Biological and Environmental Research Advisory Committee
Marriott Washingtonian Hotel
Gaithersburg, MD
October 15-16, 2012

BERAC Members Present

Gary Stacey, Chair
Dennis Baldocchi
Janet Braam
James Ehleringer
Susan Hubbard
Andrzej Joachimiak
L. Ruby Leung
Joyce Penner
Karin Remington
G. Philip Robertson
Gary Sayler
Martha Schlicher
Jacqueline Shanks
Gus Shaver
James Tiedje
Warren Washington
Huimin Zhao

BERAC Members Absent

Judith Curry
Gerald Mace
Gregory Petsko
David Randall
Herman Shugart
Judy Wall
Raymond Wildung
Minghua Zhang

About 50 others were in attendance during the course of the two-day meeting.

Monday, October 15, 2012
Morning Session

The meeting was called to order at 9:04 a.m. by Chairman **Gary Stacey**.

William Brinkman was introduced to provide an update on the activities of the Office of Science (SC).

This is an interesting time in Washington, D.C., with the election three weeks away. An enormous number of issues are to come before Congress with the funding authorizations ending at the beginning of next year. It will be a tricky time after the election. The Office is trying to put together an FY14 budget.

Gary Stacey has done a great job of repositioning the Committee and revitalizing our relationships.

The biofuel centers and the Joint Genome Institute (JGI) are going well. A major question is, what is the next big step in or level of biology? The research community needs to figure out ways to advance knowledge about plants' and microbes' effects on climate. There are impressive things going on. For six years in a row, the Arctic ice has shrunk to record summer lows. It is fascinating how complex the climate system is.

The House and Senate are trying to put together an FY13 budget. The House version is smaller than the Senate version. For BER, the current appropriation is $609.5 million. The President's request for FY13 is for $625.3 million. The House mark takes away $67.5 million of that, and the Senate mark adds $5 million to it. There will probably be a continuing resolution based on the FY12 budget and lasting through March 2013. The extension of tax cuts, the FY13 budget and its associated sequestration of funds, and the debt ceiling that will be reached in February will be the focus of Congress and will lend uncertainty to DOE's budget planning.

There is very strong evidence that global temperature is going up. Something needs to be done about this problem. There is a little good news, however. For the first time, the Energy Information Administration (EIA) has said that the amount of CO_2 being produced in the United States is going down a little bit. It needs to go further and further. The Arctic is really heating up. Old ice is melting before new ice is made each winter; this is a new cycle. Glaciers have receded, one more than 100 m. There is a belt in North America that is getting hotter, but warming is not uniform. Sea level is rising. Munich Re (an insurance company of insurance companies) projects the number of natural catastrophes and their cost to the global economy. The trend is going in the wrong direction.

New technology on the market to shift energy use from petroleum to electricity includes the Tesla automobile, which can travel 300 miles on a single charge. It runs on batteries and goes from 0 to 60 mph in 4.4 s. Lithium batteries used to cost $1000/kW-hr; now they cost less than $300/kW-hr. Our country is making progress. Hybrid-car sales have taken off in terms of percent of all vehicles sold: from 0% in 1999 to 3% in 2012. Solar sales are also increasing significantly in becoming competitive, but there is now a glut of solar cells on the market. A huge solar farm is being built in California.

A new capacitor will improve the electrical grid's efficiency. Forty-six energy frontier research centers in 35 states were launched in the fall of 2009. In addition, there are now three or four energy-research hubs. In these hubs, teams are organized to advance solar, biofuel, fuel-cell, and nuclear energy. They are used to clarify the goals of the fields.

One needs to figure out what to do with the carbon dioxide from petroleum, natural gas, and coal. It is now used to enhance oil recovery. A normal well removes only 15% of the petroleum; carbon dioxide makes it less viscous and easier to recover. Carbon sequestration costs $70–$80 per ton; the goal is $18 per ton. The country will need to reduce its carbon dioxide production in the next few years. It needs to make progress in this regard. Three quarters of the SC budget is expended on energy. The problem is tremendously hard.

Stacey asked which government agencies worried about water. Brickman replied the U.S. Geological Survey and the National Oceanic and Atmospheric Administration. Water is a big issue. The administration is pushing small modular reactors. They need to get going. They are safe, small, and amenable to economics of scale, *and* they can be buried.

Sayler asked where the legacy waste issue was going. Brinkman responded that the United States spends $6 billion per year on waste. It is a huge issue on the Hanford site with 54 billion gallons of plutonium-contaminated waste. DOE is building a $12 billion plant to process the waste. It is not known whether the government has its act together on it. The waste currently stored in underground tanks can contaminate the Columbia River.

Baldocchi asked if there was any research on and plans about the electric grid and its capacity. Brinkman answered that one approach is to upgrade the grid's performance. New transmission lines are needed, and reliability issues must be dealt with. One problem is trying to figure out in real time what breaks. New analytical capabilities, better software, and more-secure software are needed.

Stacey noted that, in the 1980s, there was a big surge in alternative energy funding, but it went away. He asked whether, with cheap natural gas now, a similar decrease in funding for alternative energy will occur. Brinkman said that the reserves that the nation has today are bigger than what it had in the 1980s. One should forget about natural gas. There is a bigger problem: climate. One has to get past the false hopes of fossil fuels.

Stacey asked the Committee members to introduce themselves and to comment on recent developments in their fields.

Sayler mentioned large Chinese biochemical companies being started up with support from universities.

Penner mentioned coupling aircraft contrails to global impacts.

Washington highlighted the conduct of computer simulations for the next assessment by the Intergovernmental Panel on Climate Change (IPCC), looking at black-carbon heating over India and China and its connections with the rest of the world.

Braam was working on how plants react to environmental stresses. She also pointed out a study that showed how scientists' evaluations of faculty (both male and female) with identical curriculum vitae were biased towards males in terms of salary, mentoring advice, and other measures.

Shaver spent the summer looking at forest canopies in the Arctic, finding that canopies affect energy transfer to the permafrost and that carbon exchange is the same from one type of forest to another.

Robertson had gotten a protocol validated for NO_x uptake and its correlation for carbon sequestering to be used in cap-and-trade carbon credits.

Shanks's Institute is extending experimental flux analysis to optimize fatty-acid production, coupling genomics, proteomics, and flux measurements.

Baldocchi has looked at the ecological restoration of wetlands and the costs in terms of methane production, finding that recent vegetation stimulates methane production.

Tiedje is evaluating the diversity of microbial communities in soil and the diversity in general, finding 5000 species in a gram of soil and 400 gigabases of DNA sequence. A pangenome diversity analysis expanded that to 1.2 terabases that would require 25 terabases of sequencing.

Zhao was invited to go to Singapore to mentor PhDs in metabolic engineering to produce chemicals from biomass; this is also being done in China.

Hubbard pointed out a *Science* article that looked at subsurface metagenomic analysis of 150,000 genes attributed to 80 organisms with 49 near-complete sequences, 10 times more than found in an iron-mine-drainage study; new genomically unrecognized phyla were characterized.

Leung noted that a National Research Council national strategy for advancing climate models has made recommendations. The next step is to work with modeling centers to incorporate these recommendations. A DOE workshop on community modeling and long-term predictions of integrated cycles is being held.

Ehleringer reported finishing a stable-isotopes course, studying wildfires with a mobile laboratory (lots of methane come out of such wildfires), and looking at trace gases and carbon dioxide in urban emissions (within 10%).

Joachimiak called attention to the Nobel prize in chemistry, which was won by Brian Kolbilka; a study of the recognition of lignin degradation products and how the catalytic enzymes are activated by those products; and construction of the Advanced Protein Crystallographic Facility at Argonne National Laboratory (ANL).

Remington has conducted genome analysis funded by the National Science Foundation (NSF) in the National Ecological Observatory Network (NEON) program. She noted that the Big-Data Initiative has announced its first awards.

Schlicher noted that the United States is starting its worst harvest since the 1980s. Crops are more resilient today than they were in the 1980s. A lot of surprises are occurring that will inform future crop models and climate models; corn stover is taking off as an animal feed, which resulted from the funding of R&D at the national laboratories. North Carolina State University research has found that increasing inputs in response to price movements leads to increased yield of crops.

Stacey pointed to the production of the Plant Summit report that gives a 20-year plan for plant science. A freshman research program has been established at the University of Missouri. New software has been developed for gene regulatory networks. Two populations of soybeans are being created with herbicide resistance and high vitamin production.

Sharlene Weatherwax mentioned a series of symposia at the reopening of the remodeled National Academy of Sciences. Washington added that his interview by the President of the National Academies will be broadcast on PBS stations in major cities.

David Thomassen mentioned that the meeting was being recorded and broadcast over the Internet.

Sharlene Weatherwax was asked for an update on the activities of the Office of Biological and Environmental Research (BER).

Fiscal year 2013 has just begun, and it is starting with a 6-month continuing resolution. BER finished out FY12 with $609.5 million enacted. $625.3 million was requested for FY13, but there is a disparity between the House and Senate markups of the appropriations bill. What is available is $264.3 million under the 6 month continuing resolution. A lot of tough decisions have had to be made. Under the 6-month continuing resolution both research and facilities took a hit. This limitation of funds will impact the availability of research facilities to users.

In the Office, Michael Teresinski retired, and Patrick Horan transferred to the Department of Homeland Security to be closer to family in California. Recruitments are in various stages. An Atmospheric Scientist has been selected, and the person has accepted the position. A Program Manager for the Bioenergy Research Centers (BRCs) has been selected. There are two unfilled positions for Science Assistants. A new Microbiologist will be recruited.

A new charge will be given to the Committee for a Committee of Visitors (COV) review of the Climate and Environmental Sciences Division. The input and feedback from these COVs are invaluable.

BER had a winner of the Presidential Early Career Award for Scientists and Engineers, Heileen Hsu-Kim, who works on nanoscale mercury sulfide–organic matter interactions at Duke University. Andrzej Joachimiak (BERAC) was elected as a foreign member of the Polish Academy of Arts and Sciences. Huimin Zhao (BERAC) was awarded a Guggenheim Fellowship. Jay Keasling (Director of the Joint Bioenergy Institute (JBEI) received the Heinz Award for his work to engineer “microbial factories.” And Steve Wofsy (Harvard) got the Roger Revelle Medal for his work on atmospheric sciences and terrestrial ecosystems.

BER gets the words out about its programs in a number of ways, including the Research Highlights on the BER website, which also has pages on BER programs, facilities, and research areas. BER research is also highlighted on the SC website. Program managers’ site visits, special sessions at professional society meetings, and town hall meetings at professional society meetings (to stand up new areas of science) are other ways that information about BER is communicated. These efforts produce a high-impact return on investment.

During the previous week, DOE celebrated 35 years of existence. Employees in BER who have been employees during all 35 years of DOE's existence include Arthur Katz, Karen Carlson-Brown, Kathy Holmes, and Mike Riches.

Sayler asked what percent of research is in the Small Business Innovation Research (SBIR) and Small Business Technology Transfer (STTR) programs. Weatherwax replied, a little less than 3% in FY 2012. This value is increasing to 3.65% in FY 2017.

Hubbard asked how the cap on travel to conferences would impact the field. Weatherwax said that it will have a definite impact on BER. Each conference has to go through a justification and approval process. The bar is set higher for federal and National Lab employees than for university researchers. It is important for program managers to go to scientific society meetings. They need to engage scientists in new disciplines. For some meetings, approval by the Secretary is necessary.

Stacey asked how the continuing resolution and the fiscal cliff were affecting BER. Weatherwax replied that strategic planning, input from BERAC, and input from the Office of Management and Budget (OMB) and DOE management are being used to develop funding scenarios and priorities. Something firm is needed for a target, and the Office is working with SC and OMB on that.

Robertson asked if there had been any reply from Brinkman about the letter on workshop development that the Committee had sent to him. Julie Carruthers said that the Office could not talk about the FY13 budget but that it was hoping to continue the graduate fellowships and Early Career awards.

A break was declared at 10:56 a.m. The Committee was called back into session at 11:16 a.m. Stacey noted that the three bioenergy research centers are now 5 years into their missions.

Paul Gilna was asked to present an update on the BioEnergy Science Center (BESC), one of the three Bioenergy Research Ceneters.

In 2006/7, the technology to produce biofuel from plant feedstocks consisted of concentrated-acid or dilute-acid pretreatment; engineered microbes that ferment multiple sugars; simultaneous saccharification and fermentation; added cellulase enzymes with sugar fermenting microbes; and the use of existing localized supplies of wastes or agricultural residues. The anticipated next generation consists of improved pretreatments, consolidated bioprocessing, and the use of dedicated energy crops. The envisioned advanced generation consisted of going beyond ethanol to advanced biofuels.

The Biomass to Biofuels Plan and vision in 2006 (http://genomicscience.energy.gov/biofuels/b2bworkshop.shtml) identified many barriers and transformational challenges and called for a deeper understanding of the resistance of lignocellulosic biomass to deconstruction and the genetic controls of plant composition and ultrastructure; bioenergy crop domestication and sustainability; and the structure and function of cellulases and other plant cell-wall depolymerizing enzymes. In enabling technologies, what was sought was gene-transfer methods and expression of genes in nonconventional host organisms; rapid tools for the analysis and modeling of cellular composition and physiological state; high-throughput screening methods; metabolic engineering/synthetic biology; and protein engineering/directed evolution.

One can take heart from where things are today. The Beta Renewables Crescentino Plant is set to open at the end of this calendar year in Italy and will produce 20 million gallons of ethanol per year. A North Carolina plant is planned that will use mixed feedstocks, including straw, switchgrass, and *Populus*.

BESC was formed as a multi-institutional, DOE-funded center performing basic and applied science dedicated to improving yields of biofuels from cellulosic biomass. It supports 300+ people in 17 institutions, including national laboratories, universities, nonprofits, and industry. Its major goals are to overcome recalcitrance; cut processing costs significantly; deploy better plants, microbes, and tools; and implement these in most conversion processes.

A genetic block was faced in lignin biosynthesis in switchgrass. Agrobacterium-mediated transformation of switchgrass has now been achieved. That problem has been taken even further: it is not just lignins that form a block; there is also pectin. Pectin synthesis genes have significant effects on recalcitrance and growth in *Populus* and switchgrass. This result is surprising because pectin is important in primary cell walls but represents only a small fraction of secondary walls and walls in grasses.

Regulatory transcription factors in switchgrass have also been looked at. As a result, improved feedstocks have been taken to the field and, so far, have been surviving.

BESC has been involved in the sequencing and analysis of *Setaria*.

The core concept that multiple genes control plant cell-wall recalcitrance has now been proven. The BESC transformation pipeline has been used to identify a panel of 37 candidate genes to date that affect feedstock recalcitrance. Reduced recalcitrance has been demonstrated in *Populus* and switchgrass, and dozens of lines are now in field trials.

On the microbial side of the picture, a system was studied early on to explore a single microbial gene linked to increased ethanol tolerance. Tolerance has often been described as a complex and likely multigenic trait for which complex gene interactions come into play. The simplicity of the genetic basis for this ethanol-tolerant phenotype informs rational engineering of mutant microbial strains for cellulosic ethanol production. This information was used to advance toward and exceed the ethanol-production goals.

BESC has developed genetic tools for *Caldicellulosiruptor* and published the *Caldicellulosiruptor* pan-genome study.

BESC has also been working in yeast, and a plant in Michigan is being developed on the basis of the Center's research results.

Where has BESC come to? New genetic tools have been developed for thermophyllic microbes, ethanol yields in *C. thermocellum* have been improved, and solubilization of plant cell walls has been made more effective when mediated by cellulolytic microbes. The BESC's enabling technologies include the ability to screen thousands of samples and the development of automated glycome profiling with technology that uses 384-well enzyme-linked immunosorbent assay (ELISA) plates. It has found an inhibitory effect in a new lignol and found that isosinapyl alcohol has mild inhibitory properties toward yeast and *E. coli*. It has now engineered a *C. obsidiansis* that overcomes this inhibition. In enabling technology, BESC has developed a high-throughput recalcitrance pipeline for composition and sugar release for thousands of samples per year at 4 mg per sample. The pipeline and material transfer agreement data are captured in a laboratory information management system. And glycome profiling has been validated for analyses of cell-wall structure and automated.

BESC has also been working on an outreach program that has reached more than 65,000 students.

In the future, BESC will focus on less-recalcitrant plants; advanced consolidated-bioprocessing microbes; and improved pretreatment, feedstock, and organism combinations. What is being developed is a focus on impactful recalcitrance science, a highly functioning team of world-class scientists, an emphasis on integration and collaboration, a close connection to industry, acceleration of research and technology outcomes, and a growing core of well-trained young research staff.

Timothy Donohue was asked to present an update on the Great Lakes Bioenergy Center (GLBRC).

The goals of GLBRC are to improve relevant plant traits and sustainable agronomic systems and to improve the energy conversion of cellulosic biofuels production. To achieve these goals, an integrative program on plants, deconstruction, and conversion has been developed.

Members of GLBRC include an international team of ten universities, two national laboratories and one company along with the University of Wisconsin-Madison (which is the lead) and Michigan State University (which is a major partner). The program includes about 400 scientists, students, and staff.

GLBRC works closely with the other two BRCs. The three BRCs benefit a lot from sharing of technologies and techniques and from being able to work from the farm to the bench to fuels. The GLBRC also benefits from other DOE and the U.S. Department of Agriculture (USDA) programs on campus. GLBRC looks at cropping systems, pretreated biomass, hydrolysates, and biofuels, giving a rich base of data and research products to draw on.

The sustainability area has two goals: discoveries to design sustainable biofuel production systems and development of model alternative biofuel systems at field to regional scales. It looks at the economic, environmental, and social aspects of the technology. Data from cropping systems' system-level responses to potential crops get fed into the life-cycle assessment (LCA) Simulation Modeling and Integration platform. Research highlights in sustainability include the ability to predict system responses to land-use changes, cropping system impacts on payback time, and metrics for crop perenniality and diversity.

In plants, the goal is to develop productive energy crops that can be easily processed into fuels. GLBRC has been looking at altering lignin to reduce recalcitrance, increasing the energy density of biofuel crops, manipulating hemicelluloses for improved processing and energy yield, and improving crop-plant properties for sustainable bioenergy production. Research started with gene discovery in model plants. Lignin is hard to break down. If ether bonds are replaced with ester bonds, the lignin is more easily broken down. Expressed-sequence-tag (EST) sequencing of *Angelica sinensis* roots was used to select candidate *fmt* genes and identify recombinant proteins and produce "Zip" lignin in energy crops. American Recovery and Reinvestment Act of 2009 (ARRA) funding allowed GLBRC to get equipment to identify genes to develop new lignin varieties, next-generation fuels in vegetative tissues, and improved hemicelluloses.

In deconstruction, the goal is to improve release of monomers or short oligomers from lignocellulosic biomass by improving alkaline pretreatments to open the plant cell wall polymer matrix and by improving enzyme cocktails to release useful intermediates. The research has shown that alkaline pretreatments preserve energy-rich compounds, enhance the C5 and C6 stream for conversion, and produce cellulose III (which is easier to digest). Enzyme mixtures were improved to identify a feedstock- or hydrolysate-limiting catalyst. Cooperation with the

Joint BioEnergy Institute (JBEI) allows very rapid screening of candidates. Added value co-products from lignocellulose have been identified: it is not only a potential lignin stream for fuel and co-product synthesis but also a source of additional energy. Recovery, complexity, and conversion strategies are being evaluated.

In conversion, the goal is to overcome barriers in converting lignocellulose products into biofuels and valuable co-products by improving the efficiency of biomass-to-ethanol conversion and to engineer routes to next-generation biofuels. A high-throughput screening process was set up for 587 wild strains in 6 hydrolysates, and sequencing at the Joint Genome Institute (JGI) and expression profiling of xylose utilizers was carried out. It was found that novel genes improve the xylose metabolism and stress tolerance to hydrolysate evolved. On the *E. coli* side, hydrolysate factors that aid growth were identified. Amino-acid depletion and concomitant adenosine triphosphate (ATP) demand of multiple stresses arrest *E. coli* growth in corn stover hydrolysate. Regulatory strategies were found to improve fermentation performance. It was desirable to optimize the output from a limited number of intermediates. Microbial fatty acids yield alkanes by catalytic decarboxylation of biodiesel by biological condensation with alcohols. γ-valerolactone yields alkenes without exogenous hydrogen. And ionic liquids produce sugars (for fermentation) or furans (for fuels), producing high-value co-products or fuels from lignin aromatics. This technology is being moved into the marketplace.

GLBRC has an industry perspective on its Scientific Advisory Board. In education and outreach, it has 200 students in the program who will be the leaders of tomorrow as well as K–12 student and teacher programs. It is looking at other cropping systems, feedstocks, and biofuels.

Jay Keasling was asked to present an update on JBEI.

JBEI is located in Emeryville, California and has seven partners. It provides the basic science for converting biomass to fuels. The major challenges are that cellulose and hemicellulose are occluded by lignin, that lignan is recalcitrant to depolymerization, and that widely used pretreatment processes do not effectively remove lignin. Developing better pretreatment methods is one approach to lignin recalcitrance. Another approach is to engineer plants to modify lignin.

One can limit plants' production of lignin, but the rest of the plant suffers because of collapsed cells. One can control the lignin biosynthetic pathway, resulting in engineered lignin deposition in vessels, giving 33% less lignin and an increase in sugar release by the plant. Traditional pretreatment methods involve extremes of temperature, pH, and pressure; use dilute acid, base, and lime; and produce cellulose/hemicellulose contaminated with lignin and various by-products.

JBEI has been working on ionic liquids for pretreatment that enables one to dissolve and precipitate out the fairly pure lignin and hemicellulose. The ionic liquids disrupt the hydrogen bonds in lignin. Ionic liquids release sugar in one-tenth the time compared to dilute-acid pretreatment. They work on all sorts of biomass, and one can use the same refinery for many feedstocks.

One can also engineer cell-wall deposition in fibers, filling up interstitial space with biomass rather than lignin. One can then stack these technologies together and increase sugar production by 30 to 50%. The challenges are that (1) biofuels are needed for all kinds of engines, particularly diesel and jet engines and (2) many fuel-producing organisms can only use a fraction of the sugars from biomass.

One would like to have sugars taken up by a microbe and fatty-acid ethyl esters excreted. Ethyl esters would float to the top of the reactor, be skimmed off, and be used to produce biodiesel. In experiments, however, the products were toxic to the organisms, so transcription factors were engineered to overcome this problem and to produce a four-fold increase in fatty lipids. An attempt to convert fatty acids to their ethyl esters with the *fadD* gene was not successful because the *fadD* was unstable and limited process scale-up. Bisabolane (which can be used in biodiesel) and α-pinene (which can be used in bio-jet-fuel) can also be produced by microorganisms. Bisabolane produced by this process has many of the qualities of No. 2 diesel fuel: a cetane number of 41.9, a freezing point of less than –81 °C, a clouding point at less than 78 °C, and a density of 819 g/L.

Unfortunately, these fuels are also great disinfectants, so 40 microbes were screened for solvent-resistant pumps (there are many different pumps in different microbes). Increased pinene tolerance was successfully introduced into *E. coli*.

JBEI has numerous interactions with industry, and there are many interactions between the Systems Biology Knowledgebase (KBase) and the BRC data registry.

Stacey asked how the centers interacted with other DOE facilities. Donohue replied that a lot has changed in the technology. JGI's equipment has changed dramatically. Many things that the centers wanted to do have become JGI's production capabilities. Gilna said that the centers' intellectual exchange with JGI has been intense. Keasling said that the centers also use Environmental Molecular Sciences Laboratory (EMSL) a lot as well as the resources of the National Energy Research Scientific Computing Center (NERSC).

Baldocchi asked what efforts there were in increasing light-gathering by plants. Donohue said that that is being done in the sustainability group. GLBRC is trying to increase biomass productivity by the genetic engineering of flowering and branching.

Zhao asked what criteria were used in selecting production-study hosts. Gilmore responded that there was a focus on about 10 microbes that were already being investigated, and these were narrowed down to three microbial platforms that have the best promise. All of these will be subjected to a key set of assessment criteria that are being quantified now.

Shanks asked if homologs would be looked for. Keasling replied, yes. Shanks asked how many of these plants were tested when they were engineered. Keasling said that there was a lot of repetition, and spectroscopic and microscopic characterization was conducted. Shanks asked about drought and insect effects. Keasling said that a battery of tests was used, including fungal resistance. Shanks asked if it would be beneficial if there were a phenotype facility. Keasling said, yes. Gilna said that his advisory committee said that as many traits as possible should be gotten out into the field early.

Joachimiak asked when these fuels would be economically feasible. Donohue noted that DuPont is building a plant now. The University of Tennessee is building one, too. Gilna replied that industry wanted to test BESC's results at least at the bench scale to see how they fit into proprietary efforts. Donohue noted that sponsored research was not shown in today's presentations, but it is being conducted. Industries are coming in and asking universities to evaluate new technologies that they have developed. Keasling pointed out that margins on fossil fuels are now very low. There is a lot to do. Alternative fuel technologies will not meet those margins very soon. Donohue said that these ethanol plants only make one product. Industrial plants that will take in multiple feedstocks and produce multiple products need to be developed.

Stacey pointed out that phenotyping can be tricky, depending on nitrogen and phosphorus availability to produce abilities to fix nitrogen or resist fungi. Donohue replied that a significant amount of what is learned is how different changes react to different soils. They vary with weather, the amount of biomass one can take off the field, etc. All of these factors and more will have to be worried about.

Schlicher asked what needed to be done tomorrow. Keasling answered that licensing needs to be centralized. DOE set that up correctly. Venture capitalists have wanted to license the whole portfolio. Also needed is someone to market the technologies to industries. Gilna said that the publications, inventions disclosures, etc. are showing that the centers are doing what they were intended to do. All three centers have produced a total of 1081 publications, 283 invention discoveries, 146 patent applications, and 50 licenses/options.

A break for lunch was declared at 12:38 p.m.

Monday, October 15, 2012
Afternoon Session

The meeting was reconvened at 2:00 p.m.

Huimin Zhao (BERAC) gave a science presentation.

Biotechnology has made a great impact in the medical, agricultural, and industrial sectors. Several key technical breakthroughs in biotechnology include, but are not limited to, recombinant DNA technology, monoclonal antibody, DNA sequencing, gene therapy, genomics, and synthetic biology. Synthetic biology is the deliberate design of improved or novel biological systems. It draws on principles elucidated by biologists, chemists, physicists, and engineers. It operates at several levels: the molecular level, the pathways/network level, the multi-cell level, and the organism level. The main challenges in synthetic biology include standardization, modularization, and system integration.

The Zhao group at the University of Illinois is focused on addressing two grand challenges facing society. One is related to energy and sustainability, in which microbial cell factories are being designed to produce fuels, chemicals, and medicines from renewable biomass. The other is related to human health, in which new therapeutic tools and agents are being developed for treatment of human diseases. For example, gene switches are designed to precisely regulate gene expression while gene scissors are designed for targeted genome editing and gene therapy. In addition, Zhao himself was recently invited to set up a Metabolic Engineering Research Laboratory in Singapore to develop and apply systems and synthetic-biology approaches to engineer micro-organisms capable of cost-effectively producing industrial chemicals from renewable feedstocks. In this presentation, three examples of applying synthetic biology tools were illustrated.

The first example was related to natural products. Natural products (i.e., bacteria, fungi, and plants) are a major source of new drugs. The majority of the drugs approved during the past 30 years are natural products or natural-product-derived compounds. However, the number of new drugs being approved is decreasing. The number of antibiotics approved by the FDA peaked in the 1960s. Fortunately, more than 2000 organisms have been sequenced, representing a rich source for discovery of new genes and pathways. The putative secondary metabolite gene clusters far outnumber the known secondary metabolites. To access those putative secondary metabolite gene clusters, the Zhao group has been developing a synthetic biology strategy. It starts with the analysis of genome sequences to identify a putative pathway, to synthesize DNA fragments, to assemble DNA fragments in yeast, to isolate product plasmid in *E. coli*, to verify the plasmid by restriction digestion, to express the pathway in a target heterologous host, and to identify the product. Compared to existing approaches, this new strategy enables facile heterologous expression of a biochemical pathway in any desired organism. It is also a useful tool for studying the biosynthetic mechanism, for enzyme discovery and engineering, and for pathway engineering and combinatorial biosynthesis. By testing the pathways one by one, new compounds may be discovered. A demonstration of this approach started with a known system: a silent spectinabilin pathway. Ten new constitutive promoters were identified from actinomyces, and the entire pathway was refactored by placing a promoter in front of each gene. By doing so, the silent spectinabilin pathway was successfully activated. Inspired by this proof-of-concept study, several unknown cryptic pathways are now being activated.

The second example was related to bioenergy, in which recombinant yeast strains are being designed to convert cellulosic materials to produce advanced biofuels. One main limitation in mixed-sugar fermentation is glucose repression (i.e., the utilization of pentose sugars, such as xylose and arabinose, is repressed by glucose). To overcome this limitation, an artificial cellobiose utilizing pathway consisting of a cellobiose transporter and a beta-glucosidase was successfully reconstituted in yeast, which enables the simultaneous utilization of cellobiose (a dimer of glucose) and xylose. However, both the cellobiose and xylose consumption rates and the ethanol productivity were relatively low. To address these issues, a combinatorial pathway engineering approach was developed in which a library of pathways was generated by placing a pool of promoter mutants with varying strengths in front of each pathway gene. This approach was successfully used to improve the xylose utilization rate and ethanol productivity by balancing the flux in the xylose-utilization pathway. A similar accomplishment was made for the cellobiose-utilizing pathway. One unexpected finding was that the optimized pathways are all host-dependent, which highlights one of the major challenges in synthetic biology, context dependency.

The third example involved the engineering of gene switches and gene scissors. Directed evolution was used in combination with rational design to create three orthogonal ligand-receptor pairs for use in the construction of gene switches. The utility of an engineered ligand-dependent gene switch was demonstrated in precisely regulating the expression level of an endogenous gene, the vascular endothelial growth factor gene (VEGF) that is associated with angiogenesis. In addition, a transcription-activator-like (TAL) effector nuclease (TALEN) was engineered for potential use in the treatment of sickle-cell disease. The single-point mutation causing sickle-cell disease was corrected in an induced pluripotent stem cell line derived from a human patient.

Sayler asked whether the estrogen receptor sequence shows any mutations that map onto the known snips in the human estrogen receptor. Zhao replied that they had not studied that.

Joachimiak asked how stable these constructed metabolic pathways were. Zhao said that they were relatively stable.

Stacey opened a discussion of the charge wrap-up. The charge from Brinkman was to develop a follow up to the previous long-term vision report. The Subcommittee submitted a report that has been revised twice. The third version of the report is in the hands of the Committee and was up for comments, discussion, revision, and possible approval.

Ehleringer wanted to insert sentences to reflect the need for the involvement of the national laboratories in terms of facilities and capabilities.

Hubbard asked if the Executive Summary should call out the workshop recommendations. Stacey replied, yes. Brinkman had asked for some specific recommendations, and the four actionable recommendations were put on Page 22. He asked if they should be revised and pointed out that not everything can be put in the Executive Summary.

Penner said that it was not clear to her what the focus was in the Virtual Laboratory and what was meant by "deep vertical integration." She suggested that those terms be clarified in the Executive Summary.

Joachimiak said that the report is better than what the Committee had been given before. There are facilities at the national laboratories that should be mentioned in the report, i.e., the synchrotron at Stanford, the Advanced Light Source (ALS), the Advanced Photon Source (APS), and the National Synchrotron Light Source (NSLS). Sayler asked whether that had been mentioned already. Stacey said, yes, it has; new language, however, is welcome.

Shaver pointed out that NEON should be referred to as 20 "sites" rather than "towers."

Stacey said that the justification for the charge was that the grand challenge had information on technology but lacked details. This report attempts to fill in those details but actually kicks the ball down the field and places the responsibility for selecting details on future workshops. Tiedje said that there was not much more that one could do; this charge was very broad and complex. Stacey noted that a BER Virtual Laboratory required cooperation, integration, and collaboration; that message is emphasized on the first page of the Executive Summary.

Robertson expressed an interest in making more specific the topic of the integrated field laboratory workshop that was mentioned on Page 23.

Sayler stated that this report was DOE-national-laboratory centric. These workshops should bring in the broad scientific community. Weatherwax said that DOE has a broad responsibility to the whole research community and that workshop participation is carefully balanced to be inclusive.

Tiedje asked if there should be another level of analysis of the results from the four workshops. Hubbard suggested that maybe the fourth workshop on implementation would accomplish that synthesis.

Washington asked that comments be entertained from Committee members who were not able to attend this meeting.

Tiedje moved the acceptance of the report pending approval of the additions to the Executive Summary and minor editorial changes. Remington seconded. The motion was unanimously accepted.

A break was declared at 3:15 p.m. The meeting was called back to order at 3:34 PM.

Todd Anderson was asked for an update on the Biological Systems Science Division.

The Division completed the FY12 reviews of DOE National Laboratory Scientific Focus Areas (SFAs) on foundational genomics, biofuels research (Lawrence Livermore National Laboratory), and radiochemistry and imaging (Jefferson Laboratory). Upcoming reviews include the three BRCs (not SFAs), biofuels research and the genomics science projects (Pacific Northwest National Laboratory), and the radiochemistry and imaging projects (Lawrence Berkeley National Laboratory and Oak Ridge National Laboratory).

In FY12, eight awards were made in genomic science for research in microbial systems designed for biofuels and plant systems designed for bioenergy. These complement the Early Career awards that were made. Nine awards were made in plant feedstocks genomics for bioenergy in cooperation the National Institute of Food and Agriculture of the U.S. Department of Agriculture. These awards constitute a large portion of the plant-science research funded by

BER. Five awards were made to universities in integrated nuclear medicine research and training in collaboration with the National Institutes of Health (NIH). A draft funding opportunity announcement (FOA) is being circulated that is co-funded by NIH.

A DOE Joint Genome Institute Strategic Planning Workshop was held in May 2012. It recommended that the JGI continue to sequence (new genomes, resequencing, transcriptomes etc.); that new capabilities for high-throughput functional annotation of DNA be incorporated into JGI's research portfolio; that bioinformatics capabilities be extended and improved; that DNA synthesis be used to efficiently build genes, pathways, and genomes; that automation of biological experiments be improved to match sequencing output; and that communities be organized around big coordinated projects.

JGI just completed its Strategic Plan that calls for it to take a path to transform the JGI into a next-generation genome science center, to place an emphasis on providing high-throughput functional information and analysis techniques to complement genomic data, and to incorporate new capabilities and/or partners to enhance genotype-to-phenotype interpretations. JGI just posted a call for its Emerging Technologies Opportunities Program for developing new capabilities for high-throughput functional annotation of DNA.

KBase just completed its first year and is making available almost 8000 genomes; 23,058,670 features; and 12,620 regulons. It has stood up the KBase network and user interface for cross-cutting science. It is forging new partnerships with JGI to enable metagenome assemble, analysis of sequencing-based gene function analysis and metabolic model reconciliation, and collaborative outreach and training. It is also collaboratively developing joint milestones with the BRCs to meet their mission goals with planned KBase functionality. It has made good progress on cloud connections among the participating national laboratories' user facilities.

A number of science highlights were presented:

Poplar resequencing suggests enhanced improvement strategies, looking for genetic and genomic structure in a natural population of *Populus* in relation to geographical location. Population structure and genetic variation were determined on a geographic scale. A strong correlation between latitude and genetic differentiation was shown to allow inference of the phenotypic expression–genotype relationship. The presence of extensive linkage disequilibrium supports the feasibility of genome-wide association studies, facilitating marker-assisted breeding through genomic selection and enhancing the potential for genetic improvement of *Populus* as a biofuel feedstock.

JBEI is very interested in using ionic liquids to break down lignin. A new high-temperature cellulase cocktail of switchgrass-degrading microbes has been developed to break down switchgrass at temperatures from 50 to 79 °C in the presence of 10 to 20% of the ionic liquid. The resulting hydrolysates were converted to biodiesel by an engineered *E. coli* strain, demonstrating low production inhibitory compounds to convert switchgrass to sugars.

The Great Lakes Bioenergy Research Center has a study out on whole-plant cell-wall characterization with two-dimensional nuclear magnetic resonance. The method can be used to facilitate the structural analysis of plant cell walls and offers a means to visualize the chemical constituents of primary and secondary lignified cell walls from a diverse set of plant species. It allows the characterization of biomass feedstock to tailor processing procedures.

Research at North Carolina State University on characterization of the regulation of wood formation has shown the repression of a transcription-factor family member by its splice variant, which has never before been seen in plants. This discovery helps define the process of wood formation at the molecular level, guiding research to optimize bioenergy production from biomass.

The JGI has had a lot of impactful publications. Of note are

- The Paleozoic Origin of Enzymatic Lignin Decomposition Reconstructed from 31 Fungal Genomes by Dimitrios Floudas et al. in *Science* 336, 1715 (2012)
- Defining the core *Arabidopsis thaliana* root microbiome by Derek Lundberg et al. in *Nature* 448, 86-90 (2012)
- Genomics of aerobic cellulose utilization systems in Actinobacteria by Iain Anderson et al. in *PLoS ONE* 7(6) 1-10 (2012)

Remington asked if the issues between KBase and JGI were still continuing. Anderson replied, no. KBase and JGI have laid out a reorganization and reached out to EMSL.

Washington noted that the price of sequencing has gone way down and asked whether BER should take some credit for that. Anderson replied, yes! It should take a lot of credit for that.

Sayler asked whether future solicitations in synthetic biology will be equal to or broader or larger than the first. Anderson replied that they will probably be similar, modified to balance the overall research portfolio.

Schlicher asked about the perception of success on the feedstock collaboration with the USDA and how success was defined. Anderson responded that the long-term collaboration includes joint solicitations and funding. Schlicher asked again how one measures success. Anderson said, by the amount of sequences with energy application done in collaboration with other agencies.

Gary Geernaert was asked to review the activities of the Climate and Environmental Sciences Division.

The Division is trying to build a one-culture vision. Three FOAs have been issued. The Atmospheric System Research (ASR) FOA resulted in 31 projects being selected, 27 of which were new projects. The Terrestrial Ecosystem Science (TES)/Earth System Modeling FOA will result in 12 to 18 projects being selected. The Global Climate FOA is resulting in five new starts.

A number of PI meetings have been held. A workshop on the integrated water cycle was held a few weeks before this meeting. It showed that there was no funding for hydrological modeling from the global down to regional scales. This is an opportunity for DOE.

Many SFA triennial reviews were conducted this fiscal year. Five reviews have been accepted for continuation. Responses for the last three have not been finalized yet.

In FY13, there will be principal-investigator (PI) meetings for the modeling programs, the ASR program, and the TES program. There will also be SFA triennial reviews for four programs. Two major workshops will be conducted, one on North American carbon and one on the Atmospheric Radiation Measurement (ARM) Program's cooperation with the European Union. (Europe is considering setting up a program parallel to ARM.)

ARM has installed a permanent facility in the Azores and has placed or will place mobile facilities at Oliktok, Alaska; Cape Cod, MAGIC (see below), and in the Amazon.

ARM–MAGIC will begin transects between Los Angeles and Honolulu at two-week intervals in October 2012. The goal is to study how cloud systems transition from cumulus to stratocumulus along this route of the Pacific Ocean as a function of season.

Sea-salt particles have been found to evolve in the atmosphere. They change into inorganic chlorides over a day as they dry and age. This is significant because current climate models do not consider these reactions, which may alter the optical properties and reactivity of aerosols in coastal areas, where sea salt and reactive organic emissions may be found together.

A new uncertainty-quantification framework for Community Land Model 4 reveals the sensitivity of surface flux simulations to hydrologic parameters. The purpose here is to reduce uncertainties in the connections between simulated water and energy fluxes to hydrologic parameters. This uncertainty-quantification framework will be used to constrain model parameters. There is a large sensitivity to parameters of subsurface processes, so subsurface properties must be included in the models.

Research has shown that growth and expansion of boreal shrubs have been found to enhance warming. Shrub expansion leads to atmospheric heating through two feedbacks: albedo and evapotranspiration. The strength and timing of these feedbacks are sensitive to shrub height. Tall shrubs destabilize the permafrost more substantially than do short shrubs.

The impact of ocean barrier layers on tropical-cyclone intensification was a key factor that could not be projected. How hurricane intensification occurs is being looked at. Cold "scars" that roil up behind a hurricane track have to be taken into account in projecting hurricane intensification.

A new era of meta-genomic analysis is needed to capture microbial community structure and to reveal biogeochemical function. The objective is to develop and test novel environmental meta- genomic techniques to more completely capture the microbial community structure and reveal the in situ biogeochemical function of community members. High-throughput sequencing of groundwater samples taken from the old Rifle (Colorado) Integrated Field Research Challenge (IFRC) site led to the cultivation-independent recovery of genomes. This work produced 46 near-complete microbial genomes and discovered four genomically unrecognized phyla. These results will have to be taken into account in models.

To understand land surface–subsurface properties in permafrost ecosystems, the domain must be mapped at high resolution. This is one of the first Next-Generation Ecosystem Experiments (NGEE) Arctic activities.

The DOE roadmap to advance predictability calls for community models, structure observations, and observational infrastructure. The evolution of the Earth system prediction goes from the 1970s (which considered only the ocean) to the 2010s and beyond with very-high-resolution projectionsa based on broad physical measurements.

Information from the Climate and Environmental Sciences Division (CESD) Strategic Plan, the U.S. Global Change Research Program (USGCRP) Strategic Plan, the National Research Council (NRC) report on the future of climate modeling, and BERAC reports is used to develop the path forward for the Division. In FY13 and 14, the Division is looking at advancing CESM architecture into the next generation, enhancing the "big-data" infrastructure for the next IPCC assessment, developing a roadmap for the NGEE and ARM link to ESMs, and forging interagency partnerships [e.g., with the National Oceanic and Atmospheric Administration (NOAA)].

Tiedje noted that a couple of international groups deal with permafrost. Geernaert replied that one benefit is that the University of Alaska in Fairbanks is a part of many research programs with BER and with other organizations.

Sayler asked if Geernaert was talking about collecting data at the "snowmobile" or at the remote sensing scale. Geernaert replied that he was talking about aircraft measurements and that they are looking for more input on what is appropriate. Sayler noted that Geernaert had brought up sea-salt aerosols and their 24-hour conversion and asked how much new information and predictability one got from these measurements. Geernaert replied that it depended on what the metrics were. One has to work backwards from desired capabilities. It has not been decided what the metrics should be. At this time, all kinds of metrics are being looked at. Sayler asked what level of the surface was being referred to. Geernaert responded, runoff and subsurface moisture.

Baldocchi asked what new sensors and sensor systems are needed and whether DOE was working with electrical engineers to develop them. Geernaert answered that DOE invests in instrument development, e.g., in the Small Business Innovative Research Program. Two things keep coming up: in situ soil-moisture measurements and how one maps the subsurface (electrically, with radar, or what).

Robertson noted that the KBase and other databases might be added to the roadmap. Geernaert said that that was a good point. The Earth System Grid will need to be upgraded over the next 5 to 6 years.

Wildung sent in a question via e-mail for the BRC Directors. Given Brinkman's earlier comments that the world will have fossil fuels for the foreseeable future, do the bioenergy research center directors see the ultimate benefits of their research primarily on the reduction of carbon emissions, and, if so, what efforts are under way to test the properties of alternative fuel products under development? Keasling replied that new fuels' properties and performances are tested. Carbon reduction is a benefit, as is the U.S. production of fuels. There is no possible replacement of jet fuel from natural resources. That is where alternative fuels could make a great contribution. The carbon reduction by switchgrass is positive over the years as the roots and the associated microbial community grow.

Stacey noted that Brinkman had given BERAC a new charge to conduct a Committee of Visitors (COV) review of the Climate and Environmental Sciences Division. Minghua Zhang has agreed to chair that COV.

The floor was opened for general discussion. There being none, the floor was opened to public comment. There being none, the meeting was adjourned for the day at 4:42 p.m.

Tuesday Morning, October 16, 2012

The meeting was called back into session at 8:32 a.m. by Chairman Gary Stacey. The next meeting of this Committee will be held on February 21-22, 2013.

Karl Taylor (LLNL) was asked to review the role of the Program for Climate Model Diagnosis and Intercomparison (PCMDI) in enabling climate science through coordinated modeling activities.

PCMDI's mission is to advance climate science through individual and team research contributions by performing cutting-edge research to understand the climate system and reduce uncertainty in climate model projections and to provide leadership and infrastructure for coordinated modeling activities that promote and facilitate research by others by planning and managing "model intercomparison projects" and by providing access to multi-model output.

In the 1970s and 1980s, climate-model evaluation was largely a qualitative endeavor done by modeling groups themselves. In 1991, the Atmospheric Model Intercomparison Project (AMIP) started working with about 30 modeling groups from 10 countries, engaging outside researchers in the evaluation and diagnosis of atmospheric models with prescribed ocean conditions; results were shared. In 1995, the Coupled Model Intercomparison Project (CMIP) was organized. In 2003, there was an expansion (CMIP3) to include idealized climate change, historical, and future scenario runs. Output became available by 2005. CMIP5 is now in progress, and output is starting to come in.

The program now deals with more experiments and more-comprehensive models. Output is shared with anyone, anywhere. There is increased standardization (facilitating data exchange), more model outputs, more-complete documentation of models/experiments, and new strategies for making output accessible to users. Climate modelers get together and decide the possible experiments to conduct, and PCMDI helps to forge a consensus on what experiments to perform. Currently, 59 models are available from 24 groups. The effort feeds into the IPCC process.

When the outputs of different models are compared, they display different responses. The spread is caused by differences in “scenarios” (i.e., different emissions or concentration prescriptions); differences in “radiative forcing” (radiative impact) of the changing atmospheric composition; differences in “climate sensitivity” (i.e., differences in climate feedbacks); and differences in the (equally likely) paths of unforced variability exhibited by simulations forced in the same way.

For one model and a single simulation of forced changes and unforced variability in global mean tropospheric temperature, one can get different times, depending on the realization. Projection ranges are initially dominated by model “uncertainty,” but eventually are dominated by scenario. In CMIP5, experiments are designed to address the causes of spread in projections and much more. Three suites of experiments formed the core experiments that all models were expected to conduct; other experiments were chosen for specified models on the basis of their interests and capabilities.

CMIP5 includes models initialized with the observed state. The hope is that, through initialization, the models will be able to predict the actual trajectory of “unforced” climate variations. The hypothesis is that some longer time-scale natural variability is predictable if the initial state of the system is known. The question is how long the models can accurately predict conditions beyond the observed starting conditions. This work is ongoing. Early results do not seem to show much added value.

The rich set of “long-term” experiments, drawn from several predecessor model-intercomparison projects, focuses on model evaluation, projections, and understanding. A broader range of variables is expected in the output with temporal sampling periodicity down to 3 hours.

PCMDI forges community consensus and provides detailed specifications for experiment design, the list of requested model output, and specifications for (1) the format and structure of model output and (2) the required metadata. It also provides software-infrastructure development and support to enable community analysis of CMIP results. A website is used to provide the information needed by modeling groups and users.

In the early 1990s, about 1 Gbyte of data was collected. During the late 1990s, about 500 Gbytes were collected. In 2004, about 36,000 Gbytes were collected, and in 2010 1000 to 3000 Tbytes were collected. The situation required new approaches for delivering data to users. Originally, they were shipped to central servers on 2-Tbyte hard disks, which delayed availability. There was also a fragility associated with dependence on a single server. The new approaches use a distributed data archive. Here, data are digitally transferred to index nodes. All index nodes communicate with all others. Users can see data from all over the globe. This produces a more-robust, faster, and easier-to-use system. There is also a script-driven direct-access route to the data, server-side computer services, and a security and authentication layer. In addition, PCMDI and other major data centers have replicated high-demand data sets.

CMIP facilitates more-comprehensive scrutiny of model behavior. Expertise is limited at the individual modeling groups. A broad community of experts can analyze output from multiple models with ease. Thousands of scientists have downloaded data from CMIP. To date, more than 600 publications have been registered claiming to report on CMIP3 results, and more than 250 publications have already been prepared on the basis of CMIP5 results (which have been available for only a year or so).

The multi-model perspective has already visibly demonstrated that model results are uncertain. It also provides a range of plausible projections for planners. It has been used as a cornerstone for recent IPCC reports. In the *Fourth Assessment Report*, about 75% of the 100 figures in Chapters 8 through 11 are based on CMIP3, and four of the seven figures in the Summary for Policy Makers are based on CMIP3.

CMIP establishes some benchmark experiments that allow one to gauge changes in model performance through AMIP runs, CMIP control runs, historical runs, and runs of idealized 1% per year CO_2 increases. All of these runs are done routinely on new models. Changes in CMIP model errors show that intermodel variability decreased between 2000 and 2005. Relationships between observables and projections (e.g., of snow cover) have been gleaned from CMIP results that indicate which models might be reliable. One of the CMIP5 experiments was designed to answer the question of what causes the spread in climate responses by models and whether the uncertainty is narrowing. It found that temperature approaches a new equilibrium over many decades when the CMIP5 equilibrium pre-industrial control is subjected to an abrupt quadrupling of CO_2. However, there are differences in results among the models. Another study showed that radiative forcing is proportional to the average surface temperature; from the plot of the data, one can get the equilibrium climate sensitivity from the slope of the feedback. In a study of climate sensitivity and feedback parameters, the relatively narrow scanner indicates that feedbacks, not forcing, are primarily responsible for the range of climate sensitivities. Differences in cloud feedback are responsible for a large fraction of the range of feedback strengths in model results.

CMIP5 offers opportunities to evaluate and diagnose reasons for differences in cloud feedback among models. The "satellite simulator" output is being collected for the first time. The International Satellite Cloud Climatology Project (ISCCP) instrument simulator code diagnoses (from model cloud vertical distribution and optical properties) the fraction of clouds occupying each of ISCCP's 49 cloud "categories." The data indicate what clouds provide the most important feedbacks and what clouds should be emphasized in models to reduce variability.

Using the kernel method, CMIP cloud radiative effects can be resolved into the amount, altitude, optical depth, and residual components. It was found that the spread and mean of cloud components has not changed much between CMIP3 and CMIP5. Accounting for the "fast adjustments" results in a clear consensus for a negative optical-depth feedback (i.e., optical depth increases with warming).

In a consideration of the simulation of the climatological distribution of clouds against satellite observations from two recent model ensembles, there was not much difference in results between the CFMIP1 and CFMIP2 models but large differences between the ISCCP and Moderate Resolution Imaging Spectroradiometer (MODIS) models. Some individual models, such as the U.K. Met Office models and the community atmosphere models have improved. A definite improvement occurs when models are tuned to the time-mean radiation balance. They commonly achieve this by simulating too many optically thick clouds and too few optically thin ones to offset too little cloud cover.

Models have quantitatively improved in the simulation of clouds, and CMIP has become an integral part of climate modeling. Modeling groups perform the core CMIP experiments as part of their model-improvement efforts. The IPCC continues to provide top-down incentives to provide projections based on common scenarios. The scientific benefits of providing multi-model output for community analysis are now well demonstrated. PCMDI, in cooperation with the World Climate Research Programme (WCRP), is working to establish climate-model metrics that can be used to identify merits and shortcomings of models relative to one another. It can be anticipated that there will be a CMIP6 but that it will likely not attempt to take on more than CMIP5.

With BER's support, PCMDI has made essential contributions to the success of coordinated modeling activities through research and project "management." CMIP has enabled a diverse community of researchers to evaluate models from a variety of perspectives and use model simulations in an enormous breadth of research. The ongoing uncertainty in projection accuracy stems from models' treatment of clouds, a target of BER's Atmospheric Radiation Measurement (ARM) program. A distributed data archive infrastructure has been developed that could serve other projects and scientific communities well.

Leung asked if the improvement in the simulation of cloud optical thickness was related to the simulation of aerosols or the simulation of cloud ice. Taylor replied that he thought that the latter was the case. The main changes have been in the treatment of the boundary layer and of some of the convective processes.

Sayler asked if China was a new addition to the group. Taylor said that they have been a member since 2004. Its models have improved rapidly. There is also a group in Brazil. Sayler asked what was done with the models when weaknesses were identified, whether there was a curation process or whether the models were thrown out. Taylor replied that it was not clear what had to be gotten right in the model. It has been suggested that models could be weighted according to a performance factor. There is little justification for doing that. Because one can get today's climate correct does not mean that one can project the future climate.

Baldocchi noted that a great amount of detail is needed to get the scenario correct. He asked about changes in land surface. Taylor replied that an attempt was made in CMIP5 to use them uniformly across all models, but that attempt was not completely successful. There are vast changes in land surface, and resolution plays a big role. Baldocchi pointed out that canopies need to be characterized in great detail, also.

Robertson asked if there were efforts under way on down-scaling. Taylor said that there is CORDEX [the Coordinated Regional climate Downscaling Experiment], which uses CMIP5 data to produce both dynamic and statistical downscaling over Africa.

Hubbell asked what motivated researchers to share data. Taylor responded that learning from each other was a great motivator; it has been learned that all models are flawed; governmental pressure played a role, also.

Martha Schlicher (BERAC) gave a science talk on Monsanto's accomplishments and opportunities in agriculture.

The United States has seen a four-fold increase in agricultural productivity in the past decade; this trend is being repeated around the world. The technical strides that have made this all possible are little known. The Green Revolution improved agronomics and conservation practices. Equipment was developed for planting, cultivating, harvesting, and storing corn. Biotechnology and genomics were introduced into agriculture but lagged 10 years behind medicine. Yield improvements have resulted from advancements in three major areas: biotechnology, plant breeding, and agronomic solutions. The interplay among the three extends from the laboratory to the field.

Plant breeding is a system of evolving technologies that continue to increase genetic gain. Hybrids that were developed took 11 years to get to market and produce an explosion in data and to fully inform decisions on what to pursue in the laboratory (speeding the process and saving money). The chipping revolution removes the bottleneck of hand-sampling plant tissue. People used to sample leaves with hand punches. Engineers automated the process by taking a sample from bar-coded seeds and then checking performance in the field against the identifying data.

Genomics allows testing of thousands of candidate genes for new biotech traits, such as yield and stress resistance. Thousands of candidate genes become tens of thousands of transformation events, and field trials have gone from 50 plots to plots that would encircle the globe many times. Automated genotyping is also a key enabler of massively parallel gene screening. Plants are put through an assembly-line automation that takes data daily. Integrated Farming Systems^{SM} would combine advanced seed genetics, on-farm economic practices, and software and hardware innovations to drive yield by allowing prescriptive planting to match soil diversity (i.e., soil composition, moisture, and elevation). These systems pull together genetic research, field experiments, and satellite imagery.

Opportunities for even further yield improvement are evident today (e.g., for 300 bushels per acre of corn). What has been done in U.S. agriculture can be exported. There is a 100-bushel-per-acre difference between current practice in foreign countries and current practice in the United States. Further yield improvements are possible.

Monsanto research has focused on the commercial viability of corn stover harvest (which also applies to switchgrass and other crops). When yield and insect resistance are increased, stalk size is also increased, which produces farming problems. What was needed were demonstrated and sustainable economic solutions for stover removal. Properly done, corn stover harvests will increase the value of an acre of corn. Improperly done, corn stover harvests will damage fields.

Ethanol policy has created a lot of interest in stover removal. The Renewable Fuel Standard (RFS) mandates 36 billion gallons per year of renewable fuels by 2022, with 16 billion gallons to come from cellulosic feedstocks like stover. However, averages that are published in the literature about what is actually available to sustainably remove tend to overlook local conditions that produce variability in sustainable stover removal and markets. A sustainable harvest must meet both environmental and economic requirements. Information and data are being broadly shared and developed involving coordinated field trials, commercial-scale trials, and decision support. Modeling and research have been focused on quantifying the limiting factors so that agronomic strategies can be effectively developed. A modeling framework was developed for planning quantitative soil-carbon analysis, greenhouse-gas fluxes, water-quality impacts, and crop-practice strategies. Models were in place to deal with yield, slope, etc. and how much stover could be removed. Measurements were needed. These measurements vary across fields. Ultimately, slope, rotation, previous cropping practices, and climate dictate sustainable stover removal rates. Removal rates vary across the field, so variable-removal equipment is needed. Weather varies, also. Harvest-period length, need for drying, etc. influence the resources needed for stover removal.

Stover biomass has alternative uses with alternative values for displacing coal in energy production, producing cellulosic ethanol, and producing animal feed (which offsets additional corn production). Lime treatment can improve the feed value of corn stover, improving in vitro digestibility by 30 to 50%.

Treated stalks displace a portion of corn in the diet. The grower makes an incremental $30 to $60 per acre. The cattleman makes an incremental $10 to $20 a head. Using stalks as feed effectively increases the productivity of the corn by 50 bushels per acre. Commercial operations are developing. Groups are chopping stocks, storing stover, and applying lime. Conservation planning tools have been used to estimate the field-specific stover-retention targets. Idaho National Laboratory developed an iPad application to calculate how much stover can be removed in real time as the equipment rolls across the field. The key thing is to retain soil carbon. Ongoing work focuses on variable-rate removal and on cover crops (e.g., rye crops) to provide grain, provide carbon, and lessen erosion.

Monsanto has been looking at barriers to the commercial viability of algal biofuels. It could not see a path forward but was excited about the possibilities. It reached out to ADM and Washington University in St. Louis to develop a first-principles model to elucidate what the net energy return is, how much it will cost, and what the carbon intensity is. However, one needs to get buy-in from all parties before one develops a first-principles model.

There are new applications for sequencing. Genomes in the public domain enable small startups to introduce new technologies. Public funding enables a network of about 50 collaborators to explore the structural and functional genomics of roundworms. These data inform a pipeline to develop novel products to control plant and animal parasites.

Public data also inform models for predicting crop yields. Dated global supply and demand economics and crop models are inadequate for current needs. Current efforts to project the impact of climate change on current and future crop productivity are severely hampered by the weakness of the mechanistic crop-simulation models. Policy decisions for agriculture are using black-box economic models instead of crop models. These are 30 years old and outdated. The data from field trials must find their way into models. The Agricultural Intercomparison and Improvement Project (AgMIP) was initiated to address these shortfalls by linking climate, crop, and economic models. Monsanto has donated resources (data and personnel) to improve the Decision Support System for Agrotechnology Transfer (DSSAT) Ceres Maize corn-simulation model, posting/donating selected test-mean yields from its global breeding trial database for use in model validation. Additional field phenology data (multi-site and multi-year) are needed to calibrate/validate the new model. Monsanto only works on long-row crops. Data from other crops are needed from third-party field trials.

By 2030, the world's population will grow so much that there will be another China. Grain ethanol use represents only a small portion of the overall agricultural food and feed commodity use. About 13% of the corn supply will be available for ethanol production. Grain will be there for basic needs. However, what is not seen is investment in smaller crops like cassava. Also, there is a big challenge to get people to pursue advanced degrees in agriculture. A lot of STEM (science, technology, engineering, and math) efforts need to be pursued. The development of the scientific workforce is critical.

Stacey said that his impression was that food crops were not being looked at as energy sources because of the food/fuel debate. There are questions about increased costs of food because of the diversion of grain to fuels. Schlicher replied that it comes down to bushels per acre. Fuel diversion is small, as is the quantity of food in the marketplace. Most grains are used for animal feedstocks. If corn goes to ethanol, only the starch is used; all the protein, fat, and fiber go back to the farm as animal feed displacing corn and soybean meal, and the net amount of corn used for ethanol is 16 or 17% of the crop production. In addition, stover is used as an animal feed. This past year, when 14.5 billion gallons of ethanol were produced, there was more animal feed available than in the year when only 1.5 billion gallons was produced. Switchgrass is also an animal feed. Why is it okay to use switchgrass for fuel?

Leung asked about delayed effects on the soil from the drought conditions this year and what the implications might be for multiuse crops. Schlicher responded that modelers are saying that next year will be a good year and that stocks will be built back up. Growers (in the same areas) say there are multi-year effects. The development of drought-resistant strains is needed.

Shanks asked how much advancement was being achieved on putting cover crops on fields. Schlicher replied that, if one has the economics to do it, it is easier to promote. Trials are under way. However, there isn't any quality assessment on cover-crop seed production. One cannot compare yields of rye to rye. No cover-crop seed is available this year because of the interest in cover cropping exhibited by farmers. Crop insurance does not cover cover crops.

Robertson noted that carbon is important but nitrogen content is important, also, and asked if Monsanto was doing any research at the genomic level on nitrogen use. Schlicher answered that the company's top priorities are to mitigate nitrogen use and to breed hybrids that need less nitrogen. It is also investing in nitrogen-gene research. Insurance programs should not promote nitrogen-fertilizer use. That is why Monsanto is championing cover crops.

Tiedje asked if the digestibility of corn stover was being improved genetically. Schlicher responded, no. All the work has been on the processing side. There is an opportunity for research on the genetic side. The industry has spent 15 years making stocks wind resistant. Now the emphasis is shifting.

A break was declared at 10:40 a.m. The meeting was called back into session at 10:55 a.m.

Daniel Stover was asked to report on the Workshop on Research Priorities for Tropical Ecosystems Under Climate Change, which was held June 4–5, 2012, in Bethesda, Md.

The tropics are one of the three ecosystems that should be studied. They cover 40% of the Earth's surface, dominate the carbon cycle, are vulnerable to small climate changes, regulate major climate feedbacks affecting the entire planet, and are inadequately represented in Earth system models. The workshop goals were to summarize what is and is not known about the interdependence of tropical ecosystems and climate change. The themes discussed were soil biogeochemistry and hydrology, natural and anthropogenic disturbance, tropical forest ecophysiology, and cross-cutting issues.

Forty experts in tropical ecology were pulled together in a 2-day workshop. The major themes and uncertainties discussed were temperature; precipitation; disturbance and mortality; elevated atmospheric CO_2; anthropogenic disturbances; and trace gases, aerosols, and particulate emissions. The workshop did not focus on geography but on the Neotropics (South America, Central America, and the Caribbean), Afrotropics (sub-Sahara Africa), and Indo-Malay-AustralAsia tropics (Southeast Asia).

It is not known how tropical ecosystems will respond to increasing temperatures. A 2 to 5 ^{0}C increase in tropical systems may occur by 2100. There are no analogous environments for comparison in space or time. There is a need to understand temperature thresholds and sensitivities, photosynthesis and respiration, plant allocation, soil biogeochemical processes, functional diversity, and spatial and temporal variation.

Also, it is not known how tropical ecosystems will respond to changes in rainfall. The models indicate reduced precipitation across most of the tropics. Drought vulnerability is poorly understood. There is a need to understand changes in precipitation in terms of spatial and temporal drivers and of feedbacks of drought stress and tree mortality and to improve model representation of soil depth, structure, and hydraulic properties; root systems; and stomatal regulation.

There is also a need to know how natural-disturbance events and mortality will increase as a result of climate forcings. Increased tree mortality can significantly affect the global carbon cycle and net forest CO_2 exchanges, and models do not represent this. There is a need to understand mechanisms of mortality in terms of the relationship with atmospheric convection patterns, extreme events, and shifts in vegetation.

There are a number of questions around how tropical ecosystems will respond to increasing atmospheric CO_2 concentrations. To understand these questions, there is a need to understand the response to elevated CO_2, including biogeochemical interactions. To predict the longer-term fate of carbon, models need data on leaf-level gas exchange, nutrient limitations, carbon-allocation patterns, and belowground responses. It is also uncertain whether elevated CO_2 will ameliorate drought responses.

The interactions between climate change and aerosol/particulate emissions from tropical forests are uncertain, also. Tropical forests are large sources of biological aerosols and trace gases. Key uncertainties include the physiological and climatic regulation of methane and nitrous oxide production/emission. Improved model representation of light quality, storm intensity, cloud- aerosol interactions, nutrient deposition, and ozone effects is needed.

It is uncertain how tropical forest interactions with the Earth system will shift as a result of anthropogenic disturbance and land-use change. A significant fraction of the tropical forest cover lies in areas recovering from logging or in secondary forests and land abandoned from agriculture. An improved understanding of land-use changes and hydrology, sensible and latent heat fluxes, and impacts on soil biogeochemistry is needed.

As it was for the Arctic, the overall goal of BER's decade-long investment will be to investigate tropical ecosystems, to study their feedbacks and vulnerabilities to climate change, and to improve the representation of these systems in Earth-system models.

NGEE Tropics is expected to be a model-informed field study that results in the iterative refinement of high-resolution predictive models; that is based on field studies in the most climate-sensitive tropical geographies; that provides a high scientific return on investment; and that provides a critical opportunity for collaborating and leveraging national-laboratory, university, and other federal investments.

Leung pointed out that the transition zone between the subtropics and the topics is a good place to put instrumentation. Stover said that there was discussion on where to put instrumentation, and it was decided to put it in forests.

Shaver noted that there were opportunities for comparing Arctic and tropical systems in terms of distribution and characterization. Stover agreed that this is a good opportunity for a rich collaboration to compare extremes.

Baldocchi asked how this new project would build upon the Large-Scale Biosphere-Atmosphere Experiment in Amazonia (LBA) program. Stover said that is premature to say where these investments should be made. LBA did not characterize rigorously. Small packets of data need to be brought together. Baldocchi asked if different regions of the world were used to determine trends in variables. Stover replied, they may be.

Robertson asked what timeline was being looked at. Stover answered that that is still in discussion in BER. This program is funded by FY13 funds, and there is not yet a budget for FY13.

Daniel Drell was asked to report on the Workshop on Strategic Planning for the Genomic Sciences.

The Strategic Planning for the Genomic Sciences Workshop was held May 30–31, 2012, in Washington, D.C. The rationale for the workshop is the explosion on JGI throughput with dramatically decreased costs. How would one graph the pace of gene annotation? "Annotation" is ambiguous, so the challenge was not addressed. What could a next-generation genome center evolve into, and what challenges should it be able to address to remain current and necessary?

Given that sequencing technologies have advanced so rapidly and not slowed, how might this affect the scientific scope at the DOE-JGI and its ability to stay at the forefront of exploiting these changes? The connection to the BER mission needs be articulated carefully and persuasively.

The initial assumptions of the workshop were that (1) DOE-JGI will remain an SC user facility; (2) DOE-JGI will continue to generate BER-mission-relevant sequences in larger amounts and at high quality; (3) DOE-JGI cannot do everything; it must be strategic in what capabilities it develops and how it transitions to a next-generation genome science center; and (4) rather than "reinventing the wheel", DOE-JGI should avail itself of other DOE assets, where appropriate (e.g., KBase, EMSL, etc.).

The workshop charge was to determine

1. What new scientific insights could be enabled by next-generation sequencing? What vision for DOE mission-driven biology, rooted in and building on high-throughput genomic sequencing and analysis, can be identified for the next 5 to 10 years?
2. What large-scale questions/grand challenges in systems biology, grounded in very high-throughput genomics and post-genomic analyses, will require a user facility to achieve necessary efficiencies and effectiveness and would have the highest impact and value for DOE biology?
3. What capabilities and technologies that (1) do not presently exist, (2) presently lack high-throughput capability, or (3) are not generally available to the biological research community will be required to address the most important questions in biology and to meet the needs of DOE biological science?

The outcomes of the research were sorted into six themes:

1. Continued and increasing large-scale sequencing of biologically important organisms and communities is needed.
2. Large-scale functional genomics technologies for high-throughput functional annotation, informed by global measurements of actual cellular activities rooted in genome sequencing are desperately needed.
3. Extended and improved bioinformatics methods are needed to enable integration and analysis of unprecedented quantities of data and to generate testable hypotheses critical to advancing DOE science.

4. Aggressively expanded capacity is required not only to sequence (“read”) DNA but also to synthesize (“write”) DNA, enabling scientists to manipulate genomes.
5. Improved automation of biological experiments is needed to match the throughput now prevalent in sequencing.
6. Communities of scientists should be led by the DOE-JGI and organized around key mission-relevant scientific questions (the crowd-source approach to scientific questions).

The JGI cannot do all of these but can contribute to them.

The vision for the DOE-JGI is for it to be a hypothesis-generating and validation engine for fundamental systems biology, reducing the search space for credible hypotheses. JGI does not just do computer analysis but computer analysis blended with experimental results. Exemplar science challenges include (1) designer phototrophs: engineering cyanobacteria to produce biofuels, (2) understanding interactions between microbes and climate, (3) advanced genomic capabilities for biofuel sustainability, (4) mining natural variation to improve energy capture (photosynthesis) in plants, (5) elucidating the dynamics of genomic participation in CO_2 cycling in marine environments, and (6) characterizing horizontal gene transfer (HGT).

The next steps include promoting the Emerging Technologies Opportunity Program (ETOP) at DOE-JGI; tuning the Community Sequencing Program at DOE-JGI towards challenges identified by the workshop [this has already happened]; continuing to work closely with the BRCs, KBase, and other sophisticated users to push the state of the art in next-generation genome analyses and annotation; and continuing to develop new sequencing technology(ies) as a faster-better-cheaper “hypothesis-generation engine”; promoting and encouraging the development of complementary interactions with other DOE facilities and activities; continuing to explore opportunities for the application of high-performance computation to genome analyses; building on emerging DNA synthesis capabilities to test annotations and enable biosystem-design science; and continuing to “seed” DOE-mission-focused communities of scientists to accelerate use of DOE-JGI generated sequence. This is not an exquisite list; it is initial thoughts.

A workshop report has been published along with a 10-year strategic vision. These two plans are complementary. One is the plan for JGI infrastructure, and the other was what is to be done with that infrastructure.

ETOP is currently encouraging projects to develop high-throughput functional-genomic capabilities; microfluidic molecular biology approaches; new ways to isolate high-quality DNA and RNA from fungi, microbes, and plants; functional characterization of microbes and microbial communities, including nondestructive functional characterization of single cells; and large-scale microbial phenotyping.

Remington asked how much the workshop went into the Strategic Plan. Drell replied that there were adjustments in the rationale for the next-generation genome center and how it would serve DOE missions.

Tiedje noted that the problem of annotation is huge. The reference database is so incomplete that he did not see how it can be solved soon. Drell agreed that it was a huge problem area, given the number or microbes out there, a couple of hundred or 1000 sequences will not get one too far but will put some data in that space. There are no known solutions. Tiedje asked if an intermediate approach were possible by looking for common functions and annotating at the community level. Drell replied that most of the studies are metagenomic, and that makes the problem worse. Multiple approaches are needed.

Joachimiak suggested that maybe the approach should be simplified at the beginning and that targets should be picked for characterization. Drell said that JGI is interested in doing that but does not have the resources. It would be an important direction to move in, but how to do it is difficult to perceive.

The floor was opened for comment. There was none. Stacey said that comments were coming in on the Long-Term Vision Subcommittee report, and a final version should be ready in a week.

The floor was opened to public comment. William Collins (Lawrence Berkeley National Laboratory and University of California at Berkeley) said that Taylor did a great job in service to the modeling community, and the framework will be used for a decade to come.

There being so further comments, the meeting was adjourned at 11:41 a.m.

Respectfully submitted,
Frederick M. O'Hara, Jr.
Recording Secretary
Oct. 31, 2012