



Lawrence Berkeley National Laboratory

BIOSCIENCES  
SCIENTIFIC  
STRATEGIC PLAN  
5-YEAR PROGRESS  
REPORT **2013–2018**

**2018**







Lawrence Berkeley National Laboratory

BIOSCIENCES  
SCIENTIFIC  
STRATEGIC PLAN  
5-YEAR PROGRESS  
REPORT **2013-2018**





# REFLECTIONS FROM BIOSCIENCES' CHIEF SCIENCE AND TECHNOLOGY OFFICER



Six years ago, as the Associate Laboratory Director for Lawrence Berkeley National Laboratory's Biosciences Area, I asked our staff to undertake the development of a 10-year scientific strategic plan. This request resulted in a first-of-its-kind activity for an Area at Berkeley Lab, and across the Department of Energy (DOE) National Laboratory complex. It also represented a new way of thinking for many of us in Biosciences; scientists are often trained to respond reactively to funding opportunities rather than work proactively to vision new opportunities. By establishing a strategic plan, we would be informing others — including funders, future collaborators, and policy-makers — of our future aims, and helping to set priorities for cutting-edge biological research befitting a national lab.

The first iteration of the Biosciences Strategic Plan (BSP) was released in 2013 following months of bottom-up visioning from the scientific and operations staff in Biosciences, and we shared it broadly with our many stakeholders, including with funding agencies and Congress. In 2014, we refreshed the plan to enable and accelerate implementation of the grand visions we had set forth and the plan was again updated in 2016 to reflect Biosciences-specific aspirations after organizational changes at Berkeley Lab. The plan highlights 5-year milestones and 10-year metrics towards achieving our four high-level goals in biological research for Energy, Environment, Health, and Biomanufacturing, as well as our fifth goal for the Technology innovations that underpin all that we do. This report assesses our progress fulfilling our vision, specifically toward reaching our 5-year milestones, and provides an outlook for achievement of our 10-year high-level goals.

Beyond guiding our scientific research efforts, the BSP has resulted in other significant changes within Biosciences, namely a major reorganization of the Area and the conception of our Biosciences Campus Vision. In 2015, Biosciences undertook a full reorganization of



our scientific Divisions and their operations to better reflect current research alignments, drive implementation of our strategies, reduce operational costs, and recruit new Divisional leadership. From this reorganization, we established three new scientific Divisions — Biological Systems and Engineering, Environmental Genomics and Systems Biology, and Molecular Biophysics and Integrated Bioimaging — and more fully integrated the DOE Joint Genome Institute into the Biosciences Area. The process of reorganizing the Area not only resulted in Divisions with purposeful new missions and visions aligned with our 10-year goals, it also gave us a detailed account of the interests and expertise of all our staff and our space and equipment requirements across the Area. With this information in hand, we created the Biosciences Campus Vision to enable future location of our staff from our four current locations in the East Bay to five buildings proposed for the main Berkeley Lab site.

This Biosciences campus would allow for deeper integration of our biological research with the computational, environmental, materials, and physical sciences research that occurs at Berkeley Lab, as well as new scientific discoveries that research at the intersections of these disciplines will foster. As this document goes to press, the first of the buildings, the Integrative Genomics Building, is under construction and scheduled to be completed in 2019. The second aspirational building, Biological & Environmental Program Integration Center (BioEPIC), was just approved by DOE for the first stage of consideration for possible future construction.

In 2017, I stepped down from my position of Associate Laboratory Director after shepherding Biosciences through these significant changes. I now serve as the Chief Science and Technology Officer for Biosciences, a role that allows me to think creatively about how we might build on our successes and to promote more aspirational visioning within the Area. A major focus for my current role is fundraising for the new Biosciences campus. Our objectives for this campus have their roots in the BSP and will influence our strategic planning efforts going forward.

I am thrilled by the progress outlined in this report — it exceeds my initial expectations for the success of the BSP. When we began considering such an effort in 2012, only one strategy of the twelve we described had significant programmatic funding. Now, in 2018, many of the strategies contained in the plan are supported by funded programs and others are gathering strong support from federal, state, and private entities. Additionally, we have both opened up new avenues of research at the Lab and increased program development capabilities within our staff.

I am pleased to share this progress report with you and am excited for the continued success of Biosciences research at Berkeley Lab.

Jay Keasling, Ph.D.  
*Chief Science and Technology Officer for Biosciences*



# IMPLEMENTATION TEAM

Volunteer staff from across the Biosciences Area is responsible for the implementation of the BSP. Strategy Leads are experts in related disciplines who coordinate research underlying the strategy. For the 5-Year Progress Report, Strategy Leads compiled accomplishments across Berkeley Lab, drafted summaries of progress, and assessed the outlook for achieving the 10-year metrics outlined in the BSP. The Strategy Leads who have contributed to implementation of the BSP since 2013 are listed below.

- \*Caroline Ajo-Franklin, Ph.D.
- \*Susan Celniker, Ph.D.
- Sylvain Costes, Ph.D.
- \*Adam Deutschbauer, Ph.D.
- \*Diane Dickel, Ph.D.
- \*Nathan Hillson, Ph.D.
- Mark LaBarge, Ph.D.
- \*Cynthia McMurray, Ph.D.
- \*Vivek Mutalik, Ph.D.
- \*Trent Northen, Ph.D.
- \*Corie Ralston, Ph.D.
- Sarah Richardson, Ph.D.
- \*Henrik Scheller, Ph.D.
- Ian Sharp, Ph.D.
- \*Steven Singer, Ph.D.
- Chia-Lin Wei, Ph.D.
- \*Junko Yano, Ph.D.
- \*Petrus Zwart, Ph.D.

*\*Denotes active BSP strategy lead*



# TABLE OF CONTENTS

A decorative graphic on a dark blue background. It features two lines that meet at a central point, forming a V-shape. The line on the left is a light grey color, and the line on the right is a light green color. The lines extend from the top right towards the bottom left, and from the top left towards the bottom right, meeting at a central point near the bottom of the page.



Reflections from Biosciences' Chief Science and Technology Officer	<b>2</b>
Implementation Team	<b>5</b>
How We Got Here	<b>8</b>
Adaptive Management of the Biosciences Strategic Plan	<b>16</b>
Progress Report: Energy	<b>19</b>
Progress Report: Environment	<b>25</b>
Progress Report: Health	<b>31</b>
Progress Report: Biomanufacturing	<b>35</b>
Progress Report: Technologies	<b>41</b>
Looking Ahead: Thoughts from the ALD for Biosciences	<b>46</b>
BSP Progress Report: 2013-2018 Milestone Completion Appendix	<b>48</b>



**Lawrence Berkeley National Laboratory**

BIOSCIENCES  
SCIENTIFIC  
STRATEGIC PLAN  
5-YEAR PROGRESS  
REPORT **2013-2018**

2012

**Strategic planning  
initiated; internal  
visioning workshops  
completed**

2013

**First iteration of  
the Biosciences  
Strategic Plan  
(BSP) published**

2014

**Second iteration of  
the BSP published,  
better enabling  
implementation**



# HOW WE GOT HERE

**2015**

**Full reorganization  
of Biosciences Area;  
Biosciences Campus  
Vision developed**

**2016**

**BSP refreshed to  
update Environment  
goal and add  
Technologies goal**

**2017**

**Initial milestone  
progress assessment  
undertaken**

**2018**

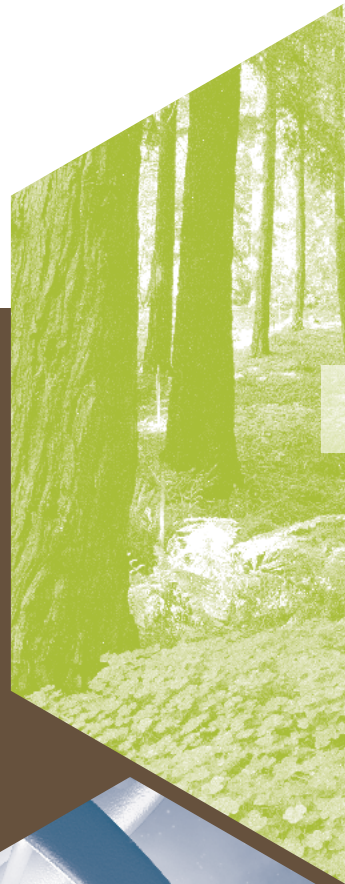
**5-year progress  
report compiled  
and released**

## MISSION

Use integrated research teams to solve national challenges in energy, environment, health, and biomanufacturing

## VISION

Berkeley Lab's Biosciences Area will lead the nation in using biology to solve energy and environmental challenges





ENVIRONMENT



ENERGY



BIOMANUFACTURING



TECHNOLOGIES



# STRATEGIC GOALS

## **Biosciences for Energy**

Develop/enable cost-competitive (economically-sustainable) and environmentally-sustainable biological and bio-inspired energy solutions capable of reducing U.S. dependence on petroleum.

## **Biosciences for the Environment**

Understand the genetic and molecular mechanisms governing the activities and ecology of organisms and multispecies communities to predict responses and harness microbes and plants for energy and environmental solutions.

## **Biosciences for Health**

Develop and apply a predictive, multiscale, integrative understanding of how biological diversity impacts responses to environmental challenges, to improve human and biome health and drive responsible economic growth.

## **Biosciences for Biomanufacturing**

Develop and demonstrate a scalable, flexible, cost-effective, and sustainable biology-based manufacturing infrastructure driven by applications in energy, health, the environment, and agriculture.

## **Technologies for Biosciences**

Develop a technology infrastructure to measure, predict, and control biological systems for solving energy, environmental, and health challenges.

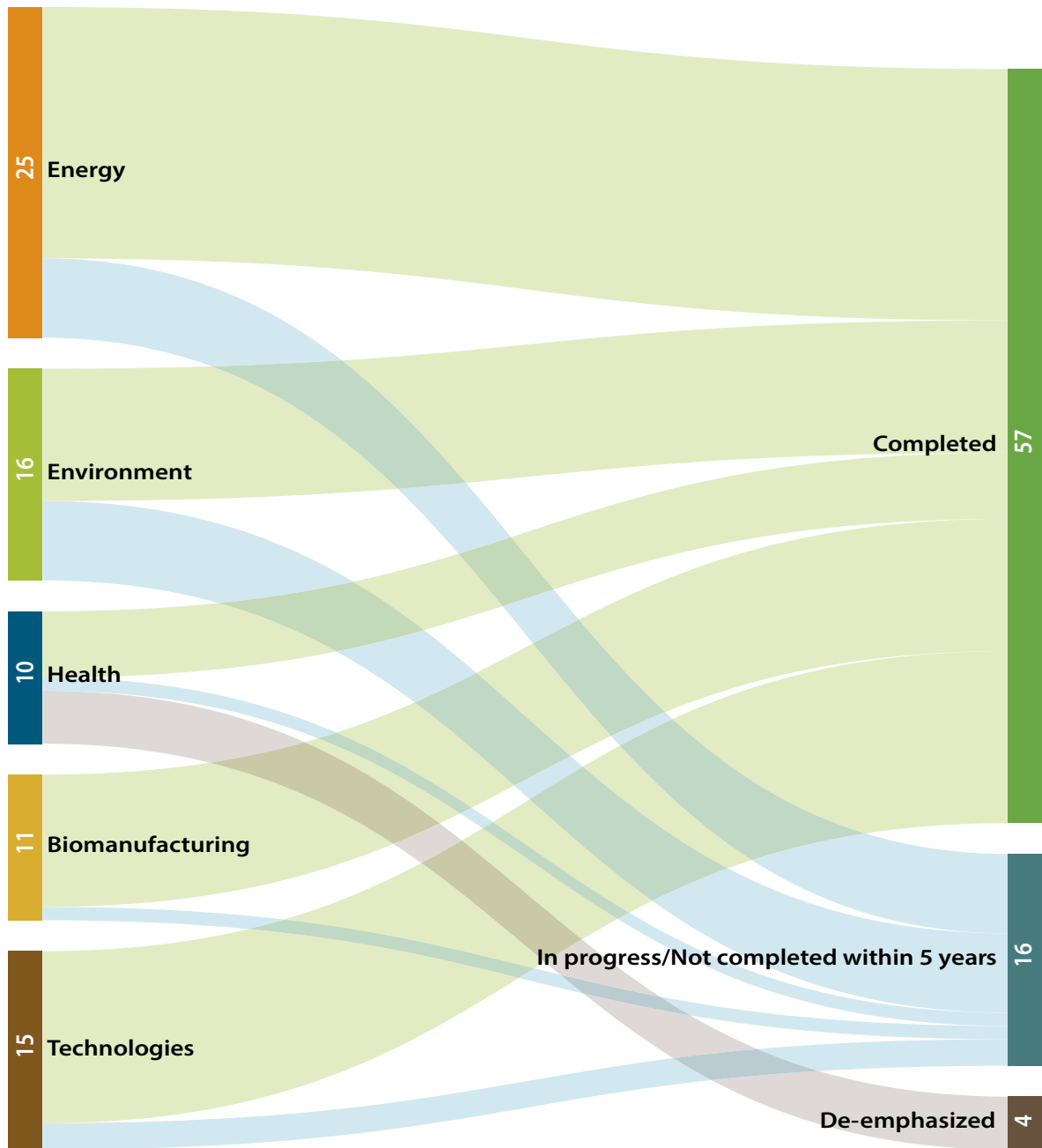
# ASSESSING OUR PROGRESS

The Biosciences Strategic Plan (BSP) identifies high-level 10-year goals for Energy, Environment, Health, and Biomanufacturing research and Technologies development in the Biosciences Area at Berkeley Lab. These goals are supported by strategies that each have a collection of 5-year milestones to be used in assessing our progress toward the goal, as well as 10-year metrics to validate achievement of the goal. This approach of using diversified strategies is designed to enable Biosciences to meet the goals through many different routes, providing insurance that if one or more strategies fail, there are additional strategic routes to follow.

For this report, Biosciences staff responsible for the implementation of the strategies (Strategy Leads) were asked to submit information from across the Area (and, in some cases, from the larger Berkeley Lab community) that described progress towards completion of the 5-year milestones outlined in the BSP. The Strategy Leads also provided outlooks for future progress toward their 10-year metrics, as well as assessments of strengths and gaps of the associated research programs. These assessments will help Biosciences identify new program development opportunities and future strategic directions for the Area.

In the Milestone Completion Appendix (p. 48), we have documented the completed 5-year milestones for each strategy, the milestones that are in progress but not yet complete after five years, and the strategy that has been de-emphasized. The progress report itself focuses on the scientific and programmatic successes that allowed Biosciences staff to achieve these milestones.

### 5-Year Milestone Assessment



The Biosciences Area’s progress towards completion of 5-year milestones by strategic goal and by milestone status after 5 years of implementation. **Left**, number of 5-year milestones for each strategic goal. **Right**, number of 5-year milestones that were completed (green), in progress but not completed within 5 years (blue) or de-emphasized (brown).



# ADAPTIVE MANAGEMENT OF THE BIOSCIENCES STRATEGIC PLAN

Biosciences began working on the first iteration of the BSP in 2012. In the five years since the release of the plan, advances in biological research, organizational changes at Berkeley Lab, and alterations in the missions and scopes of funded programs have necessitated adaptive management of the BSP and its implementation. The BSP guides the development of enduring team science programs within Biosciences and has evolved with the aspirations of scientists and management. A series of “refreshes,” in 2014 and 2016, were undertaken to strengthen the BSP to reflect those aspirations.

When the BSP was first developed, researchers from Berkeley Lab’s Earth Sciences Division were instrumental in developing the Environment goal. In 2015, this Division was expanded into the Earth and Environmental Sciences Area and began developing its own strategic framework. To better reflect a specific Biosciences Area vision, a team of Biosciences scientists refreshed and focused the Environment goal on research central to the Biosciences vision in 2016. This latest refresh outlines strategies to understand, predict, and harness the biological mechanisms that influence and respond to environmental changes. Since this refresh, Biosciences has seen program development efforts in this space transition into the funded mCAFES project and has received internal investments for the development of increasingly complicated laboratory model ecosystems.

During the first few years of research towards the Energy, Environment, Health, and Biomanufacturing goals, it became clear that technology development had a central role to play in achieving the metrics, warranting the inclusion of a specific goal for Technologies. Technology development as an aspirational strategy was specifically highlighted only in the Health section. To give a more comprehensive view of the role technologies play in underpinning the success of one or more of the aforementioned goals, four categories of technology development were identified, three of which were originally included as part of a Health strategy: structural biology, bioimaging, functional genomics, and mathematics, informatics, and computing. Since the addition of this goal, the Biosciences Area has seen an increase in investment in programs to integrate structural biology resources at the ALS and spectral phenotyping; significant functional genomics technology development; and several computational algorithm advances.

Changes in federally funded program missions will also necessitate rethinking approaches to achieving the 10-year success metrics in the BSP. For example, the Joint Center for Artificial Photosynthesis (JCAP), a DOE Energy Innovation Hub, was refocused in its renewal phase, impacting progress toward our ultimate goals.

Biosciences will undertake new program development activities to secure funding to achieve the 10-year metrics.

Staffing changes within Biosciences have also impacted milestone and metric completion. The departures of two key staff members means that Biosciences is no longer able to implement one of the strategies in the Health section. This strategy has been de-emphasized as a result and new approaches for achieving the 10-year goal have been under consideration.

This progress report serves not only as a way to track the completion of 5-year milestones, but also as an impetus to revisit strategies that may need to be refreshed in light of unforeseen challenges. Additionally, this assessment allows Biosciences to focus program development efforts towards promising avenues of research that represent core Berkeley Lab strengths and have potential for stable external funding. In 2019, Biosciences will undertake a process to update the BSP that considers revised programmatic directions and incorporation of emerging areas of interest.







# PROGRESS REPORT: ENERGY

**10-Year Goal:** Develop/enable cost-competitive (economically sustainable) and environmentally sustainable biological and bio-inspired energy solutions capable of reducing U.S. dependence on petroleum.

To achieve the Energy Goal by 2023, Biosciences' approach employs three areas of strategic focus: production of fuels from plant biomass (lignocellulosic biofuels); production of fuels from gas feedstocks (alternative biofuels); and non-biological production of liquid fuels directly from sunlight and CO<sub>2</sub> (artificial photosynthesis). These areas were chosen because (1) they collectively have potential to meet the long-term national need for sustainable cost-competitive alternatives to fossil fuels; (2) they are scientifically tractable within a 10-year span; and (3) they leverage specific facilities, organized research groups, and core competencies that exist, or can be readily assembled, within the Biosciences Area. Successful funding for the Joint BioEnergy institute (JBEI) ensures that we will be able to meet the strategic goals of the lignocellulosic energy strategy. JBEI, along with other prominent projects such as the Agile BioFoundry (ABF) and Co-Optima, has already achieved the development of a remarkable number of plant feedstocks, pretreatment processes, and microbial conversion platforms for the production of biogasoline, biodiesel, and jet biofuels and other industrial compounds that successfully replace petrochemicals. This work has led to hundreds of peer-reviewed scientific papers, dozens of patents, and numerous start-up companies.

In the past year, the Energy team has also successfully obtained funding via the Energy Biosciences Institute (EBI) for the Alternate Carbon conversion program and, in collaboration with the Advanced Biofuels and Bioproducts Process Development Unit (ABPDU), will focus on scaling the current bench scale processes to industrially relevant scales. These achievements have utilized capabilities such as gene sequencing and synthesis at the DOE Joint

## 10-YEAR Energy Goal

Develop/enable cost-competitive (economically sustainable) and environmentally sustainable biological and bio-inspired energy solutions capable of reducing U.S. dependence on petroleum.

### Energy Research Strategies to Achieve Goal

**Lignocellulosic biofuels.** Derive energy from biomass with new technologies.

**Alternative biofuels.** Engineer and scale the direct conversion of gas feedstocks to fuels by photosynthetic, methanotrophic, and chemoautotrophic microorganisms.

**Artificial photosynthesis.** Use bio-inspired reactions to create fuels directly from atmospheric CO<sub>2</sub> and sunlight.





Genome Institute (JGI) and structural biology at the Advanced Light Source (ALS). The Artificial Photosynthesis strategy team has made significant progress in better understanding photosynthesis, specifically the structure and function of photosystem II, which is the main metalloenzyme for the light-dependent reaction of photosynthesis. Recent advances by this team have resulted in several high-impact publications and helped to keep Berkeley Lab at the forefront in the research investigations of natural and artificial photosynthesis. The Energy teams are on track to achieve the 10-year Energy goal by 2023.

### **Lignocellulosic Biofuels Strategy:** Derive energy from biomass with new technologies.

The United States has abundant biomass resources; over a billion tons of feedstocks could be mobilized to replace 30 percent of U.S. needs for transportation fuels without significant impacts on human food and livestock feed production. To effectively utilize these resources, several breakthroughs must be made: improved biomass with greater amounts of fermentable sugars and valuable lignin intermediates, greater tolerance to stress, and improved nutrient acquisition; greatly improved biomass extraction and breakdown strategies; engineered microorganisms capable of converting biomass sugars and lignin to high energy density fuels and additives; and the development and demonstration of economically viable and scalable production processes for an advanced biofuel and co-product.

### **Select accomplishments relating to 5-year milestones**

- Researchers at JBEI have developed a successful method for engineering plants that makes lignins easier to cleave. This reduces the recalcitrance of lignin and improves downstream processing, allowing for the release of more convertible sugars from biomass for fuel production. This work has been translated from model laboratory plants to switchgrass, sorghum, and poplar—possible crops for future biofuels production. Two companies have licensed this technology from Berkeley Lab.
- JBEI researchers have also developed an integrated process employing ionic liquids, wherein biomass pretreatment, enzymatic digestion, and conversion to biofuel is accomplished in the same vessel. This process, which takes advantage of progress made at JBEI to reduce the cost of ionic liquid pretreatment, has been scaled up at the ABPDU. This “one-pot” process has the potential to reduce the cost of processing biomass into biofuels.
- To facilitate use of marginal lands and reduce water inputs for bioenergy crop production, JBEI scientists have been investigating drought tolerance mechanisms in plants. Drought tolerance genes identified in model plants have been transferred to switchgrass and field trials are underway to determine if this tolerance can be observed under field conditions.
- Researchers across Biosciences have probed the wealth of known metabolic pathways for plants and microbes to engineer biosynthetic pathways to produce new enzymes and fuel molecules. These efforts have resulted in enzymes that can tolerate high-temperature deconstruction processes, new pathways engineered into microbes, and new fuel molecules for biodiesel, biogasoline, and jet biofuel. By mining nature, scientists have been able to develop more efficient biological routes to important fuels.

## Outlook

In the next five years, Biosciences' achievements in this strategy will be measured by: expanding our fundamental knowledge of the plant biology that underlies biomass yield and adaptation to stress; using that knowledge to engineer biomass crops for reduced water and fertilizer inputs and more efficient deconstruction to usable intermediates; developing predictive models to facilitate biological engineering and to engineer microorganisms tailored for consumption of deconstructed biomass; and production of drop-in fuels at high yield. This strategy will be executed primarily at JBEI, the funding for which was recently renewed for five years with an expanded scope for producing cost-effective bio-based fuels and chemicals. The recently established Agile BioFoundry (ABF) is focused on improving the biological “design, build, test, and learn” process for accelerated development of microbes that can produce fuels and chemicals and will enable the development of new microbial strains. The ABPDU will continue to integrate and scale processes from bench to pre-industrial pilot scale, demonstrating potential for practical applications and providing key discoveries about how biological processes scale for future research efforts. Progress towards the 10-year metrics of demonstrated success for this strategy is already being made.

### **Alternative Biofuels Strategy: Engineer and scale the direct conversion of gas feedstocks to fuels by photosynthetic, methanotrophic, and chemoautotrophic microorganisms.**

Berkeley Lab is focused on direct microbial conversion of gas feedstocks, such as abundantly available CO<sub>2</sub>, synthesis gas (CO/CO<sub>2</sub>/H<sub>2</sub>), and waste gases, such as H<sub>2</sub>S and biogas methane, to fuels by engineered photosynthetic, methanotrophic, or chemoautotrophic organisms. The potential for direct fuel production has long been conceptually attractive, but major unsolved challenges have impeded efforts. Systems-level understanding of microbes coupled with new engineering tools hold promise to illuminate and overcome these challenges.

### **Select accomplishments relating to 5-year milestones**

- Researchers at Berkeley Lab have completed bench-scale set-ups for autotrophic growth of microbes on carbon dioxide and methane, while researchers at the ABPDU are developing bioreactors for growing microbes in these gases. Efforts at Berkeley Lab have developed new operations for fuel production from gaseous feedstocks to enable demonstration at bench and pilot scale.
- Scientists in Biosciences identified the complete set of essential genes in *Synechococcus elongatus* PCC 7942, a model organism used for studying photosynthesis and the circadian clock that is being developed for the production of fuel, industrial chemicals, and pharmaceuticals. Some of these essential genes are required for growth and metabolism of carbon dioxide. This project was a collaborative effort funded through Core and Community Sequencing Project programs at the JGI and by Laboratory-Directed Research and Development (LDRD) funding from Berkeley Lab.
- Through a Technology Commercialization Fund grant with Lanzatech, a waste gas-to-fuels company, and in partnership with Sandia National Laboratories, scientists are working to improve the throughput of genetic transformation for a syngas-metabolizing microbe.

## Outlook

The 10-year success metrics for this strategy include: using a systems-level understanding to identify key bottlenecks that limit fuel production by microbes, using that information to engineer microbes to produce fuels from gas feedstocks, and scaling production through bioreactor and process development. Recently, the Energy Biosciences Institute at UC Berkeley, with Berkeley Lab as a partner, has refocused to emphasize conversion of carbon dioxide and methane into fuels and products. Additionally, a new National Academies of Science panel has been formed to develop recommendations for a federal research agenda for waste gases. This suggests broad federal agency interest in the topic, as well as potential future support for the development of microbes that produce fuels and products. Biosciences is well positioned to expand its research into novel microbial processes for conversion of renewable (anaerobic digestion, ethanol plants) and petroleum-derived sources (oil and gas drilling, industrial waste gases) through the development of new approaches for engineering of microbes.

**Artificial Photosynthesis Strategy:** Use bio-inspired reactions to create fuels directly from atmospheric CO<sub>2</sub> and sunlight.

Over the past 50 years, basic research has steadily increased knowledge of the subtle and complex mechanisms behind natural photosynthetic systems, as well as the use of photochemical methods that mimic key steps in the process (e.g. splitting water and reducing carbon dioxide). However, significant hurdles, such as the inability to control chemical reactions on a nanometer scale and the limited understanding of photosynthesis on temporal and spatial scales, have impeded the design of solar-energy-to-fuel conversion systems with the required efficiency, scalability, and sustainability to be economically viable. Nanotechnology of inorganic materials, together with bioengineering technology that enables the manipulation of biological systems at the genomic level, make it possible to integrate living organisms with inorganic catalysis into nanoscale biohybrids. This takes advantage of the complementary strengths of abiotic and biotic systems in energy harvesting and chemical production.

## Select accomplishments relating to 5-year milestones

- There has been significant progress and success in understanding natural photosynthesis and the structure and function of photosystem II, which is the main metalloenzyme for the light reaction of photosynthesis. We have gained knowledge of its mechanisms of assembly, protection, and catalysis. This integral research will be continued beyond the 5-year milestone.
- The fundamental understanding of how photosynthesis is regulated has been used to improve photosynthesis and biomass productivity in a crop plant. Biosciences researchers targeted three genes involved in photoprotection, the process plants use to protect themselves from damage when they get more light than they can safely use. In field experiments, increasing the expression of those genes resulted in productivity increases of 14 to 20 percent in modified tobacco plants. In related work, they also recently succeeded in improving water-use efficiency by 25 percent, which could enable growth of biomass crops with less water. The molecular processes investigated in these studies are fundamental to all plants; therefore, similar improvements in other

crops are expected. This research was supported by the Bill and Melinda Gates Foundation. Any new technology licensed from this work will be made freely available to farmers in developing countries in Africa and South Asia.

### Outlook

The 10-year metrics for demonstrated success include developing and manipulating bioinspired and biohybrid artificial photosynthetic systems to produce fuels from light energy and CO<sub>2</sub>; coupling predictive models with advanced engineering to improve artificial photosynthesis; gaining fundamental understanding of the time- and space-controlled chemistry in photosynthetic enzymes and artificial systems; and using these new levels of understanding to predict improvement of microbial and plant photosynthesis. Several projects relating to photosynthetic system knowledge application to improve, predict, or mimic these systems are highly valued and funding is expected to continue in the near term. The ability to predict improvement of microbial and plant photosynthesis has potential for addressing challenges of producing energy in underdeveloped areas, which is attractive to philanthropic and foundation funders. We are actively seeking a new funding source for further development of our bioinspired and biohybrid artificial photosynthetic systems, built upon the advancement of the biohybrid research that was supported by LDRD funds. Part of the artificial photosynthesis work is carried out at JCAP, which was renewed in 2015.











# PROGRESS REPORT: ENVIRONMENT



**10-Year Goal:** Understand the genetic and molecular mechanisms governing the activities and ecology of organisms and multispecies communities to predict responses and harness microbes and plants for energy and environmental solutions.

To achieve the Environment goal by 2023, we instantiated two Biosciences Area-specific strategies in 2015 after Berkeley Lab created a separate Earth and Environmental Sciences Area to focus on advancing scientific knowledge of the integrated Earth system: (1) Advancing foundational science toward a predictive understanding of environmental organisms, and (2) Developing molecular ecosystems biology-based solutions. We believe that coupling these elements is necessary to create new classes of environmental and energy solutions. These two strategies are synergistic, requiring similar and complementary research in order to lay the groundwork for the next five years.

By linking organized research efforts and expertise in molecular microbiology, microbial ecology, and subsurface and terrestrial ecosystem science with global climate expertise, Biosciences is uniquely positioned to enhance understanding integrated environmental system behavior. Area researchers have contributed to and helped lead Berkeley Lab's Microbes to Biomes Initiative from 2014-2017. As part of that initiative, researchers aimed to develop a deep understanding of microbial, plant, and metazoan genomics and biomolecular mechanisms using integrated systems biology approaches to study the dynamics, stabilities, activities, and interactions of organisms with their communities. To leverage and extend that work, Biosciences is now pioneering the development of advanced functional genomics technologies and fabricated laboratory ecosystems (EcoFABs and EcoPods) that together will enable discovery of causal mechanisms through manipulation of the genetic, organismal, and abiotic

## 10-YEAR Environment Goal

Understand the genetic and molecular mechanisms governing the activities and ecology of organisms and multispecies communities to predict responses and harness microbes and plants for energy and environmental solutions.

### Environment Research Strategies to Achieve Goal

**Predictive Understanding of Environmental Organisms.** Discover and deeply characterize the genetic and molecular mechanisms of environmental organisms that drive and respond to environmental changes.

**Molecular Ecosystems Biology-Based Solutions.** Use biological and environmental characterization of natural and laboratory ecosystems to understand native ecosystem processes, predict responses, and harness plants and microbes for energy and environmental solutions.



system components. Since the model ecosystems will continually be compared to native ecosystems through field studies and refined to allow models to accurately represent key aspects of the native environments, they promise to provide a powerful framework for determining the necessary and sufficient components of an ecological process of interest. They also could provide test beds for examining important ecological questions such as functional redundancy, selective and neutral forces, population dynamics and adaptation to environmental changes, and perhaps give rise to a new national lab core capability.

**Predictive Understanding of Environmental Organisms Strategy:** Discover and deeply characterize the genetic and molecular mechanisms of environmental organisms that drive and respond to environmental changes.

Microbes and plants in terrestrial environments carry out a wide range of essential biogeochemical processes. Advances in DNA sequencing now enable rapid and inexpensive interrogation of genomes. As a result, vast amounts of sequence information from all types of organisms and environments are accumulating at accelerating rates. In principle, these sequence data contain the information necessary to make accurate predictions of organismal metabolism and fitness under controlled conditions. However, vital information — about the functional attributes of these sequences, and the computational models and resources necessary to make accurate predictions — is lacking. To address this challenge, a set of flexible “omic” approaches to rapidly characterize the protein and metabolite content of diverse environmental organisms is being developed. For example, new environmental metagenomics approaches are being integrated with bioinformatics, DNA synthesis, metabolomics, and cheminformatics to allow analysis and construction of biosynthesis pathways from uncultured environmental microbes in organisms that can be manipulated. This allows both for identification of the metabolites the microbes produce and examination of their biochemical ecologies within laboratory ecosystems. This functional genomic and physiological understanding will greatly enhance genomics-based modeling approaches and effective manipulation of organisms and environmental systems for environmental or energy benefits.

#### Select accomplishments relating to 5-year milestones

- Biosciences researchers have identified genes important for mediating plant-bacteria interactions. This project incorporated genome-wide fitness profiling of a lab-tractable, plant-associated bacterial strain of *Pseudomonas simiae* for its ability to colonize *Arabidopsis thaliana* roots using the random barcode transposon-site sequencing (RB-TnSeq) technology developed at Berkeley Lab. They found several hundred genes that, when mutated, impacted the ability of this bacteria to colonize plant roots *in vivo*. Many of these genes also contribute to fitness under other stress-inducing or metabolically-limiting conditions, and recent findings of this work may inspire novel strategies to manipulate plant colonization in agricultural settings.

- Scientists have developed and applied new functional genomics technologies for the discovery and validation of gene and noncoding DNA functions that impact plant fitness. By studying the pan-genome (a complete set of genes across multiple strains of a species) of the grass *Brachypodium distachyon*, they were able to identify differentially present genes that provide an advantage in response to pathogens and herbivores.
- Researchers in Biosciences have recently identified over 100 metabolites exuded by plant roots, and are now characterizing how these metabolites may be used by isolated soil microbes in culture in order to predict how they may impact microbial community structure broadly.

## Outlook

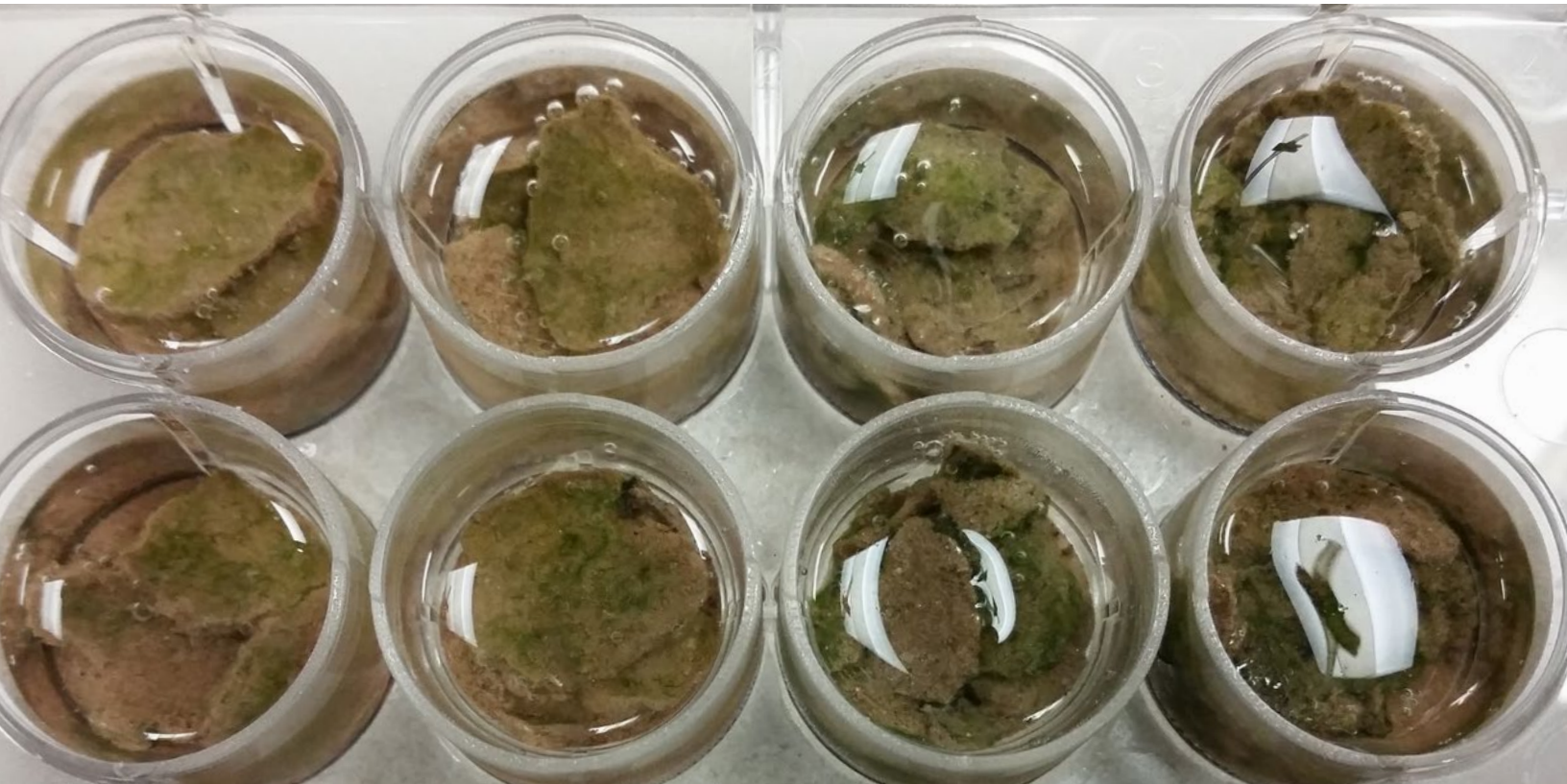
This strategy has several 10-year success metrics, nearly all of which are on track for completion. These metrics include: using model ecosystems to discover the functions of genes that mediate microbial interactions; discovering the molecular mechanisms underpinning changes in microbial communities in response to environmental contaminants; characterizing microbial isolates from field sites using multi-omics approaches; and developing computational infrastructure to transfer that knowledge to related systems and to enable predictive understanding of ecosystem function. Much of the work in this strategy is performed at the JGI, and in the Ecosystems and Networks Integrated with Genes and Molecular Assemblies (ENIGMA) Scientific Focus Area (SFA), the DOE Systems Biology Knowledgebase (KBase), and the new Microbial Community Analysis & Functional Evaluation in Soils (mCAFES) project. This work is also supported through internal Berkeley Lab investments for the development of model ecosystems, known as EcoFABs and EcoPods, which allow researchers to recapitulate key environmental parameters in the laboratory for assessing and understanding microbial and plant responses.

**Molecular Ecosystems Biology-based Solutions Strategy:** Using biological and environmental characterization of natural and laboratory ecosystems to understand native ecosystem processes, predict responses, and harness plants and microbes for energy and environmental solutions.

Improved understanding of the molecular mechanisms mediating microbial, metazoan, and plant ecologies is urgently needed to accurately predict native ecosystem processes and harness plant and microbial ecosystems for environmental clean-up and the sustainable production of biofuels and other crops. To address these critical challenges, Biosciences is developing and using model ecosystems connected to field studies and advanced bioinformatics resources to understand ecologies mediating nutrient cycling and plant-microbiome mechanisms that enhance plant growth and abiotic stress tolerance. Combined with advanced genetic and systems biology tools, these models will provide insights into microbial, metazoan, and plant genomics and elucidate mechanisms that determine factors in natural ecosystem resilience and dynamics. This knowledge can be applied for environmental clean-up and to increase bioenergy productivity and efficiency on marginal land.

### Select accomplishments relating to 5-year milestones

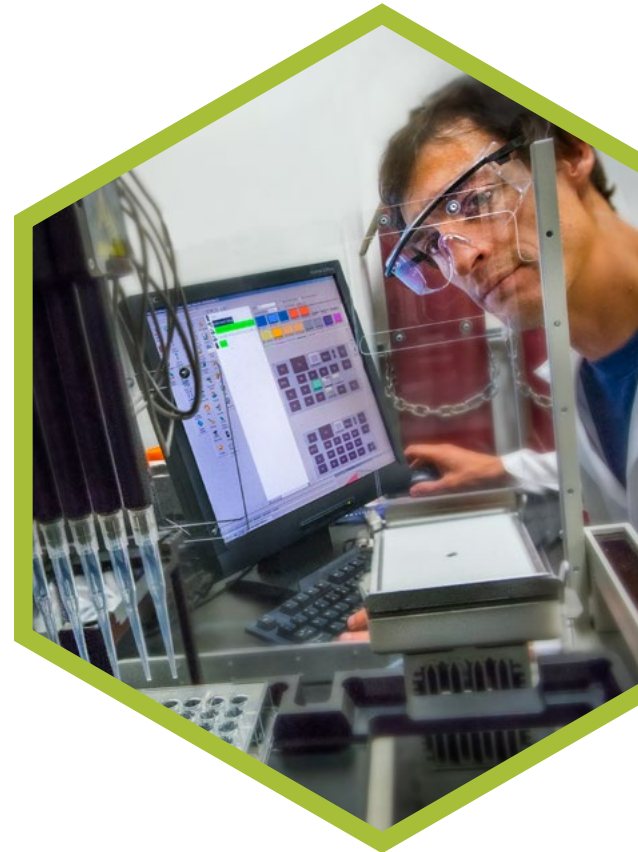
- A Biosciences team has generated a model desert ecosystem patterned on a native environment and demonstrated that it can be controlled in the laboratory to study important metabolic processes. This work is now being extended to a variety of other ecosystem types, aided by the large collection of microbial isolates amassed by the team.
- To demonstrate the ability to alter microbiome structure and soil carbon cycling, researchers genetically manipulated the plant tryptophan export pathway, which enhances the ability of growth-promoting microbes to generate the plant hormone auxin, which in turn improves plant growth. They are now building on this work to overproduce tryptophan and aromatic compounds in the roots of the model grass *Brachypodium* for similar analyses using laboratory ecosystems.
- Scientists observed discrete divisions of extracellular metabolites (exometabolite niche partitioning) among isolates of biocrust bacterial strains grown in laboratory culture. This niche partitioning enabled construction of a metabolic web model of nutrient exchange among biocrust organisms. Isolate data linked the relative abundance of four dominant bacteria to soil exometabolites in intact biocrust under two simulated conditions. Most of the soil metabolites displayed the expected relationships with organism abundance, demonstrating that integration of omics technologies can be used to develop functional links between microbial community structure and chemical composition.

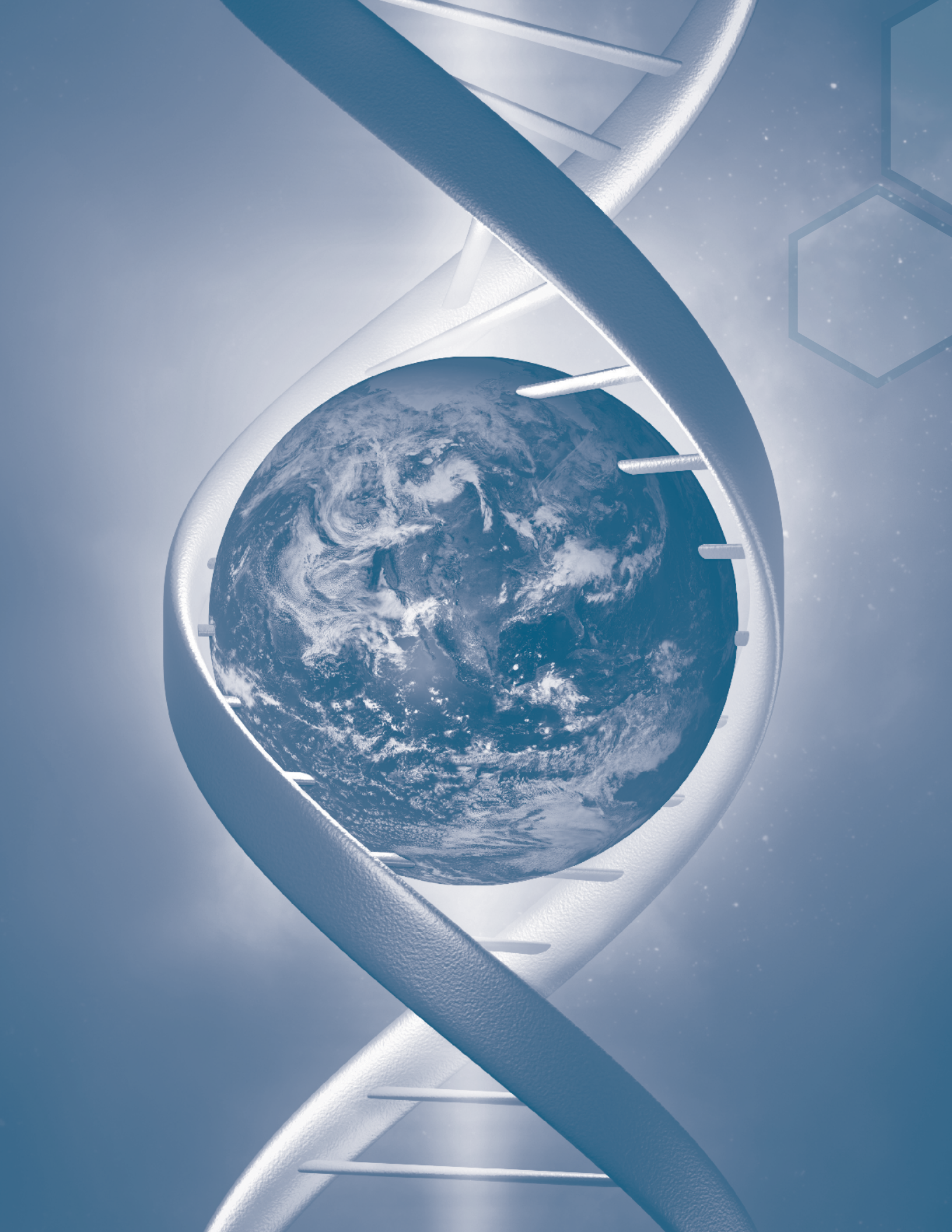




## Outlook

This strategy is on track to meet the 10-year metrics of demonstrated success, which include: identifying key environmental processes to recapitulate and validate a laboratory model ecosystem; developing and refining model predictions of native community energy and material dynamics; and harnessing the metabolic capabilities of microorganisms to increase the yield of an important crop under sub-optimal conditions. Biosciences' strong environmental programs, including JGI and ENIGMA SFA, and now the mCAFES project, will contribute significantly to achieving these metrics. Elucidating and understanding microbial functions will form the basis of tool and technology development that can be deployed for beneficial use in microbial and plant-microbial communities.









# PROGRESS REPORT: HEALTH

**10-Year Goal:** Develop and apply a predictive, multiscale, and integrative appreciation of how individual variation affects responses to environmental challenges in order to improve human and biome health, and drive responsible economic growth.

The purpose of activities to achieve the Health goal is to understand the roles played by host genetic variability, epigenetic effects, and microbiome in determining whether an individual will be sensitive or resistant to the health impacts of environmental challenges. A growing number of studies have linked the microbiome to brain health, liver function, bowel disease, and many other physiological and behavioral phenotypes. Accidental exposures of humans and animals to agricultural chemicals remain a serious problem in the United States, which accounts for 32 percent of the global pesticide market. However, the response of individual host genetic and epigenetic states, as well as its microbiome, to chemical perturbations is poorly understood.

**Biological Responses to Environmental Challenges Strategy:** Develop and deploy model systems to understand how individual genetic, epigenetic, and microbiome variation affect molecular, cellular, and organismal responses to environmental challenges, and to identify risk factors for somatic and neurological diseases.

The strategy aims to develop a mechanistic understanding of how environmental challenges, specifically chemicals, radiation, nanomaterials, diet, energy production and use, impact metazoan organisms. An important first step is defining how exposures affect biological systems in specific model organisms, from macromolecular complexes to biological outcomes. Due to the enormous complexity and multifaceted nature of biological systems, a holistic approach is needed to achieve the 10-year goal. By identifying key biological mechanisms in model

## 10-YEAR Health Goal

Develop and apply a predictive, multiscale, and integrative appreciation of how individual variation affects responses to environmental challenges in order to improve human and biome health, and drive responsible economic growth.

### Health Strategies to Achieve Goal

**Biological responses to environmental challenges.** Develop and deploy model systems to understand how individual genetic, epigenetic, and microbiome variation affect molecular, cellular, and organismal responses to environmental challenges, and to identify risk factors for somatic and neurological diseases.

**Impact of environmental challenges on human health.** Develop the knowledge and infrastructure to elucidate the immediate and long-term consequences of environmental challenges on health in diverse human populations, and to enable innovative solutions through responsible economic growth.



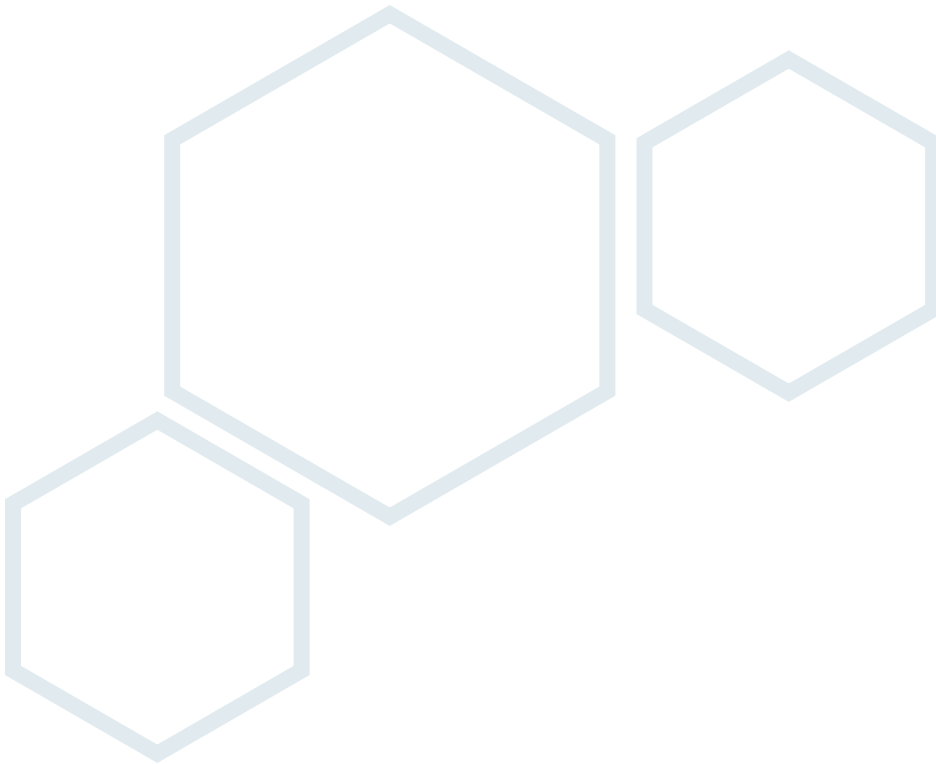
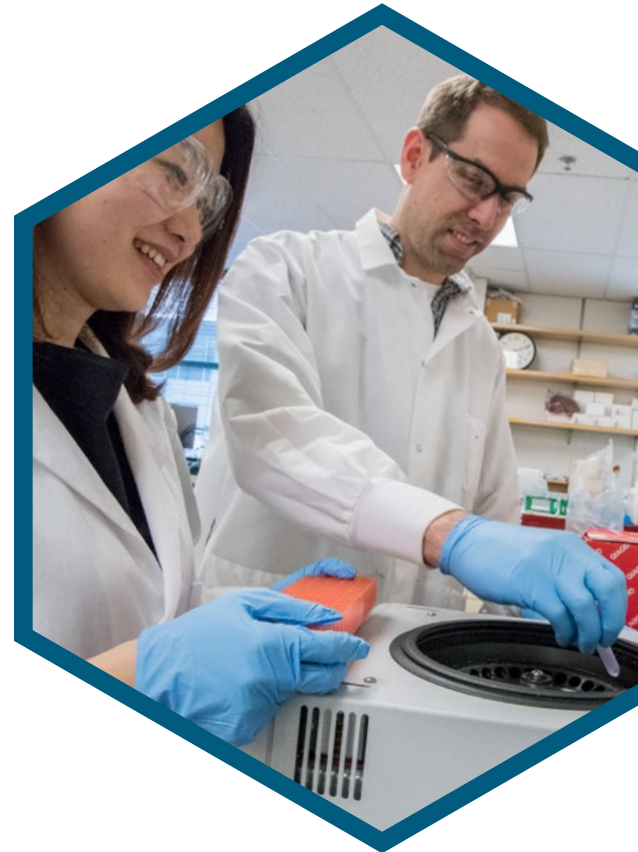
organisms, instead of merely cataloging relationships between input and output, responses of biological organisms in hypothetical situations can be predicted based upon mechanistic knowledge.

### Select accomplishments relating to 5-year milestones

- Researchers used the genetically diverse Collaborative Cross (CC) mouse system to discover that early life history impacts the gut microbiome composition, whereas dietary changes have only a moderate effect. By contrast, the composition of the gut metabolites, or metabolome, was shaped mostly by diet, with the presence of specific non-dietary metabolites explained by microbial metabolism. By performing quantitative trait analysis, they identified mouse genetic trait loci. These loci had implications for both the gut microbiome and the genetic contribution of the mouse. The scientists found that the human versions of some of the genes found in the mouse genetic loci are implicated in gastrointestinal cancer. Ongoing studies are leveraging the CC strains to discover the microbiome and genetic contributions to resistance and sensitivity in response to environmental challenges, including herbicides and radiation.
- Scientists identified genetic loci controlling motor activity and its relationship with body weight in a cohort of 365 mice across 16 CC strains. Forty-five loci affected motor activity, seven of which were also associated with body weight, suggesting a strong link at the genetic level. Genes identified in this study also overlap significantly with those related to neurological disorders and obesity in human studies. These results provide a genetic framework for studies of the connection between microbiome composition, body weight, the central nervous system, and behavior.
- Biosciences researchers also investigated the effects of neonatal and adult thirdhand smoke (THS) exposure on bodyweight and blood cell populations in a specific strain of mice. They demonstrated that neonatal THS exposure decreases bodyweight and induces persistent changes in the hematopoietic system independent of age at exposure. They also investigated the effects of short-term early exposure to THS on lung carcinogenesis in a strain of mice used in lung cancer studies. Forty weeks after THS exposure from 4 to 7 weeks of age, the mice had increased incidence of lung adenocarcinoma, and larger tumor size and number compared with controls. *In vitro* studies using cultured human lung cancer cells showed that THS exposure induced DNA damage, as well as increased cell proliferation and colony formation. RNA sequencing analysis revealed that THS exposure induced intracellular stress and activated p53 signaling. These data indicate that early exposure to THS is associated with increased lung cancer risk.
- Scientists sequenced the genomes of nine key gut microbes in the fly *Drosophila melanogaster* and used this sequence information to understand how the microbiome contributes to susceptibility to the herbicide atrazine. They found a set of candidate enzymes in the bacteria *Acetobacter tropicalis* that likely metabolize atrazine and observed that adding back *A. tropicalis* to germ-free flies enhances resistance to the herbicide.

## Outlook

The 10-year metrics for this strategy include: identifying the keystone components that mediate the impact of environmental challenges; understanding if and how the effects of major environmental stressors are transmitted through generations; integrating multiscale imaging and computation to enable predictive models for environmental challenges; and elucidating the role of microbial community interactions within biological systems. This work has been funded through federal and state programs, including the NIH's National Human Genome Research Institute **model** organism **ENCyclopedia Of DNA Elements**, (modENCODE), the Tobacco-Related Disease Research Program of California, and through internal investment at Berkeley Lab. Given the progress and expected continuation of support, this strategy is on track to meet its 10-year metrics.









# PROGRESS REPORT: BIOMANUFACTURING

**10-Year Goal:** Develop and demonstrate a scalable, flexible, cost-effective, and sustainable biology-based manufacturing infrastructure driven by applications in energy, health, environment, and agriculture.

To achieve the Biomanufacturing goal by 2023, we focus on three strategies: the development of tools to design, construct, and debug biological systems; the design and scaling of biological systems; and the creation of bio-directed materials and bio-nanosciences. These strategies address gaps that need to be met to, (1) develop sophisticated computer-aided design and fabrication tools for biological systems, (2) model and learn from engineered biological systems and processes, (3) engineer scalable, trackable, robust biological systems for a variety of applications, and (4) generate new systems by coupling biological components to chemical and physical systems for new materials such as mineral-metal nanostructures. Towards these goals, we have leveraged large ongoing projects at the Lab, such as the computational and metabolic engineering teams at the JBEI, and capabilities at user facilities, such as the analytical, gene sequencing, and synthesis at the JGI, materials fabrication and development at the Molecular Foundry, and the process demonstration at the ABPDU. Berkeley Lab researchers now lead the broad multi-lab project, Agile BioFoundry (ABF) that, together with JBEI, has made rapid progress in developing necessary standardized experiment data collection and retrosynthetic infrastructure for biosynthetic pathways. Gene discovery, DNA synthesis, and design teams at the JGI, JBEI, ABF and in Scientific Focus Areas such as ENIGMA have helped achieve many of the key goals of developing tools and paradigms of designed biological systems. At the Molecular Foundry, biohybrid systems composed of living, engineered microorganisms have been generated that can interface with electronic materials and are on track to meet the 10-year goal for a prototype multifunctional platform intended for manufacturing of two or more bio-directed materials.

## 10-YEAR Biomanufacturing Goal

Develop and demonstrate a scalable, flexible, cost-effective, and sustainable biology-based manufacturing infrastructure driven by applications in energy, health, environment, and agriculture.

### Biomanufacturing Research Strategies to Achieve Goal

**Tools to design, construct, and debug biology.** Develop computer-aided design and fabrication tools, computational, and analytical methods to model and learn from engineered biological systems and processes, and retrosynthetic infrastructure providing optimized pathways to key molecular hubs.

**Designed biological systems.** Engineer and scale-up controllable, trackable, and robust biological systems (prokaryotes, Archaea, and eukaryotes) for key energy, health, and environmental biomanufacturing applications.

**Biodirected materials and bionanosciences.** Couple biological components to chemical and physical systems to biosynthesize desired mineral/metal nanostructures.



**Tools to Design, Construct, and Debug Biology Strategy:** Develop computer-aided design and fabrication tools, computational and analytical methods to model and learn from engineered biological systems and processes, and retrosynthetic infrastructure providing optimized pathways to molecular hubs.

The technical ability to edit and insert DNA into organisms has inspired visions of a new era of synthetic biology where novel genes can be designed and constructed for useful purposes. Today, whole-genome engineering promises to enable the manufacture of increasingly complex genetic designs. However, while the advent of genome manipulation technologies has the potential to rapidly accelerate this process, progress is severely limited by the lack of knowledge of how to design and control the sophisticated gene networks and metabolic pathways in the context of an overall biomanufacturing process at relevant scales. Moreover, once a biological system has been constructed, there are limited tools to debug the system and improve upon it. Hence, tools are needed to design and fabricate biological systems, and model and learn from the systems and processes once they are constructed, as well as an infrastructure providing optimized pathways to key hub molecules in retrosynthetic design space.

#### Select accomplishments relating to 5-year milestones

- Biosciences researchers have developed a biological computer-aided design and manufacture (BioCAD/CAM) infrastructure comprised of tools for pathway retrosynthesis and host engineering. Using a web-based software tool called Experimental Data Depot, they have integrated functional genomics into the biological design process.
- Scientists used this BioCAD/CAM infrastructure to design, implement, and optimize the biomanufacture of two key product molecules: violacein and actinorhodin. The actinorhodin pathway was the largest pathway refactored at that point in time.
- Researchers developed a suite of “targets of opportunity,” a set of key retrosynthetic pathways representing very useful intermediates that enable the biomanufacture of scores of potential molecules for bioproducts.

#### Outlook

This strategy is on track to meet many of its 10-year metrics, which will be achieved by further development of a BioCAD/CAM infrastructure, establishing detailed and extensible techno-economic models for manufacturing processes and applications, and building an infrastructure for biological retrosynthesis. The Agile BioFoundry (ABF), JBEI, and the ABPDU house many of the programs that contribute to the success of this strategy. Internal Berkeley Lab investments supported the initial integration of biomanufacturing tools, and follow-on investment from DOE established the ABF as an eight-member national lab consortium. Continued engagement with the bioproducts and biofuels industry indicates that the tools developed in this strategy are greatly needed, especially by small companies in this space.



**Designed Biological Systems Strategy:** Engineer and scale up controllable, trackable, robust biological systems (prokaryotes, Archaea, and eukaryotes) for key energy, health, and environmental biomanufacturing applications.

Domestication of organisms is the process of selecting for pliability, safety, and utility in order to impart useful modifications to DNA and derive benefit for humans. Bacteria and fungi involved in fermentative food, feed, beverage, fuel ethanol, and pharmaceutical production are the best understood, safest, and most manipulated microorganisms on the planet. However, successes have been constrained by the limited availability of suitable, tractable biological systems. Adding new bacteria, Archaea, fungi, and plants that are generally regarded as safe to the stable of controllable hosts would not only advance the science of genetic manipulation, it would significantly broaden the range of products amenable to biomanufacturing. A key step in developing designed biological systems for biomanufacturing is optimizing and ensuring that these systems are scalable to relevant production levels in real world applications.

### Select accomplishments relating to 5-year milestones

- Biosciences researchers have domesticated previously intractable host bacteria for use in biomanufacturing, particularly *Rhodospiridium toruloides* and a number of actinobacteria. In addition, tools for bacterial domestication have been developed for more than 75 non-model organisms, mainly protobacteria and actinobacteria.
- Scientists have engineered plants with modified lignin that can be easily deconstructed during biomass processing. They have also engineered pathways to produce novel lignins that can be more readily processed and provide useful precursors to bioproducts. They have also identified aromatic precursors from lignin that can be used in metabolic pathways to produce chemicals of interest, and manipulated those pathways to redirect metabolic flux to the desired products.
- Researchers have introduced new pilot-scale integrated unit operations that streamline production of isopentenol, limonene, and bisabolene, demonstrating that bioprocesses developed at the bench can be successfully translated to industrially relevant production scales.

### Outlook

This strategy is on track to meet many of its 10-year success metrics. These metrics include establishing a robust protocol for host organism domestication, identifying a range of key hosts for biomanufacturing, establishing robust tools for plant engineering, and developing and demonstrating bench- and pilot-scale fermentation processes for novel bioproducts. The ABF, ABPDU, JBEI and other complementary programs in the Biosciences Area are pursuing the research that underpins these metrics. Additionally, work at the JGI and ENIGMA is enabling the identification, domestication, and use of novel non-model microorganisms for biomanufacturing. A synergistic program at University of California, Berkeley, the new Center for the Utilization of Biological Engineering in Space (CUBES), has a goal aligned with developing predictable biological systems. Many of the programs listed above have stable funding, providing a steady environment for this research.

**Biodirected Materials and Bionanosciences Strategy:** Couple biological components to chemical and physical systems to biosynthesize desired mineral/metal nanostructures.

Molecular self-assembly, the process by which molecules spontaneously adopt a desired arrangement without external guidance, underlies the construction of macromolecular assemblies that enable cells to function. Because of this inherent “programmability,” molecular self-assembly has become fundamental to certain aspects of nanotechnology and mesoscale science, and recently there has also been a surge of interest in the areas of programmable biomolecular assemblies and biodirected materials. Combining expertise in synthetic biology and nanotechnology with available tools and hardware for synthesizing and characterizing biomaterials positions Berkeley Lab as a leader able to drive advances in biomanufacturing.

**Select accomplishments relating to 5-year milestones**

- Berkeley Lab researchers have created a biohybrid system composed of a living, engineered microorganism that can interface with electronic materials. The microorganism is engineered to contain an electron transfer pathway that enables electron flow from intracellular redox carriers (e.g. NADH, menaquinone) to soluble metal ions, solid metal oxides, and a positively biased electrode. This biohybrid system can also deliver electrons from an electrode to intracellular redox species when the electrode is biased to a negative potential.
- A team of scientists led by researchers at the Molecular Foundry has discovered the details of an unconventional coupling between a bacterial protein and a mineral that allows the bacterium to breathe when oxygen is not available. The research revealed how proteins bind to mineral surfaces, which could lead to new innovations in linking proteins to other materials for bio-based electronic devices. It could also help researchers to understand and control the chemical reactions sparked by these protein-material interactions.
- Berkeley researchers have identified a novel extracellular electron transfer pathway in *Listeria monocytogenes*. It operates at different redox potential than other well-known extracellular electron transfer pathways and is widespread in fermentative organisms. This challenges views of extracellular electron transfer as a specialized respiratory process and suggests that such activity may represent a fundamental facet of microbial metabolism.



## Outlook

An important 10-year success metric — interfacing biological components to electronic apparatus to control their activity — has been demonstrated in the first five years. Likewise, interfacing active biological elements with inorganic materials has been demonstrated”, and efforts have been expanded to create interfaces with polymeric materials. One component of an infrastructure to characterize the abiotic-biotic interface has been instantiated, but the need to develop a time-resolved means of examining this interface remains. Work continues on using biological entities to biosynthesize architecturally specified, possibly self-healing, mineral-metal nanostructures and mesostructures on demand. However, this metric mirrors the central goal of a Biosciences-led, DARPA-funded project in the Engineered Living Materials portfolio. As part of this project, engineered cells have arranged metal or metal oxide nanoparticles into precise nanostructured arrays, so the current trajectory bodes well for achieving this last metric.

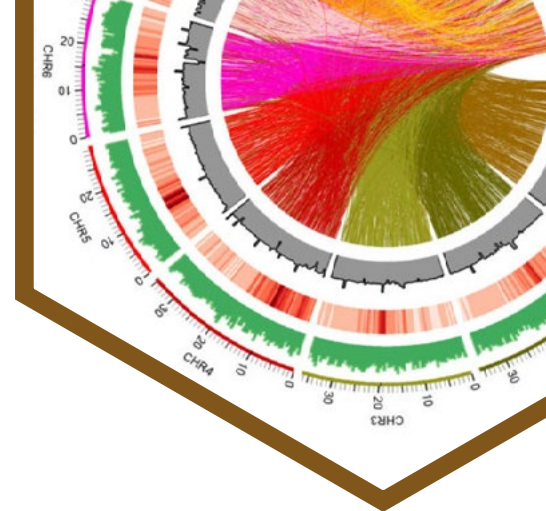








# PROGRESS REPORT: TECHNOLOGIES



**10-Year Goal:** Develop a technology infrastructure to measure, predict and control biological systems for solving energy, environmental, and health challenges.

The Technologies goal is to provide new experimental and computational tools to support the research efforts of the other BSP goals, and the research community at large. This has necessitated progress in two major areas: characterization of biological systems using structural biology, bioimaging, and functional genomics methods, and computational analysis of data. Advances in these areas will enable the new kinds of research needed for transformative breakthroughs in biomanufacturing, energy, environment, and health.

Structural biology methods are needed to provide a mechanistic understanding of the molecular machines that drive the production of biomolecules, transformation of chemicals in the environment, and destruction of faulty cells in our bodies. Biosciences researchers are enabling new approaches in structural biology by integrating methods at the DOE-supported synchrotrons, advancing rapidly development methods such as electron cryo-microscopy, and exploiting new X-ray free-electron laser (XFEL) sources. Novel imaging methods are needed to reveal the location of molecules in cells and follow their dynamics over time, and as a consequence, new non-destructive, label-free, *in situ* analysis methods are being developed and integrated with other imaging methods to provide a more complete picture of cellular activity.

While structural biology and bioimaging methods provide a wealth of information for understanding biological systems, the complete characterization of biological systems, from the cellular to organismal, requires many additional phenotyping measurements. Biosciences researchers have made excellent progress in developing new technologies for high throughput enzyme assay, and transcriptome, metabolome, and proteome measurement.

## 10-YEAR TECHNOLOGIES Goal

Develop a technology infrastructure to measure, predict and control biological systems for solving energy, environmental, and health challenges.

### Technologies for BioSciences to Achieve Goal

**Structural Biology; Bioimaging;  
Functional Genomics; Mathematics,  
Informatics, and Computing.**



These have been combined with genomic information to discover new biological activities and rapidly phenotype cells. It is anticipated that these technologies will be central to future research across the Biosciences area, for both uncovering new biological pathways, and exploiting them in engineering.

Finally, there has been great progress in developing new computational methods needed to analyze the complex data generated by all of the research being performed to realize the goals of the BSP. These include the analysis of new experimental data types from free-electron laser and electron microscopes, supported by funding from the National Institutes of Health and the Advanced Scientific Computing Research program; the latter aimed at exploiting exascale computing resources. In the coming years a major effort will be to develop new algorithms that bring together genomic, imaging, and functional genomic data types across multiple resolution ranges through computational models, and in the process generate new knowledge of biological systems.

### **Structural Biology Strategy**

The workhorse technique of crystallography has been used to delineate over one hundred thousand molecular structures to date. As a mature and accessible method, crystallography has additionally benefitted from internationally accepted databases to store solved structures, and numerous computational programs designed to aid in structure solution and visualization. As has been proved with the crystallographic databases, as data are shared more widely, science is enabled more broadly. In the case of protein crystallography, access to a greater number of previously solved structures gives a higher probability of success for future structures. Biosciences aims to link structural methods into a unified platform encompassing shared instrumentation, data analysis programs, and knowledge databases to gain a full picture of the conformational and functional flexibility of biological components.

#### **Select accomplishments relating to 5-year milestones**

- Biosciences scientists have established the ALS-ENABLE (Advanced Light Source Efficiently Networking Advanced BeamLine Experiments) program that will leverage the capabilities and expertise across the diffraction and scattering beamlines at the ALS. This will optimize the chances of successful structure determination for both routine and challenging structural biology problems.

### **Outlook**

Many of the 10-year success metrics have already been met or are on track to be met. These strategies are supported by research at the ALS, a DOE Office of Science National User Facility, as well as programs that include the Computational Crystallography Initiative, Diffraction Integration for Advanced Light Sources (DIALS), and the Center for Advanced Mathematics for Energy Research Applications (CAMERA). Through these efforts, Biosciences will continue to lead in technology development for biology.



## Integrated Bioimaging Strategy

Biological systems display unique behaviors, including self-organization across temporal and spatial scales ranging from atoms to organisms. Visualization provides perhaps the most powerful basis for understanding the behavior of molecular components in the context of cells, tissues, organisms, and communities. Berkeley Lab is addressing these challenges through advances in instrumentation and integrated computational strategies for large and diverse data arrays, allowing multimodal imaging across a range of length and time scales. This will build on available expertise and instrumentation for imaging cells, tissues, and organisms using fluorescent probes, radioisotopes, and electromagnetic radiation.

### Select accomplishments relating to 5-year milestones

- Biosciences researchers are establishing a program for spectral phenotyping, a non-invasive and label-free approach to identify cell types by chemical signatures. The program will integrate novel probes, infrared imaging, engineering, and computation and machine learning.
- Scientists have developed an approach to resolve and extract discrete cellular and subcellular information from heterogeneous cell populations in real time with high resolution using spectral phenotyping that integrates Fourier Transform Infrared (FTIR) spectro-microscopy with fluorescence-based imaging. Measurements can be made from milliseconds to hours and, barring instrument drift, even for days, since the irradiation source is non-destructive to the sample.

### Outlook

Many of the 10-year success metrics have already been met or are on track to be met. These strategies are supported by research at the ALS, as well as programs like CAMERA. Through new efforts to establish a multimodal imaging center and to expand Berkeley Lab's expertise in cryo-electron microscopy, Biosciences will continue to lead in technology development for biology.

### Functional Genomics Strategy

Robust, high-throughput functional genomic analyses are critical to diverse Biosciences research areas, including understanding the genomics of microbial communities, using synthetic biology to develop sustainable energy and materials, and measuring and monitoring phenotypes associated with engineered systems and biological responses to environmental exposures. Two research thrusts are the development of (1) high-throughput technologies that provide rapid feedback on the performance of engineered systems, real-time monitoring of exposures and responses, and the direct linkage of DNA sequence to function, and (2) analytical approaches that provide detailed insights into the genetic and physiological states of biological systems and link to bioimaging and computation.

### Select accomplishments relating to 5-year milestones

- A team of Biosciences researchers has developed MAGI (Metabolites and Gene Integration), a computational tool to quickly find and score consensus between metabolite identifications and gene annotations. MAGI was built to make connecting metabolomics data with genes easier for researchers. This is crucial to overcome the limitations of each and to strengthen the biological conclusions made by both.
- Until now, it has been difficult to determine live from dead microorganisms that are collected from nature. Scientists have successfully implemented **BioOrthogonal NonCanonical Amino acid Tagging (BONCAT)** in combination with single-cell and/or single aggregate sorting and DNA sequencing to identify active microbes and their predicted pathways within microbiomes.

### Outlook

Many of the 10-year success metrics have already been met or are on track to be met. These strategies are supported by research at JGI, a DOE Office of Science National User Facility, as well as programs that include JBEI and ENIGMA. By expanding Berkeley Lab's expertise in functional genomics through new projects like mCAFES, Biosciences will continue to lead in technology development for biology.

### Mathematics, Informatics, and Computing Strategy

Mathematics, informatics, and computing play a central role as biology continues to evolve from an anecdote-based, descriptive field of science into a quantitative discipline relying on, and driven by, large data sets. Research in mathematics, informatics, and computational sciences form an integral part of the technology strategies. Methods are being developed to extract maximal information from weak data, enabling the analysis of experiments from free electron lasers and electron microscopes. New algorithms integrate experimental information of multiple types and new methods that enable automated knowledge generation from complex data types can be used to construct mathematical models that explain a wealth of experimental observations.

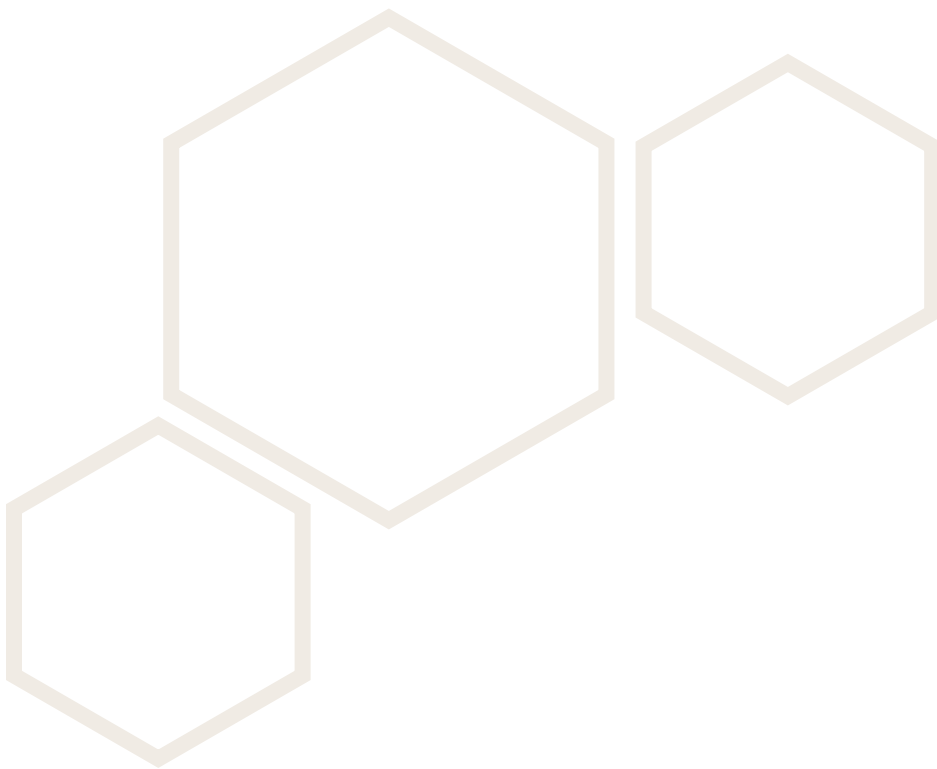
### Select accomplishments relating to 5-year milestones

- New algorithms for the analyses of data obtained from several techniques used to study the biophysical properties and structure of biological macromolecules (e.g., X-ray crystallography, small-angle/wide-angle X-ray scattering (SAXS/WAXS), and tomography) are under active development. Biosciences researchers are at the forefront, developing new diffraction integration software to meet the data analysis requirements presented by advances in the field. This is true for data collected at recently established XFEL light sources, which are used to gather structure and dynamic macromolecular snapshots and can determine molecular electronic states when coupled with X-ray spectroscopy. Novel algorithms are also being written for analyzing data collected from near-atomic resolution cryo-electron microscopy, electron tomography, and correlated X-ray scattering.

## Outlook

Many of the 10-year success metrics have already been met or are on track to be met. These strategies are supported by research at the ALS and JGI, as well as programs that include Computational Crystallography Initiative, CAMERA, JBEI, and ENIGMA. Through efforts to expand Berkeley Lab's expertise in data science, Biosciences will continue to lead in technology development for bioinformatics.

Biosciences began developing the first iteration of the BSP in October of 2012. In the five years since the release of the plan in May 2013, there has been no deviation from the 10-year goals established at that time.



# LOOKING AHEAD: THOUGHTS FROM THE ALD FOR BIOSCIENCES



As the new Associate Laboratory Director for Biosciences, I am thrilled to see the progress that Biosciences has made in the last five years. When I joined Berkeley Lab in 2012, the BSP was just an idea. Our efforts to build an inclusive, bottom-up scientific strategic plan that reflected a vision for Biosciences that was both aspirational and achievable have resulted in new scientific programs and projects — such as Agile BioFoundry, mCAFES, and Finding Engineering Linked Indicators (FELIX) — strengthened our established research, forged new collaborations, and seeded new concepts that will be the funded programs of the future.

In addition to the impressive scientific progress made across the Biosciences Area, we've also made a serious effort to focus on stewardship of our resources, including our people, and to improve our diversity, equity, and inclusion practices across the Area. Cultivating talent and promoting inclusion is central to the creation of a successful work environment driven by a diversity of thought partners working toward shared objectives. Currently, we are designing an outreach effort focused on establishing relationships with historically Hispanic-serving institutions in the greater Bay Area. The objective is to introduce scientists and administrators from those organizations to the research and fellowship opportunities available for educators and students at the Lab. We know that our success depends upon our ability to create a community that brings together people with diverse backgrounds, points of view, and approaches to problem solving. By assessing gender pay equity and developing a mentorship program for everyone in all job classes in the Area, we have made important progress in our stewardship aspirations over the last few years.

When I look forward to the next five years of biological research at Berkeley Lab, I see further integration, including a broad partnership with the Computing Sciences Area (CSA), which will help develop new core capabilities and significantly accelerate progress



toward our shared objectives. The variety and scale of data generation is growing significantly across the Biosciences Area. Some examples of this include data from multi-omics experiments, biomolecular structure and function studies, biological system population genetics, biologically influenced ecosystem dynamics, and an expanding array of fermentation processes. I foresee an expansion of specialized experts who will develop Area-specific data science approaches dedicated to our goals. Starting now with a growing partnership that brings together the JGI, KBase, and the National Energy Research Scientific Computing (NERSC) center, our teams are developing novel paths toward enabling biological investigations with high performance computing platforms to derive new insights from the growing wealth of biological data. In addition to our partnership with CSA, Biosciences has a long-standing collaboration with the Earth and Environmental Sciences Area through programs like ENIGMA and the new mCAFES project. New collaborations with the Energy Sciences Area (ESA) — including the Molecular Foundry User Facility and the Materials Sciences Division — will link Biosciences' biomanufacturing expertise with ESA's capabilities for predictive design and the development of innovative materials to create novel bio-derived materials.

The BSP forged a common sense of purpose and goals for the Biosciences Area in 2013. In 2015, we executed a comprehensive reorganization of the Area, creating new Divisions with intentional missions and visions aligned with the mission of DOE, and with the ambitions described in the BSP. As a consequence, we gained detailed insight into who we are and what we want to achieve, as well as how many we are and what space and equipment we require to carry out our work. These insights positioned us in 2015 to develop a vision for a Biosciences Campus on the Lab's main site in Berkeley where we might all be brought together from the three main off-site locations throughout the East Bay at which we currently work. The progress made over the last five years is a testament to our willingness to collaborate across distances to achieve our shared objectives. As this report goes to press, the first envisioned building of the Biosciences Campus Vision, the Integrative Genomics Building (IGB), is under construction and is scheduled to be completed in 2019; approximately 250 Biosciences Area researchers from JGI and KBase will move into IGB to establish a solid Biosciences presence at the main site. Progress is also being made on two additional future buildings that we hope to see come to fruition in the 2025 timeframe. Our co-location at the main Berkeley Lab site will offer improved proximity to key Berkeley Lab personnel, assets, and expertise. I anticipate a future where our nucleation not only enables new multidisciplinary research within Biosciences and with other Berkeley Lab Areas, but greatly accelerates our ability to deliver new scientific and technological advances for the nation.

Berkeley Lab's Biosciences Area is a leader in the advancement of biological science relevant to national-scale challenges in energy, environment, health, biomanufacturing, and technology development. Biosciences strives to align its strategic plan to these national scale challenges by outlining team science approaches to research in partnership with our sponsors, including the Department of Energy.

This report describes the hard work of nearly 650 Biosciences staff in four main locations over a period of five years. I am grateful for their efforts and am excited to support them in their efforts for the next five years and beyond.

Mary E. Maxon, Ph.D.

*Associate Laboratory Director for Biosciences*

# BSP PROGRESS REPORT: 2013-2018 MILESTONE COMPLETION APPENDIX

## Key:

✓	Completed
+	In Progress — Not Completed within 5 Years
x	De-emphasized

## Energy

### Lignocellulosic biofuels

- ✓
  - Cell-wall biosynthesis and assembly elucidated through identification of new genes, alleles, and metabolic pathways controlling cell-wall recalcitrance, sugar and lignin content, and fermentation inhibitors.
    - Secondary cell walls engineered to have more C6 sugars and fewer C5 sugars.
    - The cell wall was engineered to have easily cleavable or altered lignin.
    - Biomass traits have been engineered from model plant systems to potential bioenergy crops.
  - Tools developed to determine metabolite levels and metabolic bottlenecks in plants
  - Knowledge of drought tolerance in model crops advanced through identification of new genes, alleles and metabolic pathways
  - New pretreatment methods developed that reduce cost and efficiently fractionate lignocellulose into targeted lignin and sugar output streams.
  - Enzymes discovered and developed for optimal performance under pretreatment/saccharification conditions (temperature, pressure, presence of ionic liquids).
  - New enzymes and cofactors discovered and engineered for biomass deconstruction that are tolerant of pretreatment regimens.

- ✓
  - Efficient enzymes capable of depolymerizing lignin into aromatic hydrocarbons discovered to create a strong value stream for lignin.
  - Models developed to predict how modifications to secondary cell-wall biosynthesis and degradation to improve biomass yields developed.
  - Predictive models to describe release of sugars from the plant secondary cell wall developed.
  - Hydrocarbon biosynthetic pathways and associated transporters engineered into microbes to convert sugars to transportation fuels.
  - Native hydrocarbon biosynthetic pathways in plants and microbes described.
  - Predictive models to describe metabolic fluxes developed and used to predict bottlenecks in biosynthetic pathways in microorganisms.
  - New bench- and pilot-scale unit operations and integrated processes developed and demonstrated for the production of a lignocellulosic biofuel.

#### **Alternative biofuels**

- ✓
  - Developed and implemented gas-fed bioreactor processes and associated systems and upstream and downstream unit operations for fuel production to enable demonstration at bench and pilot scale.
  - Identified essential genes required for growth and CO<sub>2</sub> metabolism in a photoautotroph.
  - Developed synthetic biology toolbox related to hydrocarbon biofuels production in a chemoautotrophic host.
  - Converted synthesis gas to hydrocarbon biofuels under anaerobic conditions.

#### **Artificial photosynthesis**

- ✓
  - Developed an advanced mechanistic understanding of photosynthesis in plants and microbes.
    - Identified the determinants of efficient photosynthesis in plants, algae, and bacteria.
    - Defined the repair mechanisms of the photosynthetic apparatus.
  - Explored options for reaching mA/cm<sup>2</sup> currents between artificial systems and cellular organisms.
- +
- Synthesized membranes capable of separating carbon-based fuels from oxygen.

- +
  - Demonstrated capability for photocatalyzing conversion of CO<sub>2</sub> to a carbon-based fuel beyond CO and formic acid.
  - Designed the first prototype devices for testing components (catalysts, light harvesters, membranes, interfaces, etc.) as an integrated system.
  - Demonstrated a photosystem for unassisted CO<sub>2</sub> reduction by H<sub>2</sub>O under membrane separation on the nanoscale.
  - Developed a mechanistic understanding of light-driven H<sub>2</sub>O oxidation on a robust Earth abundant catalyst.
  - Performed an analysis of components, materials and chemical inputs, and hardware designs to provide information on manufacturability, life-cycle costs, and reusability to ensure the system's scalability.

## Environment

### Predictive Understanding of Environmental Organisms

- ✓
  - Used model ecosystems and bacterial mutant fitness profiling to discover new genes mediating microbial interactions under environmental constraints.
  - Developed and applied new functional genomics technologies for discovery and validation of gene and noncoding regulatory DNA functions that impact the fitness of plants and microorganisms under multiple environmental conditions.
- +
  - Used exometabolomic profiling of groundwater and soil bacteria to model how soil metabolites shape microbial community structure.
  - Discovered the molecular basis of plant-growth promotion for three bacteria, each from a different phylum, to determine if these mechanisms are conserved.
- ✓
  - Used plant mutants with defined microbiomes to discover plant genes that select for beneficial microbiomes.
  - Identified over 100 metabolites exuded by plant roots and characterized how these metabolites are used by isolate soil microbes and how they impact microbial community structure.
  - Developed technologies for genome editing, mating, and selection to gain genetic mastery of model microorganisms, plants, and metazoans and applied these strategies to understanding key biological processes in the environment.

### Molecular EcoSystems Biology Based Solutions

- ✓
  - Constructed model desert ecosystems that recapitulate key aspects of native ecosystems for controlled analysis of microbial metabolic processes.



- + • Used synthetic biology tools to construct reporter microbes and used these with advanced imaging technologies to determine where and when metabolites are being produced within a microbial community.
- ✓ • Elucidated the metabolic strategies of key environmental microbial community members and the functions of specific genes that are important for community metabolism through a combination of systems biology, physiological analysis, functional genomics, and high-throughput microbial isolation.
- + • Used manipulation of environmental variables and spatially defined sequencing to determine the environmental controls on spatial distribution of microbial communities in soil environments.
- ✓ • Applied understanding of resource competition in a model ecosystem to accurately predict soil metabolite composition and community structure.
- + • Constructed model agroecosystems that recapitulate key aspects for controlled analysis of the plant-soil-microbiome processes.
- ✓ • Demonstrated the ability to alter microbiome structure and soil carbon cycling through targeted modification of plant genes.
- Discovered at least one novel microbial metabolite beneficial to the plant host and confirmed through introduction of the biosynthetic pathway into a model plant-associated microbe.
- Developed a “data ecosystem” computational infrastructure that enabled integration of microbial and multi-cellular systems biology modeling for interactions in microbiomes, especially in native and model ecosystems.

## Health

### Biological responses to environmental challenges

- ✓ • Identified genetic, epigenetic, transcriptomic, metabolomic, proteomic, microbiome, and phenotypic responses (molecules, cells, tissues, and organisms) to two environmental challenges (e.g., anthropogenic pollutants such as heavy metals and synthetic organic compounds) in model microbial communities, biomes, and eukaryotic organisms (*Drosophila*, rats, and mice).
- Determined how the effects of these two environmental challenges are influenced by genetic and epigenetic variation, and whether they are transmitted across cell generations and trans-generationally.
- Demonstrated technologies for high-throughput characterization of macromolecular complexes acting in environmental responses.

- ✓ • Integrated data to develop and test mechanistic and predictive models of two environmental responses, including microbiome/host interactions.

- +
- Formulated a list of predictive bioindicators for fitness that includes genes, epigenetic markers, proteins, metabolites, and microbiome components.

### **Impact of environmental challenges on human biology**

- X • Developed prototype body-on-a-chip devices that represent at least two biomimetic tissues or tissue-states (normal vs. diseased), fabricated from single source normal primary human cells and extracellular matrices. Validated relevance to in vivo tissues at the levels of architecture, gene, and protein expression patterns.
- Determined if at least one of the environmental challenges identified in model systems affect human biomimetic tissues in a predictable manner.
- Completed phenotypic analyses of human blood samples, focusing on the impact of diet and different stages of pregnancy, and the roles of genetics, epigenetics, transcripts, metabolites, and the microbiome.
- Formulated a list of key bioindicators for human health and disease that include genes, epigenetic markers, transcripts, proteins, metabolites, and microbiome components.

## **Biomanufacturing**

### **Tools to design, construct, and debug biology**

- ✓ • BioCAD/CAM infrastructure developed comprising one or more tools each for:
  - Pathway retrosynthesis and host engineering for production titers, rates, and yields under industrially relevant conditions
  - Integrating functional genomics data into the design process
  - Learning from characterization results to inform the design process
- Small-scale (< 2 L) physical simulation of a (> 100 L) large-scale reactor developed to aid strain optimization
- Biomanufacture of two key product molecules designed, implemented, and optimized
- Biological routes to ten key retrosynthetic molecular intermediates established

### **Designed biological systems**

- +
- A broad host range domestication (amenable to engineering) protocol designed for use in non-model organisms.

- ✓ • Domesticated one previously intractable host.
  - Used functional genomics to identify host systems responsive to domestication and manufacture scale up.
- + • Proposed a means for identifying and tracking the engineered host in different environments and assessing its impact.
- ✓ • Improved biosynthesis of at least one product at previously unattainable yields.
  - Demonstrate that lessons learned apply to an unrelated host.
- Developed and demonstrated new bench- and pilot-scale unit operations and integrated processes for the production of a novel bioproduct.
- Engineered plants that produce modified lignin that can be easily transformed into a useful commodity chemical.
  - Identified aromatic precursors that can be used in metabolic pathways to efficiently produce chemicals of interests.- Manipulated plant metabolism to redirect metabolic flux toward desired products (e.g. commodity chemicals, aromatic precursors, novel monolignols).
  - Engineered monolignol pathways to produce novel lignin that are efficiently deconstructed and are more readily processed to commodity chemical.

### **Biodirected materials and bionanosciences**

- ✓ • Prototyped a biohybrid system that interfaces active biological elements with other chemical, physical, or electronic materials.
  - Demonstrated molecularly-defined extracellular electron transfer to metals, metal oxides, and electrodes.
  - Demonstrated molecularly-defined electron transfer from an electrode to intracellular species.
  - Identified at least two electron transfer pathways to extracellular acceptors that operate at different redox potentials.
- Structurally characterized an example of a redox active molecules that functions at the abiotic/biotic interface.
- Developed a prototype multifunctional platform intended for manufacturing of two or more biodirected materials.

### **Technologies**

#### **Structural biology**

- + • Establishment of a national biosciences cryo-EM facility
- ✓ • Integration of scattering and diffraction structural methods at the Advanced Light Source

### Bioimaging

- ✓
  - Establishment of a center for integrated bioimaging.
  - Multiple imaging modalities applied to imaging biological systems from the nm to mm length scale and the msec to hour time scale.
  - New light microscopy technologies, including new contrast probes and labeling chemistries, integrated into existing imaging systems.
  - Model-based algorithms developed for combining information across multiple imaging methods and integrating functional data.
  - Collaborations with Berkeley Lab mathematics, informatics, and computing researchers result in big data analysis methods applied to several challenging biological problems.

### Functional genomics

- ✓
  - Demonstrated effectiveness of an automated chip-based mass spectrometry platform with enhanced throughput and analytical chemistry capabilities.
  - Demonstrated advanced technologies for high-throughput functional genomic analyses that are tightly integrated with computational resources.
  - Applied comparative gene expression coupled with metabolomic, proteomic, and fitness data for biochemical discovery and testing of genomic predictions.
  - Demonstrated technologies to identify active metabolic pathways within complex multicellular systems.

### Mathematics, informatics, and computing

- ✓
  - Novel data analyses methods developed for existing methods, such as X-ray crystallography, small and wide-angle X-ray scattering (SAXS / WAXS), and X-ray tomography.
  - New applied mathematical and algorithmic methods developed for the analysis of the emerging methods that make use of X-ray free electron lasers (XFEL), near atomic cryo-electron microscopy, electron tomography, and correlated X-ray scattering.
  - New methods developed that bring together genomic, imaging, and functional genomic data types across multiple resolution ranges through computational models, in the process generating new knowledge of biological systems.
- +
- Biological activities, at the atomic, cellular, and organismal level predicted through the use of integrated analysis and computation.



