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**Morning Breakout Sessions Output**
- Productive Light Absorption
- Genetic Engineering
- Exploiting Evolution

**Afternoon Technical Improvisation Session Output**
- C4 Plant
- Aquatic Phototroph
- “Model” Organism

Wrap-up Session                                                          | 64   |
ARPA-E BACKGROUND
ARPA-E’s Mission

- Find and fund high-risk, high-impact projects
- Identify and promote revolutionary advances in fundamental sciences
- Accelerate transformational technologies or create new technologies where none currently exist
- Translate scientific discoveries and cutting-edge inventions into technological innovations
- Bridge gaps in the energy innovation pipeline

To enhance the economic and energy security of the U.S.

To ensure U.S. technological lead in developing and deploying advanced energy technologies

Reduce Energy-Related Emissions

Reduce Energy Imports

Improve Energy Efficiency

Mission
ARPA-E was created with a vision to bridge gaps in the energy innovation pipeline

**What ARPA-E will do**
- Seek high impact science and engineering projects
- Invest in the best ideas and teams
- Will tolerate and manage high technical risk
- Accelerate translation from science to markets
- Proof of concept and prototyping

**What ARPA-E will NOT do**
- Incremental improvements
- Basic research
- Long term projects or block grants
- Large-scale demonstration projects
What is an ARPA-E project?

- High impact on ARPA-E mission areas
- Disruptive, innovative technical approaches & new learning curves
- • Best-in-class people & teams containing scientists and engineers;
  • Attract the US intellectual horsepower to energy R&D
- Strong impact of ARPA-E funding relative to private sector
Technology Readiness Levels

**TRL 9**: Actual technology system qualified through successful mission operations.

**TRL 8**: Actual technology system completed and qualified through test and demonstration.

**TRL 7**: Technology prototype demonstration in an operational environment.

**TRL 6**: Technology demonstration in a relevant environment.

**TRL 5**: Technology validation in relevant environment.

**TRL 4**: Technology validation in laboratory.

**TRL 3**: Analytical and experimental critical function and/or characteristic proof-of-concept.

**TRL 2**: Technology concept and/or application formulated.

**TRL 1**: Basic principles observed.
WORKSHOP CONTEXT
Current photosynthetic efficiency is low

Photosynthesis:

\[ \text{CO}_2 + \text{H}_2\text{O} \rightarrow \text{C}_6\text{H}_{12}\text{O}_6 \]

Fermentation:

\[ \text{C}_6\text{H}_{12}\text{O}_6 \rightarrow 2\text{CO}_2 + 2\text{C}_2\text{H}_5\text{OH} \]

One third of the carbon captured is \textit{not} converted into fuel.

Hall & Rao, 1999
Agriculture and aquaculture seem to be diametrically opposed: Should we go after the middle ground?
Genetic engineering of organism metabolism and other processes offers promise to improve overall efficiency

Kebeish et al., Nature Biotechnology 25, p593 (2007)
Rapid conventional genetics has been the traditional successful route, but takes time.

Teosinte-to-maize

- Human-applied breeding
- 9,000 years, countless individual selection steps, but evidently successful
PRESENTATION SUMMARIES
“What is the maximum efficiency that photosynthesis can convert solar energy into biomass?”

-Don Ort (UIUC, USDA)

- The “green revolution” has been driven mostly by improvements in partition efficiency (harvest index), $\varepsilon_p$, and interception efficiency, $\varepsilon_i$. There is little room for improving them further. There are many opportunities, however, for improving the photosynthetic conversion efficiency, $\varepsilon_c$

- Monteith Equation: $Y\left(\text{MJ}/m^2\right) = 0.487 \cdot S_t \cdot \varepsilon_i \cdot \varepsilon_c \cdot \varepsilon_p$

<table>
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<th></th>
<th>C3 Plants (%)</th>
<th>C4 Plants (%)</th>
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<tr>
<td>Theoretical maximum</td>
<td>4.6</td>
<td>6.0</td>
</tr>
<tr>
<td>Maximum observed</td>
<td>2.4</td>
<td>3.7</td>
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<tr>
<td>Average observed</td>
<td>0.7</td>
<td>1.2</td>
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- Opportunities to raise the theoretical upper limit
  - Re-engineer RuBisCO (up to 25% increase in C3 $\varepsilon_c$)
  - Bypass photorespiration (up to 13% increase in C3 $\varepsilon_c$)
  - Convert C3 plants to C4 (up to 30% increase in C3 $\varepsilon_c$)
  - Design photosystems with two different action spectra (unknown increase in $\varepsilon_c$)

- The same fundamental principles drive aquatic and land-based photosynthesis: photosynthesis is NOT inherently more efficient in either

“Photosynthesis to Fuels”
- Tasios Melis (UC Berkeley)

- 2D problem in light utilization
  - (1) Over-absorption and wasteful dissipation of excess sunlight at the surface
  - (2) Short range of PAR (400-700 nm) – possible to extend to 950nm
  - Tools needed: new technologies for genetic engineering of chloroplasts, microalgae, and cyanobacteria (Lacking for most microalgae except Chlamydomonas and several cyanobacteria)

- Carbon partitioning from biomass to fuel production must be addressed
  - 80-85 % of CO₂ can go toward sugar/biomass production
  - Fuel/biomass is a useful metric and is currently low (20%)
  - Tools needed: metabolic engineering approaches to alter carbon flux
“What it takes to feed and fuel the world”
- Ganesh Kishore (Malaysian Life Sciences Capital Fund Ltd)

- Global food and nutrition security is under immense pressure; emerging economies are most vulnerable
- Technology has vast potential to meet not only global demands for food and nutrition but also sustainable energy production
- Genetic intensity coupled with optimum nourishment of crop is key to improving productivity
- Cost of bringing new technologies to the market continues to escalate – financing innovation and commercialization is critical
- A science-based, transparent, globally harmonized regulatory and trade policies are essential
- Agriculture infrastructure needs to be built and, on- and off-farm losses have to be reduced in emerging economies

Actual breeding plus cultural practice gain:
Corn yields continue to advance


- 50-80% of total costs related to feedstock
- Agriculture and industrial biotechnology are intimately linked
- All agriculture is local – no “one size fits all” solution
- Doing more with less is an imperative, not a choice!
- Genetic intensity coupled with optimum nourishment of crop is key to improving productivity
MORNING BREAKOUT SESSIONS

Productive Light Absorption
Metabolic Engineering
Exploiting Evolution
General Targets and Assumptions for Potential Solutions

For the purposes of this workshop, a biofuel:
- Has at least the volumetric energy density of ethanol (90 MJ/gallon)
- Is a liquid at room temperature, and is generally compatible with existing distribution infrastructure.

Baseline Financial and Operating Assumptions
- **Land**: This is essentially the cost of solar photons
  - Photochemical spectrum is the AM 1.5 solar radiation spectrum characteristic of the continental US as described in [http://rredc.nrel.gov/solar/spectra/am1.5/](http://rredc.nrel.gov/solar/spectra/am1.5/)
- **Large-scale production is supported by the North American climate**
- **Water**: Cost depends on its source
  - Fresh water from rainfall, at no cost but depends on location
  - Ground water follows the cost of irrigation
  - Sea water is provided by pipeline to the location
- **Carbon**: Atmospheric CO₂ (400 ppm) is available at no cost. Supplemental CO₂, if needed, is provided by post-combustion capture, at a fixed price of $80 per tonne.
- **Capital costs of processing will be modeled on ethanol (DOE OBP) from field to liquids**

Proposed Target Metrics
- The efficiency of photon capture and conversion at scale needs to be twice that of ethanol from corn
- Fully-capitalized production costs need to be no more $0.01/MJ
Questions for all breakout sessions

- **What are the quantitative (theoretical) limits of the technology?**
  - If the technology improvement works at 100%, what would the impact on fuel yield be?
  - What factors might reduce this impact, and how might they be quantified?

- **How can we reduce these ideas to practice?**
  - What is the TRL (technology readiness level)?
  - Is the idea science, engineering, or both?
  - What tools/techniques are needed to move the technology up the TRL scale?

- **Is a technology breakthrough in a 3-5 year timeframe realistic?**
  - What are the aspects of the technology that constrain development?

- **Are there advances in related fields that could shorten the timeline?**
MORNING BREAKOUT SESSIONS

Productive Light Absorption

Metabolic Engineering

Exploiting Evolution
Questions for Productive Light Absorption Breakout

Expanding absolute absorption

• Increasing the productive absorption spectrum can be achieved in different ways. We are aware of energy transducing pigments such as proteorhodopsin (in the visible) and chlorophyll d (in the near IR).
  • What are their engineering limitations? How might they be integrated and what organisms would be most suitable for incorporation?
    • Proteorhodopsin
    • Chlorophyll d
  • Are there similar pigments that might improve light capture?
• How large an impact will canopy architecture have? Can lessons from nature be used to improve the efficiency of deployed photobioreactors for unicellular organisms?

Balancing light flux

• Antenna size, and other physical features of the photosystems, can be engineered or adapted. What organisms have shown improved energy capture from reduced antenna sizes? Can these observations be generalized? Can these features be used productively to improve yields in the field?
• Photoprotection signaling pathways involving photoreceptors, reactive oxygen species, redox state changes, and changes in thylakoid pH seem to be so intertwined with other cell functions and that they simply can’t be tinkered with in any meaningful way. Is this accurate? If not, what would the practical consequences be?

Improving Reaction Center Efficiency

• What techniques exist to emphasize productive electron flow pathways? (e.g., cyclic versus linear electron flow)
• What can be changed to improve the photon-to-carbon fixation connection?

Are there approaches that we have not considered?
Productive light absorption breakout

- **Opportunities**
  - **Manipulation of antenna size in crop plants (TRL ~3-4)**
    - **Positives:** Enhances light distribution through canopy while reducing unproductive non-photochemical quenching
    - **Challenges:** It is not clear what amount of chlorophyll is needed; Optimal antenna sizes will depend on environment/light conditions
  - **Bypass photorespiration (TRL ~2-3)**
    - **Positives:** reduces energy required to recycle glycolate back to the C3 pathway thereby increasing overall efficiency, demonstrated in *Arabidopsis*
    - **Challenges:** translation to other C3 plants

- **Metrics Proposed**
  - Doubling of light absorption efficiency
  - 8-9% theoretical efficiency is possible

- **Tools Needed**
  - Computational/CAD system for analysis and modeling of flux balance, the physics of cell movement, and changes in antenna size, pigments, etc.
  - A standardized approach to evaluating and ranking the available options leveraging existing models and generating new models (i.e. there are too many ways to approach a problem right now)
  - Screening tools to assemble a library of modules (antenna size, reaction center, etc.) that can be “plugged-in” to figure out the ideal system of components
Productive light absorption breakout

- Basic science/engineering ideas (not in technology realm yet)
  - Engineer a mismatch in the action spectra of PSI and PSII
    - **Positives**: two photosystems don’t compete for the same photons
    - **Challenges**: Hard to control where new chromophores will go when introduced, Photosystems are highly conserved, uncertain how to change them

- Change/expand the photosynthetic active spectrum with rhodopsins, chlorophyll d, etc.
  - **Benefits**: ~50% of incident radiation is outside the photosynthetic active spectrum, this will capture some of that light
  - **Challenges**: integration will be difficult (difficult to control where they will go), many parts must be transplanted, other processes may compete with the higher proton motive force (redox reactions)

- Engineer light-powered processes that contribute to efficiency (i.e. CO₂–concentrating mechanism)
  - **Benefits**: modular design, avoids engineering highly-conserved photosystems
  - **Challenges**: not clear how to implement, don’t have the needed tools

- Integration of artificial photocatalysts or photovoltaics for biohybrid systems
  - **Benefits**: robust chromophore is less prone to photobleaching, can be more easily tuned to a specific wavelength range
  - **Challenges**: very little understanding on how to integrate into living systems
Productive light absorption breakout

- **Additional Comments**
  - Photobioreactors would benefit from insights in plant canopy (i.e. long thin absorbers are better than large ponds/raceways)
  - Agricultural production has a 1000 year history, but algae is version 1.0 (ponds, tubes, etc.) and has a lot of room to improve in economics and scalability
  - How flexible is photosynthetic metabolism? You can get the majority of carbon uptake go to your desired product rather than growing the plant by using metabolic engineering
  - There are certain things you can do with any organism, no organism, or some organisms
  - 85% of CO$_2$ put into the atmosphere every year is from biofuels (~8Gt), the same amount is taken out by crop plants. A lot more CO$_2$ is emitted from industrial sources

- **Primary Output Topics**
  - Antenna size
  - Reaction center
  - Broadening the absorption spectrum
  - Reflection versus productive absorption
  - Maximized efficiency by minimizing saturation
  - CO$_2$ fixation reaction and efficiency
  - Hybrid approaches
  - Biomass limitations - carbon partitioning
  - Continuous culture/secretion
  - Proteorhodopsin
  - Phase-assisted sequence searching
  - RuBisCO bypass – shift flux to other metabolic cycles
  - Library of modules (“cassettes” of functionality) for integrating changes
MORNING BREAKOUT SESSIONS

Productive Light Absorption

Metabolic Engineering

Exploiting Evolution
Questions for Metabolic Engineering Breakout

Improved CO₂ Fixation

• In theory, carbon uptake can be improved in different ways.
  • At what point is photosynthesis “CO₂ limited”? Why?
  • How would metabolism need to be adjusted for increased energy input?
• Photorespiratory bypass, RuBisCo and Calvin cycle optimization have been suggested. What are the engineering limitations to these approaches?
• Are there higher efficiency carboxylases and pathways in nature that might not have been accessed by modern photosynthetic organisms? Can they be made viable in the presence of O₂?

Divertible Flux

• Given the overhead energy costs of photosynthesis, what is the maximum energy flux that an organism can divert toward fuel molecules? Is there an accepted “minimal genome” for an engineered chassis?

Siphoning Off Intermediates

• Fatty acids, terpenes, and terpenoids, as well as infrastructure-compatible alcohols such as butanol, seem equally accessible from metabolic engineering. Is this accurate?
• What are the key metabolic convergence points, i.e. what are the high energy intermediates in primary metabolism that serve as flux bypass points? What enzymatic processes can be emphasized or de-emphasized to improve overall biofuel production efficiency? How predictive are metabolic models?

Are there approaches that we have not considered?
Metabolic Engineering Breakout

• Broad Issues of Discussion
  ▪ The most economic option may not be the most efficient
  ▪ We need to worry more about raising operational efficiency rather than theoretical efficiency, we are still too far from the existing theoretical
  ▪ Making biomass versus making fuel directly
    ▪ Will likely come out ahead if you can make the fuel in the organism
    ▪ Can make anything out of biomass, but need low cost/dry ton
  ▪ What we don’t know is pretty vast in organisms we’re looking at
  ▪ No metabolic pathway winner – many fuel molecules may come out
    ▪ Develop molecules that are significantly different from petroleum
    ▪ What benefits do biofuels have to offer that petroleum doesn’t?
  ▪ Need to identify the pathway efficiency to produce a target compound
  ▪ The longer the process, the more inefficient (in general), intercepting intermediates is a way to overcome this
  ▪ A reverse engineering approach may shed more light on the tools needed
    ▪ Pick a fuel molecule, then figure out how to produce it (“we need to pick the moon we’re shooting for”)
    ▪ We should at least define the ideal characteristics of the biofuel we want
  ▪ Targeted research (non-basic) is needed in gene regulation and expression mechanisms
    ▪ How much is coming up with the recipe versus characterization?
    ▪ The research is mostly an adaptation of existing techniques which is more on the side of technology than basic research
  ▪ The decision to go with batch or continuous processes depends on the organism you are using – there is no winner a priori
Metabolic Engineering Breakout

Potential Metrics
• Max theoretical carbon fixation is 25g carbon fixed/g chlorophyll/hr
  • Reality is 12g carbon fixed/g chlorophyll/hr
  • 75g carbon/m²/day, but reality is only 1/3 of that
  • Would be transformative to go to 25g, but not 10x
• 100 billion gallons of fuel is needed for energy independence
• Technology that requires 3-5 years of development needs to be compatible with current infrastructure
• Target compounds for production – fatty acids, isoprenoids
• $0.10 or less per pound sugar or oil (Biomass program)
  • A lower number would be better ($0.02/gal)

Tools Identified
• Current metabolic models for “hybrid” genomes are not sufficient but necessary
• Go to E. coli models and try to predict behavior with kinetic data
• Need models – flux analysis, feedback mechanisms on organism level
  o Part of the process but unlikely to spark breakthroughs
• Better gene regulation, expression
• Better silencing
• Can we take promoters of genes up-regulated 100-300 fold and use them to drive fuel production?
• Stationary isotopic labeling in plants
• Combinatorial approach to engineering pathways

Discussion Topics
• Carbon Delivery
  • Bicarbonate could be used in aquatic systems for carbon delivery
• Biomass processing
  • Dewatering is a big problem, better when fuel phase separates (i.e. hydrogen in algae production)
Discussion Topics (continued)

- **Algae**
  - Continuous, not limited to life cycles
  - If hydrocarbons are produced, they get eaten immediately
  - Can go from 1 to 5% terpenoids in 5 years (models can get it there faster)
  - RNAi doesn't work well in algae
  - Cyanobacteria already concentrate CO₂ efficiently
  - It is feasible to engineer cyanobacterial processes into C4 plants?

- **Other sources for reducing equivalents**
  - Would need a massive amount of a carbon source
  - Water splitting organism
  - Water is best source, other sources are impractical

- **Redirecting carbon flux**
  - 100 gigatons carbon/year – most goes to food, not enough left
  - The mass balance needs to be shifted
  - What are the consequences of changing things? (detrimental effects) It’s difficult to switch from biomass to fuel easily

- **Changing metabolism**
  - Need to know what we want to do first, start with a tractable organism
  - Metabolic engineering is doable if you know the roadmap
  - It is clear where the convergence points are
  - Need to get pyruvate and acetyl-CoA
  - Transforming diatoms is too high risk, but we shouldn’t rule out certain organisms just because we don’t yet have the tools we need

- **Specific pathways we should focus on? Which ones should we transplant?**
  - Need to know the efficiency of a particular pathway
  - Terpene and butanol synthesis are well known

- **Separate goals into two issues:** improve fuel synthesis, then metabolically engineer the pathways to get to that fuel
MORNING BREAKOUT SESSIONS

Productive Light Absorption

Metabolic Engineering

Exploiting Evolution
Questions for Exploiting Evolution Breakout

Consequences of Natural Selection
• Some organisms naturally export high-energy molecules such as terpenes.
  • Are any of these organisms viable as production species?
  • Could pathways identified in these species be transplanted into viable industrial organisms?
  • Are there environmental conditions that favor carbon fixation but NOT biomass production?
• Are there extremophile species that offer insight on more effective pathways for fuel production, e.g., through life at low [CO₂] or low light?
• Are there approaches that exploit sexual reproduction as a way of enforcing fuel production? Can hormone pathways be diverted for induction (without adding significant costs)?

Exploiting Artificial Selection
• Are there strategies that would allow faster screening, when the phenotype is fuel production at maturity?
• Are there culture conditions that would render GMOs non-viable in nature (e.g., requirement for continuous illumination)? Are there conditions that would render opportunistic organisms non-viable in the field?
• What strategies can be used to prevent trait reversion in rapidly growing cultures?

Are there approaches that we have not considered?
Exploiting Evolution Breakout

Opportunities (Two primary discussions)

- How can existing natural systems be leveraged? (force changes through breeding)
  - Allelic diversity in plants is not fully known, so metagenomic studies are needed
  - Highly conserved proteins usually cannot be evolved because mutations are not tolerated (e.g. photosystems)
  - Selection and phenotyping strategies need to be expanded
  - Crops should be engineered to deal with all processing stresses – growth to extraction of desired product

- What can be done using directed evolution?
  - Import new pathways into cyanobacteria/single-celled organisms
  - Evolve pathway for optimization using mutagenesis, selection schemes
  - This is more challenging in higher plants so create a validated single-cell photosynthetic organism as a construct to evolve pathways to be translated into plants
    - Can be used for discovery – gene screening, metagenomic analysis
  - A rational engineering approach is needed to improve protein performance using structure data
  - Introduced pathways may prove toxic to the cell – modifications will have to be made to reduce toxicity
    - Complete pathways must be incorporated into a single-celled organism at one time (not gradually) as this may reduce buildup of potentially toxic intermediates
    - Introducing pathways incrementally (traditional plant breeding for traits) will not be successful
  - In plants, there are multiple layers of cross talk that can shut down processes (e.g. sucrose inhibition), it may be better to introduce complementary parts of the pathway and then breed the strains together to get the full pathway into one organism.
  - Paired systems – figure out what happened in a modified microbe, then translate that to a plant
Exploiting evolution breakout

Tools Identified

- Model system for marine algae
- A way to deal with large protein complexes
- Metagenomic studies needed to discover new useful genes and alleles
- Selection schemes/platforms needed that are not organism or gene specific
- Next generation sequencing to discover the existing genetic diversity (comparable to pharmacogenomics)
- Metabolome profiling in different parts of the plant
- Combination of synthetic and molecular biology to incorporate novel metabolic pathways into photosynthetic organisms
- Sequencing of entire genomes rapidly and inexpensively with newly available tools
  - Large scale studies to identify desirable traits in plants are possible
  - Allows generation of expression and epigenetics profiles
- Many tools available already for engineering plants, microalgae, and cyanobacteria but getting beyond proof of principle is a challenge

Other Topics of Discussion

- Plant breeding needs to be global to match climates
  - Bamboo and oil palm (not in US), or castor (can be grown in US) are potential biofuel crops
- Using oil producing crops reduces water requirement
- Marine algae
  - Pigment systems can be altered
  - Model system does not exist and needs to be created
  - More expensive due to infrastructure costs
  - In algal bioreactors, flocculation may avoid centrifugation needs
- Make new molecules other than traditional sugars that can be converted later into some other high value product
Exploiting evolution breakout

Challenges/Problems

• Environmental conditions influence gene expression – have to manage lifecycle of photosynthesis
• Algal systems
  • CO₂ availability for algal systems
    • Atmospheric carbon concentration is too low
    • CO₂ diffusion into water is limiting
  • It is better to have a biofilm/pond but then you face contamination problems and evaporation
• Light penetration is an issue if you engineer strains to use wavelengths above 700nm
• Interactions between photosynthetic organisms and the “host” organism can modify biochemical pathways.
  • Example of coral – fixed carbon is secreted when the photosynthetic organism is inside, it doesn’t when it is outside – can this interaction be defined at the molecular level and used as a tool?
• Methods developed on plant lab strains do not always work in crops in the field
• More basic research is needed to understand the biology of endophytes
AFTERNOON TECHNICAL IMPROVISATION SESSION

C4 Plant
Aquatic Phototroph
“Model” Organism
Afternoon Technical Improvisation Session

Scenario presented to attendees:

In this exercise, participants in the morning breakout sessions are split into three teams. Each team has a similar improvisational task, with a slightly different outcome. This is not a competition; you may feel free to call upon members of other teams for technical information, as needed. The common thread:

Imagine a direction. Imagine this: As of tomorrow, your team is appointed by the President and given an essentially unlimited budget and access to all levels of the Nation’s public and private resources. Your singular goal, sanctioned by the Congress and subject to broad popular support, is to develop and scale an innovative photosynthetic process for the production of liquid biofuels. You are given the following goal: In just ten years, your team is to develop a commercial process to supplement the Nation’s transportation fuel needs, at the scale equivalent to 2.5 million barrels per day (i.e., 100 million gallons per day capacity) with low-cost biofuel. The process needs to break even at $0.01/MJ, fully capitalized.

Step into the future. It’s now ten years later, December 3, 2020, and the President has just concluded a press conference, trumpeting the success of the project, and praising your team’s "foresight", "outstanding teamwork" and "well-reasoned development of breakthrough technologies". One major breakthrough was the design of a revolutionary photosynthetic organism capable of using both natural light sources (i.e., sunlight) and atmospheric carbon dioxide as feedstock to efficiently produce a high-energy form of combustible liquid fuel for transportation.

Now, look back from the future, and tell us all a story. On the same day as the President’s announcement, your team is being interviewed for an exclusive, in-depth piece to be published in the New York Times. This piece will provide the “back story” to the announced success. The Times reporter has been gracious enough to provide you with a set of questions in advance (next page). Immediately after the interview, you will face the media with a brief presentation based on these questions.

[NOTE: After the exercise, your slides will be presented to the group for critical evaluation, and will be part of the public output of the Workshop.]

In the next 90 minutes, you should:

• Identify a team leader to keep the group on task, to referee disputes, and to lead the presentation.
• Improvise a plausible story line to answer the Times reporter’s questions, referencing the morning’s session and its findings for support. Be QUANTITATIVE where possible!
• Develop a short presentation for the group based on these answers, and be prepared to defend your answers to critics. [See PowerPoint template provided. This is flexible; you need not present slides that aren’t relevant to your points.]

The organizers of the Workshop are looking for insight, not answers, so have FUN!
Questions from the New York Times Science Editor:

As the details of your team’s achievement have come to light, it has become apparent that you have created a truly remarkable and useful organism.

• Describe the organism in simple terms. What did you see as the features and benefits of this particular solution?
• Walk me through the major milestones associated with the development and deployment of the organism. In particular, compare what your organism can do now with what the source organism did, in terms of both light and CO₂ utilization. How did the new features enable you to reduce costs at scale?
• Why do you think this approach was successful, where others have failed?

When starting out, you devised a successful project plan despite substantial technical uncertainty.

• What particular features of the organism/approach attracted you?
• What problems did you anticipate when contemplating this path?
• What was the most significant technical hurdle you were able to predict?

Early on, the project fell behind schedule, missing a few key milestones. Some criticized your choices, saying that the approach was “ill-conceived”. Every technically advanced project uncovers the unexpected, but, had you changed course, we’d be calling it a “failure” today.

• What did you learn in the early phases of the project that supported “doubling down” after this apparent setback?
• What did you do to put the project back on track?

Your newly operational production facility is particularly impressive!

• Describe the site and approach you are using for production.
• Compare your system to the “corn-based ethanol” model developed in 2010 [provided on the laptop]. Where were you able to reduce costs? What uncertainties could have been avoided if you’d spent more time planning?
AFTERNOON TECHNICAL IMPROVISATION SESSION

C4 Plant

Aquatic Phototroph

“Model” Organism

The following is the output presented by the group to all workshop attendees for discussion and debate
THE INNOVATIVE ORGANISM

Source Species: Miscanthus

Primary Features of modified C4 Organism
- accumulates massive amounts of sucrose throughout the season in vegetative tissues
- efficient post-harvest conversion of sucrose to long chain alcohols by improved endosymbionts in planta
- 1000 gals biofuel/acre from 36.5M acres per year

Benefits
- accumulates 10 tons dry matter per acre
- increased efficiency of water use
- increased photosynthetic efficiency
- increased carbon dioxide sequestration
- less need for nitrogen

Liquid Biofuel(s)
- long chain alcohols for use in combustion, diesel & aviation engines
**Development**
- Identify sucrose accumulation trait
- Breed Miscanthus lines that accumulate sucrose in stem
- Identify tractable endophyte of Miscanthus
- Introduce inducible pathway for converting sucrose to long chain alcohol

**Scaling/Deployment**
- Phase 1: parallel development of Miscanthus and endophyte lines
- Phase 2: integration and field testing of Miscanthus/endophyte pair
- Phase 3: optimize (cane mill) in silage and fermentation systems
1. Identify sucrose accumulation trait

2. Convert Miscanthus to accumulate sucrose

3. Identify tractable native endophytes of Miscanthus

4. Establish productive plant-microbe symbioses

5. Select for endophyte fuel resistance
• This organism was an attractive choice because perennial, low N requirement, high water use efficiency, high productivity, broad temperature range

• We selected the approach because high IP and fuel potential

• We thought the biggest problem would be endophyte identification, fermentation pathway design and toxicity
THE SETBACK

- Started on sweet sorghum
- Switched to cold-tolerant perennial for various reasons
- At 1M acre scale up all died
- Write renewal proposal to get back on track
THE PRODUCTION PLANT

Diagram what your production plant looks like

- **Feedstock Harvesting**
- **On farm/co-op Processing**
- **Fuel transport for distribution**

How scaled costs are reduced
- Capital/O&M – localized so very low

Production Capacity
Scales with size of processing facility
COST COMPARISON TO CORN-BASED ETHANOL MODEL

**Corn-EtOH Model**
(assuming 100 million gal/yr plant)

- Uses 225,000 acres of farmland
- $3.89 per bushel of corn
- $1.45/gal feedstock costs
- $0.31/gal capital expenses
- $0.36/gal operating expenses

**Corn-EtOH Model total cost**
$2.12/gal total cost
$0.0265 per MJ

**New Organism**

- uses only 100,000 acres
- lower farming costs
- ~$1.50 per bushel eq
- $0.72/gal feedstock
- $0.14/gal capital cost
- $0.10/gal operating cost
- $0.96/gal costs
- 40% higher energy density
- $0.01/MJ

~50% reduction in feedstock costs
~50% reduction in capital expenses
~66% reduction in O&M expenses

57% reduction in total production cost
AFTERNOON TECHNICAL IMPROVISATION SESSION

C4 Plant

Aquatic Phototroph

“Model” Organism

The following is the output presented by the group to all workshop attendees for discussion and debate
THE INNOVATIVE ORGANISM

Source Species:
- Thermotolerant Cyanobacteria chassis
- Source organisms/genes for engineering production organism
  - Photon capture
  - Energy conversion efficiency
  - Carbon fixation
  - Nitrogen utilization

Primary Features
Eurythermal
Splits water
Uses wastewater or saline water
Rapid metabolism
N adaptive; P uptake optimized
Genetic/Synthetic toolbox
Directly secretes/produces fuel
3000 gal fuel/hectare/yr
Self-separating product

Benefits vs Source Organism
- High photosynthetic efficiency
- Adaptation: product tolerant
- Evolved enzymes, eg Rubisco or alternate pathways, for improved carbon fixation efficiency and minimization of photorespiration
- Fuel production decoupled from growth
- Self-separating product
PROJECT MILESTONES & TIMELINE

Development
- Engineer photosynthetic capture and conversion efficiency
- Develop process robustness
- POC product synthesis
- Develop and scale process

Scaling/Deployment
- Yr 1-3: Design, construct production organism
- Yr 3: Lab scale organism POC **250 gal/hectare/yr**
- Yr 1-3: Reactor POC/Economics/thermal management/insolation metrics/water/gas
- Yr 3: Integrate organism/reactor at small scale
- Yr 4: Pilot Process @ **1 hectare 1000gal/hectare/yr**
- Yr 5-8: Optimize process @ **1000 hectare 2000-3000 gal/hectare/yr**
- Yr 7-10 Deploy process **12MM hectares @ 2.5MM bbl/day**
KEYS TO SUCCESS

- Overriding photorespiration; optimizing CO$_2$ delivery @ atmospheric concentration

- Efficient use of solar insolation by organism; light capture and conversion

- Efficient, high yield conversion to product in a low CAPEX/OPEX industrial process
FEATURES OF THE APPROACH

- This organism was an attractive choice because we could engineer its primary photo- and CO₂ capture.

- We thought the biggest problem would be photon capture and CO₂ conversion efficiency.
What happened: difficulty in optimizing carbon delivery

What had you learned in the earliest phases that helped you to stick to the plan: How to engineer our platform organism

What did you do to get back on track; we used our toolbox to construct solutions
How scaled costs are reduced
• Capital: by improving productivity 10X
• O&M: improved 10X

Production Capacity
• 5MM gal/1000 hectare
• 2.5MM bbl/da @ 12MM hectare
COST COMPARISON TO CORN-BASED ETHANOL MODEL

**Corn-EtOH Model**  
(assuming 100 million gal/yr plant)

- Uses 225,000 acres of farmland
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- $0.36/gal operating expenses

$2.12/gal total cost  
$0.0265 per MJ

**New Organism**

- No feedstock cost, harvesting, processing
- No displacement of agriculture
- 10x less land use; non-arable
- No need for “cheap sugar”
- No fertilizer, eg N
- No need for dewatering downstream processing
- Produces high value coproducts
- 10x higher productivity of high energy density, fungible fuel

90% reduction in feedstock costs  
90% reduction in capital expenses  
90% reduction in O&M expenses  
90% reduction in total production cost
**ENERGY CONTENT/PERFORMANCE COMPARISON TO CORN ETHANOL MODEL**

<table>
<thead>
<tr>
<th>Corn-EtOH Model</th>
<th>New Organism</th>
</tr>
</thead>
<tbody>
<tr>
<td>21.14 MJ/L (Low Heating Value)</td>
<td>• Fuel 40MJ/L</td>
</tr>
<tr>
<td>80.02 MJ/gal</td>
<td>• 120 MJ/gal</td>
</tr>
<tr>
<td>443 gallons/acre land use</td>
<td></td>
</tr>
</tbody>
</table>

**New Organism has 110% energy content compared to EtOH**

Land use of new organism is 10% of corn-EtOH land use.
AFTERNOON TECHNICAL IMPROVISATION SESSION

C4 Plant

Aquatic Phototroph

“Model” Organism

The following is the output presented by the group to all workshop attendees for discussion and debate
### THE INNOVATIVE ORGANISM

#### Primary Features
- Mixotrophic, can also use waste
- Can turn growth off, all energy goes into product (=usable fuel)
- Has engineered photosystems that don’t waste absorbed light as heat
  - (at optimal point on light dose response curve)
- Has enhanced absorption spectrum (not just 700 nm)
- Can store reducing equivalents
- Tolerant to biofuel
- Can resist predators, Can survive in open system, precludes invasion (optional) (or low capex)
- Blocks contaminants
- Harvesting: organism separates from product & feedstock flow
- Physical system supplies CO₂ and withdraws product (addressing diffusion problems)
- Nitrogen fixers (nice to have)
- Need to address: growth, containment, scalability etc.

#### Relevant problem
- Mixotrophic, -
- Can turn growth off, -
- Not obvious how to accommodate variations in sunlight
- Compatibility of systems
- NADPH is current form
- Hydrophobic compounds are toxic
  - Predators eat organism (or fuel)
  - Contamination
- Harvesting: separates from product
- Diffusion problems for delivery of CO₂ feedstock

#### Benefits vs Source Organism
- Efficiency
- Scalability

#### Why is this revolutionary?
- Solutions will be revolutionary
- Will need protein engineering, new metabolic engineering

#### Source Species: Bacterium (or yeast); (not clear that this is fundamental)

#### Liquid Biofuel(s)
**Development:** 3 years for genetic modules
~3 years for integration

**Scaling/Deployment:** 5 years (demonstration plant)
(See underlined elements on introductory slide)
This organism was an attractive choice because…
   – It could be engineered in a modular way

We selected the approach because…
   – We wanted to make every photon count

We thought the biggest problem would be… (see next slide)
THE SETBACK

- What happened?
  - Systems integration problems (combining genetic modules)
  - Genetic stability was harder to get than anticipated
  - Contamination
  - Feedstock delivery & product withdrawal ran into physical limits
- What had you learned in the earliest phases that helped you to stick to the plan?
  - All genetic modules worked at some level
- What did you do to get back on track?
  - Next-generation metabolic flux analysis
  - Total genome synthesis
  - Engineered organism to fit physical system
  - Clever directed evolution for optimization
THE PRODUCTION PLANT:
Same as photosynthetic bacterium

Diagram what your production plant looks like

Feedstock
Harvesting

Processing

Etc.

How scaled costs are reduced
• Capital
• O&M

Production Capacity
**COST COMPARISON TO CORN-BASED ETHANOL MODEL**

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### New Organism

- XX% reduction in feedstock costs
- XX% reduction in capital expenses
- XX% reduction in O&M expenses

- XX% reduction in total production cost
**Corn-EtOH Model**

- 21.14 MJ/L (Low Heating Value)
- 80.02 MJ/gal
- 443 gallons/acre land use

**New Organism**

- New Organism has 140% energy content compared to EtOH (i.e. alkane)
- Land use of new organism is 2% of corn-EtOH land use

**Key Calculations**

- Output = 100M gallons/day = spec.
  = 140 gigawatts = output value of fuel (worth $44B/year)
- Land use = 75km x 75 km (at 8% efficiency)
- (20,000 gal/acre*year)
- Assume 20 year lifetime
- Half of income $\rightarrow$ running plant
- Half is profit
- Plant is worth $190B (NPV at 10%)
  (i.e. plant should cost <$190B)
  $\rightarrow$ $36/m^2$
- Land
WRAP-UP SESSION

December 3

What have we learned?
What can we do that will be revolutionary?
How will we tell that we’ve succeeded? (metrics)
High Level Observations

- Improving both the operational and theoretical efficiency of photosynthesis are worthwhile, but many believe focus should be directed towards addressing existing operational deficiencies first.

- Developing more efficient light-to-fuel conversion is largely predicated on diverting metabolic pathways away from biomass growth and toward production of a high value product (shifting the mass balance), but this is currently a very preliminary effort.

- Two approaches have been suggested moving forward: 1) Do not constrain the discussion to one liquid fuel over another, 2) start from the production of a specific fuel molecule and work backwards to optimize its production.

- Currently, it is not immediately clear what genes or pathways would be desirable to target for transplantation; targeted (applied) research is needed in gene regulation, expression mechanisms, and metagenomic analysis.

- The changes discussed are part of a complex systems engineering problem – many changes have to be made simultaneously for the system to work. A breakthrough will come from putting many things together in unexpected ways.
High Level Observations (continued)

- Modification of photosystem absorption profiles is a fascinating idea that has been conceptualized for years, but is a long way from an engineered system.

- Metabolic engineering could have the largest impact possible but optimized systems are more of a vision than a practiced reality.

- Operations performed at the molecular engineering level should be integrated with breeding programs.

- There are opportunities to translate unicellular processes to multi-cellular organisms.

- Agriculture is highly local, there is no “one size fits all” strategy – different crops will have to be designed for different regions.

- Computation modeling and CAD tools are inadequate or do not exist but are a necessary tool moving forward.
What are the “moon shot” projects?

...and how will we know we succeeded? (i.e. What metrics define success?)

- What is possible in a 3-5 year project?
  - Altering photosynthetic antenna size
  - Altering how the organisms are displayed (thin films, on plates, gas phase versus liquid phase, etc.) to address capital costs
  - What are the intermediate milestones? (sugar is a good “currency” to use as a metric)
  - Developing tools – genomics, proteomics
  - We have to have organisms ready to go in 3-4 years to have a pilot, production plants within 10 years
  - Improve efficiency of carbon assimilation, carbon concentration (effective in C3 plants)

- What is not?
  - To be completely free of fossil fuels in 3-5 years

- What tools do you need?
  - Standardized way of looking at the available options (leveraging existing models), 8-15 models need to be constructed
  - A consistent way of calculating photons to miles?
  - How we can leverage what has been accomplished in the food industries?
    - food is inexorably linked to this discussion