. *Frontiers in Astronomy and Space Sciences*, *8*. https://doi.org/10.3389/fspas.2021.734343

Johnston, T. G., Yuan, S.-F., Wagner, J. M., Yi, X., Saha, A., Smith, P., Nelson, A., & Alper, H. S. (2020). Compartmentalized microbes and co-cultures in hydrogels for on-demand

bioproduction and preservation. *Nature Communications*, *11*(1), 563. https://doi.org/10.1038/s41467-020-14371-4

Jones, H. W. (2016). *Using the International Space Station (ISS) Oxygen Generation Assembly (OGA) Is Not Feasible for Mars Transit*. https://ntrs.nasa.gov/api/citations/20160014553/downloads/20160014553.pdf

Kacira, M & Ling, P.P. (2001). *Design And Development Of An Automated And Non–Contact Sensing System For Continuous Monitoring Of Plant Health And Growth*. https://doi.org/10.13031/2013.6231

Kaminski, M. M., Abudayyeh, O. O., Gootenberg, J. S., Zhang, F., & Collins, J. J. (2021). CRISPR-based diagnostics. *Nature Biomedical Engineering*, *5*(7), 643–656. https://doi.org/10.1038/s41551-021-00760-7

Kasiviswanathan, P., Swanner, E. D., Halverson, L. J., & Vijayapalani, P. (2022). Farming on Mars: Treatment of basaltic regolith soil and briny water simulants sustains plant growth. *PLOS ONE*, *17*(8), e0272209. https://doi.org/10.1371/journal.pone.0272209

Katsoulakis, E., Wang, Q., Wu, H., Shahriyari, L., Fletcher, R., Liu, J., Achenie, L., Liu, H., Jackson, P., Xiao, Y., Syeda-Mahmood, T., Tuli, R., & Deng, J. (2024). Digital twins for health: A scoping review. *Npj Digital Medicine*, *7*(1), 1–11. https://doi.org/10.1038/s41746-024-01073-0

Keeter, B. (2022). *Scientists Grow Plants in Lunar Soil—NASA*. https://www.nasa.gov/humans-in-space/scientists-grow-plants-in-lunar-soil/

KEGG. (2024). *KEGG: Kyoto Encyclopedia of Genes and Genomes*. https://www.genome.jp/kegg/

Keller, E. (2024a). *BioNutrients Flight Experiments—NASA*. https://www.nasa.gov/centers-and-facilities/ames/ames-science/ames-space-biosciences/bionutrients-flight-experiments/

Keller, E. (2024b). *Multi-Drug Resistant Bacteria Found on ISS Mutating to Become Functionally Distinct—NASA*. https://www.nasa.gov/centers-and-facilities/ames/ames-science/ames-space-biosciences/multi-drug-resistant-bacteria-found-on-iss-mutating-to-become-functionally-distinct/

Kellner, M. J., Koob, J. G., Gootenberg, J. S., Abudayyeh, O. O., & Zhang, F. (2019). SHERLOCK: Nucleic acid detection with CRISPR nucleases. *Nature Protocols*, *14*(10), 2986–3012. https://doi.org/10.1038/s41596-019-0210-2

Kerckhof, F.-M., Sakarika, M., Van Giel, M., Muys, M., Vermeir, P., De Vrieze, J., Vlaeminck, S. E., Rabaey, K., & Boon, N. (2021). From Biogas and Hydrogen to Microbial Protein Through Co-Cultivation of Methane and Hydrogen Oxidizing Bacteria. *Frontiers in Bioengineering and Biotechnology*, *9*. https://doi.org/10.3389/fbioe.2021.733753

Khan-Mayberry, N., James, J. T., Tyl, R., & Lam, C. (2011). Space Toxicology: Protecting Human Health During Space Operations. *International Journal of Toxicology*, *30*(1), 3–18. https://doi.org/10.1177/1091581810386389

Kilkenny & Russell. (2022, September 7). *NASA Manages Astronaut Health with Effective Diagnostics Research—NASA*. https://www.nasa.gov/centers-and-facilities/glenn/nasa-manages-astronaut-health-with-effective-diagnostics-research/

Kim, E. R., Joe, C., Mitchell, R. J., & Gu, M. B. (2023). Biosensors for healthcare: Current and future perspectives. *Trends in Biotechnology*, *41*(3), 374–395. https://doi.org/10.1016/j.tibtech.2022.12.005

Kobsa, S., & Saltzman, W. M. (2008). Bioengineering Approaches to Controlled Protein Delivery. *Pediatric Research*, *63*(5), 513–519. https://doi.org/10.1203/PDR.0b013e318165f14d

Kocalar, S., Miller, B. M., Huang, A., Gleason, E., Martin, K., Foley, K., Copeland, D. S., Jewett, M. C., Saavedra, E. A., & Kraves, S. (2024). Validation of Cell-Free Protein Synthesis Aboard the International Space Station. *ACS Synthetic Biology*, *13*(3), 942–950. https://doi.org/10.1021/acssynbio.3c00733

Korman, T. P., Sahachartsiri, B., Li, D., Vinokur, J. M., Eisenberg, D., & Bowie, J. U. (2014). A synthetic biochemistry system for the in vitro production of isoprene from glycolysis intermediates. *Protein Science*, *23*(5), 576–585. https://doi.org/10.1002/pro.2436

Kulshreshtha, A., Sharma, S., Padilla, C. S., & Mandadi, K. K. (2022). Plant-based expression platforms to produce high-value metabolites and proteins. *Frontiers in Plant Science*, *13*. https://doi.org/10.3389/fpls.2022.1043478

Kumar, L., & Gaikwad, K. K. (2023). Advanced food packaging systems for space exploration missions. *Life Sciences in Space Research*, *37*, 7–14. https://doi.org/10.1016/j.lssr.2023.01.005

Küppers, M., Pain, C., Kereszturi, Á., & Hargitai, H. (2021). Regolith. In H. Hargitai & Á. Kereszturi (Eds.), *Encyclopedia of Planetary Landforms* (pp. 1–15). Springer. https://doi.org/10.1007/978-1-4614-9213-9_293-1

Lamprecht-Grandío, M., Cortesão, M., Mirete, S., de la Cámara, M. B., de Figueras, C. G., Pérez-Pantoja, D., White, J. J., Farías, M. E., Rosselló-Móra, R., & González-Pastor, J. E. (2020). Novel Genes Involved in Resistance to Both Ultraviolet Radiation and Perchlorate From the Metagenomes of Hypersaline Environments. *Frontiers in Microbiology*, *11*. https://doi.org/10.3389/fmicb.2020.00453

Lee, N., Shin, J., Park, J. H., Lee, G. M., Cho, S., & Cho, B.-K. (2016). Targeted Gene Deletion Using DNA-Free RNA-Guided Cas9 Nuclease Accelerates Adaptation of CHO Cells to Suspension Culture. *ACS Synthetic Biology*, *5*(11), 1211–1219. https://doi.org/10.1021/acssynbio.5b00249

Lee, P., Bubeck, S., & Petro, J. (2023). Benefits, Limits, and Risks of GPT-4 as an AI Chatbot for Medicine. *New England Journal of Medicine*, *388*(13), 1233–1239. https://doi.org/10.1056/NEJMsr2214184

Lee, S. M., Dong, T. S., Krause-Sorio, B., Siddarth, P., Milillo, M. M., Lagishetty, V., Datta, T., Aguilar-Faustino, Y., Jacobs, J. P., & Lavretsky, H. (2022). The intestinal microbiota as a predictor for antidepressant treatment outcome in geriatric depression: A prospective pilot

study. *International Psychogeriatrics*, *34*(1), 33–45. https://doi.org/10.1017/S1041610221000120

Levrier, A., Karpathakis, I., Nash, B., Bowden, S. D., Lindner, A. B., & Noireaux, V. (2024). PHEIGES: All-cell-free phage synthesis and selection from engineered genomes. *Nature Communications*, *15*(1), 2223. https://doi.org/10.1038/s41467-024-46585-1

Lew, E. T., Yuen, J. S. K., Zhang, K. L., Fuller, K., Frost, S. C., & Kaplan, D. L. (2024). Chemical and sensory analyses of cultivated pork fat tissue as a flavor enhancer for meat alternatives. *Scientific Reports*, *14*(1), 17643. https://doi.org/10.1038/s41598-024-68247-4

Lewis, R. (2023). *Space Food Systems—NASA*. https://www.nasa.gov/directorates/esdmd/hhp/space-food-systems/

Li, C.-T., Eng, R., Zuniga, C., Huang, K.-W., Chen, Y., Zengler, K., & Betenbaugh, M. J. (2023). Optimization of nutrient utilization efficiency and productivity for algal cultures under light and dark cycles using genome-scale model process control. *Npj Systems Biology and Applications*, *9*(1), 1–12. https://doi.org/10.1038/s41540-022-00260-7

Li, C.-T., Yelsky, J., Chen, Y., Zuñiga, C., Eng, R., Jiang, L., Shapiro, A., Huang, K.-W., Zengler, K., & Betenbaugh, M. J. (2019). Utilizing genome-scale models to optimize nutrient supply for sustained algal growth and lipid productivity. *Npj Systems Biology and Applications*, *5*(1), 1–11. https://doi.org/10.1038/s41540-019-0110-7

Li, H., Dai, C., & Hu, Y. (2024). Hydrogels for Chemical Sensing and Biosensing. *Macromolecular Rapid Communications*, *45*(2), 2300474. https://doi.org/10.1002/marc.202300474

Li, J., Scarano, A., Gonzalez, N. M., D'Orso, F., Yue, Y., Nemeth, K., Saalbach, G., Hill, L., de Oliveira Martins, C., Moran, R., Santino, A., & Martin, C. (2022). Biofortified tomatoes provide a new route to vitamin D sufficiency. *Nature Plants*, *8*(6), 611–616. https://doi.org/10.1038/s41477-022-01154-6

Li, J., Wang, C., Liang, W., & Liu, S. (2021). Rhizosphere Microbiome: The Emerging Barrier in Plant-Pathogen Interactions. *Frontiers in Microbiology*, *12*. https://doi.org/10.3389/fmicb.2021.772420

Liew, F. E., Nogle, R., Abdalla, T., Rasor, B. J., Canter, C., Jensen, R. O., Wang, L., Strutz, J., Chirania, P., De Tissera, S., Mueller, A. P., Ruan, Z., Gao, A., Tran, L., Engle, N. L., Bromley, J. C., Daniell, J., Conrado, R., Tschaplinski, T. J., … Köpke, M. (2022). Carbon-negative production of acetone and isopropanol by gas fermentation at industrial pilot scale. *Nature Biotechnology*, *40*(3), 335–344. https://doi.org/10.1038/s41587-021-01195-w

Lim, C. T. (2020). Future of health diagnostics. *VIEW*, *1*(1), e3. https://doi.org/10.1002/viw2.3

Lin, M. T., Salihovic, H., Clark, F. K., & Hanson, M. R. (2022). Improving the efficiency of Rubisco by resurrecting its ancestors in the family Solanaceae. *Science Advances*, *8*(15), eabm6871. https://doi.org/10.1126/sciadv.abm6871

Lindsey, A. J., Thoms, A. W., Dancer, J., & Gross, M. (2021). Evaluation of Algae-Based Fertilizers Produced from Revolving Algal Biofilms on Kentucky Bluegrass. *Agronomy*, *11*(7), Article 7. https://doi.org/10.3390/agronomy11071288

Liu, Y., Xu, H., Yu, C., & Zhou, G. (2021). Multifaceted roles of duckweed in aquatic phytoremediation and bioproducts synthesis. *GCB Bioenergy*, *13*(1), 70–82. https://doi.org/10.1111/gcbb.12747

Liu, Z., Lompe, K. M., Mohseni, M., Bérubé, P. R., Sauvé, S., & Barbeau, B. (2020). Biological ion exchange as an alternative to biological activated carbon for drinking water treatment. *Water Research*, *168*, 115148. https://doi.org/10.1016/j.watres.2019.115148

Lockhart, S. (2018). *Recycling in Space: Waste Handling in a Microgravity Environment Challenge - NASA*. https://www.nasa.gov/missions/station/recycling-in-space-waste-handling-in-a-microgravity-environment-challenge/

Luo, L., Manda, S., Park, Y., Demir, B., Sanchez, J., Anantram, M. P., Oren, E. E., Gopinath, A., & Rolandi, M. (2023). DNA nanopores as artificial membrane channels for bioprotonics. *Nature Communications*, *14*(1), 5364. https://doi.org/10.1038/s41467-023-40870-1

Luo, S., Lin, P. P., Nieh, L.-Y., Liao, G.-B., Tang, P.-W., Chen, C., & Liao, J. C. (2022). A cell-free self-replenishing CO2-fixing system. *Nature Catalysis*, *5*(2), 154–162. https://doi.org/10.1038/s41929-022-00746-x

Lutzmayer, S. (2023). *Pharma's Frozen Assets*. https://www.iqvia.com/-/media/iqvia/pdfs/library/white-papers/iqvia-pharmas-frozen-assets_final.pdf

Lynch, J. B., & Hsiao, E. Y. (2023). Toward understanding links between the microbiome and neurotransmitters. *Annals of the New York Academy of Sciences*, *1524*(1), 10–16. https://doi.org/10.1111/nyas.14993

Ma, Y., Xu, A., & Cheng, Z.-M. (Max). (2021). Effects of light emitting diode lights on plant growth, development and traits a meta-analysis. *Horticultural Plant Journal*, *7*(6), 552–564. https://doi.org/10.1016/j.hpj.2020.05.007

Macário, I. P. E., Veloso, T., Frankenbach, S., Serôdio, J., Passos, H., Sousa, C., Gonçalves, F. J. M., Ventura, S. P. M., & Pereira, J. L. (2022). Cyanobacteria as Candidates to Support Mars Colonization: Growth and Biofertilization Potential Using Mars Regolith as a Resource. *Frontiers in Microbiology*, *13*. https://doi.org/10.3389/fmicb.2022.840098

Maeki, M., Uno, S., Niwa, A., Okada, Y., & Tokeshi, M. (2022). Microfluidic technologies and devices for lipid nanoparticle-based RNA delivery. *Journal of Controlled Release*, *344*, 80–96. https://doi.org/10.1016/j.jconrel.2022.02.017

Mahmoudi, M., Landry, M. P., Moore, A., & Coreas, R. (2023). The protein corona from nanomedicine to environmental science. *Nature Reviews Materials*, *8*(7), 422–438. https://doi.org/10.1038/s41578-023-00552-2

Majeed, A., Muhammad, Z., Ullah, R., & Ali, H. (2018). *GAMMA IRRADIATION I: EFFECT ON GERMINATION AND GENERAL GROWTH CHARACTERISTICS OF PLANTS–A REVIEW*. https://www.pakbs.org/pjbot/papers/1531145645.pdf

Manning, C. G. (2023). *Technology Readiness Levels—NASA*. https://www.nasa.gov/directorates/somd/space-communications-navigation-program/technology-readiness-levels/

Mapstone, L. J., Leite, M. N., Purton, S., Crawford, I. A., & Dartnell, L. (2022). Cyanobacteria and microalgae in supporting human habitation on Mars. *Biotechnology Advances*, *59*, 107946. https://doi.org/10.1016/j.biotechadv.2022.107946

Marcellin, E., Angenent, L. T., Nielsen, L. K., & Molitor, B. (2022). Recycling carbon for sustainable protein production using gas fermentation. *Current Opinion in Biotechnology*, *76*, 102723. https://doi.org/10.1016/j.copbio.2022.102723

Marova, I., Kundrat, V., Benesova, P., Matouskova, P., & Obruca, S. (2015). Use of biodegradable PHA-based nanofibers to removing microorganisms from water. *2015 IEEE 15th International Conference on Nanotechnology (IEEE-NANO)*, 204–206. https://doi.org/10.1109/NANO.2015.7388958

MarsOasis. (2024). *NASA TechPort—Project Data*. https://techport.nasa.gov/view/102871

Maurya, A., Sharma, D., Partap, M., Kumar, R., & Bhargava, B. (2023). Microbially-assisted phytoremediation toward air pollutants: Current trends and future directions. *Environmental Technology & Innovation*, *31*, 103140. https://doi.org/10.1016/j.eti.2023.103140

Mayton, H. M., Walker, S. L., & Berger, B. W. (2021). Disrupting Irreversible Bacterial Adhesion and Biofilm Formation with an Engineered Enzyme. *Applied and Environmental Microbiology*, *87*(13), e00265-21. https://doi.org/10.1128/AEM.00265-21

McNerney, M. P., Doiron, K. E., Ng, T. L., Chang, T. Z., & Silver, P. A. (2021). Theranostic cells: Emerging clinical applications of synthetic biology. *Nature Reviews Genetics*, *22*(11), 730–746. https://doi.org/10.1038/s41576-021-00383-3

McNulty, M. J., Xiong, Y. (Mary), Yates, K., Karuppanan, K., Hilzinger, J. M., Berliner, A. J., Delzio, J., Arkin, A. P., Lane, N. E., Nandi, S., & McDonald, K. A. (2021). Molecular pharming to support human life on the moon, mars, and beyond. *Critical Reviews in Biotechnology*, *41*(6), 849–864. https://doi.org/10.1080/07388551.2021.1888070

Meade, E., Hehir, S., Rowan, N., & Garvey, M. (2022). Mycotherapy: Potential of Fungal Bioactives for the Treatment of Mental Health Disorders and Morbidities of Chronic Pain. *Journal of Fungi*, *8*(3), Article 3. https://doi.org/10.3390/jof8030290

Medford et al. USPTO 11,692,199 B2. July 2023. https://ppubs.uspto.gov/dirsearch-public/print/downloadBasicPdf/11692199?requestToken=eyJzdWIiOiI3YjA4ZjM5YS0xZTNjLTRmZjEtYTI2OS1kNTJmMGIyOTUzZjIiLCJ2ZXIiOiJhZjI5MTQwMC01ZWFkLTQwZTUtYmNlYy1kM2VkZDg4NWExZmQiLCJleHAiOjB9

Messmer, T., Klevernic, I., Furquim, C., Ovchinnikova, E., Dogan, A., Cruz, H., Post, M. J., & Flack, J. E. (2022). A serum-free media formulation for cultured meat production supports bovine satellite cell differentiation in the absence of serum starvation. *Nature Food*, *3*(1), 74–85. https://doi.org/10.1038/s43016-021-00419-1

Metzger, P. T., Britt, D. T., Covey, S., Schultz, C., Cannon, K. M., Grossman, K. D., Mantovani, J. G., & Mueller, R. P. (2019). Measuring the fidelity of asteroid regolith and cobble simulants. *Icarus*, *321*, 632–646. https://doi.org/10.1016/j.icarus.2018.12.019

Meyer, C. (2003). *Lunar Regolith.* https://curator.jsc.nasa.gov/lunar/letss/regolith.pdf

Microgravity Investigation for Thin Film Hydroponics. (2024). *NASA TechPort—Project Data*. https://techport.nasa.gov/view/106694

Mimee, M., Nadeau, P., Hayward, A., Carim, S., Flanagan, S., Jerger, L., Collins, J., McDonnell, S., Swartwout, R., Citorik, R. J., Bulović, V., Langer, R., Traverso, G., Chandrakasan, A. P., & Lu, T. K. (2018). An ingestible bacterial-electronic system to monitor gastrointestinal health. *Science*, *360*(6391), 915–918. https://doi.org/10.1126/science.aas9315

miniPCR Bio. (2024). *Welcome to miniPCR bio*. https://www.minipcr.com/

Mishra, A., Ntihuga, J. N., Molitor, B., & Angenent, L. T. (2020). Power-to-Protein: Carbon Fixation with Renewable Electric Power to Feed the World. *Joule*, *4*(6), 1142–1147. https://doi.org/10.1016/j.joule.2020.04.008

Mitić, R., Cantoni, F., Börlin, C. S., Post, M. J., & Jackisch, L. (2023). A simplified and defined serum-free medium for cultivating fat across species. *iScience*, *26*(1), 105822. https://doi.org/10.1016/j.isci.2022.105822

Molitor, B., Mishra, A., & Angenent, L. T. (2019). Power-to-protein: Converting renewable electric power and carbon dioxide into single cell protein with a two-stage bioprocess. *Energy & Environmental Science*, *12*(12), 3515–3521. https://doi.org/10.1039/C9EE02381J

Monje, O., Richards, J. T., Carver, J. A., Dimapilis, D. I., Levine, H. G., Dufour, N. F., & Onate, B. G. (2020). Hardware Validation of the Advanced Plant Habitat on ISS: Canopy Photosynthesis in Reduced Gravity. *Frontiers in Plant Science*, *11*. https://doi.org/10.3389/fpls.2020.00673

Moon, T. S. (2022). SynMADE: Synthetic microbiota across diverse ecosystems. *Trends in Biotechnology*, *40*(12), 1405–1414. https://doi.org/10.1016/j.tibtech.2022.08.010

Morrison, K. D., Reiss, M. B., Tanner, T. D., Gollott, T. R., Loots, G. G., & Collette, N. M. (2024). The application of synthetic antibacterial minerals to combat topical infections: Exploring a mouse model of MRSA infection. *Scientific Reports*, *14*(1), 1762. https://doi.org/10.1038/s41598-024-52082-8

Mortazavi, S. M. J., Said-Salman, I., Mortazavi, A. R., El Khatib, S., & Sihver, L. (2024). How the adaptation of the human microbiome to harsh space environment can determine the chances of success for a space mission to Mars and beyond. *Frontiers in Microbiology*, *14*. https://doi.org/10.3389/fmicb.2023.1237564

Mortimer, J. C., & Gilliham, M. (2022). SpaceHort: Redesigning plants to support space exploration and on-earth sustainability. *Current Opinion in Biotechnology*, *73*, 246–252. https://doi.org/10.1016/j.copbio.2021.08.018

Möslinger, M., Ulpiani, G., & Vetters, N. (2023). Circular economy and waste management to empower a climate-neutral urban future. *Journal of Cleaner Production*, *421*, 138454. https://doi.org/10.1016/j.jclepro.2023.138454

Mulinti, P., Diekjürgen, D., Kurtzeborn, K., Balasubramanian, N., Stafslien, S. J., Grainger, D. W., & Brooks, A. E. (2022). Anti-Coagulant and Antimicrobial Recombinant Heparin-Binding Major Ampullate Spidroin 2 (MaSp2) Silk Protein. *Bioengineering*, *9*(2), Article 2. https://doi.org/10.3390/bioengineering9020046

Mulinti, P., Shreffler, J., Hasan, R., Dea, M., & Brooks, A. E. (2021). Infection Responsive Smart Delivery of Antibiotics Using Recombinant Spider Silk Nanospheres. *Pharmaceutics*, *13*(9), Article 9. https://doi.org/10.3390/pharmaceutics13091358

Müller, J. T. I., Rattenbacher, B., Tell, K., Rösch, C., Welsch, T., Maurer, M., Sperl, M., & Schnellenbach-Held, M. (2023). Space hardware for concrete sample production on ISS "MASON concrete mixer." *Npj Microgravity*, *9*(1), 1–9. https://doi.org/10.1038/s41526-023-00304-0

Mullin, A. C., Slouka, T., & Oza, J. P. (2022). Simple Extract Preparation Methods for E. coli-Based Cell-Free ExpressionCell-free expression (CFE). In A. S. Karim & M. C. Jewett (Eds.), *Cell-Free Gene Expression: Methods and Protocols* (pp. 51–64). Springer US. https://doi.org/10.1007/978-1-0716-1998-8_2

Murphy, T. W., Sheng, J., Naler, L. B., Feng, X., & Lu, C. (2019). On-chip manufacturing of synthetic proteins for point-of-care therapeutics. *Microsystems & Nanoengineering*, *5*(1), 1–12. https://doi.org/10.1038/s41378-019-0051-8

Nadersha, S., & Aly Hassan, A. (2022). Biodesalination and treatment of raw hypersaline produced water samples using indigenous wastewater algal consortia. *Desalination*, *528*, 115638. https://doi.org/10.1016/j.desal.2022.115638

Nangle, S. N., Ziesack, M., Buckley, S., Trivedi, D., Loh, D. M., Nocera, D. G., & Silver, P. A. (2020). Valorization of CO2 through lithoautotrophic production of sustainable chemicals in *Cupriavidus necator*. *Metabolic Engineering*, *62*, 207–220. https://doi.org/10.1016/j.ymben.2020.09.002

NASA Science Editorial Team. (2022). *4 Agencies Select 8 Research Projects to Extend Longevity of 3D Tissue Chips to 6 Months—NASA Science*. https://science.nasa.gov/missions/station/4-agencies-select-8-research-projects-to-extend-longevity-of-3d-tissue-chips-to-6-months/

National Academies of Sciences. (2024). *Read "Foundational Research Gaps and Future Directions for Digital Twins" at NAP.edu*. https://doi.org/10.17226/26894

National Aeronautics and Space Administration (NASA). (2008). *Experiments Advance Gardening at Home and in Space | NASA Spinoff*. https://spinoff.nasa.gov/Spinoff2008/ch_3.html

National Aeronautics and Space Administration (NASA). (2016). *Additive Manufacturing*. https://www.nasa.gov/wp-content/uploads/2015/04/additive_mfg.pdf

National Aeronautics and Space Administration (NASA). (2017) *Environmental Control and Life Control Systems (ECLSS)*. https://www.nasa.gov/wp-content/uploads/2020/10/g-281237_eclss_0.pdf?emrc=67ffdc

National Aeronautics and Space Administration (NASA). (2019a). *Deep-Space Food Science Research Improves 3D-Printing Capabilities | NASA Spinoff*. https://spinoff.nasa.gov/Spinoff2019/ip_2.html

National Aeronautics and Space Administration (NASA). (2019b). *3.001 MEDICAL KIT-CONTENTS AND REFERENCE. https://www.nasa.gov/wp-content/uploads/2015/03/medical_kit_checklist_-_full_release.pdf*

National Aeronautics and Space Administration (NASA). (2019c). *Veggie Fact Sheet. https://www.nasa.gov/wp-content/uploads/2019/04/veggie_fact_sheet_508.pdf*

National Aeronautics and Space Administration (NASA). (2023). *Commercial Low-Earth Orbit Destination (CLD) Concept of Operations.* https://ntrs.nasa.gov/api/citations/20230002770/downloads/ATTACHMENT%201%20CLDP-WP-1101_ConOps_Final.pdf

National Aeronautics and Space Administration Office of the Chief Health and Medical Officer (NASA OCHMO). (2023a). *OCHMO-TB-002 Environmental Control & Life Support System (ECLSS): Human-Centered Approach*. https://www.nasa.gov/wp-content/uploads/2023/07/eclss-technical-brief-ochmo.pdf

National Aeronautics and Space Administration Office of the Chief Health and Medical Officer (NASA OCHMO). (2023b). *OCHMO-TB-004 Carbon Dioxide (CO2)*. https://www.nasa.gov/wp-content/uploads/2023/12/ochmo-tb-004-carbon-dioxide.pdf

National Aeronautics and Space Administration Office of the Chief Health and Medical Officer (NASA OCHMO). (2023c). *OCHMO-TB-006 Pharmaceutical Care*. https://www.nasa.gov/wp-content/uploads/2023/12/ochmo-tb-006-pharmaceuticals.pdf

National Aeronautics and Space Administration Office of the Chief Health and Medical Officer (NASA OCHMO). (2023d). *OCHMO-TB-027 Spaceflight Toxicology*. https://www.nasa.gov/wp-content/uploads/2023/12/ochmo-tb-015-spaceflight-toxicology.pdf

National Aeronautics and Space Administration Office of the Chief Health and Medical Officer (NASA OCHMO). (2023e). *OCHMO-TB-015 Water-Human Consumption*. https://www.nasa.gov/wp-content/uploads/2023/12/ochmo-tb-027-water.pdf

National Aeronautics and Space Administration Techport (NASA TechPort). (2024). *NASA TechPort—Project Data*. https://techport.nasa.gov/view/97036

National Aeronautics and Space Administration Human Research Program (NASA HRP). (2016). *HRR - Risk—Risk of Radiation Carcinogenesis*. https://humanresearchroadmap.nasa.gov/Risks/risk.aspx?i=96

National Institutes of Health (NIH) Human Microbiome Project. (2024). *NIH Human Microbiome Project—Home*. https://hmpdacc.org/

National Institutes of Health (NIH). (2022). *Personalized Medicine*. National Institutes of Health (NIH). https://www.nih.gov/about-nih/what-we-do/nih-turning-discovery-into-health/personalized-medicine

National Institute of Standard and Technology (NIST). (2024). Human Gut Microbiome Reference Material. *NIST*. https://www.nist.gov/programs-projects/human-gut-microbiome-reference-material

Naz, N., Harandi, B. F., Newmark, J., & Kounaves, S. P. (2023). Microbial growth in actual martian regolith in the form of Mars meteorite EETA79001. *Communications Earth & Environment*, *4*(1), 1–9. https://doi.org/10.1038/s43247-023-01042-7

Negus, S. S., & Banks, M. L. (2018). Pharmacokinetic–Pharmacodynamic (PKPD) Analysis with Drug Discrimination. In J. H. Porter & A. J. Prus (Eds.), *The Behavioral Neuroscience of Drug Discrimination* (pp. 245–259). Springer International Publishing. https://doi.org/10.1007/7854_2016_36

Ng, R. N., Grey, L. J., Vaitekenas, A., McLean, S. A., Rudrum, J. D., Laucirica, D. R., Poh, M. W.-P., Hillas, J., Winslow, S. G., Iszatt, J. J., Iosifidis, T., Tai, A. S., Agudelo-Romero, P., Chang, B. J., Stick, S. M., & Kicic, A. (2021). Development and validation of a miniaturized bacteriophage host range screening assay against antibiotic resistant *Pseudomonas aeruginosa*. *Journal of Microbiological Methods*, *190*, 106346. https://doi.org/10.1016/j.mimet.2021.106346

Nguyen, P. Q., Soenksen, L. R., Donghia, N. M., Angenent-Mari, N. M., de Puig, H., Huang, A., Lee, R., Slomovic, S., Galbersanini, T., Lansberry, G., Sallum, H. M., Zhao, E. M., Niemi, J. B., & Collins, J. J. (2021). Wearable materials with embedded synthetic biology sensors for biomolecule detection. *Nature Biotechnology*, *39*(11), 1366–1374. https://doi.org/10.1038/s41587-021-00950-3

Nikkhah, A., Rohani, A., Zarei, M., Kulkarni, A., Batarseh, F. A., Blackstone, N. T., & Ovissipour, R. (2023). Toward sustainable culture media: Using artificial intelligence to optimize reduced-serum formulations for cultivated meat. *Science of The Total Environment*, *894*, 164988. https://doi.org/10.1016/j.scitotenv.2023.164988

Odermatt, E. K., Funk, L., Bargon, R., Martin, D. P., Rizk, S., & Williams, S. F. (2012). MonoMax Suture: A New Long-Term Absorbable Monofilament Suture Made from Poly-4-Hydroxybutyrate. *International Journal of Polymer Science*, *2012*(1), 216137. https://doi.org/10.1155/2012/216137

Oh, J. Y., Kim, H. S., Palanikumar, L., Go, E. M., Jana, B., Park, S. A., Kim, H. Y., Kim, K., Seo, J. K., Kwak, S. K., Kim, C., Kang, S., & Ryu, J.-H. (2018). Cloaking nanoparticles with protein corona shield for targeted drug delivery. *Nature Communications*, *9*(1), 4548. https://doi.org/10.1038/s41467-018-06979-4

Olabi, A. a., Lawless, H. t., Hunter, J. b., Levitsky, D. a., & Halpern, B. p. (2002). The Effect of Microgravity and Space Flight on the Chemical Senses. *Journal of Food Science*, *67*(2), 468–478. https://doi.org/10.1111/j.1365-2621.2002.tb10622.x

Omidfar, K., Riahi, F., & Kashanian, S. (2023). Lateral Flow Assay: A Summary of Recent Progress for Improving Assay Performance. *Biosensors*, *13*(9), Article 9. https://doi.org/10.3390/bios13090837

O'Neil, P. (2024). *ISS National Lab-Sponsored Investigation Aims to Expand Genes in Space™ Toolkit on Station*. https://www.issnationallab.org/release-ng20-genes-in-space-minipcr/

Ortiz, M., Leung, P. M., Shelley, G., Jirapanjawat, T., Nauer, P. A., Van Goethem, M. W., Bay, S. K., Islam, Z. F., Jordaan, K., Vikram, S., Chown, S. L., Hogg, I. D., Makhalanyane, T. P., Grinter, R., Cowan, D. A., & Greening, C. (2021). Multiple energy sources and metabolic strategies sustain microbial diversity in Antarctic desert soils. *Proceedings of the National Academy of Sciences*, *118*(45), e2025322118. https://doi.org/10.1073/pnas.2025322118

Ostrov, N., Jimenez, M., Billerbeck, S., Brisbois, J., Matragrano, J., Ager, A., & Cornish, V. W. (2017). A modular yeast biosensor for low-cost point-of-care pathogen detection. *Science Advances*, *3*(6), e1603221. https://doi.org/10.1126/sciadv.1603221

Oubre, C., Arbeille, P., Bailey, S., Basner, M., Bouzsein, M. L., Boyd, S. K., Clement, G. R., Gordon, G., Hughson, R. L., Ivkovic, V., Kuro-o, M., Levine, B. D., Liphardt, A., Macias, B. R., Norcross, J., Rivas, E., Shelhamer, M., Stahn, A. (2024). *CIPHER*. Retrieved September 26, 2024, from https://www.nasa.gov/mission/station/research-explorer/investigation/?#id=8413

Overbey, E. G., Kim, J., Tierney, B. T., Park, J., Houerbi, N., Lucaci, A. G., Garcia Medina, S., Damle, N., Najjar, D., Grigorev, K., Afshin, E. E., Ryon, K. A., Sienkiewicz, K., Patras, L., Klotz, R., Ortiz, V., MacKay, M., Schweickart, A., Chin, C. R., … Mason, C. E. (2024). The Space Omics and Medical Atlas (SOMA) and international astronaut biobank. *Nature*, *632*(8027), 1145–1154. https://doi.org/10.1038/s41586-024-07639-y

Oxford Nanopore Technologies. (2024). *MinION portable nanopore sequencing device*. https://nanoporetech.com/products/sequence/minion

Pandey, V. C., & Bajpai, O. (2019). Chapter 1 - Phytoremediation: From Theory Toward Practice. In V. C. Pandey & K. Bauddh (Eds.), *Phytomanagement of Polluted Sites* (pp. 1–49). Elsevier. https://doi.org/10.1016/B978-0-12-813912-7.00001-6

Pardee, K., Green, A. A., Takahashi, M. K., Braff, D., Lambert, G., Lee, J. W., Ferrante, T., Ma, D., Donghia, N., Fan, M., Daringer, N. M., Bosch, I., Dudley, D. M., O'Connor, D. H., Gehrke, L., & Collins, J. J. (2016). Rapid, Low-Cost Detection of Zika Virus Using Programmable Biomolecular Components. *Cell*, *165*(5), 1255–1266. https://doi.org/10.1016/j.cell.2016.04.059

Park, J. C., & Im, S.-H. (2020). Of men in mice: The development and application of a humanized gnotobiotic mouse model for microbiome therapeutics. *Experimental & Molecular Medicine*, *52*(9), 1383–1396. https://doi.org/10.1038/s12276-020-0473-2

Parrotta, L., Guerriero, G., Sergeant, K., Cai, G., & Hausman, J.-F. (2015). Target or barrier? The cell wall of early- and later-diverging plants vs cadmium toxicity: differences in the response mechanisms. *Frontiers in Plant Science*, *6*. https://doi.org/10.3389/fpls.2015.00133

Parry, W. (2023). *How a Human Smell Receptor Works Is Finally Revealed*. Quanta Magazine. https://www.quantamagazine.org/how-a-human-smell-receptor-works-is-finally-revealed-20230501/

Pasitka, L., Cohen, M., Ehrlich, A., Gildor, B., Reuveni, E., Ayyash, M., Wissotsky, G., Herscovici, A., Kaminker, R., Niv, A., Bitcover, R., Dadia, O., Rudik, A., Voloschin, A., Shimoni, M., Cinnamon, Y., & Nahmias, Y. (2023). Spontaneous immortalization of chicken fibroblasts generates stable, high-yield cell lines for serum-free production of cultured meat. *Nature Food*, *4*(1), 35–50. https://doi.org/10.1038/s43016-022-00658-w

Patel, J. B. (2001). 16S rRNA gene sequencing for bacterial pathogen identification in the clinical laboratory. *Molecular Diagnosis*, *6*(4), 313–321. https://doi.org/10.1054/modi.2001.29158

Patterson et al. (2021). *TEI-REX*. https://www.iarpa.gov/research-programs/tei-rex

Pawlowski, M., Ortmann, D., Bertero, A., Tavares, J. M., Pedersen, R. A., Vallier, L., & Kotter, M. R. N. (2017). Inducible and Deterministic Forward Programming of Human Pluripotent Stem Cells into Neurons, Skeletal Myocytes, and Oligodendrocytes. *Stem Cell Reports*, *8*(4), 803–812. https://doi.org/10.1016/j.stemcr.2017.02.016

Perez-Pinera, P., Han, N., Cleto, S., Cao, J., Purcell, O., Shah, K. A., Lee, K., Ram, R., & Lu, T. K. (2016). Synthetic biology and microbioreactor platforms for programmable production of biologics at the point-of-care. *Nature Communications*, *7*(1), 12211. https://doi.org/10.1038/ncomms12211

Pérez-Santaescolastica, C., Munekata, P. E. S., Pateiro, M., Domínguez, R., Misihairabgwi, J. M., & Lorenzo, J. M. (2021). Chapter 1 - Modern Food Production: Fundaments, Sustainability, and the Role of Technological Advances. In J. M. Lorenzo, P. E. S. Munekata, & F. J. Barba (Eds.), *Sustainable Production Technology in Food* (pp. 1–22). Academic Press. https://doi.org/10.1016/B978-0-12-821233-2.00003-4

Petry, H., Brooks, A., Orme, A., Wang, P., Liu, P., Xie, J., Kretschmer, P., Qian, H. S., Hermiston, T. W., & Harkins, R. N. (2008). Effect of viral dose on neutralizing antibody response and transgene expression after AAV1 vector re-administration in mice. *Gene Therapy*, *15*(1), 54–60. https://doi.org/10.1038/sj.gt.3303037

Pittia, P., Blanc, S., & Heer, M. (2023). Unraveling the intricate connection between dietary factors and the success in long-term space missions. *Npj Microgravity*, *9*(1), 1–7. https://doi.org/10.1038/s41526-023-00331-x

Portnoy, V. A., Bezdan, D., & Zengler, K. (2011). Adaptive laboratory evolution—Harnessing the power of biology for metabolic engineering. *Current Opinion in Biotechnology*, *22*(4), 590–594. https://doi.org/10.1016/j.copbio.2011.03.007

Puig, J., Knödlseder, N., Quera, J., Algara, M., & Güell, M. (2021). DNA Damage Protection for Enhanced Bacterial Survival Under Simulated Low Earth Orbit Environmental Conditions in Escherichia coli. *Frontiers in Microbiology*, *12*. https://doi.org/10.3389/fmicb.2021.789668

Purkayastha, D., & Sarkar, S. (2023). Performance evaluation of black soldier fly larvae fed on human faeces, food waste and their mixture. *Journal of Environmental Management*, *326*, 116727. https://doi.org/10.1016/j.jenvman.2022.116727

Quijano-Rubio, A., Yeh, H.-W., Park, J., Lee, H., Langan, R. A., Boyken, S. E., Lajoie, M. J., Cao, L., Chow, C. M., Miranda, M. C., Wi, J., Hong, H. J., Stewart, L., Oh, B.-H., & Baker, D.

(2021). De novo design of modular and tunable protein biosensors. *Nature*, *591*(7850), 482–487. https://doi.org/10.1038/s41586-021-03258-z

Rasor, B. J., Karim, A. S., Alper, H. S., & Jewett, M. C. (2023). Cell Extracts from Bacteria and Yeast Retain Metabolic Activity after Extended Storage and Repeated Thawing. *ACS Synthetic Biology*, *12*(3), 904–908. https://doi.org/10.1021/acssynbio.2c00685

REBYOTA. (2022). Ferring receives U.S. FDA approval for REBYOTA™ (fecal microbiota, live-jslm) – A novel first-in-class microbiota-based live biotherapeutic. *Ferring Global*. https://www.ferring.com/ferring-receives-u-s-fda-approval-for-rebyota-fecal-microbiota-live-jslm-a-novel-first-in-class-microbiota-based-live-biotherapeutic/

Rech, E. l., & Arber, W. (2013). Biodiversity as a source for synthetic domestication of useful specific traits. *Annals of Applied Biology*, *162*(2), 141–144. https://doi.org/10.1111/aab.12013

Reginato, P., Salmon, S., & Caiati, A. (2023). Lowering the Km of carbonic anhydrase to facilitate capture of CO2. *Homeworld Collective*. https://doi.org/10.21428/23398f7c.2d556855

Revellame, E. D., Aguda, R., Chistoserdov, A., Fortela, D. L., Hernandez, R. A., & Zappi, M. E. (2021). Microalgae cultivation for space exploration: Assessing the potential for a new generation of waste to human life-support system for long duration space travel and planetary human habitation. *Algal Research*, *55*, 102258. https://doi.org/10.1016/j.algal.2021.102258

Reynolds, R., Little, M. P., Day, S., Charvat, J., Blattnig, S., Huff, J., & Patel, Z. S. (2021). Cancer incidence and mortality in the USA Astronaut Corps, 1959–2017. *Occupational and Environmental Medicine*, *78*(12), 869–875. https://doi.org/10.1136/oemed-2020-107143

Rickard, J. (2023). *Newly Identified Algal Strains Rich in Phosphorous Could Improve Wastewater Treatment*. https://www.nrel.gov/news/program/2023/newly-identified-algal-strains-rich-in-phosphorous-could-improve-wastewater-treatment.html

Riley, L. A., Payne, I. C., Tumen-Velasquez, M., & Guss, A. M. (2023). Simple and Rapid Site-Specific Integration of Multiple Heterologous DNAs into the Escherichia coli Chromosome. *Journal of Bacteriology*, *205*(2), e00338-22. https://doi.org/10.1128/jb.00338-22

Rink, S., & Baeumner, A. J. (2023). Progression of Paper-Based Point-of-Care Testing toward Being an Indispensable Diagnostic Tool in Future Healthcare. *Analytical Chemistry*, *95*(3), 1785–1793. https://doi.org/10.1021/acs.analchem.2c04442

Rodrigues, J. S., & Lindberg, P. (2021). Metabolic engineering of *Synechocystis* sp. PCC 6803 for improved bisabolene production. *Metabolic Engineering Communications*, *12*, e00159. https://doi.org/10.1016/j.mec.2020.e00159

Roh, Y. H., Lee, C. Y., Lee, S., Kim, H., Ly, A., Castro, C. M., Cheon, J., Lee, J.-H., & Lee, H. (2023). CRISPR-Enhanced Hydrogel Microparticles for Multiplexed Detection of Nucleic Acids. *Advanced Science*, *10*(10), 2206872. https://doi.org/10.1002/advs.202206872

Rothschild, L. J., Averesch, N. J. H., Strychalski, E. A., Moser, F., Glass, J. I., Cruz Perez, R., Yekinni, I. O., Rothschild-Mancinelli, B., Roberts Kingman, G. A., Wu, F., Waeterschoot, J., Ioannou, I. A., Jewett, M. C., Liu, A. P., Noireaux, V., Sorenson, C., & Adamala, K. P. (2024).

Building Synthetic Cells—From the Technology Infrastructure to Cellular Entities. *ACS Synthetic Biology*, *13*(4), 974–997. https://doi.org/10.1021/acssynbio.3c00724

Ruff, S. E., Humez, P., de Angelis, I. H., Diao, M., Nightingale, M., Cho, S., Connors, L., Kuloyo, O. O., Seltzer, A., Bowman, S., Wankel, S. D., McClain, C. N., Mayer, B., & Strous, M. (2023). Hydrogen and dark oxygen drive microbial productivity in diverse groundwater ecosystems. *Nature Communications*, *14*(1), 3194. https://doi.org/10.1038/s41467-023-38523-4

Sable et al. (2016). *Does Lettuce Taste Different in Space?* https://www.issnationallab.org/does-lettuce-taste-different-in-space/

Saha, K., Sontheimer, E. J., Brooks, P. J., Dwinell, M. R., Gersbach, C. A., Liu, D. R., Murray, S. A., Tsai, S. Q., Wilson, R. C., Anderson, D. G., Asokan, A., Banfield, J. F., Bankiewicz, K. S., Bao, G., Bulte, J. W. M., Bursac, N., Campbell, J. M., Carlson, D. F., Chaikof, E. L., … Zhou, J. (2021). The NIH Somatic Cell Genome Editing program. *Nature*, *592*(7853), 195–204. https://doi.org/10.1038/s41586-021-03191-1

Sakama, S., Kurusu, K., Morita, M., Oizumi, T., Masugata, S., Oka, S., Yokomizo, S., Nishimura, M., Morioka, T., Kakinuma, S., Shimada, Y., & Nakamura, A. J. (2021). An Enriched Environment Alters DNA Repair and Inflammatory Responses After Radiation Exposure. *Frontiers in Immunology*, *12*. https://doi.org/10.3389/fimmu.2021.760322

Salehi, M. (2022). Global water shortage and potable water safety; Today's concern and tomorrow's crisis. *Environment International*, *158*, 106936. https://doi.org/10.1016/j.envint.2021.106936

Santhoshkumar, P., Negi, A., & Moses, J. A. (2024). 3D printing for space food applications: Advancements, challenges, and prospects. *Life Sciences in Space Research*, *40*, 158–165. https://doi.org/10.1016/j.lssr.2023.08.002

Santomartino, R., Averesch, N. J. H., Bhuiyan, M., Cockell, C. S., Colangelo, J., Gumulya, Y., Lehner, B., Lopez-Ayala, I., McMahon, S., Mohanty, A., Santa Maria, S. R., Urbaniak, C., Volger, R., Yang, J., & Zea, L. (2023). Toward sustainable space exploration: A roadmap for harnessing the power of microorganisms. *Nature Communications*, *14*(1), 1391. https://doi.org/10.1038/s41467-023-37070-2

Santomartino, R., Zea, L., & Cockell, C. S. (2022). The smallest space miners: Principles of space biomining. *Extremophiles*, *26*(1), 7. https://doi.org/10.1007/s00792-021-01253-w

Sauret-Güeto, S., Frangedakis, E., Silvestri, L., Rebmann, M., Tomaselli, M., Markel, K., Delmans, M., West, A., Patron, N. J., & Haseloff, J. (2020). Systematic Tools for Reprogramming Plant Gene Expression in a Simple Model, Marchantia polymorpha. *ACS Synthetic Biology*, *9*(4), 864–882. https://doi.org/10.1021/acssynbio.9b00511

Schulz, L., Guo, Z., Zarzycki, J., Steinchen, W., Schuller, J. M., Heimerl, T., Prinz, S., Mueller-Cajar, O., Erb, T. J., & Hochberg, G. K. A. (2022). Evolution of increased complexity and specificity at the dawn of form I Rubiscos. *Science*, *378*(6616), 155–160. https://doi.org/10.1126/science.abq1416

Schwander, T., Schada von Borzyskowski, L., Burgener, S., Cortina, N. S., & Erb, T. J. (2016). A synthetic pathway for the fixation of carbon dioxide in vitro. *Science*, *354*(6314), 900–904. https://doi.org/10.1126/science.aah5237

Scoles, S. (2023). *Mars Needs Insects—The New York Times*. https://www.nytimes.com/2023/11/27/science/mars-needs-insects.html

Sears, R. G., Rigoulot, S. B., Occhialini, A., Morgan, B., Kakeshpour, T., Brabazon, H., Barnes, C. N., Seaberry, E. M., Jacobs, B., Brown, C., Yang, Y., Schimel, T. M., Lenaghan, S. C., & Neal Stewart Jr., C. (2023). Engineered gamma radiation phytosensors for environmental monitoring. *Plant Biotechnology Journal*, *21*(9), 1745–1756. https://doi.org/10.1111/pbi.14072

Seely, K. D., Kotelko, C. A., Douglas, H., Bealer, B., & Brooks, A. E. (2021). The Human Gut Microbiota: A Key Mediator of Osteoporosis and Osteogenesis. *International Journal of Molecular Sciences*, *22*(17), Article 17. https://doi.org/10.3390/ijms22179452

Sengupta, P., Muthamilselvi Sivabalan, S. K., Singh, N. K., Raman, K., & Venkateswaran, K. (2024). Genomic, functional, and metabolic enhancements in multidrug-resistant Enterobacter bugandensis facilitating its persistence and succession in the International Space Station. *Microbiome*, *12*(1), 62. https://doi.org/10.1186/s40168-024-01777-1

Sentinel Environmental. (2024). *Community Characterization—Sentinel Environmental*. https://www.senviron.com/community-characterization/

Service, R. (2023). *Crops grown without sunlight could help feed astronauts bound for Mars*. https://www.science.org/content/article/crops-grown-without-sunlight-could-help-feed-astronauts-bound-mars

Shinde, A., Illath, K., Kasiviswanathan, U., Nagabooshanam, S., Gupta, P., Dey, K., Chakrabarty, P., Nagai, M., Rao, S., Kar, S., & Santra, T. S. (2023). Recent Advances of Biosensor-Integrated Organ-on-a-Chip Technologies for Diagnostics and Therapeutics. *Analytical Chemistry*, *95*(6), 3121–3146. https://doi.org/10.1021/acs.analchem.2c05036

Shukla, S., Eber, F. J., Nagarajan, A. S., DiFranco, N. A., Schmidt, N., Wen, A. M., Eiben, S., Twyman, R. M., Wege, C., & Steinmetz, N. F. (2015). The Impact of Aspect Ratio on the Biodistribution and Tumor Homing of Rigid Soft-Matter Nanorods. *Advanced Healthcare Materials*, *4*(6), 874–882. https://doi.org/10.1002/adhm.201400641

Siebert, D., Eikmanns, B. J., & Blombach, B. (2022). Exploiting Aerobic Carboxydotrophic Bacteria for Industrial Biotechnology. In A.-P. Zeng & N. J. Claassens (Eds.), *One-Carbon Feedstocks for Sustainable Bioproduction* (pp. 1–32). Springer International Publishing. https://doi.org/10.1007/10_2021_178

Sillman, J., Nygren, L., Kahiluoto, H., Ruuskanen, V., Tamminen, A., Bajamundi, C., Nappa, M., Wuokko, M., Lindh, T., Vainikka, P., Pitkänen, J.-P., & Ahola, J. (2019). Bacterial protein for food and feed generated via renewable energy and direct air capture of CO2: Can it reduce land and water use? *Global Food Security*, *22*, 25–32. https://doi.org/10.1016/j.gfs.2019.09.007

Simon, M., Latorella, K., Martin, J., Cerro, J., Lepsch, R., Jefferies, S., Goodliff, K., Smitherman, D., McCleskey, C., & Stromgren, C. (2017). NASA's advanced exploration systems Mars

transit habitat refinement point of departure design. *2017 IEEE Aerospace Conference*, 1–34. https://doi.org/10.1109/AERO.2017.7943662

Singh, V. K., & Seed, T. M. (2019). The efficacy and safety of amifostine for the acute radiation syndrome. *Expert Opinion on Drug Safety*, *18*(11), 1077–1090. https://doi.org/10.1080/14740338.2019.1666104

Singha, B., Rawat, B. S., Venkataraman, R., Nair, T., Rosenn, E. H., & Soni, V. (2023). Gut microbiome associated dysbiosis: Limited regimens and expanding horizons of phage therapy. *Aspects of Molecular Medicine*, *2*, 100029. https://doi.org/10.1016/j.amolm.2023.100029

Skrivergaard, S., Young, J. F., Sahebekhtiari, N., Semper, C., Venkatesan, M., Savchenko, A., Stogios, P. J., Therkildsen, M., & Rasmussen, M. K. (2023). A simple and robust serum-free media for the proliferation of muscle cells. *Food Research International*, *172*, 113194. https://doi.org/10.1016/j.foodres.2023.113194

Smith, S. M., & Zwart, S. R. (2021). Nutrition as Fuel for Human Spaceflight. *Physiology*, *36*(5), 324–330. https://doi.org/10.1152/physiol.00011.2021

Smith, S. M., Zwart, S. R., Block, G., Rice, B. L., & Davis-Street, J. E. (2005). The nutritional status of astronauts is altered after long-term space flight aboard the International Space Station. *The Journal of Nutrition*, *135*(3), 437–443. https://doi.org/10.1093/jn/135.3.437

Smith, S. M., Zwart, S. R., & Douglas, G. L. (2021). *Human Adaptation to Spaceflight*.

Speight, G., Schneider, W. F., Toom, K. T. (2024). *Exploration ECLSS: Brine Processor System*. https://www.nasa.gov/mission/station/research-explorer/investigation/?#id=8102

Sperl, M., Schnellenbach-Held, M., Welsch, T., Tell, K., Müller, J., Vievers, Y. (2024). *Concrete Hardening*. https://www.nasa.gov/mission/station/research-explorer/investigation/?#id=8538

Sravan, J. S., Matsakas, L., & Sarkar, O. (2024). Advances in Biological Wastewater Treatment Processes: Focus on Low-Carbon Energy and Resource Recovery in Biorefinery Context. *Bioengineering*, *11*(3), Article 3. https://doi.org/10.3390/bioengineering11030281

Stahl-Rommel, S., Jain, M., Nguyen, H. N., Arnold, R. R., Aunon-Chancellor, S. M., Sharp, G. M., Castro, C. L., John, K. K., Juul, S., Turner, D. J., Stoddart, D., Paten, B., Akeson, M., Burton, A. S., & Castro-Wallace, S. L. (2021). Real-Time Culture-Independent Microbial Profiling Onboard the International Space Station Using Nanopore Sequencing. *Genes*, *12*(1), Article 1. https://doi.org/10.3390/genes12010106

Stark, J. C., Jaroentomeechai, T., Moeller, T. D., Hershewe, J. M., Warfel, K. F., Moricz, B. S., Martini, A. M., Dubner, R. S., Hsu, K. J., Stevenson, T. C., Jones, B. D., DeLisa, M. P., & Jewett, M. C. (2021). On-demand biomanufacturing of protective conjugate vaccines. *Science Advances*, *7*(6), eabe9444. https://doi.org/10.1126/sciadv.abe9444

Stephenson, M. J., Reed, J., Brouwer, B., & Osbourn, A. (2018). Transient Expression in Nicotiana Benthamiana Leaves for Triterpene Production at a Preparative Scale. *Journal of Visualized Experiments (JoVE)*, *123*, e55617. https://doi.org/10.3791/58169

Stirling, F., Naydich, A., Bramante, J., Barocio, R., Certo, M., Wellington, H., Redfield, E., O'Keefe, S., Gao, S., Cusolito, A., Way, J., & Silver, P. (2020). Synthetic Cassettes for pH-Mediated Sensing, Counting, and Containment. *Cell Reports*, *30*(9), 3139-3148.e4. https://doi.org/10.1016/j.celrep.2020.02.033

Stout, A. J., Rittenberg, M. L., Shub, M., Saad, M. K., Mirliani, A. B., Dolgin, J., & Kaplan, D. L. (2023). A Beefy-R culture medium: Replacing albumin with rapeseed protein isolates. *Biomaterials*, *296*, 122092. https://doi.org/10.1016/j.biomaterials.2023.122092

Stout, A. J., Zhang, X., Letcher, S. M., Rittenberg, M. L., Shub, M., Chai, K. M., Kaul, M., & Kaplan, D. L. (2024). Engineered autocrine signaling eliminates muscle cell FGF2 requirements for cultured meat production. *Cell Reports Sustainability*, *1*(1), 100009. https://doi.org/10.1016/j.crsus.2023.100009

Styer, A., Pettinga, D., Caddell, D., & Coleman-Derr, D. (2024). Improving rice drought tolerance through host-mediated microbiome selection. Manuscript submitted for publication. *eLife*, *13*. https://doi.org/10.7554/eLife.97015.1

Sukenik, S. C., Karuppanan, K., Li, Q., Lebrilla, C. B., Nandi, S., & McDonald, K. A. (2018). Transient Recombinant Protein Production in Glycoengineered Nicotiana benthamiana Cell Suspension Culture. *International Journal of Molecular Sciences*, *19*(4), Article 4. https://doi.org/10.3390/ijms19041205

Svendsen, C. & Sharma, A. (2024). *Stellar Stem Cells Mission 1*. https://www.nasa.gov/mission/station/research-explorer/investigation/?#id=9052

Sychla, A., Stach, C. S., Roach, S. N., Hayward, A. N., Langlois, R. A., & Smanski, M. J. (2024). *High-throughput investigation of genetic design constraints in domesticated Influenza A Virus for transient gene delivery* (p. 2024.02.14.580300). Manuscript submitted for publication. bioRxiv. https://doi.org/10.1101/2024.02.14.580300

Szydlo, K., Ignatova, Z., & Gorochowski, T. E. (2022). Improving the Robustness of Engineered Bacteria to Nutrient Stress Using Programmed Proteolysis. *ACS Synthetic Biology*, *11*(3), 1049–1059. https://doi.org/10.1021/acssynbio.1c00490

Tabor, A. (2021, December 7). *Microbial Tracking—NASA*. https://www.nasa.gov/centers-and-facilities/ames/ames-science/ames-space-biosciences/microbial-tracking/

Tang, H., Rising, H. H., Majji, M., & Brown, R. D. (2022). Long-Term Space Nutrition: A Scoping Review. *Nutrients*, *14*(1), Article 1. https://doi.org/10.3390/nu14010194

Tender, L. (2024). *Biomanufacturing: Survival, Utility, and Reliability beyond Earth*. https://www.darpa.mil/program/biomanufacturing-survival-utility-and-reliability-beyond-earth

Tesei, D., Jewczynko, A., Lynch, A. M., & Urbaniak, C. (2022). Understanding the Complexities and Changes of the Astronaut Microbiome for Successful Long-Duration Space Missions. *Life*, *12*(4), Article 4. https://doi.org/10.3390/life12040495

Thumsi, A., Martínez, D., Swaminathan, S. J., Esrafili, A., Suresh, A. P., Jaggarapu, M. M. C., Lintecum, K., Halim, M., Mantri, S. V., Sleiman, Y., Appel, N., Gu, H., Curtis, M., Zuniga, C., & Acharya, A. P. (n.d.). Inverse-Vaccines for Rheumatoid Arthritis Re-establish Metabolic and

Immunological Homeostasis in Joint Tissues. *Advanced Healthcare Materials*, *n/a*(n/a), 2303995. https://doi.org/10.1002/adhm.202303995

Tibbetts, C. (2023). *Transforming flies into degradable plastics*. American Chemical Society. https://www.acs.org/pressroom/newsreleases/2023/august/transforming-flies-into-degradable-plastics.html

Tibocha-Bonilla, J. D., Kumar, M., Zengler, K., & Zuniga, C. (2023). Integrating Metabolic Modeling and High-Throughput Data to Characterize Diatoms Metabolism. In *The Mathematical Biology of Diatoms* (pp. 165–191). John Wiley & Sons, Ltd. https://doi.org/10.1002/9781119751939.ch7

Timm, A. C., Shankles, P. G., Foster, C. M., Doktycz, M. J., & Retterer, S. T. (2016). Toward Microfluidic Reactors for Cell-Free Protein Synthesis at the Point-of-Care. *Small*, *12*(6), 810–817. https://doi.org/10.1002/smll.201502764

Translational Research Institute for Space Health (TRISH). (2024). *What Is TRISH? | BCM*. https://www.bcm.edu/academic-centers/space-medicine/translational-research-institute/what-is-trish

Tsaneva, M., & Van Damme, E. J. M. (2020). 130 years of Plant Lectin Research. *Glycoconjugate Journal*, *37*(5), 533–551. https://doi.org/10.1007/s10719-020-09942-y

Tyagi, G., Lahoti, M., Srivastava, A., Patil, D., Jadhav, U. U., & Purekar, A. S. (2024). Bioconcrete-Enabled Resilient Construction: A Review. *Applied Biochemistry and Biotechnology*, *196*(5), 2901–2927. https://doi.org/10.1007/s12010-023-04427-8

United States Department of Agriculture (USDA). (2024). *BioPreferred|Biobased Products*. https://www.biopreferred.gov/BioPreferred/faces/pages/BiobasedProducts.xhtml

University of Applied Sciences Anhalt. (2020). *3D Printing Bioplastics – materiability*. https://materiability.com/portfolio/3d-printing-bioplastics/

Urbina, J., Patil, A., Fujishima, K., Paulino-Lima, I. G., Saltikov, C., & Rothschild, L. J. (2019). A new approach to biomining: Bioengineering surfaces for metal recovery from aqueous solutions. *Scientific Reports*, *9*(1), 16422. https://doi.org/10.1038/s41598-019-52778-2

Utsunomia, C., Ren, Q., & Zinn, M. (2020). Poly(4-Hydroxybutyrate): Current State and Perspectives. *Frontiers in Bioengineering and Biotechnology*, *8*. https://doi.org/10.3389/fbioe.2020.00257

van der Meer, J. R., & Belkin, S. (2010). Where microbiology meets microengineering: Design and applications of reporter bacteria. *Nature Reviews Microbiology*, *8*(7), 511–522. https://doi.org/10.1038/nrmicro2392

van Haasteren, J., Li, J., Scheideler, O. J., Murthy, N., & Schaffer, D. V. (2020). The delivery challenge: Fulfilling the promise of therapeutic genome editing. *Nature Biotechnology*, *38*(7), 845–855. https://doi.org/10.1038/s41587-020-0565-5

Vanhoudt, N., Horemans, N., Wannijn, J., Nauts, R., Van Hees, M., & Vandenhove, H. (2014). Primary stress responses in *Arabidopsis thaliana* exposed to gamma radiation. *Journal of Environmental Radioactivity*, *129*, 1–6. https://doi.org/10.1016/j.jenvrad.2013.11.011

Vanni, C., Schechter, M. S., Acinas, S. G., Barberán, A., Buttigieg, P. L., Casamayor, E. O., Delmont, T. O., Duarte, C. M., Eren, A. M., Finn, R. D., Kottmann, R., Mitchell, A., Sánchez, P., Siren, K., Steinegger, M., Gloeckner, F. O., & Fernàndez-Guerra, A. (2022). Unifying the known and unknown microbial coding sequence space. *eLife*, *11*, e67667. https://doi.org/10.7554/eLife.67667

Venkataraman, S., Khan, I., Habibi, P., Le, M., Lippert, R., & Hefferon, K. (2023). Recent advances in expression and purification strategies for plant made vaccines. *Frontiers in Plant Science*, *14*. https://doi.org/10.3389/fpls.2023.1273958

Veos. (2024). *Veos zet bloed om in drinkbaar water met nieuwe zuiveringsinstallatie*. Veos. https://www.veos.be/en/node/59

Warfel, K. F., Williams, A., Wong, D. A., Sobol, S. E., Desai, P., Li, J., Chang, Y.-F., DeLisa, M. P., Karim, A. S., & Jewett, M. C. (2023). A Low-Cost, Thermostable, Cell-Free Protein Synthesis Platform for On-Demand Production of Conjugate Vaccines. *ACS Synthetic Biology*, *12*(1), 95–107. https://doi.org/10.1021/acssynbio.2c00392

Watson, N., Kessler, J. L., Torrez, C., Fisher, R. T., Stanley, T. M. (2024). *NASA TechPort—Project Data*. https://techport.nasa.gov/view/154402

Wei, S., Bian, Y., Zhao, Q., Chen, S., Mao, J., Song, C., Cheng, K., Xiao, Z., Zhang, C., Ma, W., Zou, H., Ye, M., & Dai, S. (2017). Salinity-Induced Palmella Formation Mechanism in Halotolerant Algae Dunaliella salina Revealed by Quantitative Proteomics and Phosphoproteomics. *Frontiers in Plant Science*, *8*. https://doi.org/10.3389/fpls.2017.00810

Werkheiser, M. J., Moore, D. F., Petersen, E. M., Grossman, K. D. (2024). *NASA TechPort—Project Data*. https://techport.nasa.gov/view/116413

Westenberg, R., & Peralta-Yahya, P. (2023). Toward implementation of carbon-conservation networks in nonmodel organisms. *Current Opinion in Biotechnology*, *81*, 102949. https://doi.org/10.1016/j.copbio.2023.102949

White, N. J., & Wenthe, A. (2023). Managing Hemostasis in Space. *Arteriosclerosis, Thrombosis, and Vascular Biology*, *43*(11), 2079–2087. https://doi.org/10.1161/ATVBAHA.123.318783

Williamson, J., Wilson, J. P., Robinson, K., & Luong, H. (2023). *Status of ISS Water Management and Recovery*. https://ntrs.nasa.gov/api/citations/20230006217/downloads/ICES%202023-097%20Status%20of%20ISS%20Water%20Management%20and%20Recovery.pdf

Woo, S.-G., Averesch, N. J. H., Berliner, A. J., Deutzmann, J. S., Pane, V. E., Chatterjee, S., & Criddle, C. S. (2024). *Isolation and Characterization of a Halomonas Species for Non-Axenic Growth-Associated Production of Bio-Polyesters from Sustainable Feedstocks* (p. 2024.03.28.587248). Manuscript submitted for publication. bioRxiv. https://doi.org/10.1101/2024.03.28.587248

World Health Organization (WHO). (2024). *Developing Norms and Standards*. https://www.who.int/teams/health-product-policy-and-standards/standards-and-specifications/norms-and-standards

Xiong, Y., Hirano, H., Lane, N. E., Nandi, S., & McDonald, K. A. (2022). Plant-based production and characterization of a promising Fc-fusion protein against microgravity-induced bone density loss. *Frontiers in Bioengineering and Biotechnology*, *10*. https://doi.org/10.3389/fbioe.2022.962292

Yan, X., Zhang, Q., Ma, X., Zhong, Y., Tang, H., & Mai, S. (2023). The mechanism of biomineralization: Progress in mineralization from intracellular generation to extracellular deposition. *Japanese Dental Science Review*, *59*, 181–190. https://doi.org/10.1016/j.jdsr.2023.06.005

Yates, K., Berliner, A. J., Makrygiorgos, G., Kaiyom, F., McNulty, M. J., Khan, I., Kusuma, P., Kinlaw, C., Miron, D., Legg, C., Wilson, J., Bugbee, B., Mesbah, A., Arkin, A. P., Nandi, S., & McDonald, K. A. (2024). Manuscript submitted for publication. *Nitrogen Accountancy in Space Agriculture*. https://doi.org/10.21203/rs.3.rs-4004743/v1

Yates, M. D., Ma, L., Sack, J., Golden, J. P., Strycharz-Glaven, S. M., Yates, S. R., & Tender, L. M. (2017). Microbial Electrochemical Energy Storage and Recovery in a Combined Electrotrophic and Electrogenic Biofilm. *Environmental Science & Technology Letters*, *4*(9), 374–379. https://doi.org/10.1021/acs.estlett.7b00335

Yates, M. D., Mickol, R. L., Vignola, A., Baldwin, J. W., Glaven, S. M., & Tender, L. M. (2024). Performance of a combined electrotrophic and electrogenic biofilm operated under long-term, continuous cycling. *Biotechnology Letters*, *46*(2), 213–221. https://doi.org/10.1007/s10529-023-03450-3

Yeh, H.-H. J., Brown, C. B., & Jeng, F. J. (2005). *Tool for Sizing Analysis of the Advanced Life Support System* (MSC-23506). https://ntrs.nasa.gov/citations/20110016485

Yuan, P., Cui, S., Liu, Y., Li, J., Du, G., & Liu, L. (2020). Metabolic engineering for the production of fat-soluble vitamins: Advances and perspectives. *Applied Microbiology and Biotechnology*, *104*(3), 935–951. https://doi.org/10.1007/s00253-019-10157-x

Zampieri, G., Campanaro, S., Angione, C., & Treu, L. (2023). Metatranscriptomics-guided genome-scale metabolic modeling of microbial communities. *Cell Reports Methods*, *3*(1), 100383. https://doi.org/10.1016/j.crmeth.2022.100383

Zawada, J. F., Burgenson, D., Yin, G., Hallam, T. J., Swartz, J. R., & Kiss, R. D. (2022). Cell-free technologies for biopharmaceutical research and production. *Current Opinion in Biotechnology*, *76*, 102719. https://doi.org/10.1016/j.copbio.2022.102719

ZBiotics. USPTO 10849938B2. December 2020. https://patentimages.storage.googleapis.com/8f/b9/8f/09e832038e7da8/US10849938.pdf

ZBiotics. (2024). *ZBiotics | Genetically Engineered Probiotics People Love*. ZBiotics. https://zbiotics.com/

Zermas, D., Teng, D., Stanitsas, P., Bazakos, M., Kaiser, D., Morellas, V., Mulla, D., & Papanikolopoulos, N. (2015). Automation solutions for the evaluation of plant health in corn fields. *2015 IEEE/RSJ International Conference on Intelligent Robots and Systems (IROS)*, 6521–6527. https://doi.org/10.1109/IROS.2015.7354309

Zhalnina, K., Louie, K. B., Hao, Z., Mansoori, N., da Rocha, U. N., Shi, S., Cho, H., Karaoz, U., Loqué, D., Bowen, B. P., Firestone, M. K., Northen, T. R., & Brodie, E. L. (2018). Dynamic root exudate chemistry and microbial substrate preferences drive patterns in rhizosphere microbial community assembly. *Nature Microbiology*, *3*(4), 470–480. https://doi.org/10.1038/s41564-018-0129-3

Zhang, Q., Cao, W., Liu, Z., Liu, Y., Zhang, H., Meng, H., Meng, G., & Zheng, J. (2023). Performance and mechanisms of urea exposure for enhancement of biotransformation of sewage sludge into volatile fatty acids. *Bioresource Technology*, *388*, 129776. https://doi.org/10.1016/j.biortech.2023.129776

Zhu, R., Fang, Y., Li, H., Liu, Y., Wei, J., Zhang, S., Wang, L., Fan, R., Wang, L., Li, S., & Chen, T. (2023). Psychobiotic Lactobacillus plantarum JYLP-326 relieves anxiety, depression, and insomnia symptoms in test anxious college via modulating the gut microbiota and its metabolism. *Frontiers in Immunology*, *14*. https://doi.org/10.3389/fimmu.2023.1158137

Zimmer, C. (2024). What Makes Tiny Tardigrades Nearly Radiation Proof. *The New York Times*. https://www.nytimes.com/2024/04/12/science/tardigrades-moss-piglets.html

Zoghlami, A., & Paës, G. (2019). Lignocellulosic Biomass: Understanding Recalcitrance and Predicting Hydrolysis. *Frontiers in Chemistry*, *7*. https://doi.org/10.3389/fchem.2019.00874

Zuñiga, C., Levering, J., Antoniewicz, M. R., Guarnieri, M. T., Betenbaugh, M. J., & Zengler, K. (2018). Predicting Dynamic Metabolic Demands in the Photosynthetic Eukaryote Chlorella vulgaris. *Plant Physiology*, *176*(1), 450–462. https://doi.org/10.1104/pp.17.00605

Zwart, S. r., Kloeris, V. l., Perchonok, M. h., Braby, L., & Smith, S. m. (2009). Assessment of Nutrient Stability in Foods from the Space Food System After Long-Duration Spaceflight on the ISS. *Journal of Food Science*, *74*(7), H209–H217. https://doi.org/10.1111/j.1750-3841.2009.01265.x