EBRC Engineering Biology Research Consortium

Enabling Defense Applications through Engineering Biology

A Technical Roadmap

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EBRC Engineering Biology Research Consortium

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Enabling Defense Applications through Engineering Biology: A Technical Roadmap

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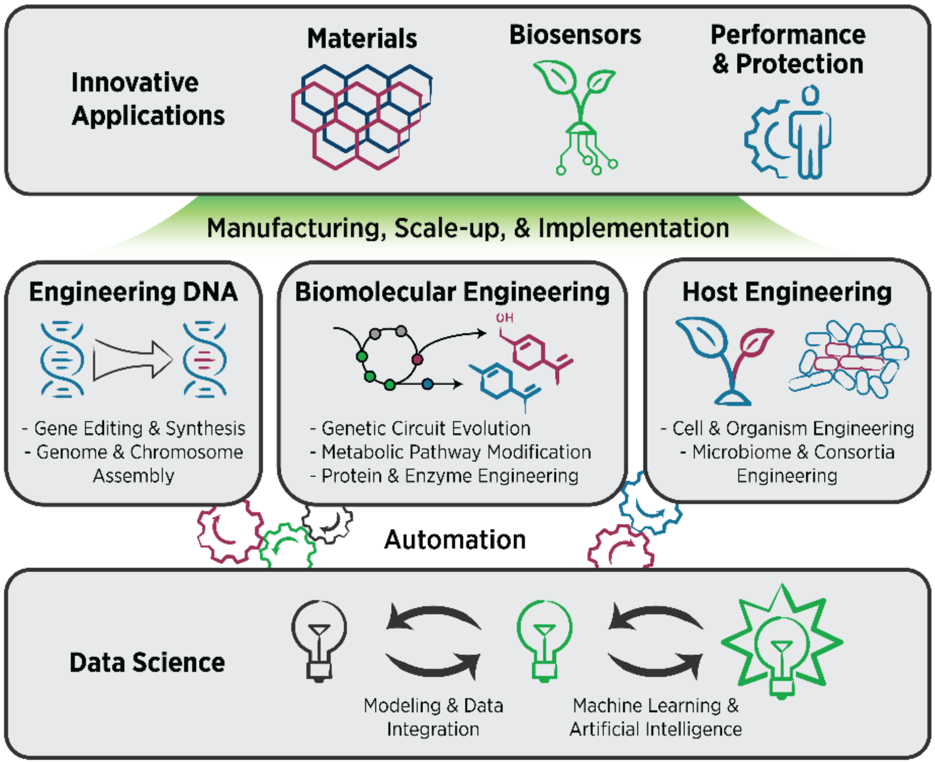
Executive Summary

We must be prepared for to operate in increasingly complex and dynamic environments. To maintain and enhance our capacity, technological advances can be leveraged to overcome challenges presented by these environments. Engineering biology is the convergence of advances in chemistry, biology, computer science, and engineering that enables us to go from idea to product faster, cheaper, and with greater precision than ever before. Engineering biology tools have the potential to provide transformational capabilities to humans and systems. Commonly used tools in engineering biology include modification of DNA and genetic material, use of microorganisms (*e.g.*, bacteria and yeast) to produce chemicals and materials, and adaptation of plants and animals to confer new abilities and modify performance. Taking advantage of these tools and technologies, engineering biology can transcend current constraints to build new materials for diverse systems, enable new methods for sensing, monitoring, and communicating, augment performance, protect the human from harsh environments and combat mission states, and build resilience to evolving threats.

To realize this potential, we must anticipate and generate significant progress and advancements in engineering biology through targeted research and development. The roadmap highlights scientific and engineering capabilities that can be achieved through various milestones over three time periods. The short-term milestones (2-5 years) are anticipated to build on current capabilities, existing infrastructure, facilities, and resources. The mid-term (5-10 year) and long-term (10+ year) milestones would require (and thus, result in) significant technical advancements, and stem from dedicated programs and resources. The milestones outlined in this roadmap are focused primarily on early stage research: TRL 1-4; 6.1-6.3. Additional strategic development efforts will be needed to advance translation of technologies into desired applications.

Achieving the milestones and capabilities detailed in this roadmap will lead to new tools and technologies that can be applied toward making humans and systems more resilient, better prepared, and better protected. Technical achievements will allow the utilization of diverse resources (including waste streams) for the production of specialty chemicals and materials, such as coatings or surfaces that can repair or regrow after sustaining damage, and systems for water filtration, desalination and purification. Engineered biosensor systems can better enable the ability to discriminate, identify, track, and target a wide variety of friends and foe in cluttered, multi-dimensional, multi-domain battlespace, through technologies such as skin- or soil-based detection and indication of pathogens, chemicals, or radiation. Integration of engineering biology with humans and systems can allow continuous and intelligent operation under adverse conditions across time



ENGINEERING BIOLOGY FOR MILITARY APPLICATIONS and space. An example of this is employing engineered gut or skin microbiomes to enable improved tolerance to harsh environments (*i.e.,* high-temperature or low-pressure), enhanced resistance to fatigue, or sustained activity.

Current national and global engineering biology research and development has the possibility of transforming military systems and mission spaces.

This roadmap is an independent study and does not confer DoD endorsement.

Technical Summary

Engineering biology – a convergence of biology, chemistry, computer science, and engineering – has the potential to transform relevant systems and mission spaces. Engineering biology comprises many powerful technologies, ranging from manipulating the bases in DNA, to modifying a cell for production of a specific compound, to altering the composition of a natural biome. Advancements in engineering biology enable gene synthesis, editing, and the assembly of chromosomes and genomes and the engineering of genetic circuits, biomolecules, and pathways, redesigning the building blocks and functional foundations of a cell. Engineering biology allows for modification, adaptation, evolution, and construction of cell-free systems, chassis, organisms, and consortia, enabling new systems for production and specialized activities, outputs, and interactions. Data science tools enable and advance biological data modeling and integration, machine learning, and artificial intelligence leading to novel, more intricate, and more robust engineered biological systems. The convergence of these engineering biology technologies and information science aims to unlock more rapid, innovative, and diverse applications than we can currently recognize or realize. Taking advantage of these tools and technologies, engineering biology can transcend current constraints to build new materials for relevant systems, enable new methods for sensing, monitoring, and communicating, and augment performance and protect the human from harsh environments, combat mission states, and build resilience to evolving threats.

The roadmap highlights scientific and engineering capabilities that can be achieved through reaching various milestones over time. Short-term milestones are objectives intended to be reached with current funding paradigms, existing infrastructure, facilities, and resources, and are attainable within 2-5 years. The mid-term (5-10 years) and long-term (10+ years) milestones are more ambitious achievements that require increased funding and resources, and new or improved infrastructure, but result in significant technical advancements. In addition to this strategic development, efforts are needed to advance translation of nascent tools and technologies into applications at scale and enable manufacturing and production for unique needs. Despite these needs, engineering biology can revolutionize conventional approaches to manufacturing and scale-up and the accessible space in materials and bioproducts.

Advancements in engineering biology can bring about new structures and functions and reduce cost and time of manufacturing and production. However, to realize this potential, we must anticipate and generate significant progress toward overcoming technical challenges in engineering biology. Therefore, the roadmap comprises topical sections embodying an increasing complexity of technical challenges, layering scientific and engineering capacities through implementation of advanced engineering biology tools and technologies. These technical challenges are captured as Enabling Capabilities, which represent significant technical achievements that will revolutionize the way the engineering biology is applied to major challenges.

The roadmap for engineering of biomolecules and cells for the production of biologics – including small molecules, enzymes, and active cells – and materials captures advancements in technologies necessary to engineer and dynamically synthesize molecules and polymers, including those not found in nature, and introduce novel functions and properties. Further, through engineering of cellular metabolism, modification and adaptation of biological pathways and circuits, and enabling control over the spatial and temporal architecture and organization of biological components, cells, and systems, achievement of the Enabling Capabilities can lead to the synthesis of high-performance, dynamic, and low-cost materials, sensors, and protective and performance enhancive products. Long-term milestones aim to enable these production capacities in relevant environments at point-of-need, real-time and in situ. Many of these capabilities represent foundational and fundamental advancements in engineering biology and application can be well-tuned to diverse defense needs. Biologics and products of engineering biomolecules and cells can be used toward applications in specialty chemicals, fuels, coatings, and surfaces; novel sensing, monitoring, and reporting systems for humans and the environment, and materials with greater levels of complexity and functionality for diverse settings.

Furthermore, this engineering can enable utilization of more diverse feedstocks or waste-streams, and has the potential to decrease footprint, costs, and time-to-implementation.

Engineering of biological systems advances the scale of tuning biomolecules and cells toward more complex systems comprised of multiple cells and species as organisms, microbiomes, and consortia. These engineered systems have potential to alter relevant environments or the ability of humans and systems to adapt to and function within those environments. Engineering of organisms, such as microbial consortia and plants, results in a roadmap to acquire greater control over spatial and temporal behaviors, the robustness of systems to withstand harsh conditions and respond quickly to local changes, and the ability to design and model these systems for diverse events and outcomes. Key milestones toward making these advancements are acquisition of the tools and ability to exercise elimination of organisms or the termination of function – such as through biological kill-switches - to ameliorate the potential of undesired changes or persistence, which must be attained before deployment of engineered systems can be realized to ensure security and safety. The resulting systems can be utilized for bioproduction, on demand and in the field, of materials, chemicals, and other products for system support and protection; to detect status indicators or fluctuations in environment or human performance and respond accordingly in a designed and predictable manner, such as producing a stand-off signal or a beneficial biomolecule; and effecting the local environment to decrease or mitigate threats, enhance conversion of local resources, or provide signature management or obfuscation benefits.

Deployment of engineered biological and bio-enabled systems into the environment will require the ability to integrate environment and system information, whether those signals are biotic or abiotic. This requires realizing Enabling Capabilities that describe biological systems that can take in and store complex, dynamic information, integrate and transform those signals, and provide an output that is selective and modulated for the information it is meant to provide. The roadmap highlights technical advancements in biological pathways and circuits that perform robustly to amplify signals and incorporate orthogonal and non-canonical components, cell-to-cell and cell-to-environment (both micro- and macro-environment) communication in relevant organisms, and machine learning to advance analytics, computation, and decision-making of biologically-sensed or -reported signals. Integrated biological systems incorporate biosensors, materials, and multiplexed data to selectively sense, process, and respond to environmental cues and can be used to detect and determine physical and chemical threats, report timing and location and movement, and integrate physiological signals to sense, assess, and react to plant, animal, and human status and stress.

By combining engineering biology with materials, sensing, and other platforms, these tools can be used to improve and enhance human and system performance. Complex platforms combining multi-scale biologics with materials and machines can enhance the way these systems operate and enable dynamic response, novel signal integration, and modulation of physiology and environment. The roadmap describes milestone advancements in cell circuits and networks, patterning and assembly of consortia, and designing and modeling of interfaces in support of Enabling Capabilities for production of living materials, functionalized interfaces, and bioelectronics. Engineering biology can facilitate production of advanced biopolymers, materials that self-repair or reconfigure to environmental cues, and sensing systems that take advantage of the massively paralleled data processing power of microbes. The resulting systems can play diverse roles in application, including filtration and decontamination, weathering and damage through self-healing and anti-corrosion, provide obscuration and camouflage, and amplify remote or minute information and signals.

Achieving the milestones and capabilities detailed in this roadmap will: 1) allow the utilization of diverse resources (including waste streams) and production of specialty chemicals and materials for protection, coatings, fuels, infrastructure and agile basing, and to sustain and enhance human performance, among other products that are needed in an available and affordable manner; 2) better enable the ability to discriminate, identify, track, and target a wide variety of friends and foe in cluttered, multi-dimensional, multi-domain battlespace; and 3) allow humans and systems to operate continuously and intelligently under adverse conditions across time and space.

This roadmap is an independent study and does not confer DoD endorsement.

1 - INTRODUCTION

Engineering biology has immense potential to transform relevant systems and mission spaces. Advancements in engineering biology can bring about new structures and functions and reduce cost and time of manufacturing and production. Engineering biology can transcend current constraints to sustain and augment performance, protect the human from harsh environments, strategically integrate electronics and communications, combat mission constraints, and build resilience to evolving threats. However, to realize this potential, we must anticipate and generate significant progress in engineering biology tools and technologies. This roadmap for engineering biology for relevant environments details technical advancements and achievements over the next 20 years that will enable transformational applications.

Engineering biology, an expansion on synthetic biology, comprises many powerful technologies: gene synthesis, editing, and the assembly of chromosomes and genomes; the engineering of genetic circuits and biomolecules, such as proteins, and subsequent pathway engineering; modification, adaptation, evolution, and construction of cell-free systems, chassis, organisms, and consortia; and data science tools that enable and advance biological data modeling and integration, machine learning, and artificial intelligence. The convergence of these engineering biology technologies and information science aims to unlock more rapid, innovative, and diverse applications than we can currently recognize or realize (Figure 1).

To illustrate the application of engineering biology in diverse environments, the roadmap considers the use of engineered organisms or processes to manufacture specialty chemicals and materials that are needed in an available and affordable manner with atomic-level precision; the interfacing of biological systems to create composite, hybrid, hierarchical, and living materials; and the use of biomolecules, cell-free systems, cells, organisms and consortia to act as tools and technologies to maintain persistent situational awareness, augment human performance and protection, sustain environmental intelligence, understand physiological markers and molecular biosignatures, and for data storage. Furthermore, the roadmap highlights the integration of bio-based sensors and bio-interfaced and bio-composed materials with relevant systems, including electronics and infrastructure, to further enhance knowledge and control over the mission and environment. These bio-integrations will better enable the ability to discriminate, identify, track, and target a wide variety of friends and foe in cluttered, multi-dimensional, multi-domain battlespace.

The roadmap comprises the engineering of molecules and cells for the production of materials and biological systems for relevant environments. Engineered biology has the potential to dramatically speed initiation and implementation of projects and production from basic design and proof-of-principle to industrial manufacturing scale. This will require advancements and innovations in protein, metabolic, and host engineering, contributing to materials, cells, and consortia with improved and novel composition and function. Engineering biology and the engineering of biological systems can provide robust, dynamic shaping of diverse environments through deployable organisms and currently unrealized/unobtainable materials that can assemble and disassemble at-will, on-cue, or under defined environments and timescales to maintain or modify an environment. Bio-enabled materials have a plethora of products and applications including specialty chemicals and precursors, materials for protection, coatings, fuels, infrastructure and agile basing, and health monitoring and performance.

Engineering of these foundational components will enable the sensing, processing, and integration of active or passive environmental cues and trigger designed, multifaceted responses. Engineering biology can produce a greater diversity of sensors that can integrate and process complex signatures to provide decision-enabling data for discrete and dynamic outputs. In particular, the monitoring, recording, and modification of biomarkers and *in vivo* biological processes could be enabled by engineering biology tools and technologies, greatly impacting human physiology and performance. This can allow the human or system to operate continuously and intelligently under adverse conditions across time and space. By strategically bringing these

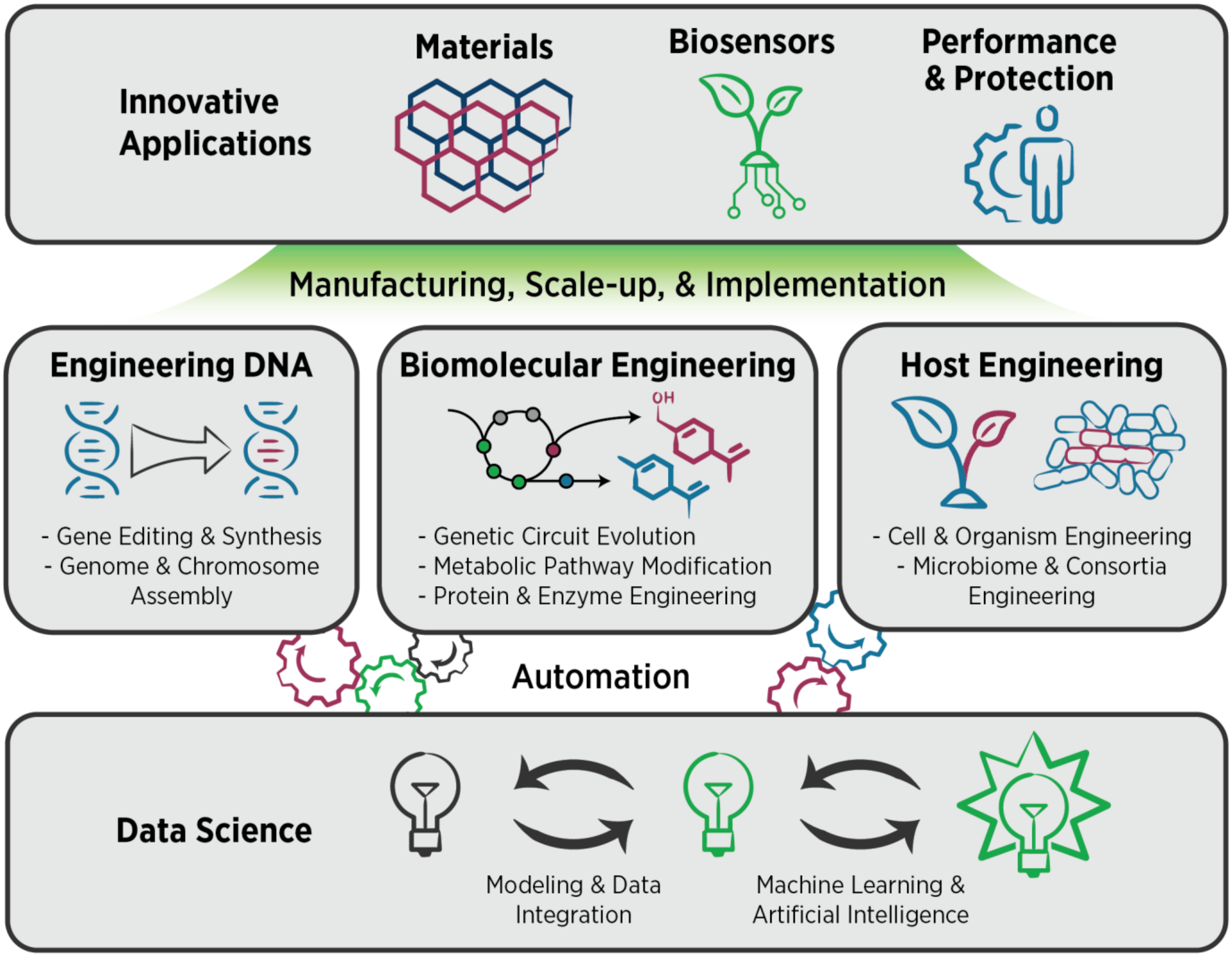


Figure 1. Engineering Biology for Military Applications. *The achievement of innovative applications in materials, sensing, and human performance and protection can have significant transformative impact on military environments and operations. These applications can be realized through manufacturing, scale-up, and implementation of advanced capabilities in engineering biology. Engineering biology comprises fundamental and emerging tools and technologies in genetic engineering and genome assembly and modification, biomolecular engineering, and the engineering of biological chassis and cellular communities. Automation of these engineered biological systems, coupled with transformational tools in data and information sciences, can revolutionize the way we solve significant challenges with biology.*

engineered biological systems together with materials, electronics, and other living systems, complex platforms are enabled to further improve and enhance performance.

In addition to strategic development of foundational engineering biology tools and technologies, efforts are needed to advance translation of nascent tools and technologies into applications at scale, by bridging the current materials, sensors, and performance requirements with possible biology-based or biology-enabled solutions. Furthermore, the development of capabilities enabling manufacturing and scaled production of engineering biology processes for unique needs, such as use in diverse settings, is needed. Engineering biology can enable heretofore unrealized products and properties but requires new avenues for scale-up and manufacturing that are distinct from existing production lines. The engineering biology solutions guided by the roadmap will need to be made efficient (optimized processing and separations, reduction in off-target pathways, minimizing contaminants and defects) and organisms and cell-free systems must be designed with scale-up and manufacturing in mind from the earliest stages of R&D. Despite this, engineering biology itself has the potential to revolutionize conventional approaches to manufacturing and scale-up and the accessible space in materials and bioproducts.

1.1 - Roadmap Architecture

This roadmap is organized into four topical sections, each comprising three to four Enabling Capabilities. The topical sections embody an increasing complexity of technical challenges, layering scientific and engineering capacities through implementation of advanced engineering biology tools and technologies. The Enabling Capabilities are intended to represent significant technical achievements that will revolutionize the way the engineering biology is applied to major challenges. Each Enabling Capability has a brief synopsis and contextual framing followed by technical milestones organized by engineering biology technical themes.

Milestone Timepoint Descriptions[1]

Construction of the technical roadmap is accomplished through delineation of current capabilities and short-term, mid-term, and long-term milestones. Short-term milestones are intended to signify objectives that can be reached with current or recently implemented funding paradigms and existing infrastructure, facilities, and resources, and are likely attainable within 2-5 years. The mid-term and long-term milestones are expected to be more ambitious achievements that require (and thus, result in) significant technical advancements, increased funding and resources, and/or new and improved infrastructure. Mid-term milestones are anticipated to be attainable within 5-10 years; long-term milestones are anticipated to be attainable within 10-20 years.

Definitions of Technical Themes

The definitions of the engineering biology technical themes – Engineering DNA, Biomolecular Engineering, Host Engineering, and Data Science - found in this roadmap have been derived from *Engineering Biology: A Research Roadmap for the Next-Generation Bioeconomy*. These definitions are used to describe the tools and technologies fundamental to research and engineering encompassed by the field. These are not clear-cut definitions, some overlap in tools and techniques between themes is anticipated and expected.

Engineering DNA[2]

Gene Editing, Synthesis, and Assembly focuses on the development and advancement of tools to enable the production of chromosomal DNA and the engineering of entire genomes. Advancements are needed in the design and construction of functional genetic systems through the synthesis of long oligonucleotides, assembly of multiple fragments, and precision editing with high specificity.

Biomolecular Engineering

Biomolecule, Pathway, and Circuit Engineering focuses on the importance, challenges, and goals of engineering individual biomolecules themselves to have expanded or new functions. Successful progress would be demonstrated by production of functional macromolecules on demand from both natural and non-natural

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[1] Adapted from *Engineering Biology: A Research Roadmap for the Next-Generation Bioeconomy* (Engineering Biology Research Consortium (2019); https://doi.org/10.25498/E4159B).

[2] It is anticipated that the fundamental advancements in gene editing, synthesis, and assembly described in *Engineering Biology: A Research Roadmap for the Next-Generation Bioeconomy* will significantly contribute to the achievement of the goals and Enabling Capabilities found in this roadmap.

building blocks, targeted design of complex circuits and pathways, and control over the dynamics of regulatory systems.

Host Engineering

Host and Consortia Engineering spans the development of cell-free systems, synthetic cells, single-cell organisms, multicellular tissues and whole organisms, and microbial consortia and biomes. Development of robust cell-free systems capable of diverse reactions, domestication and use of many single-cell hosts, targeted modification of multicellular organisms, and manipulation of microbial consortia.

Data Science

Data Integration, Modeling, and Automation focuses on robust, systematic use of the design, build, test, learn (DBTL) methodology to create complex systems. Progress requires a purpose-built computational infrastructure that supports DBTL biological processes, the ability to predict design outcomes, and optimize manufacturing processes at scale.

2 - APPLICATION NEEDS IN CONTEXT

While technical advancements in engineering biology are cross-cutting and the development of applications requires coordinated and multilateral research and production, engineering biology can also cater to specific contexts. The following reflects select applications of engineering biology to address needs in each Service:

2.1 - Army

In the Army context, engineering biology-enabled materials have applications for protection, performance augmentation and sustainment, and infrastructure to enable mission completion. This includes advancements in adaptive and responsive coatings, biological concretes, composites, biocompatible and (on-demand) biodegradable materials, engineered probiotics, hydration technologies, signature management, obscurants, ballistic/blast and environmental protection, protection against corrosion, agile basing, and (precursors for) point-of-need manufacturing for decreased logistic demand and secure supply chain. Beyond these materials needs, engineering biology capabilities may include: detection, reporting, and neutralization of chemical hazards, including (bio)chemicals in soil (at depth), water, biofluids, explosives, and pathogens; detection and reporting of physical hazards, including radiation at various wavelengths; detection and reporting of physiological stress and other biomolecules; and, detection, recording, and reporting of threats that have

Table 1. *Engineering biology solutions for U.S. Army applications.*

Army	Example Application	Engineering Biology Solution
Materials	Self-healing materials	Microbiomes embedded within coatings, fibers, or films that regrow in response to damage
	Signature management	Biofabricated materials with microstructures leading to controllable optical properties
	Water purification and desalination	Engineered microbiome filters or biologically derived filter materials
Sensing	Detection of pathogens in air or soil	Novel biosensors and reporters for recognized pathogens
	Detection and decontamination of hazardous chemicals (e.g., chemical weapons, toxic industrial chemicals (TICs), toxic industrial materials (TIMs))	Engineered cells or consortia with novel enzymatic degradation pathways
	Real-time physiological biomarker monitoring	Engineered microbiomes with specific performance biomarker receptors
Human Performance & Protection	Increased endurance	Human microbiomes engineered to produce cofactors for energy metabolism
	Enhanced immune response and pathogen resistance	Human microbiomes designed to neutralize pathogens through on demand production of immunomodulatory biomolecules
	Skin-based detection and diagnostic systems	Human skin microbiomes manipulated to sense and report environmental signals

moved over a particular location in the field. To support capabilities required to accomplish the Army mission, engineering biology may enable advancements in protective systems that include protective garments and suits, filtration systems, decontamination and remediation systems, and overarching systems such as tents, vehicles, and shared systems housing groups engaged in mission. Human performance needs could also be addressed through engineering biology tools and technologies - including sense and respond sentinel organisms, engineered commensals/tailored consortia with novel functions, and on-demand mission-specific nutrition - contributing to maintenance of cognitive, physical, and physiological homeostasis in response to environmental stressors such as sleep deprivation, dehydration, thermal regulation, sensory awareness, among others.

2.2 - Navy

Navy has specific needs for materials and sensors that operate in the marine environment and for enhancing individuals for maximum performance under Navy operating conditions, including exposure, pressure scenarios experienced by diving units, and mental fatigue on long missions in subs and surface ships. Navy has many needs for materials with potential to be addressed with engineering biology, including: lightweight and power-generating materials; composite materials, including natural composites such as wood, with increased strength and pressure resistance, or functions such as filtration; electronic materials that are resistant to radiation; and responsive materials that can detect and report ship damage. Materials for the Navy must be corrosion and fouling-resistant in the context of seawater, riverine, and other aqueous environments. Specific applications of

Table 2. *Engineering biology solutions for U.S. Navy applications.*

Navy	Example Application	Engineering Biology Solution
Materials	Corrosion-resistant and non-fouling coatings	Biofilms engineered for diverse aqueous environments
	Novel optical materials	Protein-based lenses with greater resolving power than achievable with glass
	Radiation- and fire-resistant fabrics	Biobased molecules to replace heavy and toxic materials currently used
Sensing	Detection of changes in maritime environments (e.g., ppm chemicals, pressure)	Whole-cell sensors of chemical effluent
	Biometrics of divers and other warfighters in high stress environments	Living biosensors that may be ingested or incorporated into wearable devices
	Detection of a wide range of signatures with high sensitivity and specificity	Biobased sensor arrays that detect subtle environmental anomalies in ports and other maritime installations
Human Performance & Protection	Mitigation of physiological effects of a high pressure environment	Engineered ear, nose, and throat (ENT) microbiomes
	Protection from water-borne pathogens	Engineered ingestible probiotics
	Improved cold tolerance for divers	Engineered microbiomes to enhance energy metabolism by increasing cofactor supply

engineering biology may contribute to protection from water contaminants and wearable coatings for exposure to marine environments. Additionally, engineering biology can enable autonomous, microbial energy-powered distributed sensor networks. Potential Navy applications of sensors and biological systems include monitoring for port and harbor security or monitoring the environment for hazards.

2.3 - Air Force

For Air Force needs, materials enabled by engineering biology have a range of applications such as agile basing, sustainment, energetic compounds, adhesive materials, human performance, and aeromedicine. This includes specific examples such as advanced fuels for hypersonics, optical materials to counter directed energy systems, coatings and materials for air and space applications, monomers and precursors for high-temperature materials, antimicrobials, sentinel materials, cold-chain stabilized materials, biological concretes, composites, biodegradable materials, and other biocompatible materials. Air Force sensing and monitoring for airmen, infrastructure, and systems is focused on identifying and exploiting novel molecular biosignatures. This

Table 3. *Engineering biology solutions for U.S. Air Force applications.*

Air Force	Example Application	Engineering Biology Solution
Materials	Runway construction in theater with reduced logistic costs, time, and energy needs	Biobased cement production
	Platforms with longer canopy life	Coatings and adhesives resistant to biodegradation
	Detection, identification and quantification of biocontamination for risk assessment in aerospace systems and infrastructure	Biological sensing elements for detection and differentiation of diverse microbial and viral contaminants to enable tailored decontamination strategies and biodeterioration control
Sensing	Presence of toxic industrial chemicals (TICs) and toxic industrial materials (TIMs) in an operational environment	Novel biosensors and reporters for chemicals of interest
	Detection, identification and quantification of contamination for risk assessment in aerospace systems and infrastructure	Biological sensing elements for detection and differentiation of diverse microbial and viral contaminants to enable tailored decontamination strategies and biodeterioration control
	Detection of material fatigue prior to failure	Embedded biosensors with colorimetric reporters to highlight tiny cracks in aircraft canopies and windshields
Human Performance & Protection	Augment or enhance pilot resistance to fatigue	Personalized microbiomes that support sustained activity and focus, and/or stimulate wakefulness
	Ability to extract more energy from food sources	Probiotics that allow for higher nutritional uptake at high altitudes
	Sustain warfighter performance in operational conditions	Engineered microbes to sense and respond to mitigate operational stressors

includes identification of biomarkers to monitor physical and cognitive performance of airmen, such as continuous assessment of a person's state, and dynamic, responsive interventions to expand the performance envelope and mitigate exposures. Further, engineering biology can be applied to enhance and sustain performance and increase cognition and resistance to stressors, such as fatigue. Beyond the human, Air Force advancements in engineered biosensors may enable exploitation of new molecular tools for biosignature detection and identification of TICS/TIMS for environmental monitoring.

3 - ENGINEERING MOLECULES AND CELLS: Production of biologics and materials for relevant applications.

There is great opportunity for engineering biology to transform the landscape. Research and development in protein and metabolic engineering will enable synthesis and production of chemicals, polymers, and biomolecules for a myriad of uses in relevant environments. Diversification of biomolecules through engineering and utilization of non-natural components and properties can generate novel functions not currently possessed by natural systems. Likewise, engineering biology can increase stability of biologics; decrease time and cost of production; reduce or prevent production of toxic intermediates, products, and byproducts; and help to maintain functionality in diverse environments. Engineering biology technologies can enable complex materials with hierarchical structures, dynamic properties, and programmed functionalities, and composites that can serve to protect, obscure, and filter, while increasing performance and expanding manufacturing opportunities in austere, point-of-use, and far-forward environments.

The basis of such capabilities is advancements in fundamental engineering of nucleic acids, biomolecules, and cells. Engineering biology allows for the adaptation of genomes, design of new proteins, adjustment of metabolic circuits and cell-signaling pathways, two- and three-dimensional assembly of biomolecules and cells, and production of new products, materials, and chemicals. Rapid synthesis of DNA, utilization of gene editing tools, and high-throughput sequencing allow scientists and engineers to discover and direct the basic activities of cells and organisms. Likewise, circuit and pathway engineering can reconfigure the products of a single cell, whether for direct use of a biomolecule or chemical, or for targeted cellular behavior. Advancements in data science, machine learning, artificial intelligence, and automation enable efficiencies and innovation in design, modeling, and integration of biological data.

Enabling Capabilities:

- Engineering DNA, proteins, and proteomes to support the dynamic synthesis of molecules and polymers.
- Enabling the diversification of designed and engineered biomolecules not found in nature, leading to novel functionalities and properties.
- The ability to engineer cellular metabolism to rapidly produce desired biomolecules and enable the synthesis of materials, sensors, and biosystems.
- Control of biological self-assembly to enable the synthesis of high-performance, dynamic, and responsive materials.

3.1 - Enabling Capability: Engineering DNA, proteins, and proteomes to support the dynamic synthesis of molecules and polymers.

Nucleic acid and protein engineering are foundational to engineering biology. DNA and protein engineering can enable the production of new monomers and polymers and improve the cost and/or performance of existing chemicals and materials. These engineered nucleic acids and biomolecules can contribute to: the generation of new biological sensing-and-reporting systems; to the production of specialized molecules, cells, and biosystems to support human performance; and to the synthesis of materials with programmed heterogeneity, self-assembly at different length scales, and new chemistries and material properties. In addition, improvements in DNA and protein engineering can enable more efficient engineering of secondary metabolism for production of small molecules, including material precursors (*e.g.*, polymer monomers), by design. Advancements in engineering biology tools can create precise and scalable assembly of materials with limited defects, thus enabling the development of materials ideal for miniaturization and for compatibility with the person, their mission constraints (*e.g.,* weight, temperature), and adoption for use. Additional examples of

Table 4. *Engineering biology milestones toward achieving the engineering of DNA, proteins, and proteomes to support the dynamic synthesis of molecules and polymers.*

Engineering DNA, proteins, and proteomes to support the dynamic synthesis of molecules and polymers.			
Milestones	**Short-term**	**Mid-term**	**Long-term**
Engineering DNA	Incorporate bio-orthogonal nucleic acids into sequences and produce non-canonical amino acids.	Extend the length of synthesized and assembled sequences to 100-1,000 kilobase-pair systems.	Assemble and construct synthetic genomes at chromosomal-length on demand and at low cost.
Biomolecular Engineering	Build biopolymers, protein-polymer hybrids, and chimeric constructs with increasing levels of rational design, function, and interaction.	Identify and characterize new enzyme classes without directed evolution; further engineer orthogonal translation systems.	Synthesize or produce full-length synthetic proteins via cell-free systems on-demand; Design de novo functional proteins in real time, in situ.
Host Engineering	Engineer hosts and cell-free systems to utilize non-canonical amino acids.	Engineer specialized hosts for directed protein/ enzyme evolution and advancements in point-of-need biomanufacturing of single products.	Synthesize desired biomolecules and customized organisms with synthetic proteomes in the field, on demand.
Data Science	Improve characterization, design, and modeling of proteins and multi-protein complexes.	Design any enzyme from scratch, computationally, with limited previous sequence information.	Design protein enzymes de novo with novel catalytic chemistries, substrate targets, and precisely engineered K_m, K_{cat}.

materials enabled by protein engineering include sustainable and renewable infrastructure, surfaces and coatings, and protective materials such as lenses and filters.

Engineering DNA

Advancements in gene editing, synthesis, and assembly underpin the use of engineering biology for nearly all applications. With the expanded use of efficient DNA engineering tools, such as CRISPR, the ability to modify and synthesize novel genes, sequences, and eventually genomes, is rapidly accelerating. Current DNA synthesis allows for 5,000 base-pair fragments and assembly of sequences up to 20,000 base-pairs, with a number of reliable methods of plasmid and chromosomal engineering. Short-term and mid-term milestones extend the length of synthesized and assembled sequences to 100-1,000 kilobase-pair systems. Short-term milestones also include further incorporation of bio-orthogonal nucleic acids and production of non-canonical amino acids, allowing for expanded expression of synthetic proteins containing non-canonical amino acids. Long-term milestones encourage on-demand and low-cost chromosomal-length DNA assembly and construction of synthetic genomes for expression of synthetic proteins, which may include many non-canonical amino acids. Long-term milestone attainment includes engineered synthetic genomes with up to 50% of genome content directly functioning as sensors and regulators for specified triggers.

Biomolecular Engineering

Current biomolecular engineering enables production of polymers and proteins from canonical amino acids; however, behavior and interactions of engineered proteins are often unpredictable or unknown. Some biofunctionalized, cell-free scaffolds are sufficiently stable and available for use for pharmaceutical production, water purification, or sensing. Short-term milestones enable more advanced biopolymers and protein-polymer hybrids and chimeric constructs built with increasing levels of rational design and greater confidence in function and interaction. An expansion of the genetic code will enable increasing encoding of proteins with non-canonical amino acids. Mid-term milestones allow for identification and characterization of new enzyme classes without directed evolution and further engineering of orthogonal translation systems. On-demand biofunctionalization of general use substrates (*e.g.*, currently produced surface coatings) is feasible. Longer-term milestones will enable on-demand synthesis or production of full-length synthetic proteins via cell-free systems and *in situ*, real-time design of *de novo* functional proteins.

Host Engineering

Currently, there are a number of chassis and some cell-free systems that can efficiently produce biomolecules, polymers, and proteins from designed sequences; however, there is significant need for new secretory mechanisms and improvement in production strains. Short-term milestones also include engineering of hosts and cell-free systems to utilize non-canonical amino acids. Mid-term milestones expect to produce more specialized hosts for directed protein/enzyme evolution and advancements in point-of-need biomanufacturing of single products (*e.g.*, chemicals, monomers, simple polymers). This will build into tailored scale-up of engineered organisms and consortia for relevant settings and increased biosynthesis and secretion of large quantities of protein or polymer from engineered hosts and cell-free systems. Long-term milestones seek achievements in on-demand, in-field synthesis of desired biomolecules and customized organisms with synthetic proteomes.

Data Science

Currently, protein engineering and structural modeling software is mediocre and requires specialized training. There is also a need for improved computational protein design and larger functional genomics screens to populate the permissible chemistry space. Short-term milestones seek improvements in characterization, design, and modeling of proteins and multi-protein complexes, advancing into real-time protein modeling. Mid-term milestones include computation design of any enzyme from scratch with limited previous sequence information. Long-term milestones seek the ability to design protein enzymes *de novo* with novel catalytic chemistries, substrate targets, and precisely engineered K_m, K_{cat}.

3.2 - Enabling Capability: Enabling the diversification of designed and engineered biomolecules not found in nature, leading to novel functionalities and properties.

Building off the capability to engineer biomolecules to support the synthesis of molecules and materials, the tools and technologies of engineering biology are quickly expanding the breadth of biology beyond what occurs naturally and expanding an already diverse array of applications. Coupled with recent advancements in cell-free technologies and progress in synthetic cells, engineering biology can enable biomolecules with controlled chirality, new elemental components, and enable function in non-aqueous solvents. The potential of such tools and technologies includes the design and production of unique or non-natural biomolecules, cells, and materials.

Table 5. *Engineering biology milestones toward enabling the diversification of designed and engineered biomolecules not found in nature.*

Enabling the diversification of designed and engineered biomolecules not found in nature, leading to novel functionalities and properties.

Milestones	Short-term	Mid-term	Long-term
Biomolecular Engineering	Improve engineering of biomolecules and ribosomes for producing novel, non-canonical peptides and abiological polymers.	Engineer circuits responsive to non-canonical biomolecules; Develop tools for epigenetic control of synthetic genomes and post-translational modifications of non-canonical peptides.	Design and produce non-canonical biomolecules with specialized functions not constrained by known biochemistry on demand.
Host Engineering	Engineer hosts and cell-free systems to utilize non-canonical amino acids.	Produce cell-free extracts and composite cell-free systems for optimization of novel small molecules and materials.	Design and produce reconfigurable cells (synthetic cells) with core biosynthesis machinery capable of handling novel biochemistries and orthogonal genetic codes.
Data Science	Advance data mining and -omics tools.	Increase modeling and computational design of biomolecules, protein folding, and cell-free systems and their interactions.	Improve predictability and optimization of models for biomolecules with non-canonical chemistry and the desired products.
Engineering DNA	Utilize expanded genetic codes, via advancing engineering and implementation of bio-orthogonal nucleic acids and non-canonical amino acids.		

Engineering DNA

The utilization of expanded genetic codes, via bio-orthogonal nucleic acids and non-canonical amino acids, is likely to advance along similar lines as the tools and technologies for gene editing, synthesis, and assembly for natural genetic systems.

Biomolecular Engineering

Currently, adoption of non-canonical amino acids and synthetic proteins is limited, and focused on expanding the addressable chemical space. Short-term milestones include improved engineering of biomolecules and ribosomes for producing novel, non-canonical peptides and abiological polymers, through novel translation pathways that can accommodate an expanded library of nucleotides. Improved discovery and engineering methods (directed evolution and computational design and modeling) will establish new orthogonal parts across taxa. Mid-term milestones will see expansion of circuits responsive to non-canonical biomolecules and expanded tools for epigenetic control of synthetic genomes and post-translational modifications of non-canonical peptides. Long-term milestones aim for on-demand design and production of non-canonical biomolecules with specialized functions not constrained by known biochemistry.

Host Engineering

Cell-free system technology is expanding rapidly, but current functionalities are limited; however, cell-free platforms and synthetic cells are likely to be at the forefront of enabling the expansion of biological systems beyond nature. Short-term milestones will see the engineering of hosts and cell-free systems to utilize non-canonical amino acids as required. Mid-term milestones seek to expand the production of cell-free extracts and composite cell-free systems for optimization of novel small molecules and materials. Long-term milestones aim to design and produce reconfigurable cells (synthetic cells) with core biosynthesis machinery capable of handling novel biochemistries and orthogonal genetic codes.

Data Science

The development and production novel biomolecules will require significant advancements in data science, particularly modeling and machine learning. Currently, data to predict and design novel biomolecules and functionalities is very limited and short-term milestones focus significantly on advancements in data mining and -omics tools. Mid-term milestones expand to increased modeling and computational design of biomolecules, protein folding, and cell-free systems and their interactions. Long term milestones improve on the predictability of those models for biomolecules with non-canonical chemistry and the desired products, as well as optimization. Long-term, advancements in machine learning and artificial intelligence will significantly speed and improve accuracy of novel genetic codes, biochemistries, cell-free systems, and synthetic cells that can be produced through engineering biology.

3.3 - Enabling Capability: The ability to engineer cellular metabolism to rapidly produce desired biomolecules and enable the synthesis of materials, sensors, and biosystems.

In combination with nucleic acid and protein engineering, the adaptation and modification of metabolic pathways can enable more creative and robust production of molecules and polymers. Further, engineered production chassis can be prepositioned to enable this production on demand and in the field using available resources. Metabolic engineering can also enable the transformation of diverse feedstocks, including wastes and unique local biomass, into relevant biomolecules and materials, allowing designed biological systems to perform necessary tasks autonomously. Engineering cellular metabolism can generate systems with the potential to decrease the industrial footprint, secure logistics and supply chains, and accomplish agile manufacturing at the point of need.

Engineering DNA

To achieve this Enabling Capability, general advancements in gene editing, synthesis, and assembly will be required. For more on this topic, see *Engineering Biology: A Research Roadmap for the Next-Generation Bioeconomy* (EBRC, 2019).

Biomolecular Engineering

Engineering biology has enabled simple modifications of metabolic pathways, typically modifying a single enzyme pathway, or introduction of a construct to produce a single biomolecule, but this is mostly limited to known pathways. Short-term milestones look to expand on the ability to modify more pathways. This will be accomplished through goals to expand this to production of a biomolecule via introduction of a circuit construct from an unknown or hypothetical pathway, meanwhile significantly increasing the libraries of non-repetitive control elements, logic gates, standardized parts, orthogonal ribosomes, non-canonical amino acids, and interaction driven switches with minimal crosstalk. Mid-term milestones aim to increase the number of biomolecules that can be produced through the insertion of a single, more complex gene construct (including

Table 6. *Engineering biology milestones toward achieving the ability to engineer cellular metabolism to rapidly produce desired biomolecules and enable the synthesis of materials, sensors, and biosystems.*

The ability to engineer cellular metabolism to rapidly produce desired biomolecules and enable the synthesis of materials, sensors, and biosystems.

Milestones	Short-term	Mid-term	Long-term
Biomolecular Engineering	Expand production of a biomolecule via introduction of a circuit construct from an unknown or hypothetical pathway.	Increase the number of biomolecules that can be produced through the insertion of a single, more complex gene construct.	Engineer cells or cell-free systems that can utilize substrates from relevant environment feedstocks and produce numerous and potentially novel biomolecules.
Host Engineering	Improve tools for engineering non-model organisms and increase the number of “domesticated” chassis strains and cell-free platforms.	Develop robust, relevant hosts, cell-free systems, and consortia for production in non-traditional manufacturing pipelines.	Expand the number and type of production platforms (species, systems, consortia) and conditions under which they can operate with sense-and-respond pathways for on-demand production.
Data Science	Refine existing pathway design tools; More efficiently integrate multi-omics for more complete metabolic pathway models.	Produce larger datasets and autonomous systems to rapidly predict engineering products; Create predictive algorithms for metabolic circuit and pathway design based on desired product.	Design and integrate metabolic pathways from the ground up and utilize machine learning prediction for self-optimizing organisms, consortia, and cell-free systems.
Engineering DNA	To achieve this Enabling Capability, general advancements in gene editing, synthesis, and assembly will be required. For more on this topic, see Engineering Biology: A Research Roadmap for the Next-Generation Bioeconomy (EBRC, 2019).		

the use of non-canonical amino acids), expanding the range of products from a single host species or cell-free system. Mid-term milestones also seek to broaden the catalog of enzymes that utilize specific small molecule substrates, increasing the flexibility in feedstock utilization. Long-term milestones aim to engineer cells or cell-free systems that can utilize or recycle substrates from wastes or other I relevant environment feedstocks, and to produce numerous and potentially novel biomolecules via engineered metabolic pathways.

Host Engineering

Currently, there are a handful of production chassis that are already used in industrial settings, but very few relevant environment strains. There is also limited capacity for rewiring cellular metabolism for growth on atypical substrates (such as plastic). Short-term milestones look to improve tools for engineering non-model organisms and increase the number of “domesticated” chassis strains or the number of cell-free platforms for biomolecule production. Short-term milestones aim to engineer microbial communities (2+ organisms) for biosynthesis to reduce metabolic load and reduce toxicity. Mid-term milestones look to expand the substrates and feedstocks for cultivation, including the use of non-potable water sources (seawater, wastewater) as growth matrices. Mid-term milestones also aim to develop robust, relevant hosts, cell-free systems, and consortia for production in non-traditional manufacturing pipelines, with the goal of cultivating organisms that can survive more challenging environmental conditions. Long-term milestones include further expanding the number and type of production platforms (species, systems, consortia) and conditions under which they can operate and enveloping sense-and-respond pathways for on-demand or triggered production.

Data Science

Currently, there are rather limited databases for genes, proteins, and metabolites available to the public; however, genome sequencing and annotation from traditional and environmental samples can be readily performed. Still, while valuable, there is still limited utility since enzymes and metabolic pathways are often not fully characterized, leading to inaccurate annotations and predicted function. Synthetic gene circuits are built manually or by newly developed pathway design tools. Short-term milestones seek to refine these existing pathway design tools, including more that are available as open source. Through increased high-throughput screening, there should arise better integration of multi-omics for more complete metabolic pathway models and better machine learning discovery. Mid-term milestones seek larger datasets and autonomous systems to feed in genomics and experimental data to rapidly predict engineering products, as well as predictive algorithms for metabolic circuit and pathway design based on desired product and considering increased complexity of chassis organisms. Human-out-of-the-loop automated experiments based on rational or combinatorial designs (*i.e.*, human led), will lead to wholly automated experiments, with advanced 'computationally rational' experimental design (no human involvement in experiment design or interpretation of results). Long-term milestones aim to use commercially available pathway design tools for ground-up metabolic pathway design and integration and machine learning prediction of self-optimizing organisms, consortia, and cell-free systems that can automatically adapt to different feedstocks.

3.4 - Enabling Capability: Control of biological self-assembly to enable the synthesis of high-performance, dynamic, and responsive materials.

A key application of biomolecule, particularly protein, engineering and the interactions between cells is the attainment of structure and physical material. As in nature, biomolecules and organisms can be engineered to dynamically self-assemble (or disassemble), with designed and controlled two-, three-, and four-dimensional structures to create unique and complex materials. Controlled biological self-assembly could result in unique materials synthesis across numerous platforms to include polymerization of biofabricated monomers and material processing for coatings, filters, and fabrics. Furthermore, a unique aspect of bio-based and bio-enabled materials is the potential for responsiveness to internal or extrinsic cues. Such materials could be formed to contain gradients, sense and respond properties, and designed heterogeneity, and enable, for example, self-healing and defect management or environmental reporting.

Engineering DNA

To achieve this Enabling Capability, general advancements in gene editing, synthesis, and assembly will be required. For more on this topic, see *Engineering Biology: A Research Roadmap for the Next-Generation Bioeconomy* (EBRC, 2019).

Biomolecular Engineering

Currently, there are a few examples of biomolecular self-assembly, such as vesicle production and peptide display, but there is generally little control over cell-cell assembly or bulk material formation. Short-term milestones include an increased number of tools to enable cell-cell patterning (based on naturally occurring systems) and improved abilities to conduct directed nucleic acid and protein polymerization. Mid-term milestones aim to enable improved control over molecules and extracellular structures that order cell-to-cell

Table 7. *Engineering biology milestones toward achieving control of biological self-assembly to enable the synthesis of dynamic and responsive materials.*

Control of biological self-assembly to enable the synthesis of dynamic and responsive materials.

Milestones	Short-term	Mid-term	Long-term
Biomolecular Engineering	Increase number of tools to enable cell-cell patterning; Increase capability to perform directed nucleic acid and protein polymerization	Improved control over ordering of cell-to-cell assembly; Expand libraries of functional biomolecules that predictably self-assemble or drive self-assembly.	Program and/or responsively control the morphology hierarchical self-assembled structures; Program heterogeneity of materials.
Host Engineering	Engineer cells that can be used as materials; Advance templated bacteria growth on designed scaffolds.	Engineer self-assembling biological materials that maintain persistency; Enable control over three-dimensional cell assembly within a consortium.	Enable dynamic cell assembly within consortia with both temporal and spatial control; Automate material fabrication from cells and consortia.
Data Science	Improve design parameters for heterologous expression of desired biomolecules involved in self-assembly; Develop predictive models of cellular self-assembly.	Develop predictive models of self-assembly of simple systems linked to high-throughput data development from materials formation from biology.	Utilize artificial intelligence and machine learning to develop predictable approaches to controllably disassemble and reassemble hierarchical structures.
Engineering DNA	To achieve this Enabling Capability, general advancements in gene editing, synthesis, and assembly will be required. For more on this topic, see Engineering Biology: A Research Roadmap for the Next-Generation Bioeconomy (EBRC, 2019).		

assembly and expanded libraries of functional biomolecules that predictably self-assemble or drive self-assembly. Mid and long-term milestones aim for predictable and reversible biomolecular self-assembly of materials and ordering of cells and improved understanding of down-stream processing for efficient purification, polymerization, and material processing into final material form factors. Long-term milestones also seek to attain programmable and/or responsive morphological control of hierarchical self-assembled structures and programmed heterogeneity of materials, including coatings, composite gradients, and hydrogels.

Host Engineering

While the ability to engineer some biomolecular structuring for materials currently exists, the complexity of systems for cellular ordering has significantly limited the use of cells for materials to a limited number of model organisms, particularly without any underlying abiotic scaffold. Short-term milestones seek to advance the engineering of cells that can be used for/as materials, and to advance templated bacteria growth on designed scaffolds. Mid-term milestones aim to achieve self-assembling biological materials that persist when required and greater control over cell assembly within a consortium, specifically control over three-dimensional structure. Long-term milestones aim for dynamic cell assembly within consortia with both temporal and spatial control (four-dimensional materials) and cells and consortia that can be engineered for automated material fabrication and sense-respond scenarios. Long-term milestones also include the use of synthetic cells to construct ordered materials.

Data Science

Currently, some libraries contain sequence data with associated structural properties and there are some rudimentary modeling programs that can predict shapes from known structural rules for DNA. Short-term milestones aim to improve the understanding of design parameters for heterologous expression of desired biomolecules involved in self-assembly and predictive models of self-assembly of simple cellular or multicellular systems. Short-term milestones also seek to improve analytics linking biology to material properties (going from genotype to phenotype). Mid-term milestones aim to build the knowledgebase of biomolecular and protein interactions necessary for self-assembly and to develop predictive models of self-assembly of simple systems linked to high-throughput data development from materials formation from biology. Long-term milestones seek to use artificial intelligence and machine learning to develop predictable approaches to controllably disassemble and reassemble hierarchical structures.

4 - ENGINEERING BIOLOGICAL SYSTEMS: Advanced organisms, microbiomes, and consortia for diverse environments.

Engineered biological systems, such as human, soil, or marine microbiomes, have immense potential to provide direct benefit to human performance, help to secure the mission space, and accelerate adaptation to adverse environments. Building off the engineering of nucleic acids, proteins, and other biomolecules, engineering biology can enable robust cells, organisms, and consortia that contribute to the production or degradation of (bio)chemicals, materials, and other products, and can themselves act as the material, sensor, or system. By utilizing engineering biology technologies, multicellular organisms, biomes, and consortia can be tuned to provide complex signals and reactions to environmental cues. Engineering of the human microbiome can significantly impact performance and protection by enabling faster or more robust adaptation to relevant environments and supply sustenance and physical support. Engineered consortia can assist in altering or adapting an environment and do so in ways that are persistent or adaptive as necessary. Regardless, adoption of engineered biology towards environmental deployment requires an inculcation of safe, secure, and contained biological systems, designed with this in mind from DNA sequence to data utilization.

Enabling Capabilities:

- Engineering easily deployed biological systems with robust operation that function in diverse environments.
- Constructing and maintaining stable engineered microbiomes for human performance and protection.
- The ability to engineer consortia for the manipulation of relevant environments.

4.1 - Enabling Capability: Engineering easily deployed biological systems with robust operation that function in diverse environments.

Advancements in engineering biology can enable more robust biological systems that can be readily deployed to solve diverse challenges. These systems – whether biomolecules, cell-free systems, cells, organisms, or consortia – can be engineered to detect, reduce, and mitigate threats; serve as on-demand, agile bioproduction mechanisms; or provide products or materials to sustain and support the relevant operation and environment. Through engineering, biological systems can persist in the environment through unique means of longevity, have temporal- or spatial-dependent behaviors, and withstand extreme environments or fluctuations in environmental conditions. Such advancements will contribute to more robust situational awareness and better decision making for the relevant environment. However, environmental deployment also requires introducing and maintaining biological mechanisms to secure synthetic organisms against unintended release or spread, alteration and transfer of genetic information, and protection from other unintended threats to the system.

Engineering DNA

Engineering and adaptation of biological systems for robust environmental deployment requires some unique modifications of DNA, including alterations that allow (or limit) for environmental persistence, and a better understanding of horizontal gene transfer. Short-term milestones include building genetic systems with non-repetitive genetic parts for evolutionary robustness and overall fitness and design of genetic systems that avoid mutations, such as single nucleotide polymorphisms, insertions, or deletions. Longer-term goals include design of genetic systems with built-in modules to immediately verify functionality and robust, controllable genetic circuits.

Table 8. *Engineering biology milestones toward engineering easily deployed biological systems with robust operation that function in diverse environments.*

Engineering easily deployed biological systems with robust operation that function in diverse environments.			
Milestones	**Short-term**	**Mid-term**	**Long-term**
Biomolecular Engineering	Expand libraries of enzymes and biomolecular pathways for diverse environments.	Engineer robust cellular logic circuits capable of long-term persistence and performance in stabilized environments.	Engineer circuits and pathways used as triggered kill-switches or to activate time-dependent behaviors; Enable performance- and quality-monitoring autonomous biomolecular sense and response pathways.
Host Engineering	Diversify and domesticate microbial strains for relevant environments.	Engineer persistent cells with reactive and responsive functionalities, defined cell-cell communication pathways, and controlled nutrient resourcing.	Enable reprogramming of microbes and consortia in the context of complex and dynamic environments, like soil.
Data Science	Develop predictive modeling of survivability and validation of sensing activity over time.	Predict optimal biomolecular pathway design and implementation and pathway activity in diverse environments.	Enable machine learning and artificial intelligence for design, modeling, and integration of more complex biosensing systems and signature detection and response.
Engineering DNA	Engineer and adapt biological systems for robust environmental deployment, including alterations that allow for environmental persistence; understand and engineer horizontal gene transfer.		

Biomolecular Engineering

Currently, there are a number of proteins and other biomolecules that can be added to bio-enabled materials or systems that act to maintain stability under normal conditions. Additionally, tools exist to encapsulate biomolecules in naturally occurring or synthetic materials to help systems maintain stability and robustness. Short-term milestones include expanding the libraries of enzymes and biomolecular pathways for diverse environments and continuing to engineer biomolecules that can mitigate cold-chain requirements. Advancements in cell-free systems are also likely to speed deployment of biomolecules and biomolecular manufacturing platforms. Mid-term milestones include advancements in robust cellular logic circuits capable of long-term persistence and performance in stabilized environments, and advancements in sensing pathways for a wider range of environments. Long-term milestones include circuits and pathways that can be used as triggered kill-switches or to activate time-dependent behaviors, and performance- and quality-monitoring autonomous biomolecular sense and response pathways.

Host Engineering

Currently, there exists a small number of host strains that have robust function or can persist in challenging environments. Specialized tools have been developed to engineer and optimize specialized strains, but the quantity of such systems is limited. Additionally, advancements in cell-free systems have the potential to significantly enhance the use of biologics in adverse environments. Short-term milestones seek to diversify and domesticate microbial strains for diverse environments, including identifying environmental organisms capable of field-based conversion of local carbon sources to necessary products and materials. Mid-term milestones aim for inducible and controlled sporulation or arrested metabolism; and persistent engineered cells with reactive and responsive functionalities, defined cell-cell communication pathways, and controlled nutrient resourcing. Mid- and long-term milestones will advance microbial consortia for multi-strain biofilms and consortia capable of converting local, mixed carbon sources to necessary products and materials. Long-term milestones aim to enable *in situ* reprogramming of individual organisms and consortia to respond quickly to local environments and engineering dependencies in biological systems that are restricted by encapsulation or triggered at the cell-material interface.

Data Science

Bioinformatics and data modeling and validation tools are likely to allow for increased predictability of bio-enabled systems and advance integration of engineered biology with the environment. Current databases of biological systems and organisms found in relevant environments help to enable predictive machine learning for a small number of organisms and pathways. Short-term milestones will enable predictive modeling of survivability and validation of sensing activity over time. Further registry of microbes and microbiomes will enable more robust design and implementation. Mid-term milestones will allow better prediction of optimal biomolecular pathway design and implementation and functionality in diverse environments and better tools to establish and mature non-model organisms. Long-term milestone improvements in machine learning and artificial intelligence will allow for design, modeling, and integration of more complex biosensing systems and signature detection and response.

4.2 - Enabling Capability: Constructing and maintaining stable engineered microbiomes for human performance and protection.

Microbes and microbiomes are important for manipulating the environment, as well as monitoring and actuating a person's physiology. The microbiome, through its vast genetic repertoire and host adaptation, lends itself to augmenting performance through engineering circuits that can sense operator status (including fatigue, stress, and environmental and biological hazards) and respond appropriately. The use of microbial consortia for the 'actuation' of human health and performance will require the development of reliable, controllable and predictable methods for the engineering of pathways for producing small molecules and biopolymers. Because of the individual nature of human responses to interventions, accompanying efforts in standardization and acquisition of data, as well as predictive modeling will be needed to understand personalized correlations between microbiomes and human hosts. Applications include skin microbiome engineering to neutralize environmental hazards; and using laboratory models to understand how microbial production of natural or engineered effectors influence human performance metrics like cognition and endurance.

Engineering DNA

In addition to fundamental advancements in gene editing, synthesis, and assembly, this Enabling Capability will be achieved through a more robust understanding of, and the ability to better control and engineer,

Table 9. *Engineering biology milestones toward constructing and maintaining stable engineered microbiome communities for human performance and protection.*

Constructing and maintaining stable engineered microbiome communities for human performance and protection.

Milestones	Short-term	Mid-term	Long-term
Engineering DNA	Enable a more robust understanding of horizontal gene transfer among members of a consortia.	More effectively control and engineer horizontal gene transfer among members of a consortia.	Simultaneously edit the genomes of most or all consortia members.
Biomolecular Engineering	Assess and understand biomolecular triggers for substrate utilization and production pathways.	Engineer biomolecular pathways for the production of performance-associated compounds and response to environmental perturbations.	Manipulate biomolecular pathways in an interdependent and targeted manner, leading to greater control over individualized microbiomes.
Host Engineering	Expand relevant strain identification and modeling beyond gut microbiome, with emphasis on transplantation and signaling.	Engineer microbiomes (gastrointestinal or other) via in situ genetic modification.	More effectively control spatial and temporal microbiome composition and function; Produce functional microbiomes de novo.
Data Science	Acquire large-scale metagenomic and microbiome data from warfighters in response to the environment.	Enable standardized analyses and reproducible -omics pipelines for microbiomes; Develop computational tools for quantifying and predicting consortia-level phenotypes.	Enable rapid and efficient predictive modelling for on-demand microbiome phenotypes; Develop tools for designing individual personalized mission-specific microbiome modifications.

horizontal gene transfer among members of a consortia. The ability to simultaneously edit the genomes of consortia members will also advance our capabilities.

Biomolecular Engineering

Selective metabolic engineering of human microbiome strains will enable more effective manipulation of microbiomes for protective and performance functions. Current knowledge of microbial effector molecules for the human microbiome is rather limited; however, engineering of enzymatic pathways for complex carbohydrate conversion can be achieved. Short-term milestones include building on assessment and understanding of biomolecular triggers for substrate utilization and production pathways. Mid-term milestones will build on this through the engineering and advancement of engineered biomolecular pathways for the production of performance-augmenting compounds (*e.g.*, cofactors and enzymes for energy metabolism, neuro- or immuno-modulators, antioxidants, etc.) and response to environmental perturbations. Long-term milestones aim to improve directed biomolecule retention or elimination and on-demand production through engineered microbiomes and enable interdependent, targeted pathway manipulation leading to greater control over individualized microbiomes that can accelerate adaptation, augmentation, or survivability.

Host Engineering

Current capabilities of manipulating human microbiome are limited, and much research revolves around recreation of simplified communities derived from samples and identification and simple modifications of biome microbes that may serve useful purposes for sustainment and enhancement. Short-term milestones aim to expand relevant strain identification and modeling beyond gut microbiome, with emphasis on transplantation and signaling within the microbiome. Identification of individual microbiome differences will better enable advancements. Mid-term milestones include microbiome engineering (gastrointestinal or other) via *in situ* genetic modification, but likely limited to the engineering of a single strain or limited consortia members. Long-term milestones include expanded control over spatial and temporal microbiome composition and function, and *de novo* design and production of microbiomes, particularly those that can produce compounds that impact human performance or provide protection.

Data Science

Currently research focuses on identifying and building databases of microbiome strains and composition. Short-term milestones focus on a continuance of this, along with large scale acquisition of metagenomic and microbiome data from individuals in response to relevant environments, and standardization of experimental data to feed predictive modeling. Mid-term milestones include standardized analyses and reproducible metagenomic and transcriptomic pipelines for microbiomes, and development of computational tools for quantifying and predicting consortia-level phenotypes. Further advancements will enable predictive models of nutrient-microbiome interactions, human-consortia compatibility analyses, and artificial intelligence and machine learning tools for individualized microbiome solutions. Long-term milestones include rapid and efficient predictive modelling of consortia and hosts for on-demand phenotypic outcomes (such as a specific physical and/or cognitive state), predictive tools to personalize nutrition for tailored mission performance (*e.g.*, task-based nutritional needs), and data acquisition and computational tools for designing individual personalized mission-specific microbiome modifications.

4.3 - Enabling Capability: The ability to engineer consortia for the manipulation of relevant environments.

Understanding and engineering how organisms behave in the context of their surroundings can have unique defense benefits. Applications such as the modification of local environments and the conversion of local resources into precursors useful for distributed materials production require an ability to use multiple strains in coordination and an understanding of the effects of micro- and macro-environments on larger, complex biosystems. The information required to engineer microbiomes and consortia in the field is very different than engineering a single host's microbiome and requires an exceptional level of control over the engineered system. Engineered consortia must be able to interact within the biome and with the environment and must maintain stability and function over time. The ability to eliminate or terminate function of engineered microbes *in situ* to ameliorate the potential of long term or undesired changes in the environment is also required. Further, consortia engineering requires the acquisition of a much broader set of environmental and biological data as well as different analytical methods and modeling capabilities. However, the understanding and ability to carefully and controllably manipulate environmental microbiomes presents a great opportunity for altering local environments to serve relevant needs.

Engineering DNA

In addition to fundamental advancements in gene editing, synthesis, and assembly, this Enabling Capability will be achieved through a more robust understanding of, and the ability to better control and engineer,

Table 10. *Engineering biology milestones toward achieving the ability to engineer consortia for the manipulation of relevant environments.*

Ability to engineer consortia for the manipulation of relevant environments.

Milestones	Short-term	Mid-term	Long-term
Engineering DNA	Enable a more robust understanding of horizontal gene transfer among members of a consortia.	More effectively control and engineer horizontal gene transfer among members of a consortia.	Simultaneously edit the genomes of most or all consortia members.
Biomolecular Engineering	Encapsulate and integrate synthetic constructs, specifically sensing pathways for relevant threats.	Integrate engineered consortia with surfaces and other organisms within the environment.	Enable distance observation through biofilms and consortia.
Host Engineering	Create broader libraries of minimally dependent consortia that could have field applications.	Modify microbiomes in situ via the controlled introduction of engineered consortia in select species.	Develop tools to engineer consortia with defined or controlled functionalities, or to conduct localized modification of naturally occurring consortia.
Data Science	Increase complexity of modeling and machine learning and artificial intelligence to identify naturally occurring pathways and interactions to predict desired functionalities.	Enable predictive modeling for multi-input and environmental impacts on consortia behavior and data-driven design and engineering of minimal consortia.	Enable rapid and efficient predictive modeling and subsequent designed modification of environmental consortia for functionality.

horizontal gene transfer among members of a consortia. The ability to simultaneously edit the genomes of consortia members will also advance our capabilities.

Biomolecular Engineering

Currently, there exist few relevant sensing and response pathways that can be engineered into naturally occurring hosts, often only a single species within a consortium. Short-term milestones aim to advance the integration of synthetic constructs, specifically sensing pathways. Doing so within a wider array of hosts is likely to advance sense and response capabilities within consortia. This is likely to come about through short-term creation and further adaptation of toolkits for biofilm formation from diverse organisms. Mid-term milestones include integration of engineered consortia with surfaces and other organisms within the environment. Long-term milestones include standoff signaling through biofilms and consortia, including radiofrequency and, potentially quantum, signaling.

Host Engineering

Current limitations in the libraries and toolsets for engineering naturally occurring hosts limits our ability to create consortia with desired capabilities. We are still mostly limited to engineering a single species within a consortium and often only species that are commonly cultivated in the laboratory. Short-term milestones aim to create broader libraries of minimally dependent consortia that could have field applications. Mid-term engineering milestones are likely to include limited *in situ* microbiome modification via the controlled introduction of engineered consortia, such as into limited generation (sterile) arthropods. This is also likely to expand orthogonal conjugation systems or horizontal gene transfer abilities across microbial species. Long-term milestones include *in situ* host modification tools to produce specific microbe functionalities in pre-existing microbial communities and eventually control over environmental microbiomes and consortia. Introduction of engineered consortia with defined or controlled functionalities, or localized modification of naturally occurring consortia, could enable systems capable of transformation of any local mixed feedstock into materials or products to support relevant needs.

Data Science

To enable relevant consortia, there is a need to leverage growing databases of organisms found in relevant environments and increasing understanding of the dependencies and interrelationships of relevant biomes. We currently have limited data to support predictive modeling of consortia, particularly in response to environmental factors. Short-term milestones aim to expand such data sets to allow increased complexity of modeling and coupling with machine learning and artificial intelligence to identify naturally occurring pathways and interactions to predict desired functionalities. These advancements will lead to mid-term milestones of predictive modeling for multi-input and environmental impacts on consortia behavior and data-driven design and engineering of minimal consortia. Long-term milestones include rapid and efficient predictive modeling and subsequent designed modification of environmental consortia for on-demand functionality.

5 - INTEGRATING THE ENVIRONMENT: Sensing and responding to human and environmental signals.

A key enabling role of engineering biology in relevant environments is the ability to exploit organisms to provide information about their status and surroundings to adapt accordingly. Engineering biology tools and technologies can help to maintain persistent situational awareness, sustain environmental intelligence, and discriminate and modulate molecular signatures and biomarkers. Biology can be used to detect and determine physical and chemical threats, such as radiation or explosives, report timing and location and movement, and integrate physiological signals to sense, assess, and react to plant, animal, and human stress. Biomolecules, cells, and multicellular organisms and consortia can all serve as sensors and be tuned to deliver discrete, readable responses, both in proximity and at distance. Bio-based sensors can exist as stand-alone devices or be coupled to materials, electronics, or infrastructure to provide added capability and function.

Cells and organisms constantly process information from the outside world and adapt their behavior accordingly. Further, biology can be engineered to selectively sense, process, and respond to chemical, physical, and mechanical cues from the environment. By modifying the genetics, circuits, pathways, and internal and external components of cells, biological systems can be designed to take in a myriad of signals, integrate and transform those signals, and perform or produce a desired output, communicating a response. Through these processes, engineered biology can report information that enables faster, smarter decision-making in relevant environments.

Enabling Capabilities:

- The ability to provide decision-quality information through rapidly customized engineered biological sensors and sensor arrays.
- Coupling biosensing and signal processing to sense, report, and respond to desired signatures.
- Engineering integrated biosensor reporter systems that communicate information at stand-off distances through multiple channels.
- Engineering biology tools and technology to measure, record, and modulate mammalian biomarkers and physiology.

5.1 - Enabling Capability: The ability to provide decision-quality information through rapidly customized engineered biological sensors and sensor arrays.

Engineering biology has the potential to enable persistent, self-contained, autonomous bio-based sensors; more specifically, this includes sensors based on the high sensitivity and specificity of living organisms and distributed networks, and sensors that do not rely on conventional battery power or placement. To the extent that many of these sensors will either be part of natural microbes or introduced into new organisms, extended capabilities for organismal engineering will be needed, including improvements in DNA synthesis and construction, vectors and operating systems that can be used across many different chassis, and understanding how these chassis may interact with one another in larger consortia. Better understanding of how to construct microbial sensors and consortia *in situ* will require further information about endogenous microbiomes, and how they can potentially be engineered by the direct introduction of DNA into the host and/or by horizontal transfer. Depending on application, sensor recovery may not be required, and sensors may be disposable or degradable, but most sensor systems must be robust and operate in diverse environments. As an example of such technology this will enable, an engineered organism (plant or microbe) to detect environmental perturbations.

Table 11. *Engineering biology milestones toward achieving the ability to provide decision-quality information through rapidly customized engineered biological sensors and sensor arrays.*

The ability to provide decision-quality information through rapidly customized engineered biological sensors and sensor arrays.

Milestones	Short-term	Mid-term	Long-term
Biomolecular Engineering	Enable import of known, functional receptor and transcription factor systems into novel hosts; Design and couple biological recognition element activities for interfacing with materials and other sensor systems.	Engineer multicomponent sensing suites and logic gates for environmental/ physiological signals; Engineer enzymes for increased substrate promiscuity and catalytic efficiency.	Incorporate non-canonical amino acids to diversify sensors; Engineer circuits and pathways for mechanical/chemical sensing of physiological and environmental reporter signals.
Host Engineering	Expand toolsets used to deploy sensing pathways and modalities; Engineer cells and cell-free systems to express two-component sensing systems.	Diversify modular membrane sensors across relevant strains; Develop sensors for living cells that are in a minimal, or arrested, metabolic state.	Engineer cells and consortia with many sensors working simultaneously as arrays; Engineer real-time temporal sensing of human biomarkers.
Data Science	Develop computational tools to more thoroughly investigate binding/pathway activity and suggest engineering designs for biological recognition elements and improved sensor pathway performance.	Develop tools for multiplexing signals to improve information quality and classification accuracy and analytic integration of sensor systems for decision making.	Enable predictive modeling and computer aided design of engineered biosensors.
Engineering DNA	To achieve this Enabling Capability, general advancements in gene editing, synthesis, and assembly will be required. For more on this topic, see Engineering Biology: A Research Roadmap for the Next-Generation Bioeconomy (EBRC, 2019).		

Engineering DNA

To achieve this Enabling Capability, general advancements in gene editing, synthesis, and assembly will be required. For more on this topic, see *Engineering Biology: A Research Roadmap for the Next-Generation Bioeconomy* (EBRC, 2019).

Biomolecular Engineering

Current biomolecular engineering tools and technologies have significantly advanced our ability to generate proteins and other biomolecules that could act as sensors; however, there remains a significant amount of work to diversify and functionalize biosensors for relevant environments. Biomolecular engineering milestones will seek to advance the number and selectivity of sensors that function simultaneously, or side-by-side, whether within a single cell or across an organism or consortia. Short-term milestones aim to import known, functional receptor and transcription factor systems into novel hosts, and design and couple biological recognition element activities for interfacing with materials and other sensor systems. Short-term developments of RNA or protein-based biosensors to detect target pathogens or threats is also anticipated. Mid-term milestones aim to develop and improve multicomponent sensing suites and logic gates for biological computation of environmental/physiological signals and improved engineering of transcription factors for sensing capabilities, enzymes for increased substrate selectivity and catalytic efficiency, and two-component systems for community and cellular signal amplification. Long-term milestones will seek incorporation of non-canonical amino acids to diversify sensors and development of circuits and pathways for mechanical/chemical sensing of physiological and environmental reporter signals to achieve sensing modalities integrating complex samples and data streams (*e.g.*, samples containing organic material, geographic data, temporal data, etc.).

Host Engineering

Currently, a number of model microbe strains and some simple cell-free systems can be used for synthetic sensing systems, but we lack the ability to engineer as sensors broad ranges of environmental strains, consortia, or complex sensing systems. Short-term milestones aim to expand the toolsets used to deploy sensing pathways and modalities in a greater number of strains and engineer cells and cell-free systems to express two-component sensing systems. Mid-term milestones seek a diversity and modularity of membrane sensors across relevant strains. Mid-term milestones also aim to develop sensors for living cells that are in a minimal, or arrested, metabolic state. Long-term milestones aim to engineer cells and consortia with many sensors working simultaneously together within an array (sensor arrays). Long-term milestones also seek real-time temporal sensing of biomarkers to detect and monitor physiological status and systems biology.

Data Science

Databases of membrane proteins and signaling pathways, and computational approaches to identify potential receptors and transcription factors, continue to grow, as do complementary -omics measurements for mining biological data from natural organisms from relevant environments that will lead to increased numbers of useful biosensors. Short-term milestones aim to develop computational tools, including machine learning, to more thoroughly investigate binding/pathway activity data and suggest engineering designs for biological recognition elements and improved sensor pathway performance. Mid-term milestones seek to develop tools for multiplexing signals to improve information quality and classification accuracy and analytic integration of sensor systems for decision making. Mid-term milestones will also advance model-based design of biological circuits for simultaneous and ratiometric detection and monitoring of multiple biomarkers. Long-term milestones aim for predictive modeling of chemical classes and biosensors from databases; computer aided design of engineered biosensors that minimizes the need for screening or directed evolution; and robust, on-demand training and utilization of models for designing autonomous sensing devices and utilizing their information outputs.

5.2 - Enabling Capability: Coupling biosensing and signal processing to sense, report, and respond to relevant signatures.

Sensing, processing, and subsequent reporting of environmental signals can have applications from deep ocean, to soils, to humans and systems. Furthermore, biological systems can be used to detect diverse signatures, such as radiation, acoustics, and electromagnetism, and potentially do so in a multimodal manner that also captures time and space. This will be enabled by implementation of customized genetic constructs, novel sensing molecules and peptides, and intracellular pathways and circuits that facilitate rapid decision making. Additional advancements will be needed in design and engineering parallel processing across multiple cells, feedback and feed-forward motifs for improved signal processing, and improved signal amplification within cells and across consortia. The majority of existing biological sensors show exquisite specificity but lack the ability to generate a signal that provides actionable information in real time. Retaining the specificity

Table 12. *Engineering biology milestones toward enabling the coupling of biosensing and signal processing to sense, report, and respond to DoD-relevant signatures.*

Coupling biosensing and signal processing to sense, report, and respond to DoD-relevant signatures.

Milestones	Short-term	Mid-term	Long-term
Biomolecular Engineering	Engineer sense and response signal cascades for cell-free systems; Engineer circuits and processing capabilities that are responsive to known signatures.	Engineer novel sensors for relevant chassis and ability to detect mechanical and physical stimuli; Advance signal processing circuitry to generate designed responses.	Engineer sensors that can amplify signals across a precise range; Enable robust circuit performance in some relevant environments; Enable rapid, responsive synthesis of output signals.
Host Engineering	Develop reliable methods for engineering hosts capable of sense and response and cell-to-cell communication.	Engineer modular membrane sensors and robust cell-to-cell communication in relevant strains, to enable multiplexed signal processing across cell arrays.	Enable and amplify known biological sense and respond systems in the field via engineering of relevant chassis strains.
Data Science	Enable artificial intelligence and machine learning for determining sensing pathways and appropriate circuit design.	Enable predictive, model-based biological circuit design in relevant hosts and environments; Develop tools for the integration of sensing system inputs and outputs for decision making.	Enable machine learning for modeling and design of signal integration, amplification, and response across single cells and consortia in relevant environments.
Engineering DNA	To achieve this Enabling Capability, general advancements in gene editing, synthesis, and assembly will be required. For more on this topic, see Engineering Biology: A Research Roadmap for the Next-Generation Bioeconomy (EBRC, 2019).		

inherent to biology while providing a rapid signal will require engineering of novel systems that may include biological signal amplification or a bio-electronic interface.

Engineering DNA

To achieve this Enabling Capability, general advancements in gene editing, synthesis, and assembly will be required. For more on this topic, see *Engineering Biology: A Research Roadmap for the Next-Generation Bioeconomy* (EBRC, 2019).

Biomolecular Engineering

Current biomolecular engineering technologies to enable sensing and processing through biological systems is limited to circuits that can process a small number (1-2) inputs and outputs and some nominal oscillator circuits for temporal control, working in only one or two host strains. Short-term milestones seek advancements in sense and response signal cascades for cell-free systems and increases in the number of circuits and their processing capabilities that are responsive to known signatures. Mid-term milestones will advance sensing capabilities, including novel sensors for relevant chassis and ability to detect mechanical and physical stimuli. Mid-term milestones also aim to advance signal processing circuitry to generate designed responses, including novel catalytic activity and production and/or secretion of desired biomolecules. Long-term milestones will enable sensors that can amplify signals across a precise range, robust circuit performance in some relevant environments, and rapid, responsive synthesis of bioproduct.

Host Engineering

Current work focuses on the isolation, characterization, and cultivation of host strains from relevant environments and conditions that could be used for signal processing in the field. Short-term milestones aim to develop more reliable methods for engineering hosts capable of sense and response and cell-to-cell communication. Mid-term milestones aim to achieve modular membrane sensors and robust cell-to-cell communication in relevant strains, to enable multiplexed signal processing across cell arrays. Long-term milestones seek to achieve known biological sense and respond systems in the field via engineering relevant chassis strains, and further enabling responsive engineered cells and biological systems capable of recognizing and storing environmental data. Long-term milestones also aim to achieve chassis strains that will amplify both natural and -synthetic biosignatures in a range of environments.

Data Science

Current capabilities include data-driven circuit design via platforms like Cello and modeling on cells using a small number of sensors and regulators in simple control loops to control cellular processes. Short-term milestones seek artificial intelligence and machine learning for determination of sensing pathways and appropriate circuit design. Mid-term milestones aim to enable predictive, model-based biological circuit design in relevant hosts and environments and analysis tools for the integration of sensing system inputs and outputs for decision making. Long-term milestones include machine learning for modeling and design of signal integration, amplification, and response across single cells and consortia in relevant environments.

5.3 - Enabling Capability: Engineering integrated biosensor reporter systems that communicate information at stand-off distances through multiple channels.

Sensor systems must be robust and operate in diverse and adverse environments and must do so in ways that are not overtly obvious to foreign parties. The ability to use proteins and small molecules to generate fluorescent and luminescent signals is well established. Improvements in the design and construction of biological molecules and systems will allow expansion of the spectrum of signals that can be generated by bio-based sensors and expand the potential applications for these systems while evading adversary detection. Advancements must be made in biological reporter systems that can record and store environmental signals and data and then report the data over multiple channels – such as IR, UV, and spatiotemporal pattern generation – likely using orthogonal and non-natural pathways and mechanisms. All of this will also rely on the ability to safely and securely produce and disperse engineered organisms at scale into diverse environments.

Engineering DNA

To achieve this Enabling Capability, general advancements in gene editing, synthesis, and assembly will be required. For more on this topic, see *Engineering Biology: A Research Roadmap for the Next-Generation Bioeconomy* (EBRC, 2019).

Table 13. *Engineering biology milestones toward engineering integrated biosensor reporter systems that communicate information at stand-off distances through multiple channels.*

Engineering integrated biosensor reporter systems that communicate information at stand-off distances through multiple channels.

Milestones	Short-term	Mid-term	Long-term
Biomolecular Engineering	Improve enzyme production for efficient expression, stability, and localization of reporter signals; Improve pathways and circuits for signal amplification.	Engineer network-scale reporters that can generate signals in multiple channels, to enable multi-modal sensing/reporting.	Engineer orthogonal reporter systems and temporal control.
Host Engineering	Enable robust engineering of relevant environmental bacteria, viruses, plants, and animals, including organisms that are currently recalcitrant to culture.	Develop tools and technologies to modify species in the field; Engineer multiple organisms within the same biome for distributed sensing and reporting capabilities.	Engineer biosensor reporter systems of organisms and consortia relevant for desired environments, that persist as needed.
Data Science	Advance machine learning and other engineering technologies to develop instruments that are better able to sense and interpret noisy signals.	Advance analytic integration of sensor systems and multiplexing signals for improved decision making and information accuracy and quality.	Enable computational and modeling tools to optimize dynamic signal acquisition from engineered organisms.
Engineering DNA	To achieve this Enabling Capability, general advancements in gene editing, synthesis, and assembly will be required. For more on this topic, see Engineering Biology: A Research Roadmap for the Next-Generation Bioeconomy (EBRC, 2019).		

Biomolecular Engineering

Most biological systems are currently capable of a range of chemical outputs, including odorants and chromophores, and some are capable of optical outputs, such as bioluminescence and fluorescence; to do so, known reporter systems are isolated from natural systems and repurposed for applied use. Short-term milestones aim to improve genetic (codon) optimization and enzyme production for efficient expression, stability, and localization of reporter signals. Simultaneously, improvements of signal amplification are needed, both in sensor and reporter systems, through improvements in efficient genetic circuits and cell-to-cell communication. Mid-term milestones will improve on engineering of reporter proteins to accentuate natural reporter activities and to generate signals not currently present in nature, and to introduce more temporal control over dynamic reporting signal. Mid-term milestones aim to achieve network-scale reporters that can generate signals in multiple channels, to enable multi-modal sensing/reporting. Long-term milestones aim to produce non-natural reporter systems that use channels orthogonal to natural biology, to convey more information about the sensed environment, and further temporal control to enable real-time reporting. Finally, long-term milestones aim to advance orthogonal reporter systems and temporal control.

Host Engineering

Currently, most hosts that could be engineered as reporters are microbial, though there is a growing library of domesticated strains of bacteria, viruses, fungi, plants, and fish. Short-term milestones aim to expand the range of chassis that can be engineered to include more relevant environmental bacteria, plants, and animals, including improving the ability to robustly engineer organisms that are currently recalcitrant to culture. Mid-term milestones aim to develop tools and technologies to modify these species *in situ*, or in the field. Mid-term milestones also seek to develop tools for engineering multiple organisms (*e.g.*, a plant and a soil microbe) within the same biome for distributed sensing and reporting capabilities and advancing engineering of cells that can persist in the environment utilizing local nutrients and communicate with the surroundings. Long-term milestones aim to create biosensor reporter systems of organisms, including multi-species communities, relevant for environments that are persistent as needed.

Data Science

Tools and technologies are needed to predict, model, and integrate data to produce robust sensing and reporting organisms, but new tools and instruments to detect and interpret biological reporting signals are also needed. While efforts will be made to improve the robustness and amplification of biological signals, short-term milestones aim to use machine learning and other engineering technologies to develop instruments that are better able to sense and interpret noisy signals. Mid-term milestones seek to advance analytic integration of sensor systems and multiplexing signals for improved decision making and information accuracy and quality. Mid-term milestones also seek to co-develop sensing and data processing instruments and software specifically for signals produced by engineered organisms. Long-term milestones aim to develop sensors capable of dynamically tuning range and thresholds to respond to signals produced by engineered organisms through computational and modeling tools to optimize signal acquisition.

5.4 - Enabling Capability: Engineering biology tools and technology to measure, record, and modulate mammalian biomarkers and physiology.

The ability to continually sense, interpret and respond to dynamic changes in physiological status - including physical, behavioral and cognitive states - environmental conditions, mission demands, and threats will maintain and enhance survivability and lethality under relevant stress conditions. Sentinel cells and organisms can be engineered to monitor physiological status, with real-time communication to *ex vivo* devices, and elicit specific responses to attenuate an induced degradation of health and performance. The use of sentinels and/or engineered organisms will require the development of new genetic tools for non-traditional, anaerobic organisms, and utilization of non-toxic systems to promote or modify microbiome homeostasis. The ability to sense and respond to physiological breakdown before it manifests in a person’s innate biology will allow leadership to build resiliency to this failure through medical intervention, a reassignment of duties, or simple rest, preserving unit readiness and longevity in the field.

Engineering DNA

To achieve this Enabling Capability, general advancements in gene editing, synthesis, and assembly will be required. For more on this topic, see *Engineering Biology: A Research Roadmap for the Next-Generation Bioeconomy* (EBRC, 2019).

Biomolecular Engineering

While there exists significant numbers of known biomarkers, detection methods for these biomolecules are often significantly limited in their use non-invasively and in the field, and the effects of stress on biomarker

Table 14. *Engineering biology milestones toward enabling tools and technology to measure, record, and modulate mammalian biomarkers and physiology.*

Engineering biology tools and technology to measure, record, and modulate mammalian biomarkers and physiology.

Milestones	Short-term	Mid-term	Long-term
Biomolecular Engineering	Enable low- and medium-throughput sensing, recording, and reporting of biomolecular signatures in cells and tissues.	Engineer robust, synergistic-dependent pathway activation for targeted production of performance biomarkers.	Engineer biomolecule sensors that respond to desired biomarkers at environmentally relevant levels; Engineer circuits and pathways for continuous recording and dynamic response.
Host Engineering	Identify, detect, and characterize beneficial and detrimental biomarkers for biosensor design.	Engineer microbiomes and commensal organisms to sense changes in physiology or detect toxins or performance-degrading metabolites.	Engineer cells and consortia that can detect and respond to select biomarkers in real-time, including orthogonal control elements for host cell manipulation or augmentation.
Data Science	Develop libraries and datasets of biomarkers and biosensors that can be used with machine learning to decipher homeostasis vs. dysbiosis.	Integrate -omics based signatures for microbiome characterization and predictive modeling of cells and tissues with or without an exposure/infection.	Develop spatiotemporal maps of homeostasis and stress biomarkers, integrating all biomarker signatures for predictive, holistic mathematical modeling of individuals over time in relevant environments.
Engineering DNA	To achieve this Enabling Capability, general advancements in gene editing, synthesis, and assembly will be required. For more on this topic, see Engineering Biology: A Research Roadmap for the Next-Generation Bioeconomy (EBRC, 2019).		

expression are not as widely known. There currently exists a few wearable monitors with a limited set of specific enzyme assays for detecting these biomarkers in sweat and blood. Short-term milestones seek tools for low- and medium-throughput sensing, recording, and reporting of biomolecular signatures in cells and tissues, including sensor circuits for multiplexed signatures. Better understanding and selection of relevant biomarker sensors and receptors will also advance this technology. Mid-term milestones seek robust, synergistic-dependent pathway activation for protection and performance circuitry, including epigenetic regulation, for targeted production of performance biomarkers. Long-term milestones seek to advance protein engineering of biomolecule sensors that respond to desired biomarkers at environmentally relevant levels and development of biomolecular circuits for continuous recording and response of dynamic processes of whole tissues, and eventually, individuals. Long-term milestones also aim to advance biomolecular circuits and pathways that can respond to emergent threats with on-demand metabolic outputs or compound production.

Host Engineering

Currently, systems are in place that aim to understand, observe, and measure host response to physiological stressors (including chemicals and biomolecules) and identify biomarkers unique to the human microbiome under homeostasis. Microbe and cell-free system engineering is also currently enabling some mammalian biomarker modeling and sensor assessment. Short-term milestones aim to identify beneficial microbes and metabolites that will inform development of more efficient physiological biosensors. Likewise, detection and characterization of human microbiome-linked biomarkers under stress or threat will advance our capabilities of modulating host response. Mid-term milestones seek to engineer microbiomes and commensal organisms to sense changes in physiology in both humans and animals or detect toxins and pathogens or performance-degrading metabolites. Long-term milestones aim for engineered cells and consortia that can detect and respond to select biomarkers in real-time, including orthogonal control elements for host cell manipulation or augmentation. Long-term milestones also seek advancements in biotic-abiotic interfaces (*e.g.*, consortia-material systems) that seamlessly connect biological sensors and actuators to living systems.

Data Science

Currently, there is limited sensor integration with biomolecular, cellular, or tissue profiles, combining physical sensors and processing of data to assess physiological status. Short-term milestones aim for datasets of biomarkers that can be used with machine learning to decipher homeostasis vs. dysbiosis and generation of a validated list of biosensors for human biomarkers, with bioinformatic prediction of useful sensors and metabolic pathways. Mid-term milestones aim for integration of all signatures (-omics based: genomics, genome epigenetics, transcriptomics, proteomics, metabolomics,) for predictive modeling ranging from cells to tissues to organisms, with or without an exposure/infection. Mid-term milestones also seek de-oprhanization of otherwise unknown biomarker receptors and sensors and predictive modeling of circuits and pathway responses under homeostasis conditions. Mid- to long-term milestones seek to capture consortia variation between individuals and dynamic responses to evolving environments. Long-term milestones aim to achieve machine learning to build spatiotemporal maps of homeostasis biomarkers and stress/threats and integration of all biomarker signatures for predictive, holistic mathematical modeling of individuals over time in an relevant environment.

6 - ENABLING COMPLEX PLATFORMS: Combining biology with 'other' to improve and enhance human and system performance.

Engineering biology can enable a myriad of complex platforms. With significant advancement, we can utilize engineered biological systems to dramatically enhance our current capabilities and improve the performance and operation of systems and humans. Materials and machines interfaced with biology can provide rapid, dynamic response to activity and environment; can modulate and modify surfaces, circuits, and physiology; and can integrate signals and stimuli to enhance performance in new and unimagined ways. Functional living materials and bio-composites, and the potential in strategically integrating electronics and biology, can greatly enhance transportation, physical infrastructure, and wearables by adapting to, or circumventing, the environment, repairing and replacing defects and damage, and providing additional protection.

Understanding and engineering of biological circuits underpins most of this functionality. Metabolic activity, cell-to-cell communication, and integration with the surrounding environment – sensing, biocompatibility, three-dimensional space filling – are all areas where research and application of engineering biology will enable these complex, biotic/abiotic platforms. In this space in particular, advancements in materials science, machine learning and artificial intelligence, and data integration will be key to achieving these capabilities.

Enabling Capabilities:

- The ability to modulate and control dynamic response of functional living materials.
- Engineering of biotic/abiotic interfaces and interactions for enhanced systems.
- Optimizing microbes for integration with bioelectronic circuits.

6.1 - Enabling Capability: The ability to modulate and control dynamic response of functional living materials.

Functional living materials can play diverse roles in many applications, including healing or self-repair, response and reconfiguring to environmental cues, and/or signal amplification. The combinatorial power of using biological systems to sense and respond to defined signals and using the biomolecules or cells themselves as a material, will require a wider range of biosensors and deployable organisms, advancements in signal transduction and cell-to-cell communication, control over cellular patterning and architectures, and better data modeling tools to evaluate complex, real-world circuit and system needs. Engineered living materials for deployment in defense systems can contribute to applications such as windshields that can self-repair cracks, produce colored indicators of where relevant damage has been sustained, or can be resurfaced and restored by a simple sprayover of living cells.

Engineering DNA

To achieve this Enabling Capability, general advancements in gene editing, synthesis, and assembly will be required. For more on this topic, see *Engineering Biology: A Research Roadmap for the Next-Generation Bioeconomy* (EBRC, 2019).

Biomolecular Engineering

Biomolecular engineering currently allows for some simple sensing modalities of non-biological signatures and attachment of some peptides or other biomolecules onto abiotic substrates. However, the pathways that result

Table 15. *Engineering biology milestones toward enabling the ability to modulate and control dynamic response of functional living materials.*

The ability to modulate and control dynamic response of functional living materials.			
Milestones	**Short-term**	**Mid-term**	**Long-term**
Biomolecular Engineering	Engineer increased expression of known sensing peptides at the cell surface.	Increase modularity of membrane-associated sensor proteins; Improve receptor-ligand signal transduction and amplification.	Embed computation via biochemical reaction networks; Engineer cell-to-cell signaling via designer biomolecules that amplify signal across a precise dynamic range.
Host Engineering	Enable multicellular two- and three-dimensional patterning and printing.	Enable three-dimensionally patterned living materials that can sense and respond to known biosignatures and some environmental stimuli.	Engineer responsive architectures via four-dimensional cell patterning; Design consortia that can sense and respond, including the amplification of natural and synthetic signals.
Data Science	Enable computational modeling to understand interactions of biological systems with material and design of biotic response to external stimuli.	Advance analytics and modeling to better understand multi-scale and multi-component signaling within living materials.	Advance designs for relevant contexts and advance machine learning and artificial intelligence for predictive modeling for material development.
Engineering DNA	To achieve this Enabling Capability, general advancements in gene editing, synthesis, and assembly will be required. For more on this topic, see Engineering Biology: A Research Roadmap for the Next-Generation Bioeconomy (EBRC, 2019).		

in cell-to-cell signaling are mostly limited to those found in nature. So, while we can nominally create basic living materials, they currently have limited functionality. Short-term milestones aim to expand sensing capabilities, particularly at the surface, through engineering increased expression of known sensing peptides. Mid-term milestones aim to improve signaling between cells within a living material, including by increasing modularity of membrane-associated sensor proteins, and improve the transduction of signaling through advancements in receptor-ligand signal transduction and amplification and orthogonal recognition between cells. Long-term milestones seek biochemical reaction networks that carry out embedded computation, enabling reconfigurable living materials and highly insulated cell-to-cell signaling via designer biomolecules that also amplify signal across a precise dynamic range to create a fully living, responsive material.

Host Engineering

Currently, the production of functional living materials is limited mostly to two-dimensional cell patterning. Short-term milestones aim to expand multicellular two- and three-dimensional patterning and printing. Mid-term milestones aim to achieve three-dimensionally patterned living materials that can sense and respond to known biosignatures and some environmental stimuli, requiring improved cell-to-cell communication. Long-term milestones seek to achieve responsive architectures through four-dimensional cell patterning, requiring engineering of consortia that can sense and respond between individual cells and the larger engineered material, including the amplification of natural and synthetic signals. Long-term milestones aim toward materials that are capable of self-repair.

Data Science

Current data analytics and tools are limited to understanding and limited modeling of sensing, regulating, and response activities within biological systems. Short-term milestones aim to improve computational modeling to understand interactions of biological systems with material and design of biotic response to external stimuli. Mid-term milestones aim to achieve more advanced analytics and modeling to better understand multi-scale and multi-component signaling within living materials to improve signal transduction and amplification. Long-term milestones seek to achieve quick, facile living material design for desired contexts and advancements in machine learning and artificial intelligence for predictive modeling for material development with self-repair response.

6.2 - Enabling Capability: Engineering of biotic/abiotic interfaces and interactions for enhanced systems.

Through augmenting surfaces on platforms and systems to integrate engineered biology, the interfaces have the potential to serve relevant and advantageous functions, such as filtration and decontamination, combat weathering through self-healing and anti-corrosion, and provide obscuration and camouflage. To do so, there is a need for more advanced and defined biopolymer production, modalities to control the stability, assembly, and spacing of biomolecules and cells, and organized engineered consortia. The interface between biology and

Table 16. *Engineering biology milestones toward the ability to engineer biotic/abiotic interfaces and interactions for enhanced warfighter systems.*

Engineering of biotic/abiotic interfaces and interactions for enhanced warfighter systems.			
Milestones	**Short-term**	**Mid-term**	**Long-term**
Biomolecular Engineering	Engineer biomolecules to functionalize surfaces, with control over conformation, density or spacing, and biomolecule activity.	Design biomolecules that confer desired and advantageous functionalities to the physical interface.	Precisely, predictably, and robustly functionalize biotic-abiotic substrates through engineered biomolecules.
Host Engineering	Expand libraries of organisms that can be used to functionalize sensor surfaces, such as by secreting proteins, small molecules, or biofilms.	Optimize compatibility with abiotic surfaces of warfighter systems; Effectively functionalize surfaces through biomolecule secretion.	Engineer coordinated consortia to sequentially functionalize or assemble abiotic surfaces in patterns or layers.
Data Science	Advance modeling and prediction of binding and reaction kinetics and biomolecule conformation at biotic-abiotic interfaces.	Enable artificial intelligence and machine learning advancements in interface design and performance in controlled environments.	Enable predictive designs of multiple biotic-abiotic interface components that can autonomously assemble and interact for a desired application.
Engineering DNA	Incorporate non-canonical amino acids to improve interfacing capability, including the utilization of DNA or XNA as the structural interface material itself.		

devices can also enable bioelectronics with engineered cells that can interpret and transduce signals between surrounding tissues and electronic components.

The convergence of man or animal and machine will facilitate two-way, high-speed data transfer at the cellular level, enhancing the real-time response to stimuli, allowing autonomous adaptation, and expanding therelevant space. Once two-way data transfer has been perfected, the ability to read and write environmental data will evolve, fundamentally changing the way we communicate, learn, and respond.

Engineering DNA

In addition to fundamental advancements in gene editing, synthesis, and assembly, this Enabling Capability will be achieved through incorporation of non-canonical amino acids to improve interfacing capability, including the utilization of DNA or XNA as the structural interface material itself.

Biomolecular Engineering

Towards the production of functional biotic-abiotic interfaces, small libraries exist of biological molecules that can interface with abiotic surfaces. Short-term milestones aim to expand these libraries, particularly to include relevant surfaces, and seek the ability to engineer biomolecules to functionalize surfaces, with control over conformation, density or spacing, and biomolecule activity. Stability and robustness of engineered biomolecules in controlled, moderate environments (for example, exposure to weathering, UV) without disruption to interface would also be sought in the short-term. Mid-term milestones aim for the ability to design biomolecules that confer relevant and advantageous functionalities to the physical interface, such as for applications in decontamination, camouflage, self-healing, and anti-corrosion. This will be achieved by advancements in self-assembling lattices or other structures dependent on application. Long-term milestones aim to achieve controllable, bidirectional transduction of biochemical signals to optical, electrical, or magnetic signals, and advancements in functionalities such as self-reconfiguration and self-repair. Long-term milestones seek development of precise, predictable, and robust biotic-abiotic substrate functionalization through engineered biomolecules, leading to greater automation and manufacture.

Host Engineering

Currently, a very limited number of cell types or strains can be functionalized for use at biotic-abiotic surfaces. Short-term milestones aim to expand these libraries of chassis systems, in particular organisms that can be used to functionalize sensor surfaces, such as by secreting proteins, small molecules, or biofilms. Mid-term milestones aim to optimize compatibility with abiotic surfaces (*e.g.*, metals, ceramics), including engineering hosts to survive in relevant environments and effectively functionalize surfaces, such as through biomolecule secretion. Long-term milestones aim to engineer consortia to sequentially – or in otherwise coordinated fashion – functionalize or assemble abiotic surfaces in patterns or layers, contributing to directed material properties, surface self-repair or reconfiguration.

Data Science

Current data modeling and design capabilities primarily focus on predicting DNA and peptide binding to surfaces. Short-term milestones aim to advance modeling and prediction of binding and reaction kinetics and biomolecule conformation at biotic-abiotic interfaces and produce annotated libraries of biomolecule structure and activity sequences. Mid-term milestones seek artificial intelligence and machine learning-enable advancements in interface design and performance in controlled environments. Long-term milestones aim to produce predictive designs of multiple biotic-abiotic interface components that can autonomously assemble and interact for a desired application.

6.3 - Enabling Capability: Optimizing microbes for integration with bioelectronic circuits.

Bacteria are naturally able to form electrical connections with minerals and electrodes through extracellular biomolecules, including proteins, polymers, and small molecules. The tools of engineering biology can define the design rules to port these properties to organisms of interest to create new sensor devices that report using electrical current, new conductive materials, and even nano-scale living microprocessors that can be multiplexed to form living integrated circuits. Such devices take advantage of the massively paralleled data processing power of microbes, their self-assembling and self-healing properties, and ability to form biofilms. In addition, bacteria that interact with metals, or have metal co-factors that respond to electromagnetic fields, may passively engage with electronic devices by acting as scaffolds for nucleation of conductive materials (for example, copper nanoparticles) or alter magnetic fields for sensing applications.

Engineering DNA

There is some evidence that DNA itself is conductive and could be used to create synthetic polymer matrices to be utilized in bioelectronic circuits, requiring customized DNA synthesis. In addition to fundamental advancements in gene editing, synthesis, and assembly, this Enabling Capability will be achieved through synthesis and assembly of sequences that code for conductive proteins and polymers, with engineering to confer tunable redox properties based on ligand sites and non-native metal cofactors and formation of conductive biofilms.

Table 17. *Engineering biology milestones toward optimizing microbes for integration with bioelectronic circuits.*

Optimizing microbes for integration with bioelectronic circuits.

Milestones	Short-term	Mid-term	Long-term
Biomolecular Engineering	Engineer extracellular or membrane-bound biomolecule sensors that can transmit/transform electrical signals.	Advance interfacing of responsive bio-circuits with material scaffolds and creating material coatings that encapsulate electrically conductive biomolecules.	Engineer charge-carrying proteins that generate redox signals not currently present in nature and associated sensing circuits.
Host Engineering	Engineer cells to produce real-time response current; Increase number of host strains capable of engineered extracellular electron transfer and interface with abiotic surfaces.	Engineer consortia capable of cell-to-cell communication via electrons through a designed conductive cellular matrix.	Enable three-dimensional hybrid devices that incorporate electronics and any number of designed chassis strains to function in desired environments.
Data Science	Advance electrode arrays and instrumentation to better detect electrical signals produced by living cells.	Enable detection using ultrasensitive, ultra-small arrays, capable of real-time signal detection in noisy environment settings.	Develop distributed networks of autonomous sensors that enables the processing of cellular information to create spatial and temporal maps of events.
Engineering DNA	Synthesize and assemble sequences that code for conductive proteins and polymers, with engineering to confer tunable redox properties based on ligand sites and non-native metal cofactors.		

Biomolecular Engineering

Current engineering biology capabilities enable genetic circuits and biochemical pathways for the production of biomolecules that can interface with electronics; for example, soluble redox mediators are known to trigger cellular redox sensors creating a two-component system with cells and oxidizing electrodes. Short-term milestones aim to expand the number of genetically encoded circuits for electronic interfaces and the number charge carrying biomolecules (DNA, proteins, and small molecules) that can be synthesized as part of electron transfer conduits for selectivity on sensor surfaces, such as to create patterned biological electronics. Short-term milestones also seek improvement in engineering of extracellular or membrane-bound biomolecule sensors that can transmit/transform electrical signals. Mid-term milestones will advance interfacing responsive biocircuits with material scaffolds and creating material coatings that encapsulate electrically conductive biomolecules, as well as advance design rules for fast and reversible electrical sensing in redox proteins, leading to rapid on/off, reversible functionality for electrical signaling at biointerfaces. Long-term milestones include engineering charge carrying proteins that generate redox signals not currently present in nature and associated sensing circuits, materials with biosensor systems that can amplify signals across a precise dynamic range, and biomolecules and bioelectronic circuits designed to control and integrate with devices.

Host Engineering

There are a very limited number of domesticated microbial hosts that can be engineered to produce an electrical current in response to (bio)chemical input. Short-term milestones aim to advance this engineering such that these cells can produce current in real-time response to stimulation, to expand the number of host strains capable of engineered extracellular electron transfer, and to better engineer organisms to interface with abiotic surfaces, specifically in precise patterns necessary for effective electronics. Mid-term milestones seek to enable engineered cells that can produce overlapping electrical and biological outputs and consortia of microbes capable of cell-to-cell communication via electrons through a designed conductive cellular matrix. Mid-term milestones also aim to enable coatings or surface materials with engineered cells capable of fast post-translational sensing necessary for electronic interfaces. Long-term milestones aim to enable three-dimensional hybrid devices that incorporate electronics and any number of designed chassis strains to function in relevant environments. This will require cells that are capable of sensing and amplifying natural and synthetic signatures in a range of environments and cellular state (*e.g.*, arrested metabolism, in spore-form) using electronic and ionic conductivity.

Data Science

Currently, enabling microbial electronics (and other types of bioelectronics) are restricted by the ability to detect signal and decipher outputs because of high signal-to-noise ratio. Short-term milestones aim to advance electrode arrays and instrumentation to better detect electrical signals produced by living cells. Mid-term milestones aim to enable this detection using ultrasensitive, ultra-small arrays, capable of real-time signal detection in noisy settings. Long-term milestones aim to develop distributed networks of autonomous sensors with device-to-device communication that enables the processing of cellular information from many locations to create spatial and temporal maps of events occurring in various field settings.

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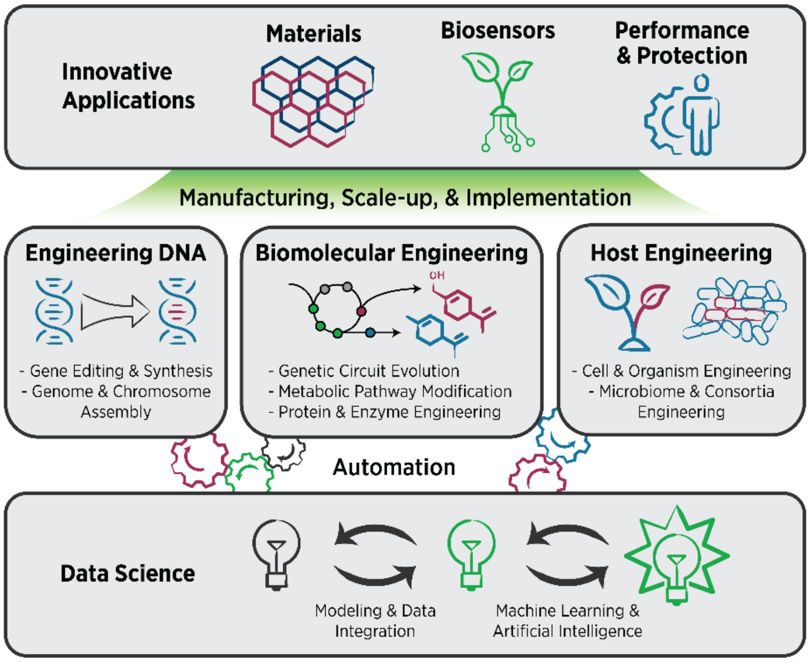
ENABLING DEFENSE APPLICATIONS THROUGH ENGINEERING BIOLOGY
A Technical Roadmap

Executive Summary

We must be prepared to operate in increasingly complex and dynamic environments. To maintain and enhance desired capacity, technological advances can be leveraged to overcome challenges presented by these environments. Engineering biology is the convergence of advances in chemistry, biology, computer science, and engineering that enables us to go from idea to product faster, cheaper, and with greater precision than ever before. Engineering biology tools have the potential to provide transformational capabilities to the human and their systems. Commonly used tools in engineering biology include modification of DNA and genetic material, use of microorganisms (*e.g.*, bacteria and yeast) to produce chemicals and materials, and adaptation of plants and animals to confer new abilities and modify performance. Taking advantage of these tools and technologies, engineering biology can transcend current constraints to build new materials for relevant systems, enable new methods for sensing, monitoring, and communicating, and augment performance and protect the human from harsh environments, combat mission states, and build resilience to evolving threats.

To realize this potential, we must anticipate and generate significant progress and advancements in engineering biology through targeted research and development. The roadmap highlights scientific and engineering capabilities that can be achieved through reaching various milestones over three time periods. The short-term milestones (2-5 years) are anticipated to build on current capabilities, existing infrastructure, facilities, and resources. The mid-term (5-10 year) and long-term (10+ year) milestones would require (and thus, result in) significant technical advancements, and stem from dedicated programs and resources. The milestones outlined in this roadmap are focused primarily on early stage research: TRL 1-4; 6.1-6.3. Additional strategic development efforts will be needed to advance translation of technologies into desired applications.

Achieving the milestones and capabilities detailed in this roadmap will lead to new tools and technologies that can be applied toward making people and systems more resilient, better prepared, and better protected. Technical achievements will allow the utilization of diverse resources (including waste streams) for the production of specialty chemicals and materials, such as coatings or surfaces that can repair or regrow after sustaining damage and systems for water filtration, desalination and purification. Engineered biosensor systems can better enable the ability to discriminate, identify, track, and target a wide variety of friends and foe in cluttered, multi-dimensional, multi-domain battlespace, through technologies such as skin- or soil-based detection and indication of pathogens, chemicals, or radiation. And integration of engineering biology with humans and systems can allow them to operate continuously and intelligently under adverse conditions across time and space, such as through employing engineered gut or skin microbiomes that enable improved tolerance to harsh environments like high-temperature or low pressure and enhance resistance to fatigue or support sustained activity.



ENGINEERING BIOLOGY FOR MILITARY APPLICATIONS

ENABLING DEFENSE APPLICATIONS THROUGH ENGINEERING BIOLOGY
A Technical Roadmap

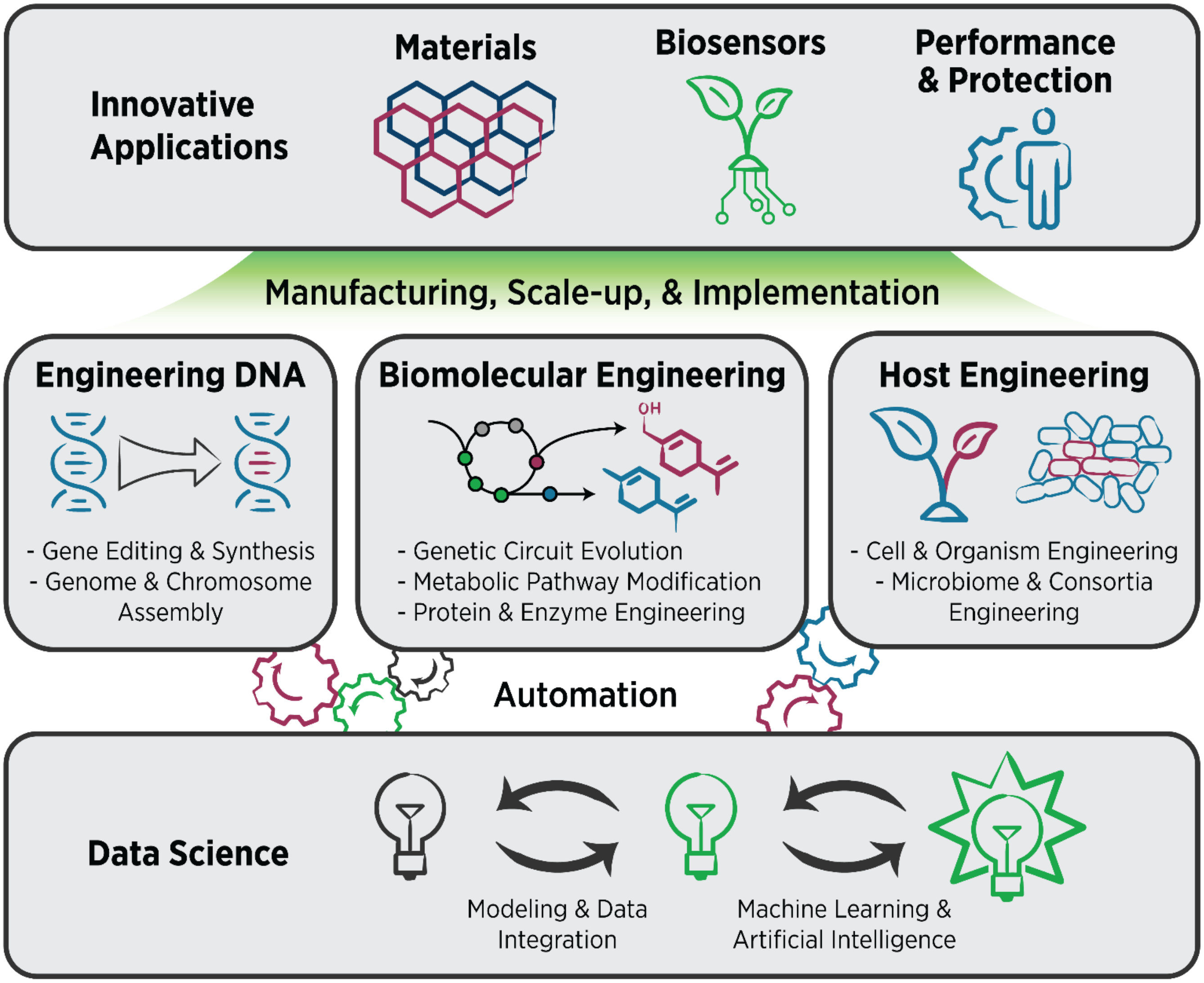
Technical Summary

Engineering biology – a convergence of biology, chemistry, computer science, and engineering – has the potential to transform relevant systems and mission spaces. Engineering biology comprises many powerful technologies, ranging from manipulating the bases in DNA, to modifying a cell for production of a specific compound, to altering the composition of a natural biome. Advancements in engineering biology enable gene synthesis, editing, and the assembly of chromosomes and genomes and the engineering of genetic circuits, biomolecules, and pathways, redesigning the building blocks and functional foundations of a cell. Engineering biology allows for modification, adaptation, evolution, and construction of cell-free systems, chassis, organisms, and consortia, enabling new systems for production and specialized activities, outputs, and interactions. Data science tools enable and advance biological data modeling and integration, machine learning, and artificial intelligence leading to novel, more intricate, and more robust engineered biological systems. The convergence of these engineering biology technologies and information science aims to unlock more rapid, innovative, and diverse applications than we can currently recognize or realize. Taking advantage of these tools and technologies, engineering biology can transcend current constraints to build new materials for relevant systems, enable new methods for sensing, monitoring, and communicating, and augment performance and protect the human from harsh environments, combat mission states, and build resilience to evolving threats.

The roadmap highlights scientific and engineering capabilities that can be achieved through reaching various milestones over time. Short-term milestones are objectives intended to be reached with current funding paradigms, existing infrastructure, facilities, and resources, and are attainable within 2-5 years. The mid-term (5-10 years) and long-term (10+ years) milestones are more ambitious achievements that require increased funding and resources, and new or improved infrastructure, but result in significant technical advancements. In addition to this strategic development, efforts are needed to advance translation of nascent tools and technologies into applications at scale and enable manufacturing and production for unique needs. Despite these needs, engineering biology can revolutionize conventional approaches to manufacturing and scale-up and the accessible space in materials and bioproducts.

Advancements in engineering biology can bring about new structures and functions and reduce cost and time of manufacturing and production. However, to realize this potential, we must anticipate and generate significant progress toward overcoming technical challenges in engineering biology. Therefore, the roadmap comprises topical sections embodying an increasing complexity of technical challenges, layering scientific and engineering capacities through implementation of advanced engineering biology tools and technologies. These technical challenges are captured as Enabling Capabilities, which represent significant technical achievements that will revolutionize the way the engineering biology is applied to major challenges.

The roadmap for engineering of biomolecules and cells for the production of biologics – including small molecules, enzymes, and active cells – and materials captures advancements in technologies necessary to engineer and dynamically synthesize molecules and polymers, including those not found in nature, and introduce novel functions and properties. Further, through engineering of cellular metabolism, modification and adaptation of biological pathways and circuits, and enabling control over the spatial and temporal architecture and organization of biological components, cells, and systems, achievement of the Enabling Capabilities can lead to the synthesis of high-performance, dynamic, and low-cost materials, sensors, and protective and performance enhancive products. Long-term milestones aim to enable these production capacities in relevant environments at point-of-need, real-time and in situ. Many of these capabilities represent foundational and fundamental advancements in engineering biology and application can be well-tuned to diverse defense needs. Biologics and products of engineering biomolecules and cells can be used toward applications in specialty



ENGINEERING BIOLOGY FOR MILITARY APPLICATIONS

chemicals, fuels, coatings, and surfaces; novel sensing, monitoring, and reporting systems for humans and the environment, and materials with greater levels of complexity and functionality for relevant settings. Furthermore, this engineering can enable utilization of more diverse feedstocks or waste-streams, and has the potential to decrease footprint, costs, and time-to-implementation.

Engineering of biological systems advances the scale of tuning biomolecules and cells toward more complex systems comprised of multiple cells and species as organisms, microbiomes, and consortia. These engineered systems have potential to alter relevant environments or the ability of humans and systems to adapt to and function within those environments. Engineering of organisms, such as microbial consortia and plants, results in a roadmap to acquire greater control over spatial and temporal behaviors, the robustness of systems to withstand harsh conditions and respond quickly to local changes, and the ability to design and model these systems for diverse events and outcomes. Key milestones toward making these advancements are acquisition of the tools and ability to exercise elimination of organisms or the termination of function – such as through biological kill-switches - to ameliorate the potential of undesired changes or persistence, which must be attained before deployment of engineered systems can be realized to ensure security and safety. The resulting systems can be utilized for bioproduction, on demand and in the field, of materials, chemicals, and other products for system support and protection; to detect status indicators or fluctuations in environment or human performance and respond accordingly in a designed and predictable manner, such as producing a stand-off signal or a beneficial biomolecule; and effecting the local environment to decrease or mitigate threats, enhance conversion of local resources, or provide signature management or obfuscation benefits.

Deployment of engineered biological and bio-enabled systems into the environment will require the ability to integrate environment and system information, whether those signals are biotic or abiotic. This requires realizing Enabling Capabilities that describe biological systems that can take in and store complex, dynamic information, integrate and transform those signals, and provide an output that is selective and modulated for the information it is meant to provide. The roadmap highlights technical advancements in biological pathways and circuits that perform robustly to amplify signals and incorporate orthogonal and non-canonical components, cell-to-cell and cell-to-environment (both micro- and macro-environment) communication in relevant organisms, and machine learning to advance analytics, computation, and decision-making of biologically-sensed or -reported signals. Integrated biological systems incorporate biosensors, materials, and multiplexed data to selectively sense, process, and respond to environmental cues and can be used to detect and determine physical and chemical threats, report timing and location and movement, and integrate physiological signals to sense, assess, and react to plant, animal, and human status and stress.

By combining engineering biology with materials, sensing, and other platforms, these tools can be used to improve and enhance human and system performance. Complex platforms combining multi-scale biologics with materials and machines can enhance the way these systems operate and enable dynamic response, novel signal integration, and modulation of physiology and environment. The roadmap describes milestone advancements in cell circuits and networks, patterning and assembly of consortia, and designing and modeling of interfaces in support of Enabling Capabilities for production of living materials, functionalized interfaces, and bioelectronics. Engineering biology can facilitate production of advanced biopolymers, materials that self-repair or reconfigure to environmental cues, and sensing systems that take advantage of the massively paralleled data processing power of microbes. The resulting systems can play diverse roles in relevant applications, including filtration and decontamination, weathering and damage through self-healing and anti-corrosion, provide obscuration and camouflage, and amplify remote or minute information and signals.

Achieving the milestones and capabilities detailed in this roadmap will: 1) allow the utilization of diverse resources (including waste streams) and production of specialty chemicals and materials for protection, coatings, fuels, infrastructure and agile basing, and to sustain and enhance human performance, among other products that are needed, in an available and affordable manner; 2) better enable the ability to discriminate, identify, track, and target a wide variety of friends and foe in cluttered, multi-dimensional, multi-domain battlespace; and 3) allow the human and relevant systems to operate continuously and intelligently under adverse conditions across time and space.

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