

# INTEGRATING CO<sub>2</sub> INTO BIOMANUFACTURING WORKSHOP REPORT

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### EXECUTIVE SUMMARY

Mitigating climate change stemming from human activities that emit greenhouses gases, primarily carbon dioxide (CO<sub>2</sub>), is a central global challenge. Reducing current CO<sub>2</sub> emissions as well as removing CO, from the air is critical to prevent global warming from increasing beyond 2°C. Much of the research and development on mitigating CO<sub>2</sub> emissions at their point of origin have been focused on capturing and sequestering  $CO_2$ . However,  $CO_2$  mitigation is also an opportunity to incorporate CO, into the bioeconomy through sustainable biomanufacture of biofuels, biochemicals and long-lived bioproducts. Biological systems are uniquely capable of capturing and converting CO<sub>2</sub>, so incorporating CO<sub>2</sub> as a feedstock into the biomanufacturing enterprise provides a promising opportunity to establish a new sector in the US bioeconomy that directly mitigates climate change. CO<sub>2</sub> biomanufacturing, which encompasses biological CO<sub>2</sub> conversion as well as conversion of reduced products derived from CO<sub>2</sub>, including: carbon monoxide (CO), formate, and methanol can leverage both plants and microbes for bioproduction. Therefore it is valuable to consider both plants and microbes together in developing a research agenda for biomanufacturing, because they have related CO, uptake mechanisms and CO, fixation pathways. Biodesign principles, systems biology techniques and heterologous production pathways for plants and microbes that have been developed for the conversion of plant biomass can be reimagined to improve the ability of plants and microbes to use CO<sub>2</sub> more efficiently and produce biofuels, bioproducts and biomaterials.

On November 24, 2020, Lawrence Berkeley National Laboratory (Berkeley Lab) hosted a virtual workshop on biomanufacturing using CO, to identify ways to integrate the study of biological CO, conversion in plants and microbes. The workshop participants were asked to evaluate current gaps and challenges in biological CO, conversion, focusing on three main areas: CO, uptake, CO, fixation, and bioproducts that can be obtained from CO<sub>2</sub>. For CO<sub>2</sub> uptake, capture of atmospheric  $\mathrm{CO}_{_2}$  was identified as a unique property of biological systems. Opportunities to deploy carbon concentrating mechanisms (CCMs) in biomanufacturing and discovery of new CCMs that may be present in soil chemoautotrophic microbes were discussed. The possibility to improve plant biomass yields through engineering CO<sub>2</sub> uptake was identified as a compelling research challenge. Participants discussed improvements in current CO, fixation pathways like the Calvin-Benson-Bassham (CBB) cycle, exploiting alternative CO, fixation pathways found in microbes, and designing de novo pathways. The identification of microbial hosts to exploit these alternative and synthetic pathways was identified as a promising research direction. Products from CO, were divided into two categories: carbon-neutral, for biofuels and commodity chemicals, and carbonnegative, for biomaterials that can be engineered to sequester carbon for long time scales, either in the terrestrial biosphere or in the built environment. For hybrid bio-electrochemical systems, further understanding of C1 microbial metabolism and integration of electrodes and microbes in single bioreactors by harmonizing rates and minimizing toxic by-products, were discussed.

### ABOUT THE WORKSHOP

On November 24, 2020, Lawrence Berkeley National Laboratory (Berkeley Lab) hosted a virtual workshop on Integrating CO, into Biomanufacturing to identify ways to integrate the study of biological CO<sub>2</sub> conversion in plants and microbes with the purpose of developing systems that can generate biofuels, bioproducts and biomaterials that can mitigate climate change. The workshop leverages Laboratory-Directed Research and Development investments in biological CO, conversion and work on biosystem design of plants and microbes performed in the Joint BioEnergy Institute and Joint Genome Institute. The workshop also leverages work performed at the Joint Center for Artificial Photosynthesis (JCAP) and Liquid Sunlight Alliance (LiSA) on photoelectrochemical water-splitting and CO<sub>2</sub> reduction. This workshop brought together researchers from the units in the Biosciences Area at Berkeley Lab (Biological Systems and Engineering, Environmental Genomics and Systems Biology, Molecular Biophysics and Integrated Bioimaging and Joint Genome Institute) Berkeley Lab's Chemical Sciences Division in the Energy Sciences Area and the Energy Technologies Area. This workshop is an initial effort to establish research directions for integrating CO, into biomanufacturing focused on CO, bioconversion and we look forward to engaging with other national laboratories, academic partners, and private industry to advance this critical area.

The workshop focused on science drivers and research areas for integrating  $CO_2$  into biomanufacturing, dividing the topic into:  $CO_2$  uptake,  $CO_2$  fixation, products from  $CO_2$ , and hybrid approaches (electrochemical-biological) to  $CO_2$  conversion. The workshop was framed by overview talks from Biosciences Area leadership and researchers involved in biological  $CO_2$  fixation. These talks were followed by three breakout sessions that asked participants to identify gaps in  $CO_2$ conversion, possible research directions and articulate what success looks like in the short, near and long-term.

24 attendees took part in the workshop and the outputs of the workshop will be **1**) a workshop report describing the participants' discussions, **2**) a position paper providing perspective on biological CO<sub>2</sub> conversion and, **3**) concepts for new research programs.

This workshop was funded with internal Biosciences Strategic Programs Development funds.

### INTRODUCTION

Anthropogenic climate change is a global issue of enormous magnitude that threatens human civilization. Carbon dioxide  $(CO_2)$  generated from human activities, primarily the combustion of fossil fuels, energy-intensive agriculture, and construction, is the most important contributor to climate change. Reducing current  $CO_2$  emissions as well as removing  $CO_2$  from the air is critical to prevent global warming from increasing beyond 2°C. There has been substantial recent interest in developing technologies to capture  $CO_2$  from point sources, such as ethanol production facilities and power plants, and from atmospheric  $CO_2$ . Some of these technologies will be deployed to capture  $CO_2$  and sequester it by storage in geologic formations, others capture  $CO_2$  and use the captured  $CO_2$  for chemical synthesis. However, current conversion technologies are inadequate to capture and convert  $CO_2$  from point sources and the air efficiently or economically.

An alternative way to think about the problem of  $CO_2$  mitigation is as an opportunity to incorporate  $CO_2$  into sustainable biomanufacturing. Since transportation fuels and commodity chemicals are multi-carbon compounds, there are large global markets for products that can be made from  $CO_2$ . Biological systems are uniquely capable of capturing and converting  $CO_2$ , so incorporating  $CO_2$  into biomanufacturing provides a promising opportunity to establish a new sector in the US bioeconomy that directly mitigates climate change. The introduction of  $CO_2$  into biomanufacturing strategies that have been established based on starch and lignocellulosic-derived sugars.

 $CO_2$  biomanufacturing can leverage both plants and microbes for bioproduction. Plants require  $CO_2$  to grow and therefore bioproducts in plants naturally are synthesized from  $CO_2$ . Therefore, biodesign improvements to  $CO_2$  uptake and fixation linked to targeted manipulation of metabolic pathways will yield bioproducts that can be extracted or incorporated into soil upon plant senescence. These bioproducts may be carbon neutral if used as fuels or commodity chemicals or carbon negative if sequestered in long-lived materials or soils. Microbes, including bacteria, archaea and microalgae, have a variety of different strategies to fix  $CO_2$ , including photosynthetic and chemoautotrophic (non-photosynthetic)  $CO_2$  fixation. This variety in host and mechanism will lead to flexible strategies for biomanufacturing that can be adapted to different or different in developing a research agenda for  $CO_2$  biomanufacturing, because they have related  $CO_2$  uptake mechanisms,  $CO_2$  fixation pathways and can be engineered with the same heterologous pathways for biomanufacturing.

The rapid reduction and expanding deployment of renewable electricity provides a potential pathway to use electrons to generate reductants for microbial  $CO_2$  conversion. Currently, the most attractive option is to convert electricity to hydrogen gas (H<sub>2</sub>), which can serve as a reductant for both aerobic and anaerobic chemoautotrophs. However, H<sub>2</sub> is poorly soluble in aqueous solution, therefore CO, formate, and methanol may be alternative substrates to H<sub>2</sub> or  $CO_2$  for microbial conversion that are freely soluble in aqueous solution.

Biodesign principles, systems biology techniques and heterologous production pathways for plants and microbes that have been developed to improve the conversion of biomass through sugar and aromatic intermediates can be reimagined to improve the ability of plants and microbes to use CO<sub>2</sub> more efficiently and produce biofuels, bioproducts and biomaterials.

### WORKSHOP INSIGHTS

The workshop participants were asked to evaluate current gaps and challenges in biological  $CO_2$  conversion, focusing on three main areas:  $CO_2$  uptake,  $CO_2$  fixation, and bioproducts that can be obtained from  $CO_2$ . The participants included both microbial and plant biologists, so approaches to improving  $CO_2$  conversion and possible synergies between these two platforms were explored. The participants also considered hybrid approaches that combined electrochemical, photoelectrochemical, and biological conversion through C1 intermediates: carbon monoxide (CO), formate, and methanol.

#### **Science Drivers and Research Opportunities**

#### CO<sub>2</sub> Uptake

Air Capture of  $CO_2$ : Capturing and concentrating  $CO_2$  from the atmosphere is essential for removing  $CO_2$  from air. Chemical methods are being developed to perform air capture of  $CO_2$ ; however, achieving efficient and cost-effective  $CO_2$  air capture remains a significant challenge. A variety of plants and microbes can grow by concentrating  $CO_2$  for autotrophic growth. Understanding these carbon concentrating mechanisms (CCMs), along with approaches to improve their efficiency, will enable improved biological  $CO_2$  air capture that can be applied to draw down  $CO_2$  from the atmosphere and integrate into biomanufacturing.

Photosynthetic microbes, including cyanobacteria and algae, have developed carbon concentrating mechanisms to deliver low concentrations of  $CO_2$  from air using microcompartments, referred to as carboxysomes (cyanobacteria) and pyrenoids (algae), to concentrate  $CO_2$  in close physical proximity to the key  $CO_2$ -fixing enzyme ribulose biphosphate carboxylase (Rubisco). This CCM mechanism is also found in some chemolithoautotrophic bacteria. Certain land plants have developed CCM mechanisms, the C4 pathway (tropical grasses) and the Crassulacean acid metabolism (arid plants), that are distinct from the microbial systems and involve malate as carrier of  $CO_2$  to Rubisco. A key fundamental science gap in understanding CCM mechanisms is to discover whether there are additional CCM mechanisms that may be employed by plants and microbes. In support of this hypothesis, microbes that have alternative  $CO_2$  fixation pathways lack known CCMs, though many of them are capable of growing autotrophically in air. A key engineering gap is that the principles for constructing CCMs are not known.

 $CO_2$  Capture from Point Sources:  $CO_2$  from point sources, such as ethanol production (90-99%  $[CO_2]$ ), refinery offgas (50%  $[CO_2]$ ) concrete production, and power plant effluent (3-14%  $[CO_2]$ ), are both substantial sources of  $CO_2$  emission and opportunities to co-locate facilities for biological  $CO_2$  conversion. An important knowledge gap is to understand the effect of contaminants in these gas streams on biological  $CO_2$  conversion and how inhibitors in these gas mixtures can be overcome.

**Research opportunities:** Designing strategies for introduction of CCMs into plants and microbes has the potential to improve  $CO_2$  uptake and conversion, leading to advances in plant biomass productivity and biotechnological applications that can convert gas streams with low concentrations of  $CO_2$ . This design strategy will require understanding how the components of

traditional CCMs (based on carboxysomes and pyrenoids) function to concentrate  $CO_2$  and whether these functional elements can be transferred to plants and microbes that do not have these capabilities. The compartments that house the components of the CCMs may be repurposed to combine catalysis with sequenctial separation for reactions beyond Rubisco-based  $CO_2$  fixation. This membrane can be used to confine  $CO_2$  (e.g. separation) with other  $CO_2$ - fixing catalysts (synthetic or natural) that do not suffer from the shortcomings of Rubisco. Discovery of new, non-carboxysome-based CCMs in microbes may identify complementary functional elements that can also be introduced into plants and microbes. Soils may harbor a large reservoir of these CCMs as soil autotrophs need to respond to widely fluctuating concentrations of  $CO_2$ . These noncarboxysome-based CCMs also may be important in improving  $CO_2$  conversion in microbes with alternative or synthetic  $CO_2$  fixation pathways. An important step in the research area is to identify model systems that are sources of CCMs and target hosts to develop engineering principles for installation of these CCMs. These hosts could include crops and associated model plants (e.g. *Arabidopsis thaliana* and poplar) and chemoautotrophic bacteria that have been demonstrated to produce biofuels and bioproducts from  $CO_2$ .

#### CO, Fixation

**Calvin-Besson-Bassham (CBB) Cycle and Rubisco:** All plants and the vast majority of microbes use the CBB cycle to fix  $CO_2$  into biomass. The central enzyme in this cycle, ribulose biphosphate carboxylase, Rubisco, is a slow enzyme that functions as an oxygenase as well as a carboxylase. In addition, the CBB cycle is requires high levels of NAD(P)H and ATP to function. To mitigate the oxygenase bypass of Rubisco, plants and microbes have developed photorespiration pathways to recycle the products of Rubisco oxygenase activity. The carboxysome-based CCMs described above are also adaptations to the poor activity of Rubisco, concentrating  $CO_2$  in the vicinity of the enzyme to enhance kinetics. While the CBB cycle is ubiquitous, its poor efficiency remains a significant challenge in deploying  $CO_2$  biomanufacturing in plants and microbes.

**Alternative CO<sub>2</sub> fixation pathways:** Bacteria and archaea have developed alternative CO<sub>2</sub> fixation pathways, some which are less energy intensive than the CBB pathway and use carboxylases that have higher rates and lower oxygen sensitivity compared to Rubisco. However, these alternative pathways are often present in slow-growing microbes that are difficult to genetically manipulate, limiting our understanding of these alternative pathways and complicating the implementation of these pathways in more tractable hosts. An additional complication is that the most energy efficient fixation pathways, the Wood-Ljungdahl pathway and the reverse TCA cycle, have Fe-S proteins with pronounced oxygen sensitivity as part of each pathway.

**Synthetic pathways:** The drawbacks associated with the CBB pathway and the alternative  $CO_2$  fixation pathways have led to proposals for synthetic pathways that combine features of  $CO_2$  fixation pathways that minimize energy expenditure and maximize  $CO_2$  fixation rates. These pathways have been demonstrated *in vitro* but have not been achieved *in vivo*.

**Research opportunities:** There are multiple approaches to redesign  $CO_2$  fixation to generate plants and microbes that convert  $CO_2$  more rapidly and more efficiently than native systems. Large research efforts have focused on improving the kinetics of Rubisco and eliminating its oxygenase activity. However, comparatively less work has been done on improving the overall CBB cycle by diverting intermediates in the pathway through reactions that consume less ATP and NAD(P)H, which will improve the efficiency of  $CO_2$  conversion. This improvement will increase biomass yield in plants and lower the amount of reductant needed for microbial transformation of  $CO_2$ . A related approach integrates alternative  $CO_2$  fixation pathways into existing autotrophs that use the CBB cycle. These alternative pathways can take advantage of intermediates generated in the CBB cycle or in central metabolism. An excellent example of this approach has been demonstrated in cyanobacteria, where a novel pathway, the malyl-glycerate cycle, integrates  $CO_2$  carboxylation reactions from central metabolism into a  $CO_2$  fixation pathway that complements the native CBB cycle.

For microbes, potential hosts that have alternative  $CO_2$  fixation pathways may be domesticated by uncovering conditions for successful transformation and genetic manipulation. These new hosts can serve as alternative to current microbial hosts for  $CO_2$  conversion, which predominantly use the CBB cycle. These alternative  $CO_2$  fixation pathways can also be expressed in a model host such as *E. coli*, which would allow production of a varied suite of biofuels and bioproducts from  $CO_2$ . In both cases, substantial research would need to be conducted to develop these hosts with alternative  $CO_2$  fixation pathways for application in biomanufacturing. It is possible that entirely synthetic biological  $CO_2$  fixation pathways can be demonstrated *in vitro*, then transferred to an *in vivo* model systems; however, implementation of this approach will require extensive remodeling of native metabolism and finely tuned heterologous protein expression.

For all of these approaches described in the  $CO_2$  fixation section, the biosystem design approaches will require complementary systems biology studies, 13C isotopic tracing and metabolic modeling to identify potential bottlenecks when existing  $CO_2$  fixation pathways will be altered or when new pathways will be introduced. A combination of experiment and modeling will be crucial to successfully improving biological  $CO_2$  fixation.

#### **Products from CO**,

**Heterologous pathways and hosts:** A wide array of biofuels and bioproducts have been produced in microbes from biomass-derived sugars. In principle, these products can also be directly from  $CO_2$ ; however, methods to express the heterologous proteins in these pathways in plants and  $CO_2$ -converting microbes are not well-established. Controlled expression of heterologous proteins is critical to achieve production titers relevant to biomanufacturing. Host selection for products will also depend on native metabolisms as well as the ability of the host to be integrated into a biomanufacturing process.

**Biofuels and commodity chemicals:** The largest markets for  $CO_2$ -based products are in biofuels and commodity chemicals. Generating these products by combining light or renewably-derived reductants and  $CO_2$  using engineered microbes is a promising method for carbon neutral biomanufacturing that does not require agricultural production. Significant improvements in titer, rate and yield are required to establish this pathway for biofuels and bioproducts. Also, strategies where intermediates are produced biologically from  $CO_2$  (ethanol, isobutanol) and then converted to final products by catalysis need to be considered alongside complete biological conversion.

**Long lived materials:** An attractive goal for biomanufacturing is to generate bio-based materials that are capable of long-term carbon sequestration. Plants can be engineered to produce recalcitrant carbon compounds (lignin, suberin) in soils, directly sequestering  $CO_2$  fixed through photosynthesis. Microbes can be engineered to produce precursors for polymers that can be incorporated into building materials. Examples of these polymers include carbon fiber, which can be produced from bio-based 3-hydroxypropionate, and polyurethanes, which can be produced from bio-based polyols. Both of these polymers can be used as building materials, which will facilitate long-term storage of carbon derived from  $CO_2$ .

**Research opportunities:** A critical decision in biomanufacturing with  $CO_2$  is to determine whether the bioproduct is more efficiently and cost-effectively produced in plants or in microbes. This determination will involve techno-economic analysis (TEA) of the proposed process as well as life cycle assessment (LCA) to identify the process with lowest emissions.

Conversion of CO<sub>2</sub> to products is an inherent property of plants as CO<sub>2</sub> is their only substrate. Therefore, the ability to divert intermediates in CO<sub>2</sub> fixation and reengineer central metabolic pathways are essential for developing strategies to generate bioproducts from CO<sub>2</sub>. Initial plant bioproduct research will likely need to be conducted in model plants with relatively short generation times such as *Arabidopsis* or *Brachypodium* to accelerate discovery. However, these CO<sub>2</sub> conversion technologies will ultimately need to be deployed in crops to enable bioproduction at scale. Therefore, the choice of bioproduct and plant will be influenced by agronomic considerations. TEA/LCA will guide whether bioproducts will be molecules that may be stored in the soil or will be harvested for applications in biomanufacturing. Bioproducts introduced into soil will need to be tested to measure their persistence and to determine any adverse environmental impacts. Plant bioproducts for biomanufacturing can be accumulated in specific plant tissue from which they can be easily extracted. Successful bioproduct generation from CO<sub>2</sub> in plants will require advances in plant biosystems design, including engineering of plant metabolic pathways and synthetic biology tools for localizing bioproducts in plant tissues.

For microbes, a number of factors need to be considered for  $CO_2$  bioproduction, based on the requirements of substrate availability (light, electricity,  $H_2$ ), cost and scale. These considerations will dictate whether a photosynthetic microbe or non-photosynthetic microbe is chosen for bioproduction. If a non-photosynthetic system is chosen, the decision to choose an anaerobic host or aerobic host also needs to be made. Anaerobic autotrophs convert  $CO_2$  with high efficiencies; however they have limited product ranges (2-4 carbon chains) and are difficult to engineer. Aerobic autotrophs can produce a wider variety of products (2-18 carbon chains) and are more straight-forward to engineer, but are less efficient at converting  $CO_2$ , requiring reductant consumption for ATP generation.

#### Hybrid Electrochemical and Biological Approaches

**Microbial C1 metabolism:** Microbes possess metabolic pathways for conversion of the products of  $CO_2$  reduction: CO, formate, and methanol. These C1 intermediates are an alternative to direct biological  $CO_2$  conversion, and have the advantage of being highly soluble in aqueous solution, which obviates the need for CCMs. Conversion of these intermediates has recently been scaled for biomanufacturing: Lanzatech has commercialized the anaerobic conversion of CO to ethanol from steel mill off-gas and ICI commercialized the conversion of methanol, obtained from natural gas, to single cell protein. Therefore, these C1 intermediates may be more viable for biomanufacturing in the near term than direct bioconversion of  $CO_2$ . Pathways for conversion of these C1 intermediates have been expressed in model organisms (*E. coli, S. cerevisiae*); however these heterologous systems perform poorly compared to native hosts, suggesting that further systems biology studies of these hosts are needed to make the C1 pathways broadly viable for production.

**Bioelectrochemical reactors:** The C1 intermediates can be produced by electrochemical reduction of  $CO_2$  using renewable sources of electricity (photovoltaics, wind, hydroelectric). An intriguing variation on biological  $CO_2$  is a hybrid approach where  $CO_2$  is converted electrochemically to C1 intermediates in the same bioreactor that the biological conversion occurs. This process configuration combines advantages of both the chemical and biological systems but requires alignment in scales and rates to be deployed successfully.

**Research opportunities:** Fundamental understanding of the conversion of C1 intermediates derived from CO<sub>2</sub> (CO, formate, and methanol) is still not sufficient to reconstitute these metabolic pathways in model hosts at rates and biomass yields that are comparable to native systems, suggesting that further systems biology investigations of microbes that convert these C1 compounds will provide new insights into their metabolism. These three intermediates are also toxic to microbes, so identifying mechanisms to alleviate this toxicity is also necessary. There are opportunities, especially with CO and formate, to directly integrate electrochemical and biological conversion in one bioreactor. These hybrid bioreactors will require the rates of electrochemical and biological system mitigated.

### **Summary: Research Opportunities**

#### CO, Uptake

- Understand how components of traditional CCMs function to concentrate CO<sub>2</sub> to transfer to plants and microbes and build compartments based on these components for other microbial conversion.
- Discover new, non-carboxysome-based CCMs in microbes that may have complementary functional elements that can also be introduced into plants and microbes.
- Identify model systems that are sources of CCMs and target hosts to develop engineering principles for installation of these CCMs.

### CO<sub>2</sub> Fixation

- Improve the efficiency of the overall CBB cycle by diverting intermediates in the pathway through reactions that consume less ATP and NAD(P)H.
- Integrate alternative CO<sub>2</sub> fixation pathways into existing autotrophs to take advantage of intermediates generated in the CBB cycle or in central metabolism.
- Domesticate hosts that have alternative CO<sub>2</sub> fixation pathways and/or express these alternative pathways in a model host such as *E. coli*.

#### **Products from CO**,

- Develop TEA/LCA-based decision tree to determine whether a bioproduct is more efficiently and cost-effectively produced in plants or in microbes from CO<sub>2</sub>.
- Divert intermediates in CO<sub>2</sub> fixation and reengineer central metabolic pathways to generate bioproducts from CO<sub>2</sub> in plants.
- Establish whether a microbially produced bioproduct is generated under photosynthetic or chemosynthetic conditions and whether it should be produced in aerobic or anaerobic chemoautotrophs.

#### Hybrid Electrochemical and Biological Approaches

- Further systems biology investigations of microbes that convert these C1 compounds (CO, formate, and methanol) will provide new insights into their metabolism.
- Integration of electrochemical and biological conversion in one bioreactor.

# APPENDIX 1: BERKELEY LAB FACILITIES AND PROGRAMS RELEVANT TO CO<sub>2</sub> BIOMANUFACTURING

The **Joint Genome Institute** (JGI) performs large-scale sequencing of DNA and RNA and computational genomic analyses, and will support both biosystems design and systems biology effort for  $CO_2$  biomanufacturing. Discovery of new CCMs and new  $CO_2$  fixation pathways will rely on JGI tools.

**The Advanced Biofuels and Bioproducts Development Unit** (ABPDU) is a bioprocess scale-up facility that enables early stage innovation by performing process science to transfer fermentation and downstream purification technologies from microtiter plates to bioreactors at 100s of liters. The ABPDU currently has small scale facilities for gas fermentation that can be scaled to support CO<sub>2</sub> biomanufacturing.

**The Agile BioFoundry** (ABF) integrates industrially relevant production microbes, advanced tools for biological engineering and data analysis, and robust, scaled-up processes for integrated biomanufacturing. The ABF has collaborative projects on  $CO_2$  biomanufacturing with private companies and the tools developed by the ABF will be adapted for  $CO_2$  biomanufacturing.

The **Joint BioEnergy Institute** (JBEI) is a U.S. Department of Energy Bioenergy Research Center dedicated to developing advanced biofuels — liquid fuels derived from the solar energy stored in plant biomass that can replace gasoline, diesel and jet fuels. Researchers are using the latest tools in molecular biology, chemical engineering, computational and robotic technologies to transform biomass into biofuels and bioproducts. JBEI has developed high-throughput microfluidic systems that can be used for large-scale screening of samples to help develop diagnostic probes.

The **Liquid Sunlight Alliance** (LiSA) will establish the science principles by which durable coupled microenvironments can be co-designed to efficiently and selectively generate liquid fuels from sunlight, water, carbon dioxide, and nitrogen. Founded in 2020, LiSA is one of two projects in the Fuels from Sunlight Energy Innovation Hub funded by the U.S. Department of Energy, Office of Science, Basic Energy Sciences.

### APPENDIX 2: WORKSHOP PARTICIPANTS

Steven Singer, Senior Scientist, Biological Systems and Engineering Division Katy Christiansen, Head, Strategic Programs Development Group, Biosciences Area Mary Maxon, Associate Lab Director, Biosciences Area Eric Sundstrom, Research Scientist, Biological Systems and Engineering Division Deepika Awasthi, Project Scientist, Biological Systems and Engineering Division Patrick Shih, Faculty Scientist (UC-Davis), Biological Systems and Engineering Division Blake Simmons, Division Director, Biological Systems and Engineering Division Aymerick Eudes, Research Scientist, Environmental Genomics and Systems Biology Division Esther Singer, Research Scientist, Environmental Genomics and Systems Biology Division Lauren Jabusch, Postdoc, Environmental Genomics and Systems Biology Division Susannah Tringe, Division Director, Environmental Genomics and Systems Biology Division Nicholas Sauter, Senior Scientist, Molecular Biophysics and Integrated Bioimaging Division Junko Yano, Senior Scientist, Molecular Biophysics and Integrated Bioimaging Division Setsuko Wakao, Research Scientist, Molecular Biophysics and Integrated Bioimaging Division Kris Niyogi, Faculty Senior Scientist (UC-Berkeley), Molecular Biophysics and Integrated Bioimaging Division Cheryl Kerfeld, Guest Faculty (Michigan State), Molecular Biophysics and Integrated Bioimaging Division Michelle Chang, Faculty Scientist (UC-Berkeley), Molecular Biophysics and Integrated Bioimaging Division Yasuo Yoshikuni, Staff Scientist, Joint Genome Institute Nigel Mouncey, Director, Joint Genome Institute Frances Houle, Senior Scientist, Chemical Sciences Division Peter Agbo, Staff Scientist, Chemical Sciences Division Chris Chang, Faculty Scientist (UC-Berkeley), Chemical Sciences Division Corinne Scown, Staff Scientist, Energy Analysis & Environmental Impacts Division Millicent Firestone, Deputy Program Manager for the Office of National & Homeland Security

### APPENDIX 3: WORKSHOP AGENDA

Integrating CO<sub>2</sub> into Biomanufacturing November 24, 2020 1:00-4:30 pm Zoom/Miro

Торіс	Speaker	Time
Purpose of the workshop/Vision for CO <sub>2</sub> Biomanufacturing	Steve Singer	1:00-1:10 pm (10 minutes)
How CO <sub>2</sub> biomanufacturing aligns with U.S. and global bioeconomy strategies	Mary Maxon	1:10-1:20 pm (10 minutes)
History of the CARBON Big Idea	Blake Simmons	1:20-1:25 pm (5 minutes)
Engineering photosynthesis	Patrick Shih	1:25-1:32 pm (7 minutes)
Engineering plants for CO <sub>2</sub> capture	Aymerick Eudes	1:32-1:39 pm (7 minutes)
CO <sub>2</sub> biomanufacturing LDRD	Eric Sundstrom	1:39-1:46 pm (7 minutes)
BREAK		1:46-1:56 pm (10 minutes)
Sustainable CO <sub>2</sub> biomanufacturing TEA/LCA	Corinne Scown	1:56-2:06 pm (10 minutes)
Introduction to Miro and practice exercise	Katy Christiansen	2:06-2:21 pm (15 minutes)
Discussion 1: Maximizing capture and use of CO <sub>2</sub> by plants and microbes		2:21-3:06 pm (45 minutes)
BREAK		3:06-3:16 pm (10 minutes)
Discussion 2: Maximizing efficiency of CO <sub>2</sub> transformation into products		3:16-4:01 pm (45 minutes)
STRETCH		4:01-4:05 pm (4 minutes)
Discussion 3: Beyond biology: integration of biology and electrochemistry		4:05-4:25 pm (20 minutes)
Wrap up and next steps		4:25-4:30 pm (5 minutes)

