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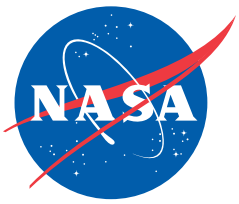

Workshop Report on What are the Potential Roles for Synthetic Biology in NASA's Mission?

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Report of a workshop
sponsored by and held at
NASA Ames Research Center
Moffett Field, California
on October 30-31, 2010

March 2011

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Executive Summary

A weekend workshop entitled "What are the Potential Roles for Synthetic Biology in NASA's Mission?" was held at NASA Ames Research Center on October 30-31, 2010 to discuss ways in which the emerging field of synthetic biology could revolutionize NASA's mission. Approximately 100 representatives from government, industry, and academia were in attendance.

The fast pace of biological discovery started with the solving of the structure of DNA published by Watson and Crick in 1953. This marked the birth of molecular biology and began what we now know as the century of biology. The rapid pace continued with the discovery of restriction enzymes in the 1970s allowing the cutting and pasting of DNA. In the 1980s the polymerase chain reaction (PCR) was invented allowing sequences of DNA to be copied. DNA sequencing began in the 1970s and the genetic code began to be stored as a digital one. The 1990s saw the development of rapid ways to sequence DNA, to discover genes, and to understand DNA variants. The first human genome was sequenced in 2000. Synthetic biology began with early genetic switches and oscillators in 2000, followed in 2009 with a technical tour de force—the creation of the first bacterial cell with a synthetically replicated genome by the J. Craig Venter Institute (JCVI). This achievement is noteworthy because it proved that it was now possible to begin with digital code and 'boot up' an organism from it. This also established the bidirectional connection between biology and information technologies, potentially putting biology on an exponential rate of discovery. Given this trajectory, 2010 seemed like the right time to ask how synthetic biology, defined as the design and construction of new biological functions and systems not found in nature, could impact NASA's mission.

The applications that were highlighted for the synthetic biology community were biological in-situ resource utilization, biosensors, biomaterials and self-building habitats, human health, and life support for long duration spaceflight. It was clear that synthetic biology has the potential for game changing breakthroughs in all these areas. The potential advantages of synthetic bioprocessing over physical or chemical processing include reduced upmass, power, and the ability to do in-situ manufacturing and processing with reduced reliance on hazardous chemicals. Since DNA doesn't weigh much, it would be possible to bring a "toolkit" containing a large collection of genes, enzymes, regulatory networks, sensors, structural proteins, etc. Alternatively, it may be possible to synthesize genetic constructs in space. Synthetic biology could then give NASA missions the ability to create a wide range of novel materials on site, as they are needed. Thus, adding a diverse synthetic biology tool set to the mission would add little weight, but could be invaluable in utilizing resources on arrival.

Long duration spaceflight and living on the surface of another planet such as Mars is extremely challenging. For NASA to successfully explore other worlds, we are going to have to take advantage of revolutionary new approaches that synthetic biology affords. For example, it provides the foundation to revolutionize plant growth systems for space exploration. Plants could be re-engineered to provide nutritionally better food, or transgenic plants could be developed with greater stress tolerance by modifying them to express less ethylene. Eventually, it may be pos-

sible to reprogram plants to synthesize their own power-receiver/photon-emitter packets and convert locally available energy sources for internal light generation with high quantum efficiency in space. One can even consider going beyond plants as a food source. Standard photosynthetic production of food from green plants requires significant surface areas and is inefficient with respect to light, CO_2, and mineral use. Metabolic engineering of photosynthetic bacteria has the advantage that sunlight and CO_2 are efficiently used and that waste products such as stems, roots etc., are not produced. We now have the ability to modify the odor, color, and taste of microorganisms, and consistency as well. This makes it conceivable that genetically modified cyanobacteria could function as a palatable food source on long-duration spaceflights.

In planning future long-term, remote, and perhaps unmanned missions taking advantage of *In-Situ* Resource Utilization (ISRU), synthetic biology may provide new strategies in areas such as biological regolith reduction, trash/waste processing, hydrocarbon fuel production processes, and metal production. Bioleaching of minerals is a naturally occurring process on Earth. Synthetic biology offers the possibility of designing microorganisms that can accomplish bioleaching more efficiently and with specific adaptations to extraterrestrial environments. It is possible that most the metals needed for self-sufficiency could be mined from basalt. This clearly has significant implications for biomining planets, moons, and asteroids.

One aspect of astrobiology research is the study of extremophiles (microorganisms that can live in different extremes such as temperature, pH, salinity, radiation, etc.) Synthetic biology offers the potential to take these evolutionary adaptations and transfer them to other species of interest. Exploring the toolkit of evolved organisms and metabolic pathways on Earth will unleash the full potential of synthetic biology in space. Since low-temperature, radiation and desiccation tolerant organisms do not generally induce weathering of minerals at high rates, synthetic biology can be employed to either introduce biochemical pathways for weathering into environmentally tolerant organisms or to add environmental adaptations to organisms that are known to weather minerals. Key steps would be to up-regulate either photosynthesis or acid production to increase weathering, to enhance tolerance to desiccation (for storage), and to enhance tolerance to ionizing radiation to improve “space worthiness”. If we could design microbes with specific capabilities, this might even provide a pathway to terraforming Mars into a habitable planet. This would require super microbes with specific properties, all of which would have to be resistant to high ultraviolet light and oxidant concentration, desiccation, cold, and perchlorate.

The workshop keynote speaker, Dr. J. Craig Venter, noted that while NASA currently performs genetic (phenotype) selection for space missions, in the future they will be able to use genomics to screen for traits that are compatible with life in space, such as inner ear changes that eliminate motion sickness, rapid bone regeneration, DNA repair, a strong immune system, and small stature. He discussed the possibility of creating a synthetic metabiome for space travelers, in other words, replacing the thousands of microbes in the human body with a well-defined microbial community. Potential benefits could be the elimination of disease organisms that cause infections and dental decay, methanogens and sulfur producers, and organisms associated with body odor. Each space traveler would have the same metabiome resulting in a healthier environment for long durations in space.

Nobel prize-winning physicist, Richard Feynmann, wrote the following phrase on his blackboard before he left his office at the California Institute of Technology for the last time: "What I cannot create, I do not understand." This quote is so relevant to the emerging field of synthetic biology that it was included as part of the watermark in the 1.08 Mbp (million base pairs) *Mycoplasma mycoides* JCVI-syn1.0 genome (recently created at JCVI). Synthetic biology is a discipline that will lead to an ability to first engineer and then understand life on a molecular scale. This workshop identified many ways where synthetic biology could revolutionize NASA's science and exploration missions. The challenge going forward is to make this a reality.

Workshop Report on What are the Potential Roles for Synthetic Biology in NASA's Mission?

Dr. Stephanie Langhoff[1], John Cumbers[2], Dr. Lynn Rothschild[1], Dr. Chad Paavola[1], and Dr. Simon P. Worden[1]

Introduction

Recognizing the recent advances in our capability to re-engineer organisms on Earth and the potential of this technology in NASA's mission, we held a workshop entitled "What are the Potential Roles for Synthetic Biology in NASA's Mission?" The workshop was held at Ames Research Center on 30-31 October 2010 and was co-sponsored by the National Academies Keck Futures Initiative. It is part of a series of informal weekend workshops hosted by Center Director Pete Worden.

The Program Organizing Committee, which included Stephanie Langhoff (co-chair), Lynn Rothschild (co-chair), John Cumbers (co-chair), Drew Endy, Chad Paavola, and George Church, was responsible for the selection of speakers. A key goal of the workshop was to understand if synthetic biology can provide a more efficient, lower cost, practical approach to specific challenges in space.

Dr. Pete Worden, ARC Center Director, kicked off the workshop with a welcome and explanation of why Ames Research Center (ARC) and the National Academies Keck Futures Initiative were hosting the workshop. He noted that ARC's interest in synthetic biology follows from a rich history of scientific contributions in fundamental biology, exobiology, and astrobiology.

Dr. Worden noted that NASA does primarily three things and synthetic biology could potentially play a significant role in all three. First NASA does science and has made some really exciting discoveries in the last 20 years with such observatories as Hubble. These discoveries will continue in the future with results from the Kepler mission about the frequency of Earth-like planets in the habitable zone around other stars. Astrobiology, which seeks to understand the origin, evolution, distribution, and future of life in the Universe, has made significant progress, in part, because of our ever improving ability to observe and understand life on a molecular scale. Secondly, NASA helps life on Earth in fields like aeronautics and environmental monitoring from an array of Earth Observing Satellites. Synthetic biology could help make aviation greener by designing mechanisms or organisms to produce biofuels, and sensors to better monitor climatic effects. The final mission is the human exploration of the solar system. It is here that synthetic biology could have a profound effect, for example, in designing better methods of long-term spaceflight and improving our ability to live on another planet.

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Dr. Lynn Rothschild, a research scientist at ARC, further elaborated how synthetic biology could impact NASA's missions. She began by proclaiming this as the century of biology, because of the phenomenal pace of discovery and number of technical breakthroughs. Coupled with the diversity of four billion years of evolution, we now have a powerful toolkit for NASA. She defined synthetic biology as the design and construction of new biological functions and systems not found in nature. In aeronautics, synthetic biology has the potential to make aviation greener by designing better mechanisms for making biofuels. In the area of exploration, synthetic biology could impact how we make spacecraft materials, how we develop life support and make food, and how we do *In-Situ* Resource Utilization (ISRU). Finally, synthetic biology potentially impacts NASA's science objectives by helping to understand the origin and evolution of life, alternative biologies for life elsewhere, and the future of life in the Universe. For example, we may use synthetically produced microbes for on-demand biomining, habitat construction, or drug production.

Dr. Rothschild ended by discussing some of the activities that will be supported by the NASA synthetic biology initiative. The initiative vision is to harness biology in reliable, robust, engineered systems to support NASA's exploration and science missions, to improve life on Earth, and to help shape NASA's future. Some of the milestones in the 2010-2015 innovation phase are to demonstrate how synthetic biology can impact bioplastics synthesis, waste bioprocessing, and biological fuel systems. There will be opportunities for select researchers as affiliated groups, collaborators, and advisors. The call for research fellows beginning in 2011 has already been issued. There may be opportunities for students in the International Genetically Engineered Machine competition (iGEM)—details are currently being worked out.

I. Why Take Biology to Space: Past and Present

I.1 Hardware Requirements for Biotechnology in Space

John Hines, Chief Technologist at NASA Ames Research Center, discussed the hardware requirements for biotechnology in space over the next 50 years, with exploration goals of traveling to the Moon, Mars, and asteroids. Synthetic biology has the potential to revolutionize our approach to sustaining life in space. While we have taken familiar biological organisms into space and engineered environments for them, in the future we will engineer biological systems to make them suited to extraterrestrial environments. Everything you may need must be taken with you on a long-duration spaceflight. The hardware requirements for space synthetic biology include elements for providing specimen habitat, sample handling, process monitoring, and process control, or in other words, a multipurpose synthetic microbial bioreactor. The potential advantages of synthetic bioprocessing over physical/chemical processing include reduced upmass, power, and the ability to do *in-situ* manufacturing and processing with reduced reliance on hazardous chemicals. Therefore, synthetic biology has the potential for game-changing breakthroughs for sustaining life in space.

Mr. Hines discussed the microsatellite free-flyer project that can be used to study *in-situ* bioanalytical technologies in space. Advantages of these fully autonomous, self-contained free flyers are their many possible configurations that enable them to address multiple research scenarios. There are many launch opportunities into different orbital trajectories due to their small mass and volume. *In-situ* real time analysis is currently possible and sample return is feasible. He discussed some of the current microsatellite projects at ARC, such as Pharmasat-1, whose science goal is to measure the effects of antifungal agents on yeast. The International Space Station (ISS) is also an excellent platform for research in disciplines such as biotechnology and plant research. Very capable research facilities are already available on the ISS. In addition to freezers and incubators, there is the Commercial Generic Bioprocessing Apparatus (CGBA), the Advanced Biological Research System (ABRS), and the European Modular Cultivation System (EMCS).

Mr. Hines ended his presentation by discussing the space technology development approach in the Office of the Chief Technologist (OCT). The current program contains three development steps: (1) early-stage innovation where creative ideas are nurtured; (2) game-changing technology where the feasibility of early-stage ideas is proven; and (3) crosscutting capability demonstrations where new technology capability is matured to a flight readiness status. He noted that synthetic biology is an emerging innovative, game-changing bioengineering discipline with potential applications to NASA's mission. For example, it provides the foundation to revolutionize plant growth systems for space exploration, and it is a potential technology for biomining planets, moons, and asteroids.

I.2 MELiSSA: An Approach to Using Biological Systems for Life Support in Space

Dr. Francesc Godia, professor at the Universitat Autònoma de Barcelona (UAB), presented an overview of the MELiSSA project, the life support system developed by an international consortium lead by the European Space Agency. Inspired by a lake ecosystem, the MELiSSA loop proposes the use of different types of microorganisms and higher plants to develop a biological life support system, combining within one system food generation, water re-utilization, atmosphere regeneration, and waste recycling. In order to build such a system, it is necessary to combine the capacities of biological systems with the design, construction, operation, and control of the appropriate bioreactor systems to ensure the continuous operation of each one of the elements of the complete loop in a sustained and reliable mode over long operation periods. To support a crew of six for a 1000-day Mars mission, estimates for the metabolic consumables are 30,000 kg (132,000 kg including hygiene water) far in excess of current launch capabilities.

The MELiSSA concept is illustrated in figure 1. It is conceived as a closed life-support system based on the assembly of biological and physico-chemical processes. Each compartment performs a specific task within the loop. The goals are the recovery of food, water, and oxygen from waste and CO_2. The MeliSSA pilot plant at UAB is the primary European facility for life-support ground demonstrations, but the partners contributing to the research are widely distributed. The research problem is made more tractable by breaking the closed-loop life support system into various compartments. Research within each compartment seeks to progress from basic design and characterization (bench-scale system) to operation, monitoring, and control (pilot scale). Integration of the individual compartments into the MEliSSA loop is possible only after the individual compartments are developed at pilot scale, the associated control laws are understood, and the interfaces, associated sensors, sampling protocols, and quality control procedures are in place. Progressive integration proceeds in a step-by-step approach to close the gas-liquid-solid loops and the elemental mass balances. The final result is the integration and demonstration of the complete MELiSSA loop in the pilot plant. The challenge is to make all of the compartments work together for an extended period of time.

Dr. Godia went into some detail about the research that is in progress for each of the separate compartments. The details are beyond the scope of this workshop report. However, the audience was impressed by the maturation of the MELiSSA concept and the actual full-scale demonstration of the life-support system.

There was a question about whether using rats in the simulation introduced a second microbial environment (i.e., the microbes inside the rats). The facility uses rats (one person = ~40 rats) to simulate human breathing. The rats are fed a normal diet, not the food grown within the facility. It was noted that the biome within the facility or the rats was not characterized well enough to definitively answer the question.

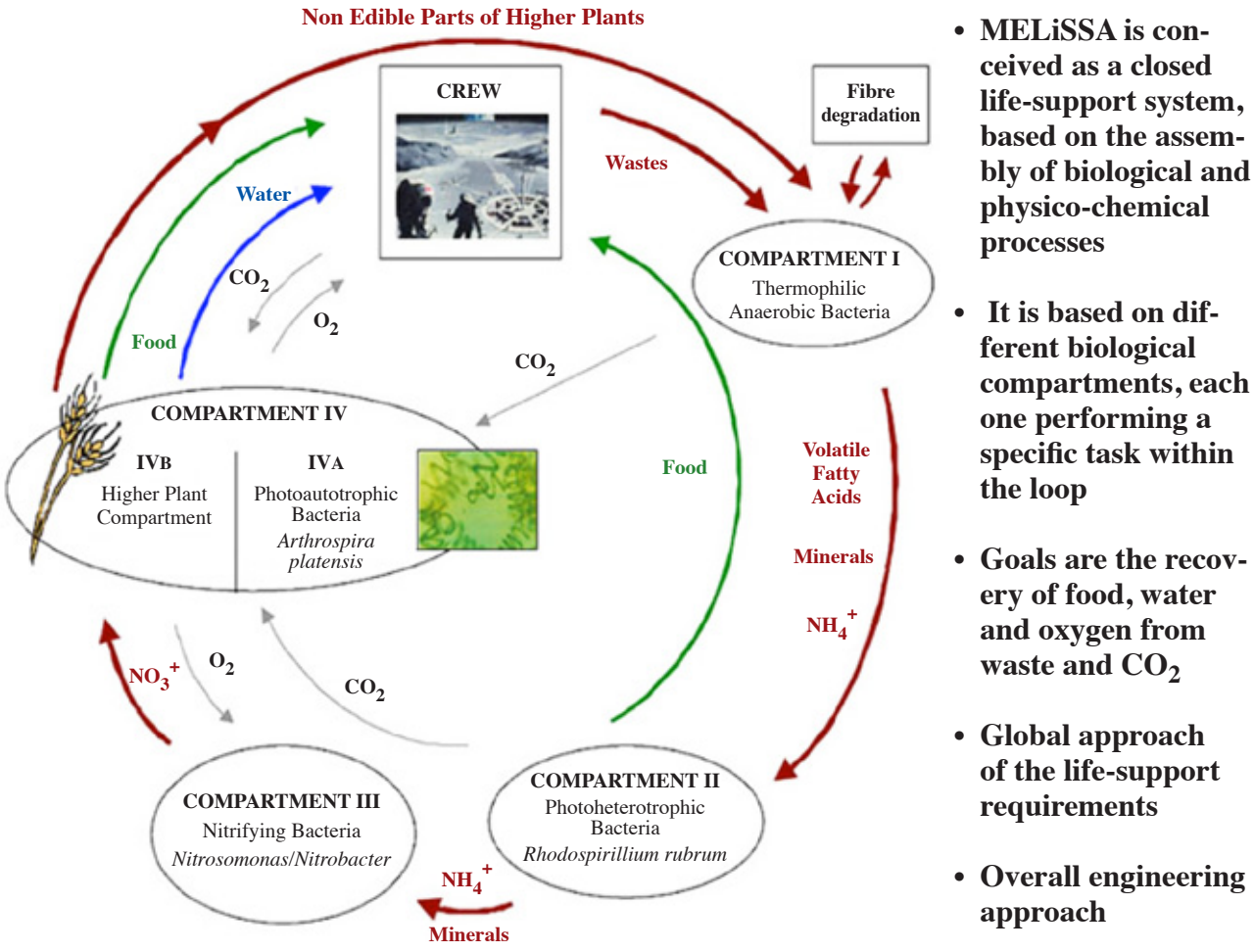


Figure 1. An illustration of the MELiSSA concept: a closed life-support system based on the assembly of biological and physico-chemical processes.

I.3 Environmental Control and Life-Support Systems on the International Space Station

Monsi Roman, a microbiologist and Life-Support Project Manager at the Marshall Space Flight Center (MSFC), discussed the Environmental Control and Life Support Systems (ECLSS) on the International Space Station (ISS). While these systems have been in operation for more than 10 years, it took at least that long to design, test, and develop them. The ECLSS team at NASA's MSFC was responsible for the task and is currently part of the NASA team that is working on the design and development of the life-support systems that will be used by the crew during long-duration missions away from Earth.

Ms. Roman provided an overview of the current systems and the plans for future physical-chemical systems on the ISS. The key functions of the ECLSS are summarized in figure 2. The challenge is to deal with the effluents such as CO_2, urine, feces solids, and to provide for the needs of the astronauts such as oxygen, clean water, food solids, etc. Some of the elements in the life-support loop include waste water/urine recovery and processing, waste management, temperature and humidity control, CO_2 removal, CO_2 reduction, and oxygen generation.

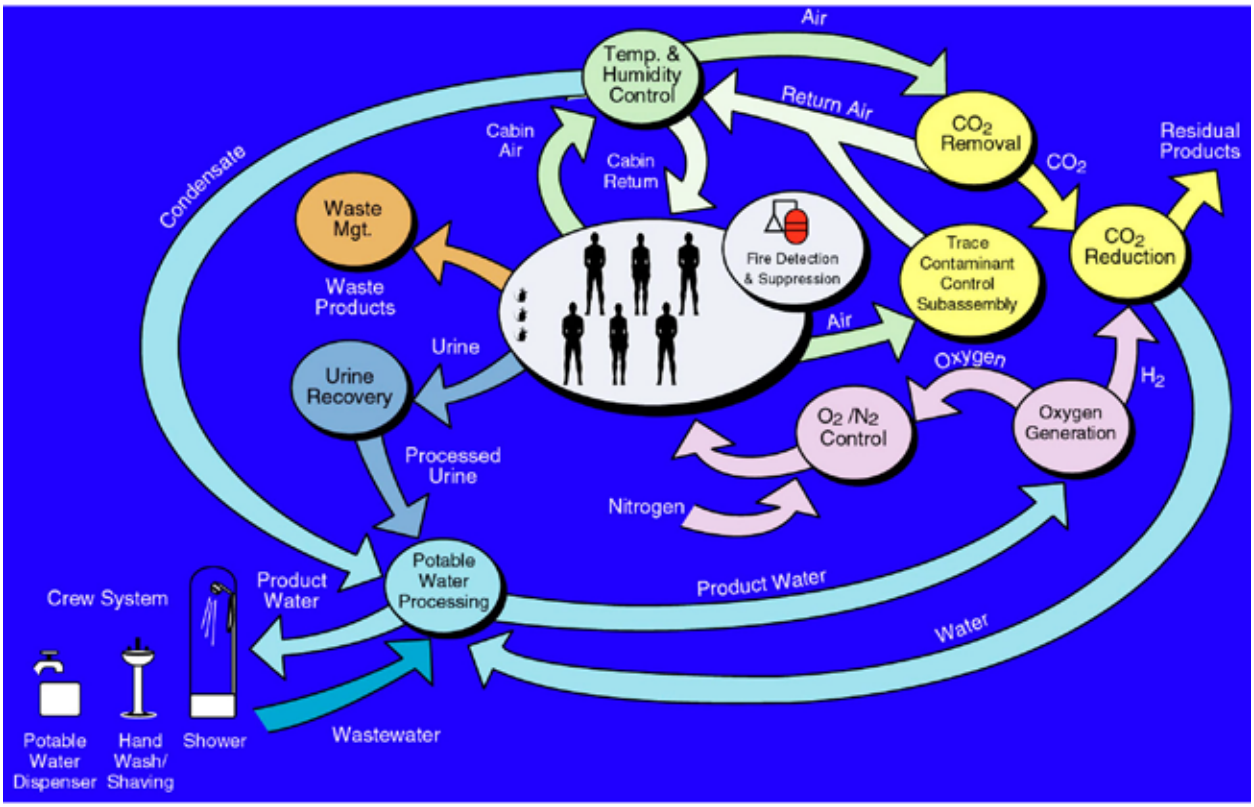


Figure 2. The key functions of the environmental control and life-support systems on the International Space Station (ISS).

Challenges for ECLSS on the ISS include the need to design the systems for a long life, the requirement to have a triple containment system for some components, control of microbial growth in stored pretreated urine, slow flow rates, dead legs in the system, limitations on the use of antimicrobials, microbial control in stored potable water tanks, and to develop a plan for handling by-products and waste. In addition, there are effects due to microgravity and/or living in a closed "can", such as permeation of cabin gases (CO_2, ammonia, etc) through the flexible Teflon hoses, the design of robust pumps, valves and tanks, and back contamination in the water systems. Gas does not separate from liquids in microgravity. There is also significant calcium loss in microgravity, which makes the processing of urine for recycling a challenge, because it increases the percentage of solids. Control of microorganisms in potable water is more of a challenge in microgravity. All of these challenges depend on crew size and level of exertion, which varies substantially. She described briefly some of the ECLSS on board the ISS, for example, the oxygen generator that uses electrolysis and the trace contaminant control system that uses a thermal catalytic oxidizer and a carbon bed.

Ms. Roman concluded her presentation by discussing some of the microbial challenges in space. A key problem is that the biome aboard the ISS has not been well characterized due to several challenges that include lack of microbial identification equipment in the ISS because of funding limitations and no priority for sample returns unless there is a problem in the Station. Even when the samples can be returned for ground analysis, the holding time between the sample collection and sample analysis can be days, and most of the time, the samples are not refrigerated making the ground analysis "suspect".

Finally, she discussed some of the NASA's challenges with the development of new microbial monitoring technologies. These include limitations on equipment size and power, the use of non-hazardous reagents, calibration issues, and the cleaning and disinfection of the sample collection areas. Her presentation provided a great introduction to the current state of the art and the challenges that were found during the design and testing of ECLSS.

Questions focused on whether we could characterize the biome on the ISS and whether we could use synthetic biology to engineer a better biome.

I.4 Bio/Tech/Eng Synergisms Needed to Enable Productive and Affordable Plant Growth in Space

Dr. Cary Mitchell, professor of horticulture at Purdue University, discussed what is needed to enable productive and affordable plant growth in space. He noted that there has been a lack of systematic pre-investigation of underlying principles governing plant responses to real spaceflight conditions. This has made it difficult to separate the effects of microgravity on plant growth from other effects such as vibration. This is beautifully illustrated in figure 3, where he compares three plants all grown at 1g. The plant on the left is never shaken, the middle plant is shaken for a few seconds once a day, and the plant on the right is shaken twice a day. Fundamental questions regarding the productivity of plants in space have still not been answered definitively despite 30 years of NASA life science programs.

Dr. Mitchell highlighted recent advances in micro- and nano-scale sensor technology that can aid in developing plant flight hardware that more effectively avoids designs that limit plant productivity (e.g., root-zone hypoxia and ethylene-induced leaf epinasty). For example, engineering artificial mechanical environments using *in planta* nano-accelerometers can counteract negative microgravity effects. Micro/nano devices could be developed for tissue detection and monitoring of oxygen and ethylene concentrations, nutrient content, vibration profile, and photosynthetic responses. Life-support plant research needs to determine the productivity potential of candidate crop species as a function of acceleration (e.g., 0.16 (lunar), 0.38 (Martian), compared to 1.0g (Earth)). Also needed is a study of the radiation tolerance and shielding requirements for candidate crop species. He recommended that a good approach to the design of the life-support system would be to minimize the Equivalent System Mass (ESM). This metric takes into account the mass, volume, power, and cooling of the system as well as both crew time and mission duration. Thought should be given to how synthetic biology could reduce ESM. For example, plants can be re-engineered to provide nutritionally better food or transgenic plants could be developed with greater stress tolerance by modifying them to express less ethylene.

Figure 3. Three tomato plants all grown at 1g. The plant on the left is never shaken, the middle plant is shaken for a few seconds once a day, and the plant on the right is shaken twice a day.

A major tall pole threatening the future use of photosynthetic plants in space for both fundamental space biology as well as bioregenerative life-support purposes is the energetic cost of providing light for plant growth. Present lighting technologies are far too power and energy consuming, and generate copious waste heat. However, solid-state LED technology is very promising due to cool photon-emitting surfaces, custom selectability of emission spectra, and increasingly efficient light generation as the technology develops rapidly. One means of increasing light-capture efficiency is to reduce shading of lower leaves by upper leaves by replacing overhead LED arrays with intracanopy or lightsicle arrays of thermally cool LED light emitters. However, all sources that generate light external to plants have inherent crop quantum-efficiency limitations. A promising future approach to circumvent some of the quantum inefficiencies is to surgically insert micro-scale LED chips with receivers and wireless power receivers into plant tissues and energize them externally with low-power sources that don't create much waste heat. This proof of concept would be followed by uptake of nano-scale power-receiver/photon-emitter packets that would distribute to molecular recognition sites within plants (e.g., chloroplast outer membranes) and generate photosynthetic radiation internally in response to external excitation. Eventually, synthetic biology would reprogram plants to synthesize their own power-receiver/photon-emitter packets and convert locally available energy sources (IR, radio, UV, cosmic-galactic) for internal light generation with high quantum efficiency in space. Such synthetic biology approaches would virtually eliminate a tall pole that has kept bioregenerative life support for distant crewed missions on NASA's back burner for the past 30 years.

Dr. Carey's presentation made a strong case for two things—first the need to determine the effects of altered gravity and radiation on plants with carefully designed experiments, and secondly, that plant productivity and nutritional quality could be significantly enhanced with modern technology including genetic engineering and synthetic biology.

II. Why Take Biology to Space: Future

II.1 Photosynthetic Production of Food Molecules from Bacteria

Dr. Jeffrey Way, a staff scientist at Harvard University's Wyss Institute, discussed one of the key issues of space travel, namely the efficient production of nutrients that are both palatable and provide some sensory stimulation. Standard photosynthetic production of food from green plants requires significant surface areas and is inefficient with respect to light, CO_2, and mineral use. Metabolic engineering of photosynthetic bacteria has the advantage that sunlight and CO_2 are efficiently used and that waste products such as stems, roots etc., are not produced. To address whether cyanobacteria could be engineered to produce and secrete organic primary metabolites that are used in the food industry, he discussed using a synthetic biology approach to engineer *Synechococcus elongatus* PCC7942 to express genes encoding an invertase and a glucose facilitator, which then mediate secretion of glucose and fructose. Similarly, expression of lactate dehydrogenase and lactate transporter-encoding genes allowed lactic acid accumulation in the extracellular medium. Expression of the relevant transporter was essential for secretion. Production of these molecules was further improved by expression of additional heterologous enzymes. These results indicate that photosynthetic bacteria can be engineered to produce and secrete high-value products. He concluded that there is significant room for improvement in cyanobacterial production using traditional metabolic engineering.

Dr. Way discussed the issues of providing sensory stimulation of the nutrients on long-duration spaceflights. Although bacteria can provide an efficient source of nutrients, there are currently issues of palatability. He discussed the work of the Harvard iGEM 2010 and previous teams to use genetic engineering to create plants and bacteria with "designer" flavors, odors, and colors. Previous iGEM teams have created systems to express methyl salicyate, a mint-smelling compound, and isoamyl acetate, a banana scent. Color is another aspect that can be engineered. For example, engineering the accumulation of the pigments lycopene and beta-carotene can produce color.

Dr. Way concluded by noting promising research that NASA could pursue in the area of synthetic biology. For example, NASA could pursue dual-use space/terrestrial life support technologies, such as the solar-driven production of nutritional chemicals and other useful commodity chemicals. The production of palatable bacterial food would be a technology that is commercially viable on Earth. Futuristic ideas such as making photosynthetic astronauts that would not require food was also touched upon.

A question following the presentation was how do you engineer for texture? His response was that it might be accomplished by crosslinking, but improving texture could be challenging.

II.2 Desirable Traits in a Bioleaching Microbe for In-Situ Resource Recovery

Dr. Frank Roberto, biochemist at the Idaho National Laboratory, discussed some of the terrestrial applications of microbial-mediated mineral dissolution, commonly referred to as bioleach-

ing, which is estimated to facilitate the extraction and recovery of approximately 25% of the world's annual copper production. A more engineered and limited attack by microbes on gold ores, termed bio-oxidation, accounts for a significant amount of gold produced from refractory gold ores. Chemolithotrophic microbes mediate these processes through the oxidation of reduced iron and sulfur species. Ferric iron and sulfuric acid, resulting from biological oxidation, have been demonstrated to catalyze the breakdown of hard rock minerals and to release metals such as copper and gold. Other base metals, including zinc, nickel, and lead, are leading candidates for future bioleach operations. Other feasible candidates for bioleaching include platinum, silver, palladium, gallium, rhodium, lithium and uranium.

While bioleaching of minerals is a naturally occurring process that has been exploited by man for thousands of years, process conditions, including pH, temperature, [Fe^{3+}], oxygen, and heavy/otherwise toxic ions can significantly impact bioleaching rates. A recent NASA workshop (Lunar Regolith Biomining; NASA/CP-2008-214564) concluded that ISRU of regolith minerals would not be feasible until sufficient oxygen and carbon dioxide are available as waste products from manned presence. In planning future long-term, remote, and perhaps unmanned missions geared towards ISRU, synthetic biology may provide new strategies for strain improvement to increase the efficiency of biological approaches. He concluded his presentation by discussing what an extraterrestrial bioleaching operation might look like on lunar, Martian, or asteroid missions.

A question following the talk was whether mineral enrichment was required to make bioleaching feasible. The response was that some enrichment was required, but minerals such as olivine are available on Mars. The Moon would probably be more difficult to mine because of its lack of differentiation.

II.3 Synthetic Microbes and Rocks—Geomicrobiology for Human Space Settlement

Dr. Charles Cockell, professor at the Open University in the United Kingdom (UK), discussed how geomicrobiology could be used in space exploration and settlement. Microorganisms that break down rocks can be used in biomining to extract essential elements for manufacturing processes and ISRU. This ability to weather rocks might also be used to ameliorate regolith for crop growth and to produce substrate from regolith for microbial growth in bioregenerative life support systems. These applications and others depend on linking the industrial process with optimum microbial performance. Synthetic biology offers the possibility of designing microorganisms that can accomplish these tasks more efficiently and with specific adaptations to the extraterrestrial environment. Since space tolerant species are generally not fast growing or good at weathering, he discussed some of the steps that would be required to build a "super-weathering microbe". Key steps would be to up-regulate either photosynthesis or acid production to increase weathering, to enhance tolerance to desiccation (for storage), and to enhance tolerance to ionizing radiation to improve "space worthiness". This last step might be achieved by using genes from known space-tolerant organisms. In constructing this synthetic microbe, it would be desirable to maintain a high growth rate.

In the later part of his presentation, he described a conceptual industrial process for rock elemental extraction in extraterrestrial environments using synthetic/engineered microorganisms. Of par-

ticular interest is the application of this process to extracting useful elements from crustal material that is undifferentiated into rich ores. He described how synthetic biology could be used to drive the basalt economy. All of the metals needed for self-sufficiency could be mined from basalt. He discussed how the manipulation of metal sequestration genes might be used to generate new approaches to biomining. The extraction of different metals may be possible by creating metal sequestration microbes and then using fluorescence or color tagging to achieve separation.

Questions were centered around whether the weathering rates would be sufficiently fast to work in an actual environment, for example on Mars. Basalt can be composed of a glassy and crystalline structure. Biology prefers basalt glass, because the quenched basalt has a homogeneous distribution of elements. It was also noted that when metals are sequestered within the cell, concentrations must be kept low enough to prevent poisoning the cell.

II.4 The Synthetic Cell: From the Mind, to Life, to Space

Dr. Michael Montague, a staff scientist at the J. Craig Venter Institute, discussed the institute's notable accomplishment of creating a synthetic cell (defined as a cell operating off a genome that is 100% the result of human design decisions). In itself the cell that was made is useless (it exists in nature) except as a proof of concept. He used the analogy of the Eiffel Tower, which was useless except as a proof of concept of steel trellis construction that is now in wide use.

Creating a bacterial cell that is driven by a wholly synthetic genome involves the development of several techniques of synthetic DNA construction spanning several orders of magnitude of size. The initial assembly strategy is an isothermal process known as "Gibson Assembly". (See youtube video at http://www.youtube.com/watch?v=WCWjJFU1be8 for entertaining exposition of the process). This process takes small pieces of DNA (oligonucleotides) and joins overlapping ends together in a single reaction.

Before proceeding further he explained why they chose to use mycoplasma in their proof of concept. These very small bacteria have small genomes (0.58-1.5 million base pairs), can be grown in pure culture, and have no cell walls. *Mycoplasma genitalium* has the smallest genome of any free-living bacterium (580 Kbp (thousand base pairs)). However, since this bacterium grows so slowly they used *Mycoplasma mycoides* (1080 Kbp) in their final experiments.

The next technical challenge was reached as the assemblies approached the size limit of cloning into *E. coli* (300 KB). To combine larger pieces of DNA they used a technique called Transformation Associated Recombination (TAR) cloning using yeast as the host. They demonstrated a one-step assembly in yeast of 25 overlapping DNA fragments to form a complete synthetic *Mycoplasma genitalium* genome. The final achievement was the synthesis of the 1.08 million base pair chromosome of a modified *Mycoplasma mycoides* genome and its implantation into a DNA-free bacterial shell of *Mycoplasma capricolum*. The synthetic cell is called *Mycoplasma mycoides* JCVI-syn1.0, and is the proof of principle that genomes can be designed in the computer, chemically made in the laboratory, and transplanted into a recipient cell to produce a new self-replicating cell controlled only by the synthetic genome (Science 2 July 2010: Vol. 329, no. 5987, pp. 52-56).

Dr. Montague ended by discussing metal extraction using bioleaching. Bioleaching bacterial communities are amenable to metagenomic sampling and whole genome cloning offers the possibility for massively upscaled metagenomic sampling. He proposed scoring populations of bacterial communities for their capability to liberate desired metals from asteroidal or lunar simulants. Then use TAR-cloning and metagenomic sampling to characterize and sequence the best-scoring populations. This should lead to the identification of genes that may be involved in the liberation of desired metals.

When asked how long it would take to construct the *Mycoplasma mycoides* genome from scratch again, he replied less than a year. Other questions related to the limitations of Gibson assembly and TAR cloning.

II.5 Synthetic Biology: Duct Tape for a Mars Mission

Dr. John Mulligan, founder and chairman of Blue Heron Biotechnology, discussed the concept of bringing a synthetic biology toolkit on a long duration spaceflight to make some of the physical materials you need in flight. Inspired by the example of Apollo 13, he suggested bringing flexible general use tools that give you the ability to respond to unexpected problems. Since DNA doesn't weigh much, you could bring a toolkit containing a large collection of genes, enzymes, regulatory networks, sensors, structural proteins, etc. Synthetic biology could then give NASA missions the ability to create a wide range of novel materials on site, as they are needed. A combination of simple on board molecular biology and a comprehensive "gene toolkit" could allow space travelers to create new materials based on information received from Earth. This would allow scientists and engineers on Earth to intercede by designing a new enzyme, a biosynthetic pathway, an antibody or a protein drug in response to an unplanned event.

A key to the strategy is to bring the largest possible diversity, for example, a wide variety of enzymes and a few thousand single amino acid variants of each. He suggested that while enzymatic gene synthesis based on libraries of pre-built oligonucleotides might be viable, *de novo* gene synthesis may be beyond the scope of what can be done in space, considering the difficulty of synthesizing oligonucleotides. He concluded that adding a diverse synthetic biology tool set to the mission would add little weight or complexity, but could be invaluable in counteracting an unexpected emergency in transit or on the surface of another world.

This approach would require one new technology—a self-sustaining molecular biology toolkit that can produce all of the consumables that it uses. It might include protocols based on crude purification of enzymes and reagents, for example, producing antibiotics using fermentation and agar from plants. Some of the molecular biology equipment needed in space includes re-useable pipets and plasticware, a pocket thermocycler, incubator, gel box, and low-speed centrifuge.

A question was raised as to whether problems related to space such as microgravity affecting fluid flow would complicate synthesis in space. Also, could the process be sufficiently automated or would you have to have a trained biologist on the mission? He responded that the procedures could probably be sufficiently simple or sufficiently automated to avoid needing a trained biologist.

II.6 In-Situ Resource Utilization (ISRU): State of the Art and the Potential for Biology

Gerald Sanders, Lunar Surface Systems ISRU manager at Johnson Space Center, spoke about the current state-of-the-art ISRU at NASA and how synthetic biology might play a role. He defined ISRU to be that which involves any hardware or operation that harnesses and utilizes "*in-situ*" resources to create products and services for robotic and human exploration. ISRU involves five major areas: (1) resource characterization and mapping; (2) civil engineering and surface construction; (3) *in-situ* manufacturing and repair; (4) mission consumable production; and (5) *in-situ* energy generation, storage, and transfer. The goal of ISRU is to enable affordable, flexible, and sustainable exploration. For ISRU to be viable, it must have mass and cost payback and the mission and crew risk reduction must outweigh any increased risk of the ISRU system. The top-level ISRU development and integration strategy recognizes the need for achievable and visible successes, while taking an evolutionary approach in development and missions. He made an analogy to the MELiSSA project that has a number of compartments that have to work together in unison. All steps need to be considered when evaluating ISRU concepts as illustrated in the space ISRU "mining" cycle shown in figure 4.

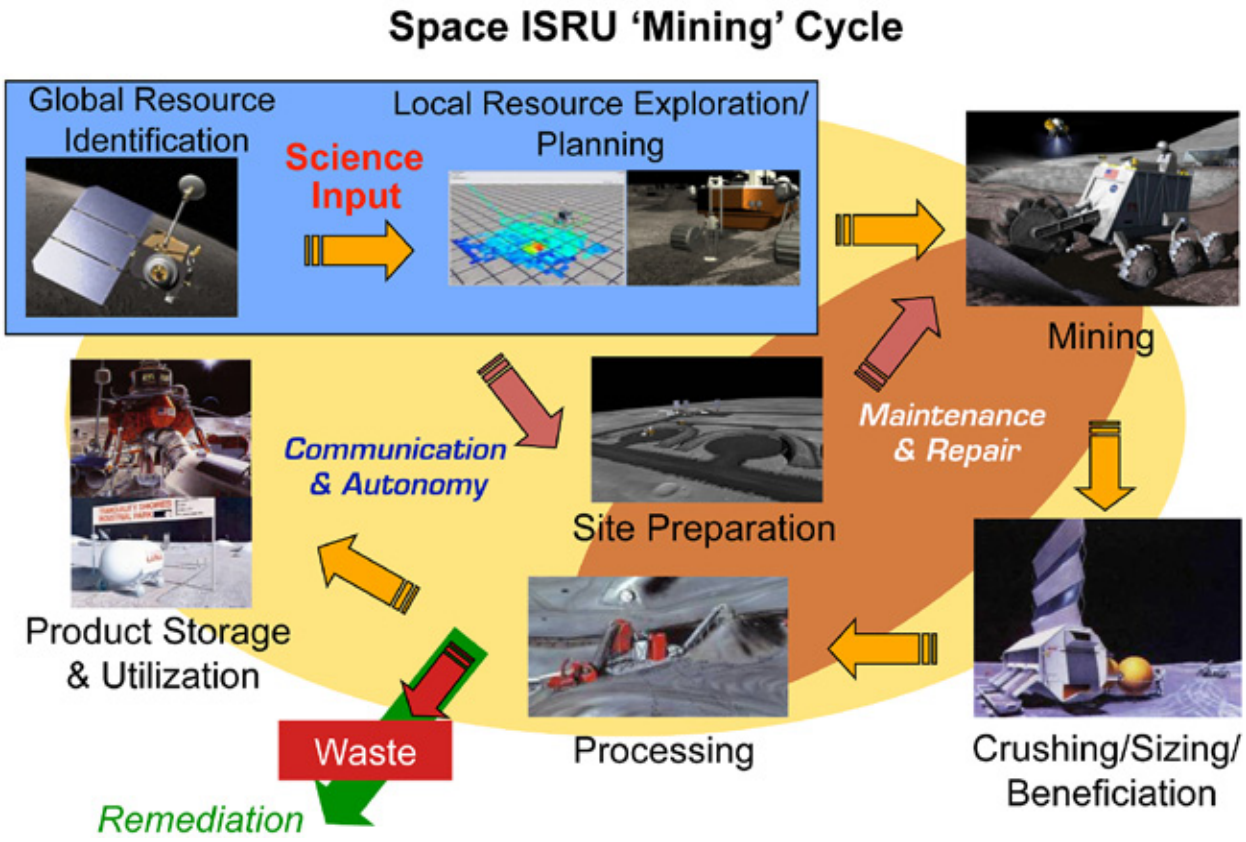


Figure 4. Illustration of the steps and interactions that need to be considered in an ISRU "mining" cycle.

Mr. Sanders showed NASA's ISRU breakdown structure for future research. He felt that synthetic biology could play a significant role in areas such as biological regolith reduction, beneficiation (separation of ore into mineral and gangue), trash/waste processing, hydrocarbon fuel production processes, and metal production. He provided some analogies to better help understand the requirements of ISRU systems. For example, to produce 10 metric tones (MT) of O_2 per year would require the excavation of a soccer field (110 by 65 meters) to a depth of 0.6 to 8 cm for an extraction efficiency of 1 to 14%. Oxygen extraction efficiency depends on both the process and location. For processing of oxygen and metal extraction from lunar regolith, he compared the techniques of hydrogen reduction, carbothermal reduction, and molten electrolysis. A prototype hydrogen reduction system has been demonstrated to have 1% efficiency. Carbothermal reduction and molten electrolysis have higher efficiencies. Which technique would be implemented would depend in large part on location. The challenge for synthetic biology is to develop processes that compete favorably with the chemical processes just described.

The first question was what happens if it breaks. The response was to design with redundancy and simplicity and with the knowledge that the system must work day after day in extreme environments. To the question of where can synthetic biology help ISRU; he responded extraction of metals from ore, trash processing, and alternative fuel production.

III. Working Groups on Applications of Synthetic Biology to NASA's Mission

Group 1: Biological In Situ Resource Utilization

Much of the group discussion was aimed at explaining ISRU capabilities that have been considered for lunar and Mars robotic and human exploration missions and what role they played. Once a capability was discussed, the group would brainstorm synthetic biology processes that might provide the same capability. However, because of the wide and diverse range of potential resources and the ISRU capabilities considered, the group felt that without a detailed systems analysis and a trade study of capabilities that only the near-term or 5-year timeframe could be addressed. The problem is highly location dependent, for example, the basic resources on the Moon and Mars differ significantly. Some of the mission ideas that could be addressed in the near term include surface hardening to produce, for example, a landing pad, energy capture using batteries or solar arrays, soil production and detoxification, and trade studies of existing and future capabilities. Initial work should address critical needs such as fuel, O_2, and basic construction of habitat. The group discussed some of the work needed, such as separation techniques, systems analysis, and high-level logistics, but because of time constraints, did not address the longer term.

Group 2: Biosensors

The biosensor group began by discussing current capabilities. These include two-component signal transduction using ions, peptides, molecules, etc. Current biosensors are based on periplasmic binding proteins, olfactory sensors, RNA (riboswitches), DNA (aptamers), and temperature sensing with RNA folding. Life detection can be carried out using molecules that are universal in life as we know it, such as nucleosides, nucleotides, amino acids, and lipopolysaccharides (LPS). Some of these analytes are also produced by abiotic mechanisms, in which case it is necessary to distinguish biogenic from non-biogenic analytes by a property like stereochemistry.

In the 5-year timeframe research on the ISS and robotic missions on near solar system objects are possible. There is a need to develop a list of phenomena that need to be sensed such as radiation, stress and damage, and then compare this list with current sensing capabilities to determine what research and development is needed to fill the gap. Since organisms may behave differently in space, ground research needs to be validated in the appropriate environments.

In the 15-year timeframe, missions to the Moon and high-Earth orbits are possible. Longer-term issues such as sustainability, auxotrophy, and adaptation of sensors should be addressed. Since it will be necessary to maintain communities of bacteria for long periods of time, research is needed to improve consortium monitoring (multiple generations or ~900 days). It would be beneficial to have the capability to simulate various gravities by centrifuge, and to determine what can live in orbit, on the moon, or on Mars.

In the 30-year timeframe some of the technologies that are conceivable include sensors that are multifunctional, that is, capable of simultaneously sensing, for example, radiation, O_2, and toxics. Both early warning detection systems and dynamic self-modifying systems on buildings and space ships are possible, as well as implantable sensors. In this timeframe issues such as genetic stability will have to be addressed.

Group 3: Biomaterials and Self-Building Habitats

For the near term, the group discussed the use of microorganisms to grow construction materials. More specifically following on the work of Ginger Dosier to use *Sporosarcina pasteurii,* a nonpathogenic, common-soil bacterium to induce the production of calcite through a chemical reaction, thereby fusing loose aggregate. A hardened material is formed in a process known as Microbial Induced Calcite Precipitation (MICP). This material acts as a binder, similar to Portland cement within concrete, and exhibits physical properties similar to those of natural sandstone. This process can take advantage of locally sourced sand and aggregate material to reduce the weight of materials requiring delivery to the site. MICP coatings retard water absorption and exhibit self-healing properties. MICP does not shrink during the curing process and can work with a variety of aggregates. Cementation can be evidenced in less than 24 hours, depending on cell, urea, and $CaCl_2$ concentrations. Biomanufacturing would eliminate the need for costly expendable formwork. In the 5-year timeframe, the most promising course of action would be to explore Earth applications and alternative materials and binders. One needed capability is to find sustainable sources of Ca and urea.

In the 15-year timeframe consideration should be given to co-cultivation with photosynthetic and nitrogen fixing organisms. One should look at surface modifications and at developing large 3D patterning devices. In the 30-year timeframe, research should focus on self-patterning bio matrices and super-composites with new properties. Needed capabilities include a toolkit for rejigging matrices. Applications of these methods should be tried on Mars and other non-terrestrial locations.

Group 4: Synthetic Biology and Human Health

The synthetic biology and human health group looked at mission ideas and needed capabilities in the next 5-30 years. The current capability is conventional small light-weight commercial-off-the-shelf medical technology adapted for space use. Within 5 years it should be possible to test synthetic biology techniques in microgravity on the ISS, develop a repertoire of sensing elements incorporated into a synthetic organism, and carry out a microbial ecology assessment of the biome on the ISS. A needed capability is a toolkit that can analyze for specific DNA sequences. In the 15-year timeframe, therapies such as reprogrammable drug delivery patches, the engineering of probiotics as radiation protectants, and the development of synthetic biology elements for delivery of therapeutics for acute radiation exposure are possible. This would require the development of patch technologies for space applications, and encapsulation technologies for implantation of devices that could function autonomously in the body. In the 30-year timeframe,

gene knockdown and stem cell therapies are possible to counteract damages from the space environment. Another far reaching possibility is preventive reprogramming of the crew genome. This latter possibility would have to deal with both regulatory and ethical issues. The development of RNAi technologies would also be needed in the 30-year timeframe. Additional work that could be done on the ground includes identifying the basis for individual variability and susceptibility to diseases and large-scale testing of bio-building blocks for their susceptibility to radiation and other space factors.

Group 5: Life Support for Long Term Space Travel and Habitation

The life-support group recommended doing trade studies to analyze future potential benefits of synthetic biology to help identify needs and prioritize research. Projects that are feasible in a 5-year timeframe include studying microorganisms cultured in microgravity to assess the viability and stability of genetically altered organisms in space. Fermentation processes should have some priority. We should continue developing algae as a nutrition source using classical molecular biology techniques. Other research projects include looking at the scaling necessary for O_2 and CO_2 recycling as a function of crew size, micro-ecology studies with mixed populations, and finding ways to fully degrade human and other organic wastes. These studies will require platforms such as the ISS and near-Earth satellites, as well as appropriate bioreactors.

In a 15-year timeframe, we should develop a small-scale, semi-closed-loop system on Earth incorporating waste remediation, food, and fuel production. We should develop modified organisms that improve filtration, concentration, and processing, and that have improved tolerance to radiation and microgravity. These may be engineered organisms specifically designed and adapted for extreme environments. We should continue to explore novel techniques for energy efficient growth of nutritional plants. Some of the needs in the longer term are full-scale, closed-loop systems with higher organisms (mammals), a synthetic biology tool set for engineered nutrition and safety, and a modular habitat that is transportable. Key goals are full food production without resupply, processing, storage, capability for waste mineralization, and full biological control over O_2 and CO_2.

IV. How Does Space Synthetic Biology Pick up on the Broader Agenda?

IV.1 Foundational Synthetic Biology Technologies: Parts Registries, BioCAD

Dr. Nathan Hillson is the Director of Synthetic Biology at the Joint BioEnergy Institute (JBEI) Fuels Synthesis Research Division. He coordinates and directs the development of the JBEI biological parts registry, the characterization and standardization of biological parts, the computer-aided design of biological pathways and circuits invoking the standardized parts, and the automated assembly of the pathways and incorporation thereof into microbial hosts. Their core mission is the conversion of biomass into clean biofuels.

He discussed synthetic biology as an integrated process (see figure 5). The recent emergence of foundational technologies, including repositories of biological parts, biological computer-aided design (BioCAD) tools, and automated DNA-assembly methods, promises to greatly facilitate the execution of synthetic biology tasks and to increase the scope of what is readily experimentally achievable. He discussed the advanced search features of JBEI registry of biological parts. An open source version of the registry is available on the JBEI public registry website: https://public.jbeir.org. He also discussed their DeviceEditor, which is a drag-and-drop BioCAD canvas that visually represents and captures DNA assembly design schematic details.

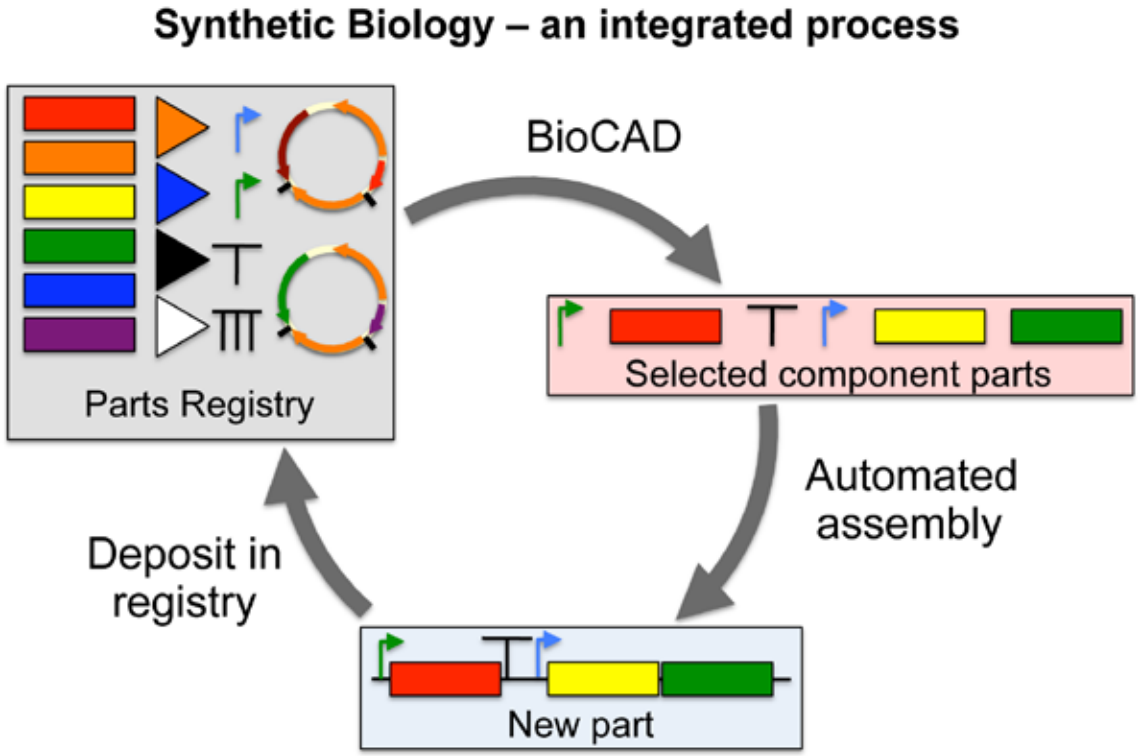


Figure 5. Illustration of synthetic biology as an integrated process involving the creation of a parts registry, BioCAD design using existing parts, and the automated assembly of new parts.

Dr. Hillson discussed the challenge of DNA assembly. The traditional assembly strategy is not standardized and is difficult to automate. The BioBrick approach is a standardized assembly strategy that is automatable, but it leaves a scar in the sequence. He compared the Sequence and Ligation-Independent Cloning (SLIC), Gibson assembly, and Circular Polymerase Extension Cloning (CPEC) methods. These three methods provide automatable, scarless, and largely sequence-independent, multipart DNA assembly. Given a list of biological parts to assemble, their DNA assembly design automation software j5 manages the assembly process. Both j5 and the DeviceEditor are available on line at no cost to academic, non-profit, and government labs. The forthcoming integration of the JBEI registry of biological parts, the DeviceEditor, and j5 with liquid-handling robotics and microfluidics platforms, promises to reduce the cost, labor, and error-prone tedium of the cloning process, allowing researchers to focus on the design and assay of biological devices, rather than on their construction.

In the discussion following it was noted that microfluidic devices could be used to reduce time, size, and cost. He noted that efforts were underway to integrate the j5 software with other research groups. The advantage of using scarless assembly methods was emphasized.

IV.2 Genome Engineering and Biosensors

Dr. Vatsan Raman, postdoctoral fellow in Professor George Church's laboratory at the Harvard Medical School, discussed some of the work going on in their lab. The ability to engineer microorganisms to serve the needs of humankind is at the heart of synthetic biology. Microbes can be harnessed for fuel production, complex chemical synthesis, bioremediation, and several other industrially important applications. Nature has evolved enormous genomic diversity such that only those that possess the "correct genetic make-up" are selected to survive in their ecological niche. In order to engineer microbes for synthetic purposes, it is necessary to accelerate microbial evolution by rapidly modifying their genome to generate billions of combinatorial genetic variants within hours to days. Dr. Raman and colleagues have developed a method called Multiplex Automated Genome Engineering (MAGE) to enable large-scale engineering of the *E-Coli* genome by targeted oligonucleotide insertions using phage lambda red machinery. Using lambda red's homologous recombination mechanism, MAGE can generate over 4 billion combinatorial genetic variants per day. They have applied MAGE to improve the production of lycopene, an industrially important compound, over five-fold by modifying the promoters and ribosome-binding sites of the genes involved in the lycopene pathway.

The power of genome engineering can be truly realized if the genetic diversity of MAGE can be coupled with selection of the desired genotypes. To select a favorable pool of mutants, we need to develop small-molecule biosensors inside the cell so that the selection marker is expressed when the desired molecule is detected. They are developing a library of protein-based biosensors that can regulate transcription. The LacI/GalR family of transcription factors is an ideal candidate for biosensors. These transcription factors comprise ligand-binding and DNA-binding protein domains. Upon binding its putative ligand, the protein undergoes a conformational change, depresses the DNA binding site, and enables transcription. The LacI/GalR family is known to bind to a wide variety of small molecules. They would like to redesign the ligand-binding site of this protein so that it responds to a ligand of choice.

By combining genetic diversity (by MAGE) and selection (by biosensors), the principles of synthetic biology can be used for the production and optimization of industrially important compounds, drugs, detection of toxins, and several other applications.

IV.3 Synthetic Biology and Reshaping Plant Form

Dr. Jim Haseloff of the University of Cambridge discussed how synthetic biology is providing a conceptual and practical framework for the systematic engineering of gene expression and behavior in microbes, facilitating the design of novel regulatory networks, including synthetic oscillators, switches, logic gates, intercellular signaling systems and metabolic pathways. Synthetic biology approaches also show great potential for the engineering of multicellular systems. It is feasible to consider creating new tissues or organs with specialized biosynthetic or storage functions by remodeling the large and relatively well-understood distribution of existing cell types. Of all multicellular systems, plants are the obvious first targets for this type of approach. Plants possess indeterminate and modular body plans, have a wide spectrum of biosynthetic activities, can be genetically manipulated, and are widely used in crop systems for production of biomass, food, polymers, drugs and fuels.

Current genetically modified (GM) crops generally possess new traits conferred by single genes, and expression results in the production of a new metabolic or regulatory activity within the context of normal development. However, cultivated plant varieties often have enlarged flowers, fruit organs or seed, and are morphologically very different from their wild-type ancestors. Recent genetic studies have provided detail of the molecular processes underlying plant development. The next generation of transgenic crops will contain small gene networks that confer self-organizing properties, with the ability to reshape patterns of plant metabolism and growth, with the prospect of producing neomorphic structures suited to bioproduction.

Morphogenesis, the biological process that causes an organism to develop its shape, is a cellular process driven by interplay between gene expression and a growing network of cell interactions. Dr. Haseloff discussed some of the genetic, microscopic, and software tools, such as in *planta* high-resolution cytometry, that provide a clear visualization of individual cells inside living plant tissues. He discussed the empirical rules that describe cell division such as cell plate formation that occurs normal to the growth axis (Hofmeister's rule), and that cell plate formation occurs at right angles to existing walls (Sachs' rule). Also discussed were models for the regulation of cell division and cellular automata models for plant morphogenesis.

Applications for synthetic biology include cell autonomous genetic circuits with self-regulating properties and morphogenetic circuits with self-organizing properties. He concluded by discussing the benefits of rational design. Modern plants have evolved by many generations of human selection and breeding. They differ mainly in the number and proportion of cells that contribute to different tissues of an organ. Engineering of intercellular logic could provide simple and predictable tools for altering plant form.

Plenary Talk on Synthetic Genomics

Dr. J. Craig Venter, Founder and President of J. Craig Venter Institute (JCVI), gave the plenary talk entitled "Synthetic Genomics" to the combined audience of the space settlement and synthetic biology workshop groups. His talk focused on five important questions: (1) What is life?; (2) Can we digitize it?; (3) How extensive is it?; (4) Can we pare life down to its most basic components?; and (5) Can we regenerate life or generate new life out of the digital world? He discussed the recent achievement of JCVI of chemically synthesizing a 1.08 Mbp modified *Mycoplasma mycoides* genome and transplanting it into another bacterium, *Mycoplasma capricolum*, to create a new *Mycoplasma mycoides* bacterial cell controlled only by the synthetic genome. This landmark achievement in engineering biology provides a proof of concept that it is now possible to start with the digital code and create a synthetic organism.

Dr. Venter gave an historical perspective of the events leading up to their recent landmark achievement. The mid 1980s marked the beginning of the conversion of the genetic code, expressed in terms of the four base pairs (adenine, thymine, guanine, and cytosine) into digital code. The 1990s saw the development of rapid ways to sequence DNA, discover genes and to understand DNA variants. He discussed complementary DNA sequencing, expressed sequence tags (ESTs) and the human genome project. Using new mathematical algorithms that they developed, the first full genome was sequenced in 1995, the fruit fly genome was sequenced in 1998-1999, and the first human genome was sequenced in 2000.

The first full diploid genome sequence of an individual human (J. Craig Venter) was reported in 2007. Comparing the two sets of genes that he received from his parents, he found differences as large as 0.5%. These variations impacted genes as well. He discovered that 44% of his protein coding genes had greater than one heterozygous variant, 29% of his genes had greater than one non-synonymous variant, and 15% of his genes had more than one non-coding transcriptional variant. When they compared the genomes of unrelated humans, differences of 1-3% were observed when all the insertions and deletions were included, which is a factor of ten greater than previously thought. These significant differences between individual genomes can have a significant effect on the efficacy of different drugs. Since the cost of sequencing a human genome is decreasing rapidly, human genomes are pouring into the database at an ever increasing rate, allowing comparison of genomes from different human populations.

Dr. Venter noted that while NASA currently performs genetic (phenotype) selection for space missions, in the future they will be able to use genomics to screen for traits that are compatible with life in space, such as inner ear changes that eliminate motion sickness, rapid bone regeneration, DNA repair, a strong immune system, and small stature. He discussed the possibility of creating a synthetic metabiome for space travelers, in other words, replacing the thousands of microbes in the human body with a well defined microbial community. Potential benefits could be the elimination of disease organisms that cause infections and dental decay, methanogens and sulfur producers, and organisms associated with body odor. Each space traveler would have the same metabiome resulting in a healthier environment for long durations in space.

Dr. Venter discussed the genomics of the human microbiome. While there are approximately 100 trillion human cells in the body, there are approximately twice that many bacteria. The number of species of bacteria in both the oral cavity and the intestinal tract are on the order of one thousand. To understand the factors that contribute to infectious disease susceptibility, it is necessary to understand the human genome, the human microbiome, and their interaction. For example, to explain the six-fold increase in esophageal adenocarcinoma requires understanding the changes in the microbiota associated with this cancer. The metabolic potential of the microbiota is considerable. There are approximately 2400 different chemical compounds that we can make enzymatically from our gene set. After eating there are typically 450-550 chemicals in the blood plasma. Approximately 60% arise from human metabolism, 30% are from the digested food, but 10% are bacterial metabolites, whose role in human physiology is unknown.

Of the microbial abundance on the Earth, microbes make up over ½ of the earth's biomass, while animals account for a mere 1/1000th of the total biomass. He discussed his global ocean sampling expedition where they are performing a detailed genetic analysis on both water and air samples every 200 miles in diverse environments. They are finding an incredible diversity of bacteria in their samples. The majority of genes found in each sample of sea water are unique. While most of the mammalian genes have been found, we are still on a linear phase of gene discovery for viruses, bacteria and archaea, despite having over 50 million genes in the database. They also find significant diversity in sampled deep sea and Earth microbes, albeit with fewer mutations because they are shielded from UV radiation.

Dr. Venter began the discussion of how they created the first bacterial cell run by a synthetically replicated genome. It begins with the question of what is minimal life. *Mycoplasma gentilalium* has the smallest known genome (580 Kbp) of any bacterium capable of independent life. It has 482 protein-coding genes and 43 RNA genes. How many of these are essential or in other words, what is the smallest number of genes needed to run the cell? Approaches to determining the essential genes to operate a cell include comparative genomics and experiments such as gene knockout and genome reduction. They carried out a global transposon metagenesis of *Mycoplasma genitalium*. Transposons are small pieces of DNA that jump around in the genome. If a transposon jumps into a gene and the organism survives, then that gene is probably not essential. Knocking out genes as a means of discovering essential function also had its limitations, in part because some genes have duplicate functions. He noted that the question of whether a gene is essential or not is context-specific, it depends on environment. In the end the only way forward was to synthetically create a synthetic chromosome where they could completely control the genetic content. The technical challenge was to assemble large pieces of DNA, and then having created an entire genome, to get it to function within a cell.

Generating a synthetic genome required improving and dramatically shortening the time required to accurately assemble 5-6 Kbp segments of DNA from synthetic oligonucleotides (see the discussion of Gibson assembly and TAR cloning in section II.4). Another important aspect of the work included developing error correction methods, because even a single base pair deletion can render the genome ineffective. To put the larger pieces of DNA together they used yeast's powerful

homologous recombination system to assemble the overlapping pieces *in vivo*. In 2009 they were able to form a complete synthetic *Mycoplasma genitalium* genome in a one-step assembly in yeast of 25 overlapping DNA fragments. The enzymatic assembly of DNA molecules up to several hundred kilobases can be automated. Using these techniques they constructed the *Mycoplasma mycoides* (1.08 Mbp) in yeast as circular centromeric plasmids. However, while genome transplants from bacteria worked, transplants from yeast did not. It was discovered that methylation of the incoming genomic DNA was critical for the success of the transplantation. Methylation protected the DNA from the restriction enzymes of the host capricolum cell.

In summary, the culmination of the work at JCVI was the design, synthesis, and assembly of the 1.08 Mbp *Mycoplasma mycoides* JCVI-syn1.0 genome starting from digitized genome sequence information and its transplantation into a *Mycoplasma capricolum* recipient cell to create new Mycoplasma cells that are controlled only by the synthetic chromosome. The only DNA in the cells is the designed synthetic DNA sequence including "watermark" sequences and designed gene deletions.

Dr. Venter ended his presentation by talking about some of the potential applications of synthetic genomics. He noted that 40 million genes have been discovered to date and that these are the design components of the future. He discussed a synthetic organism design tool that could be used to construct organisms with specific properties. One application is to use genetically engineered organisms to produce carbon fuels to mitigate the rise in atmospheric CO_2. He discussed 4th generation designer fuels that were based on CO_2 as the source of carbon and the sun for energy. These synthetic cells could be engineered to produce desired outputs such as sugar, protein, biopolymers, or methane. He briefly discussed work to develop a microalgal biomass culture system to support food production and atmosphere renewal for long-duration manned spaceflight. Finally he discussed the potential of synthetic biology to rapidly produce new vaccines to combat emerging diseases. Clearly we are on the cusp of the development of major new industries based on synthetic biology.

The initial question concerned the potential for bioterror such as the release of pathogens. His response was that pathogens would be relatively hard to create. He noted that everything in his laboratory is being designed to be unable to survive outside the laboratory. Someone asked whether there was a particular bacterial chassis that you might select for use in space. His response was no that he would take advantage of the large diversity of bacteria for specific purposes. The next question was given a technology developmental timeline, what would be the first use of synthetic biology in NASA's mission? His response was that it depended on the level of investment. With the proper investment in people and money, he felt that synthetic biology could have a major impact on NASA's mission. The next question was whether he had any plan to design for the synthesis of the membrane proteins to accommodate his synthetic genomes. He replied saying that they are considering trying to design a universal recipient cell that could host a variety of chromosomes. The final question was to what extent he was able to predict functionality from his designed genome. His response was that not everything can be done by computer and simulation at the present time. There is not a single genome for which the scientific community understands the function of every gene in it. Biology is fundamentally in a discovery mode, and is not a first principle method at the present time.

V. Lightning Talks (LT)

Since the lightning talks were very short, I have modified submitted abstracts in this section to better capture the intellectual contributions of those participating in this session.

LT-1: Computational Challenges in Design, Fabrication, and Testing of Synthetic DNA

Dr. Joel Bader, Johns Hopkins University, discussed some of the work being carried out in his laboratory. Genomes have at least the complexity of large human-engineered systems, yet the tools for designing, building, and testing genome-scale DNA sequences remain rudimentary. An improved, shareable infrastructure would accelerate our ability to explore and exploit the potential of synthetic biology. He described the suite of software tools developed in his laboratory that scale to design and synthesis of full genomes in the context of a collaborative project to create synthetic yeast. His BioStudio server automates many design tasks and provides an optimal synthetic strategy to build a full chromosome from overlapping oligonucleotides. It links down to GeneDesign for fine-scale editing of protein-coding sequence and connects to a back-end CloneQC system for validating the physical sequence of synthetic DNA. He described a new workflow component of BioStudio that tracks the flow of DNA from electronically designed sequences to physical oligonucleotides, clones, and finished genomes. This software is all open source and has been developed with the goal of easy portability and widespread use.

LT-2: Building a Toolkit for Thermophilic Cyanobacteria

Dr. Devaki Bhaya, of the Carnegie Institution of Washington, discussed her research on cyanobacteria, an ancient and ubiquitous lineage of photosynthetic prokaryotes that were partly responsible for the oxygenation of the Earth's atmosphere. Furthermore, cyanobacteria have the ability to survive some of the harshest environments on the planet, which provides critical information about ways in which phototrophs respond to stress at a mechanistic and systems level. Thus, understanding how cyanobacteria adapt and thrive in various environments is crucial from several different perspectives.

Her research has focused on microbial communities that form layered biofilms or mats in hot springs where 16S rRNA diversity has previously been correlated with environmental gradients of temperature and light. To understand the genetic and physiological basis of these observed populations, she and her collabortaors sequenced the genomes of two thermophilic nitrogen-fixing *Synechococcus* isolates that dominate at different temperatures in the mats. This information combined with metagenomics has provided many insights into genomic and metabolic diversity within these populations. In the particular context of synthetic biology, thermophilic cyanobacteria provide a useful model system, thus there is an important need to build and extend on this foundation to provide a robust toolkit for future experiments.

LT-3: Building a re-coded yeast genome powered by an army of undergraduates

Dr. Yizhi (Patrick) Cai from Johns Hopkins University (JHU) discussed the Build-a-Genome course at JHU and progress towards the first synthetic eukaryotic genome synthesis. Synthetic biology offers an excellent framework within which students may participate in cutting-edge interdisciplinary research. This new discipline offers the promise of a deeper understanding of gene function, gene order, and chromosome structure through the *de novo* synthesis of genetic information, much as synthetic approaches informed organic chemistry. While considerable progress has been achieved in the synthesis of entire viral and prokaryotic genomes, fabrication of eukaryotic genomes requires synthesis on a scale that is orders of magnitude larger. These high-throughput but labor-intensive projects serve as an ideal way to introduce undergraduates to hands-on synthetic biology research. We are pursuing synthesis of *Saccharomyces cerevisiae* chromosomes in an undergraduate laboratory setting. The Build-a-Genome course at JHU exposes students to the engineering of biology on a genome-wide scale while focusing on a limited region of the genome.

LT-4: The Search for Extra-Terrestrial Genomes (SETG): A life detection instrument with biological components

Dr. Christopher Carr, research scientist at Massachusetts Institute of Technology (MIT), spoke about SETG, a NASA-funded instrument development project based on the hypothesis that life on Mars, if it exists, may be related to life on Earth. The approximately one billion tons of rock thought to have been transported between these planets, due to meteorite impacts mainly between 3.5-4 Gya, may have carried viable microbes. If so, such microbes may continue to survive on Mars today. Thus, SETG will target life-as-we-know-it by isolating, detecting, and sequencing RNA or DNA, *in-situ* on Mars, from soil, ice, or brine samples. The most viable approaches for automating these instrument functions require biological components, including nucleotides, DNA primers, and enzymes such as polymerase and reverse transcriptase. These components must withstand a number of challenging aspects of the space environment including potential large temperature fluctuations, and a more intense and diverse radiation environment. Understanding how to safely store these components over long periods of time and how to protect them from the radiation environment will support the development of space systems with increased biological heritage. SETG's intended application is to search for life on Mars ancestrally related to life on Earth, but it can also be used in planetary protection, space medicine, and environmental health applications. Perhaps it may also have a role in monitoring space applications of synthetic as well as natural biology.

LT-5: The Biochemical Processing Unit (BPU): Rapid DNA Synthesis and Genetic Prototyping

Dr. Peter Carr at the MIT Media Lab discussed the BPU platform being developed for large-scale (up to megabase) *de novo* DNA synthesis. Beyond constructing DNA, the BPU uses that DNA along with a compact microfluidic system to provide rapid, economical, and high-throughput assessment

of genetic function. Thus far they have demonstrated advances in oligonucleotide microarray fabrication, DNA error correction, and microfluidic gene synthesis coupled to protein expression and functional assays. They are now combining these elements to produce the integrated system.

As an enabling technology, the BPU will be used to test large panels of proteins designed *in silico*, and sets of DNA parts for genetic circuit designs. This rapid prototyping and feedback will accelerate design cycles and inform engineers which components merit further development *in vivo*. For applications in personalized medicine and drug development, the BPU will be used to evaluate the effects of genetic diversity and individual biochemistry for making clinical decisions. For NASA's goals, this decoupling of genetic information from the physical DNA molecules could be put to work in multiple ways. The potential exists to take these synthetic capacities into space on long-term manned missions, providing the ability to synthesize genetic solutions on-site. Alternatively, in the case of illness during a mission, a BPU on Earth could be used to rapidly evaluate medical questions that arise for a mission participant.

LT-6: Industrial Scale Fermentation

Christopher DaCunha, senior research scientist at EdeniQ, spoke about industrial scale fermentation processes. These encompass a wide range of products including food, liquid fuels, pharmaceutical products, and active biomolecules. Upstream of the fermentation process, key steps include identifying the appropriate organisms for the system, understanding the genes of interest, the host choice and engineering parameters such as promoter choice, condon optimization, vector design, etc. The next steps include proof-of-concept and performance evaluations. Since profitability of the process is a key concern, a scaling evaluation is required to assess how cost scales with increasing infrastructure and whether bioreactors can achieve proper control of key parameters such as pH, temperature, cell mass, etc. In the fermentation process itself, key considerations include plant design, engineering implementation, and optimization of the process. Finally, downstream of the fermentation process, key issues are bulk separation, purification and concentration, recovery, and quantification.

Some of the potential NASA applications might include cycling of resources through multiple types of bioreactors or culture chambers, where the output from one is utilized as feedstock or nutrient supplement in successive reactors. The MELiSSA project is an example where elemental analysis of effluents is required to determine proper control valving, metering, and mass balancing. Lessons learned from fermentation processes could help guide the design of large-scale NASA life support systems. Another application of multiple reactors would be for an on-demand production of products such as lubricants, medicine, and nutrition supplements for NASA personnel on long-duration spaceflights.

LT-7: Beyond iGEM: a pH-Based Biosensor for Detection of Arsenic in Drinking Water

Kim de Mora, a doctoral candidate at the University of Edinburgh, discussed their arsenic biosensor that was developed for the 2006 iGEM competition. He discussed continued developments of this sensor with experimentation on the media conditions and fine tuning of the sensor parameters using changes to the growth media and experimental protocol. This biosensor was engineered by constructing a plasmid that contains the *E. coli ArsR* promoter and *ArsR* gene, followed by the *LacZ'* gene. The system functions by fermenting lactose in the presence of arsenic, where a decrease in pH can be detected by pH measurement or using a chemical indicator. Recent work has involved optimizing the system for potential field trials in parts of Europe and South East Asia that suffer from arsenic groundwater contamination issues. To this end, we have tested the system with groundwater analogues and seek to obtain water samples from affected regions in Europe and South East Asia. Recent tests using samples collected in the South of Hungary have shown that our sensor can detect arsenic in real world groundwater. In order to detect changes to the system and fine tune the composition of the media, we have designed and built a quantitative colorimetric pH assay. This assay functions by creating a time-lapse video of the sensor over 48–72 hours and analysis of the images to quantify the data. We have shown that the optimized system can detect an arsenic concentration as low as 5 ppb. This sensor would lend itself well to integration into a micro-fluidics system where the components and reaction volumes could be greatly reduced in size and weight.

LT-8: Paper-Supported 3D Cell Culture for Tissue-Based Bioassays

Ratmir Derda, postdoctoral fellow at Harvard University, discussed his work on 3D cell cultures (Derda et al. "Paper-Supported 3D Cell Culture for Tissue-Based Bioassays", Proc. Natl. Acad. Sci. (2009) 106, 18457). Simple approaches are being developed that permit generating organized synthetic communities of bacteria or mammalian cells and regulating molecular gradients inside or outside these systems. Multi-layer communities are assembled composed of multiple cell types simply by folding sheets of paper permeated by cells. Cells in these multi-layered communities form 3D tissue or 3D biofilm-like structures in which cells can proliferate, migrate, create and respond to molecular gradients of oxygen, nutrients, or signaling factors (e.g., quorum sensing molecules). Introducing layers of semi-permeable materials into defined locations inside these systems makes it possible to manipulate specific molecular gradients in these 3D communities. For example, encasing multi-layer films of cells between layers of poly(dimethoxy silane) allows the creation of communities in which gradients of gas molecules (e.g., O_2 and CO_2) and gradients of soluble nutrients can be established and controlled independently. This approach could be applied to cultures of bacteria in areas with a limited amount of water but with a sufficient gaseous atmosphere, such as remote rural areas with limited water access or the surface of Mars.

LT-9: Biologically Manufactured Building Materials

Ginger Dosier, professor of architecture at the American University of Sharjah, discussed growing construction materials by employing microorganisms. *Sporosarcina pasteurii,* a nonpathogenic, common-soil bacterium naturally found in wetlands, has the ability to induce the production of calcite through a chemical reaction, thereby fusing loose aggregate. A hardened material is formed in a process known as Microbial Induced Calcite Precipitation (MICP). This material acts as a binder, similar to Portland cement within concrete, and exhibits physical properties similar to those of natural sandstone. This form of biocementation can take less than a few days to complete. Minimal resources are required for growth because of the low embodied energy requirements. This demonstrates that MICP, in conjunction with local sand aggregate, can be used for the creation of "biologically grown" building materials, currently in brick form. This obviates the need for Portland cement mortar, as the MICP process facilitates bonding through bacterially induced precipitation. The method is efficient since minimal materials are needed for manufacture. Combining this approach with rapid manufacturing methods provides a novel and efficient approach to building structures and habitats in extreme environments such as Mars.

LT-10: The Coupled Autotrophic Nitrous Decomposition Operation (CANDO) Process: From Waste to Propulsion

Yaniv Scherson, a graduate student at Stanford University, discussed the CANDO process for N_2O production. Nitrous oxide (N_2O) is a safe and nontoxic monopropellant/oxidizer ideal for propulsion and energy generation space applications. Biological systems capable of converting nitrogenous waste into nitrous oxide present a unique opportunity for on-board or remote production of propellant and renewable energy with enriched gaseous air products. At the Stanford Aeronautics and Astronautics lab, catalytic decomposition of nitrous oxide in meso-scale monopropellant thrusters has been successfully demonstrated with bed loadings up to 15 kg/m^2/sec and c-star efficiencies up to 81%. In development at Stanford is a bioreactor system capable of converting ammonia (common nitrogen waste) into N_2O via a two-step process: (1) biotic conversion of ammonia to nitrite followed by (2) abiotic conversion of nitrite to N_2O. To date, a bioreactor system enriched with ammonia oxidizing bacteria has successfully demonstrated efficient conversion of ammonia to nitrite with very low oxygen demand, and an abiotic process has been demonstrated for efficient chemical conversion of nitrite to nitrous oxide. Converting nitrogenous waste into nitrous oxide and the subsequent catalytic decomposition into nitrogen and oxygen products would enable a clean and safe remote source of propulsion, energy, and enriched air.

LT-11: Algae to Biofuels Technology: From Metabolic Engineering to Synthetic Biology

Dr. Patrick Fu, professor at the University of Hawaii, discussed his work on biofuels. For NASA space missions, algae metabolic engineering/synthetic biology may become a useful engineering technology to provide oxygen, biofuels, and food for extraterrestrial colonization. Metabolic engineering aims at the purposeful modification of metabolic and other cellular networks to achieve

desired goals, such as overproduction of bioproducts, or redirection of intracellular carbon flows towards altered pathways. He discussed his work on genetically modifying blue-green algae for bioethanol production. Systems biology research of the *cyanobacterium Synechocystis* PCC 6803 was enabled by the reconstruction of its genome-scale metabolic network. Synthetic biology was used to modify *Synechocystis* by inserting the basic functional elements of ethanol fermentation into the cellular network. This novel, "add-on" function of ethanol production introduced into blue-green algae is designed to co-exist with their normal processes of using photosynthesis and CO_2 assimilation functions for the direct conversion of CO_2 to the biofuel and O_2.

LT-12: Measuring the Performance Benefit of Synthetic Biology Systems

Dr. Jason Held of Saber Astronautics discussed how to measure the performance of synthetic biology systems, which requires being able to quantify how change (e.g., adding some element of synthetic biology) can affect the system as a whole. This is difficult because biological systems are typically complex, difficult to measure, and exhibit emergent behaviors. Space systems are also highly interdependent, and modification to one component may have consequences to another component's design. What is needed is a method of performance analysis which models the interactions between components and can handle complexity and emergence.

He presented a logical paradigm to derive data driven variables (metrics and functional attributes) from group level mission goals. Then a non-linear state space model was used to learn the inter-variable interactions, using a combination of dynamic Baysian networks and Gaussian mixture models. The result, called a "System Map", is a set of regression matrices that allows the systems engineer to conduct several types of online system performance analysis. The System Map can provide high-level observation of performance for the group, or it may be used to estimate cause and effect for events down to individual components.

LT-13: An RNA-Based Platform for Gene Network Engineering

Dr. Julius Lucks at the University of California, Berkeley, discussed his RNA-based platform. We have entered the era of whole genome engineering, yet there remain fundamental questions of how to design complex genetic networks that create novel biological function. Many simple synthetic circuits have been constructed from various natural regulators. However, this approach suffers from the inherent barriers associated with coordinating increasing numbers of diverse components. They engineered independent, yet functionally identical, copies of a natural antisense-RNA-mediated transcription attenuator and arranged them in networks that performed essential and necessary functions of cellular gene regulation. They found that attenuator variants can be engineered to independently regulate two genes in the same cell, that tandem attenuators perform genetic logics by a simple multiplication rule, and that attenuators can be connected together in the first example of an RNA-based regulatory cascade. Their results demonstrate a simplified approach to synthetically creating sophisticated function simply by changing the network topology of independent, yet otherwise identical components.

LT-14: Metabolic Engineering of Microbes to Support Human Space Exploration in the Post-Genomic Era

Dr. Wayne Nicholson, professor at the University of Florida, discussed the work going on in his lab in metabolic engineering of microbes. Microbes are the dominant form of life on Earth and make human existence possible. It is therefore imperative that microbes form an integral part of any viable long-term human space exploration or colonization endeavor. Such microbes will need to be tailored for optimal performance of a wide variety of tasks in numerous types of off-Earth environments. In the current post-genomic era it is becoming increasingly feasible to predict the metabolic capabilities of microbes from genomic sequence data, to genetically engineer microbial cells to perform specific tasks, and to use directed evolution to adapt engineered microbes for optimal performance and robustness under off-Earth environmental conditions. Using the *Bacillus subtilis* model system, he discussed his experiences with metabolic engineering as it relates to radiation resistance, biofuel and bioplastics production, waste-stream processing, adaptive evolution, and the effects on gene expression and physiology of bacteria cultivated under lowered atmospheric pressure.

LT-15: Synthetic Biology Data Exchange Group

Dr. Herbert Sauro, professor at the University of Washington, discussed the work of the Synthetic Biology Data Exchange Group, which is a consortium of individuals from a number of U.S. universities. All engineering fields have benefited from degrees of abstraction and standardization. From the standardization of the humble nut and bolt to the standard electrical characteristics in transistor-transistor logic microcircuits, standardization is a relatively simply way to significantly increase engineering productivity. Synthetic biology is a new engineering discipline that has only recently begun to consider what kinds of standards might be useful. The exchange group aims to enable synthetic designs to be stored, described, exchanged and built using standard formats and protocols. The process will depend on the development of exchange formats, repositories of parts and software to assist in design and control of laboratory handling equipment. He described the efforts of the exchange group, in particular software developments and the Synthetic Biology Open Language Initiative.

LT-16: Bio-Nano-Info Lego Toolkit for Synthetic Space Biology

Dr. Alena Shmygelska, Carnegie Melon University, discussed her work to develop a toolkit for synthetic space biology. The living cell is an ultimate nanoscale fabrication system containing hundreds of nanomachines that can be synthetically modified. Using computational data-mining techniques, we collated the data from bio-medical databases and bio-medical literature and created a catalog of 'bio-nano parts' made by living cells. Our initial synthetic biology catalog contains a number of existing bio-nano components including nano-scale rotary and linear motors, enzymes, working assemblers, receptors, ion channels, etc. Particular emphasis was made to compile the toolbox of bio-nano parts and modules from extremophiles containing enzymes and bio-nano machinery that are stable in extreme environments.

The long-term goal of the toolkit is to enable computational re-design of biological systems for industrial-scale resource processing and utilization in space applications, for example, efficient excavation and transport of resources in extremely cold (e.g., a permanently shadowed lunar crater), dusty/abrasive, and/or micro-g environments (e.g., Moon, asteroids, comets, Mars), *in-situ* propellant manufacturing, solar power batteries from lunar and Mars regolith, and nano-scale manufacturing for life support.

LT-17: ChimeraBrick: An Extended Placeholder Standard for Operon Assembly and Tuning

Norman Wang, research scientist at the University of Hawaii, discussed the ChimeraBrick standard. Constructing successful expression vectors often requires tuning of gene regulatory modules, where too little expression poses difficulty in isolation or visualization of the desired product, while too much expression can lead to slow growth or death of the host because of excess metabolic burden. Therefore, to facilitate testing and selecting the proper BioBrick parts and/or short synthetic fragments of DNA (usually promoters, ribosomal binding sites, and transcriptional terminators), he proposed a placeholder standard that is compatible in parallel with the BioBrick BBa series of restriction enzyme site overhangs. These restriction enzyme sites are backward-compatible to fuse with parts that have been excised by BioBrick BBa restriction enzymes. The ligation of BioBrick and ChimeraBrick restriction enzyme overhangs fuse parts together, producing an uncuttable mixed site. Thus the ChimeraBrick standard provides a second set of BioBrick compatible restriction enzyme overhangs that allows nesting a maximum of two ChimeraBrick placeholder sites within a traditional BioBrick BBa part. It features idempotent assembly just like the BioBrick BBa standard, and has some backward compatibility for incorporating and interchanging with BioBrick BBa parts and plasmids.

VI. What New Scientific Questions Arise from Combining Synthetic Biology and Space Missions?

VI.1 Why Not Pursue Synthetic Ecologies?

Dr. Roger Brent, now at the Fred Hutchinson Cancer Research Center, discussed why NASA should consider pursuing synthetic ecologies in its exploration mission. He was excited to be at the workshop for in his words "what do we stand for as a people if not for exploring space?" He gave a special thanks for Francesc Godia for his fine discussion of the MELiSSA project, as it provided an illustration of what he intended to speak about.

To set the stage, he presented an overview of how the field of biology has evolved. Before synthetic biology evolved as a self-identified field, it was clear that *fabrication* presented technical, but not conceptual problems. The first problem is the design of new parts that were never touched by evolutionary history. True *de nova* protein synthesis of simple individual protein parts was only successful in the 1980s, but is now routine. A second, still more severe problem, is *composition* of individual parts into new functional assemblies (aka devices). It is possible that protein parts which make up existing biological systems have a small number of key attributes and functional interactions. If so, engineers could more fully enumerate and quantify the interactions between parts, and then generate a standardized parts list to be stored in a registry. However, for protein components that come from evolved biological systems, the functions of the components and their functionally important interactions are usually not known exhaustively. Those functions that are known cannot always be quantified. Similarly, many of functional interactions among protein components in evolved biological systems are likely unknown, and most of these interactions embody 3.5 billion years of historical accident. For these reasons, devices composed from current standardized parts rarely work as intended without considerable troubleshooting. Thus the difficulty of the composition of devices from standard parts is considerable. Creation of an enzyme active site is barely possible with 2010 technology. Protein design from scratch is still a dream. Nature figured out how to do it, but over a very long time.

Given the difficulties that composition from evolved parts will continue to present for the development of *de novo* devices and for organisms to achieve engineering goals, an alternative for NASA is to pursue synthetic ecologies for things like the provision of stuff and terraforming. It is not clear that the functional interactions among components of ecosystems will be simpler than functional interactions among the protein components of cellular biological systems and devices. But there are some reasons to think that they might. Ecosystems have not had enough time to develop complicated interdependencies. Ecosystems are sustaining, that is they keep themselves on track with time. This concept could equally be applied to ecosystems on Mars or on board a spaceship. He contrasted this self-regulating capability to the MELiSSA project, where considerable control and regulation was required to keep the system running properly.

The key question that was raised was whether synthetic biology could be applied to existing ecosystems, such as the one on the ISS? His response was that we ought to characterize the microbial ecology on the ISS and then engineer it to suit NASA's needs

VI.2 Synthetic Extremeophiles and the Limits of Life

Dr. Lynn Rothschild discussed what is known about extremophiles and what they tell us about the limits for life. She made the important point that there is already on Earth an incredible toolkit of evolved organisms and metabolic pathways. There is a large commercial application for extremeophiles—be they thermophiles (extreme temperatures), halophiles (extreme salinity), or alkaliphiles and acidophiles (extreme pH). She noted that while evolution has created a large diversity of life, it is not limitless. There are three classes of constraints: formal constraints that result from the physical laws of nature, historical constraints that reflect the result of evolutionary history, and developmental constraints, such as the differentiation of cell types. Extremophiles help us learn about these evolutionary constraints. Space poses an even greater challenge for organisms because of extremes in atmospheric composition, gravity, vacuum, temperature, nutrient sources and radiation.

Dr. Rothschild showed some examples of extremophiles. The temperature limits for different forms of life are shown in figure 6. The archaea *Methanopyrus kandleri* currently holds the temperature record at 121ºC. Similarly there are organisms that can live in a range of pH from boiling

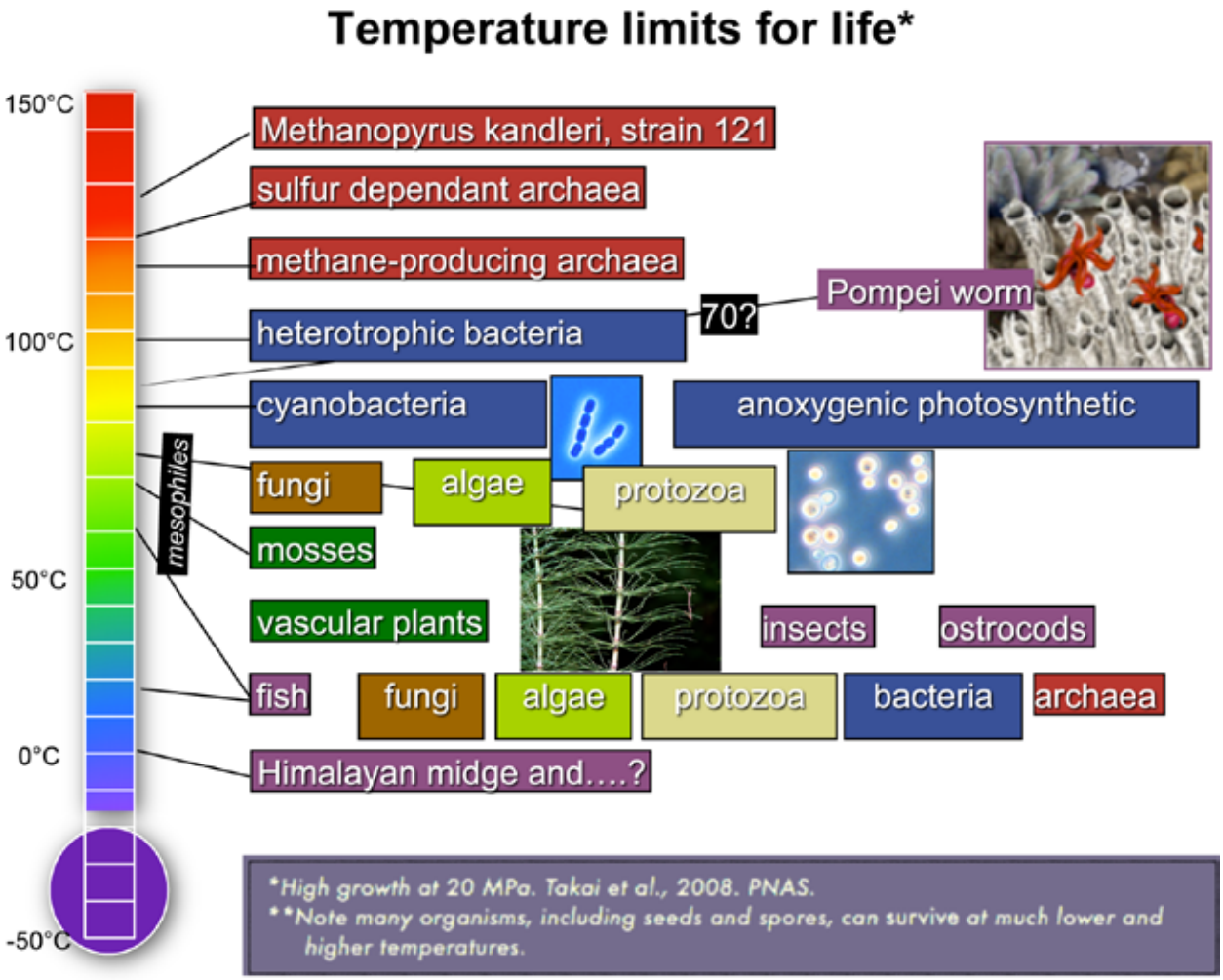


Figure 6. The temperature limits for different forms of life.

acid to strong bases. There are halophiles that can survive in pure salt and organisms that can survive extreme desiccation. One of the greatest challenges that space presents is an extreme radiation environment. Unlike Earth, high-energy ionizing radiation is an issue outside of the protective ozone layer. Nevertheless there are bacterium such as *D. radiodurans* and *Chroococcidiopsis* that can survive high levels of radiation. She showed several examples of how extremophiles adapt to extremes. Generally the adaption is relatively small, because life is lazy and finds an optimal means of survival. For example, acidophiles maintain a neutral pH by either having a strong proton pump or through having low proton membrane permeability. She concluded her talk by discussing the potential of "biomining" the genome of extremophiles. Why not take some of these amazing evolutionary adaptations and transfer them to species of interest? Exploring the toolkit of evolved organisms and metabolic pathways on Earth will unleash the full potential of synthetic biology in space. One such organism that offers promise is *Chroococcidiopsis*, which appears in nearly all extreme environments.

The first question dealt with whether multicellar organisms could be extremeophiles. She noted that metazoans can be extremophiles and even humans can deal with high levels of O_2. To the question of have we looked at the evolution of the interaction of different extremophiles, she responded that microbial mats were a perfect example. It was asked if we had a facility at Ames that could mimic Martian conditions. She responded not currently, but that carrying out research under these conditions would be worthwhile. Finally she made a strong plea that evolution is not a nuisance—it provides us with an amazing toolkit that we should use to our advantage.

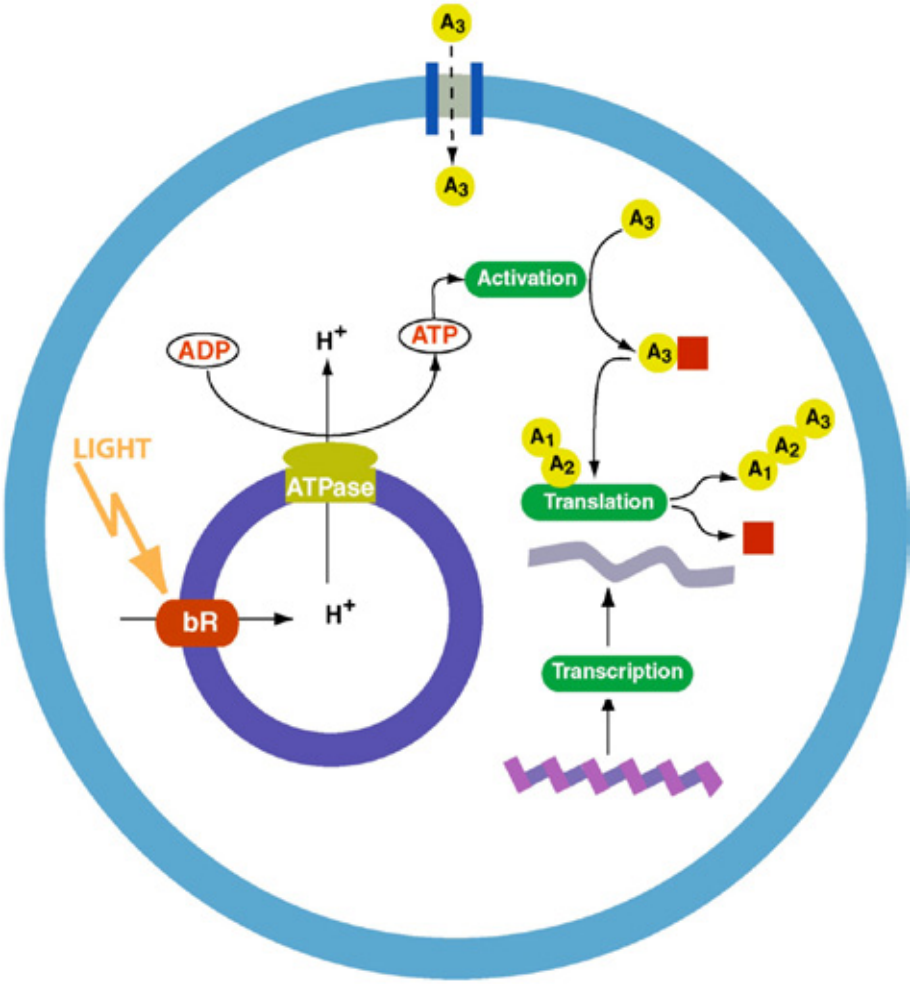
VI.3 Artificial Cells for Space Applications

Dr. Andrew Pohorille, research scientist at NASA Ames Research Center, discussed the creation of artificial cells for space applications. He discussed six different but related concepts of cell types. Specifically, he discussed re-engineered cells, which are cells tuned for a specific metabolism, minimal cells that have a minimal genome or minimal function, protocells that are very simple cell-like structures on the early evolutionary pathway, synthetic cells, artificial cells that have biological functions, but are put together differently than in nature, and functionalized liposomes, artificial cell-like structures that do not replicate. He mentioned the protocell website (http://www.protocell.org/) for a link to the research efforts to understand and harness the basic principals of chemical living systems. He enumerated the traits of artificial cells, which are closely related to the criteria for a minimal living system. These traits include template-directed synthesis of information polymers, transduction of external energy to drive chemical reactions, catalytic activity coupled to regulation, a boundary membrane, a mechanism for division, and mechanisms to communicate with and respond to the environment.

He noted that all classes of protein structures that exist in nature have been identified. Since many other protein structures appear to be possible, why are they not observed in nature? One possibility is that the initial set of protein structures were selected through extensive "evolutionary pruning" Alternatively, the initial set of protein structures could have been selected through "evolutionary accident". To distinguish between these possibilities he discussed results of studies that combine *in vitro* protein evolution with computational design. As an illustration, he showed the first protein that has no biological ancestry, but instead was evolved from a random sequence of amino acids.

This protein binds adenosine triphosphate (ATP) but has sequence and structure different from ATP-binding proteins in nature. In another example, a protein has been evolved that has novel structure and joins two fragments of RNA, a function not performed by biological systems. These examples demonstrate enormous opportunities for creating artificial cells endowed with proteins designed for specific functions that might extend beyond the capabilities of biological systems, for example, for *in situ* resource utilization and building of new materials.

To illustrate that creating artificial cells remains a challenge even if their components have already been tested and shown to work separately, he discussed an attempt to build a system in which energy transduction is coupled to cell growth. The energy transduction system consisted of two proteins: *bacteriorhodopsin* (BR) that takes light to pump protons and TF_0-TF_1 ATPase that uses the proton gradient to generate ATP. ATP, in turn, was used to synthesize the building material for cell walls from fatty acid precursors. This process is shown in Figure 7. Despite its apparent simplicity, the system never worked because BR and ATPase require Mg^{2+} ions to work, but Mg^{2+} ions complex with fatty acids causing them to precipitate out. This was because in biological systems concentrations of Mg^{2+} ions are different in different cellular environments, but similar segregation was not achieved in the design of the artificial system.



Pohorille A. and Deamer, D. (2001) Artificial cells: Prospects for biotechnology, Trends Biotechnol., 20:123-128.

Figure 7. The energy transduction system consisting of two proteins: *bacteriorhodopsin* (BR) that takes light to pump protons, and TF_0-TF_1 ATPase that uses the proton gradient to generate ATP.

Dr. Pohorille ended by discussing the need to determine if terrestrial life (or the artificial cells we create) can expand into environments in space and adapt to these conditions over time. If terrestrial life can survive in space then it is probably not unique to Earth. To successfully re-engineer life to survive and thrive beyond Earth, we first need to establish its limits in space. A question was asked about how the metal availability, which changed with redox conditions on the early Earth, might have affected the evolution of proteins and more specifically, whether he had looked at novel metal/protein fold combinations. He noted that metals not only play a functional role but also help stabilize the structure of proteins. It is likely, therefore, that metals were important in the evolution of proteins from the outset.

VI.4 Role of Synthetic Biology in Developing Specialized Cyanobacteria for Long-Term Space Flight

Dr. Louis Sherman, professor at Purdue University, discussed their research on cyanobacteria. The genomic plasticity of cyanobacteria makes it an excellent system for the analysis of photosynthesis and metabolism, genetic diversity, and the role of gene duplication and evolution. Cyanobacteria have important potential applications to NASA's missions, since along with plants they can provide both food and oxygen as part of a bioregenerative life-support system for long spaceflights and at colonies on Mars. Their initial work was performed with the unicellular, diazotrophic cyanobacterium *Cyanothece* sp. ATCC 51142, which fixes nitrogen when combined nitrogen is limiting, and evolves O_2 during light-driven photosynthesis. The *Cyanothece* strains have an important attribute of temporally separating the oxygen-sensitive nitrogenase activity into the dark and performing photosynthesis during the day (see figure 8). During photosynthesis, fixed CO_2 is stored in large glycogen granules that are used as a substrate for respiration in order to help protect the nitrogenase during the night. Thus, the cell is a natural bioreactor for the storage of solar energy with subsequent utilization at a different time. Recent work has shown that these organisms can convert this stored energy into specific biofuels such as H_2, lipids and alkanes.

Synthetic biology can be used to alter cyanobacteria in a number of ways, such as altering and augmenting metabolite production or storage for specific purposes. Examples of some of the ways that *Cyanothece*, and a model cyanobacterium *Synechocystis* sp. PCC 6803, can be manipulated for such purposes were shown. These included anaerobic growth of the strains and alteration of the Photosystem II reaction center. Secondly, creating a two-component regulatory system by putting one copy of the gene on the chromosome and a second on a plasmid. These operons are now regulated differently and have significant impact on the relationship of photosynthesis and heterotrophic metabolism. In addition, a mutant in this operon grows better anaerobically. Finally, he showed how a gene knockout system in a strain of *Cyanothece* could be used to improve H_2 production.

A question was raised concerning how low O_2 conditions could be maintained, since O_2 was produced in growth. He noted that the system is not strictly anaerobic, but that the O_2 never gets above 0.1%. He was also asked whether he had changed the diurnal cycle. He replied that the diurnal cycle can be modified from 6 hr light to 24 hr light, but that nitrogenase activity and H_2 production continue to oscillate, but damp with time.

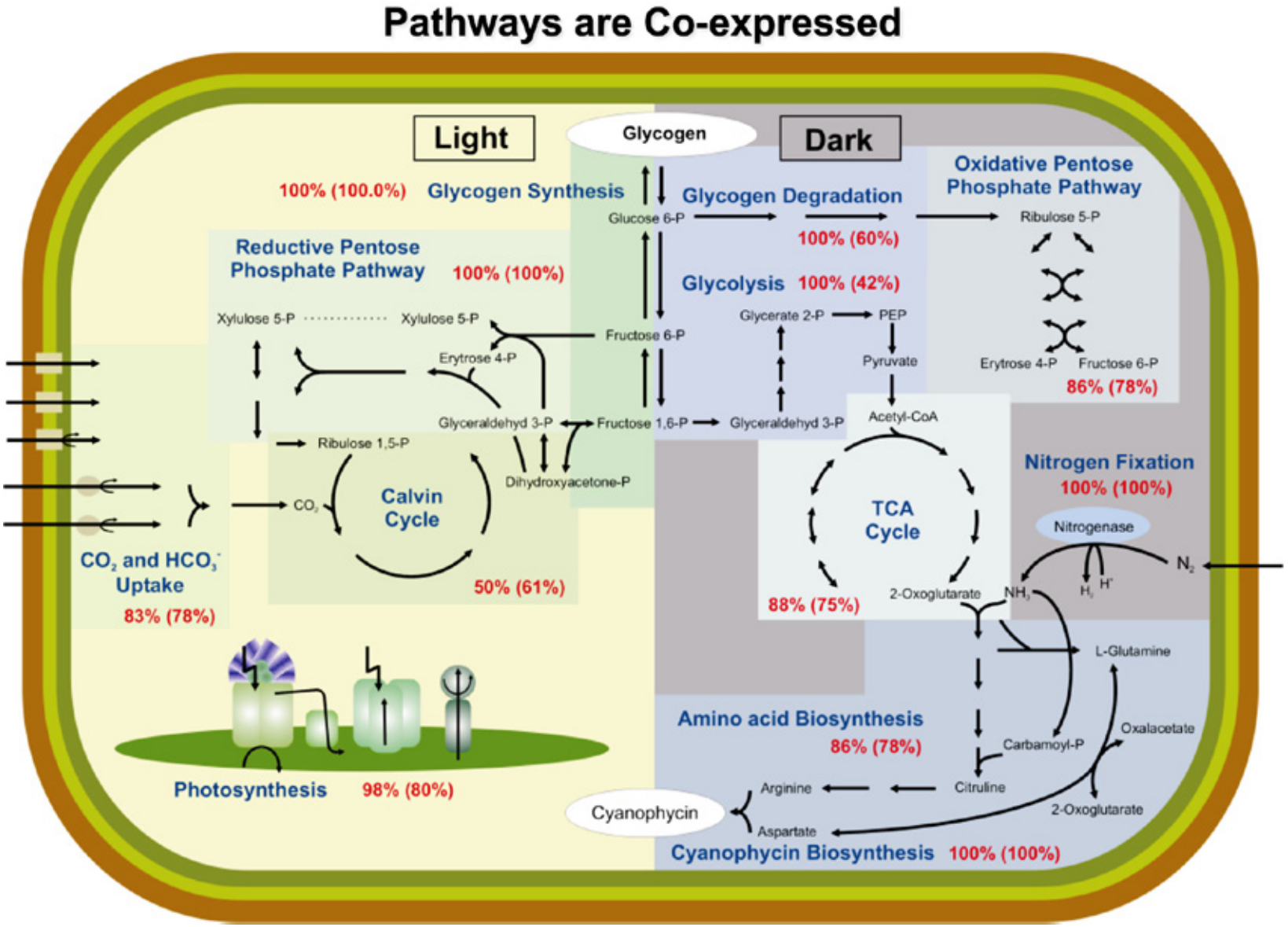


Figure 8. The light and dark pathways of cyanobacterium Cyanothece.

VII. What are the Broader Ethical and Societal Implications of Engineered Life in Space?

VII.1 Ethical Issues in Synthetic Biology

Jacob Moses of The Hastings Center spoke about the ethical issues that synthetic biology raises. His presentation drew from an interdisciplinary project at The Hastings Center that has engaged scientists, engineers, philosophers, social scientists, public policy experts, and theologians in a sustained examination of the ethical and moral concerns at stake in synthetic biology.

Ethics considers both the potential benefits and harms. It is important to consider these now as synthetic biology seeks to develop into a field of consequence. Its engineering orientation has invited many to imagine whole new classes of applications, from the biofuels to pharmaceuticals. Indeed, it is precisely these potentially significant consequences that have also spurred discussion about the potentially significant risks to human welfare and environmental health. He divided the concerns into two classes: physical concerns about consequences and non-physical or moral concerns.

Some of the physical concerns include bioterror, the possibility that new techniques in synthetic biology could enable bad actors to resurrect bad germs. This has low probability, but potentially catastrophic consequences. However, any tool of consequence can lead to bad uses. Biosafety is another concern. This includes accidental release and planetary protection (discussed in detail in later talks). Generally there is widespread agreement that physical harms are valid concerns, but there are different estimates about their likelihood and importance; how best to weigh and address these risks remains a large question.

The non-physical concerns are more difficult to articulate, but include our understandings about life on earth, the human relationship to the natural world, as well as scientific freedom, justice, and access to the benefits of technology. For example, one of the concerns about synthetic biology is that it is an inappropriate role for humans to create artificial life. This concern could draw in part from religious arguments, but it could also stem from an environmental view that sees life as ineffable or special. However, there are also nonphysical reasons for advancing synthetic biology, for example, the intrinsic benefit of the excitement about the "existential pleasures of engineering" or working together on difficult problems even if tangible benefits are not immediately forthcoming. A specific example is the iGEM competition that occurs annually.

He ended the presentation by discussing the range of potential policy responses from restrictive regulations to further discussion and study about the meaning and implications of this work. He cited a 2010 public opinion poll that showed a significant increase in those opting for "the risks will outweigh the benefits" category after hearing a short description of the technology. The appropriate policy may well depend on the potential for harm. For example, the act of intentionally releasing a known pathogen into nature should be treated differently than careful laboratory research employing safeguards.

Some of the questions dealt with the poll and the somewhat disturbing finding that more people went from undecided to feeling that the harms outweigh the benefits after hearing a short synopsis of the technology. Was this just a question of individuals needing better education or suggestive of a deeper value difference that cannot be completely closed by providing additional information? Also, it was suggested that moral and psychological issues are often intertwined. Another suggestion was that part of the problem could be understood as a part of larger tensions between nature and human technology.

VII.2 Safeguarding the Crew and Engineering Systems for Human Missions

Dr. Kasthuri Venkateswaran of the Jet Propulsion Laboratory discussed engineering systems for human missions. He gave examples of what NASA's needs, gaps, expectations, and requirements are for human habitation of other planets. Microbial detection and mitigation systems will be paramount to prolong the longevity of human habitation on other planets. Since life-support processes will promote the proliferation and colonization of microbes, we need validated environmental monitoring systems and control strategies. Such systems are crucial to preserve acceptable microbial burden levels in human compartments, ensure negligible interference of false-positives with life-detection experiments, and to prevent the inadvertent exposure of humans to extraterrestrial materials.

Planetary protection policies derive from international treaties whose goal is "to preserve our ability to study other worlds as they exist in their natural states; to avoid contamination that would obscure our ability to find life elsewhere—if it exists; and to ensure that we take prudent precautions to protect Earth's biosphere in case it does." Mandates are in place to minimize the likelihood of catastrophic outcomes as a result of human-associated cross-contamination between solar system bodies. To meet planetary protection obligations, NASA needs an integrated microbial monitoring system validated in a terrestrial Mars analog environment. Such a system is essential for human missions to comply with requirements to avoid harmful contamination and thereby facilitate the search for extraterrestrial life. The proposed integrated microbial monitoring system will bolster confidence in, and lend support to, planetary protection efforts, hardware reliability, and sustained crew health. By forewarning human explorers of any significant fluctuations in microbial burden, the system allows the crew to take immediate actions to significantly diminish any threat to crew health, or deterioration of the habitation module resulting from bio-corrosion. This approach will strive to directly integrate the technologies proposed herein with those being developed for robotic sample return missions, thereby providing a cradle-to-grave planetary protection implementation capability for human exploration.

A question was asked about whether we could add a barcode to identify Earth microbes. His response was that this would be problematical considering that humans are inhabited by 10^{14} microbes. It was noted that planetary protection policies do not apply in Low-Earth Orbit (LEO). Thus the need for monitoring for planetary protection reasons is only required beyond LEO.

VII.3 Astrobiology at the Maker Faire

Dr. Chris McKay, planetary scientist at NASA Ames Research Center, addressed long-term issues where synthetic biology could contribute to NASA's exploration mission. Specifically, he suggested using synthetic biology to reconstruct Martian life from the fragments preserved in ancient ice, and to create five types of super-microbes that could survive on Mars. These objectives are consistent with the overarching goal of astrobiology and society to enhance the richness and diversity of life in the Universe. The implied activities are to search for a second genesis of life on other worlds and to expand life from Earth.

Dr. McKay made the point that ethically our search for a second generation of life on another world should be done in a manner that is biologically reversible. He noted that at least three possibilities exist for past life on Mars: (1) there was no life; (2) it was related to Earth life (i.e., is on the tree of life (see common ancestor in figure 9)); and (3) it was a second genesis unrelated to Earth (see aliens on far right in figure 9). It is this third possibility that raises the greatest ethical issues. He noted that the best place to find life was to deeply drill in the ancient ice that still retains its crustal magnetism. One would not expect to find anything alive due to radiation and thermal

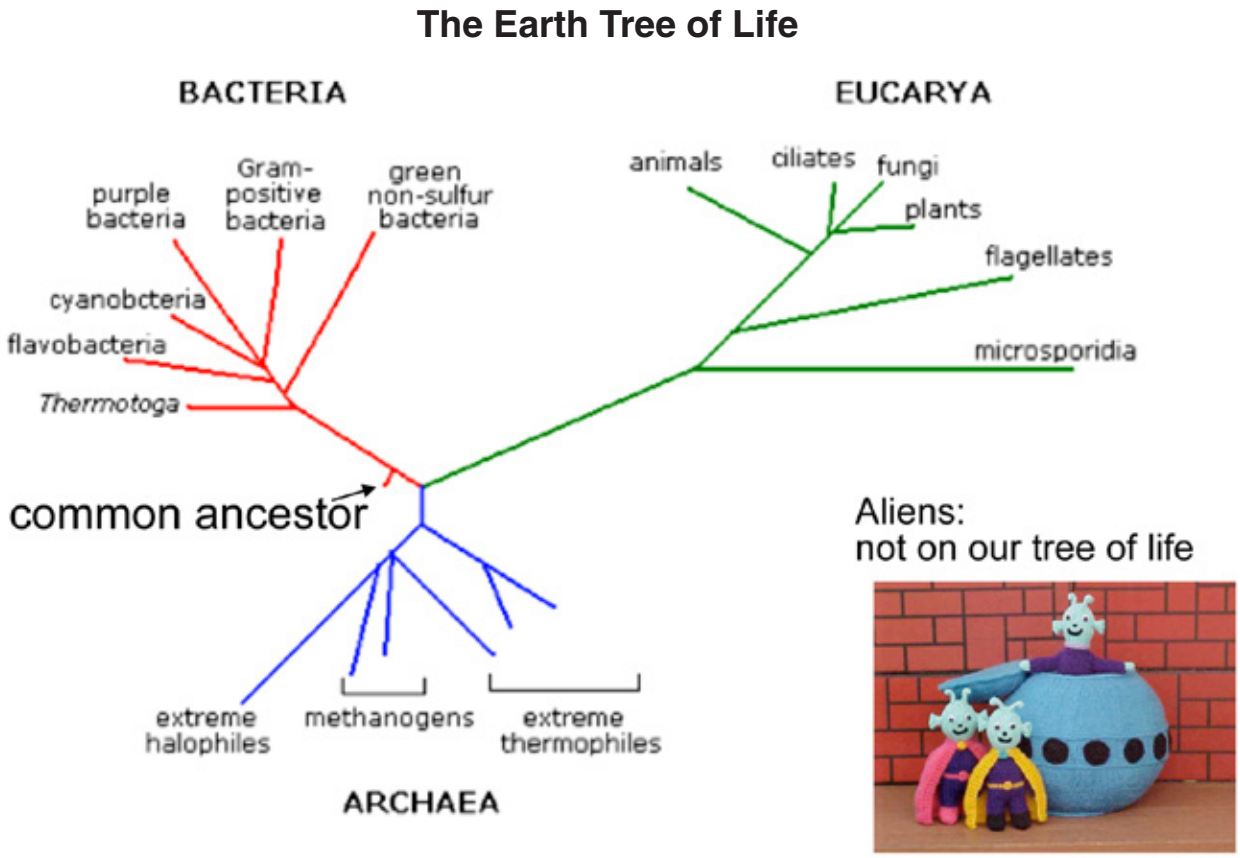


Figure 9. The Earth tree of life.

decay, but synthetic biology could restore the genome from the fragments of dead life. Preserving a second genesis is a worthy pursuit for several reasons. It derives from the fundamental, ethical principles related to the value of life and the value of diversity in life; there is a utilitarian benefit that comes from direct study of a second genesis, and restoring life and a biosphere to a dead world is a worthy goal for space-faring people.

Dr. McKay talked briefly about how synthetic biology could play a role in terraforming Mars. The first step is to determine if life from Earth can grow on Mars. A near-term mission would be to try to use the Martian soil and atmosphere for a plant growth module. A longer-term goal would be to determine if Mars could be restored to habitability. An optimal strategy for warming the planet would be to release a combination of perfluorcarbons (PFCs) (e.g., CF_4, SF_6, C_2F_6 and C_3F_8). It would take an estimated 100 years to warm the planet, but 100,000 years to produce ample O_2. This is where his second request for five types of super microbes comes in. First, all of the super microbes would have to be resistant to high ultra-violet light and oxidant concentration, low water, cold, and perchlorate. The five types of super microbes needed are super-weathering, organic producers, wood-making (O_2 releasing), PFC makers, and N_2 fixing at low N_2 pressure.

This thought provoking talk produced a lively discussion. He was asked if it was necessary to determine if a second genesis exists before we terraform. His response was to emphasize the importance of exploring in a bioreversible manner. Drilling down into an aquafer or in the ice would potentially be non-reversible. Current standards of planetary protection would not be adequate—medical standards are required. Considering the history of mankind, why would one expect that the exploration of Mars could be done in a controlled manner? Mars is not an environment where individuals can go on their own (a good analogy is Antarctica where man's footprint on the continent has been controlled). Mars does not have a magnetic field—does this pose an issue for terraforming? Probably not, Mars once had a thick atmosphere. Why construct life from fragments in the ice if there is an aquafer? Good question, but this is probably not going to happen. We will be lucky to find something dead for a billion years.

VIII. How Does Space Synthetic Biology Pick up on the Broader Agenda?

VIII.1 Self-Sufficient Life Below the Planet Surface: A Chassis for Survival in Energy Poor Environments

Dr. Adam Arkin, professor at the University of California at Berkeley, espoused the capability of bacteria to survive in diverse environments. Bacteria are capable of environmentally transformative processes to extract energy from the environment, to produce complex chemicals and materials from simple building blocks, to transform soil and water, and to self-organize into superstructures. In addition, their simplicity makes them relatively easy to engineer. He talked in some detail about what environmental genomics reveals about a single slowly evolving species in a gold mine deep beneath the surface. This work is published by T.C. Onstott at Princeton University. These self-sustaining bacteria live in rocks deep below the surface and draw their energy from chemicals produced by the radioactive splitting of water molecules.

To be more specific, the analysis was based on a DNA sample from fracture water collected at a depth of 2.8 kilometers in a South African gold mine. It was sequenced and assembled into a single complete genome. One bacterium composes over 99.9% of the microorganisms inhabiting the fluid phase. The bacterium was named *Candidatus Desulforudis Audaxviator*, meaning "bold traveler" motivated from Jules Verne's "Journey to the Center of the Earth." Its genome indicates a motile, sporulating, sulfur reducing chemoautotrophic thermophile. It is capable of fixing both nitrogen and carbon. It is an example of a natural ecosystem that appears to have its biological component encoded within a single genome. An illustration of the bacterium is shown in figure 10. The genome contains 2.3 million base pairs and 2241 Open Reading Frames (ORFs). It is about half the size of *Escherichia coli*, but has considerable machinery. It is capable of transport both into and out of the cell. The bacterium is extremely slow growing and because of that there are few mutations. Based on an analysis of the Single Nucleotide Polymorphisms (SNPs), they estimated that the subsurface residence time for the fracture water was in excess of 3-5 million years.

The final aspect of the presentation considered whether this bacterium would be capable of living in the subsurface of Mars where they would be protected from the harsh UV light. On the positive side, sulfates have been shown to be widespread on the surface of Mars and sulfate-brine solutions have been correlated with recent water activity. On the negative side, it is much colder on Mars than in the South African gold mine and there may be insufficient hydrogen to power the key energetic reactions. Nevertheless, subsurface sulfate reducers are a possible chassis for terraforming and for the biotransformation of the environment to produce chemicals, fuels, and materials for human use on other planets. This potential is increased by our ability to engineer these microbes.

There was a question about how these bacteria came to be in the gold mine. The current theory is that they evolved somewhere else and perhaps circulated through cracks in the rocks. There was a question about whether the radiation would cause water to split within the cell. His response

was that they survive the radiation by assembling into biofilms. Someone asked if you could grow the bacteria faster. He responded negatively saying that this was an object lesson in how to build a self-sustaining ecosystem.

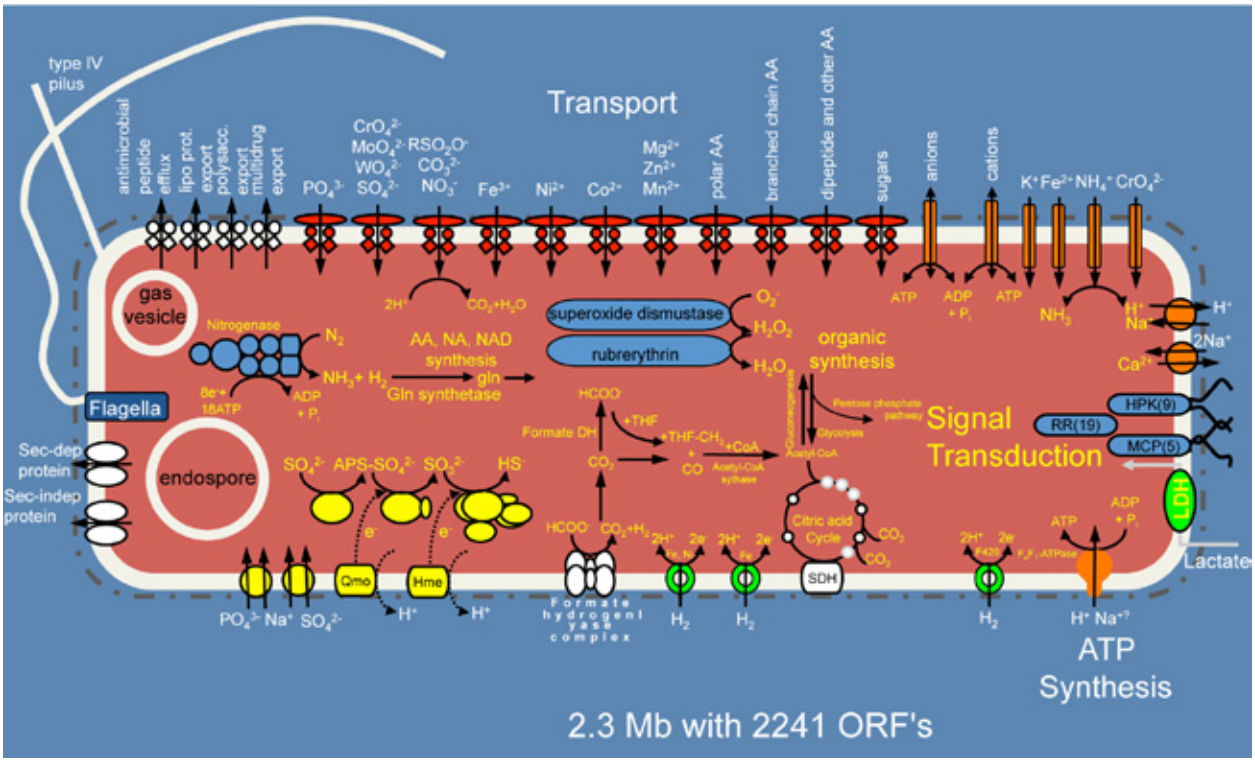


Figure 10. Illustration of the bacterium named *Candidatus Desulforudis Audaxviator*.

VIII.2 Modular Design of New Biological Functions in Lower Metazoans

Dr. Chris Anderson, professor at the University of California at Berkeley, discussed the design of a new model to engineer lower phagocytic metazoans. He started the discussion by recalling that biological solutions are always in competition with what can be done with physical or chemical solutions. For example, you can genetically modify trees so that they can live in Martian soil and atmosphere, or alternatively, you can bring a greenhouse to grow them under Earth-like conditions. Similarly, with bacteria you have an option to genetically modify them to live under Martian conditions or protect them in a bioreactor. The latter solution requires the machinery to purify and post-process the bacteria.

While there are some things you can process in self-contained boxes, such as self-isolating chemicals, most things are more difficult to separate. He suggested that solving problems like these will require new strategies of design, new methodologies of fabrication, and most likely, starting organisms distinct from the familiar yeast, *E. coli,* and mammalian chassis used in most synthetic biology projects. One particular class of organisms that may satisfy the needs of these applications

is lower metazoans. For example, in figure 11 a process is depicted whereby dirty water is purified. What organism do you put on the frit to affect the separation? Bacteria, which form biofilms, are insufficient, because they do not provide an impenetrable barrier. However, lower phagocytic metazoans, such as *trichoplax adhaerens* depicted in figure 12 may be the solution. It has four unique cell types and an epithelial layer on both the top and bottom. These metazoans have many favorable characteristics. For example, they are relatively fast growing, can grow in salt water, and they can eat algae, bacteria and debris. All have exposed and robust cytoplasmic membranes and some have plastids (organelles), and some form epithelia. They have the useful property of being able to be dessicated and quickly recover with the addition of water. Finally, they are capable of a G0 state, which means they can be metabolically active, but not dividing.

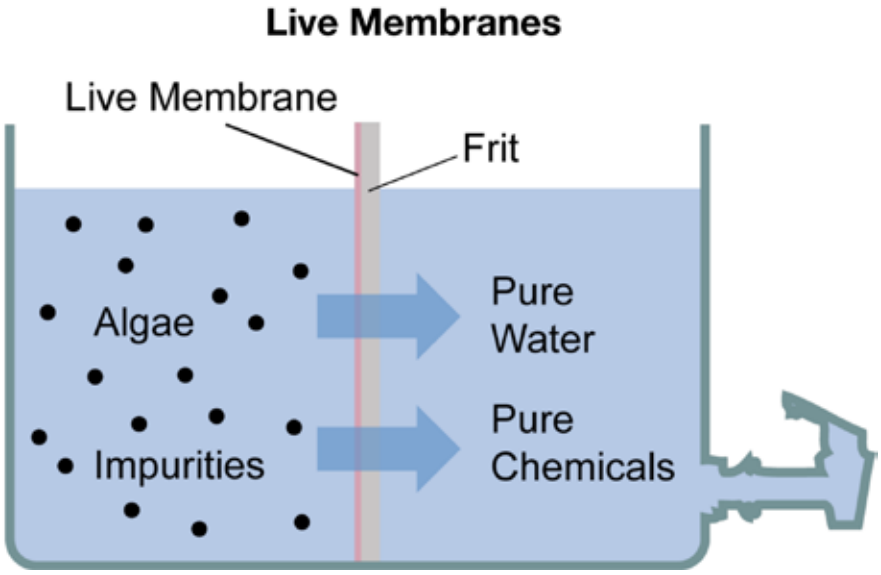


Figure 11. Depiction of a process whereby dirty water is purified.

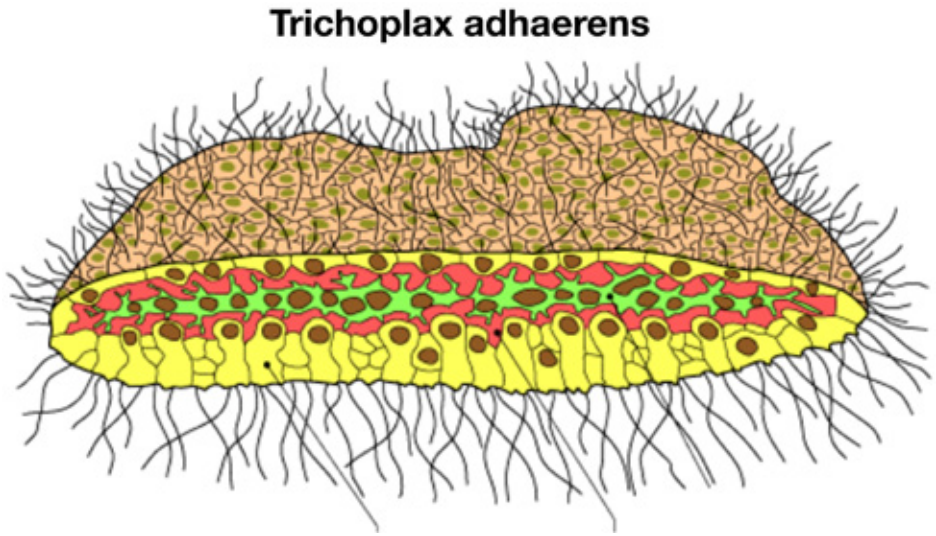


Figure 12. Depiction of trichoplax adhaerens, a lower phagocytic metazoan.

The issue with using them is that they are currently genetically intractable. The remainder of his talk focused on the effort in his laboratory to open up metazoans for genetic manipulation. Specifically, he described a payload delivery device for delivering proteins or DNA inside the membrane. Starting with a highly engineered bacterium (*E coli*), they use this as a vehicle for invading a Mammalian cell. The final result of the invasion process is a bacteria inside a cell inside a membrane. The issue is then how to pop all the membranes except the last one. They use a self-lysis device and a vaculole-lysis device to accomplish getting through the membranes. This work potentially opens up another class of organisms that can be genetically modified to accomplish specific tasks.

VIII.3 Synthetic Transcription Factors based on Engineered Zinc Finger Arrays for the Construction and Regulation of Gene Networks

Dr. Mo Khalil, postdoctoral fellow at Boston University, talked about the work being carried out in the James J. Collins laboratory. He started by discussing the need for expanding and diversifying toolkits for regulatory parts. He discussed a genetic toggle switch where the gene product of one state is used to repress the other state. He noted that the composition of parts is important, and that considerable fine-tuning of parts is required to achieve the desired behavior. Three principal goals of synthetic biology are the design of logical forms of control, modularity (i.e., the ability to deconstruct networks into individual parts), and the development of diverse platforms from archaea to bacteria to eucarya. The key forms of cellular control include logic gates, switches, and dynamical systems, such as oscillators and filters. The inspiration for these forms of cellular control exists in nature to a large degree. He gave several classical examples of cellular control that exist in the literature. Key design criteria include programmability (to be able to understand or tune interactions between components), orthogonality (or the ability to tune specificity), and cooperativity.

The remainder of the talk was focused on engineering zinc finger proteins, which are a promising source of synthetic components that can be used as vehicles for targeting recombination, controlling transcriptional activity, and making circuit interconnections. In particular, the Cys_2-His_2 zinc finger domain, which is the most common and best characterized structural motif found in eukaryotic transcription factors, has been successfully harnessed as a scaffold for creating customized DNA-binding domains. Pools of such designed zinc fingers have been arrayed and subjected to a combinatorial-based selection method, termed OPEN (Oligomerized Pool ENgineering), to identify context-specific and highly optimized zinc finger arrays.

Dr. Khalil discussed the work in the laboratory to develop a synthetic platform for constructing artificial transcription factors (TFs) using zinc finger arrays, and to use them to build synthetic gene networks. Within this platform, their TFs display targeted gene activation from chromosomally-integrated synthetic promoters with minimal cross activation. Orthogonal and programmable TFs developed from this system will be key to the construction of complex next-generation networks, which are currently limited by the small repertoire of commonly-used regulatory components, and to the synthetic regulation of biological networks within higher-order (i.e., mammalian) systems.

The first question addressed the difficulty of getting zinc fingers to work in a synthetic system. He stated that selection methods have been the key limiting factor, and that this work shows promise because it merges a new, successful selection method with a plug-and-play synthetic system. The

second question concerned the types of orthogonality and whether some types were more important than others. His response was that it depends on the system and how tight the orthogonality needed to be.

VIII.4 Programmable Synthetic Systems and Materials in Synthetic Biology

Dr. Randall Hughes, postdoctoral fellow at the University of Texas at Austin, spoke about the creation of novel biological materials by augmenting their interactions with substrates or by incorporating new building blocks to change their function. He described their gene construction facility that works off a protein fabrication automation methodology. The design of synthetic schemes, oligonucleotide synthesis and databasing, and generation of robotic operations scripts are all automated in custom software. Total throughput with existing equipment is approximately 100-150 kilobases per week of novel genes.

One of the things that the gene construction facility is being used for is biosensor discovery and development. By taking advantage of bacterial metabolic sensing capabilities, bacteria can be modified to serve as platforms for sensing small molecules. He showed how a phosphonate binding protein could be modified and optimized to give greater sensitivity for detection. The application of protein fabrication automation technology to scan for unnatural amino acids was briefly discussed. He also showed how the application of bio-prospecting and synthetic gene construction could be used for antibody repertoire discovery. They were able to create approximately 200 recombinant antibody genes in just three weeks. This rapid discovery is only possible with high-throughput DNA sequencing and analysis.

The final discussion topic was nucleic acid operating systems, which touched on the capabilities that biology presents in doing computation. Some characteristics of biological systems are that all units (cells) hold and execute the same program, the units have limited memory and bandwidth, and they have no knowledge of position, so that only local communication is possible. Biological systems are best described by the term “amorphous computing”, which refers to computational systems that use very large numbers of identical, parallel processors, each having limited computational ability and local interactions. They have previously built amorphous computers, but the address space is ridiculously small. He showed an image of Charles Darwin made on a bacterial lawn using a light-directed genetic circuit. The image in figure 13 shows the resolution that is possible. This first bacterial photograph was part of an iGEM project at the University of Texas.

Dr. Hughes showed that the address space available to nucleic acids can be exploited to execute interesting algorithms. From an operating system perspective, the only biologically relevant systems that make sense are nucleic acids. From a synthetic biology perspective, nucleic acids are the only parts that are truly modular, composable, and scalable. Unfortunately, we actually want to do things with cells, the current unit of biological replication. He discussed protein-nucleic acid mimics that might be useful as programmable interaction tools. This would enable the monitoring of protein-protein interactions. One of the ultimate goals is to make a synthetic hormone that regulates gene networks.

Levskaya A., *et al. Nature* **2005** 438:441-2

Figure 13. An image of Charles Darwin made on a bacterial lawn using a light directed genetic circuit. Original publication source is Nature (438 (7067), 441-442, 2005).

IX. Research priorities: where do we go from here?

The final session was co-chaired by Dr. Pete Worden, ARC Director, and Professor Drew Endy of Stanford. The purpose of the session was to summarize key findings of the workshop and to discuss how we could maintain the momentum of the workshop. Dr. Worden began the discussion by making three points. The first dealt with what could be done in the near future, such as develop biosensors to understand the biome on the ISS, conduct experiments to help us tailor life off the planet using either the ISS or nanosatellites, and research on how to produce products such as edible food from bacteria that would promote long-duration spaceflight. Secondly, he discussed the establishment of a synthetic biology program at ARC, with opportunities for research fellows, and the possibility of having an iGEM team to work on NASA inspired projects. Finally, he indicated an interest in participating in the synthetic biology meeting at Stanford next summer and was exploring having a follow-on satellite meeting at ARC. He emphasized that the long-term goals of the synthetic biology research effort at ARC are to improve life on Earth and to extend and to find life on other worlds.

Dr. Endy indicated that he felt that NASA could contribute to the field in three basic areas—policy, tool development, and education. NASA could join with other agencies such as NIH to develop policy. He felt that NASA could contribute in such areas as planetary protection and biosecurity. NASA could also help build better tools to facilitate reprogramming something in space or on the Moon using transmission from Earth. He thought that developing a DNA toolkit for NASA's missions was a good idea. Responding to the fact that more people are inclined to fear synthetic biology after being briefed about its capabilities, he suggested that there may be an education deficit in America. NASA engages people of all ages and could help educate people about the potential good that synthetic biology could do for mankind.

Dr. Worden responded to Dr. Endy's comments. He addressed the need to balance mission specific needs with the development of foundational capabilities. He was looking at community development such as going to meetings, publishing in journals, and developing wikis. He thought it worthwhile to set up a working group to see what NASA could do in the area of policy and education. He mentioned the possibility of developing an external committee to advise NASA.

When the discussion was opened up, the first question from the floor was whether synthetic biology was the correct term for the proposed research effort given the political ramifications. Other terms that were suggested included molecular biology, systems biology, synthetic biotechnology, and adaptive biotechnology. Drs. David Bergner and Lynn Rothschild were given the specific action of forming a committee to determine the best term to use.

Another discussion topic that again arose in this final session was the unique opportunity to study the biome on the ISS. A better understanding of the current biome is needed before synthetic biology could be used to improve the bacterial communities. Monsi Roman took the action to form a committee to study this issue further.

Dr. Worden ended by discussing his desire to bring students to ARC and to give them problems in synthetic biology to solve. There is consideration for having a summer program next year in concert with the meeting at Stanford. He noted that students get excited about flying things, and that opportunities to fly something the size of a shoe box would likely manifest in the next 12-18 months. He challenged the community to come up with worthwhile experiments. The workshop ended with Dr. Worden wishing everyone God speed in your endeavors and Happy Halloween.

“Happy Halloween”

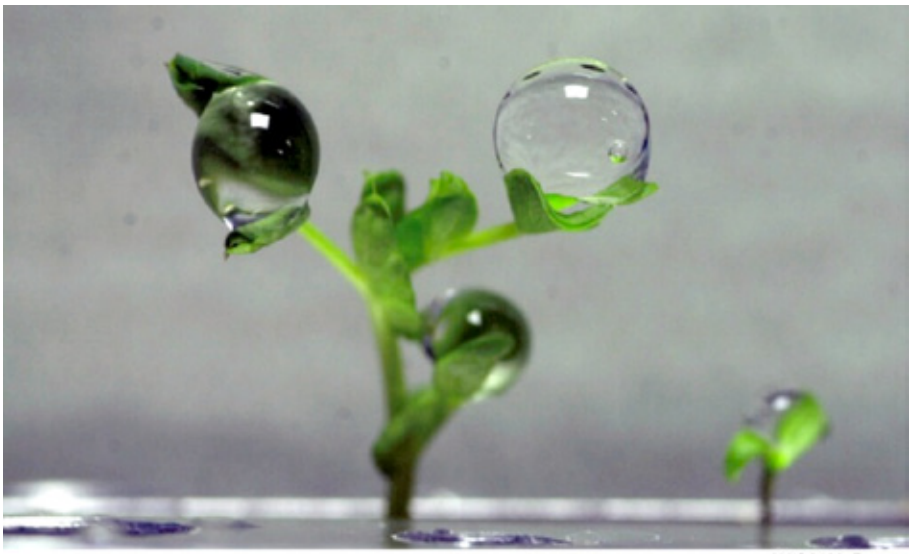


Figure 14. Picture of a plant with water droplets taken on the International Space Station. Physics manifests differently in microgravity.

Figure 15. Depiction of the bacterium, *Candidatus Desulforudis Audaxviator*, meaning “bold traveler”. It doesn’t really feed off radiation, but it draws its energy from chemicals produced by the radioactive splitting of water molecules.

Figure 16. The liverwort *Marchantia polymorpha*, descendant of the first land plants that helped terraform Earth.

AGENDA

What are the potential roles for synthetic biology in NASA missions?				
Agenda: Venue is 583C except Saturday evening.				
Saturday October 30th, 2010				
7.45-8.30	**Registration & breakfast**			
8.30-9.00	**Welcome & introductions:**			
	Logistics	Stephanie	Langhoff	NASA Ames, Chief Scientist
	Why this workshop?	Pete	Worden	NASA Ames, Center Director
	NASA's missions and the proposed relationship with synthetic biology	Lynn	Rothschild	NASA Ames, Astrobiology
	Introductions			
9.00-10.30	**1: Why take biology to space: past and present**	**Chair: John Hogan, NASA Ames**		
	Hardware requirements for biotechnology in space	John	Hines	NASA Ames
	MELiSSA: an approach to using biological systems for life support in Space	Francesc	Godia	Universitat A. de Barcelona
	Environmental Control & Life Support Systems on the International Space Station	Monsi C.	Roman	NASA Marshall SFC
	Bio/Tech/Eng Synergisms Needed to Enable Prod. & Aff. Plant Growth in Space	Cary	Mitchell	Purdue
10.30-10.45	**Break**			
10.45-12.45	**2: Why take biology to space: Future**	**Chair: Joel Bader, Johns Hopkins University**		
	Photosynthetic production of food molecules from bacteria	Jeffrey	Way	Harvard University
	Desirable traits in a bioleaching microbe for in situ resource recovery	Frank	Roberto	Idaho National Laboratory
	Synthetic microbes and rocks – geomicrobiology for human space settlement	Charles	Cockell	The Open University, UK
	The Synthetic Cell: From the Mind, to Life, to Space.	Michael	Montague	J Craig Venter Institute
	Synthetic Biology: Duct Tape for a Mars Mission	John	Mulligan	Founder, Blue Heron Biotech
	In Situ Resource Utilization (ISRU), state of the art and the potential for biology.	Jerry	Sanders	NASA Johnson Space Center
12.45-1.45	**Lunch**			
1.45-2.45	**3: Working groups on applications of synthetic biology to NASA's mission:**			
	Group 1: Biological in situ resource utilization	Jerry	Sanders	NASA Johnson Space Center
	Group 2: Biosensors	Chad	Paavola	NASA Ames, Bioengineering
	Group 3: Biomaterials and self-building habitats	Jim	Haseloff	Cambridge University
	Group 4: Synthetic biology & human health	Fathi	Karouia	NASA Ames
	Group 5: Life support for long term space travel & habitation	Cary	Mitchell	Purdue University
2.45-3.10	Feedback from working groups	**Break**		
3.10-3.30	**Break**			
3.30-4.45	**4: How does space synthetic biology pick up on the broader agenda?**	**Chair: George McArthur IV, Virginia Commonwealth U.**		
	Foundational synthetic biology technologies: parts registries, BioCAD	Nathan	Hillson	JBEI (Joint Bioenergy Institute)
	Genome engineering and biosensors	Srivatsan	Raman	Harvard University
	Synthetic biology and reshaping plant form	Jim	Haseloff	Cambridge University
5.00-6.00	**Synthetic Genomics - (located in building 3)**	Craig	Venter	JCVI
6.00-6.30	Brainstorming with Q & A			
6.45-8.15	**Informal dinner and reception in NASA Exploration Center**			
8.15-9.15	**Lightning talks (3-4 mins each)**			
	Computational Challenges in Design, Fabrication, and Testing of	Joel	Bader	Johns Hopkins University
	Building a tool kit for thermophilic cyanobacteria	Devaki	Bhaya	Carnegie Institution for Science
	Building a re-coded yeast genome powered by an army of undergraduates	Patrick Yizhi	Cai	Johns Hopkins University
	Searching for Extra-Terrestrial Genomes: A life det. instrument with bio. components	Chris	Carr	MIT
	The Biochemical Processing Unit (BPU): rapid DNA synthesis and parts prototyping	Peter	Carr	MIT
	Industrial scale fermentation	Christopher	DaCunha	EdenIQ
	Beyond iGEM: a pH-based biosensor for detection of arsenic in drinking water	Kim	de Mora	University of Edinburgh
	Paper-supported 3D cell culture for tissue-based bioassays	Ratmir	Derda	Harvard University
	Biologically Manufactured Building Materials	Ginger	Dosier	American University, UAE
	The Coupled Autotrophic Nitrous Decomposition Operation (CANDO)	Yaniv	Dror Scherson	Stanford University
	Algae to Biofuels Technology: from Metabolic Engineering to Synthetic Biology	Patrick	Fu	University of Hawaii
	Measuring the Performance Benefit of Synthetic Biology Systems	Jason	Held	Saber Astronautics
	An RNA-Based Platform for Gene Network Engineering	Julius	Lucks	UC Berkeley
	Metabolic eng. of microbes to support human space exploration in the post-genomic era	Wayne	Nicholson	University of Florida
	Synthetic Biology Data Group	Herbert	Sauro	University of Washington
	Bio-Nano-Info Lego Toolkit for Synthetic Space Biology	Alena	Shmygelska	Carnegie Melon University
	ChimeraBrick: An Extended Placeholder Standard for Operon Assembly & Tuning	Norman	Wang	University of Hawaii at Manoa
9.00-10.30	**Bar & poster session + define brainstorming sessions on whiteboards for following morning.**			

AGENDA

Sunday October 31st, 2010				
8.00-8.30	**Breakfast**			
8.30-10.00	**6: What new scientific questions arise from combining syn bio and space missions?**	**Chair: Roger Brent, Hutchinson Center, Seattle**		
	Why not pursue synthetic ecologies?	Roger	Brent	Hutchinson Center, Seattle
	Synthetic extremophiles and the limits of life	Lynn	Rothschild	NASA Ames
	Artificial Cells for Space Applications	Andrew	Pohorille	NASA Ames
	Role of syn bio to develop specialized cyanobacteria for long-term space flight	Louis	Sherman	Purdue University
10.00-10.30	Break			
10.30-11.45	**7: What are the broader ethical and societal implications of engineered life in space?**	**Chair: Margaret Race, SETI Institute**		
	Ethical Issues in Synthetic Biology	Jacob	Moses	The Hastings Center
	Safeguarding the Crew and Engineering Systems for Human Missions	Kasthuri	Venkateswaran	Jet Propulsion Lab
	Astrobiology at the Maker Faire	Chris	McKay	NASA Ames
11.45-12.30	**8: Brainstorming Working Groups**			
12.30-12.45	Feedback from brainstorming working groups			
12.45-1.45	**Lunch**			
1.45-3.15	**9: How does space synthetic biology pick up on the broader agenda?**	**Chair: Peter Carr, MIT**		
	Self-sufficient life below the planet surface: A chassis for survival in energy poor env.'s	Adam	Arkin	UC Berkeley
	Modular design of new biological functions in lower metazoans	J Chris	Anderson	UC Berkeley
	Syn. transcription factors from eng. zinc finger arrays for the const. of gene networks	Ahmad	Khalil	HHMI, Boston University
	Programmable Synthetic Systems and Materials in Synthetic Biology	Randall	Hughes	University of Texas Austin
3.15-4.00	**Discussion: Research priorities & agenda: where do we go from here?**	Pete	Worden	NASA Ames, Center Director
		Drew	Endy	Stanford University
4.00	Close			

List of participants for the Synthetic Biology Workshop

	Name	Affiliation
1.	Anderson, Chris	University of California, Berkeley
2.	Arkin, Adam	University of California, Berkeley
3.	Bader, Joel	Johns Hopkins University
4.	Bebout, Brad	NASA Ames
5.	Bergner, David	NASA Ames
6.	Bhattacharya, Sharmila	NASA Ames
7.	Bhaya, Devaki	Stanford University
8.	Bonaccorsi, Rosalba	NASA Ames
9.	Braxton, Lewis	NASA Ames
10.	Brent, Roger	Fred Hutchinson Cancer Research Center
11.	Bubenheim, David	NASA Ames
12.	Buchan, Bill	NASA Ames
13.	Cai, Patrick	Johns Hopkins University
14.	Carr, Chris	Massachusetts Institute of Technology
15.	Carr, Peter	Massachusetts Institute of Technology
16.	Catalina, Maria	Moon Mars Atacama Research Stations
17.	Chen, Ying-Ja	University California, San Francisco
18.	Cockell, Charles	Open University
19.	Correll, Randy	Ball Aerospace Technologies Corporation
20.	Couch, Jennifer	National Institutes of Health
21.	Cowell, Mackenzie	DIYbio.org
22.	Cumbers, John	NASA Ames
23.	DaCunha, Christopher	Eden iQ
24.	Daniels, Matthew	NASA Ames
25.	De Mora, Kim	The University of Edinburgh
26.	Derda, Ratmir	Harvard University
27.	Dosier, Ginger	American University of Sharjah
28.	Dyson, Esther	Edventure
29.	Endy, Drew	Stanford University
30.	Fleming, Erich	SETI Institute
31.	Garside, Brion	Graphic Artist
32.	Gilmore, Josh	Joint BioEnergy Institute
33.	Godia, Francesc	Universitat Autònoma de Barcelona
34.	Goldhaber, Sam	Graphic Artist
35.	Grace, Mike	NASA Ames
36.	Griko, Yuri	NASA Ames
37.	Haseloff, Jim	Cambridge University
38.	Held, Jason	Saber Astronautics Australia Pty Ltd
39.	Hessel, Andrew	Q Squared
40.	Hillson, Nathan	Joint BioEnergy Institute- LBL

	Name	Affiliation
41.	Hines, John	NASA Ames
42.	Hogan, John	NASA Ames
43.	Howard, Russell	Oakbio Inc.
44.	Hughes, Randall	University of Texas at Austin
45.	Huh, Jin	University of California, Berkeley
46.	Ishkhanova, Galina	NASA Ames
47.	Jan, Darrell	Jet Propulsion Laboratory
48.	Karcz, John	NASA Ames
49.	Karouia, Fathi	NASA Ames
50.	Katz, Leonard	SynBERC, University of California, Berkeley
51.	Kelly, Jason	Ginkgobioworks
52.	Khalil, Ahmad	Boston University
53.	Kliss, Mark	NASA Ames
54.	Kraft, Daniel	Stanford University
55.	Krieg-Dosier, Ginger	American University
56.	Langhoff, Stephanie	NASA Ames
57.	Loftus, David	NASA Ames
58.	Lucks, Julius	University of California, Berkeley
59.	Ludmila, Kisseleva-Eggleton	Expression College for Digital Arts
60.	Mancinelli, Rocco	NASA Ames
61.	Marshall, Will	NASA Ames
62.	Martin, Gary	NASA Ames
63.	McArthur, George	Virginia Commonwealth University
64.	McKay, Chris	NASA Ames
65.	Mitchell, Cary	Purdue University
66.	Montague, Michael	J. Craig Venter Institute
67.	Morrison, David	NASA Ames
68.	Moses, Jacob	The Hastings Center
69.	Mulligan, John	Blue Heron Biotechnologies
70.	New, Michael	NASA Headquarters
71.	Nicholson, Wayne	University of Florida
72.	Paavola, Chad	NASA Ames
73.	Partridge, Harry	NASA Headquarters
74.	Pengcheng (Patrick) Fu	University of Hawaii at Manoa Honolulu
75.	Perez-Mercader, Juan	Harvard University
76.	Pilcher, Carl	NASA Ames
77.	Pohorille, Andrew	NASA Ames
78.	Race, Margaret	SETI Institute
79.	Raman, Srivatsan	Harvard University
80.	Rappaport, Alain	Medstory
81.	Reddy, Michael	NASA Headquarters
82.	Reinsch, Sigrid	NASA Ames
83.	Reiss-Bubenheim, Debra	NASA Ames

	Name	Affiliation
84.	Roberto, Frank	Idaho National Laboratory (INL)
85.	Roman, Monsi	NASA MSFC
86.	Rothschild, Lynn	NASA Ames
87.	Sanders, Gerald	NASA JSC
88.	Santos, Orlando	NASA Ames
89.	Sauro, Herbert	University of Washington
90.	Scherson, Yaniv	Stanford University
91.	Schipper, John	NASA Ames
92.	Selch, Florian	Carnegie Mellon University
93.	Sherman, Louis	Purdue University
94.	Shetty, Reshma	Gingko Bioworks
95.	Shmygelska, Alena	Carnegie Mellon University
96.	Smolke, Christina	Stanford University
97.	Swan, Melanie	MS Futures Group
98.	Tarjan, Dan	University of California, Berkeley
99.	Venkateswaran, Kasthuri	Jet Propulsion Laboratory
100.	Venter, Craig	J. Craig Venter Institute
101.	Wang, Norman	University of Hawaii
102.	Way, Jeffrey	Harvard University
103.	Worden, Pete	NASA Ames

A group photo of the participants at the workshop on "What are the Potential Roles for Synthetic Biology in NASA's Mission?" held October 30-31, 2010 at NASA Ames Research Center.