EBRC Engineering Biology Research Consortium

Engineering Biology & Materials Science

A Research Roadmap for Interdisciplinary Innovation

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5885 Hollis Street, 4th Floor, Emeryville, CA 94608
Phone: +1.510.871.3272 Fax: +1.510.245.2223

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Roadmap Contributors

(affiliations at time of contribution)

Production of this roadmap was led by Emily R. Aurand

Bryn Adams (DEVCOM Army Research Lab)
Jamel Ali (FAMU-FSU College of Engineering)
Jennifer Andrew (University of Florida)
Paul Ashby (Lawrence Berkeley National Lab)
Rana Ashkar (Virginia Tech)
Mariah Austin (University of Texas at Austin)
Michael Blaisse (Zymergen)
James Carothers (University of Washington)
Sifang Chen (University of Washington)
Amy Congdon (Biofabricate)
Tara Deans (University of Utah)
Jared DeCoste (Army Research Lab)
Domitilla Del Vecchio (Massachusetts Institute of Technology)
Melik Demirel (Pennsylvania State University)
Steve Evans (Re-Knowvate LLC)
Mike Fero (TeselaGen Biotech)
M.G. Finn (Georgia Institute of Technology)
Elisa Franco (University of California, Los Angeles)
David Gay (University of California, Santa Barbara)
Wendy Goodson (Air Force Research Laboratory)
Samuel Gowland (Northwestern University)
Jingjiao Guan (FAMU-FSU College of Engineering)
Maneesh Gupta (Air Force Research Laboratory)
Michael Hammerling (Northwestern University)
Germano Iannacchione (National Science Foundation; Worcester Polytechnic Institute)
Farren Isaacs (Yale University)
Justin Jahnke (DEVCOM Army Research Laboratory)
Michael Jewett (Northwestern University)
Alex Juminaga (LanzaTech)
Lauren Junker (BASF)
Neha Kamat (Northwestern University)
Jay Keasling (University of California, Berkeley)
Nancy Kelley-Loughnane (Air Force Research Lab)
Michael Köpke (LanzaTech)
Aditya Kunjapur (University of Delaware)
Suzanne Lee (Biofabricate)
Nancy Lin (National Institute of Standards and Technology)
Allen Liu (University of Michigan)
Shadi Mamaghani (AAAS S&T Policy Fellow, National Science Foundation)

Andrea Markelz (SUNY Buffalo)
Joshua A. Orlicki (DEVCOM Army Research Laboratory)
Javin Oza (California Polytechnic State University)
Merja Penttilä (VTT)
Edward Perkins (US Army Engineer Research and Development Center)
Brian Pfleger (University of Wisconsin-Madison)
David Ross (National Institute of Standards & Technology)
Lynn Rothschild (NASA Ames Research Center)
Rebecca Schulman (Johns Hopkins University)
Seunghyun Sim (University of California, Irvine)
Emilie Siochi (NASA Langley Research Center)
Michael Smanski (University of Minnesota)
Victoria Soghomonian (Virginia Tech)
Kevin Solomon (Purdue University; current affiliation University of Delaware)
Deepti Tanjore (Lawrence Berkeley National Laboratory)
Danielle Tullman-Ercek (Northwestern University)
Jordan Villa (University of Texas at Austin)
Fuzhong Zhang (Washington University in St. Louis)

EBRC
Emily Aurand, Director of Roadmapping and Education
Eric Lee, Postdoctoral Fellow
Becky Mackelprang, Postdoctoral Fellow
Angela Lee, Administrator
Douglas Friedman, Executive Director

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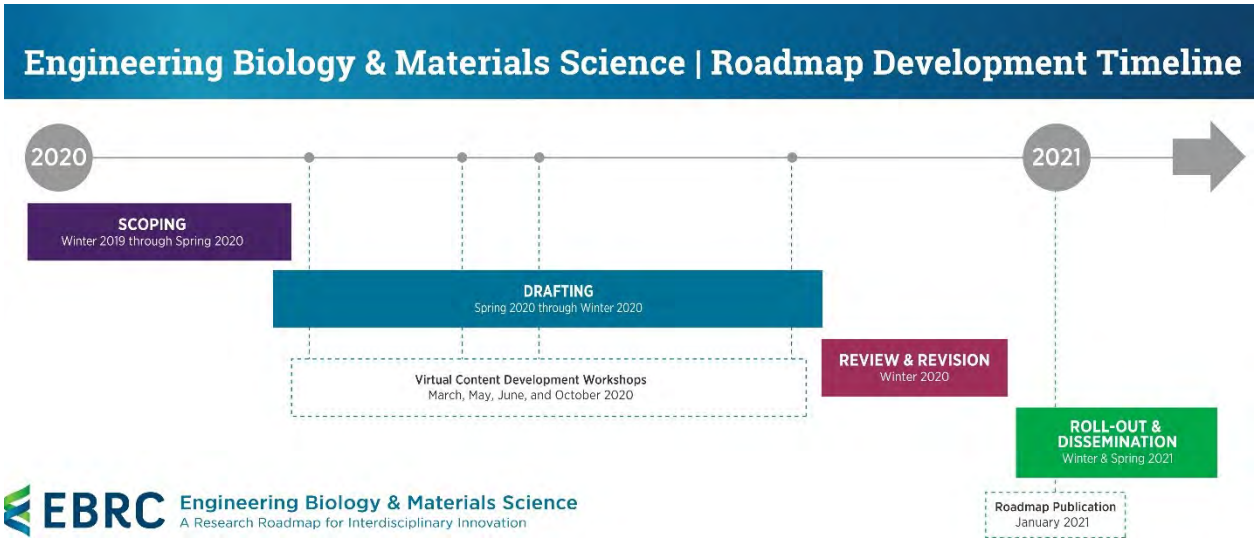
Overview

Topic Selection and Roadmap Development

EBRC's roadmapping is an evergreen activity. Following the release of our 2019 technical research roadmap *Engineering Biology*[1], the EBRC community expressed interest and enthusiasm toward focusing our efforts in 2020 on roadmapping select topics, highlighting two research spaces - microbiomes and materials - where intersection with advancements in synthetic/engineering biology have the potential to transform the tools, applications, and products used and generated over the next 20 years. The selection of the materials topic proved to be incredibly timely, following the convening of the Division of Materials Research's three Square-Table workshops. These Square-Table workshops brought together experts in the synthetic biology and materials science fields to discuss trends and innovations and identify challenges and bottlenecks toward novel materials and material properties intertwined with synthetic biology. These workshops generated enthusiasm in the future of materials from synthetic/engineering biology and primed participants to contribute to EBRC's roadmap. To develop this roadmap, we engaged more than 60 past Square-Table participants, EBRC members, students and postdocs, and other experts from the materials science and engineering biology communities, with the aim of producing a technical roadmap that serves both the synthetic biology and materials science fields. These individuals dedicated significant time and effort toward this roadmap's production, and we are grateful for their efforts.

The entirety of this roadmap was produced during the global SARS-CoV-2/COVID-19 pandemic, forcing our community of collaborators to adjust to working remotely, with all the distractions and stresses that our new lives afforded. In a departure from EBRC's established roadmapping process – which is primarily conducted through intensive, in-person writing workshops – this roadmap is the product of eight virtual, videoconference workshops and many individual working hours, and adaptation of our previous processes of review and revision. However, the resulting roadmap is still anticipated to provide the research community and stakeholders in both materials science and engineering biology fields with an inspirational pathway towards interdisciplinary innovation.



EBRC's roadmapping is an iterative process of brainstorming, discussion, drafting, review, and revision. Roadmap contributors participate in workshops and collaborative writing sessions, building on the work of their colleagues and bringing new ideas and approaches to each strategy laid out in the roadmap's milestones and technical achievements. The roadmap incorporates elements of EBRC's other roadmaps (accessible at https://roadmap.ebrc.org), while accommodating different topics and the nuances and novelties of the intersection of materials science and engineering biology. EBRC's roadmaps are intended to serve as a resource for the research community, to inspire innovation and build a collective strategy towards advancing science and engineering, and for policymakers, funders, and other stakeholders interested in understanding the opportunities and potential of this research.

Roadmap Components

Technical Themes - The roadmap consists of four technical themes that encompass the tools and technologies that are envisioned to enable materials from engineering biology. The technical themes are: Synthesis, Composition & Structure, Processing, and Properties & Performance. The theme structure encourages easy navigation through different topics of the roadmap, clustering similar scientific and engineering ideas. Each technical theme consists of the *Roadmap Elements* (further described below).

The following are descriptions of each of the technical themes within the context of this roadmap:

Synthesis - The generation of material components via engineered biology; primary production or creation of material components. Includes utilizing or exploiting engineered biology to produce monomers, polymers, biomolecules, and macromolecules that serve as components of a material (bulk or otherwise).

Composition & Structure - The design or control over the components of a material through engineering - either via biological activity or through engineering of the biological component - and the two- and three-dimensional space these components occupy. This includes engineering of the interactions within a material, such as the biotic-abiotic interface and embedding of biomolecules, enzymes, and cells. Also included is the engineering of the physical and bulk characteristics of a material, such as biomolecule (*e.g.,* protein) structure and three-dimensional architecture of a material.

Processing - The engineering of biology to conduct "unit operations" to build or destroy materials through polymerization and degradation, templating, patterning and printing. This includes engineering the biological extrusion or secretion of materials, material deposition, and self-assembly and -disassembly. Processing also includes engineering biology-based technologies, tools and systems (*e.g.,* cell-free systems) to manufacture, recover, and purify materials. Includes engineering biological materials to function in non-natural environments and extreme conditions.

Properties & Performance - The engineering of dynamic characteristics and activities of materials, including sensing and response, communication and computation, and self-

repair through the incorporation or activation of biocomponents. This includes the engineering of materials to provide signals and store and release energy or information through an engineered biological component and the engineering of dynamic interactions between the biological and abiotic components of a material. Properties & Performance also considers challenges in tools, methods, and technologies for characterizing dynamic activity and performance of living materials and materials that incorporate biocomponents.

Roadmap Elements - The roadmap elements consist of a cascading hierarchy of breakthrough capabilities, milestones, bottlenecks, and potential solutions. Each technical theme has a number of breakthrough capabilities, visionary, 20+ year aspirations which represent the long-term objectives in each thematic space. Milestones at 2, 5, 10, and 20 years (2022, 2025, 2030, and 2040, respectively*) chart the path toward achieving the breakthrough capability and each milestone is elaborated by anticipated or imagined bottlenecks and creative potential solutions. The 2-year and 5-year milestones are intended to signify objectives that can be reached with current or recently implemented funding programs, as well as existing infrastructure and facilities resources. The 10-year and 20-year milestones are expected to be more ambitious achievements that may require (and thus, result in) significant technical advancements and/or increased funding and resources and new and improved infrastructure. (*Though released in January 2021, this roadmap was written almost exclusively during 2020, thus we have chosen to retain these earlier timepoints.)

Included in the roadmap are breakthrough capabilities from EBRC's 2019 roadmap *Engineering Biology: A Research Roadmap for the Next-Generation Bioeconomy.*[1] While written in the context of advancing the field of engineering biology, the EBRC Materials Roadmapping Working Group leading this roadmapping project felt that the technical achievements elaborated in these breakthrough capabilities and their milestones directly contribute to achieving advancements in materials from engineering biology. This content has been incorporated as reference and, when pertinent, will be provided with context for its inclusion in this roadmap.

Application Sectors - The roadmap consists of five application sectors to illustrate the potential applications at the intersection of materials and engineering biology: 1) Industrial Biotechnology; 2) Health & Medicine; 3) Food & Agriculture; 4) Environmental Biotechnology; and 5) Energy. These application sectors and the associated societal challenges are captured from *Engineering Biology*[1] and represent a broad consideration of significant economic and social roadblocks towards advancing the way we live and thrive. Within each application sector we highlight a number of exemplar applications of materials from engineering biology that will help us overcome these pervasive societal challenges, such as enabling and establishing a cleaner environment, supporting the health and well-being of growing populations, and accelerating innovation and economic viability of industry. We further identify potential discrete technical achievements necessary to obtain those exemplar applications. These exemplar applications and technical achievements reflect and tie together the advancements envisioned in the roadmap's technical themes.

The Application Sector content is built from the top-down: considering recognized societal challenges, roadmap contributors consider the myriad options and creative opportunities for materials from engineering biology to contribute to solutions to overcome those challenges, and then the technical advancements that help to enable those solutions.

Glossary - The convergence of the fields of materials science and engineering biology is a nascent space. As such, researchers in these fields are just beginning to develop intersecting technologies and collaborative concepts. As the tools and technologies combine, there is a need to develop more common language between the fields. To that end, we have developed a glossary for the key terms and concepts in this roadmap. The glossary is specific to the context of this roadmap, but was developed with input from the community in both fields.

The final production release of this roadmap includes an interactive website, hosted within the roadmap.ebrc.org domain, a printable PDF version, and associated figures and graphics. Feedback or questions about this roadmap or EBRC's other roadmaps and roadmapping activities can be directed to roadmapping@ebrc.org.

Introduction

History of Materials Science and Engineering Biology

Materials science emerged as a coherent discipline around in the 1960s, when academic departments in metallurgy and ceramics expanded their focus beyond the materials important in the 19th and early 20th century. In the United States, the Advanced Research Projects Agency (ARPA) played a crucial role in the growth of materials science (initially referred to as materials sciences, reflecting the highly interdisciplinary nature of its origin), by funding a series of university-based laboratories in the 1960s, some of which are still highly active today. Their mission was to “expand the national program of basic research and training in the materials sciences."[2] Although fundamentally synonymous with condensed matter physics, the distinguishing feature of materials science is the understanding of material properties for their engineering. The field now encompasses every class of materials from ceramics, polymers, semiconductors, magnets, biomaterials, soft-matter, nanomaterials, and composites. In recent years, materials science has become even more interdisciplinary, bringing together physics, chemistry, mathematics, biology, and engineering. Advancements in research have driven the development of new experimental tools as well as demonstrated the growing need for computational modeling and machine learning to discover and understand new phenomena of condensed matter, find and develop new materials, predict their properties, and benefit the world around us. This is encapsulated in the mission of the Materials Genome Initiative,[3] which is a multi-agency initiative for the coordination of policy, resources, and infrastructure supporting U.S. institutions for the discovery, manufacture, and deployment of advanced materials.

The field of engineering biology (also referred to as synthetic biology) has revolutionized the way that we study and apply living systems. Engineering biology leverages chemistry, engineering, and computer science to design and construct new biomolecules and cells and transform existing biological systems. Engineering biology is built off four guiding principles: 1) We have advanced the tools and technologies to build functional biological systems from their basic parts, allowing us to model and test systems, learning from observations; 2) By manipulating systems at the molecular level, we can create better, or entirely novel, biological components and systems; 3) By designing and building biological systems, rather than only observing natural evolution, we can make them easier to study and interact with; and 4) Biology can be used as a technology to process information, produce energy, manufacture chemicals, and fabricate materials.

As a field, engineering biology has only existed for roughly 20 years,[4] and is still considered an emerging discipline. However, strong foundational advances have quickly moved the field into a place where we can consider the influence engineering biology might have on other fields of science and engineering, and on applications to serve society. The potential for interfacing living and engineered biomolecules and cells with fabrics, electronics, and plastics can generate myriad new products, imbuing advanced capabilities, extending signaling and communication ranges, and solving industrial, health, agricultural, and environmental challenges. Breakthroughs in genetic and metabolic circuit engineering have helped us control cellular growth and function, define production from cells and biosystems, and manipulate or

dictate how cells communicate. Incorporation of novel and non-native chemistries, amino acids, and biomolecules has greatly expanded the possibilities of what biology can hold and harness.

Engineering and producing materials from biology requires capitalizing on our ever-expanding abilities to engineer bacteria, fungi, and other cellular and cell-free systems to generate novel materials. Innovative companies such as Bolt Threads, Spiber, Ecovative Design, and bioMASON are delving into this space and demonstrate engineering biology's capacity to mimic or replace known materials. Yet, most of this space is nascent and there is enormous potential to produce yet-unimagined materials from biology.

The Intersection of Biology and Materials

The engineering of biology can extend to the engineering of materials and devices that are made by and incorporate living matter. Biology already produces materials that we use and consume every day, such as wood and cotton. Biology also produces materials that we have yet to fully harness but hold incredible potential, such as spider silk, mycelia, and silica. Beyond this, engineering biology can enable wholly novel materials by incorporating new and non-natural chemistries, conformations, and functions. By leveraging engineering biology tools and techniques, materials science capabilities, and other technologies, we have the potential to generate or reproduce natural and novel materials under controlled conditions and study them in the laboratory, and the potential to scale and apply these materials to solve persistent challenges. Biomaterials are inherently adaptable and subject to evolution-selection and via engineered biology, can be endowed with new chemistries, tunable properties, and be armed with the ability to sense and self-repair.

To exploit these material opportunities, we need to better understand their composition, how they are generated and destroyed, and how their properties - strength, elasticity, conductivity, among others - evolve and change over the life of the material or biological component. Interfacing cells and biomolecules to abiotic materials also requires a better understanding of and ability to manipulate surface structures and dynamics, interactions and attachments, and communication pathways.

Current research in engineering biology can directly contribute to the design and production of materials. Biopolymer synthesis, protein engineering, and incorporation of non-native or unnatural nucleotides and amino acids can enable a wider range of biomolecule-based materials.[5] Engineering circuits, pathways, and cell-free systems can expand the types and structures of materials produced and the environments in which production can occur.[6] The incorporation of cells and biomolecules can expand the functions of existing materials by providing new avenues for signaling and response, material generation and repair, and data processing and storage.[7] The traditional definition of biomaterials focuses on materials for medical applications; however, a broader definition of biomaterials is emerging as materials derived or inspired from biology or materials developed for biological applications. This broader definition reflects the importance of biomaterials beyond traditional medical applications to include such venues as new structural materials (*e.g.,* bioconcrete and biopolymers), new coatings and surface treatments, new fuels and lubricants (biologically sourced and renewed), new sensors and diagnostics, new materials for the food/water/energy nexus, and even new information storage and processing (biocomputers). For example, the new area of *active matter* has largely been spearheaded by exploring new biopolymer or bio-inspired liquid crystalline

systems far-from-equilibrium. This area is at the forefront of both fundamental physics and biology. Another example is the development of new extracellular matrices (ECMs) that have only recently allowed for the inclusion of living cells in what would have been traditionally considered a bulk material.

Challenges for Innovation and Advancement

Despite this potential, we must build on our understanding of the properties of biology when used as or incorporated into materials. We must bring about advancements in the characterization of biomolecules, cells, and biosystems that correlate or align to measurements and traits of abiotic materials like metals and plastics. The dynamic nature of biology contributes directly to out-of-equilibrium behaviors and conditions that require new tools and techniques to quantify and control. Processing and scaling of biology for materials applications is also a significant challenge, particularly as properties of biosystems change as they scale. And as we build and strengthen the bioeconomy, sustainability moves to the forefront, and we must consider how engineered biology can enable materials that are renewable and recyclable, designing and controlling biodegradation.

The challenges of integrating biology with materials science stems from incorporation of the fundamental unit of biology, the cell. Bio-inspiration or bio-mimicry of materials design has a long history and as techniques and capabilities expand, new and ever more exciting developments of novel materials will surely emerge. However, the incorporation of cells into a material matrix blurs the line between materials and devices by adding functionality and programmability to materials. Biocompatibility, transduction of signals, and control of biological systems when interfaced with materials are all crucial elements that are poorly understood, even for natural systems. This is made even more challenging because of the dynamic and active nature of incorporating biomolecules and cells in any application. This results in a highly multi-dimensional and time-dependent data space. There currently does not exist a fundamental understanding of emergent order far-from-equilibrium to guide this exploration. This current state of affairs, where we have developed tools and materials to see the possible intersection of materials and biology but not the pathway to do so, has emphasized the need for development of new theory and computational tools, such as machine learning.

Roadmap to Interdisciplinary Innovation

Through explicit, long-term breakthrough capabilities for scientific and technological achievement, discrete milestones over 20 years, and associated bottleneck challenges and select potential solutions to those bottlenecks, this roadmap aims to describe the potential for innovation and advancements at the intersection of engineering biology and materials science. Further, the roadmap envisions creative and ambitious material solutions to persistent societal challenges that leverage the opportunities and advantages of harnessing and integrating engineered biology. The roadmap provides a high-level path for research and development (and inherently, for funding, investment, and infrastructure) to enable a future of advanced materials.

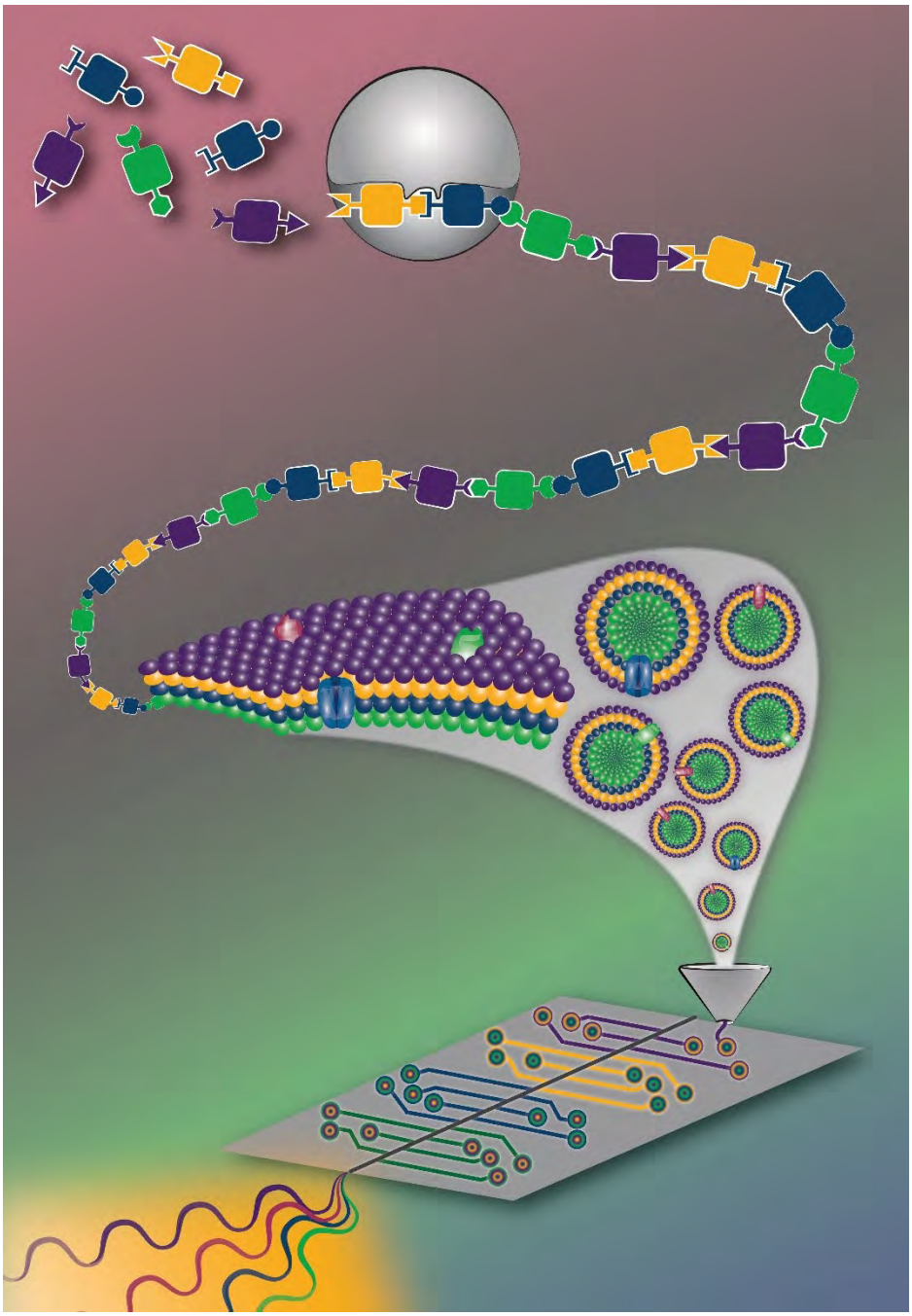


Figure 1. Technical advancements to accelerate innovation at the intersection of engineering biology and materials science. Demonstrating the continuity between each of the roadmap's technical themes and how they build upon one another, we begin with material synthesis of monomers, natural or synthetic, through sequence-defined polymerization of components. Control over composition and structure enables a higher order structure and overall architecture of the interface that is programmable. With *de novo* prediction of membrane dynamics, components can be precisely distributed throughout the membrane to direct interactions and containment of cells and biomolecules, or other abiotic components. Processing enables further control and specification of materials through deposition or printing, here onto a biosensing platform. Such programmable patterning is a key feature that enables multiplexed sensing in complex biological environments, demonstrating how further advancements in engineering biology and materials can contribute to novel dynamic activity and performance.

Technical Themes

The roadmap consists of four technical themes that encompass the tools and technologies that are envisioned to enable materials from engineering biology. Construction of the technical roadmap is accomplished through delineation of milestones at 2, 5, 10, and 20 years and each milestone is elaborated by anticipated or imagined bottlenecks and creative potential solutions. The 2-year and 5-year milestones are intended to signify objectives that can be reached with current or recently implemented funding programs, as well as existing infrastructure and facilities resources. The 10-year and 20-year milestones are expected to be more ambitious achievements that may require (and thus, result in) significant technical advancements and/or increased funding and resources and new and improved infrastructure. The breakthrough capabilities therefore represent the visionary 20+ year aspirations for the technical themes.

Synthesis

We consider *Synthesis* as the primary production or creation of material components. In this roadmap, the focus is on the generation of material components via engineered biology. This includes utilizing or exploiting engineered biology to produce monomers, polymers, biomolecules, and macromolecules that serve as components of a material (bulk or otherwise). Key challenges in Synthesis include high-yield production of protein-based natural materials, such as spider silk and elastin, from engineered biology; alternative biological production systems and utilization of natural and non-natural nucleic and amino acids; and enabling resynthesis or recycling of materials to enable greater sustainability.

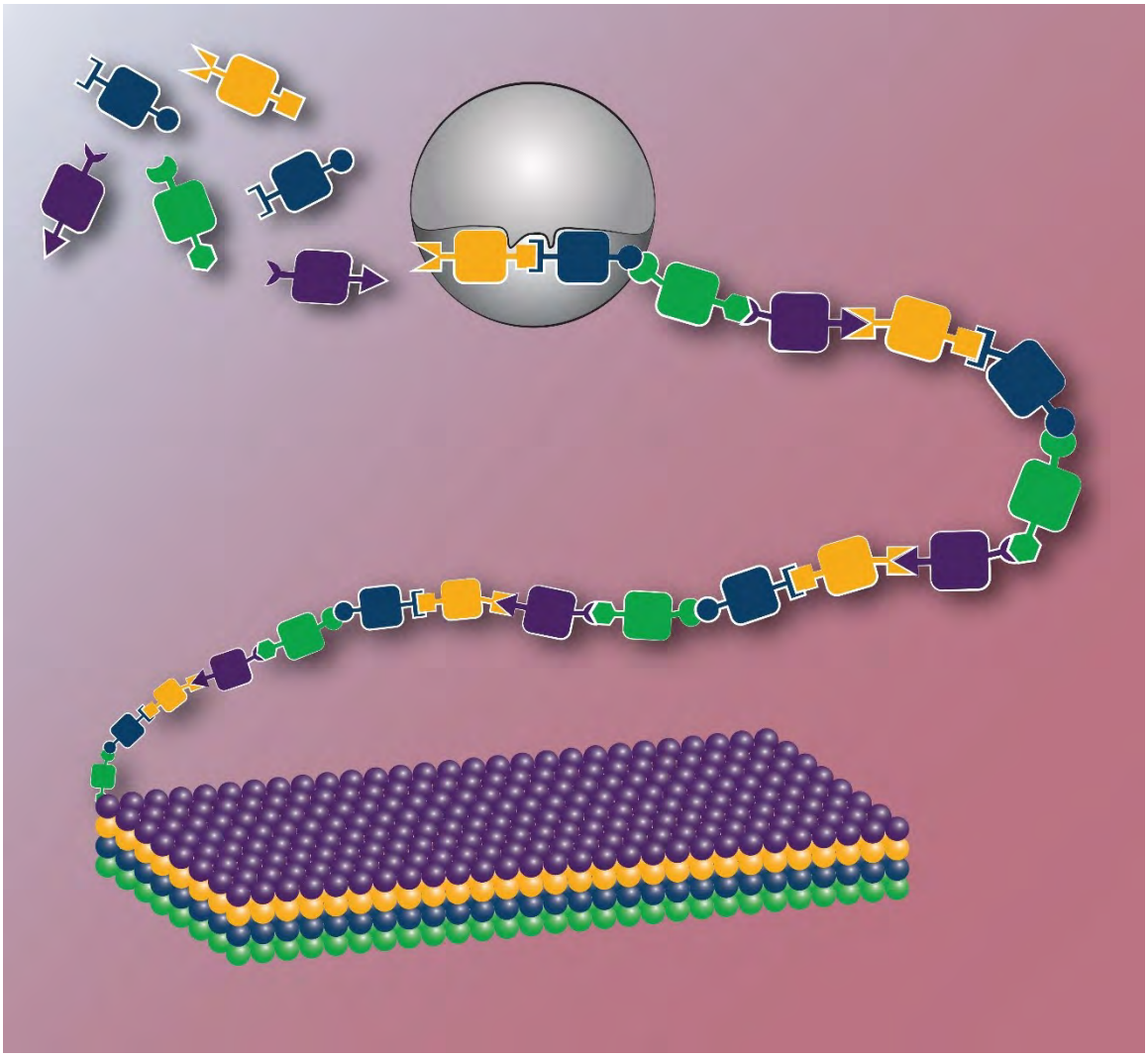


Figure 2. Engineering biology has the capability to transform the materials generated through biosynthesis and the catalysts which facilitate their formation. In this depiction, novel monomers with orthogonal reactivity enable sequence-defined polymerization and the precise control of monomer order provides programmed higher order structure. Equally important, this synthesis is carried out by robustly engineered enzymes able to recognize non-natural monomers.

Breakthrough Capability: Biological synthesis and/or polymerization of non-natural and/or abiotic chemical monomers, excluding amino acids.[8, 9, 10]

- **2022 milestone: Identify and engineer enzymes to recognize and polymerize conventional chemical monomers (*e.g.,* acrylamide).**
    - [Bottleneck]: Limited library of employable enzymes that can be used to produce polymers in a variety of environments.
        - [Potential Solution]: Identify the target, based on protein structure and known biological roles, and develop screening strategy for directed-evolution.
- **2022 Milestone: Develop metabolic pathways to yield chiral cyclic monomers amenable to ring-opening polymerization.**
    - [Bottleneck]: Compatibility of aromatic, cyclic monomers with cell interior may require cell-free synthetic approaches to be developed.
        - [Potential Solution]: Engineering of synthetic lipid bilayer systems for synthesis processes.
    - [Bottleneck]: Prediction of materials properties lags behind protein folding, inorganic material property forecasting.
        - [Potential Solution]: Requires continued investment in computational models of soft materials, meso-scale tool development for capturing impacts of processing conditions and kinetically trapped structures.
- **2022 Milestone: Demonstrate side chain modification of polymeric scaffolds by biological reactions catalyzed by living cells.**
    - [Bottleneck]: Dissolving conventional polymers in a condition where an enzyme can operate (*i.e.,* temperature, oxygen, buffer, cofactors like ATP); in order for this to work, the polymer chains should dissolve well in a medium (ideally water) so that enzymes can approach.
        - [Potential Solution]: *De novo* engineering of an enzyme that is functional in multiple environments, such as design of an active site that doesn't require extensive folding structure in water.
        - [Potential Solution]: "Harden" enzymes to maintain activity in non-aqueous environments.[11]
    - [Bottleneck]: Development of strain chassis that can access and modify polymeric scaffolds under survivable conditions.
        - [Potential Solution]: Engineering of extracellular matrix and enzyme secretion pathways to enable modification of polymeric scaffolds outside of cells.
- **2025 Milestone: Enable vinyl monomer production by engineering metabolic pathways for *in situ* polymerization reactions.**
    - [Bottleneck]: High-yield production of vinyl monomer by enzymatic cascade has yet to be achieved.
    - [Potential Solution]: Targeted directed evolution of enzymes.
- **2025 Milestone: Expand the library of chemical monomers polymerizable by evolved enzymes.**

    - [Bottleneck]: Testing enzyme variants *in vivo* is slow due to transformation and cell growth requirements.
        - [Potential Solution]: Cell-free prototyping of enzyme variants allows much higher-throughput exploration of protein space for identification of variants with favorable functionalities.
- **2025 Milestone: Engineer complex orthogonal translation systems, including engineered orthogonal ribosomes, for site-directed incorporation of novel (non-amino acid) chemistries into polymers.[12]**
    - [Bottleneck]: Ribosomes do not natively polymerize most non-L-α-amino-acid substrates.
        - [Potential Solution]: Use RNA modeling/design to remodel the peptidyl transferase center (PTC) of the ribosome to accommodate new amino acids.[13]
        - [Potential Solution]: Evolve the ribosome toward incorporation of new chemistries in a cell-free environment.[14]
    - [Bottleneck]: Requires construction of well-coordinated biological systems in cells to function effectively.
        - [Potential Solution]: Development of genome-engineering tools that improve the throughput of designing, building, and testing effective orthogonal translation systems.
        - [Potential Solution]: Use and develop new genomic engineering and molecular evolution strategies to permit continuous diversification of biomolecular systems and multi-gene pathways for systems engineering and optimization.
- **2030 Milestone: Enable complex polymer production (*e.g.,* co-polymers, sequence-controlled polymers, brushed polymers) with enzymatic systems.**
    - [Bottleneck]: Monomer and polymer solubility limit potential for bio-catalysis, especially in aqueous systems.
        - [Potential Solution]: Explore cell-free or bi-phasic conditions to drive reactions in organic solvents or at the water-solvent interface.
    - [Bottleneck]: Inability to produce sequence-controlled polymers with both natural and synthetic chemistries.
        - [Potential Solution]: Engineer biological systems that can polymerize materials with biological and abiotic monomers, such as organisms with open coding channels, engineered translation systems that can encode diverse monomers, engineered ribosomes that can catalyze new bond formation and polymerization, and synthetic organelles that can serve as dedicated sites for polymerization of synthetic biomaterials isolated from the cell and prevent cytotoxicity.

Breakthrough Capability: Hybrid chemical and biological synthesis methods.[15, 16]

- **2025 Milestone: Characterize and improve the enzymology of orthogonal translation systems (OTSs) for site-directed incorporation of novel chemistries into polymers.**

    - [Bottleneck]: A variety of OTSs exist for the incorporation of over 100 non-canonical chemistries into proteins; however, their utility for applications remains unclear.
        - [Potential Solution]: Perform thorough characterization of OTS expression, solubility, enzymology, for K_m, K_{cat}, and orthogonality.
        - [Potential Solution]: Engineer enhanced orthogonality, specificity, and efficiency into OTS to permit multi-site incorporation of two or more synthetic chemistries into the same polymer.
- **2030 Milestone: Enable hybrid flow chemistry-biocatalytic synthesis systems.**
    - [Bottleneck]: Limited library of catalytic enzymes that would be stable at high temperatures and/or in the absence of water.
        - [Potential Solution]: Design thermophilic catalytic enzymes for use in flow reactors.
        - [Potential Solution]: Explore alternative solvents as stabilization agents (*e.g.,* ionic liquids).
- **2030 Milestone: Full integration of heterogeneous chemical and biological catalysis in modular platforms that are stable after long-term storage and facilitate product switching.**
    - [Bottleneck]: Long-term storage of biological entities typically requires a cold chain for stability, thus more robust systems that realize long-term storage in ambient temperature and humidity range is needed.
        - [Potential Solution]: Innovations in genetic engineering of cell-based and cell-free transcription/translation, consortia and hybrid biological platforms to enable manufacturing flexibility.
- **2040 Milestone: Enable retrobiosynthesis of materials with desired properties, such as thermal conductivity or elasticity.[5, 17]**
    - [Bottleneck]: Mapping of traditional materials properties to biomolecules and self-assembled/hybrid systems.
        - [Potential Solution]: Databases with materials properties for characterized systems.
    - [Bottleneck]: Paucity of data about emergent properties of materials from biocomponents.
        - [Potential Solution]: Computational prediction of properties in assembling and assembled systems.
    - [Bottleneck]: Isolation of materials from complex cellular systems.
        - [Potential Solution]: Cell-free systems that enable unique protein tagging and purification.

Included below are select breakthrough capabilities from our 2019 roadmap, *Engineering Biology*[1] (Milestones at 2021, 2024, 2029, and 2039). While these breakthrough capabilities were written in the context of advancing the field of engineering biology, the technical achievements elaborated in these breakthrough capabilities and their milestones are likely to directly contribute to achieving advancements in materials from engineering biology. This

content has been incorporated as reference and, when pertinent, is provided with context for its inclusion in this roadmap (as Notes).

Engineering Biology breakthrough capability: PCR, reverse transcription, cellular replication, and transcription of fully unnatural nucleotide-containing genes of up to 400 base pairs.

- **2021: Identification of "missing" functionality or functionalities in ATGC base pairs.**
- **2024: Improved *in vitro* manipulation of unnatural nucleic acids.**
- **2024: Expansion of unnatural nucleotide toolkit.**
- **2029: Biosynthesis of unnatural nucleotides.**
- **2039: Organisms capable of full replication, maintenance, and transcription of a plasmid or artificial chromosome made up entirely of unnatural bases.**

Engineering Biology breakthrough capability: Expanded genetic code systems for translation of >100-amino acid proteins containing fully-unnatural amino acids, and proteins with at least four, distinct unnatural amino acid building blocks.[8, 9]

Note: Implementing unnatural amino acids for materials synthesis will require advancements in translation system engineering, to enable a broader production range from ribosomes. Furthermore, biosynthesis of sequence-defined synthetic biopolymers in which new chemistries (synthetic amino acids or synthetic monomers made-up of non-natural backbones) can be encoded in a template-directed manner, is likely to require the engineering or repurposing of organisms with multiple open coding channels (recoded genomes).

- **2021: Create proteins that are capable of gaining new, therapeutically-useful activities through unnatural amino acids.**
- **2024: Efficient biosynthesis of proteins containing three or more distinct unnatural amino acid building blocks.**
- **2029: Biosynthesis of unnatural amino acids.**
- **2039: Templated biosynthesis and evolution of new polymers with large user-selected sets of unnatural building blocks *in vivo*.**

Engineering Biology breakthrough capability: Ability to rationally engineer sensor suites, genetic circuits, metabolic pathways, signaling cascades, and cell differentiation pathways.

Note: the engineering of circuits and pathways will be necessary for the synthesis of material components from engineered biology. The engineering of sensor suites and signaling cascades will also be important for the dynamic behaviors of materials, particularly living materials and composite materials that incorporate cells. More on engineering dynamic activities of materials can be found in the **Properties & Performance** Technical Theme.

- **2021: Reliable engineering of genetic circuits with more than 10 regulators for sophisticated computations.**
- **2024: Reliable engineering of novel, many-enzyme pathways utilizing combinations of bio-prospected enzymes with well-characterized kinetics.**
- **2024: Five-time improvement and expansion of inducers/promoters for model organisms that respond to environmental inputs and any intracellular metabolite.**

- **2024: Utilize machine-learning approaches to use the vast amount of un-curated literature results within pathway design.**
- **2029: Creation of optogenetic tools for *in vivo* RNA post-transcriptional control to allow for easy control of any gene expression process through mRNA.**
- **2029: Reliable expression of redesigned synthases to produce secondary metabolites (*e.g.,* polyketides, non-ribosomal peptides)**
- **2029: Computational design of protein-ligand and RNA-ligand interfaces suitable for engineering protein-based or RNA-based sensors.**
- **2039: Simultaneous, tunable, timed expression of many transcription factors controlling mammalian cell state.**

Engineering Biology breakthrough capability: Ability to build and control small molecule and monomer biosynthesis inside cells by design or through evolution.

Note: in the context of synthesizing materials from engineering biology, design and evolution of cells to build and control the synthesis of monomers, in addition to small molecules, will be important.

- **2021: Identify model organisms for performing specific types of chemistries or organisms that have native precursor biosynthesis pathways for specific classes of molecules.**
- **2021: Precise temporal control of gene expression for well-studied systems.**
- **2024: Construct a limited number of model host organisms for synthesizing all-natural products.**
- **2024: Construction of single-cell organisms for production of unnatural derivatives of natural products.**
- **2024: Temporal control over multiplexed regulation of many genes in parallel.**
- **2029: Software and hardware for optimizing titer, rate, and yield of any product produced by any host.**
- **2039: On-demand construction of single cell organisms for production of nearly any molecule of interest, including organic chemicals and polymers.**

Engineering Biology breakthrough capability: Ability to manufacture any targeted glycosylated protein or metabolite using cell-free biosynthesis.

Note: contributors to this roadmap have indicated that the 2024 milestone “Production of bacterial glycoconjugate vaccines in cell-free systems” would gain significant relevance and value by including the production of bacterial glycoconjugate therapeutic antibodies in cell-free systems, in addition to vaccines.

- **2021: Ability to build modular, versatile cell-free platforms for glycosylation pathway assembly.**
- **2024: Expanded set of glycosylation enzyme-variants that efficiently install eukaryotic glycans.**
- **2024: Production of bacterial glycoconjugate vaccines in cell-free systems.**
- **2029: Expanded set of enzymes capable of glycosylating metabolites *in vitro*.**

- **2029: Cell-free pipelines to produce and assess the functionality of diverse, human glycosylated protein therapeutics.**
- **2039: Ability to produce any glycosylated protein therapeutics and vaccines at the point-of-care in less than one week.**

Engineering Biology breakthrough capability: Production and secretion of any protein with the desired glycosylation or post-translational modifications.

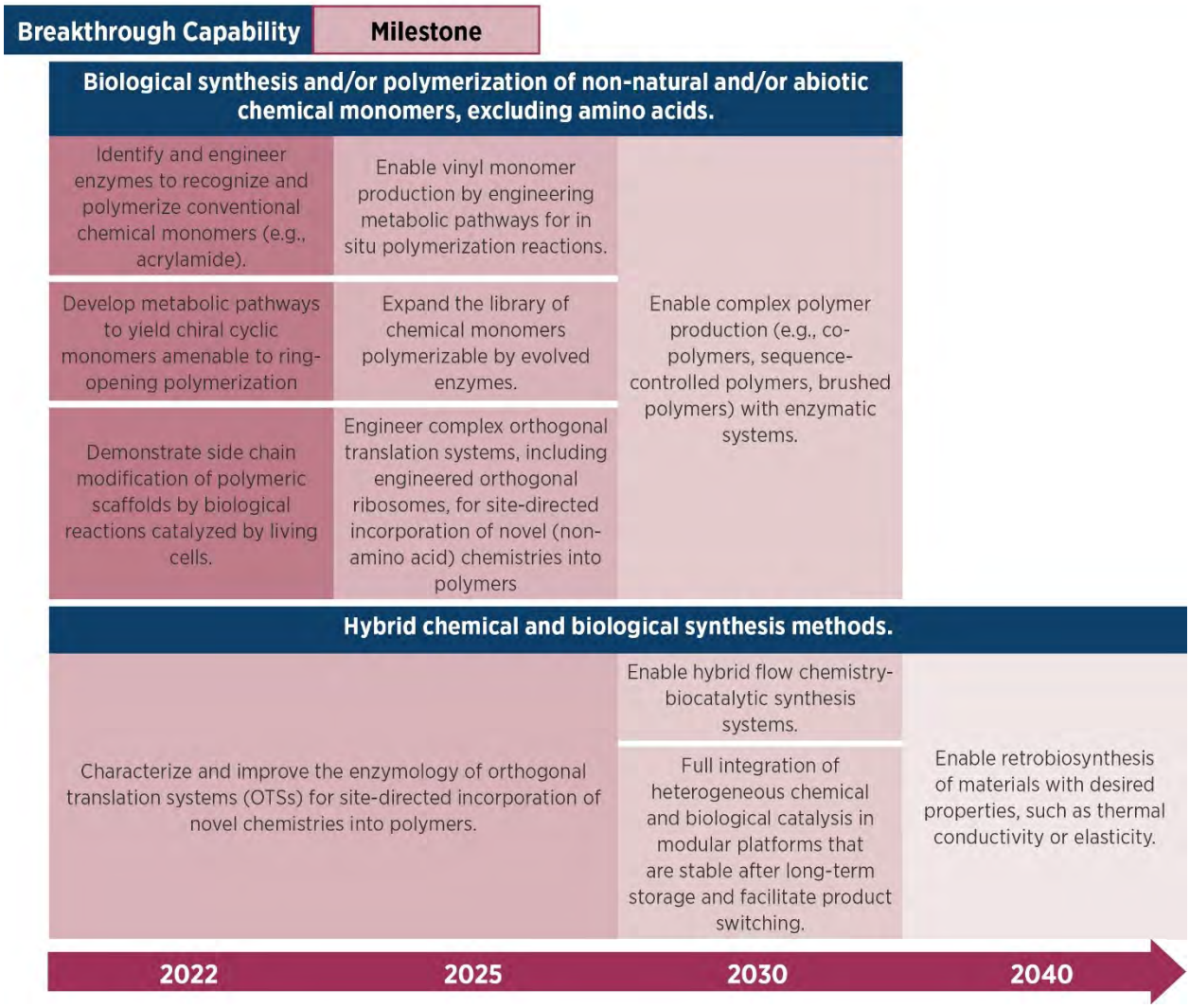
Note: in the context of synthesizing materials from engineering biology, this breakthrough capability can include the production and secretion of any small polypeptide, in addition to protein, with the desired modifications, and non-ribosomal protein production. Post-translational modifications that are likely to greatly contribute to protein and polypeptide synthesis for materials include phosphorylation, acetylation, and methylation, among other less-common modifications.

- **2021: One or more microbial hosts capable of producing laboratory-scale quantities of small polypeptides.**
- **2024: A few microbial hosts capable of secreting functional versions of proteins with no post-translational modifications.**
- **2039: Ubiquitous control of post-translational modification (including glycosylation of multiple sites with multiple sugars) in a diverse array of hosts.**

Engineering Biology breakthrough capability: Long-lasting, robust, and low-cost cell-free system for protein synthesis and biomanufacturing.

- **2021: Identify reagent instabilities in cell-free systems across multiple organisms and all biological kingdoms.**
- **2024: Alleviate reagent instabilities and prolong the half-life of cell-free reagents from a few hours to several days using inexpensive substrates.**
- **2024: Avoid inhibition (poisoning) of cell-free reactions by byproducts or the desired products.**
- **2029:Stabilize catalysts to facilitate cell-free reactions on the order of weeks.**
- **2039: Robust and scalable production of cell-free systems that last for weeks.**

ENGINEERING BIOLOGY & MATERIALS SCIENCE SYNTHESIS



Composition & Structure

The design or control over the components of a material through engineering – either via biological activity or through engineering of the biological component – and the two and three dimensional space these components occupy (four-dimensional dynamic activity is found in **Properties & Performance**). This includes engineering of the interactions within a material, such as the biotic-abiotic interface and embedding of biomolecules, enzymes, and cells. Also included is the engineering the physical and bulk characteristics of a material, such as biomolecule (*e.g.,* protein) structure and three-dimensional architecture of a material.

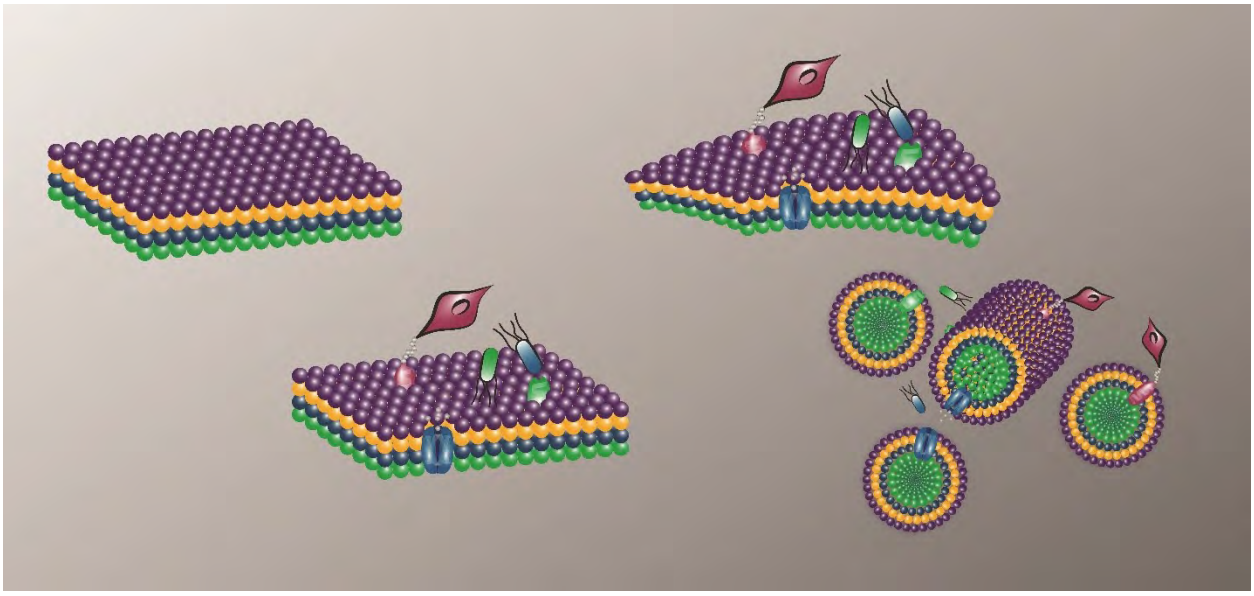


Figure 3. Prediction and control over the architecture and dynamics of systems combining abiotic and biotic materials is a multifaceted challenge. Rational design of synthetic membranes will enable control over the placement and function of membrane components, such as proteins, thus providing an environment for engineering the biotic-abiotic interface when cells and other biomolecules are incorporated. Furthermore, synthetic membranes can fold and reform into controlled architectures like tubes and micelles, as depicted.

Breakthrough Capability: Enable containment of biological materials.

- **2022 Milestone: Engineer libraries of polymeric scaffolds that enable confinement of living cells or cell-free systems within materials.**
    - [Bottleneck]: Molecular principles for polymeric scaffolds that enable confinement are lacking (*e.g.,* interaction with the cell surface, molecular cue to manipulate cellular behavior, minimum pore size that prevents cell division).
        - [Potential Solution]: Systematically vary chemical scaffolds and develop a rigorous analysis flow to interrogate cellular biological state within materials.
    - [Bottleneck]: Polymeric scaffolds that can be produced or maintained by incorporated cells based on the target and environmental conditions.
        - [Potential Solution]: Engineer non-canonical polymeric monomers or systems that can be produced or degraded by incorporated cells.

        - [Potential Solution]: Repurpose cellular metabolic pathways to produce and secrete monomers for polymeric scaffolds *in situ*.
- **2025 Milestone: Directed compartmentalization within cellular materials.**
    - [Bottleneck]: Limited set of well-characterized compartments in cells to use as models.
        - [Potential Solution]: Characterize compartment types in a wider set of bacteria, particularly non-model hosts.
        - [Potential Solution]: Engineer "universal" compartments that could be used in any organism.
- **2030 Milestone: Engineer cellular sensing capability (*e.g.,* edge detection) that mimics self-containment in natural systems.**
    - [Bottleneck]: Coupling environmental stimuli to growth-limiting responses.
        - [Potential Solution]: Engineer metabolic pathways that couple sensing of subtle environmental cues (*e.g.,* oxygen gradients or mechanical environment) to growth-limiting molecular switches, like lysis proteins.
- **2040 Milestone: Ability to grow or print multifunctional, multicomponent biological materials with specific substructures and functions.**
    - [Bottleneck]: Predictable control of stratification/layering and growth of substructures of functional cell types.
        - [Potential Solution]: Innovation in manipulating cell cycles combined with additive manufacturing effort.

Breakthrough Capability: Achieve the desired extracellular matrix (ECM) in a multicellular (hybrid, composite, or living) material.

- **2022 Milestone: Identify biological receptors, sensors, and actuators amenable for engineering at the cell-ECM interface.**
    - [Bottleneck]: Cellular input signals are still poorly defined (*e.g.,* cellular sensing of soft versus hard surfaces are not well understood).
        - [Potential Solution]: Identify and manipulate mechanosensitive pathways that govern rigidity sensing.
    - [Bottleneck]: Receptor engineering is still somewhat limited.
        - [Potential Solution]: Standardize the approach for engineering and testing of chimeric receptors and include adhesion receptors and more signaling receptors.
- **2025 Milestone: Repurpose dynamic cytoskeletal networks and non-native analogs as an ECM material with dynamic assembly/disassembly behavior to program cell phenotype.**
    - [Bottleneck]: Engineering multicomponent ECM with dynamic cellular cues.
        - [Potential Solution]: Engineer *in vitro* assembled cytoskeleton networks decorated with adhesive ligands to support cell spreading and migration.
- **2025 Milestone: Gain molecular understanding of the interaction of living cells with abiotic materials.**

    - [Bottleneck]: Understanding of behavior of biological components within materials and design principles for composite formulation with biological components are lacking.
        - [Potential Solution]: Catalog behaviors of various types or strains of living cells with various synthetic scaffolds.
- **2025 Milestone: Develop high-sensitivity methods to characterize and quantify ECM chemistry and structure *in situ* in order to confirm the desired ECM is achieved.**
    - [Bottleneck]: Quantitative *in situ* methods are lacking beyond fluorescence microscopy.
        - [Potential Solution]: Improve imaging technologies to encompass hyperspectral imaging, polarization imaging, and/or neutron scattering.
- **2030 Milestone: Integrate ECM and cells in a spatially organized manner in tissue-like constructs.**
    - [Bottleneck]: Incorporating different cell types, biomolecules, and additional components while retaining functionality within the same platform remains a challenge.
        - [Potential Solution]: Development of a versatile 3D bioprinting platform that templates cells in the desired ECM environment.
- **2040 Milestone: On-demand production of functional living, hybrid, and composite materials.**
    - [Bottleneck]: Currently, there are no design principles to guide the design of materials that accounts for the complexity of biology.

Breakthrough Capability: De novo design and/or prediction of membrane dynamics.

- **2022 Milestone: Develop computational methods to accurately predict physical properties of biological membranes and location of integrated transmembrane proteins as a function of membrane composition.**
    - [Bottleneck]: Computational studies of membrane protein location have, to date, not been confirmed experimentally in many cases.
        - [Potential Solution]: Validate membrane protein sorting and location as a function of protein identity, physical features, in both synthetic and cellular membranes.
- **2025 Milestone: Design of natural and non-natural membrane proteins that sort and localize into regions (*i.e.,* domains) of cellular and synthetic membranes.**
    - [Bottleneck]: Delivery of membrane proteins into synthetic membranes may require native protein machinery and also depends on composition of the membrane.
        - [Potential Solution]: Decipher which membrane proteins can be spontaneously integrated and functional in synthetic membranes and which require native chaperone systems.
        - [Potential Solution]: Catalog leader sequences of membrane proteins have high propensity to be incorporated into endoplasmic reticulum fragments for membrane protein synthesis.

        - [Potential Solution]: Systematic testing with different membrane composition and charges to determine rule for membrane protein incorporation with synthetic membrane.
- **2030 Milestone: Control of cell-free system location and reactivity within artificial membranes and control of the location of orthogonal DNA/RNA/protein synthesis machinery in living cells.**
    - [Bottleneck]: Controlling reaction of cell-free systems powered by internal metabolism in a spatially controlled manner.
        - [Potential Solution]: Leverage liquid-liquid phase separation or protein-cage systems for compartmentalizing distinct reactions to control overall dynamics and functions of artificial membranes.
- **2040 Milestone: Designed trafficking of cell-free synthesized proteins in synthetic membranes.**

Breakthrough Capability: Engineer the biotic/abiotic interface.

- **2022 Milestone: Engineer cell surface biomolecular interaction scaffolds to facilitate association (*e.g.,* adhesion, binding) with abiotic materials.**
    - [Bottleneck]: Lack of reference or control materials with known biotic/abiotic interfaces to facilitate development and comparison of interfaces.
        - [Potential Solution]: Characterization of bacterial biofilms as example of biotic/abiotic interface.
    - [Bottleneck]: Understanding of structure-property relationship between a synthetic scaffold and living cell.
        - [Potential Solution]: Compatibility testing of libraries of polymeric or proteinaceous scaffolds for mechanical property and cellular viability.
    - [Bottleneck]: Ability to measure binding affinity between biotic and abiotic materials.
        - [Potential Solution]: Develop tools for accurate and reproducible measurements, such as surface plasmon resonance and simultaneous atomic force microscopy (AFM)/confocal microscopy (where AFM is used to measure the interface and the immediate “area” around it while the confocal portion measures or monitors what is happening in the bulk).
        - [Potential Solution]: Development of microfluidic and robotic systems for improved screening platforms.
- **2022 Milestone: Develop nano- and meso-structured (bio)templates (*e.g.,* DNA, viral capsids, proteins) with controllable topological, mechanical, and functional properties to direct nanomaterial synthesis and to manipulate interfacial interactions with proximate biological systems (*i.e.,* cells or tissues).[18]**
    - [Bottleneck]: Limited knowledge of how topography and template mechanics triggers compositional and functional responses in proximate cell membranes.
        - [Potential Solution]: Develop an understanding of how DNA/RNA nanostructure size, shape, and membrane receptor affinity influences internalization into cells.

    - [Bottleneck]: Limited knowledge of how surface characteristics drive nanomaterial synthesis.
        - [Potential Solution]: Develop an understanding of how surface features and biomolecule folding interact with abiotic molecules to drive deposition on the surface.
    - [Bottleneck]: Interactions that drive self-assembly of biological templates are too weak to withstand industrial processing conditions for large scale synthesis of diverse materials.
        - [Potential Solution]: Understand and control protein-protein interactions that drive template assembly.
        - [Potential Solution]: Investigate how external stimuli, light (any part of the electromagnetic spectrum), or electric or magnetic fields may guide the assembly.
- **2025 Milestone: Enable bioconjugation methods to combine biotic and abiotic materials.**
    - [Bottleneck]: Lack of cost-effective, efficient, scalable, bio-compatible, site-directed bioconjugation chemistries.
        - [Potential Solution]: Develop non-canonical amino acids capable of biocompatible conjugations.
        - [Potential Solution]: Optimize reaction conditions for non-canonical amino acid mediated bioconjugation.
        - [Potential Solution]: Evaluate context-dependency (*i.e.,* specific protein *versus* specific polymer) for optimal bioconjugation chemistries and optimal reaction conditions.
        - [Potential Solution]: Develop computational tools for modeling protein-polymer interfaces.
- **2030 Milestone: Enable the complete characterization of the molecular interface between materials and living cells.**
    - [Bottleneck]: Methods to characterize the molecular interface between materials and living cells.
        - [Potential Solution]: High-performance liquid atomic force microscopy, Raman imaging, and/or electron tomography for visualization of the interface.
        - [Potential Solution]: Spectroscopic techniques to probe molecular signature at the interface.

Included below are select breakthrough capabilities from our 2019 roadmap, *Engineering Biology*[1] (Milestones at 2021, 2024, 2029, and 2039). While these breakthrough capabilities were written in the context of advancing the field of engineering biology, the technical achievements elaborated in these breakthrough capabilities and their milestones are likely to directly contribute to achieving advancements in materials from engineering biology. This content has been incorporated as reference and, when pertinent, is provided with context for its inclusion in this roadmap (as Notes).

Engineering Biology breakthrough capability: De novo prediction of RNA structure, protein structure, and complexes of DNAs/RNAs and proteins from primary sequence and the ability to make accurate predictions of mutability and effect of mutations from structure.

- **2021: Reliably predict (*i.e.,* greater than a 50% success rate) the structure of 300-amino acid proteins and 200-nucleotide RNA domains within 5 Ångstroms from primary sequence.**
- **2021: Improve force-field and backbone-sampling algorithms and include capabilities to capture force-fields of post-transcriptionally- and post-translationally-modified nucleosides and amino acids.**
- **2024: Reliable *de novo* prediction (*i.e.,* greater than a 50% success rate within 5 Ångstrom root mean-square deviation) of RNAs and proteins containing non-canonical structures (including irregular protein loops and RNA aptamers).**
- **2024: Routine design of ligand binding sites and/or aptamers for custom ligands with a greater than 50% success rate.**
- **2029: Routine prediction of structures for 500-amino acid proteins and 200-nucleotide RNA domains within 3 Ångstrom.**
- **2029: Design proteins and RNAs that fold correctly 50% of the time and RNA-protein complexes that form correctly 20% of the time.**
- **2029: Modeling and design of chromatin states that can be manipulated to change function.**
- **2039: Routine prediction of structures for 3,000-amino acid proteins (*e.g.,* polyketide synthases), protein-protein and RNA-protein interactions, and protein and RNA-protein complexes (*e.g.,* re-engineered ribosomes, spliceosomes).**
- **2039: Routine prediction of RNA and protein function from structure.**

Engineering Biology breakthrough capability: De novo design and/or prediction of macromolecular dynamics and dynamic macromolecular structures.

- **2029: Incorporate co-transcriptional (for RNA) and co-translational (for protein) processes (and including cellular factors that participate in these processes) into design algorithms.**
- **2029: Design of intrinsic regulatory control into biomolecules (*e.g.,* allostery).**
- **2029: Design of dynamic and responsive protein-RNA nanomachines.**
- **2029: Routine design of large proteins, beta topologies, membrane proteins, and loops.**
- **2029: Routine design of protein complexes.**
- **2039: Routine design of enzymes with high activities (*i.e.,* $k_{cat}/K_M > 10^5$ 1/M*s).**
- **2039: Modeling and design of dynamic RNA nanomachines that can engage with and manipulate the chromatin states of living systems.**
- **2039: Modeling and design of dynamic DNA-RNA-protein condensates that can expand beyond the functionality of natural condensates (ex: heterochromatin, mediator and Pol II nuclear condensates that govern transcription initiation).**

Engineering Biology breakthrough capability: Ability to control and/or define the function of an engineered microbial community/biome.

- **2021: Ability to combine species with specialized functions to enable the production of desired products.**
- **2024: Assembly of consortia to produce desired molecules/products, considering community-level metabolic flux.**
- **2025 Milestone: Engineer defined consortia established by synthetic cross-feeding and inter-species dependencies to collectively achieve programmable function enabled by a microbial community**
- **2029: Plug-and-play assembly of consortia to produce desired molecules/products from specific starting materials, considering community level metabolic flux and organism-to-organism communication. For example, developing consortia of different microbial species that are grown/fermented together to create a desired product.**
- **2039: On-demand assembly of consortia that are programmed to respond dynamically, such that they can use different feedstocks, metabolize toxins or toxic byproducts, or produce different products in response to endogenous (system) or exogenous (user) cues.**

Engineering Biology breakthrough capability: Ability to characterize and control the three-dimensional architecture of multicellular systems.

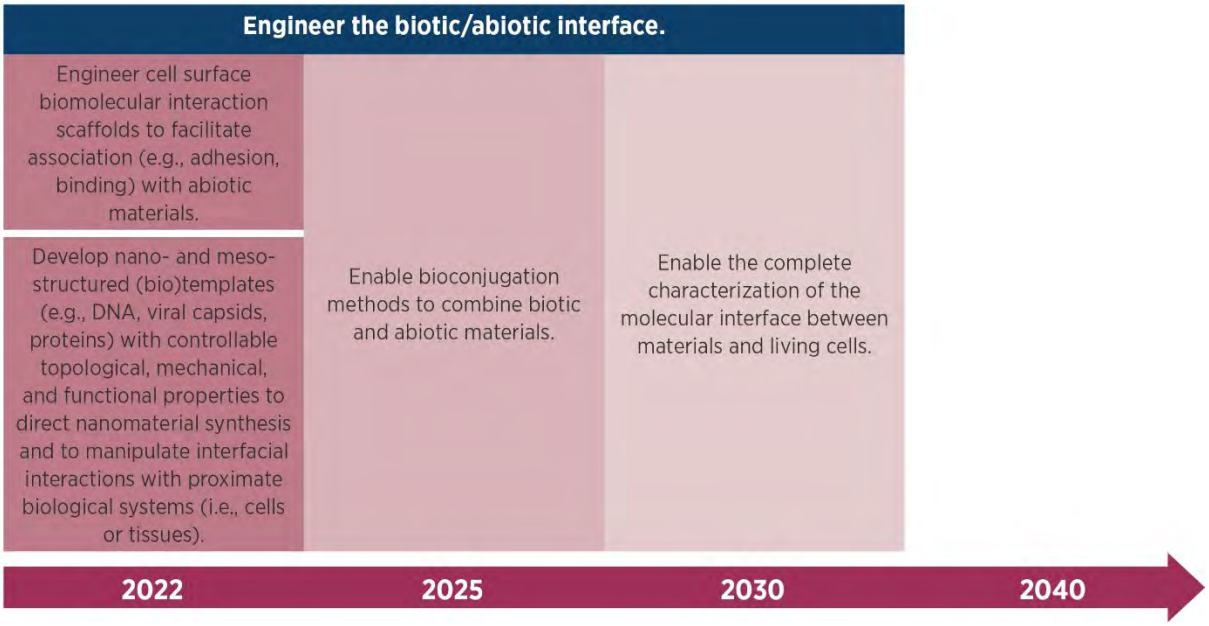
- **2021: Characterize existing tissue components and standardize measurements to evaluate function.**
- **2024: Identification of novel 3D scaffold designs that can lead to desirable cellular properties.**
- **2029: Create modular, synthetic communication circuits that can be implemented in tissues to allow for control of new or existing cellular communication systems.**
- **2039: Bottom-up design and construction of whole organs at the centimeter-length scale.**

Engineering Biology breakthrough capability: Ability to characterize, manipulate, and program the three-dimensional architecture of the biome.

- **2021: Use of existing technologies (including metagenomics, transcriptomics, proteomics, and mass spectrometry) to better understand the species composition and collective components of microbial communities and consortia.**
- **2024: Non-destructive, 3D visualization of microbial communities from a broad range of environments.**
- **2029: Ability to manipulate the 3D architecture of natural or engineered communities using external inputs (such as molecules, temperature, or pH).**
- **2039: Programmed communities that self-assemble into a desired 3D architecture.**

ENGINEERING BIOLOGY & MATERIALS SCIENCE COMPOSITION & STRUCTURE

Breakthrough Capability	Milestone			
Enable containment of biological materials.				
Engineer libraries of polymeric scaffolds that enable confinement of living cells or cell-free systems within materials.	Directed compartmentalization within cellular materials.	Engineer cellular sensing capability (e.g., edge detection) that mimics self-containment in natural systems.	Ability to grow or print multifunctional, multicomponent biological materials with specific substructures and functions.
Achieve the desired extracellular matrix (ECM) in a multicellular (hybrid, composite, or living) material.			
Identify biological receptors, sensors, and actuators amenable for engineering at the cell-ECM interface.	Repurpose dynamic cytoskeletal networks and non-native analogs as an ECM material with dynamic assembly/disassembly behavior to program cell phenotype.	Integrate ECM and cells in a spatially organized manner in tissue-like constructs.	On-demand production of functional living, hybrid, and composite materials.
	Gain molecular understanding of the interaction of living cells with abiotic materials.		
	Develop high-sensitivity methods to characterize and quantify ECM chemistry and structure in situ in order to confirm the desired ECM is achieved.		
De novo design and/or prediction of membrane dynamics.			
Develop computational methods to accurately predict physical properties of biological membranes and location of integrated transmembrane proteins as a function of membrane composition.	Design of natural and non-natural membrane proteins that sort and localize into regions (i.e., domains) of cellular and synthetic membranes.	Control of cell-free system location and reactivity within artificial membranes and control of the location of orthogonal DNA/RNA/protein synthesis machinery in living cells.	Designed trafficking of cell-free synthesized proteins in synthetic membranes.
2022	2025	2030	2040


Engineer the biotic/abiotic interface.
Engineer cell surface biomolecular interaction scaffolds to facilitate association (e.g., adhesion, binding) with abiotic materials.
Develop nano- and meso-structured (bio)templates (e.g., DNA, viral capsids, proteins) with controllable topological, mechanical, and functional properties to direct nanomaterial synthesis and to manipulate interfacial interactions with proximate biological systems (i.e., cells or tissues).
Enable bioconjugation methods to combine biotic and abiotic materials.
Enable the complete characterization of the molecular interface between materials and living cells.
2022
2025
2030
2040

Processing

The engineering of biology to conduct “unit operations” to build or destroy materials through polymerization and degradation, templating, patterning, and printing. This includes engineering the biological extrusion or secretion of materials, material deposition, and self-assembly and -disassembly. Processing also includes engineering biology-based technologies, tools and systems (*e.g.,* cell-free systems) to manufacture, recover, and purify materials. Includes engineering biological materials to function in non-natural environments and extreme conditions.

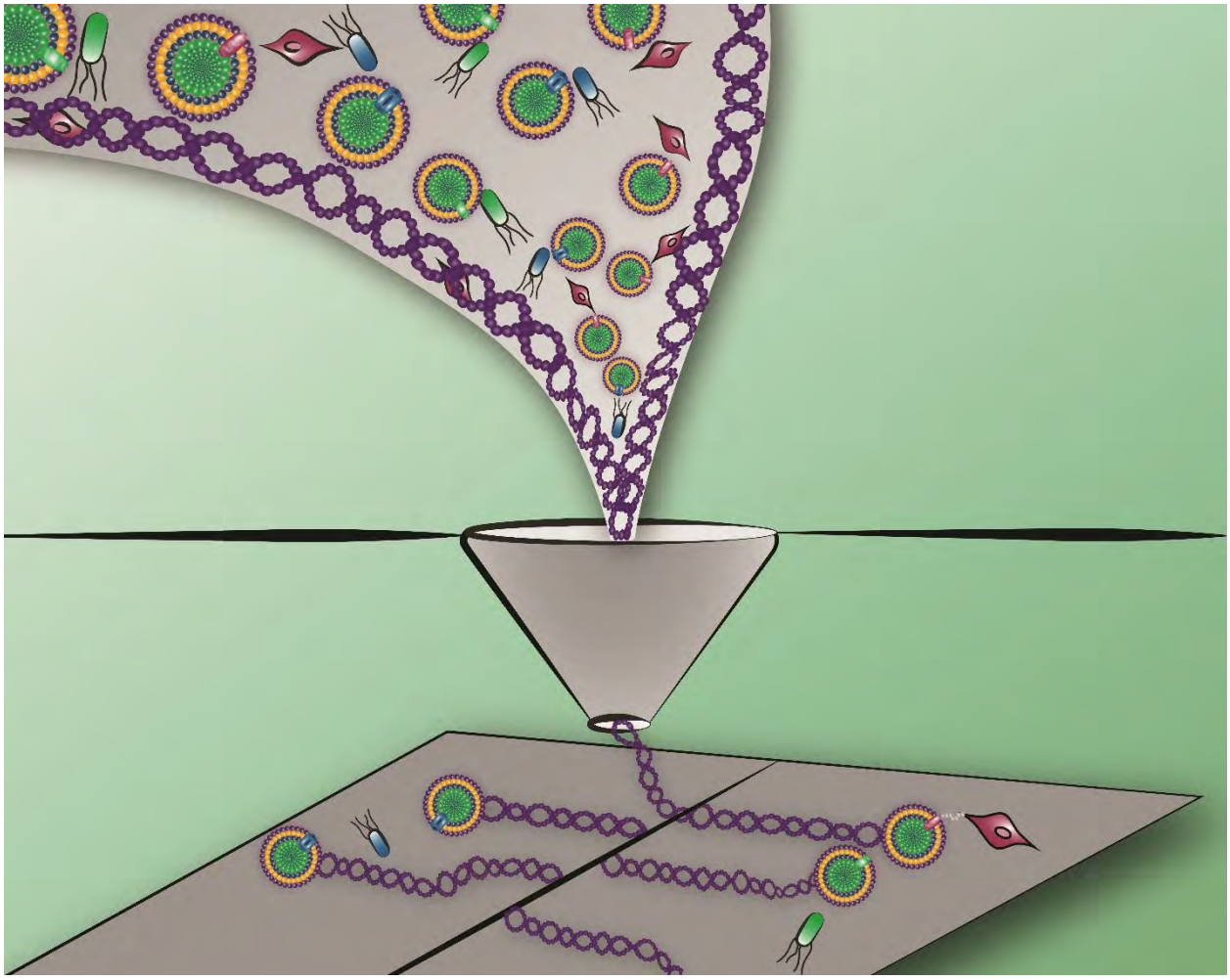


Figure 4. Advancements in processing of materials with, or directed by, biocomponents can enable new characteristics and functionalities. Depicted here, synthetic polymers with differing dimensionality are sorted and printed in a manner that localizes cells on a surface. The complex patterning of bioactive material is enabled by polymers that provide protection through a printing process. Processing of materials systems (*e.g.,* extracellular matrix mimetics) in a manner that can distinguish and sort various components of the system for precise deposition into patterns provides a degree of control and reproducibility not available in natural systems.

Breakthrough Capability: Enable secretion of monomers or polymers without destruction of cells.

- **2022 Milestone: Enable efficient protein/polypeptide secretion, including proteins containing non-canonical amino acids.**

    - [Bottleneck]: Secretion systems in model organisms are limited in yield and sequence requirements.
        - [Potential Solution]: Develop better tools for protein secretion and for polymer formation in non-model organisms (*e.g., Bacillus subtilis*).
    - [Bottleneck]: Limited number of chassis used for non-canonical amino acid incorporation.
        - [Potential Solution]: Expand orthogonal translational systems to other organisms such as *Bacillus subtilis*, an efficient protein secretor.
- **2022 Milestone: Advance bioprocess techniques used in fermentation to isolate secreted hydrophobic materials.**
    - [Bottleneck]: Limited secretion products have resulted in under-exploration of *in situ* separation methods to improve production.
        - [Potential Solution]: Test techniques such as organic overlay, used for *in situ* small molecule extraction.
- **2025 Milestone: One-pot fermentation and conjugation or macromolecular assembly for streamlined separation.**
    - [Bottleneck]: Few conjugation, functionalization, and/or assembly strategies well-suited to occur in or around cells.
        - [Potential Solution]: Identify or design new coupling chemistries that function in fermentative environments.
- **2030 Milestone: Design and engineer eukaryotic chassis and secretion systems that allow compartmentalization and timing of synthetic pathways, taking inspiration from developmental biology.**
    - [Bottleneck]: Incomplete understanding of, and design tools, for complex regulation that controls spatiotemporal patterning in biological systems.
        - [Potential Solution]: Identify a series of model materials with design features that require spatiotemporal patterning and compartmentalization, to quantify progress.
- **2040 Milestone: Incorporate coordinated consortia of production chassis that can build (*in situ*) complex patterned materials from diverse unit-level materials (*i.e.,* diatom deposition of silica combined with bacterial protein functionalization).**
    - [Bottleneck]: Lack of cooperativity in growth and material production and patterning in organisms that do not come by it naturally; inconsistent growth medium requirements and replication rates that confound such coordinated consortia.
        - [Potential Solution]: Engineer mutualistic dependencies between disparate organisms that link growth and development, providing the framework on which to build spatiotemporal coordination biomaterial synthesis and deposition.
        - [Potential Solution]: Template inorganic structures with unique stoichiometry and potential to access novel ceramics or catalysts.

Breakthrough Capability: Ability to control self-assembly and disassembly of biomolecule-based or -embedded materials.

- **2022 Milestone: Development of materials design approach that allows for programmatic control of self-assembly and disassembly.**
    - [Bottleneck]: Design rules of self-assembly are not fully elucidated.
        - [Potential Solution]: Development of experimental and computational frameworks to support materials discovery for biomolecule-based and/or embedded materials (*e.g.,* machine learning, computational biology, and protein-folding algorithms).
- **2025 Milestone: Understand the mechanisms of cellular remodeling of the extracellular matrix (ECM) and dynamic interactions between cells and templating materials.**
    - [Bottleneck]: Fundamental gaps in knowledge about how specific protein sequences and tissue inhibitors affect cellular properties and cell-matrix interactions.
        - [Potential Solution]: Controlled engineering of 3D networks that resemble the ECM.
        - [Potential Solution]: Standardized characterization of cellular properties in natural and engineered networks with site-specific cellular/protein interactions.
    - [Bottleneck]: Gaps in knowledge remain about how specific physical properties of ECM (stiffness, porosity, viscoelasticity) affect cell functions from different cell types.
        - [Potential Solution]: Enable more thorough characterization of ECM physical properties.
- **2040 Milestone: Ability to control and direct hierarchical self-assembly to achieve desired material architecture, properties, or functions.**
    - [Bottleneck]: Limited understanding of the complex multiscale interactions that drive self-assembly.
        - [Potential Solution]: Molecular-level investigations of the self-assembly of stimuli-responsive biopolymers or amphiphiles with conformational adaptability.
    - [Bottleneck]: Understanding of the interactions of biocomponents with the substrate, including covalent, ionic, and van der Waals interactions.
        - [Potential Solution]: Characterize the bonding in categories of the type of interaction, with the aim that similar biocomponents will bond similarly to the same substrate.
    - [Bottleneck]: Tunable protein-based stimulus-responsive nanostructures.
        - [Potential Solution]: Characterize and engineer existing proteins that are responsive to stimuli (*e.g.,* light, pH, mechanical stimuli).

Breakthrough Capability: Ability to control molecular and macromolecular deposition, patterning, and remodeling on biotic and abiotic surfaces.

- **2022 Milestone: Increase capability to deposit or pattern biomolecules on various substrates.**
    - [Bottleneck]: Lack adhesive biological and/or synthetic moieties that allow for specific interactions.
        - [Potential Solution]: Engineer a series of orthogonal membrane proteins on living cells to create autonomous patterns on surfaces.
    - [Bottleneck]: Limited characterization methods for patterned/deposited surfaces.
        - [Potential Solution]: Establish and expand dimension, thickness, periodicity, and spectroscopic methods.
- **2022 Milestone: Control proteins on the outside of an organism for selective reactivity with material precursors for the creation of size and shape controlled materials.**
    - [Bottleneck]: Understanding of amino acid and traditional chemical reactivity to build controlled structures.
        - [Potential Solution]: Full characterization of the suites of materials produced to date and analyze with artificial intelligence and machine learning techniques.
- **2025 Milestone: Ability to build robust interfaces between living systems and semiconducting/electronic surfaces to sense and manipulate biological processes.**
    - [Bottleneck]: Biological materials do not withstand the processing conditions that semiconductors do.
        - [Potential Solution]: Stepwise generation of hybrid systems to account for the fragility of biocomponents.
        - [Potential Solution]: Depending on application/desired outcome, inorganic semiconductor may not be needed, and a more biocompatible one, such as an organic semiconductor, may be used with processing conditions closer to that of biomaterials.
    - [Bottleneck]: Molecular platforms to transmit electrical signals between cells and electronic materials are not universal and maybe difficult to integrate with signal transduction pathways.
        - [Potential Solution]: Develop interchangeable tools to allow for integration of common electrical signals (*e.g.,* redox, ion conduction).
- **2025 Milestone: Produce templated materials of atypical biological shapes or size scales.**
    - [Bottleneck]: The utilization of what we already know in templating materials on biological systems to produce the material size and shapes desired.
        - [Potential Solution]: Utilize techniques such as DNA origami or very selective chemistry to combine biological materials into non-natural shapes and sizes (*e.g.,* combining linear phage into the vertices of a pyramid).

        - [Potential Solution]: Manipulate an organism to autonomously form a desired size or shape.
        - [Potential Solution]: Use bilayers, micelles, and vesicles to constrain shape.
        - [Potential Solution]: Use a patterned and sacrificial substrate to constrain systems to certain shapes.
        - [Potential Solution]: Use asymmetric polymeric materials (*e.g.,* tapered bottlebrushes similar to scaffolding proteins) as molecular units for templating and scaffolding.
- **2030 Milestone: Engineer living organisms that change their surface properties upon response to an external stimuli, in order to template desired materials on demand.**
    - [Bottleneck]: Multiple biological processes need to work in tandem, molecular precursors need to be present and accessible, and the kinetics of many processes need to be improved.
        - [Potential Solution]: Improve engineered communication between singular intracellular functions.
        - [Potential Solution]: Perform processes on an intelligent substrate, such as one that can monitor activity of components or precursors and update components' information to each other.
- **2040 Milestone: Controllably form on-demand macroscale materials templated on biological organisms with desired properties.**
    - [Bottleneck]: Controlled interactions between individual materials would be required in order to stack materials in a desired array without outside stimuli.
        - [Potential Solution]: Innovation in additive manufacturing (*e.g., in situ* printing of a defined layer of materials directly onto a living organism).

Breakthrough Capability: Enable biomolecule and cellular patterning and printing under diverse conditions.

- **2022 Milestone: Develop technology to spatially place single cells at defined locations with high spatial resolution in two dimensions, as the biological pattern for the material.**
    - [Bottleneck]: Maintaining the structure and function of cells in the template.
        - [Potential Solution]: 'Ink-jet printing' of bacterial inoculum using acoustic liquid-handling.
        - [Potential Solution]: 'Photo-masking' enabled selection of spatially-resolved surviving organisms from a mixed population using light.
        - [Potential Solution]: Imprint lithography to generate a scaffold with spatially differentiated layers or regions through the step of coating these regions with an appropriate chemical.
- **2022 Milestone: Establish 3D-printing methods for producing biological (living) composite materials.**
    - [Bottleneck]: Cellular behavior in printing conditions (in shear field, solidification, or polymerization) remains to be explored.

        - [Potential Solution]: Develop an analysis flow for systematic studies on the relevant variables (*e.g.,* printing condition and viability, scaffold molecular composition and viability, viability and motility relationships with metabolic activity).
        - [Potential Solution]: Develop methodology to assess cellular viability during or shortly following printing.
    - [Bottleneck]: Engineering microstructures within the printed biological composite materials.
        - [Potential Solution]: Enable block-copolymer, shear-induced pre-alignment.
        - [Potential Solution]: Introduce acoustic, magnetic, or electric fields into synthesis protocols.
- **2025 Milestone: Capability to print microbes and/or grow desired three-dimensional communities from two-dimensional patterns under various environmental conditions.**
    - [Bottleneck]: 'Externally' defined 3D patterns (*i.e.,* those determined by printing) are not currently reinforced by intracellular genetic circuits that preserve patterning in different environments.
        - [Potential Solution]: Introduce asymmetric control of cell growth and reproduction, such that one class of microbes constrains physiology of other community members in a consortium.
- **2030 Milestone: Maintain desired microbial community structural organization over time.**
    - [Bottleneck]: Engineering persistence of designed and structured 3D cellular communities over time.
        - [Potential Solution]: Control individual strains in a mixed-culture pattern with feedback circuits from environmental sensors to preserve deposited patterns in diverse environmental conditions.
- **2040 Milestone: Print cellular structures on demand on any surface.**
    - [Bottleneck]: Need to enable a wider variety of cell types that can be printed.
        - [Potential Solution]: Further characterization of multi-species and multicellular communities and structures.

Breakthrough Capability: Engineer cells to produce materials in environments optimal for the ex vivo material.

- **2022 Milestone: Develop additional polymerization strategies (*e.g.,* peroxidase-catalyzed, reversible or irreversible covalent couplings) for abiotic and non-natural monomers that can occur in aqueous or fermentative environments.[19]**
    - [Bottleneck]: Many of these enzymes/couplings function only in conditions incompatible with protein and cell stability.
        - [Potential Solution]: Investigate associated enzyme family members under a broader range of conditions.
- **2022 Milestone: Engineer minimal cells for production and processing of biological materials (*e.g.,* chromosome-free bacterial cells).**

    - [Bottleneck]: Expanding types and host chassis for creating minimal cell systems is required.
        - [Potential Solution]: Explore types and host chassis for creating minimal cell systems and test their resilience, robustness, and performance in/with materials.
- **2025 Milestone: Engineer thermophilic microorganisms for producing biomass or biomolecules to produce materials in/at higher temperatures.**
    - [Bottleneck]: Engineering of non-canonical organisms for isolation of novel molecules.
        - [Potential Solution]: Determine synthesis chassis for extremophile organisms that allows production of unique molecules.
- **2030 Milestone: Manage biological complexity and interactions in experimental model systems to determine the ideal host species for a product.**
    - [Bottleneck]: Building of models with experimental data from a wide range of organisms.
        - [Potential Solution]: Import of useful functionalities from less conventional organisms into other chassis.
- **2030 Milestone: Production and processing of biological materials in the absence of water.**
    - [Bottleneck]: Biological structure-function-property relationships are driven by solvophobic/hydrogen bonding interactions; very difficult to translate to other solvent systems.
        - [Potential Solution]: Explore polar, non-volatile solvent systems for ability to maintain enzymatic function, structure in non-aqueous environments; initial substrates may include dimethyl sulfoxide, ethylene glycol, and low-molecular-weight polyethylene glycols.
        - [Potential Solution]: Develop predictive capability for folding correlated to solvent dielectric, protein analogs.

Breakthrough Capability: Enable robust processing of materials using cell-free systems.

- **2022 Milestone: Expand cell-free systems to different bacterial species and mammalian cell types.**
    - [Bottleneck]: Standardization of cell lysates collection and optimization of reaction conditions.
        - [Potential Solution]: Systems biology approaches (*e.g.,* proteomics, metabolomics, transcriptomics) to analyze cell-free expression systems to assess and identify limiting factors.
- **2025 Milestone: Use cell-free systems for large scale production of complex biologics including proteins with post-translational modifications.**
    - [Bottleneck]: Large-scale culture may require industry scale equipment not readily available in academic labs.
        - [Potential Solution]: Develop continuous bioprocessing strategies for routine, large-scale production of cell-free systems.
    - [Bottleneck]: Post-translational modifications are difficult to precisely control.

        - [Potential Solution]: Engineer systems in which cell-free synthesized proteins go through a series of desired modifications by passing through microfluidic channels with enzymes at specific locations to modify the proteins passing through, akin to a conveyer belt in a manufacturing process.
        - [Potential Solution]: Develop batch processes with multiple distinct enzymes together that enables synthesis of homogeneous glycoproteins.
- **2030 Milestone: Establish cell-free "distributed processing" for sustainable feedstock utilization.**
    - [Bottleneck]: Compartmentalization is typically lacking in cell-free systems.
        - [Potential Solution]: Engineer encapsulation into cell-free systems or minimal cells to permit material separation from enzymes or cofactors for optimized utilization.
- **2030 Milestone: Augment materials with freeze-dried cell-free systems for on demand, real-time diagnostics.**
    - [Bottleneck]: Humidity and reaction stability limit use.
        - [Potential Solution]: Develop abiotic materials that can maintain biological conditions.
- **2040 Milestone: Establish cell-free systems for on-demand and personalized biomanufacturing platforms.**
    - [Bottleneck]: High costs limit adoption.
        - [Potential Solution]: Develop strategies based on energy substrates and strain engineering to reduce costs an order of magnitude from present day.
    - [Bottleneck]: Reaction stability constrains utility in resource limited settings.
        - [Potential Solution]: Develop materials that stabilize cell-free systems for extended periods of time while freeze-dried (>1 year).
- **2040 Milestone: Develop carbon-optimized cell-free bioconversions for the industrial scale production of materials.**
    - [Bottleneck]: Most bioprocesses still use reduced-carbon compounds like glucose.
        - [Potential Solution]: Capture carbon from CO_2 fixation pathways.
        - [Potential Solution]: Combine electrolyzers with cell-free systems to fuel cost-effective, manufacturing strategies at large-scale.
    - [Bottleneck]: Co-factor regeneration and stability is a concern.
        - [Potential Solution]: Develop new stable cofactors.
    - [Bottleneck]: Enzyme stability is limiting.
        - [Potential Solution]: Develop continuous bioprocesses to replenish catalysts.
        - [Potential Solution]: Develop materials to stabilize catalysts for one month continuous operation.

Breakthrough Capability: Enable selective component and material degradation through engineering biology (e.g., degradation of bonds that are particularly susceptible to hydrolytic degradation, enabled by enzymes).

- **2022 Milestone: Identify and engineer enzymes (*e.g.,* esterase) to interact and degrade polymeric substrates with tunable performance.**
    - [Bottleneck]: Studies of enzymatic degradation of polymeric scaffolds exist, yet their performance and substrate scope remain to be improved and engineered for various contexts; current approaches involve trial and error and oftentimes substrates are not selective but instead responsive to a variety of enzymes.
        - [Potential Solution]: Develop predictive models that help develop structure-property relationships in enzymatically degradable systems that allow for development of materials with increased selectivity.
- **2022 Milestone: Identify enzymes capable of interaction with, and degradation of, both hard and soft segments of polymer systems.**
    - [Bottleneck]: Complete degradation of polymers (*e.g.,* ester and carbonyl bonds) may require multi-enzyme systems or chimeric enzymes.
        - [Potential Solution]: Enable engineering of expanded classes of enzymes, including incorporation of non-canonical and/or non-natural amino acids.
- **2022 Milestone: Design and engineer cellular half-life and patterning for persistence in materials.**
    - [Bottleneck]: Spore-forming microbes and associated tools have been underdeveloped for synthetic biology applications.
        - [Potential Solution]: Engineer spore-display and genetic circuit tools for responsive functionalization of spores to abiotic interfaces or within abiotic materials.
- **2025 Milestone: Engineer enzymes capable of degrading or reversing click chemistry reactions used to conjugate proteins and polymers, while leaving the polymer and protein intact.**
    - [Bottleneck]: Click chemistries used for bioconjugation through the use of non-canonical amino acids are infrequent/nonexistent in nature, enzymes that can specifically degrade/reverse a click reaction have not been identified or developed.
        - [Potential Solution]: Scour sequence databases for DNA sequences that could encode enzymes with needed putative activities.
        - [Potential Solution]: Pursue a protein engineering effort to generate an enzyme capable of degrading or reversing a click reaction.
- **2025 Milestone: Engineer living cells to produce and secrete the target enzymes for modulating polymeric scaffold *in situ*.**
    - [Bottleneck]: Requires development of feedback loops for new materials.
        - [Potential Solution]: Studies to explore dynamic equilibria exhibited by actin filaments, application to motility and force generation; develop principles for governing dynamics, required kinetics, expand from baseline understanding.

- **2025 Milestone: Enable force application and specific extracellular matrix (ECM) binding ('AND gate') to trigger ECM degradation.**
    - [Bottleneck]: Control of sense-response activity for extracellular matrices.
        - [Potential Solution]: Identification of signaling nodes that receive input from force sensing (*i.e.,* via a mechanosensitive channel) and ECM binding that can be coupled to secretion of matrix metalloproteinases.
- **2025 Milestone: Enable aqueous biomolecules to interact at high affinity with existing insoluble polymers.**
    - [Bottleneck]: Degradative enzymes are often intolerant of solvents required to solubilize synthetic polymers.
        - [Potential Solution]: Design strategies to functionalize enzyme surfaces for increased solvent tolerance (*e.g.,* via non-canonical amino acids such as fluoro-phenylalanine, post-translational modifications, or synthetic modification).[20]
        - [Potential Solution]: Enable a co-polymer approach to enzyme stabilization.[11]
- **2030 Milestone: Engineer polyspecific enzymes that can break specific classes of chemical bonds between heteroatoms (*e.g.,* C-O, C-N), also potentially targeting unsaturation (*e.g.,* metathesis).**

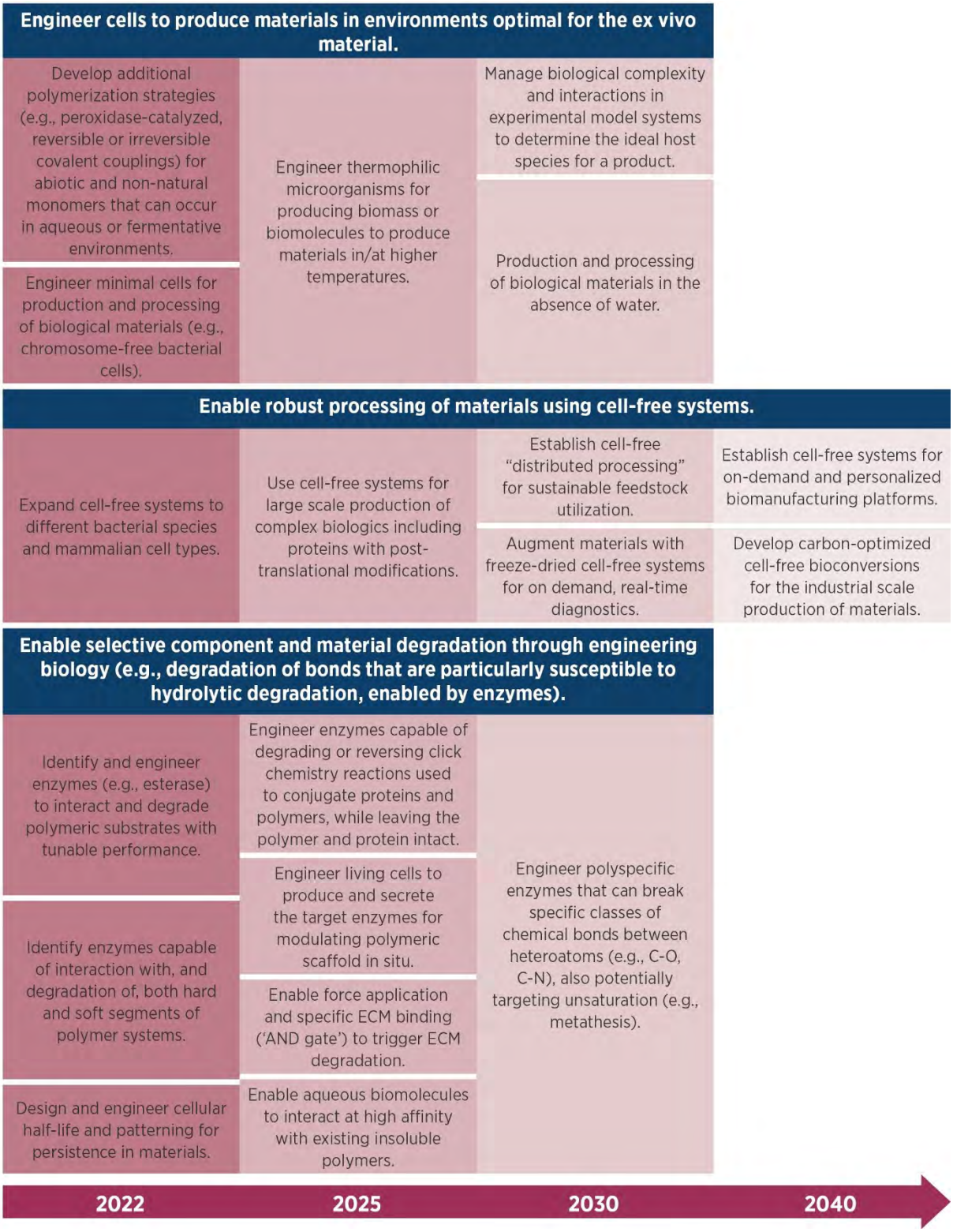
Breakthrough Capability: Industrial infrastructure and accelerated downstream processing of biocomponent-containing materials.

- **2022 Milestone: Enable systems that operate in semi-continuous modes with modular units designed using process-intensification principles to accelerate transport and reaction processes.**
    - [Bottleneck]: Efficient product isolation and purification requires biocatalysts compatible with advanced materials and systems engineering strategies spanning multiple scales.
        - [Potential Solution]: Bioreactors with cells suspended in extruded pluronic hydrogels with tunable rates of substrate and product diffusion.
        - [Potential Solution]: 3D-printed biocompatible nanostructures encapsulating engineered cell- or cell-free biocatalysts.
- **2022 Milestone: Establishment of multiple (5+) pilot scale facilities (300-10,000 L) for the production of biomolecules and biomaterials; such facilities each need to enable fermentation, downstream processing, and feedstock supply.**
    - [Bottleneck]: Currently there is not a large economic incentive for industry to iterate at this scale.
        - [Potential Solution]: Government and academic investments to produce such facilities.
- **2025 Milestone: Process monitoring and control for modular and on-site (*i.e.,* point-of-need) biomanufacturing, incorporating domain expertise.**
    - [Bottleneck]: Characterization of performance metrics for materials within context of application.

        - [Potential Solution]: Spectroscopy methods that enable high-throughput analysis of materials characterization.
- **2025 Milestone: Establishment of a robust network (25+) of pilot scale facilities for the production of biomolecules and biomaterials.**
    - [Bottleneck]: Currently there are very few facilities in the US that provide fermentation and downstream processing capabilities.
        - [Potential Solution]: Regional facilities that enable the utilization of various feedstocks and increase accessibility.
    - [Bottleneck]: Pilot scale facilities need to be self-sustaining.
        - [Potential Solution]: A robust number of academic, industrial, and government led projects continuing for the next decade will allow for such facilities to gain traction.
- **2030 Milestone: Full integration of unit operations for homogeneous biological and heterogeneous chemical and biological catalysis with separations.**
    - [Bottleneck]: Coordinated technologies and platforms for bioprocessing.
        - [Potential Solution]: Optimization and molecular design of hydrogel materials, bioreactor platforms and integration of separations.
        - [Potential Solution]: Incorporate process monitoring and control schemes.
- **2030 Milestone: Development of point of need production of biomolecules and biomaterials.**
    - [Bottleneck]: Insufficient demand for such infrastructure due to the way supply chains are handled and the fact that the bioeconomy is still a small section of the overall industrial base.
        - [Potential Solution]: Development of less expensive and more modular equipment will increase accessibility of fermentation and downstream processing equipment to be stood up at the point-of-need.
- **2040 Milestone: Quick infrastructure stand-up at point-of-need for large scale production of biomolecules and biomaterials.**
    - [Bottleneck]: The investment to stand up a biomanufacturing facility with downstream and feedstock processing is timely and costly.
        - [Potential Solution]: Development of chemical engineering processes that are more flexible and modular for downstream processing.
        - [Potential Solution]: Development of fermentation equipment that is disposable or recyclable.
        - [Potential Solution]: High titer yields would require a much smaller scale of production.

ENGINEERING BIOLOGY & MATERIALS SCIENCE PROCESSING

Breakthrough Capability	2022 Milestone	2025 Milestone	2030 Milestone	2040 Milestone
Enable secretion of monomers or polymers without destruction of cells.				
	Enable efficient protein/ polypeptide secretion, including proteins containing non-standard amino acids.	One-pot fermentation and conjugation or macromolecular assembly for streamlined separation.	Design and engineer eukaryotic chassis and secretion systems that allow compartmentalization and timing of synthetic pathways, taking inspiration from developmental biology.	Incorporate coordinated consortia of production chassis that can build (in situ) complex patterned materials from diverse unit-level materials (i.e., diatom deposition of silica combined with bacterial protein functionalization).
	Advance bioprocess techniques used in fermentation to isolate secreted hydrophobic materials.			
Ability to control self-assembly and disassembly of biomolecule-based or -embedded materials.				
	Development of materials design approach that allows for programmatic control of self-assembly and disassembly.	Understand the mechanisms of cellular remodeling of the extracellular matrix (ECM) and dynamic interactions between cells and templating materials.	Ability to control and direct hierarchical self-assembly to achieve desired material architecture, properties, or functions.	
Ability to control molecular and macromolecular deposition, patterning, and remodelling on biotic and abiotic surfaces.				
	Increase capability to deposit or pattern biomolecules on various substrates.	Ability to build robust interfaces between living systems and semiconducting/ electronic surfaces to sense and manipulate biological processes.	Engineer living organisms that change their surface properties upon response to an external stimuli, in order to template desired materials on demand.	Controllably form on-demand macroscale materials templated on biological organisms with desired properties.
	Control proteins on the outside of an organism for selective reactivity with material precursors for the creation of size and shape controlled materials.	Produce templated materials of atypical biological shapes or size scales.		
Enable biomolecule and cellular patterning and printing under diverse conditions.				
	Develop technology to spatially place single cells at defined locations with high spatial resolution in two dimensions, as the biological pattern for the material.	Capability to print microbes and/or grow desired three-dimensional communities from two-dimensional patterns under various environmental conditions.	Maintain desired microbial community structural organization over time.	Print cellular structures on demand on any surface.
	Establish 3D-printing methods for producing biological (living) composite materials.			


Engineer cells to produce materials in environments optimal for the ex vivo material.
Develop additional polymerization strategies (e.g., peroxidase-catalyzed, reversible or irreversible covalent couplings) for abiotic and non-natural monomers that can occur in aqueous or fermentative environments.
Engineer minimal cells for production and processing of biological materials (e.g., chromosome-free bacterial cells).
Engineer thermophilic microorganisms for producing biomass or biomolecules to produce materials in/at higher temperatures.
Manage biological complexity and interactions in experimental model systems to determine the ideal host species for a product.
Production and processing of biological materials in the absence of water.
Enable robust processing of materials using cell-free systems.
Expand cell-free systems to different bacterial species and mammalian cell types.
Use cell-free systems for large scale production of complex biologics including proteins with post-translational modifications.
Establish cell-free "distributed processing" for sustainable feedstock utilization.
Augment materials with freeze-dried cell-free systems for on demand, real-time diagnostics.
Establish cell-free systems for on-demand and personalized biomanufacturing platforms.
Develop carbon-optimized cell-free bioconversions for the industrial scale production of materials.
Enable selective component and material degradation through engineering biology (e.g., degradation of bonds that are particularly susceptible to hydrolytic degradation, enabled by enzymes).
Identify and engineer enzymes (e.g., esterase) to interact and degrade polymeric substrates with tunable performance.
Identify enzymes capable of interaction with, and degradation of, both hard and soft segments of polymer systems.
Design and engineer cellular half-life and patterning for persistence in materials.
Engineer enzymes capable of degrading or reversing click chemistry reactions used to conjugate proteins and polymers, while leaving the polymer and protein intact.
Engineer living cells to produce and secrete the target enzymes for modulating polymeric scaffold in situ.
Enable force application and specific ECM binding ('AND gate') to trigger ECM degradation.
Enable aqueous biomolecules to interact at high affinity with existing insoluble polymers.
Engineer polyspecific enzymes that can break specific classes of chemical bonds between heteroatoms (e.g., C-O, C-N), also potentially targeting unsaturation (e.g., metathesis).
2022
2025
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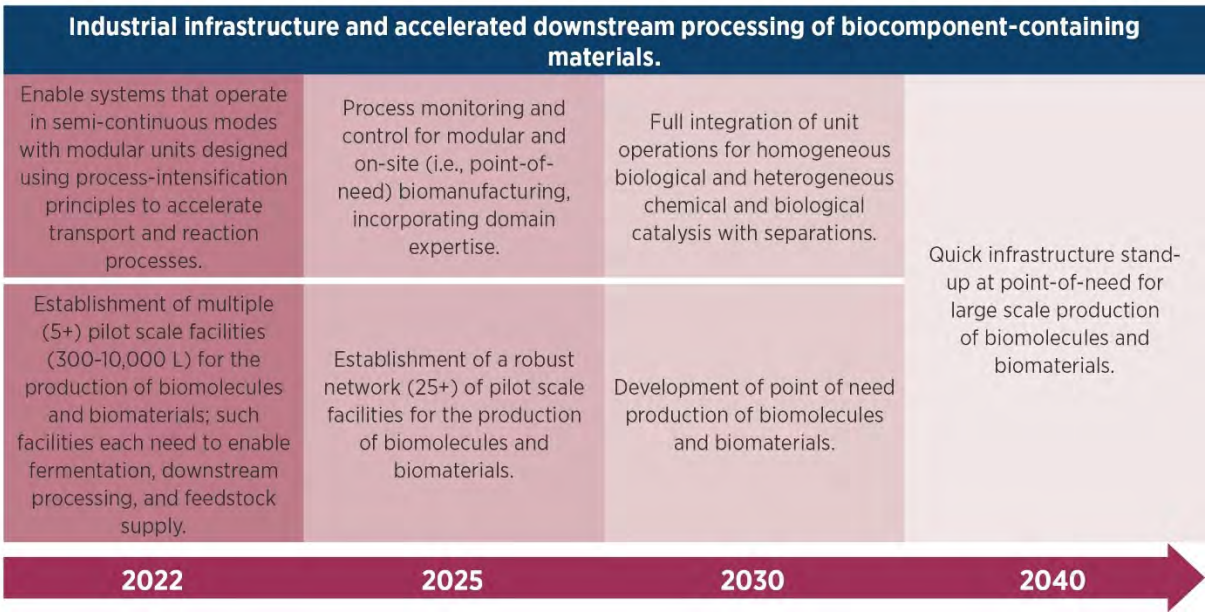

Industrial infrastructure and accelerated downstream processing of biocomponent-containing materials.
Enable systems that operate in semi-continuous modes with modular units designed using process-intensification principles to accelerate transport and reaction processes.
Process monitoring and control for modular and on-site (i.e., point-of-need) biomanufacturing, incorporating domain expertise.
Full integration of unit operations for homogeneous biological and heterogeneous chemical and biological catalysis with separations.
Quick infrastructure stand-up at point-of-need for large scale production of biomolecules and biomaterials.
Establishment of multiple (5+) pilot scale facilities (300-10,000 L) for the production of biomolecules and biomaterials; such facilities each need to enable fermentation, downstream processing, and feedstock supply.
Establishment of a robust network (25+) of pilot scale facilities for the production of biomolecules and biomaterials.
Development of point of need production of biomolecules and biomaterials.
2022
2025
2030
2040

Properties & Performance

The engineering of dynamic characteristics and activities of materials, including sensing and response, communication and computation, and self-repair through the incorporation or activation of biocomponents. This includes the engineering of materials to provide signals and store and release energy or information through an engineered biological component and the engineering of dynamic interactions between the biological and abiotic components of a material. *Properties & Performance* also considers challenges in tools, methods, and technologies for characterizing dynamic activity and performance of living materials and materials that incorporate biocomponents.

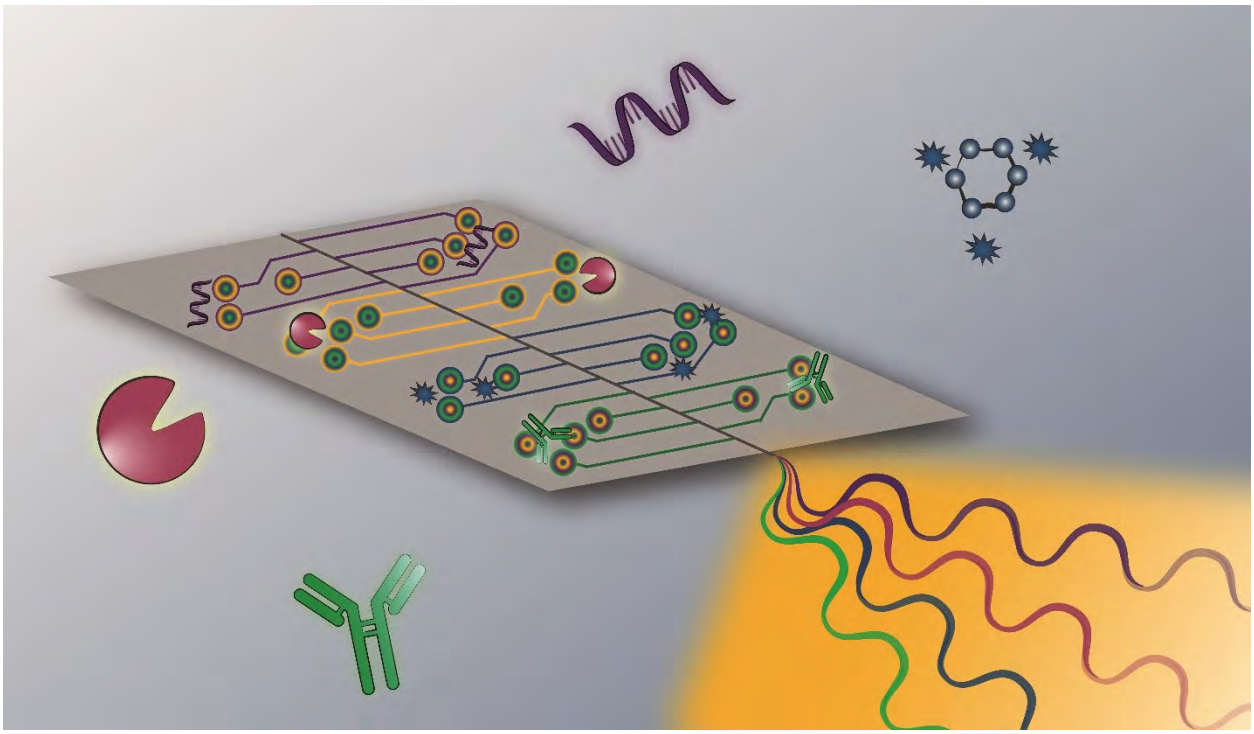


Figure 5. Materials can be enabled to dynamically sense and transmit information through distinctively engineered biocomponents in the system. Here, a biosensing circuit made out of synthetic biomolecules is patterned onto a surface. Small adjustments in the sequence and identity of the sensing components makes it receptive to a variety of biological matter like RNA (purple), enzymes (red), reactive chemical species and environmental factors (blue), and specific antibodies (green). The multiplex sensor is able to simultaneously interact with these complex cues and generate measurable output that quantifies each component in the system.

Breakthrough Capability: Enable self-regulating living materials by introducing feedback loops to maintain performance, to adapt to fluctuating environmental conditions, and to demonstrate out-of-equilibrium behaviors.

- **2022 Milestone: Utilize DNA and RNA nanostructures to build components and materials that dynamically reorganize in a stimulus-dependent manner.**
    - [Bottleneck]: *De novo* engineering of nucleic acid assemblies that interface with biological environments remains challenging.

        - [Potential Solution]: Develop modular nucleic acid assemblies with easily reconfigurable interfaces that form in physiological conditions and are robust to degradation.
        - [Potential Solution]: Develop peptide/protein-DNA, peptide/protein-RNA conjugates that can introduce functionalities to the reconfigurable interfaces.
        - [Potential Solution]: Develop nucleic acid structures that have programmable material/mesh features but reduced nanoscale complexity/rigidity, minimizing complex folding protocols and that would be compatible with physiological production.
        - [Potential Solution]: Integrate and control nucleic acid production and assembly with biological or biomaterial signals.
        - [Potential Solution]: Engineer synthetic proteins and polymers comprising of synthetic chemistries that enable tunable structural, chemical, and biophysical properties.
- **2022 Milestone: Enable genetic circuit design that links environmental stimuli and output, such as intracellular proteins determining the cell's mechanical properties, or secreted proteins that take part in composite material or extracellular matrix.**
    - [Bottleneck]: It is challenging to obtain molecular materials with predictable responses, for example graded response (predictable response to input change) and homeostatic response (minimal effects of perturbations such as changes in temperature or nutrient).
        - [Potential Solution]: Introduction of negative feedback genetic circuits to cells to allow for self-regulation of expression of specific proteins participating in multi-component materials.
        - [Potential Solution]: Use of RNA or *de novo* proteins to enable integral feedback control of expression of material components.
    - [Bottleneck]: Lack of molecular feedback architectures tailored to orchestrate multi-component material expression.
        - [Potential Solution]: Expand integral feedback control circuits for multi-output regulation.
        - [Potential Solution]: Nested, hierarchically organized feedback loops for expression of complex materials.
    - [Bottleneck]: Scarcity of models and theoretical tools to guide the design of complex material responses that are robust to uncertain processes and perturbations, including growth rate variability, resource fluctuations, temperature and pH.
        - [Potential Solution]: Develop data-driven models linking material response (output) to a range of undesired perturbations (disturbances).
        - [Potential Solution]: Develop model-based strategies to minimize the effects of disturbances, test them experimentally, and revise.

- **2022 Milestone: Ability to rapidly prototype novel components for feedback circuits including sensors, transducers, logic gates, and actuators with precisely defined characteristics in model organisms.**
    - [Bottleneck]: Poorly matched components degrade signal transmission and/or host cell fitness.
        - [Potential Solution]: Use cell-free systems to prototype new components, but this will not address host cell fitness.
        - [Potential Solution]: Develop a platform to transfer devices developed in cell-free extracts to model organisms and validate their operation and fitness.
    - [Bottleneck]: Poor robustness to context changes within and outside of the cell (*i.e.*, behavior of an assembled feedback logic circuit is often different than predicted, due to parts and subsystems being context-dependent).
        - [Potential Solution]: High-throughput methods to engineer or refine part performance in the context of host cell and assembled circuit.
        - [Potential Solution]: Develop/refine "contactless" measurements (*e.g.,* impedance spectroscopy) to measure overall features, from shape of trace, and distinguish contributions from properties like interface, bulk, or charge transfer.
- **2025 Milestone: Engineering of feedback circuits to maintain homeostasis via post-translational modification of proteins for cellular materials.**
    - [Bottleneck]: Increased control over protein post-translational modification is needed.
        - [Potential Solution]: Development of enzyme scaffolds that can better control the sequence and specificity of protein post-translational modifications.
- **2025 Milestone: Rapidly create new logic gates with precisely defined characteristics in a broad range of organisms.**
    - [Bottleneck]: The same circuit works very differently in different chassis and under different conditions.
        - [Potential Solution]: Make designs robust for high portability across conditions and organisms.
        - [Potential Solution]: Develop more high-throughput methods to engineer and/or refine part performance in new organisms.
        - [Potential Solution]: Establish machine-learning methods to predict change in part performance across different conditions and organisms.
- **2025 Milestone: Enable more complex logic gate processing of multiple extrinsic signals by cells and link to a biological function.**
    - [Bottleneck]: Compatibility issues with different extrinsic signals and whether they need to be orthogonal to what the cells naturally sense and respond to.
        - [Potential Solution]: Use a consortium of cells, each specific for sensing a particular signal and have a central processing strain that then combines the signals from the different sensing cells.

    - [Bottleneck]: Interference between orthogonally-designed inducer/promoter pairs over limited resources for protein synthesis and expression.
        - [Potential Solution]: Isolate orthogonal pathways and make sensory pathways more robust to cellular and extracellular contexts.
        - [Potential Solution]: When possible, attach a secondary "signal" or "reaction" to one pathway over another that can be measured independently and without interference from other pathways.
- **2025 Milestone: Engineer feedback mechanisms to respond to different stimuli for the planned operation.**
    - [Bottleneck]: Genetic feedback mechanisms act over a longer timescale and are not amenable to short term cellular processes and can require active degradation of protein products.
        - [Potential Solution]: Adopt protein post-translational modifications for controlling protein activity or sequestration for modulating their functions.
- **2030 Milestone: Embed active reaction mechanisms in extracellular matrix (ECM) materials.**
    - [Bottleneck]: There are limited reactions (adhesion, degradation, light sensitivity) that are currently available for engineered ECM materials.
        - [Potential Solution]: Develop materials with reactive/adaptive capabilities.
        - [Potential Solution]: Develop hybrid ECM materials (*e.g.,* with semiconductors or metals).
- **2030 Milestone: Incorporate control circuits in cells that offset the effect of a particular stimulus to limit or impede response.**
    - [Bottleneck]: Temporal response to stimulus (dosage and duration) is still not well understood.
        - [Potential Solution]: Make use of completely *de novo* proteins for cell signaling.

Breakthrough Capability: Enable materials with the ability to self-repair.

- **2022 Milestone: Enable a broader range of naturally self-repairing biomolecules to be used as material biocomponents, including proteins and amino acids.**
    - [Bottleneck]: Limited chemistry with existing natural materials.
        - [Potential Solution]: Enable a wider array of engineered synthetic biological materials, including non-canonical amino acids and *de novo* designed proteins.
    - [Bottleneck]: Repair mechanism might cause deterioration of properties to prevent utility of self-repairing material (*e.g.,* repair mechanism may introduce defects into the pristine material so it loses necessary performance characteristics for intended application).
        - [Potential Solution]: Improve and increase high-throughput characterization of biocomponents and materials.
- **2025 Milestone: Improve and optimize structure-property-performance metrics of biosynthetic materials (*e.g.*, time of repair, strength, stimuli-range).**

    - [Bottleneck]: Limitation in the range of physical properties that can be attained in natural materials.
        - [Potential Solution]: Design sequence-defined synthetic biopolymers comprising combinations of natural and synthetic monomers such that structure-property-performance metrics can be defined, evolved and tuned with an expanded chemical palette toward programmable function.
    - [Bottleneck]: Rate of repair in natural systems may be too slow for practical use.
        - [Potential Solution]: Better understanding and ability to control rate of re-synthesis or re-polymerization in biotic systems and materials.
- **2030 Milestone: Engineer circuits within cells that allow their functionality to adapt to changing conditions, to recover from failures, and to reconfigure functionality based on need.**
    - [Bottleneck]: Cells need a way to estimate environmental conditions and to predict how they may be changing.
        - [Potential Solution]: Engineer suites of biosensor molecules for use in target strain chassis that allow cellular sensing of relevant environmental conditions.
- **2030 Milestone: Mimic natural self-repair (as in living organisms) for creating self-healing in abiotic and composite materials that is autonomous and self-powered.**
    - [Bottleneck]: Materials need to be able to self-produce more of the material for repair.
        - [Potential Solution]: Create microenvironments within the finished materials that activate dormant microbes upon damage and exposure to stimulus (*e.g.,* oxygen) to synthesize replacement materials.
    - [Bottleneck]: Need for genetic and metabolic circuits that can respond to signals in a controlled, predictable manner.
        - [Potential Solution]: Engineer rapid signaling cascades to trigger damage and self-repair responses in materials.
- **2040 Milestone: Engineer synthetic cell systems that can create a hierarchical self-healing composite material that operates (senses, responds, communicates, and computes) at broadband frequencies.**

Breakthrough Capability: Enable materials that sense, encode, and store multimodal, multiplexed environmental signals.

- **2022 Milestone: Assemble tailored cell sensor and actuator arrays and consortia using 3D printing or other top-down methods.**
    - [Bottleneck]: Prediction and validation of function of designed cell consortia/arrays; true orthogonality among different sensory pathways is often a challenge as the expected or predicted function is often different from the observed function.
        - [Potential Solution]: Ensure true orthogonality/modularity among different sensory pathways to enable interference-free multiplexing of many different inputs processing, through isolating sensory pathways from

sharing common enzymes and resources, from cellular circuitry, and from growth rate variability.

- **2022 Milestone: Rapidly create new sensors with precisely defined characteristics.**
    - [Bottleneck]: Lack of methods for quantitative, precise engineering of sensors, such as the defined, individualistic response.
        - [Potential Solution]: Engineer surfaces to interface between biocomponents and, for example, semiconductor technologies.
- **2022 Milestone: Enable transfer of information from biological sensor to machine readable form and vice-versa, to reliably interpret biological signaling.**
    - [Bottleneck]: Bidirectionally crossing the electron-photon and ion transport energy mismatch without loss of fidelity and in a way that allows uniform information readout for data accumulation and AI processing.
        - [Potential Solution]: Utilization of redox reactions (*e.g.,* soxR system and redox biomolecules).
        - [Potential Solution]: New semiconductor intermediate interfaces with ion signaling; for example, coupling a biorelevant ion signal to phonons, and then to an electron band-gap.
- **2022 Milestone: Enable cellular or cell-free system responses within 2-3 minutes from stimulus exposure.[21]**
    - [Bottleneck]: Many bacterial or cell-free sensors use gene expression to process signals, resulting in significantly delayed (1-2 hours) responses.
        - [Potential Solution]: Bypass gene expression and instead use re-engineered signal transduction pathways, which can process a stimulus within minutes.
        - [Potential Solution]: Couple stimulus to known chemistry with colorimetric output.
        - [Potential Solution]: Couple protein activity to stimuli-responsive materials (*e.g.,* light sensitive protein dimerization) to engineer material functionalization and/or rapid cellular responses.
- **2025 Milestone: Adapt proteins that have evolved stimuli-responsive functions into biomaterials to achieve novel sensing and signaling properties.**
    - [Bottleneck]: We lack the understanding of structure-function properties of proteins in the context of polymeric biomaterials.
        - [Potential Solution]: Pursue characterization with a few ‘model’ systems and evaluate the site-specificity of non-canonical amino acid enabled bioconjugations onto polymers to understand what part(s) of a given protein should be conjugated to a polymer to maximize efficiency and retain function.
        - [Potential Solution]: Evaluate the role of post-translational modification of proteins in retaining/achieving novel biomaterials properties.
        - [Potential Solution]: Determine how to scale protein functions to larger scales needed in materials; this requires scale-dependent understanding of protein function depending on their host medium.

        - [Potential Solution]: Advance molecular dynamics simulations to accelerate the synthesis and design of biomaterials that are adequately suited for the incorporation of functional proteins.
- **2025 Milestone: Record multiple types of time- and space-domain events using libraries of biomolecular signals (*e.g.,* RNA or proteins) to store different types of signals for readout by, for example, high-throughput sequencing.**
    - [Bottleneck]: Storage of information that is robust to cell growth/operations, including characterization of multiple signals (chemical, pH, temperature, mechanical).
        - [Potential Solution]: Enable storage of information by creating toggle switches, where the system's state can be flipped by transient stimuli at will.
        - [Potential Solution]: Utilize CRISPR-like technologies to create a permanent change in DNA; however, this is not reversible/reconfigurable.
- **2025 Milestone: Develop means to use ion-based communication via excitable membranes in addition to chemical communication for faster, more location specific communication.**
    - [Bottleneck]: Enabling micro- and nano-scale communication within a wider range of biological systems.
        - [Potential Solution]: Induction of cytoskeletal programs to direct growth of protruding structures for cell morphologies (*i.e.,* axons or dendrites) that interface with membranes.
        - [Potential Solution]: Creation of sets of protein factors that can import and traffic vesicles in response to external signals, to direct changes in gene expression or other long-duration cell changes.
        - [Potential Solution]: Integrate fine electrometers, such as Single-Electron Transistor, or (sub)micro-scale Hall effect sensors, with biocomponents.
- **2025 Milestone: Ability to engineer new surface receptors for sensing and cell-to-cell signaling.**
    - [Bottleneck]: Keeping receptors "alive" during sustained use applications.
        - [Potential Solution]: Develop and engineer new porous or networked materials that are surface compatible with a wide variety of receptors.
        - [Potential Solution]: Develop new planar surfaces, such as a 2D array of electrical sensors, with similar properties as the network but with much higher efficiencies to compensate for the much lower surface area; the advantage of planar architecture is the easy incorporation into microfluidic devices.
- **2025 Milestone: Development of novel mechanisms for storage of signals in biomolecules (*e.g.,* polypeptides).**
    - [Bottleneck]: Long-term, robust and stable data storage in biological systems.
        - [Potential Solution]: Encoding of information into DNA (*i.e.,* via CRISPR) in order to obtain a permanent storage of information.
- **2030 Milestone: Ability to engineer new hetero-complex surface receptors for multiplexed sensing sensing and cell-to-cell signaling.**

- [Bottleneck]: Unknown design rules for making easily customizable receptors that can either be multi-responsive themselves or can be deposited onto a patterned surface with other receptors without cross-interference.
    - [Potential Solution]: An easily reconfigurable “test-bed” or chip that can rapidly go through a wide variety of potential receptor candidates for both multiplexed behavior or patterned arrays, lowering the cost and time to test new sensing materials.

- **2030 Milestone: Custom integration of signals over different time and spatial scales by cells or cell-free circuits, to process inputs and direct cellular response.**
    - [Bottleneck]: Integrating information shared between cells that is noisy, relative or variable, and highly localized.
        - [Potential Solution]: Develop distributed algorithms for reliably integrating information that are simple enough to be implemented within cells as biochemical or bio-electrical programs.
        - [Potential Solution]: Use a consortium of cells each with a specific sensing capability and utilizing a distributed sensing and centralized memory approach to integrate signals.
    - [Bottleneck]: Within a multi-strain type of setup, multiple strains will need a way to co-exist together while keeping/adapting their ratios as appropriate.
        - [Potential Solution]: Creation of robust population controllers that work across environmental conditions (*e.g.,* pH, temperature, nutrients).
    - [Bottleneck]: Sensing and signal integration in either bacterial or cell-free synthetic biology is highly fragile to environmental conditions (*e.g.,* nutrients, temperature, pH).
        - [Potential Solution]: Determine environmental conditions typical of applications that match up with bacterial/cellular viability.
    - [Bottleneck]: It is challenging to elicit a robust response for signals that may have different amplitudes and duration.
        - [Potential Solution]: Simultaneous comparative measurements; for example, in one set-up measure one type of signal (*e.g.,* low amplitude, high gain), in another set-up, a different type of signal (*e.g.,* slow or fast signal).

- **2040 Milestone: Engineer materials with sentinel sensor networks that can sense and integrate numerous signals.**
    - [Bottleneck]: Design of networks that can integrate more than one or two types of signals.
        - [Potential Solution]: Develop computational methods able to parse the multiple forms of data signaling.
    - [Bottleneck]: Ability to have numerous sensors (of the correct scale) integrating data with minimal crosstalk to reduce false signals/data being used in the sensing/monitoring/feedback scheme.
        - [Potentials Solution]: Engineer sensors that can effectively function within a “small relevant volume” where, by necessity, many chemical reactions are happening at the same time.

    - [Bottleneck]: Transfer of information from biological sensors to abiotic systems.
        - [Potential Solution]: Design functional materials that can bridge to biological systems; for example, electrically responsive materials that can integrate information with bioelectric systems (*e.g.,* nervous system).
    - [Bottleneck]: Real-time sensing with living cells and/or cell-free systems.
        - [Potential Solution]: Remove gene expression from the sensing process and instead use signal transduction or other similarly fast processes.
- **2040 Milestone: Develop new forms of communication from biological systems, such as light (including infrared and radio frequency) or mechanical force (*e.g.,* sound) production.**
    - [Bottleneck]: Lack of natural systems to be exploited or used as the basis for further engineering.
        - [Potential Solution]: Rational design of biological elements, mimicking synthetic systems with desired properties.

Breakthrough Capability: Enable biological control through abiotic materials.

- **2022 Milestone: Develop cell-like materials that can secrete molecular information.**
    - [Bottleneck]: Stimuli-responsive release and/or transport of biological molecules (*e.g.,* cytokines, transcription factors, hormones).
        - [Potential Solution]: Engineer stimuli-responsive chemical scaffolds to encapsulate/release biological molecules.
        - [Potential Solution]: Development of synthetic cell systems capable of secretion.
- **2022 Milestone: Utilize stimuli-responsive proteins (*e.g.,* light-responsive, pH-responsive, temperature-responsive, mechanosensitive) and demonstrate their use in augmenting performances of polymeric materials.**
    - [Bottleneck]: Incorporating reactive components into custom synthesized structural proteins and assembled in a desired structure.
        - [Potential Solution]: Understand the structure-property relationship between the synthesized protein and the assembled material system.
        - [Potential Solution]: A structural “test-bed” to incorporate new proteins in various configurations to work out design-rules for utilizing stimuli-responsive proteins.
        - [Potential Solution]: Explore proteins that are known to have large scale actuation ability when they are self-assembled into supramolecular complexes.
        - [Potential Solution]: Explore different types of elastin-like polypeptides that can be deformed elastically and incorporate them into polymeric materials.
- **2025 Milestone: Incorporate a variety of natural proteins that sense abiotic stimuli into biological composites and demonstrate combinatorial sensing.**
    - [Bottleneck]: Emulating the sensing mechanisms and dynamic interplay between functional proteins and their native lipid environment.

        - [Potential Solution]: Understand the design rules and collective lipid dynamics involved in the regulation of protein functions using high-resolution structural and dynamical characterization methods.
    - [Bottleneck]: Integration of functional membrane proteins into synthetic membrane materials.[22]
        - [Potential Solution]: Development of cell-free strategies for synthesizing membrane proteins.
        - [Potential Solution]: Explore membrane protein behavior and function in non-lipid-based amphipathic material; neutron spectroscopy would be useful here, as this can "mask" different parts of protein and amphipathic material through labeling, allowing the study of behavior and structure at the same time.
- **2025 Milestone: Engineer arrested metabolism or programmed cell death upon task completion, by either external stimuli or by the cell sensing that the environmental condition to be monitored is not present any longer.**
    - [Bottleneck]: Identifying pathways that can be effectively used to couple an non-endogenous input to halting cell growth or leading to cell death.
        - [Potential Solution]: Use of genetic circuits with sensing/feedback mechanisms orthogonal to natural cell pathways that lead to cell death upon a defined signal.
        - [Potential Solution]: Development of chimeric receptors for wiring desired signal to programmed cell death.
- **2030 Milestone: Establish key design principles for polymeric scaffolds or cell-like materials to augment biological functionality.**
    - [Bottleneck]: Aiding and augmenting biological functionality through synthetic materials.
        - [Potential Solution]: Controlled activity of signaling molecules, engineering living cells to reconfigure chemical/physical context, and design feed-back loops or co-dependent cellular systems.
    - [Bottleneck]: Enabling biocomponent persistence within the material.
        - [Potential Solution]: Renewable or regenerative hosts that can encode performance and provide signaling for adaptation of the other components in the materials system.
- **2040 Milestone: Develop polymeric scaffolds or cell-like materials that can augment functions of natural living cells.**
    - [Bottleneck]: Mimicking the diverse functions of natural systems on demand.
        - [Potential Solution]: Designing the architecture and material compliance, sensing, and other properties of biological systems within polymeric scaffolds and other materials.

Breakthrough Capability: Utilize biology to enable chemical, thermal, kinetic, and electrical storage and release from materials.

- **2022 Milestone: Engineer more efficient biomolecules and strain chassis that can store and release energy.**

- [Bottleneck]: Limited chemistry with existing natural chassis.
  - [Potential Solution]: Engineer orthogonal translation systems to incorporate amino acids with non-native R-groups, new classes of amino acids, and/or new monomers altogether.
  - [Potential Solution]: Use hybrid biological-chemical synthesis methods (*e.g.,* click chemistry) to incorporate non-biological chemistries into biological substrate molecules.
- [Bottleneck]: Limited biological materials that can trap, store, and damp kinetic energy (*e.g.,* trapping high speed objectives).
  - [Potential Solution]: Bio-prospect natural materials with high energy damping capacities.
  - [Potential Solution]: *De novo* design of protein materials with predictable strength, toughness, and high energy damping capacity.

- **2022 Milestone: Develop biological structures to localize and release dynamically abiotic components.**
  - [Bottleneck]: Difficulty in selecting components from a complex environment due to non-specific interactions.
    - [Potential Solution]: Identify optimal structural components (types of peptides or nucleic acid motifs) and their material features (*e.g.,* density, operation temperature) for selective capture/release.
- **2025 Milestone: Engineer energy storage and release from biocomponents in response to environmental stimuli.**
  - [Bottleneck]: Directing genetic and metabolic circuit activity in response to environmental stimuli.
    - [Potential Solution]: Develop tightly controlled, stimuli-responsive, high-yield secretion machinery and couple it with the synthesis of biomolecule-of-interest.
- **2030 Milestone: Engineer materials that allow energy (*i.e.,* thermal, chemical, electrical) storage and release to a broad range of stimuli on-demand.**
  - [Bottleneck]: Engineering the energy storage and release mechanism.
    - [Potential Solution]: Design and build energy storage mechanisms enabled by biocomponents (*e.g.,* anisotropic, capacitor-like structure that is self-organized by biocomponent within the material).
- **2040 Milestone: Capability to combine multiple storage-release mechanisms within a single material.**

Breakthrough Capability: Tools and techniques for characterizing material biocomponent dynamics.

- **2022 Milestone: Develop automation and other high-throughput methods for testing cellular sense-response across a range of conditions and with conditions that change in time of cells in suspension.**
- **2025 Milestone: Enable visualization of cellular behavior (*e.g.,* viability, morphology, locomotion) upon materials deformation.**

    - [Bottleneck]: Current techniques do not enable *in situ* correlation of cellular behavior upon material distortion.
        - [Potential Solution]: Combine indentation/microrheology set-up with high-resolution imaging of living cells.
- **2025 Milestone: Develop automation and other high-throughput methods for testing cellular sense-response across a range of dynamic conditions in solid (biotic or composite) materials.**
- **2030 Milestone: Develop techniques to measure cell properties and function in the presence of abiotic material components, which often interfere with current measurement methods.**
    - [Bottleneck]: Cost and availability of precursors to create observable isotopically substituted systems.
        - [Potential Solution]: Depending on the system, isotopic substitution may make certain parts of the system "invisible" to neutron scattering techniques.
- **2030 Milestone: Enable techniques to determine the conformational and configurational entropy in kinetically-trapped biological materials at molecular scale, to design and control function and property.**
    - [Bottleneck]: Biocomponents currently must exist primarily in dynamic fluid environments that are difficult to capture.
        - [Potential Solution]: Use hydrostatic pressure to probe biomaterial properties.
- **2030 Milestone: High-throughput methods to test sense-response materials *in situ* in a range of realistic use environments.**
- **2030 Milestone: Develop characterization methods for understanding cellular behavior (locomotion, deformation, viability) upon shear stress.**

Breakthrough Capability: Tools and technologies to measure materials properties and performance that operate at biological throughput and scale.

- **2025 Milestone: Develop appropriate modeling techniques for materials that include living cells that correlate well with experimentally observed properties.**
    - [Bottleneck]: Materials with biocomponents are inherently different from conventional homogeneous elastic/viscoelastic materials.
        - [Potential Solution]: Develop datasets of standard materials properties of living cells.
- **2025 Milestone: Models that can learn from different classes of experiments used to test material properties.**
    - [Bottleneck]: Data are not available for dynamic activities.
        - [Potential Solution]: Sourcing of data from physics/materials datasets to predict bio-material behavior.
- **2025 Milestone: Structure-property mapping via high-throughput screening of materials with biocomponents.**
    - [Bottleneck]: Currently available high-throughput techniques are not designed to map structure-property relationships of biologics.

        - [Potential Solution]: Modify existing techniques (*e.g.,* ssNMR, femtosecond optics, fluorescence) or create novel techniques to measure dynamics.
- **2025 Milestone: Realization of high-throughput methods for physical property characterization (*e.g.,* adhesion, tensile strength).**
    - [Bottleneck]: The quantity of materials experimentally produced is significantly smaller than the quantity needed for existing testing methods.
        - [Potential Solution]: Characterization methods that have the ability to utilize smaller sample sizes.
        - [Potential Solution]: Pilot scale-up of materials more readily realized.
    - [Bottleneck]: Testing in biologically relevant environments (*e.g.,* in the presence of water, salts) and maintaining cell viability.
        - [Potential Solution]: Characterization methods that can be accomplished with samples in standardized formats for biological samples (*e.g.,* 96 well plates).
- **2030 Milestone: Develop high-resolution and three-dimensional imaging methods to capture subtle differences in biological components within materials.**
    - [Bottleneck]: Improve and develop conventional microscopy techniques for this purpose.
        - [Potential Solution]: Expansion microscopy designed to visualize subcellular context of living cells within materials.
        - [Potential Solution]: EM tomography repurposed to be utilized for visualizing cell and materials interface.
- **2040 Milestone: *In vivo* characterization of materials produced in real time.**
    - [Bottleneck]: Most characterization techniques rely on methods that can only be utilized after the material has been produced as they may have deleterious effects on the material production.
        - [Potential Solution]: Genetic systems to allow for developmental type studies of hierarchical materials assembly *in vivo.*
- **2040 Milestone: Expansion of 2D measurement methods to bulk anisotropic 3D structures.**
    - [Bottleneck]: Limitations of visualization tools and technologies.
        - [Potential Solution]: Hybrid AFM and confocal imaging; femtosecond optics to measure multiple layers.

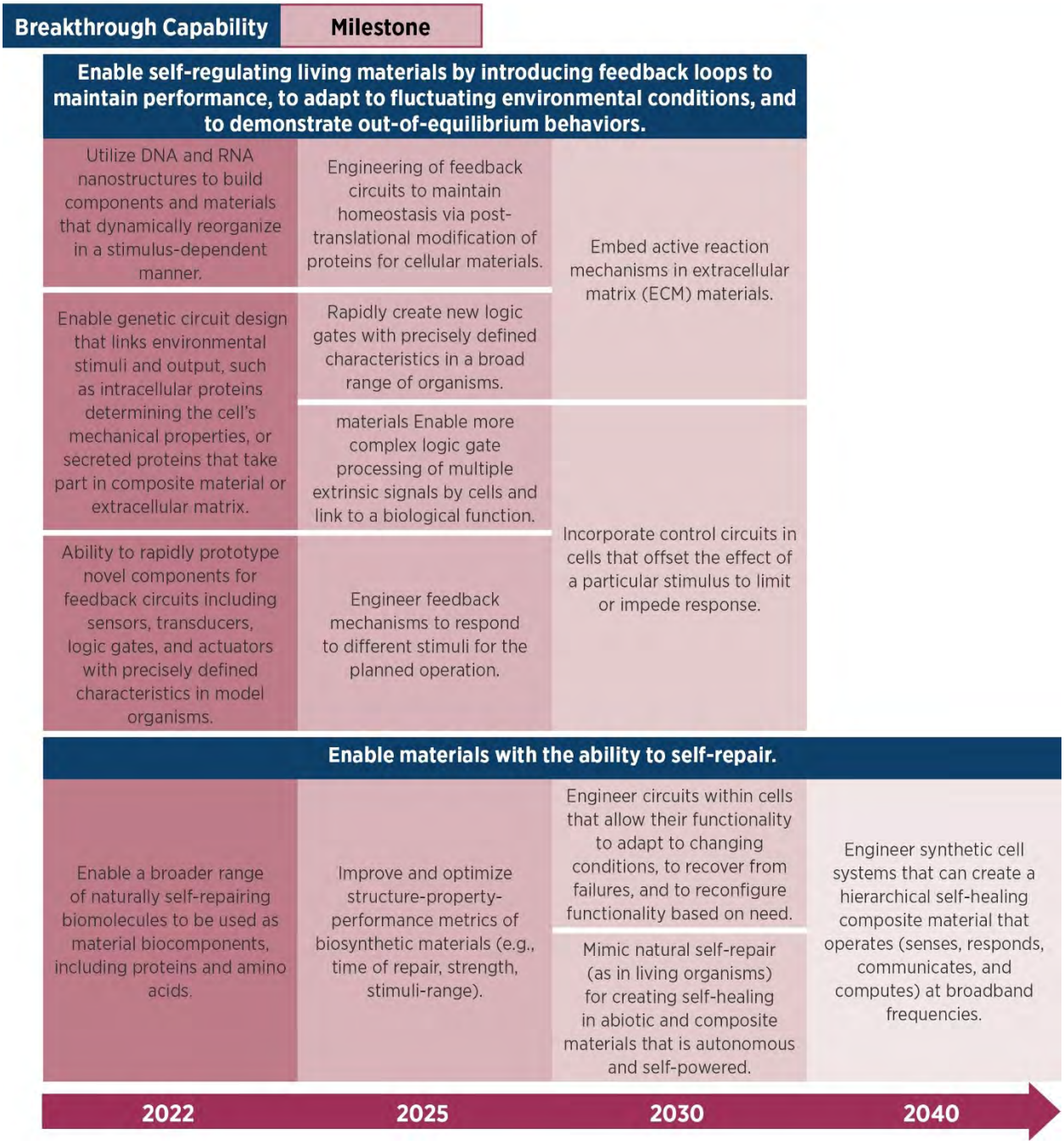
Included below are select breakthrough capabilities from our 2019 roadmap, *Engineering Biology*[1] (Milestones at 2021, 2024, 2029, and 2039). While these breakthrough capabilities were written in the context of advancing the field of engineering biology, the technical achievements elaborated in these breakthrough capabilities and their milestones are likely to directly contribute to achieving advancements in materials from engineering biology. This content has been incorporated as reference and, when pertinent, is provided with context for its inclusion in this roadmap (as Notes).

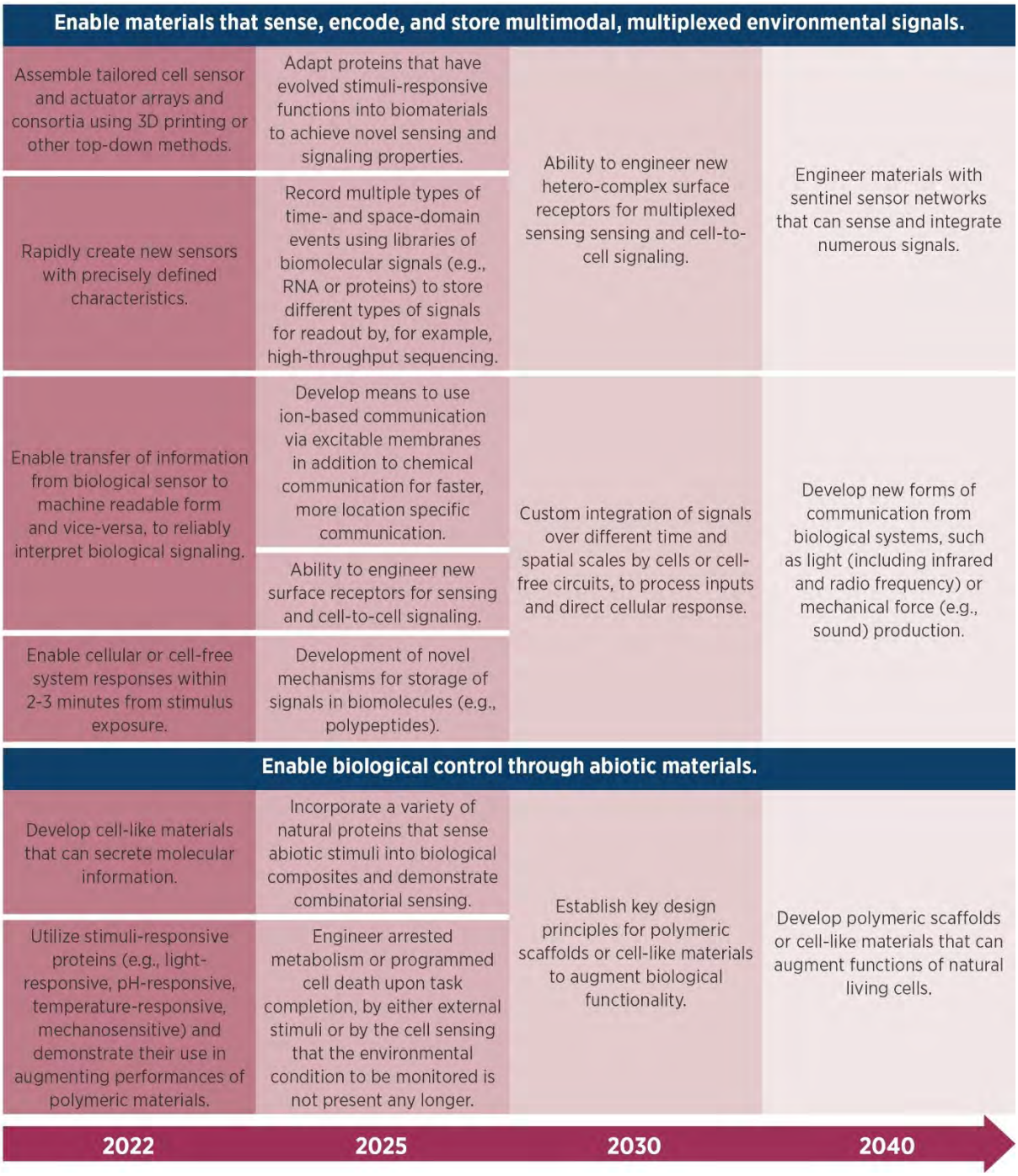
Engineering Biology breakthrough capability: Ability to control cell-to-cell communication between different species.

Note: of particular importance for enabling dynamic materials is the ability to engineer signaling and sensing from surface-to-surface through cell-to-cell contact. We envision this as a 2025 milestone. A major bottleneck to this biotechnology is that the ligand-receptor pairs that are functionally expressed across different species remains unknown, but could serve as a general library for advancing this engineering biology. Potential solutions to this bottleneck include: identifying and testing common ligand-receptor pairs that are retained across species; and/or identifying ligand receptor pairs that can be functionally expressed in non-native hosts.

- **2021: Tightly-controlled promoter-response regulator systems that enable intra- and inter-species cellular communication.**
- **2024: Synthetic cell-to-cell communication elements and networks that function in a broad range of host organisms.**
- **2029: Signal-response pathways that function in synthetic communities of 5-10 organisms, employing a variety of pathway types and host species.**
- **2039: Ability to produce engineered microorganisms that can reliably invade and coexist within a complex community and manipulate the consortium/biome function and behavior.**

ENGINEERING BIOLOGY & MATERIALS SCIENCE PROPERTIES & PERFORMANCE




Enable materials that sense, encode, and store multimodal, multiplexed environmental signals.
Assemble tailored cell sensor and actuator arrays and consortia using 3D printing or other top-down methods.
Adapt proteins that have evolved stimuli-responsive functions into biomaterials to achieve novel sensing and signaling properties.
Ability to engineer new hetero-complex surface receptors for multiplexed sensing sensing and cell-to-cell signaling.
Engineer materials with sentinel sensor networks that can sense and integrate numerous signals.
Rapidly create new sensors with precisely defined characteristics.
Record multiple types of time- and space-domain events using libraries of biomolecular signals (e.g., RNA or proteins) to store different types of signals for readout by, for example, high-throughput sequencing.
Enable transfer of information from biological sensor to machine readable form and vice-versa, to reliably interpret biological signaling.
Develop means to use ion-based communication via excitable membranes in addition to chemical communication for faster, more location specific communication.
Ability to engineer new surface receptors for sensing and cell-to-cell signaling.
Custom integration of signals over different time and spatial scales by cells or cell-free circuits, to process inputs and direct cellular response.
Develop new forms of communication from biological systems, such as light (including infrared and radio frequency) or mechanical force (e.g., sound) production.
Enable cellular or cell-free system responses within 2-3 minutes from stimulus exposure.
Development of novel mechanisms for storage of signals in biomolecules (e.g., polypeptides).
Enable biological control through abiotic materials.
Develop cell-like materials that can secrete molecular information.
Incorporate a variety of natural proteins that sense abiotic stimuli into biological composites and demonstrate combinatorial sensing.
Establish key design principles for polymeric scaffolds or cell-like materials to augment biological functionality.
Develop polymeric scaffolds or cell-like materials that can augment functions of natural living cells.
Utilize stimuli-responsive proteins (e.g., light-responsive, pH-responsive, temperature-responsive, mechanosensitive) and demonstrate their use in augmenting performances of polymeric materials.
Engineer arrested metabolism or programmed cell death upon task completion, by either external stimuli or by the cell sensing that the environmental condition to be monitored is not present any longer.
2022
2025
2030
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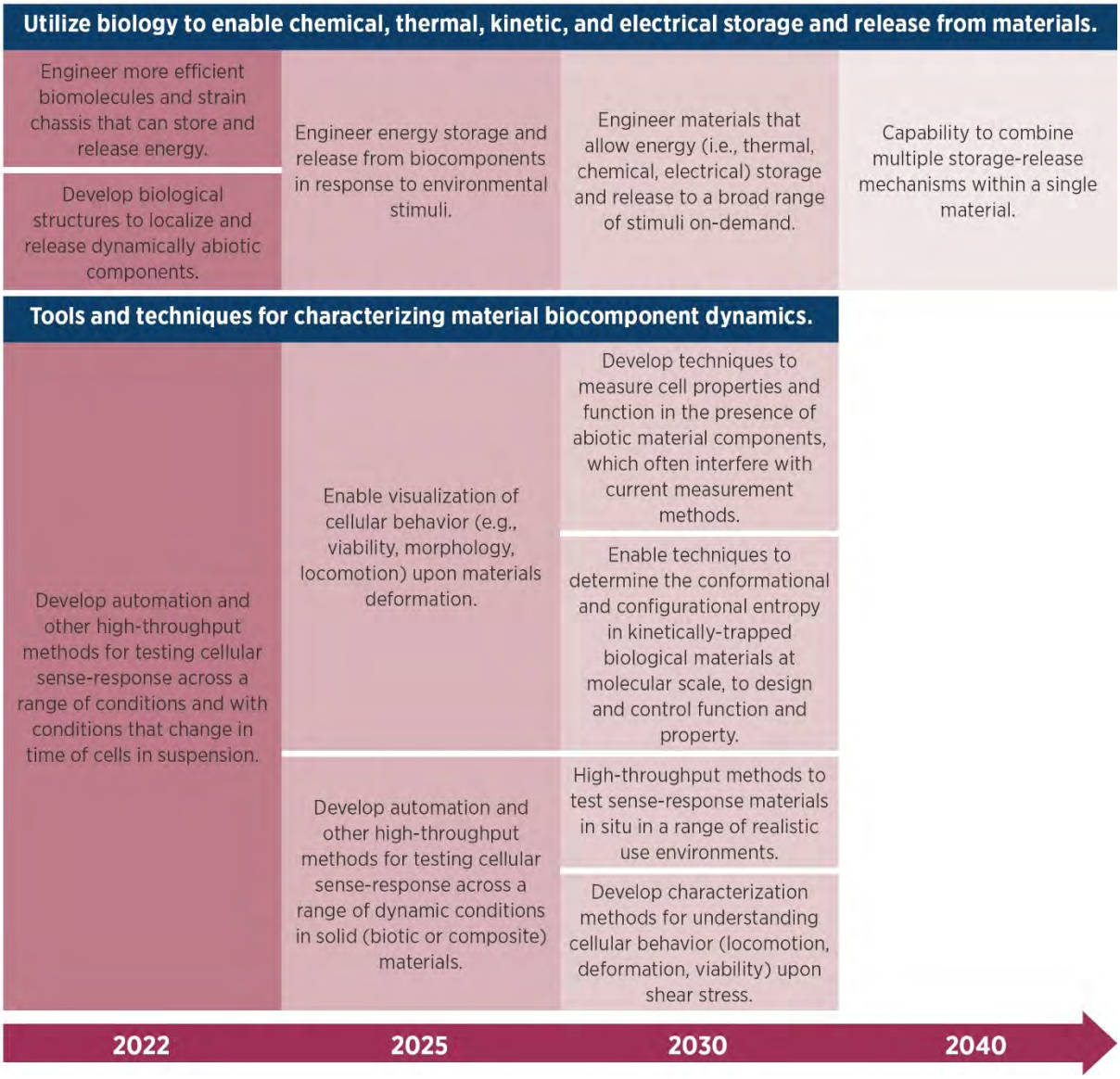
Utilize biology to enable chemical, thermal, kinetic, and electrical storage and release from materials.
Engineer more efficient biomolecules and strain chassis that can store and release energy.
Develop biological structures to localize and release dynamically abiotic components.
Engineer energy storage and release from biocomponents in response to environmental stimuli.
Engineer materials that allow energy (i.e., thermal, chemical, electrical) storage and release to a broad range of stimuli on-demand.
Capability to combine multiple storage-release mechanisms within a single material.
Tools and techniques for characterizing material biocomponent dynamics.
Develop automation and other high-throughput methods for testing cellular sense-response across a range of conditions and with conditions that change in time of cells in suspension.
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2022
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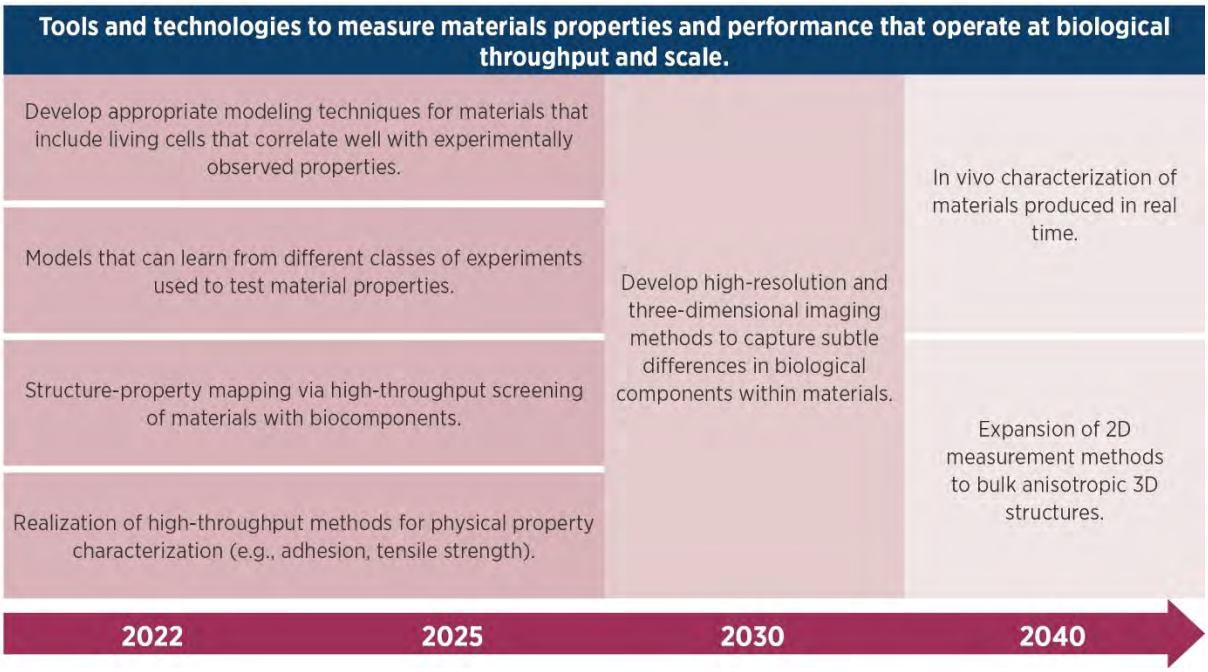
Tools and technologies to measure materials properties and performance that operate at biological throughput and scale.
Develop appropriate modeling techniques for materials that include living cells that correlate well with experimentally observed properties.
Models that can learn from different classes of experiments used to test material properties.
Structure-property mapping via high-throughput screening of materials with biocomponents.
Realization of high-throughput methods for physical property characterization (e.g., adhesion, tensile strength).
Develop high-resolution and three-dimensional imaging methods to capture subtle differences in biological components within materials.
In vivo characterization of materials produced in real time.
Expansion of 2D measurement methods to bulk anisotropic 3D structures.
2022
2025
2030
2040

Application Sectors

The roadmap consists of five application sectors to illustrate the potential applications at the intersection of materials and engineering biology; these application sectors and the associated societal challenges are captured from *Engineering Biology*[1] and represent a broad consideration of significant economic and social roadblocks towards advancing the way we live and thrive. Within each application sector we highlight a number of exemplar applications of materials from engineering biology that will help us overcome these pervasive societal challenges, such as enabling and establishing a cleaner environment, supporting the health and well-being of growing populations, and accelerating innovation and economic viability of industry. We further identify potential discrete technical achievements necessary to obtain those exemplar applications. These exemplar applications and technical achievements reflect and tie together the advancements envisioned in the roadmap's technical themes.

Food & Agriculture

Food & Agriculture focuses on the tools and technologies impacting how we feed the Earth's people and animals. Innovative materials have the potential to impact the way we grow, harvest, and distribute food, by protecting agriculture and feed from pests and the effects of weather and climate change and through preserving foods following harvest or processing as they are transported, stored, and sold. Advancements in engineering biology and materials science also have the potential to further revolutionize biology-based materials that are themselves food, such as plant-derived meats.

Challenge: Sustainably produce more food for a growing global population.

- ➔ **[Exemplar Application]: Packaging materials or coatings for foods that indicate spoilage or protect foods from spoilage.**
  - Technical Achievement: Engineer ethylene absorbing microbial coatings that can be applied to a broad variety of foods.[23]
  - Technical Achievement: Engineer materials with inherent sensing capabilities that recognize and indicate the presence of pathogens or spoilage metabolites, such as chemically-triggered coatings that release anti-bacterial agents or sentinel microbes.
  - Technical Achievement: Create packaging that incorporates engineered pathogen predators, including bacteriophages an predatory bacteria (i.e. BALOs), that prey on microbes that drive spoilage.[7]
  - Technical Achievement: Engineer materials that produce optical mass read-out signals (such as by changing surface absorption or reflectivity).
- ➔ **[Exemplar Application]: Materials for crop pest control.**
  - Technical Achievement: Engineer biodegradable crop-coating materials with pest-deterrent biological components, such as pheromones or baculoviruses.[24]
  - Technical Achievement: Engineer tree, plant, or soil coatings or coverings that can sense and respond to pest presence with mass read-out capability, such as changes in IR reflectivity, to be detected by drone or satellite.
- ➔ **[Exemplar Application]: Degradable weed control/suppression cover materials.**
  - Technical Achievement: Engineer biodegradable hydrogels that incorporate herbicides or microbial sentinels that inhibit growth of undesirable plants.
  - Technical Achievement: Engineer derivatized polysaccharide sprayable polymer coatings that inhibit early stage biofilm formation.
- ➔ **[Exemplar Application]: Materials that retain water, protect from extreme weather, or provide nutrients and/or soil amendments in the field.**
  - Technical Achievement: Seed-coating polymers that can be applied prior to planting to protect and enhance growth of emerging seedlings.
  - Technical Achievement: Engineer materials/capsules that release cargo in response to analyte (such as pathogen or fertilizer) detection.
  - Technical Achievement: Engineer materials that incorporate aquaporins to release water or ion channels to remove excess salt.[25]

- Technical Achievement: Engineer biodegradable coatings that alter freezing point (such as incorporating antifreeze proteins) or prevent evaporation in response to weather change.

➔ **[Exemplar Application]: Reduce demand for nitrogen fertilizers and ammonia synthesis.**

- Technical Achievement: Establish materials to support engineered rhizosphere microbiomes to enhance the rate of localized N_2 fixation.
- Technical Achievement: Develop materials for delivering microbial cultures (such as algae and cyanobacteria) to serve as agricultural fertilizers.

Challenge: Increase and improve the nutritional content and value of food.

➔ **[Exemplar Application]: Advance alternative proteins and cellular or cultivated meats.**

- Technical Achievement: Improve prototyping matrices for more intricate biomolecule and cellular scaffolding.
- Technical Achievement: Advance the incorporation of additional nutritional components besides proteins in lab-grown meats.
- Technical Achievement: Use biologically-derived polymers (*e.g.,* cellulose, hyphae, mycelia) to improve textures of plant-based proteins.

➔ **[Exemplar Application]: Materials to detect and control ripening.**

- Technical Achievement: Engineer materials that produce or incorporate cyclodextrins, cyclodextrin-like molecules.
- Technical Achievement: Engineer the application of interface materials for stems or surfaces of harvested crops that would seal or provide a means to introduce desired chemicals for the control of ripening.
- Technical Achievement: Materials to detect and report biomolecules associated with over-ripening/spoilage in food storage facilities (*e.g.,* ethylene detecting materials in apple warehouses).

Environmental Biotechnology

Environmental Biotechnology focuses on materials to monitor and mitigate the effects of climate change and build and interact more efficiently and sustainably with our surroundings. The materials highlighted in this sector may help us to better adapt to hotter, drier lands, and more extreme weather. *Environmental Biotechnology* considers materials that incorporate biocomponents to sense and respond to greenhouse gases and enable carbon capture. Integrating engineered biology and materials can advance water and air filtration, contributing to resource recovery, bioremediation, and decontamination. Engineering biology can also contribute to more resilient and sustainable materials for the built environment.

Challenge: Address and mitigate climate change.

- ➔ **[Exemplar Application]: Materials that can regulate, or are adaptive to, heat and moisture.**
  - ◆ Technical Achievement: Advanced stimuli-responsive polymers or hydrogels with tunable functionality and environmental adaptability.
  - ◆ Technical Achievement: Engineer flexible thermoelectric or heat converting materials to transform environmental heat into other useful forms of energy.
  - ◆ Technical Achievement: Engineer soft materials and membranes incorporating the adaptability of extremophiles to harsh environmental conditions.
  - ◆ Technical Achievement: Advance computational modeling of hierarchical and functional properties of candidate soft materials under extreme conditions.
- ➔ **[Exemplar Application]: Materials to enhance carbon capture from the atmosphere.**
  - ◆ Technical Achievement: Engineer structured surfaces for seeding photosynthetic biofilms that maximize light penetration and energy capture per unit surface area.
  - ◆ Technical Achievement: Engineer functionalized polymeric thin films with CO_2 capturing moieties or particles (such as metal-organic frameworks) that trigger conformational or fluorescence response (*e.g.,* stress sensors) upon gas capture.
- ➔ **[Exemplar Application]: Easily-recyclable materials with desirable mechanical properties.**
  - ◆ Technical Achievement: Engineer biologically-derived materials with restricted or incomplete depolymerization that can be used to replace plastic bottles and bags, and can be easily recycled through an environmental process into new materials with similar mechanical properties.
  - ◆ Technical Achievement: Development of strains capable of growth on plastics at reasonable growth rates and organisms that convert “bad” plastic into “good” biodegradable plastic at scale.
  - ◆ Technical Achievement: Engineer biomaterials that degrade into environmentally-friendly products both aerobically and anaerobically.

Challenge: Expand tool sets for bioremediation and resource recycling.

- ➔ **[Exemplar Application]: Materials for detection and removal of contaminants.**

- Technical Achievement: Engineer stimuli-responsive, biodegradable polymers with on-demand self-healing properties, enabling active repair of fouled or damaged filter membranes.
- Technical Achievement: Demonstrate cell-free sensors, embedded in shelf-stable materials that can detect water contaminants at EPA limits (*e.g.,* lead, fecal coliforms).
- Technical Achievement: Demonstrate cell-free sensors, embedded in shelf-stable materials that can robustly report on contaminants within 15 minutes.[26]
- Technical Achievement: Enable multiplexed detection of 10 or more chemical contaminants (excluding RNA) in a single device.
- Technical Achievement: Engineer novel biomaterials that incorporate obligate hydrocarbonoclastic microbes (*i.e.,* "oil-eating" bacteria).[27]

➔ **[Exemplar Application]: Materials for bioremediation of environments.**

- Technical Achievement: Synthesize porous materials completely or mainly from refuse (*e.g.,* plants, tree bark, shrimp shells); similar to molecular sieves (zeolites) or metal-organic frameworks, these materials may function for diverse purposes (*e.g.,* CO_2 sequestration, water purification, metal uptake, sensors).
- Technical Achievement: Develop mechanism-based toolkit to approach material breakdown coupled to genetic circuits/chassis engineering to provide new platforms (*e.g.,* hydrolysis, singlet oxygen generation, photo-driven cleavage) for remediation.

Challenge: Enable sustainable, more environmentally-friendly materials and infrastructure development.

➔ **[Exemplar Application]: Stiff, strong, porous, low-density materials for infrastructure applications.**

- Technical Achievement: Enable detailed characterization and greater control of material properties of biologic components.
- Technical Achievement: Enable biologically-driven tailor-ability of materials for infrastructure.
- Technical achievement: Design durable, porous, self-repairing surface coatings (*e.g.,* asphalt replacements) to reduce flooding.

➔ **[Exemplar Application]: Cross-laminated biofilms and wood-like materials.**

- Technical Achievement: Enable greater cellulose secretion with controlled crystallinity to impart reinforcement/toughness in response to specific cues.
- Technical Achievement: Engineer specialized lignin that better interacts with adhesives.

➔ **[Exemplar Application]: Materials that can actively adapt to changing environmental conditions.**

- Technical Achievement: Engineer radiation-resistant materials, such as by incorporating *Deinococcus radiodurans* or enabling radiation-resistance in other microbes, to enhance weatherability.[28]

- Technical Achievement: Protein-based materials that can store and release heat reversibly could be used to melt snow/ice (*e.g.,* ice-structuring and ice-nucleating proteins).

Industrial Biotechnology

Industrial Biotechnology focuses on the intersection of materials and engineered biology for robust, sustainable, and efficient manufacturing and production. Engineering biology can contribute to materials that enable better separations and resource uptake and recycling. Materials incorporating engineered biology may make production more environmentally-friendly or cost-effective. Engineering biology also holds the potential to advance industry and the economy through interfacing with abiotic materials and technologies, such as electronics, fabrics, and packaging, to create new systems and devices.

Challenge: Enable next-generation production through sustainable, cost-competitive, flexible, and efficient manufacturing processes.

- ➔ **[Exemplar Application]: Processes that enable circularity by utilizing complex waste resources that cannot be recycled with existing technologies.**
  - ◆ Technical achievement: Enable efficient breakdown of complex waste plastics (particularly mixed waste streams or multi-layer plastics such as those found in carpets or shoes) by engineered enzymes (*e.g.,* PETases) or hybrid chemical-biological processes (*e.g.,* gasification and fermentation) to make virgin materials.
- ➔ **[Exemplar Application]: Materials that enable low-cost, low-energy separation of bioproducts from aqueous solutions.**
  - ◆ Technical Achievement: Identify, develop, and demonstrate separation membranes composed of biologically produced monomers/materials.
  - ◆ Technical Achievement: Identify, develop, and demonstrate biocompatible solvents/adsorbents capable of extracting molecules from fermentation broths.
- ➔ **[Exemplar Application]: Materials for biomining and uptake of metals.**
  - ◆ Technical Achievement: Develop a broader range of biological systems (*e.g.,* viral, bacterial, fungal-based) engineered to accumulate metals.
  - ◆ Technical Achievement: Tune biological systems for metal uptake, particularly bioaccumulation of only desired products (*e.g.,* rare earth or precious metals).
  - ◆ Technical Achievement: Use natural biological polymers harnessed from biomining and functionalize with synthetic chemistries to endow tunable chemical and biophysical properties.

Challenge: Scalable production of novel and existing products that are more sustainable and economically- and environmentally-friendly.

- ➔ **[Exemplar Application]: Biocomputers and cells and biosystems that interface, or function like, electronics.**
  - ◆ Technical Achievement: Enable selective ion channel (*e.g.,* potassium) activation in response to gene circuit initiation.
  - ◆ Technical Achievement: Enable stronger cell-electronics interfaces through novel cell surface proteins and polymers.
  - ◆ Technical Achievement: Enable swarm decision making, such as engineering large collections of cells that can apply an algorithm providing a statistical answer to a complex multivariable problem.

- Technical Achievement: Create artificial retina by integrating patterned cells or artificial cells sensitive to light with multi-electrode arrays.

➔ **[Exemplar Application]: Electrically conductive biofilms for radiofrequency shielding/cloaking.**

- Technical Achievement: Enable synthesis of bio-wires such as cytochrome chains or ferritin chains which cross link between cells.
- Technical Achievement: Engineer sensing inputs that instruct the limit of biofilm layer thickness needed for specific frequency range.
- Technical Achievement: Enable persistence of materials with biological components in harsh or extreme environments.

➔ **[Exemplar Application]: Self-repairing materials for consumer products and the built environment.**

- Technical Achievement: Engineer materials that retain their biological function during use that are triggered only as needed.
- Technical Achievement: Incorporate biocomponents to sense damage and repair through biofabrication of new material.
- Technical Achievement: Engineer materials that can trigger reorganization or alter interaction networks to fix gaps or damage in the material.
- Technical Achievement: Incorporation of microbes into construction materials (e.g., concrete, bricks) that produce mineral healing agents.

➔ **[Exemplar Application]: Stimuli-responsive adhesives.**

- Technical Achievement: Engineer controlled electrically- or magnetically-responsive materials where the physical properties are locked into the desired state.
- Technical Achievement: Engineer adhesives utilizing protein phase transitions that are reversible above and below certain temperatures.
- Technical Achievement: Develop biologically-derived adhesives that can bond together surfaces with different mechanical properties (*e.g.,* soft-to-hard surface).
- Technical Achievement: Exploit biology to engineer self-assembling adhesives that spontaneously construct graded interfaces.

➔ **[Exemplar Application]: Packaging and building materials with adjustable strengths.**

- Technical Achievement: Produce lightweight, high-tensile-strength materials built from engineered spider silk.[29]

Health & Medicine

Health & Medicine focuses on exemplar applications of materials relevant to the well-being of humans, non-human animals, and populations. Prevention and treatment of injuries, diseases, and disorders may be achieved with advancements of materials we currently research and develop, such as gels and scaffolds for tissue repair, but also novel materials that may, for example, incorporate biomolecules or cells to sense and respond to pathogens that come into contact with the body or immediate surroundings. Engineering biology-enabled materials may improve drug targeting and delivery or be used to detect and report biomarkers in ways only currently imagined. And engineering biology has the potential to produce materials that make diagnostics and medicines more accessible and equitable, such as through cell-free engineering.

Challenge: Eradicate existing and emerging infectious diseases.

- ➔ **[Exemplar Application]: Fabrics and other materials to protect against and prevent infection.**
  - Technical Achievement: Engineer systems for attaching biomolecules and biopolymers to fabrics and fibers covalently or noncovalently for stable, robust, and uniform functionalization.
  - Technical Achievement: Engineer fabrics that incorporate metallic nanoparticles or functionalized silica that act to lyse pathogens.
  - Technical Achievement: Synthesize biologically-derived fabrics that have similar or better mechanical properties (strength, toughness, elasticity) as the best petroleum-derived and agriculture-derived fabrics.
  - Technical Achievement: Engineer fabrics that incorporate antimicrobial proteins or peptides, small molecules.
  - Technical Achievement: Engineer fabrics that modulate skin microbiome composition and/or function.
  - Technical Achievement: Engineer fabrics with amphiphilic or structured surfaces that prevent bacterial/microbial growth and proliferation.
  - Technical Achievement: Engineer fabrics that can be ‘re-activated’ with biofunctionalization with formulated soak/washes in defined solutions.
  - Technical achievement: Engineer antimicrobial peptides functionalized with synthetic chemistries or conjugated to inorganic substances to enhance specificity and potency.
  - Technical Achievement: Engineer protein coatings for medical implants (pacemakers, artificial valves, joint replacements) that actively resist biofilm formation.
- ➔ **[Exemplar Application]: Materials that enable transport and storage of vaccines, medications, and diagnostics without a cold-chain, or on-site production, to maximize efficacy and affordability in remote field locations.**
  - Technical Achievement: Engineer sol-gels or similar phase-transition materials to limit diffusion of molecules in room-temperature storage conditions.
  - Technical Achievement: Engineer materials that stabilize proteins against denaturation or refold them, to remove the need for cold-chain storage.

- Technical Achievement: Engineer materials that reduce DNase-, RNase-, protease-, lipase-catalyzed degradation of vaccine and medication components.
- Technical achievement: Improve materials for 'paper strip' cell-free biological diagnostics to improve stability and shelf-life.
- Technical Achievement: Enable materials and manufacturing ability to produce and stand up remote biomanufacturing production facilities, such as construction materials that could be produced on-site via engineered biology.

➔ **[Exemplar Application]: Materials to encapsulate and disperse genetically engineered insect technologies to eradicate disease vectors.**

- Technical achievement: Engineer material coating for mosquito eggs to keep them healthy and viable in diapause at room temperature for >12 months.
- Technical achievement: Enable materials for packaging genetically engineered mosquito eggs to enable remote environmental release while protecting from predation or infection.

Challenge: Address non-communicable diseases and disorders.

➔ **[Exemplar Application]: Materials that can be used as scaffolds for organ-building and tissue engineering.**

- Technical Achievement: Enable printable biomimetic hydrogels with tunable mechanical properties and enable long-term primary cell growth and proliferation.
- Technical Achievement: Engineer soft, conductive (and/or stimulatory) biocompatible materials for repair or replacement of neural damage.
- Technical Achievement: Engineer new and existing extracellular matrix proteins as scaffolds that can be functionalized into new biomaterials with expanded and tailored properties.
- Technical Achievement: Engineer adhesives that bond to unsmooth and wet biological surfaces for tissue repair.
- Technical Achievement: Engineer protein bio-wires with enhanced conductivity to enable 'flexible electronics'.[5]
- Technical Achievement: Engineer proteins for 3D-printing of tissue scaffolds with tunable mechanical properties and controlled biological functions.
- Technical Achievement: Engineer biomaterials endowed with tunable elasticity and strength to meet the demands dynamic tissues.
- Technical Achievement: Engineer coatings for brain implants that prevent scarring of surrounding tissue and prolong implant lifetime.

➔ **[Exemplar Application]: Smart biologics with programmable functions.**

- Technical achievement: Customize pharmacodynamic properties, such as tuned stability of protein or peptide-based transcription, for different biologics to overcome the challenge of short half-lives.
- Technical achievement: Target delivery and localization, and enable specificity, of biologics to tissue or cells of interest.[30]
- Technical achievement: Engineer responsive biologics that sense environmental or metabolic cues to govern activity (*e.g.,* smart insulin that can sense and respond to changes in blood sugar).

- Technical achievement: Engineer biomolecules and cellular biologics to reduce immunogenicity.
- Technical achievement: Engineer biologics endowed with ability to encode multiple new functions.

Challenge: Address environmental threats to health, including toxins, pollution, accidents, radiation, exposure, and injury.

- ➔ **[Exemplar Application]: Eye and skin protection materials.**
  - Technical Achievement: Engineer self-renewing, ultra-thin materials resistant to UV, such as via incorporating or secreting biocomponents (*e.g.,* melanin).
- ➔ **[Exemplar Application]: Bandages that incorporate proteins or cells for accelerated/advanced wound healing.**
  - Technical Achievement: Engineering passive biological approaches to integrate stimuli in materials to enhance wound healing.
  - Technical Achievement: Engineer materials that incorporate persistent clotting factors to promote coagulation.
  - Technical Achievement: Engineer extracellular matrix proteins that exhibit bioactivity and adhesion for wound healing and surgical applications.
- ➔ **[Exemplar Application]: Deployable sensors (including enzymes, cells, and nanoparticles) to detect and report biomarkers.**
  - Technical Achievement: Engineer (bio)sensors that utilize the flow within the fluid medium as an energy source to transmit information and location.

Challenge: Promote equitable access to healthcare, patient representation in research, democratization of medicine, and the development of personalized medicines.

- ➔ **[Exemplar Application]: Materials that monitor and report patient vitals, particularly those vitals that otherwise require complex/expensive machinery (*e.g.,* magnetic resonance imaging) to monitor.**
  - Technical Achievement: Engineer stimuli-responsive materials that create sufficient contrast for high-resolution, noninvasive visualization of tissues (*e.g.,* ultrasound).
  - Technical Achievement: Develop nanoscale materials that recognize specific biomarkers for detection and/or tagging of tissues for surgical intervention.
- ➔ **[Exemplar Application]: Cell-free systems for democratizing access to medicines, materials, and diagnostics.**
  - Technical Achievement: Develop low-cost, portable, on-demand cell-free synthesis platforms to make vaccines and therapeutics in freeze-dried lysates.
  - Technical Achievement: Develop low-cost, portable, on-demand cell-free diagnostic platforms to monitor patient vitals, viral load, or environmental exposures.

Energy

Energy focuses on materials that can help to achieve reliable, affordable, clean energy and to help reduce the consumption of energy worldwide. This includes engineering biology-enabled materials that can capture, store, convert, or produce energy, including thermal, kinetic, or electrical energy (details can also be found in the technical Breakthrough Capability: Utilize biology to enable chemical, thermal, kinetic, and electrical storage and release from materials). *Energy* also considers the application of transistors and semiconductors enabled by engineered biology.

Challenge: Produce affordable and clean energy.

- ➔ **[Exemplar Application]: Materials that capture, convert, and/or produce electricity.**
  - Technical Achievement: Engineer materials that utilize traditional proton pumps to generate electrical current.
  - Technical Achievement: Biologically-based design of electrically responsive materials.
  - Technical Achievement: Develop bioinspired materials for artificial photosynthesis capable of producing energy at large scale.
- ➔ **[Exemplar Application]: Transistors and semiconductors made with biology.**
  - Technical Achievement: Engineer carboxysomes or similar organelles to synthesize multilayer semiconductor nanoparticles with tailored layer structure for specific optical emission.
  - Technical Achievement: Engineer peptide or protein-based nanowires with enhanced conductivity.
  - Technical Achievement: Advance engineering of hybrid biomaterials at the biotic/abiotic interface.[31]

Challenge: Reduce global energy consumption.

- ➔ **[Exemplar Application]: Construction materials that get stronger with usage/increased loads (such as for bridges).**
  - Technical Achievement: Design tools to link performance characteristics to biological systems that have the required characteristics, to create a directory of biological functions that may be integrated in materials design.
  - Technical Achievement: Achieve everlasting synthetic cells which can incorporate synthesizing materials for the controlled chemical reaction and deposition based on engineered sensors within the “cell wall”; the synthetic cell would be robust to hydration and temperature changes expected for the specific environment of the structure.

Glossary

Definitions and description of terms in the context of this roadmap.

Active Matter - States of matter that exist only because it is driven from equilibrium or that require a steady flow of energy through the system.

Architectured Materials (versus Engineered Materials) **-** Architectured materials are those that derive a property based on the specific arrangement of the material, usually in the form of a scaffold or other structural component. This is in contrast to Engineered Materials that usually refers to the tuning of a material bulk property such as the composition and treatments of an alloy for a particular application. Many natural materials, such as bone or shells, can be considered architectured materials.

Biobased - Materials derived from biomass (in whole or in part), that may have undergone chemical, physical, or biological treatment. Note: the United States Department of Agriculture's BioPreferred Program has a further definition of "biobased" that we find helpful, available at https://www.biopreferred.gov/BioPreferred/faces/pages/BiobasedProducts.xhtml

Biocomposite - A material consisting of two or more biotic components, or a combination of biotic and abiotic components. A biocomposite may be two- or three-dimensional and may be homogeneous or heterogeneous in composition and appearance. The subsequent performance or function of the biocomposite is improved over the function or performance of the components alone.

Biofabricated - Materials produced by living cells and microorganisms, such as bacteria, yeast, and mycelium. For further information, please see Understanding 'Bio' Material Innovation: a primer for the fashion industry.[32]

Bioinspired - Typically referring to novel (usually abiotic/non-biological materials) materials based on or inspired by biology. The advancements described in this roadmap go beyond this classic definition to include (novel, adapted, or advanced) living, biotic materials and materials that incorporate(s) engineered biology (*i.e.,* biocomposites).

Biomimicry - Material or material system built or designed to mimic a function or mechanism in biology. This is usually extending a biological design principle, biochemical reaction, or biological function to systems at very different length or time scales.

Biomaterial - The emerging definition of a biomaterial is any biological substance that has been engineered to interact with biological systems or derived from biological systems for non-biological use. Some have limited this definition as materials for medical purposes, either as a therapeutic (treat, augment, repair or replace bone or a tissue function in a body) or a diagnostic. The inclusion of materials from biology includes new small molecules (such as biofuels or enzymatic precursors), monomers, polymers/gels, mineralized composites, structured material systems, among many others, produced through engineering biology.

Biomolecule - A member of one of several major classes of biological molecules, including proteins, nucleic acids, lipids, and glycans.

Biosynthetic - Refers to molecules that can substitute for natural biochemicals in either biological processes or structure such as non-canonical lipids, nucleic acids, macro-molecules with protein-like function, biomineralization beyond carbon, calcium, or silica (*i.e.,* heavy metals).

Biotemplate - Biological components that act as the pattern or structure for the ordered or stepwise assembly of a material or material system.

Biotic and Abiotic Materials - **Biotic material**: Substance comprised of or produced by living cells or cell-free biological systems. **Abiotic material**: Substance comprised of non-living cells and not produced directly by living cells or cell-free biological systems. In this roadmap we consider tools and technology for the engineering of materials that are (entirely) biological (*e.g.*, living materials or derived from living organisms) and composites of living or biology-based or -produced (biotic or organic) components and non-living (abiotic or inorganic) components, with particular focus for the latter on tools and technologies for the interactions and interface of the components.

Cell-free System - Typically produced by isolating subcellular fractions, a cell-free system is an engineering biology tool for more controlled study of cellular reactions; simplified production of desired chemicals, biomolecules, or materials; or production in extreme or non-natural environments or with non-natural precursors or components. Cell-free expression is the use of cell-free lysate harvested from living cells (bacterial or mammalian cells) that are translationally active. Cell-free expression is used for making proteins of interests outside of living cells.

Composite - A collection of materials or elements, either in an engineered or structured fashion, that has properties which are some superposition of the individual element's properties.

Composition - The make-up or contents of a material or the result of the combination of components. This roadmap considers engineering of material composition through the design or control over the interactions between the components and how they function together, such as the biotic-abiotic interface and embedding of biomolecules, enzymes, and cells.

Engineered Materials - Material in which the bulk properties have been tuned for a particular application.

Engineering Biology - The design and construction of new biological entities such as enzymes, genetic circuits, and cells or the redesign of existing biological systems. Engineering biology builds on the advances in molecular, cell, and systems biology and seeks to transform biology in the same way that synthesis transformed chemistry and integrated circuit design transformed computing. The element that distinguishes engineering biology from traditional

molecular and cellular biology is the focus on the design and construction of core components (*e.g.,* parts of enzymes, genetic circuits, metabolic pathways) that can be modeled, understood, and tuned to meet specific performance criteria, and the assembly of these smaller parts and devices into larger integrated systems to solve specific problems. Unlike many other areas of engineering, biology is incredibly non-linear and less predictable, and there is less knowledge of the parts and how they interact. Hence, the overwhelming physical details of natural biology (*e.g.,* gene sequences, protein properties, biological systems) must be organized and recast via a set of design rules that hide information and manage complexity, thereby enabling the engineering of many-component integrated biological systems. It is only when this is accomplished that designs of significant scale will be possible. EBRC (and thus, this roadmap) uses Engineering Biology synonymous with **Synthetic Biology**.

Living or Dynamic Material - A material in which the biological component enables change with time. Typically, though not always, it consists of whole cells incorporated into a host matrix that provides the persistence of the cells and the passing of bidirectional signals and output. Incorporating biocomponents into a material system introduces additional design consideration such as nutrient uptake and metabolite clearance, and concerns about genetic drift over time.

Multiscale Materials - Engineering biology can enable materials across scales: from atomic and nanoscale materials (such as nucleic acid based materials) to macroscale materials (such as biofilms). Furthermore, the biological component of a material can enable a dynamic material to cross scales over time. There is a unique challenge in producing and characterizing biocomposites or materials derived from biology because of the dynamic and inherently uncontrolled nature of biology. In particular, macro-level properties of biomaterials can't necessarily be determined from micro-level measurements in the same way they can be from conventional materials. For example, the strength of a metal composite is going to be mostly consistent across scales (or civil engineering allows you to anticipate how the strength changes at scale), but biological materials will have emergent properties that can't necessarily be predicted from small tests.

Material vs. Device - Enabling materials through engineering biology - particularly through leveraging biology's ability to sense, integrate, and respond to local and environmental signals - can blur the lines between when the product is classified as a material or a device. For the purposes of this technical roadmap, we consider a material designed for a specific purpose or function to be a device, and focus (in the Technical Themes section of the roadmap) on the development of materials with minimal emphasis on the discrete application of the material.

Material System - A system of multiple materials integrated in a designed way that combine the different physical properties and responses of the components into a higher functional whole. This concept extends the notion of materials toward a device.

Materials Science - The study and applications of matter and its properties, as determined by composition and structure, combining principles of physics, chemistry, and engineering.

Organic/Inorganic - See **Biotic and Abiotic Materials**

Orthogonal - In a cellular system, operating independently from those processes that support the cell; that is an orthogonal translation system operates independently of the cell-supporting translation machinery and can synthesize genetically-encoded polymers with fewer constraints imposed by the need to maintain cell viability.

Performance - The engineering of dynamic activities of materials, including sensing and response for computation, communication, and self-repair. This includes the engineering of materials to provide signals or store data through an engineered biological component.

Precursor vs. Material - Like the distinction between material and device, the difference between a precursor and material may be similarly subjective. We consider a material to be the product of combined precursors, but recognize that multiple materials can be combined to make another, different material.

Processing - The engineering of biology to conduct “unit operations” to build or destroy materials through polymerization and degradation, patterning and printing. This includes engineering the biological extrusion or secretion of materials, material deposition, and self-assembly and -disassembly. Processing also includes engineering biology-based technologies, tools and systems (*e.g.,* cell-free systems) to manufacture, recover, and purify materials. Includes engineering biological materials to function in non-natural environments and extreme conditions.

Properties - The engineering of dynamic characteristics and activities of materials, including sensing and response for computation, communication, and self-repair. This includes the engineering of dynamic interactions between the biological and abiotic components of a material.

Scaffold - A structure, biologically-derived or synthesized that mimics the extracellular matrix and promotes cellular responses.

Segregation (Materials vs Biological phase segregation) - Aggregation of components based on characteristics, such as hydrophilicity/hydrophobicity or charge density. Surface segregation/presentation is a specific stratification that may take place at an interface, and result in graded composition in the material.

Self-assembly - The process in which a system’s components create an organized structure or pattern without external direction. Biomolecules readily self-assemble, both naturally and through engineered design, and can contribute significantly to ordering and patterning of materials. While self-assembly most often occurs at the molecular level, macromolecular- and cellular-level self-assembly can also facilitate material formation.

Self-healing/Self-repair - A critical functionality that may be imparted by incorporating biology into a material is the capacity to *repeatedly* self-heal or self-repair to reconstruct or replace damaged components or structures without external input/influence. Biocomposites can be engineered to sense damage and to elicit a reconstituting or repair response that, with the appropriate precursors and functional pathways, can be executed more than once.

Sensing/Detecting - This can refer to any mechanism that reacts to an external cue or stimulus and provides a signal (the response) denoting the reaction (either that it occurred or it occurred with a particular strength). Engineering biology can enable, tune, or modify this process by adapting the pathway from the target to the output measurement.

Stimuli-responsive - In biological contexts, at the cellular level or larger where the agent (bacteria or animal) senses the environment around them (*e.g.,* chemical, thermal, pressure, light) and responds to either avoid pain/death and/or seek reward/food. In a materials context, it generally refers to a designed interaction with an incident stimulus (*e.g.,* light, heat, magnetic field oscillation) that can drive a change in property.

Structure - The architecture of a material. This roadmap considers engineering of the two- and three-dimensional space a material and its components occupies and the tools and technologies necessary to control and dictate the architecture, including engineering the physical and bulk characteristics of a material. Structure also includes the arrangement and templating of material components.

Synthesis - In the context of this roadmap, the generation of material components via engineered biology; primary production or creation of material components. Includes utilizing or exploiting engineered biology to produce monomers, polymers, biomolecules, and macromolecules that serve as components of a material (bulk or otherwise).

Synthetic Biology - See **Engineering Biology**.

Synthetic (artificial) Cell - This roadmap considers synthetic cells as cell-like systems constructed from biological materials (proteins, nucleic acids, and lipids). One example is the encapsulation of cell-free expression systems expressing desired genetic circuits or proteins of interest in lipid bilayer vesicles. They can be engineered to respond to different external stimuli (*e.g.,* light, mechanical forces, small molecules). The synthetic cells do not need to be patterned completely on naturally-evolved cells, for example, the lipid bilayer could be replaced by other organic or inorganic components.

References

1. Engineering Biology Research Consortium. (2019). Engineering Biology: A Research Roadmap for the Next-Generation Bioeconomy. Retrieved from https://roadmap.ebrc.org. https://doi.org/10.25498/E4159B
2. "Interdisciplinary Laboratories for Basic Research in Materials Sciences," John Clarke Slater papers, 1908–1976, American Philosophical Society, Philadelphia, PA.
3. Materials Genome Initiative Home Page. https://www.mgi.gov/ (Accessed January 19, 2021).
4. Meng, F., & Ellis, T. (2020). The second decade of synthetic biology: 2010-2020. *Nature Communications*, *11*, 5174. https://doi.org/10.1038/s41467-020-19092-2
5. Torculas, M., Medina, J., Xue, W., & Hu, X. (2016). Protein-based bioelectronics. *ACS Biomaterials Science & Engineering*, *2*(8), 1211-1223. https://doi.org/10.1021/acsbiomaterials.6b00119
6. Vermaas, J.V., Dellon, L.D., Broadbelt, L.J., Beckham, G.T., & Crowley, M.F. (2019). Automated transformation of lignin topologies into atomic structures with LigninBuilder. *ACS Sustainable Chemistry & Engineering, 7*(3), 3443–3453. https://doi.org/10.1021/acssuschemeng.8b05665
7. Youdkes, D., Helman, Y., Burdman, S., Matan, O., & Jurkevitch, E. (2020). Potential control of potato soft rot disease by the obligate predators *Bdellovibrio* and like organisms. *Applied and Environmental Microbiology, 86*(6), e02543-19. https://doi.org/10.1128/AEM.02543-19
8. Arranz-Gibert, P., Vanderschuren, K., & Isaacs, F.J. (2018). Next-generation genetic code expansion. *Current Opinion in Chemical Biology*, *46*, 203-211. https://doi.org/10.1016/j.cbpa.2018.07.020
9. Lutz, J-F., Ouchi, M., Liu, D.R., & Sawamoto, M. (2013). Sequence-controlled polymers. *Science*, *341*(6146), e1238149. https://doi.org/10.1126/science.1238149
10. Thuronyi, B.W., Privalsky T.M., & Chang, M.C.Y. (2017). Engineered fluorine metabolism and fluoropolymer production in living cells. *Angewandte Chemie International Edition, 56*(44), 13637-13640. https://doi.org/10.1002/anie.201706696
11. Panganiban, B., Qiao, B., Jiang, T., DelRe, C., Obadia, M.M., Nguyen, T.D., Smith, A.A.A., Hall, A., Sit, I., Crosby, M.G., Dennis, P.B., Drockenmuller, E., Olvera de la Cruz, M., & Xu, Ting. (2018). Random heteropolymers preserve protein function in foreign environments. *Science, 359*(6381), 1239-1243. https://doi.org/10.1126/science.aao0335
12. Lajoie, M.J., Rovner, A.J., Goodman, D.B., Aerni, H.R., Haimovich, A.D., Kuznetsov, G., Mercer, J.A., Wang, H.H., Carr, P.A., Mosberg, J.A., Rohland, N., Schultz, P.G., Jacobson, J.M., Rinehart, J., Church, G.M., & Isaacs, F.J. (2013). Genomically recoded organisms expand biological functions. *Science*, *342*(6156), 357-360. https://doi.org/10.1126/science.1241459
13. Ribosome pilot challenge. *Eterna*. https://eternagame.org/labs/9162726 (Accessed January 6, 2021).
14. Hammerling, M.J., Fritz, B.R., Yoesep, D.J., Kim, D.S., Carlson, E.D., & Jewett, M.C. (2020). *In vitro* ribosome synthesis and evolution through ribosome display. *Nature Communications, 11*(1108). https://doi.org/10.1038/s41467-020-14705-2

15. Johnston, T.G., Yuan, S., Wagner, J.M., Johnston, T.G., Yuan, S., Wagner, J.M., Yi, X., Saha, A., Smith, P., Nelson, A., & Alper, H.S. (2020). Compartmentalized microbes and co-cultures in hydrogels for on-demand bioproduction and preservation. *Nature Communications, 11,* 563. https://doi.org/10.1038/s41467-020-14371-4
16. Amiram, M., Haimovich, A.D., Fan, C., Wang, Y., Aerni, H., Ntai, I., Moonan, D.W., Ma, N.J., Rovner, A.J., Hong, S.H., Kelleher, N.L., Goodman, A.L., Jewett, M.C., Söll, D., Rinehart, J., & Isaacs, F.J. (2015). Evolution of translation machinery in recoded bacteria enables multi-site incorporation of nonstandard amino acids. *Nature Biotechnology, 33*, 1272–1279. https://doi.org/10.1038/nbt.3372
17. Hadadi, N. & Hatzimanikatis, V. (2015). Design of computational retrobiosynthesis tools for the design of *de novo* synthetic pathways. *Current Opinion in Chemical Biology, 28*, 99-104. https://doi.org/10.1016/j.cbpa.2015.06.025
18. Cangialosi, A., Yoon, C., Liu, J., Huang, Q., Guo, J., Nguyen, T.D., Gracias, D.H., & Schulman, R. (2017). DNA sequence-directed shape change of photopatterned hydrogels via high-degree swelling. *Science, 357*(6356), 1126-1130. https://doi.org/10.1126/science.aan3925
19. Jia Liu, J., Kim, Y.S., Richardson, C.E., Tom, A., Ramakrishnan, C., Birey, F., Katsumata, T., Chen, S., Wang, C., Wang, X., Joubert, L., Jiang, Y,, Wang, H., Fenno, L.E., Tok, J.B.H., Pașca, S.P., Shen, K., Bao, Z., & Deisseroth, K. (2020). Genetically targeted chemical assembly of functional materials in living cells, tissues, and animals. *Science, 367*(6484), 1372-1376. https://doi.org/10.1126/science.aay4866
20. Deepankumar, K., Shon, M., Nadarajan, S.P., Shin, G., Mathew, S., Ayyadurai, N., Kim, B., Choi, S., Lee, S., & Yun, H. (2014). Enhancing thermostability and organic solvent tolerance of ω-transaminase through global incorporation of fluorotyrosine. *Advanced Synthesis & Catalysis, 356*(5), 993-998. https://doi.org/10.1002/adsc.201300706
21. Jung, J.K., Alam, K.K., Verosloff, M.S., Capdevila, D.A., Desmau, M., Clauer, P.R., Lee, J.W., Nguyen, P.Q., Pastén, P.A., Matiasek, S.J., Gaillard, J., Giedroc, D.P., Collins, J.J., & Lucks, J.B. (2020). Cell-free biosensors for rapid detection of water contaminants. *Nature Biotechnology, 38*, 1451–1459. https://doi.org/10.1038/s41587-020-0571-7
22. McLean, M.A., Gregory, M.C., & Sligar, S.G. (2018). Nanodiscs: a controlled bilayer surface for the study of membrane proteins. *Annual Review of Biophysics, 47,* 107-124. https://doi.org/10.1146/annurev-biophys-070816-033620
23. Awalgaonkar, G., Beaudry, R., & Almenar, E. (2020). Ethylene-removing packaging: basis for development and latest advances. *Comprehensive Reviews in Food Science and Food Safety, 19*(6), 3980-4007. https://doi.org/10.1111/1541-4337.12636
24. The Science. *Provivi*. https://provivi.com/en/the-science (Accessed January 8, 2021).
25. Medford, J., Morey, K., Kassaw, T., & Antunes, M. Synthetic desalination genetic circuit in plants. Patent application 16/492584, February 13, 2020.
26. Silverman, A.D., Akova, U., Alam, K.K., Jewett, M.C., & Lucks, J.B. (2020). Design and optimization of a cell-free atrazine biosensor. *ACS Synthetic Biology 9*(3), 671-677. https://doi.org/10.1021/acssynbio.9b00388
27. Yakimov, M.M., Timmis, K.N., & Golyshin, P.N. (2007). Obligate oil-degrading marine bacteria. *Current Opinion in Biotechnology, 18*(3), 257-266. https://doi.org/10.1016/j.copbio.2007.04.006

28. Daly, M.J., Gaidamakova, E.K., Matrosova, V.Y., Vasilenko, A., Zhai, M., Leapman, R.D., Lai, B., Ravel, B., Li, S.M., Kemner, K.M., & Fredrickson, J.K. (2007). Protein oxidation implicated as the primary determinant of bacterial radioresistance. *PLoS Biology, 5*(4), e92. https://doi.org/10.1371/journal.pbio.0050092
29. Whittall, D.R., Baker, K.V., Breitling, R., & Takano, E. (2020). Host systems for the production of recombinant spider silk. *Trends in Biotechnology*. https://doi.org/10.1016/j.tibtech.2020.09.007
30. Wang, S-T., Gray, M.A., Xuan, S., Lin, Y., Byrnes, J., Nguyen, A.I., Todorova, N., Stevens, M.M., Bertozzi, C.R., Zuckermann, R.N., & Gang, O. (2020). DNA origami protection and molecular interfacing through engineered sequence-defined peptoids. *Proceedings of the National Academy of Sciences of the United States of America, 117*(12), 6339-6348. https://doi.org/10.1073/pnas.1919749117
31. Zhao, M., Chen, Y., Wang, K., Zhang, Z., Streit, J.K., Fagan, J.A., Tang, J., Zheng, M., Yang, C., Zhu, Z., & Sun, W. (2020). DNA-directed nanofabrication of high-performance carbon nanotube field-effect transistors. *Science, 368*(6493), 878-881. https://doi.org/10.1126/science.aaz7435
32. Biofabricate and Fashion for Good. (2020). Understanding 'Bio' Material Innovation: a primer for the fashion industry. Retrieved from https://www.biofabricate.co/resources